**NANYANG
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**MINIATURIZED MOTORS: SELF-POWERED SYSTEMS
FOR WATER MONITORING AND REMEDIATION**

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Miniaturized Motors: Self-Powered Systems for Water Monitoring and Remediation

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"If you only read the books that everyone else is reading, you can only think what everyone else is thinking."

This quote is from the book, *Norwegian Woods*, by Japanese novelist Haruki Murakami. Taking on a PhD study is a road less travelled. However, it has taught me things beyond measure, and I am glad that I have made it my choice to take on this adventure. It has taught to be inquisitive and critical of data and information that now permeates around us. By embracing change as the only constant, it has gave me balance and aptitude to achieve more.

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Summary

Self-powered moving motors, which are able to carry out a plethora of operations, is an emerging field that has captivated the attention of researchers. The self-propelled motion of miniaturised motors represents the next step forward for applications in water monitoring and remediation systems. Fuelling the motion of particulates in aqueous environments results in the paradigm change from passively allowing particles to accomplish their work, to highly mobile agents that dynamically manoeuvre throughout the solution. We demonstrate the use of locomotion of motors to move in waters so that they can do work for environmental monitoring and remediation, where heavy metals can be monitored and removed using composite micromotors. Control of these self-propelled micromotors are investigated through the inherent design of the chemical fuels, such as inclusion of water as a co-reactant. The control of their locomotive behaviour of these artificial self-propelled moieties can be modulated through the change of chemical environments by tuning the pH, coupling light sources with photochromic additives, using ultrasonic manipulation with vibration of microbubbles. While monitoring the motion of self-propelled micromotors are of utmost importance, barriers remain in monitoring the motion of these autonomous moving entities due to limits of optical scrutiny. Through the utility of electrochemistry, properties of the micromotors and the environments they have been exposed to are quantified, providing an alternative pathway to independent monitoring systems. Motion of self-propelled devices traversing in aqueous environments, represents an opportunity to probe the environment and a utility to be maximised for pollutant management. We believe with the development of appropriate systems, shown in this thesis, miniaturized motors: self-powered systems for water monitoring and remediation will be realised.

Publications

1. Moo, J. G. S.; Pumera, M. Chemical Energy Powered Nano/Micro/Macromotors and the Environment. *Chem. Eur. J.* **2015**, *21*, 58-72. (Cover)
2. Moo, J. G. S.; Mayorga-Martinez, C. C.; Wang, H.; Khezri, B.; Teo, W. Z.; Pumera, M. Nano/Microrobots Meet Electrochemistry. *Adv. Funct. Mater.* **2016**, DOI: adfm.201604759. (Accepted)
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7. Moo, J. G. S.; Presolski, S.; Pumera, M. Photochromic Spatiotemporal Control of Bubble-Propelled Micromotors by a Spiropyran Molecular Switch. *ACS Nano* **2016**, *10*, 3543-3552.
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Thesis organization

The thesis is divided into 13 chapters. **Chapter 1** lists the objectives and the motivation behind each of the objectives listed. **Chapter 2** entails the introduction of self-powered miniaturised motors, the influence of environments and how they can carry out remediation work. Following which, in **Chapter 3**, it provides the context through literature review, where the current state-of-the-art environmental applications of self-propelled motors are introduced and analysed, with the control and monitoring of micromotors through electrical fields and electrochemistry.

For the first objective of using micromotors in pollutant management, **Chapters 4-6** describes the use of micromotors for detection and the removal of heavy metal ions. **Chapter 4** outlines the use of micromotor for the detection of heavy metals of lead and cadmium. Graphene oxide from different oxidation routes were used as sorbents for the removal of heavy metals and described in **Chapter 5**. In **Chapter 6**, the preliminary work on the coupling of graphene oxide in a platinum-based micromotor composite was used to remove heavy metals of cadmium.

For the second objective, from **Chapters 7-10**, we dwelled into how manipulating the conditions of the surroundings via chemical and physical means can influence and control the micromotor selectively. In **Chapter 7**, an alternative propellant of calcium carbide is investigated utilising water as a co-reactant via an encapsulation strategy. The

influence of the pH has a drastic influence on the performance of catalytic micromotors, where changes in activity and velocities were found in **Chapter 8**. Through selective utilisation of the light source with the presence of photochromic additives, the locomotive properties of single and swarms of micromotors can be tuned in **Chapter 9**. In **Chapter 10**, we explored the use of the bubble interactions in an acoustic field to manipulate micromotor motion.

For the third objective, we investigate the use of electrochemistry to monitor autonomously self-propelled miniaturised objects. In **Chapter 11**, a Marangoni-effect self-propelled capsule is monitored in a channel environment by electrochemistry. Miniaturisation of this strategy to track the motion of micron-sized catalytic bubble-propelled micromotors is accomplished in **Chapter 12**.

We conclude the thesis with a past, present and a grand vision for the future of using miniaturised systems for monitoring environments through self-propelled miniaturised systems for water bodies in **Chapter 13**.

The published version of the results shown in **Chapters 2-12** are shown in **Appendices**.

Index of Abbreviations

°C	Degree centigrade
at%	Atomic percentage
CV	Cyclic Voltammetry
DMF	<i>N,N</i> -Dimethylformamide
EDS	Energy Dispersive X-ray Spectroscopy
Eq.	Equation
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
FTIR	Fourier-Transformed Infra-Red
fps	Frame per second
GC	Glassy Carbon
GO	Graphene Oxide
h	Hour (time)
mL	Millilitre
mM	Millimolar
min	Minute (time)
μm	Micrometre
nm	Nanometre
SDS	Sodium dodecyl sulphate
SEM	Scanning Electron Microscopy
wt. %	Weight Percentage
XPS	X-ray photoelectron spectroscopy

Chapter 1

Objectives of Thesis

1.1 Objectives of Thesis

1.2 Objective 1: To Develop the Use of a Self-Propelled Miniaturized Device to Monitor and Remove Heavy Metal Ions

1.3 Objective 2: To Study the Influence of Environment and to Optimise Motor Performances

1.4 Objective 3: To Develop an Electrochemical Technique to Monitor Self-Propelled Miniaturized Systems

1.1 Objectives of Thesis

The rise of miniaturized artificial self-powered devices, demonstrating autonomous motion, have brought in new considerations from the environmental perspective. The research project addresses the interplay between these nano/micro/macromotors and the environment, recent advances and their applications towards pollution management. Such self-propelled devices are able to actuate chemical energy, into mechanical motion *in situ*, adding another powerful dimension towards solving environmental problems. Use of synthetic nano/micro/macromotors have demonstrated competence in environmental remediation, both in pollutant removal and contaminant degradation, due to motion-induced mixing. At the same time, the chemical environment exerts influence on the locomotion of the motors. These sensitized self-powered devices demonstrate capabilities in being deployed as sensors and their chemotactic behaviours, show efficacy to act as first responders towards a chemical leakage. Thus said, the notion of a self-propelling entity also entails further investigation into its inherent toxicity and possible implication as a pollutant. Development of novel independent systems to monitor their behaviours give rise to autonomous instrumentation. Future challenges and outlook of the employment of these miniaturized devices are discussed, with regards to the field of environmental remediation and monitoring, as we move towards their wider acceptance. We believe that these tiny machines will stand up to task as solutions towards environmental sustainability in the 21st century.

Environmental deterioration has been occurring at such a rapid rate that our natural resources are becoming depleted at an unsustainable rate. Water scarcity has become a pressing issue, where the pollution of water sources have drastically reduced the capacity of countries to provide for their burgeoning population.¹ Decentralization strategies, amidst rapid urbanization, allow for the rapid deployment of resources, where it is most needed.²

The rise of self-propelled miniaturized objects raises the promise of remotely powered devices that are able to do work untethered^{3,4} and reducing the contact time that ground workers have to spend handling toxic chemicals.⁵⁻⁷ These motors have demonstrated abilities of remote monitoring⁸⁻¹⁰ and remediation via rapid breakdown⁵⁻⁷ and removal,¹⁰⁻¹⁴ by using the fuel *in situ*. This removes the logistics of having to move bulky equipment to support such operations, creating an added dimension of robustness in handling environmental issues.

The pairing of autonomous self-propelled objects and nanomaterials, has provided new opportunities in the space of dynamic nanoscale motion-based environmental solutions.¹⁵⁻¹⁸ Optical observations of their locomotion and the motion-induced stirring have offered new blueprints towards solving problems entailing pollution. These have reduced reaction times and extended the reach of the active reactants to throughout the solution mixture. Furthermore, the inherent properties of the nanomaterial, with its reactive chemistry and high surface area to volume ratio, presents innovative solutions that could be exploited for the environmental cause.^{19, 20} Peripheral functionalization, have also wielded it with additional specificity and directionality, being able to home in to the site of interest with the help of an external force, such as using electromagnetic,²¹ magnetic²² or acoustic stimuli.²³

While self-propelled autonomous devices witnessed dramatic developments in the recent years, the systems monitoring their movement have not kept up and continue to rely on optical tracking which is difficult to automate and requires large processing power for analysis of recorded movies. Optical systems are also limited by transparency of the media/system and are constrained by the depth and field of view. A system that obviates these inherent limitations of optical tracking is urgently needed. Here, the remote positioning by an electrochemical method is investigated. It demonstrates efficacy in the

Chapter 1: Objectives of Thesis

determination of the location of an autonomous self-propelled capsule in continuous motion regardless of transparency or optical limitations. This novel method allows for the mapping of self-propelled motors and their surroundings, via the transmission of real-time information, in a channel environment. Such a system provides a break-through of monitoring limitations and allows for truly autonomous systems.

Hence, this thesis has three objectives that aim to use miniaturized motors as self-powered systems for water monitoring and remediation, as illustrated in Figure 1.1. Firstly, it is to develop a self-propelled miniaturized device to monitor and remove heavy metal concentrations. Secondly, it is to study the influence of the environment on the performances of the micromotor and improving their performances. Thirdly, we will be developing an alternative electrochemical technique to monitor self-propelled miniaturized system. The three objectives are shown in Figure 1.1 and described in the later part of this chapter and followed by an introduction in **Chapter 2** and a thorough literature review in **Chapter 3**.

Figure 1.1 Illustration of the challenges, objectives and solutions of the project.

1.2 Objective 1: To Develop the Use of a Self-Propelled Miniaturized Device to Monitor and Remove Heavy Metal Ions

Due to the recent rise in anthropogenic activities, such as industrial activities and indiscriminate disposal of refuse, environmental pollution has become a serious problem.¹ Detection of water pollutants is an important, but challenging task. Thus to say, to be proactive, rather than being retroactive in solving problems surrounding aquatic pollution is of utmost importance.^{2,3} Through early detection, appropriate preventive measures could be taken, without the actual need of a remediation step. The response of living organisms towards aquatic contamination has been one of the frontlines of defence against the pollution of water bodies.⁴⁻⁶ Behavioural studies and mortality rates of fishes have been demonstrated to both identify and quantify the amount of contaminants in water.^{4, 6} However, given the absence of clear guidelines, consistency and the sheer human capital and technological intensiveness required of such a set-up, the implementation is extremely difficult.

Water pollution caused by heavy metals is particularly of concern, due to the ready accumulation of these heavy metals in the eco-system and the health problems they pose.⁷ Remediative measures not only protect the natural habitat, but too prevent passing the concentration of heavy metals along the food chain, which inadvertently affects humans in the surroundings. Measures to remove these heavy metals include reduction, precipitation, membrane filtration, ion exchange and the use of adsorbents.⁸ Adsorption is amongst the most ideal method, as it not only removes the pollutants from the system, it also allows for proper disposal of such chemical hazards and its possible recovery for the conservation of materials.⁹⁻¹¹ The environment, in which the nano/micro/macromotor operates, exerts a strong influence on the activity and velocity of these self-propelled entities. Previously, it was shown that a bubble-propelled micromotor can move independently of the salt effect

which can otherwise adversely affect the self-electrophoresis mechanism.²⁴ However, recent research has shown converse results that these bubble-propelled motors and Marangoni millimetre-sized capsules are affected by the presence of ions, organic molecules, biomolecules and cells, influencing the propulsion of these autonomous self-moving objects.

The alterations of the changes in micromotor behaviour is much alike the performances of fishes in water, where decreasing speeds were found with increased concentrations of the ions. This real-time changes in locomotion of the micromotors can serve as indication of the levels of chemicals in the water. In addition, this very behaviour could be exploited via optical observations for the construction of sensors and chemotaxis for tracing and marking environmental contaminants. An opportunity to use performances of the micromotor to probe the environment is explored in **Chapter 4**.

The adsorption of chemicals on material surfaces has served as an active removal of ions, this display capabilities of such adsorption as a possible methodology of removing ions away from polluted waters. Graphene oxide, which has been shown as a promising sorbent for the removal of ions are tested as possible sorbents for the removal of heavy metals. Different routes of obtaining graphene oxide has been illustrated. The best methodology for obtaining the graphene oxide is now being studied for removal of the heavy metal ions in **Chapter 5**.

The integration of adsorption materials into a micromotor should benefit the micromotor with both sorption capabilities and the locomotion of the micromotor that is able to move throughout the solution. We conducted a bottom-up approach to use nanomaterials as sorbents for the removal of ions from the water. This allows the mixing of the solution, while providing a sponge for the removal of the pollutants. Integration of 2D materials and the combination of actively moving micromotors serve as a paradigm change for the use of nanomaterials actively moving in waters for removing pollutants.

Locomotion of graphene oxide composite micromotors and their removal of heavy metal pollutants are explored in **Chapter 6**.

An opportunity has been provided to use performances of the micromotor to probe the environment using the miniaturised self-propelled systems to detect and remove metal ions.

1.3 Objective 2: To Study the Influence of Environment and to Optimise Motor Performances

Chemical composition of the miniaturised motor itself and its surrounding environment affects the performance of the micromotor. This leads to changes in locomotive properties of the micromotor. New insight is needed to study how the chemical environment and chemical composition powers locomotion. Investigation into new composition of miniaturised devices for propulsion and manipulation of these self-propelled machines using the chemical and physical environments are found wanting. Alterations to the inherent design of motor, changes in the field effect of chemical environments, light irradiation and acoustic pressures serve as possible routes to improve motor performances.

A fuel-free autonomous self-propelled motor is investigated. The motor is powered by the chemistry of calcium carbide and utilising water as the co-reactant, through a polymer encapsulation strategy. Expulsion of acetylene bubbles powers the capsule motor. This is an important step, going beyond toxic hydrogen peroxide fuel used normally, to find alternative propellants for self-propelled machines. Locomotion of an acetylene bubble-propelled capsule is explored in **Chapter 7**.

Self-propelled miniaturized machines harness the chemical potential of their environment for movement. Locomotion of chemically-powered micromotors have been hugely dependent on the surroundings. The use of pH to alter the mobility of micromotors is investigated through the manipulation of hydrogen peroxide chemistry in different

acidity/alkalinity. The sequential addition of sodium hydroxide to increase the pH of the solution led to a consequent increase in hydrogen peroxide decomposition to oxygen bubbles. Meanwhile, addition of hydrochloric acid compromised the structural integrity of the microstructures. Such dramatic changes can result in alterations of micromotor's performances. This concept was illustrated with Janus silver micromotors and tubular bimetallic Cu/Pt micromotors. Effect of pH changes in catalytic micromotors is explored in **Chapter 8**.

Controlling the environment in which bubble-propelled micromotors operate represents an attractive strategy to influence their motion, especially when the trigger is as simple as light. We investigate spiropyrans, which isomerize to amphiphilic merocyanines under UV irradiation, can act as molecular switches that can drastically affect the locomotion of the micron-sized engines. The phototrigger could be either a point or a field source, thus allowing different modes of control to be executed. A whole ensemble of micromotors or the velocity of individual micromotors can be altered by using a field and point irradiation source respectively. Such selective manipulation, achieved here with an optical microscope and a photochromic additive in the medium, potentially eases the methodology, which can allow micro- and nano-motors to reach their full potential of not just stochastic, but directional controlled motion. Photochemical control of self-propelled micromotors using chemical environments altered by light is studied in **Chapter 9**.

While numerous roadmaps have been cast, the control of the locomotion and directionality of these miniaturized machines by creating pressure gradients in solution using acoustic fields remains elusive. Ultrasound has been used to produce stop/go motion on bubble-propelled micromotors.³³ An intricate study using high-speed camera on the interactions between the bubbles and micromotor is undertaken. The reciprocal action between oscillating bubbles aggregates and ejected microbubbles in an acoustic field can demonstrate influence on the motion of the micromotor through pressure gradients.² Long-

range translational motion can be induced into the micromotor, when repulsive forces between bubble aggregates and ejected microbubbles are manifested in an acoustic field by Bjerknes forces. Additionally, such ultrasonic pulses may demonstrate capability to change the directionality of the micromotor. Here, introduction of pulses of ultrasonic irradiation is investigated for new capabilities to control the motion of bubble-propelled micromotors. Control of bubble-propelled micromotor in ultrasound field is depicted in **Chapter 10**.

1.4 Objective 3: To Develop an Electrochemical Technique to Monitor Self-Propelled Miniaturized Systems

Monitoring the motion of self-propelled nano/micro/macrosystems have been done through optical microscopy, capture through camera and processing, which is laborious and is inherently an *ex situ* technique. However, it is only until recent times that chemically-powered units of autonomously moving objects were monitored using electrochemical techniques. With the ability to track and obtain information from miniaturized devices by electrochemistry, one can envisage an internet of things at the nanoscale with the use of networks for first-hand transmittance of data.²⁵ The use of particle-electrode impact voltammetry can be used to investigate the locomotion of the micromotor.

A system that obviates these inherent limitations of optical tracking is urgently needed. Here, the remote positioning of a Marangoni effect propelled millimetre-sized capsule by an electrochemical method is investigated. A technique to determine the location of an autonomous self-propelled capsule in continuous motion regardless of transparency or optical limitations is explored. The “cognitive” ability of the self-propelled capsule to survey positions of obstacles in the environment is also explored. This novel method allows for the mapping of self-propelled motors and their surroundings, via the transmission of real-time information, in a channel environment. Such a system provides a break-through of monitoring limitations and allows for truly autonomous systems. Electrochemical

monitoring of a Marangoni-effect propelled capsule and its interactions with the environment are investigated in **Chapter 11**.

Self-propelled micromotors, as the next frontier of smart miniaturized machines, are able to carry out a plethora of tasks and operations. To date, the evaluation of their capabilities and behaviour have been carried out by optical microscopy coupled with computationally intensive imaging software. The use of particle-electrode impact voltammetry serves as an alternative means of quantifying and qualifying their locomotion. Perturbances of the diffusion layer at the electrode interface from the locomotion of the micromotor in solution, can result in spikes that were recorded by electrochemical signals. The number of micromotors and velocities of these self-propelled objects could be evaluated and examined in real-time *in situ*. This phenomena was illustrated with Janus silver micromotors and tubular Cu/Pt micromotors. An on-site tracking of self-powered miniaturized entities propagating in solution serves as an alternative means for monitoring during their operations in environmental and biological applications. Monitoring micron-sized self-propelled objects via an electrochemical technique is explored in **Chapter 12**.

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Chapter 2

Introduction

2.1 Discovery of Self-Powered Miniaturized Motors

2.2 Nano/Micro/Macromotors Remediating the Environment

2.3 Influence of Chemical Environment on Motors

2.4 Motors Polluting the Environment

2.5 Monitoring Motion of Nano/Micro/Macromotors

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2.1 Discovery of Self-Powered Miniaturized Motors

Since the beginning of civilisation, humans have been fascinated with locomotion. The Wright brothers have replicated the wings of the birds in an airplane. The streamlined body of a fish can be found in designs of boats and ships. A machine such as a miniaturized self-powered system shows promise in moving cargo and objects in fluid. However, to recreate locomotion in miniaturized artificial objects, challenges remain.

The above are illustrations of the human endeavour to reproduce movements of the natural world in machines. Such autonomous motion are not unique in animate and living organisms. Miniaturised inanimate objects are capable of taking on autonomous motion as well. Camphor boats are able to move due to the asymmetric dissolution of camphor.¹⁻⁶ This results in the asymmetric chemical gradients, which culminates in differences in surface tensions. A dissymmetric release of organic solvent such as DMF⁷⁻⁹ and ethanol¹⁰ has also resulted in chemical gradients due to the release of organic solvent. The aforementioned chemical gradient leads to an effect known as the Marangoni effect, where differences in surface tension occur and the entity is able to move from a region of lower surface tension to a region of higher surface tension, as depicted in Figure 2.1. However, the saturation in the chemicals on a surface also results in the termination of motion.¹¹ Typically, a chemical that is stored within the materials is expended, which result in the concomitant stop of motion of the motor.¹²

Figure 2.1 Marangoni-effect propelled motor, where it is pulled from a region of lower surface tension (γ_2) to a region of higher surface tension (γ_1). Reprinted from ref. 9.

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The sizes of these miniaturised systems are typically in millimetre-sizes. Motion in micron- and nanoscale has been difficult to reproduce due to the high Reynolds number¹³ and Brownian motion.^{14,15} The first self-electrophoretic nanomotors were first demonstrated by Sen and Mallouk *et al.* where they showed autonomous motion in hydrogen peroxide.¹⁶ Later, the same team demonstrated self-diffusiophoretic motion in silver chloride micromotors that are able to move under ultraviolet illumination that were moving by secretion of chemicals that other particles can respond.¹⁷ Challenges remain due to the complex environments and viscosity of the solution in the tiniest of scale.¹⁸

Since then, the Mallouk/Sen groups have pioneered several other micro/nanomotors devices on the basis of this asymmetrical chemical gradient that allows for propulsion of the motors.¹⁹⁻²⁵ The motors designed principally runs on the mechanisms of self-electrophoresis and self-diffusiophoresis, where the basis of a spatially separated chemical gradient is required. The self-generation of a localised gradient through chemical free energy of the environment, suggests that these motors are reactive to the compounds that are present in the solution, garnering a response from these nano/microdevices.²³ This would have been ideal for the operation of motors in a chemical setting, where responsive motors can react to the changes in the solution. However, in an environmental remediative setting, the presence of a plethora of chemicals in the solution led to high ionic strengths and the increased substrate concentrations in the solution will interfere with the mechanism operating such nano/micromotors. Micromotors have been endorsed to do work in microfluidic chips, for environmental remediation and within the biological vascular systems. However, it is known that the fluids inside these environments contain high amounts of ions and other biomolecules. Thus, it is of utmost importance to test the robustness of the micromotor system using conditions that reflect the actual environment. It is noted that these motors generally propels in the order of tens of micrometres per second,²⁶ which might not generate the power used in mixings. This is in comparison to

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bubble-propelled nano/micromotors and interfacial tension-propelled devices, which have shown capable of high powers.^{27,28}

Bubble-propulsion proves to be the most effective method of motion in an array of medium due to the salt independence. Such autonomous motion was illustrated by Whitesides team, where a millimetre sized PDMS plates that was coated with platinum, was able to emit bubbles and demonstrate swarming behaviour¹². At the micrometre size, it was the pioneering work of Joseph Wang and co-workers that used a templated method to etch a hollow silver micromotor that was able to move by bubble propulsion.²⁹

Figure 2.2 Mechanisms of self-propulsion. **A** Self-electrophoresis¹⁸ **B** Self-diffusiophoresis³⁰ **C** Bubble-propulsion.²⁹

The locomotion of these devices have been based on three principal origins: (i) self-electrophoresis¹⁶ (ii) self-diffusiophoresis¹⁶ and (iii) bubble-propulsion,^{12,17} as illustrated in Figure 2.2:

- i. Self-electrophoresis occurs in nanorods and is dependent on concurrent chemical oxidation and reduction reactions at opposite ends. The flow of electrons along the

length of the device, led to the laminar flow of hydronium ions along the sides of the rod, propelling the nanorod forward.

- ii. Diffusiophoresis occurs when dissymmetric particles are also capable of generating an asymmetric concentration gradient of reaction products. The resulting accumulation of a concentration gradient in the immediate surrounding, leads to fluid flow, propelling the motor forward.
- iii. Bubble-propulsion relies on the catalytic decomposition of the substrate to gaseous products. Typically, the reaction is carried out on the decomposition of hydrogen peroxide to oxygen bubbles over a platinum catalyst. The high velocities, power output and robust behaviours in various mediums, make it suitable for applications in different environments, overcoming the inherent limitations of the preceding mechanism of propulsion in (i) and (ii).

For the study of chemical environment and the micromotors, two principal designs of micromotors have been used. The first is the template-assisted electrodeposited micromotors (ED micromotors).^{31, 32} Electrodeposited micromotors have been fabricated through a template-assisted approach. The templates range from polymer-based membranes¹² to anodized aluminium oxide membranes.^{13,14} These have been employed for the fabrication of nanorods¹⁵ and nano/microtubes.¹²⁻¹⁴ These tubular micromotors are concentric and multi-component in nature, consisting of metals or polymers. The second design is via roll-up technology, using a sacrificial polymer template with consequent e-beam deposition of metal layers. These rolled-up micromotors (RU micromotors) usually consist of multi-layers of metal that roll up, upon removal of stress from the etching of sacrificial polymer.³³

While we advocate the use of these self-propelled devices in environmental monitoring and remediation, we must remain conscious of their limitations.^{34,35} The

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environment settings that these motors are set in are very often the obstacles, debarring them from achieving the ideal performance.^{8,9,13,36-41} These have not only reduced the efficiency of the motors, but may very well be an active pollution factor that warrants further contemplation before utilization in the field.¹¹

In this study, we reflect on the recent advances of nano/micro/macromotor with the possible ramifications on the environmental and water sector. Motion-induced mixing by the motors has added an extra element of efficiency towards pollutant removal and decontamination works. The influence of the environment with regards to the performance of these devices and their corresponding applications as sensors and tracers for environmental monitoring will also be discussed. Lastly, the possible implications of environmental degradation due to self-propagated entities are raised in this review. We attempt to develop an encompassing study detailing nano/micro/macromotors and their roles in the environment, the limitations they face and the future ahead as the community forge forward towards their wider acceptance and employment. This will be illustrated in the properties of the micromotors, where the three predominant types of micromotors are illustrated.

For a clearer understanding of this field, the thesis literature review has been divided into four sections in **Chapter 3**, as shown in Figure 2.3 First, the remediation of the environment, with regards to pollutant removal and contaminant degradation due to motor enhanced mixing is discussed. Secondly, the influence of the environment on the motors will be elucidated and will be extended into the possible use of their locomotion as sensors. The chemotactic behaviour of relevant motors to detect chemicals will also be included for their roles as tracers and markers. Thirdly, the role of nano/micro/macromotor as a pollutant due to toxicity effects and its inherent self-propagating factor are considered. Fourth, a background on electrochemical methods to control and detect their motion electrochemically is reviewed. The examples listed in each section are arranged in

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increasing size order from the nano/micromotor to the macromotor, which is millimetres in size. The macromotor, continues to serve importance, due to the operational readiness of being able to cover long distances in a short time frame.⁴²

Figure 2.3 Nano/Micro/Macromotors and the Environment.

2.2 Nano/Micro/Macromotors Remediating the Environment

Converting chemical energy from the environment into mechanical energy, nano/micro/macromotors have given environmental specialists more degrees of freedom in the area of pollutant disposal onsite and off-site. The mechanical agitation brought about by the motion of the motors enables efficient mixing of the solution. This is particularly so for bubble-propelled micro/nanomachine, where the rising bubbles can serve as an additional mixing agent.^{43,44} The drag of the mixture by the micro/nano/macromotors, bring the active reactants to the source, enabling better contact and reach, for removal and degradation of pollutants.

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Two recurrent themes play in the self-propelled devices for environmental remediation, in addition to surface modifications. These are the roles of size^{18,22,25} and speed.^{27,28} At these scaled-down dimensions, the role of inertia plays a lesser role as compared to the viscous drag of the solution.¹⁸ The solution-borne motors are immersed in their own fuel, which effected the continuity of the instantaneous forces that propels the motion, since inertia no longer plays an essential role in locomotion.²⁵

Surface forces become an important factor at the miniaturised scale.¹⁸ This is proportional to the relative amount of exposed area per unit volume becoming larger. When an object is scaled down by a proportion ΔL , the volume of the object reduces by a scale of ΔL^3 , while the object's surface reduces by a scale of only ΔL^2 . The large surface area to volume ratio, allows for larger contact areas, while continuously bringing in active materials, by drag force, to regions in need of environmental remediation, allowing for facile action. As we enter the miniature stage, the average distance between self-propelled devices and the substrates becomes reduced, in comparison with a single bulk entity with equivalent mass. This aids the chemical remediation process that is going to take place with increased reach, in togetherness with increased contact area. Additionally, it is noted that physical forces, such as electrostatic interactions and Van der Waal's forces increase in the proportion of ΔL^2 and ΔL^6 respectively with reduced distances, aiding the remediative actions.

Speeds of the micromotors play an important role during remediative action.⁴³⁻⁴⁵ Orozco *et al.* demonstrated that the self-stirring effects due to the collective hydrodynamic movement of the micromotors can reach up to 200 rpm (rounds per minute) equivalent, that was in part due to the high speeds of up to $500 \mu\text{m s}^{-1}$, that were reached.⁴⁴ Soler *et al.* also demonstrated that the drag of the micromotors brought active materials to the proximity of the contaminant, allowing active mixing at increased speeds.⁴³

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In situ and onsite decontamination protocols are possible with such a set-up, as the stirring component can be brought to the source that requires remediative treatment.⁴³⁻⁴⁵ This decentralisation strategy obviates the need for big bulky equipment in conventionally externally-powered stirring devices. Instead of sending the pollutants to the large factories for remediative action, we are able to bring the “mixers” of micromotors to the sources.

Motors that simultaneously move and leach chemicals often operates on interfaces (water/air), effecting the dissipation of chemicals that aids in the environmental remediation. This is especially helpful during operations for clean-up of oil, where it remains on the water/air interface and extends over large areas. Current technology defers to the use of oil booms or chemical booms for containment, where it remains relatively sedentary and is categorically a passive mechanism.⁴⁶ The employment of motors that move by leaching chemicals, provides a new protocol for environmental clean-up, where actively moving motors are able to herd and gather oil spills for curbing the spread and that of an easier clean-up of chemicals. The use of sorption materials that are able to propagate in solution represent new frontiers of research for self-propelled micromotors for pollutant management.

Hydrogen peroxide has been ubiquitous in the context of environmental remediation for the breakdown of organic matter.⁴⁷ This is especially so in the context of advanced oxidation process, where highly reactive species of hydroxyl radicals are formed.⁴⁸ Fenton’s reactions are one of the chemical methods for producing such radicals *in situ*.^{49,50} We must remain mindful that the hydroxyl radicals remain as one of the strongest oxidation agents known. The coupling of the self-stirring motion and highly oxidative agents are attractive for the management of aqueous waste.

2.3 Influence of Chemical Environment on Micromotor

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The environment, in which the nano/micro/macromotor operates, exerts a strong influence on the activity and velocity of these self-propelled entities. Previously, it was shown that a bubble-propelled micromotor can move independently of the salt effect which can otherwise adversely affect the self-electrophoresis mechanism.²⁹ However, recent research has shown converse results that these bubble-propelled motors and Marangoni millimetre-sized capsules are affected by the presence of ions, organic molecules, biomolecules and cells, influencing the propulsion of these autonomous self-moving objects. In addition, this very behavior could be exploited via optical observations for the construction of sensors and chemotaxis for tracing and marking environmental contaminants.

Zhao *et al.* illustrated the effects of using different water matrixes from the real environment and probed their possible influence on bimetallic Cu/Pt electrodeposited (ED) micromotor.⁵¹ The activity and velocity of micromotors in natural water matrixes such as lake water and seawater were all negatively influenced. In the presence of 9 % by volume of seawater, the velocity of the micromotor reached zero. Test runs via ion chromatography confirmed that the high salt content was the principal cause of retardation in the micromotor's activity and velocity. A later report using a poly (3, 4-ethylenedioxythiophene) (PEDOT)/Pt ED micromotor was also negatively influenced by the type of water matrix and it was again shown to be most adversely influenced by seawater matrix, where the salt content was the highest.³⁷ Although the trends were similar, the magnitude of the changes were conspicuously different. It must be recognized that the design of these two configurations were inherently different, with the bimetallic Cu/Pt ED micromotor being significantly impeded by the presence of high salt concentration as compared to the polymer PEDOT/Pt ED micromotor. This is perhaps due to the heavier metal components being used in the Cu/Pt ED micromotor. Thus, we must always bear in mind the design of the micromotor during observation of locomotive events.

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In a similar light, the carrier fluid in blood contains a plethora of substances like ions in the blood electrolyte,³⁸ biomolecules,⁴¹ metabolites,³⁹ proteins in blood,⁴⁰ and red blood cells,⁵² which may affect the motion of the micromotor. Indeed, the presence of these entities were demonstrated to slow down the activity and velocity of the micromotors. For simple molecules and ions, four principal reasons have been proposed for the retardation of micromotors: (i) sensitivity of the active platinum layer in micromotor to poisoning (ii) interruption of the radical reaction during decomposition of the hydrogen peroxide through quenching (iii) the addition of ions leading to aggregation of surfactants, which increases surface tension and leading to the reduction of detachment rate of the ejected microbubbles from micromotor and (iv) the increase of viscosity of the chemical environment impeding the motion of the motors. For large biological components, in addition to viscosity, chemisorption and physisorption also impede the motion of the motors. In the literature review in **Chapter 3**, we have arranged the discussion with regards to the impediments in increasing size order: from simple ions and molecules, to large biological components and to the cellular level. Additionally, we also discussed the role of surfactants for bubble-propulsion and self-propulsion in unconventional environments.

Alterations of locomotion due to changes in the chemical environments represent opportunities to use bubble-propelled micromotor's behaviour for monitoring pollutant levels.⁵³ Changes in acidity and alkalinity in solution have been known to tune the performances of the self-propelled devices.⁵⁴ However, such bubble propulsion often involves the use of toxic hydrogen peroxide.^{31,32} Alternative fuels are needed to be developed to power the micromotors. As such, new mechanisms are needed for the propulsion of the micromotors. Furthermore, the control of micromotor still requires the direct modification of the micromotor.⁵⁵ Changes in chemical environment to induce changes in the micromotor may prove to be an attractive methodology.

2.4 Motors Polluting the Environment

Micromotors often operate in harsh environments, which may result in the corrosion of the metallic motors themselves, leaching heavy metal ions.³⁴ Furthermore, since they are proposed to work in water environments and for biological settings, related toxicity effects are crucial.³⁵ The self-propelled motion in the environments may also result in the propagation of pollutants.¹¹ These effects are important and requires further study before their applications in the real-world environments.

2.5 Monitoring Motion of Nano/Micro/Macromotors

Till date, the evaluation of nano/micro/macromotors' performances has still been reliant on the use of an optical microscope coupled with an imaging software. Optical analysis is still the most advocated utility for observation of micromotor's motion, as it allows for kinetic events to be recorded, while allowing a live feed of possible non-locomotive events.⁵⁶⁻⁵⁸ However, it is laborious from the perspective of having to use manual tracking of the autonomously moving entity. Workflow of such processes often include recording of videos before post-processing using computationally intensive tracking software. Automation in the form of tracking the self-propelled object is few and far between and it is also met with limitations due to the difficulties in achieving contrasts in different mediums.⁵⁹ There is also an inherent limitation of viewing particles that reach 200 nm in size, which is the limit of detection.⁶⁰ Furthermore, considerations that these self-propelled objects are moving in three dimensions must be put into place. Typical optical analysis will only have the motion of the micromotors being tracked in 2D. While it has been able to observe particles in a single plane, common microscopy is not ready for 3D imaging of moving objects in real-time. Evaluating objects that are moving in and out of the focal plane in the z-axis have hardly been considered. The most advanced form of scanning confocal microscopy demands the use of fluorescence spectroscopy coupled with additives, in the presence of complementary light excitation and detection sources.⁶¹⁻⁶³ Furthermore, there is a tradeoff between resolution, scanning speed and area being imaged

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as the speed is inversely proportional to area.⁶² These techniques haven been primarily used to map biological cells, typically moving by a crawling mechanism at 5.2 $\mu\text{m}/\text{min}$. This is in addition to limitations of depth and field of view and particularly the formation of bubbles which can obscure visibility but not often remarked on by practitioners in the field. More aptly put forth, having to extract samples from the bulk solution and to be placed on a microscope platform for observation is also far from ideal. This calls for the need of innovative techniques, obviating additives, to monitor locomotion of micromotors.

The use of electrochemistry can serve as an alternative means of quantifying and qualifying their locomotion, as depicted in Figure 2.4. Perturbances of the diffusion layer at the electrode interface from the locomotion of the micromotor in solution, can result in spikes that were recorded by electrochemical signals.

Figure 2.4 Utilisation of electrochemistry to monitor miniaturised devices. Reprinted from ref. 64.

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Chapter 3

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3.1. Literature Review of Nano/Micro/Macromotors and the Environment

Locomotion at the nano, micro and macro scale has been the subject of much efforts of the scientific community.¹⁻⁴ Biological soft matter, from enzymes at the nanoscale,⁵ to the micrometre-cilia in bacteria^{6,7} have been demonstrated to be capable of doing work.⁸ However, they are often unstable and not resilient to changes in the environment.⁹ Replicating how Nature achieves that level of complexity, with an added element of hardness in unconventional environments has been a constant pursuit in the fraternity.¹⁰ Synthetic hard matter shows promise in overcoming these challenges, with a combination of metals being utilized as key components in miniaturized motors.^{11,12} With the innovation of materials scientists, chemists and physicists alike, we have endowed these objects with specialized functions, performing a multitude of tasks and operations.¹³ Coupling them with biological components ranging, from simple biomolecules,¹⁴ enzymes¹⁵ to small organisms¹⁶ are also now made possible. The capacity of these self-propelled devices to perform tasks and operations have now reached multi-fold, where specificity and efficiency are seeing unprecedented levels for environmental applications.

3.2. Environmental Remediation

Figure 3.1 A) Composition of superhydrophobic layer-modified tubular micromotor for removal of oil droplet and the mechanism of a) approach b) capture and c) transport. Reprinted from ref. 17. B) Composition of self-propelled Janus microsphere for removal of oil droplet and the mechanism of a) approach b) capture and c) transport. Reprinted from ref. 18. C) a) - f) Sequential images detailing the shepherding of oil droplets by SDS-loaded Marangoni capsule to a single droplet for easy removal. Reprinted from ref. 20. D) a) Fe^{III} contaminated waters in the absence of SDS-loaded Marangoni capsule b) Flocculation of Fe^{III} contaminated waters by SDS-loaded Marangoni capsule for easy removal. Reprinted from ref. 19.

3.2.1 Pollutant Removal

Using a self-assembly strategy on gold surfaces, Guix *et al.* modified the surface of a microtubular motor with a monolayer of alkanethiol, to form the superhydrophobic layer as shown in Figure 3.1 A).¹⁷ The *n*-dodecanethiol's carbon chain length shows the highest efficacy in transporting oil droplets away from the contaminated water surface, while the micromotor is being powered by the decomposition of hydrogen peroxide to oxygen bubbles in the inner platinum layer. A similar strategy was employed on a hydrogen bubble-propelled gold coated magnesium Janus particle in water, as illustrated in Figure 3.1 B).¹⁸ The self-assembled monolayer (SAM) of octadecanethiol demonstrated a good rate for removal of oil droplets away from the surface. The Janus particle, when exposed to a real environment, is able to operate without the need of an external fuel source. The pitting mechanism of the chloride ions in the seawater prevents passivation from occurring and the macrogalvanostatic corrosion of the magnesium in the seawater media to form hydrogen bubbles propels the motor forward. Modification with a nickel layer enables magnetic manipulation in both the Janus and microtube motor for a directed movement towards contaminant source.

Polymer capsules propelled by Marangoni effect have been demonstrated to move up to 64 body lengths per second and over distances of up to 24.5 m.²⁰ Polysulfone dissolved in dimethylformamide (PSf/DMF) undergoes phase inversion and solidifies upon contact with an aqueous solution to form a polymer capsule. The porous PSf polymer capsule releases DMF asymmetrically, resulting in a difference of surface tension, which culminates in propulsion, in a phenomena otherwise termed as Marangoni effect. Additionally, these capsules can be loaded with chemicals, which demonstrate ability to remediate environmental pollutants.¹⁹⁻²¹ Zhao *et al.* demonstrated the ability of these polysulfone/DMF capsules to load the sodium dodecyl sulphate (SDS) surfactant.²⁰ These laden self-propelled capsules, on introduction into the aqueous medium, releases SDS and

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actively spreads them. As illustrated in Figure 3.1 C), on close proximity with the capsule, the oil droplet is repelled by the surfactant and gradually merged to form a large puddle, enabling an easy clean-up protocol. In a follow-up research, the positively-, negatively-charged and neutral surfactant-infused polymer capsule all showed capability towards the gathering of an oil droplet.²¹

The SDS-loaded PSf capsule also demonstrated efficacy in the removal of heavy metals Fe^{III} through precipitation, as depicted in Figure 3.1 D).¹⁹ The surfactant released by the PSf capsule converts the Fe^{III} in aqueous phase to solid precipitates. Such a motion-induced mixing with the controlled release of the surfactant, is also another important mechanism of remediation, where contaminants could be flocculated and separated away easily.

Later, using a similar concept based on Marangoni effect, Orozco *et al.* designed tubular motors from pipette tips that are capable of releasing SDS in solution.²² The release of the SDS surfactant results in an ensuing change in surface tension, exerting a force on the motor to exhibit a circular motion. These tubular motors can be easily loaded with coordination factors that result in precipitation for environmental remediation. In this fashion, the authors demonstrated that heavy-metals pollutant of Pb^{II} could also be removed using a strategy of utilizing the co-ordination agent, ethylenediaminetetraacetic acid (EDTA). Furthermore, the agents used in this exercise are environmentally friendly and the tubular motors could be recycled for subsequent usage. The controlled release of the active substrate with the consequent efficient mixing has allowed for the precipitation of heavy metals via chelation chemistry.

The utilisation of self-propelled nanoadsorbents by Jurado-Sanchez *et al.* was demonstrated for the removal of both heavy metals and organics alike.²³ Activated-carbon nanoparticles were modified with a layer of platinum, to confer locomotion by bubble-

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propulsion in hydrogen peroxide, where the microbubble tail can act as an additional mixing agent. These Janus-type motors that operate on the principles of adsorption and microfiltration through its porous structures. The autonomous self-propelled motors demonstrate efficacy in removing organics with the likes of 2, 4-dinitrotoluene, rhodamine-6-G, methyl-paraoxon and heavy metals of lead. The dynamic mixing has extended reach, higher efficiency and shorter clean-up times, in comparison to static activated-carbon nanoadsorbents, which are in need of an external mixing force.

Figure 3.2 A) Micromotor-based accelerated oxidative decontamination of chemical threats with H_2O_2 as the fuel and for *in-situ* generation of base-activated nucleophilic oxidant of OOH^- . Reprinted from ref. 24. B) Self-propelled Pt-based microtube with an outer Fe layer that dissolves in catalytic amounts to participate in Fenton reaction with hydrogen peroxide to produce hydroxyl radicals for degradation of organic pollutants. Reprinted from ref. 25. C) Enzyme releasing biocatalytic motor's degradation of pollutants with induced mixing using pipette tips with mixture of enzyme remediator (10%) and SDS propellant (90%). Reprinted from ref. 22. D) Schematic illustration of the dual-function plant (radish) motors in hydrogen peroxide, with the catalase-driven bubble propulsion and

peroxidase-based decontamination capability, for in-motion bioremediation. Reprinted from ref. 26.

3.2.2 Contaminant Degradation

Hydrogen peroxide is commonly used in self-propelled systems, due to the ease of decomposition of hydrogen peroxide to oxygen and water. However, it must also be realized that the compound itself is a strong oxidizing agent that has been used by the environmental^{27,28} and defence community for the degradation of harmful organic substances.²⁹ As illustrated in Figure 3.2 A), Orozco *et al.* extended this research with the use of base-activated peroxide anions for the motor-induced self-stirring breakdown of organophosphate nerve agents, using the bubble-propelled polymer/platinum composite micromotor.²⁴ The large scale collective motion of the micromotors led to hydrodynamic interactions for enhanced mixing. Such collective movement of the micromotors, in togetherness with the decomposition of hydrogen peroxide to oxygen bubbles, also increased the yield and speed of reaction by efficient fluid mixing. UV spectroscopic observation of the *p*-nitrophenol by-product at 400 nm indicated that the forced convection and enhanced mass transport of the bubble-propelled micromotor is the principal cause of oxidative decontamination. The subsequent exclusion of micromotors or surfactants essential for bubble-propulsion did not warrant any reduction in contaminant levels for the control experiments. The shorter timescale, lower peroxide loading and high efficiency of such a procedure is projected to have real applications in detoxifying chemical threats.

At approximately the same time, Soler *et al.* pushed for the use of micromotors in environmental remediation by using active materials from the surface of the micromotor that participate in the degradation of organic pollutants, as illustrated in Figure 3.2 B).²⁵ These rolled-up Fe/Pt tubular micromotors have a configuration such that platinum lines the inner surface and the outer layer is made of iron. Under a typical harsh condition of pH

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2.5 and 15 % H_2O_2 , iron gradually dissolves into the solution mixture. The catalytic amount of Fe^{II} participates in the Fenton reaction with hydrogen peroxide, which produces the highly oxidizing species of hydroxyl radicals. The hydroxyl radical readily breaks down the complex organic molecule of rhodamine 6G (R6G) to inorganic by-products. Active participation of the autonomous moving Fe/Pt microtubes also leads to the micromotor stirring the solution due to their motion and the streaming oxygen bubble formation from the Pt inner lining. In addition, the drag on the microtube allows the active species of the Fenton's reaction to be brought to the contaminant source. The environmental strategy of using the Fe/Pt microtubes completes the clean-up of the R6G in 5 hours and is 12 times faster as compared to the scenario when only Fe microtubes are introduced. Only minute quantities of iron is introduced to the system and they can be readily recovered using a magnet for the next batch of environmental remediation.

Photocatalytic breakdown of chemical and biological agents, using self-propelled spherical motors that operates on water as the fuel, without the addition of chemical fuels and active agents, have been demonstrated by Wang and co-workers.³⁰ The motor has a core-shell structure with an inner core of spherical magnesium scaffold as the fuel, followed by an interlayer of gold particles, before encapsulation by the active titanium oxide layer on the outside. The $\text{TiO}_2/\text{Au}/\text{Mg}$ micromotor operates via the macrogalvanostatic corrosion of magnesium in water, to produce hydrogen bubbles for propulsion. Typically, these micromotors has a lifetime of minutes due to the limited fuel packed into the device. On ultraviolet irradiation, the reactive oxygen radicals that are formed on TiO_2 surface in water, breaks down organic and biological materials alike. Continuous motion of these micromotors have been instrumental in stirring the solution of harmful agents for degradation. This continuous motion has also aided in the self-renewal of titanium dioxide surface, which would otherwise be easily passivated.

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The pipette tubular motors designed by Orozco *et al.* that was highlighted earlier for carrying chelating agents are capable of carrying bioremediation enzymes too.²² In Figure 3.2 C), the release of the active enzyme with the consequent efficient mixing allows for the facile degradation of environmentally malicious compounds to benign substances. Simultaneous employment of 6 laccase-carrying SDS motors demonstrated capability for 77.4 % degradation of 50 mL of 250 μ M 2-amino-4-chlorophenol to innocuous substances in 30 minutes. An analogous experiment for the eriochorome black-T (EB-T) azo-dye resulted in complete degradation of the pollutant, with the same parameters. A direct introduction of laccase at 10 times the concentration in a localized position does not affect the rate of degradation significantly, insinuating the importance of the motor-induced stirring of the solution. The controlled release of the active compounds followed by the motor-induced stirring is again highlighted as an important strategy for efficient reactions to occur.

Biocatalytic millimetre-sized plant tissue motors have also been employed for remediation of waters containing toxic phenolic compounds, as depicted in Figure 3.2 D).²⁶ Enzymatic bioremediation of aromatic compounds, such as guaicaol, catechol and 2-amino-4-chlorophenol represents an attractive strategy over decontaminative work by micro-organisms, due to toxicity limitations. By introducing asymmetry to the tissue compounds in the presence of hydrogen peroxide, these biomotors demonstrated propulsion, effectively increasing the induced convective fluid mixing, coupled with detoxification capabilities. The two principal enzymes of peroxidase and catalase in the radish tissue based motors worked harmoniously together for active fluid mixing and decontamination respectively. Hydrogen peroxide is the fuel for propulsion for catalase, while also being the co-substrate for the detoxification reaction for peroxidase, with both enzymes being inherently present in radish tissues. They are projected to induce wastewater decontamination works involving 1 to 600 mg L⁻¹ of phenols with about 1 to 4 tissue motors mL⁻¹, reaching complete removal

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in an hour. The motion of these tissue motors and the release of bubbles extended the reach of the enzymes across the reaction vessel, while improving mass transfer through active mixing.

3.2.3 Immobilised Micromotors: The Micropumps

Self-powered pumps, are analogous models of immobilised motors that actively inject fluid flow into the mixture. Micropumps imparts momentum to fluid circulation through the use of chemical gradients resulting in the mechanisms of self-electrophoresis, self-electroosmosis or self-diffusiophoresis,², effecting fluid flow. Recent developments have also included micropumps that are independent of the substrate materials, utilising depolymerisation reactions upon introduction of external stimuli, such as using chemical and ultraviolet factors.^{31,32} Further manipulation of the fluid flow directions can be accomplished by changing the fuel source³³ or the zeta potential of the particle and the micropump's surface.³⁴

Depolymerisation to reactive monomers that can remove inorganics from the solution are particularly interesting due to the amplification provided by the catalytic reactions. The proposition of an *in situ* production of high amounts of reactive monomers on site with portable micropumps coupled with the amplification by catalytic reactions, are especially attractive for the pumping of ambient fluid and removal of deleterious materials.

Although these reactions are catalytically driven, fluid speeds have remained low in the order of $1\text{-}10\ \mu\text{m s}^{-1}$.³² Furthermore, the pumping speeds vary with reaction rates and distance from the pumps themselves. More work is required in order for the micropumps to be fielded in real-world applications and to allow for a uniform hydrodynamic mixing throughout the solution.

3.3. Influence of the Environment on Nano/Micro/Macromotor

3.3.1 Nano/Micro/Macromotors are Influenced by the Chemical Environment

Figure 3.3 **A)** Motion of the electrodeposited micromotor(ED micromotor) in various real-world waters. **a)** lake water **b)** seawater. Reprinted from ref. 35. **B)** Motion of rolled-up micromotor (RU micromotor) in the presence of sulphur-containing molecules. **a)** DMSO and **b)** methionine. Reprinted from ref. 36. **C)** Motion of ED micromotor in blood electrolytes. **a)** NaCl **b)** phosphate buffer. Reprinted from ref. 37. **D)** Motion of ED micromotor in the presence of uric acid. Reprinted from ref. 38. Conditions in the all experiments: 3% (wt.) H_2O_2 and 1 % (wt.) SDS.

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The first works for such effects is represented by Zhao *et al.* with their work on chemical environments retarding the propulsion of micromotor.³⁶ Dimethyl sulfoxide (DMSO) exhibited strong inhibition towards the movement of the platinum-based RU micromotors. This is largely due to its role as a quencher of the hydrogen peroxide decomposition reaction as shown in Figure 3.3 B) a). The extension of the research delved into sulfur-containing simple molecules using RU micromotors, where entities such as methionine, illustrated strong poisoning effects in Figure 3.3 B) b). Poisoning effects of such sulphur-containing groups can be related to the branching of the alkyl groups in the sulphur containing groups: with the thiol in cysteine exhibiting more pronounced poisoning effects as compared to the thioether in methionine. Beyond sulphur containing moieties, Wang *et al.* used ions found in blood electrolyte to exemplify the negative effects in Figure 3.3 C).³⁷ The increasing ionic concentrations of these species exhibited debilitating effects towards the microtubular motor. The team later extended the work into the common metabolite, uric acid.³⁸ As a radical scavenger, the uric acid in Figure 3.3 D), also showed dramatic decrease for the viability of micromotors, due to the reduced capacity for radical decomposition of hydrogen peroxide in the Cu/Pt ED micromotor. Hence, the presence of these substances illustrated strong influence on the micromotor's mobility and a spectrum of simple chemicals can affect the integrity of operation of the microtubular motor in hydrogen peroxide.

Figure 3.4 A) Influence of serum albumin concentration on the velocities of ED micromotors. Reprinted from ref. 40. B) Influence of red blood cell concentration on the velocity of RU micromotors. Reprinted from ref. 39. C) Average speeds of RU micromotors in suspensions of red blood cells 10 $\times$ diluted: average speeds during 4 temperature cycles of 5 min each between 25 and 37 $^{\circ}\text{C}$. Reprinted from ref. 41. Conditions in all of the experiments: 3 % (wt.) H_2O_2 and 1 % (wt.) SDS.

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For large biomolecules and at the cellular level, it was proposed that the chemisorption or physisorption has led to the passivation of the active platinum surface and the increased viscosity of the solution too plays a determining factor in the slowdown of the micromotors.^{39, 40} The presence of proteins such as serum albumin impeded the motion of the micromotor in Figure 3.4 A).⁴⁰ In addition to the passivated platinum surface, the increased viscosity elevated the drag force on the micromotors, in accordance to Stoke's law, resulting in the retardation. Zhao *et al.*'s work with blood components of red blood cells and blood serum recasted the same light on the increment in viscosity impeding the motion of the micromotors and the inactivation of the surface via biological components. Additionally, the blockage of the opening of the microtubular motor by red blood cells through chemisorption or physisorption can occur.³⁹ This crippled the micromotor, as the fuel source is effectively cut off, resulting in the reduction of velocities in the micromotors, as observed in Figure 3.4 B). Wang *et al.* managed to circumvent the problem of the impaired ED micromotor by increasing the hydrogen peroxide in the presence of excess blood electrolyte.³⁷ However, it must be noted that high concentrations of hydrogen peroxide are toxic. Soler *et al.* later overcame the impediments of navigating RU micromotor amongst reconstituted blood in 10% blood serum in phosphate buffer at 10 X dilution, by raising the temperature of the serum to 37 °C, the body temperature, as illustrated in Figure 3.4 C).⁴¹ The team proved that the high viscosity of blood at room temperature has been one of the critical factors for the retardation of micromotors in physiological medium. The synergy of a reduction in viscosity of blood and thermal activation of micromotor at elevated temperature shows promise of propelling autonomous self-propelled objects in complex biological media.

Figure 3.5 A) Motion directionality of micromotor is influenced by Reynolds numbers. **a)** Linear motion of micromotors and **b)** circular motion of micromotor. Reprinted from ref. 42. **B)** Motion directionality of Marangoni capsule is influenced by Reynolds numbers. **a)** Linear translational **b)** rotational and **c)** non-linear translation motion. Scale bars indicate 2 cm; tracking time of 1 s. Reprinted from ref. 43.

Undeniably, viscosity can become a crucial factor in self-propelled objects under environmental conditions, where turbidity and dissolved substances play a contributing role. At the sub-millimetre or micrometre level, in the scenario of the Cu/Pt ED micromotors, the Reynolds number is influenced by the velocity due to fuel (H_2O_2) concentrations and the viscosity of the solution.⁴² Reynolds number-dependent bubble formation and the damping effect of viscosity are the principal causes for instability in a micromotor's trajectory. The transition for the style of motion from a linear to non-linear motion for a

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micromotor is found to occur at a critical Reynolds number of 10^{-3} , as shown in Figure 3.5 A).

Zhao *et al.* investigated the motion of a Marangoni polymer capsule under different Reynolds number regime.⁴³ Three different types of motion can be identified with the capsule, as illustrated in Figure 3.5 B): linear, rotational and non-linear modes. It was found that the trajectory of the system can be switched to a non-linear mode, by increasing the viscosity or Reynolds number of the solution, through the addition of viscous glycerol.

Surfactants are essential for the formation of microbubbles and their detachment in a bubble-propelled nano/micromotor. Wang *et al.*⁴⁴ and Simmchen *et al.*⁴⁵ demonstrated that the types of surfactant determine the mobility of motors, due to their relation to bubble formation on the platinum catalytic surface to power the locomotion. Wang *et al.* advocated the stabilization of charges on the platinum surface by the charge-carrying surfactant for bubble formation has led to a difference in performances. Of which, the negatively charged SDS demonstrated the best efficacy. However, Simmchen *et al.* proposed the reduction of surface tension for bubble formation by surfactants as the principal factor for propulsion to occur. Further investigation is still warranted for a clearer understanding of the role of surfactants for bubble propulsion.

Environmental remediation is often undertaken under unconventional surroundings and the differing solvent composition may affect the velocity and activity of the self-propelled objects, in addition to trajectory patterns. At the macroscale, PSf capsules were demonstrated to operate under ionic conditions and high concentrations of organic solvents, with the likes of acetonitrile, ethanol, acetic acid and DMF.²⁰ However, due to changes in surface tension in the presence of organic solvents, the velocity of the PSf capsule will be attenuated at high concentrations of these organic solvents. The presence of surfactants and oil droplets also results in changes in motion of the capsule due to negative chemotactic effects, resulting in repulsion.^{20,21} Such changes in behaviour were also observed in an

interlayer of organic and aqueous phase, where these PSf capsules demonstrate versatility in traversing between the oil and water layer, albeit at a lower velocity.⁴⁶

3.3.2 Nano/Micro/Macromotors as Environmental Sensors

One of the first instances of nanomotors demonstrating selectivity to chemicals in the environment is represented by the work by Kagan *et al.* with Au-Pt nanowire-based nanomotors, as shown in Figure 3.6 A). They exhibited sensitivity to the presence of Ag⁺ ions in the solution, via a self-diffusiophoretic mechanism.⁴⁷ The underpotential deposition of silver on Au-Pt nanowires in the presence of hydrogen peroxide allows for increased catalytic activity. This enhanced catalytic decomposition of hydrogen peroxide increased the reaction products, leading to enhanced self-diffusiophoretic locomotion. As a result, this constitutes the motion-based sensitive chemical detection of trace silver ions at the micromolar ranges, where higher speeds are recorded in the presence of silver.

In Figure 3.6 B), Orozco *et al.* illustrated the use of an artificial enzyme-powered microfish for the multiplex study of water quality.¹⁵ Exploitation of the change in propulsion behaviour in the presence of pollutants in water was used as the source of stimuli input. The catalase-immobilized microtubular PEDOT/Au microfish exhibits competence in sensing the presence of inorganic and organic pollutants, such as sodium azide, mercury ions, copper ions and aminotriazole (AT). The authors used the toxin-induced retardation of motion in the tubular micromotors and the life expectancy as gauges for poison concentrations. However, it must be noted that the methodology does not offer a selective process across the compendium of toxins covered and that the concentrations were of different orders of magnitude. It must also be noted that the presence of biological components in the motor do not ensure a long shelf-life of the device.

Selectivity of the bubble-propelled synthetic platinum-based micromotor was uncovered by Zhao *et al.* with their work with chemical environments.³⁶ It is well-known

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that thiol based groups can be chemisorbed onto surfaces by the formation of sulphur bridge on pristine platinum surface. The extension of the work used sulphur-containing thiol in cysteine as a comparison to the oxygen-containing analogue of hydroxyl group in serine. This selective poisoning by the sulphur containing derivative was illustrated in Figure 3.6 C). Suppression of velocity in the RU micromotor was not visible in the presence of 10 mM of serine. However, no moving micromotors were observed in the presence of cysteine at the same concentration.

Utilizing the sensitivity of platinum surface in the ED micromotors towards poisoning, direct selective detection of Pb^{2+} over Cd^{2+} via optical visualization of the activity and mobility of these microtubular Cu/Pt ED microfish can be realized, as depicted in Figure 3.6 D).⁴⁸ Leveraging on the LD_{50} (lethal dose for 50 % disablement of activity in micromotor), quantitative treatment of the selectivity of microfish towards heavy metal ions can be achieved, where the presence of 0.96 mM Pb^{2+} , as opposed to 8.9 mM of Cd^{2+} , is required to impair the mobility of the micromotor. The stronger poisoning of lead ions can be attributed to the stronger electronegativity of lead versus cadmium, leading to stronger adsorption and greater decrease in activity of the platinum's active sites.

There have been much progress in the detection of pH levels with the recent advent of micromotors that runs on the acid-base reactions. These were first led by the discovery of Gao *et al.* with zinc-based micromotor that moves in extreme acidic environment via hydrogen bubble-propulsion.⁴⁹ The team's later work showcased amphoteric aluminium-based motors that are able to work in both extremes of the pH.⁵⁰ With the inclusion of a palladium surface in the aluminium-based motor, coupled with the presence of hydrogen peroxide, unique velocity values could be obtained at different pH ranges. Indeed, these propulsion systems can be extended to the monitoring of industrial processes and even changes in pH of the environment. They exhibit competence to be the first indicators of industrial leakage, especially for chemical environments that uses extreme pH as part of the

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manufacturing procedures. However, it must be noted that the lifetimes of these devices are in the order of minutes, due to the limited fuel packed into the device.

Figure 3.6 **A**) Au-Pt nanowire average speed in 5% (wt.) H_2O_2 solution containing 100 μM metal-nitrate salt solutions and the motion-based chemical sensing of trace Ag^+ . Reprinted from ref. 47. **B**) Schematic depicting pollutant impeding microfish motion and the dependence of survival time upon pollutant concentration, where survival time is time taken for fish to lose directional movement and reaches minimal speed. Conditions: 2% (wt.) H_2O_2 and 5% (wt.) sodium cholate. Reprinted from ref. 15. **C**) Selectivity of RU micromotor's motion towards presence of cysteine and serine. Reprinted from ref. 36. **D**) Selectivity of ED micromotor's motion towards presence of Pb^{2+} and Cd^{2+} . Reprinted from ref. 48. Conditions in **C**) and **D**): 3 % (wt.) H_2O_2 and 1 % (wt.) SDS.

3.3.3 Tracing and Marking the Chemical Plume

Chemotactic behaviour of Pt/Au rods towards higher concentrations of hydrogen peroxide due to diffusivity has been demonstrated.⁵¹ The motion towards higher fuel concentrations of fuel has been due to the higher average displacement it experiences in regions of increased hydrogen peroxide fuel. The locomotion continues as these micromotors travel up the chemical gradient. This is the first illustration of inanimate objects demonstrating chemotactic behaviour and capabilities of moving against concentration gradients towards the fuel source.

Tracing the chemical to its source, in the event of leakage, takes a proactive stance to avert a large deluge of toxic chemicals into the environment. Micromotors have been known to demonstrate chemotactic behaviour towards fuel sources, ranging from hydrogen peroxide^{52,53} to polymeric materials of nucleotides⁵ and simple organic molecules.⁵⁴ Dey *et al.* illustrated the construction of bubble-propelled palladium-coated microspheres of polystyrene that move towards regions of higher alkali content in Figure 3.7 A).⁵³ The biased movement of these artificial devices in an external pH gradient has been attributed to the chemotactic behaviour towards hydroxide, which acts as a chemical attractant. The pH chemotaxis is able to move towards higher pH regions with increasing velocity, following the source of the alkali.

Later, Baraban *et al.* also studied the motion of two asymmetric man-made catalytic structures in channel environments: platinum-based tubular RU micromotor and Janus-type microsphere, running on the decomposition of hydrogen peroxide to oxygen, as illustrated in Figure 3.7 B).⁵² They illustrated that these artificial structures are able to move across a fuel gradient towards the source. The inherent sensing capacity to navigate in the channel environment and self-induced directed movement towards desired locations may allow it to be transformed into first responders towards chemicals, such as pollutants in water.

Figure 3.7 A) Schematic of Pd microparticle moving towards alkali-rich zone, away from the fuel-rich zone of hydrogen peroxide. Reprinted from ref. 53. B) Schematic for chemotactic attraction of catalytic micromotors in channels towards hydrogen peroxide-rich zone in a microfluidic flow cell with three merging inlets in Ψ -shape. Inlet i_1 contains hydrogen peroxide as the fuel. Micromotors are introduced through inlet i_2 . Inlet i_3 contains water. Scale bar: 5 μm . Reprinted from ref. 52. C) a) - d) Increasing angular deflection with increasing concentration of SDS at the reservoir. SDS reservoir is labelled “s” and inlet for introduction of SDS into circuit is labelled “x”. Zero-velocity points give an angle shown by the red lines. Paths along which the droplets moved are marked by dashed lines. Reprinted from ref. 19.

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A similar chemotactic behaviour of PSf capsules towards the presence of surfactant using long range interactions was also identified. Zhao *et al.* illustrated the negative chemotactic behaviour using deflection and directionality change of a SDS-loaded PSf capsule when traversing in the presence of another SDS-loaded PSf capsule, with these interactions extending long distances of several centimetres.²⁰ Under boundary conditions, optical marking of the origin for the source of chemicals can be realized in such a system using the PSf capsule, demonstrating promise of the optical checking and marking of leaks in a channel and pipe environment, as seen in the confined environment of Figure 3.7 C).¹⁹ The behaviour of PSf capsule in a circular track, with the presence of increasing surfactant concentrations in a central reservoir was studied.¹⁹ Such confinement of the PSf capsule in a circular maze resulted in monotonic increase in angular deflection of the PSf with increasing concentrations of the SDS surfactant, demonstrating capability in detection of SDS concentrations.

3.4. Nano/Micro/Macromotors Polluting the Environment

Environmental considerations of self-propelled objects and its possible implications on the surroundings are important points to note before their actualization in the field. Heavy-metals fabricated nano/micromotors under harsh conditions, together with the presence of corrosive hydrogen peroxide, insinuate that possible leaching can happen. Furthermore, cytotoxicity of such miniaturised materials must be established before the employment across wider applications can actually occur. Such long-range self-moving materials may in fact result in the increased transport and spread of pollutants. In this section, we explore the possible role of the self-propelled object being an active spreader of toxic pollutants.

Figure 3.8 A) Corrosion of concentric bimetallic Cu/Pt micromotors in various media. **a)** 7.76% H₂O₂ (fuel) and 1.15% SDS; **b)** 1.15% SDS and **c)** distilled water. Values stated in particular columns reflect the amount of the micromotors corroded within 7 days. Reprinted from ref. 57. **B)** Cell viability of A549 cells determined from the WST-8 and MTT assays after a 24 h exposure to different dilutions of the stock solution containing the nanomotors. Reprinted from ref. 59. **C)** Calculated mean squared displacement of 1, 1-diphenylhydrazine aggregates on the surface of sea water. Reprinted from ref. 60.

3.4.1 Corrosion of Micromotors

Nano/microtubular motors are often made from two or more different heavy metals, so as to introduce asymmetry to the structure of the nano/micromotors and demonstrate propulsion.^{55,56} The sequential layers of metals in electrical contact are known to exhibit a degradation phenomenon known as macrogalvanostatic corrosion in aqueous solutions, which is further exacerbated by the presence of an electrolyte.¹⁸ The origin of such a behaviour finds its basis from the different electrochemical potentials of the two metals. Such a continuous boundary between these metals results in a current flowing from the metal with a higher electrochemical potential to the metal with a lower electrochemical potential. The metal with the more positive electrochemical potential effectively dissolves, resulting in the corrosion of the metals. In the presence of the mixture of hydrogen peroxide and surfactant, concentric bimetallic tubular Cu/Pt micromotors were demonstrated to show corrosion of 1.3% Pt and 54 % Cu by weight after a week's period in Figure 3.8 A).⁵⁷ The effect is not limited to such template-assisted electrodeposited microtubes. Rolled-up microtubes, which utilized a sequential deposition of multi-layer sheets of metals showed a similar behaviour. In such circumstances, the differentially layered metals demonstrate corrosion at 3.9% for Pt, 11% for Ti, 29% for Cr and 79% for Fe by weight. Elevated levels of heavy metals are especially detrimental to human health.⁵⁸ Advocates for the use of micromotors must think in retrospect of whether the presence of such quantities of heavy metals will amount to any poisoning to the bio-environment or the natural environment.

3.4.2 Toxicity of Micro/Nanomotors

The toxicity of the nano/micromotors challenge the premise of whether such devices can be used in the environmental and biomedical context. The study of cytotoxicity in these materials are few and far between. Chng *et al.* recently demonstrated that an Au/Pt composite nanomotor fabricated via a template-assisted electrochemical method is

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cellularly benign and can find possible applications *in vivo*.⁵⁹ To draw relevance for future possible *in vivo* applications, the A549 lung epithelial cell line was used. Assays of WST-8 and MTT assay of the A549 cell line of lung epithelial cells demonstrates > 90% viability for a 24 h exposure in Figure 3.8 B). A lengthened exposure of 48 h in the presence of Au/Pt nanomotor demonstrates little change. Additionally, the nanomotor shows no distinguishable dose-dependent toxicity effects on cellular numbers too.

3.4.3 Propagation of Pollutants by Self-Propulsion

Modelling the transport of pollutants in the environmental system has often been undertaken using a passive model based on diffusion. However, it has been shown that millimetre-sized aggregates of organic molecules are able to demonstrate self-induced motion, actively interacting with its surrounding environment. Zhao *et al.* illustrated this phenomena with the use of an aromatic hydrazine molecule of 1, 1-diphenylhydrazine in different natural aqueous media. The agglomerate demonstrated transport of up to 5 orders of magnitude larger, in comparison to the standard molecular diffusion co-efficient as illustrated by the mean squared displacement of the particle in seawater media in Figure 3.8 C).⁶⁰ The asymmetric dissolution of these particles, led to the build-up of differences in surface tension, propelling them, a phenomena otherwise termed as the Marangoni effect. This alternative façade to the pollutant actively moving via a chemically induced effect provides us with valuable input for consideration with regards to the spread of environmental pollutants.

3.5 Controlling and Detecting Micromotors through Electrochemistry and Electric Fields

Figure 3.9 Schematics of electrochemical control of micromotors. **A)** Electrophoresis of a particle moving towards an oppositely charged electrode. Reprinted from ref. 61. **B)** Bipolar electrochemistry of a conducting particle moving by bubble emission.⁶² **C)** Dielectrophoresis of a particle experiencing either attraction or repulsion in an AC/DC electric field: Particles more polarizable than the media moves into the gradient of higher electric field intensities, positive dielectrophoresis (pDEP); Less polarizable particles move away from these regions, negative dielectrophoresis (nDEP). Reprinted from ref. 63. **D)** Induced-charge electrophoresis in an anisotropic Janus particle, resulting in unequal electroosmotic flows, culminating in the particle motion orthogonal to the applied electric field. Reprinted from ref. 64. **E)** Diode rectification of an AC field converted to localized DC electrophoresis, with the diode propelling with the direction of the cathode(-) facing front. Reprinted from ref. 65

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Micromotors convert energy from the surrounding environment into locomotion. Electrical fields are able to modulate energetics within the micromotors themselves or the immediate environment that they are enveloped in. Through the astute tuning of the surface charges or realizing electrochemical reactions on the interface between the media and the solid entity, propelling forces can be manifested.⁶¹⁻⁶⁸ The utility of an electrical field affects locomotive events, in which it has been demonstrated to not only change the propulsion, but illustrate guidance on the self-propelled micromotor.

Principally, both dielectric materials and conducting materials can all be subjects of interest to be manipulated. This can be accomplished through the modification of charges on the entity itself or the interfacial polarity at the electrochemical double layer, where polarity-based attractive or repulsive forces can be realized by the external driving electrodes.

The type of electric fields, define the mechanism of propulsive forces that can be realized on the object. External electrical fields can result in a redistribution of charges across the materials, leading to physical or chemical events, which have been known to control the motion of nano/micromotors and their properties, ranging from pure electrostatic forces, fluid flow to physical forces actualized by electrochemical reactions. These include the use of direct current and alternating current as external electrical fields that are used to exact autonomous motion on the object. Two types of electrical fields are of interest: DC (Direct current) electric field and AC (Alternating current) electric field. Electrophoresis and bipolar electrochemistry operates on the use of DC electric field. Separately, dielectrophoresis, induced charge electrophoresis, diode rectification to localized electrophoresis are based on the usage of an AC electric field. A summary is given in Table 3.1.

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Common parameters include the potentials applied across the electrodes, electrolytes, conductivity of the material, geometry and symmetry. Here, the discussions are divided into the type of electric fields used: DC or AC electrical fields. In addition to motion control, we will also delve into the electrochemical luminescence, their deployment as sensors, the applications of electrochemistry-modulated propulsive power to do work and remote electrical powering of electrochemical devices.

Table 3.1. Summary of electrochemical manipulations of particles for locomotion.

Particle-localized manipulation	<i>E</i> -Field	Homogeneous <i>E</i> -field	Directionality Control	Remote Electrical Powering	Particle Conductivity	Size Limit
Electrophoresis	D.C.	Yes	Yes	No	Dielectric and conductor	nm
Bipolar electrochemistry	D.C.	Yes	Yes	Yes	Conductor	μm
Dielectrophoresis	A.C./D.C.	No	Dependent on polarizability of particle	No	Dielectric and conductor	nm
Induced charge electrophoresis	A.C.	Yes	Dependent on Janus particle orientation	No	Anisotropic conducting and dielectric surfaces	nm
Diode Rectification to Localized Electrophoresis	A.C.	Yes	Dependent on diode polarity orientation	Yes	Semiconducting	nm

3.5.1. Control by Direct Current (DC) Electric Field

Figure 3.10 **A) (i)** Deformation and **(ii)** Locomotion following the movement of the cathode in 1.0 M NaOH with 0.3 mL of gallium and graphite electrode rods of diameter, 2 mm with a potential difference of 8 V. Reprinted from ref. 69. **B)** Translational motion generated with a 275- μm glassy carbon sphere in a microchannel with a 43 V cm^{-1} electric field in an aqueous solution of 7 mM HCl and 14 mM hydroquinone. Scale bar: 100 μm . Reprinted from ref. 70. **C)** Levitation of a light-emitting glassy carbon bead. Series of optical images showing a glassy carbon bead emitting electrochemical luminescence at different times during its motion. The bead was placed in a U-shaped cell, filled with 100 mM PBS buffer containing 0.5 mM $[\text{Ru}(\text{bpy})_3]^{2+}$, 100 mM TPA, and surfactants in an external electric field of 25.5 V cm^{-1} . The left image was taken under visible light and the other images were taken in the dark. Inset: Plot showing the change in height, h , of the bead as a function of time, t . Reprinted from ref. 71. **D) (i)** Scheme of a vertical rotor powered by the reduction of protons coupled to hydroquinone oxidation. **(ii)** Rotational motion of a vertical bipolar rotor, made out of an asymmetrical conductive polycarbonate sheets, in an

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electric field of 5 V cm^{-1} in 50 mM HCl and 100 mM hydroquinone. Scale bar, 0.5 cm. Reprinted from ref. 70. **E)** Still frames showing the LED-swimmer while changing the polarity of the horizontal electric field (44 V cm^{-1} in x-direction); inversion of the polarity at 4 s leads to a change in color and of the direction of the horizontal motion. Scale bar: 5 mm. Reprinted from ref. 72.

Direct current electric field is able to exert a uniform electric field across a fluid. It grants the user much ease to control the magnitude of the potential difference across the field. Exchanging the polarity of the electrodes also enables the converse reversal in motion. Furthermore, the locomotion can be easily started and stopped by turning the electric field on and off. Here, we investigate two electrochemical phenomena that can be realized in a DC field: Electrophoresis and bipolar electrochemistry.

Under the influence of a uniform electric field, electrophoresis causes the motion of the charged particles to move towards electrodes of the opposite charge, as shown in Figure 3.9A. The principal driving force for electrophoretic force is the charge on the particle and the electrophoretic mobility of the particles in the solvent under the influence of an applied electric field.⁷³ The charges can be borne on the particle itself or the enveloping electrochemical double layer surrounding the particulate. Two principal factors that affect electrophoresis are the properties of the colloidal solution and that of the electrical settings.⁷³⁻⁷⁶ This has become an established technique and protocols have been developed regarding the choice of electrolyte, pH, particle sizes, potential and current, due to the advent of electrophoretic deposition of nanomaterials.⁷³

Here, we list two examples that make use of electrophoretic phenomena to reversibly alter the locomotion of particles. Takinoue *et al.* demonstrated that micrometre-sized water-in-oil emulsion, prepared in the presence of surfactants, are capable of continuous motion.⁷⁷ The dielectric water droplet experiences an electrophoretic attraction

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towards the tungsten electrode and electrostatic repulsion upon contact. The two tungsten electrodes are staggered to allow continuous rotary motion, with exertion of potential differences at 40 V. Upon contact, charge transfer occurs and the droplet encounters repulsion due to the identical charges of the droplet with the electrode. The droplet then was attracted to the oppositely charged electrode due to electrophoretic forces. Upon contact, charge transfer occurs at the oppositely charged electrode and repulsion occurs again. The cycle can be continuous and the rotary cycle repeats itself.

Liquid metal droplets have been demonstrated to show locomotion due to electrophoretic phenomena. This was demonstrated by Liu and co-workers with a graphite electrode that is immersed into the liquid gallium amalgam, where the composite shows capability of shape deformation under voltage, due to the electrochemical reactions that take place on the metal surface.^{69,78} As the electrode is punctured into the liquid metals, the liquid amalgam and the electrode act as a single unit of electrode. When a pair of oppositely charged electrodes are used, the liquid metal is able to demonstrate shape deformation and locomotion towards the other pole of the graphite electrode, as illustrated in Figure 3.10 A. The prey-predator relationship is mimicked between the composite electrode and the electrode of the opposite polarity.⁶⁹ Furthermore, when a liquid bulk gallium amalgam is introduced as an cathode with a small capillary outlet, small spherical spheres are dispensed from the capillary tube under an electric field towards the anode.⁷⁸ It has been demonstrated to achieve micron-sized units when a fast ejection of bulk liquid gallium amalgam is executed.⁷⁹ With an electric field of 61 V m^{-1} , the liquid metal droplet reaches a velocity of 3 cm s^{-1} . The electric field generated across the different poles has been the origin of the liquid amalgam droplet showing locomotion towards the electrode of opposite polarity, in an electrophoretic phenomena.^{69,78}

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Bipolar electrochemistry allows for the powering of a conducting element, hereby termed as the bipolar electrode, that is within an electrical field generated by a D.C. current.⁶² It functions in solutions of low ionic strength as the conducting element must be more conductive than the medium itself.⁸⁰ Length of the particle defines the potential difference across the two terminus of the particle, which inadvertently affects the driving force and motion. In addition, this relationship of the maximum polarization voltage between two terminuses of the particle confined between the bipolar electrodes, is also defined by the electric field exerted by the driving electrodes.

Figure 3.9 B illustrates the setup of a conducting element that occurs between 2 driving electrodes during bipolar electrochemistry. If a substantial potential is conferred in an exerted electric field, two differing electrochemical reactions can occur across the conducting element. In an aqueous solution, hydrogen evolution reaction (HER) occurs at the cathode of the bipolar electrode, while oxygen evolution reaction (OER) occurs at the anode, as illustrated in Figure 3.9B. The production of the hydrogen gas Eq. (3.1) at one end and the oxygen gas Eq. (3.2) at the other is not equal due to stoichiometric amount of electrons that passes through the bipolar electrode. Such unequal production of gas on the bipolar electrode results in locomotion. This resulting formation and ejection of the bubbles results in the particle to move in the opposite direction of the terminus, where more vigorous hydrogen bubble production was observed. However, to increase the efficiency of the bipolar electrode during propulsion, an additive of hydroquinone, $C_6H_6O_2$ (HQ) can be added. The oxidation of HQ Eq. (3.3) precedes that of the oxidation of water, where the oxidation results in quinone, $C_6H_4O_2$ (Q) formation. The elimination of the oxygen bubbles retarding propulsion during electrochemical reaction in a bipolar electrode increases the efficiency of the unit, where the locomotion is wholly dependent on hydrogen bubble ejection.⁷⁰

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The electric field (E) applied on the object is equal to the potential applied (ΔV_1) divided by the distance (l) between the two poles of the driving electrodes, $E = \Delta V_1 / l$. Potential difference (ΔV_2) is thus exacted across the conducting element, termed as the bipolar electrode, in the electric field. The potential difference (ΔV_2) can be calculated from the product of electric field and the dimension of the bipolar electrode across its cathode and anode: $\Delta V_2 = E \times d$. If it exceeds the electrochemical potential of $\Delta V_2 = E_2^0 - E_1^0 = 1.23$ V, the redox reactions of water occurs and the bipolar electrode commences its locomotive behavior. In the presence of hydroquinone, the potential can be depressed to $\Delta V_3 = E_3^0 - E_1^0 = 0.70$ V

Micron-sized objects such as 275- μm sized glassy carbon sphere can be moved under a channel environment with an electric field of 43 V cm^{-1} , as illustrated in Figure 3.10B.⁷⁰ Directionality can be altered by switching the polarity and changes in magnitude of the potential difference alters the velocity of the micromotor.⁷² The dimensions of the micromotors and the external electric field strength defines the difference in polarity across the bipolar electrode in the unit of self-propelled micromotor. Roche and co-workers illustrated this through the manipulation of the magnitude of potential difference, affecting the velocities of the micromotor and oriented the direction by changing the polarity of the driving electrodes.⁷² The external electric field was controlled between 11 to 44 V cm^{-1} and changes in polarity was actualized to control the directional velocities of the micromotor via a bipolar electrochemistry methodology.

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Physical factors such as the confinement of the conductive particle in the capillary walls define the motion of the particle itself. This is to prevent the particle from encountering tumbling forces, which reduces the efficiency of the unit.⁸⁰ Surfactants can be added to aid the formation and release of the bubbles evolved.⁷⁰ Proton concentration,⁷⁰ OER suppressant hydroquinone,⁸¹ dimensions of particulates⁸⁰ and the inherent overpotential of the material during electrochemical reactions⁷⁰ in concern should also be accounted for. The material considerations for electrocatalytic effects is a key factor, when the choice of a substrate such as carbon or metal, is deliberated for a bipolar electrode.⁷⁰

Under confinement in a capillary tube, it allows for the accumulation of the hydrogen bubbles underneath the microparticle endowing buoyancy to the object, as shown in Figure 3.10C.⁸⁰ The continuous accumulation of the bubbles allows an upward force to be built for upward motion. This has been extensively illustrated by the Kuhn group, where hydrogen bubbles acts as buoyant forces for ascension.^{71,72,80,82} The forces work against gravity and enable the object travel in the z-axis.

This was later exemplified by Loget *et al.* who used a 1 mm conductive glassy carbon to move a polymer cargo up a glass capillary tube in an electric field of 23 V cm^{-1} .⁸⁰ The defined spaces of the capillary tube entrap the hydrogen bubbles beneath the glassy carbon bead for a buoyant force to uplift the bead and moving the cargo.

To overcome the problem of requiring the physical environment to trap gases, Roche *et al.* designed micromotors that can trap gas bubbles independently of the surrounding.⁷² A rocket-shaped motor with an escape corridor that allow the bubble to leak out, leads to an equilibrium between bubble production and escaping bubbles. This permits translational movement in the z-axis with constant acceleration with a defined amount of entrapped gases. Meanwhile, a cube-shaped motor with no escape catch, accumulates gas

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bubbles underneath the miniaturized motor, which experiences increasing driving force with time and therefore an increasing acceleration.

In addition, Roche *et al.* designed the cube shape swimmer to have two sets of metal wires that are orthogonal to each other. This allows controllable upward and horizontal motion of the device.⁷² When coupled with two sets of bipolar electrodes, the x-z motion of the micromotors can be controlled. It will not be unimaginable that with a third set of bipolar electrodes that the device can achieve motor motion in all three directions.

Horizontal and vertical asymmetry can be factored into rotors, resulting in complicated and elaborate movements.⁷⁰ Changing the shapes and materials of the object, has also enabled increased modes of action from the micromotors. Loget *et al.* designed rotors that are capable of revolving, by placing the conductive element asymmetrically on the fan-shaped rotor, pivoted by a glass stick.⁷⁰

The flaps of the fan-shaped rotor is made of conductive carbon-doped polycarbonate membrane that was covered with an insulating tape, with only the terminus exposed asymmetrically, as shown in Figure 3.10D. For the horizontal rotor, four flap-units of the exposed terminus follows a rotational symmetry, allowing electrochemical OER to occur on one side and HER on the other side of the flap. This creates momentum in electric field of 0.5 kV m^{-1} , achieving rotating speeds of 1.9° s^{-1} or 0.32 r.p.m. During rotation, when passing the central point at the axis parallel to the external driving electrodes, the electroactive parts of the rotor exchange their polarization. The formation and ejection of the bubbles along all four individual flaps result in the motion of the rotor in an anti-clockwise fashion.

For the vertical rotor, buoyancy of the bubbles produced has also been utilized as a driving force. However, the rotational symmetry of the rotor, may result in competing forces at opposite ends of the flap in the rotor. The introduction of hydroquinone to suppress

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oxygen evolution reaction to form oxygen bubbles, increases the efficiency of the vertical rotor, as illustrated in Figure 3.10D. Consequently, it also increases the amount of hydrogen bubbles produced due to increased HER with the lower ΔV that is required to drive hydrogen bubble evolution across the bipolar electrode in the rotor for hydrogen bubble production. The motion increases by two-fold, at a rotational speed of 4.2° s^{-1} or 0.70 r.p.m. An additional buoyant upward force and expansion of bubbles with increased hydrogen bubble production have been the principal origin in the vertical rotor's revolution.

A valve based on the bipolar chemistry was also created with a carbon membrane attached to a graphite in an enclosed compartment.⁸¹ Formation of hydrogen gas on a single side, with the suppression of oxygen evolution with hydroquinone allows the buoyant bubbles to gradually collect in the compartment and push up the flap of the valve, when the upward-acting forces overcome the weight of the load. However upon escaping, the buoyant forces reduced dramatically as the bubbles escape, resulting in the subsequent drop of the cap. The valve is thus closed again, repeating itself in a cyclical fashion as long as the electric field is kept on.

When Faradaic currents run through the bipolar electrode, with opposite electrochemical reactions occurring along the two different poles, it demonstrates capability of powering a small device. This demonstrates a remote powering of the unit through electric fields passing through the bipolar electrode. Such remote powering, allows far-ranged powering of a thermometer sensor and transmitting the data to a receiver.⁷²

Light emitting diodes (L.E.D.), which only allows the flow of current in one direction, allows the micromotors to be directionally labelled.⁷² Since the diodes only allow a unidirectional flow current, it represents a method of signaling the direction of motion. In this above listed example in Figure 3.10E, while moving in the horizontal direction from left to right, the LED is lighted red and the converse direction will be lighted green, as the

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polarity of the electric field is being reversed. This illustrated a micromotor with directional signaling, while also demonstrating the capability of remote sensing in the electric field direction.

Global effects of changing the polarity and magnitude of the motion of the micromotors can be achieved easily by altering the voltage across the two external electrodes in bipolar electrochemistry. This will culminate in the changes in magnitude and directionality of the micromotors. With the switching off of the electrochemical system, the motion of the micromotors can be terminated. However, the use of bipolar electrochemistry with a resulting global effect on the motion of multiple units has been limited, due to the need of entrapping bubble gases for locomotion.^{72,80}

However, the converse selective localized modulation of bipolar self-electrophoretic micromotors be accomplished through a selective usage of the electrochemical potential. Calvo-Marzai *et al.* demonstrated that with a micron-sized electrode, through the adjustment of oxygen concentration via a potentiostatic method, locomotive properties in populations of Au-Pt micromotors moving by self-electrophoresis can be altered, due to changes in surface tensions and suppression of oxygen concentrations during self-electrophoresis.⁸³ The global effect of controlling multiple micromotors by bipolar electrochemistry can be realized with a more intricate look at bipolar electrode and channel designs in the foreseeable future.

Bipolar electrochemistry has been demonstrated to manipulate the chemical composition of a solid metal. This was demonstrated with the use of a zinc dendrite that was immersed in the solution of zinc ions in a capillary tube.⁸⁴ The simultaneous deposition of zinc at cathode and dissolution of zinc at the other pole of the anode, results in locomotion, much alike a chemical wave propagation. A micrometre-sized zinc dendrite was able to reach a velocity of $80 \mu\text{ms}^{-1}$ in an electric field of 70 V cm^{-1}

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Bipolar electrochemistry coupled with electrochemical luminescence allows the self-propelled object to be monitored remotely, indicating the location of the device using light, as illustrated in the vertically rising motor in Figure 3.10C. This was first illustrated with $[\text{Ru}(\text{bpy})_3]^{2+}$ and co-reactant tri-n-propylamine (TPA) with a glassy carbon bead in a vertical capillary channel. On anodic oxidation, these substrates create intermediate states that react with each other to regenerate an excited molecular state of $[\text{Ru}(\text{bpy})_3]^{2+}$ which relaxes and releases a photon simultaneously. The light from this emission results in the orange glow of the micromotor device. Hence, this electrochemiluminescence allows the tracking of micromotor in a dark environment. Meanwhile, the cathodic end of the bipolar electrode produces hydrogen bubbles for propulsion and giving rise to buoyant forces. The orange glow is seen as moving towards the cathode of the external driving electrode. Later in another study, Sentic *et al.* used $[\text{Ru}(\text{bpy})_3]^{2+}$ in bipolar electrochemistry, but replaces the TPA with enzyme co-factor NADH. Upon expenditure of NADH to NAD^+ , it can be regenerated by glucose dehydrogenases in the presence of glucose.⁸⁵ Glucose dehydrogenase oxidizes glucose to produce NADH from NAD^+ . Thus, the bipolar electrode with the presence of increased concentrations of glucose, was able to give off an increased orange glow, demonstrating capabilities to detect glucose concentrations in the medium.⁸⁶

This effect was later extrapolated to the molecule luminol.⁸² However, this reaction now takes place in an alkaline environment in the presence of sodium hydroxide, hydrogen peroxide and luminol. The electrochemical oxidation of hydrogen peroxide to oxygen propels the micromotor, with a concomitant reaction of oxygen with luminol to give off blue electrochemiluminescence. In this case, the bipolar electrode is seen moving towards the anode of the external driving electrode in the setup. Thus, these two methodologies give the user an array of choices of tracking self-propelled micromotor in a dark environment using bipolar electrochemistry.

3.5.2 Control by Alternating Current (AC) Electrokinetics

Figure 3.11. **A) (i)** Scheme of 2D controlled motion of self-propelled micromotors sandwiched between electrodes that are perpendicular to each other in a grid. When AC voltage is applied on a bottom electrode, the micromotor moves along the bottom electrode. When the AC voltage is then switched onto a top electrode, the micromotor is uplifted and guided with pDEP and the ACEO to the top electrode and moves along it by electroosmosis. **(ii)** Trajectories of micromotors for 20 s. AC electric field (1 kHz, 8 V_{p-p}) was applied to the electrodes B1, T1, B2, T2, and B3 in sequence. Reprinted from ref. 87. **B) (i)** Optical micrographs of diode micromotor. **(i)** Overlay of image sequence with directional pathway of a self-propelling diode for a total duration of 43 s. **(ii)** Two LEDs light up and move towards the top or bottom depending on the orientation of their anodes (marked with white paint). All experiments in (i)–(ii) were carried out at electric field of $E=120 \text{ V cm}^{-1}$, frequency 1 kHz and 10^{-6} M NaCl . The scale bars represent 5 mm. Reprinted from ref. 65.

Use of AC fields to effect motion of micromotors has the benefit of avoiding electrolysis of water and competing forces in water such as DC electrophoresis across the

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two poles of the electrodes.⁶¹ There are in principal, direct or indirect pathways in which one can manipulate matter using an alternating electric field. A direct pathway includes dielectrophoresis of a particle experiencing either attraction or repulsion in an electric field: In positive dielectrophoresis (pDEP), particles more polarizable than the media moves into the gradient of higher field intensities; in negative dielectrophoresis (nDEP), less polarizable particles move away from these regions. Indirect pathways using AC electric fields include induced-charge electrophoresis and diode rectification of an AC field leading to localized DC electrophoresis. Induced-charge electrophoresis occurs in an anisotropic Janus particle, resulting in unequal electroosmotic flows. This culminates in the particle motion orthogonal to the applied electric field. Diode rectification of an AC electric field converted to localized DC electrophoresis, with the diode propelling in the direction of the cathode(-) in front.

In a non-uniform AC field, dielectrophoretic forces can manifest. This is a development of the particle being more polarizable than the media. The particles are being attracted to or repelled from the higher electric field intensity region due to interaction of induced dipole with the gradient of an AC electric field, as seen in Figure 3.9C. In positive dielectrophoresis (pDEP), particles that are more polarizable than the media are pulled along the gradient into the areas of the highest field intensities. In contrast, in negative dielectrophoresis (nDEP), particles that are less polarizable than the media are pushed away from these area. This is also especially prominent in DC fields, but can be overwhelmed by electrophoretic effects and water electrolysis at high potentials. Dielectrophoretic forces can therefore guide the motion of the particles.

Trajectory effect of the positive DEP effect can used to guide the direction of micromotor motion that was moving by electroosmotic effects.⁸⁷ This was illustrated in Au/Pt Janus particles that have been capped with gold and platinum. Instead of random motion of the micromotor, the direction of the micromotor can be directed unidirectionally.

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Coupled with another perpendicular set of electrodes sandwiching the micromotors in Figure 3.11A (i), the set-up allows for the two dimensional control of the micromotors along 2 axes, in Figure 3.11A (ii). The frequencies were controlled at 10-1000 kHz and voltages of 8 and 12 V_{p-p}.

Janus particles are particulates that have asymmetric surfaces. They form different induced electrochemical double layers due to the differing polarization, in the presence of an AC electric field, as illustrated in Figure 3.9D. At frequencies of lower than 10 kHz, the Janus particles are able to move due to this difference in polarizability across two different sides of the surface. This was exemplified in the gold coated polystyrene particle.⁶⁴ The electric field acts tangentially on the diffuse counter ions on the induced electrochemical double layer in the Janus particle, resulting an electroosmotic flow from the boundary of the gold/polystyrene modification towards the midpoint in the Janus particle. The flow across the metallic component is greater than the flow in the dielectric counterpart in the uncapped polystyrene component. This results in the direction of motion in the particle to be normal to the electric field.

Diode rectification of an alternating current (AC) field has been realized by Velez *et al.*⁶⁵ The localized DC voltage induced into the diodes from the AC field resulted in an electrophoretic motion on the diode. The particle-localized rectification of the AC electric field to direct voltage will lead to an electroosmotic ionic flux, which leads to a propelling force on the diode to be exerted in the opposite direction, as depicted in Figure 3.9E.

The polarized current direction of the diode can only flow in a specific direction under the AC electric field, depending on the alignment of the diode polarity (Figure 3.11B(i)). The corresponding current rectification phenomena can occur in a diode when it is exposed to an alternating current electric field. This results in a current flow between the anode(+) and cathode(-) of the diode. The current flow then induces an electro-osmotic

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flow of the ions at the interface of the diode and the electrolyte. With the diode held at the interface between air and water by surface tension, the electroosmotic flow powers the propulsion of the micromotor in the opposite direction. Typically, the external field of 120 V cm^{-1} and frequency of 1 kHz is used. Speeds can reach up to 8 cm^{-1} for a millimetre-sized diode. An arranged assembly of diodes can also permit for the design for rotational motion in the diodes. An encirclement of the diodes in a circular shape, with the polarity of the diodes in an anti-clockwise fashion, result in a rotary motion in an anti-clockwise manner. LEDs can also be utilized in the above scenario, resulting in light emitting devices that were powered by the AC electric fields, as illustrated in Figure 3.11B (ii). Depending on the relative orientation of the anode, the diodes can be manipulated to travel in different directions.

Miniaturization of the diodes was also realized by Wang and co-workers through the use of electrodeposition protocols, where different segments of the conductive polymers and metals were being electrodeposited.⁸⁸ 200 nm diameter segmented Schottky barrier diode nanowires such as polypyrrole-cadmium and CdSe-Au-CdSe nanowires can be achieved. In the presence of a uniform AC field, the polypyrrole-cadmium semiconductor diodes experience the rectified field-induced electroosmotic flow, demonstrating locomotion at $17.2 \text{ } \mu\text{m s}^{-1}$, at 7 body lengths per second. These diodes pave the way for use in microfluidics, where immobilized diodes can be used as pumps to introduce flow into the entrapped system.

3.5.3 Detection of Micromotors

The converse use of electrochemistry to detect the motion of artificial moving miniaturized devices has recently been demonstrated. Previously, attempts to use electrochemistry to quantify the motion of moving objects have mostly been limited to organisms such as *daphnia magna*⁸⁹ and fragmented biological units.⁹⁰ In one of the first

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instances of using electrochemical methods to track a moving man-made miniaturized object, Compton *et al.* demonstrated the use of an interdigitated electrode to survey positions of a millimetre-sized basalt ball moved by magnetic forces.⁹¹ Later, motion of water droplets in a microfluidic chip were detected by Fisher and co-workers using an on-chip electrode set-up.⁹²

However, it is only until recent times that chemically-powered units of autonomously moving objects was proposed to be able to be monitored by electrochemical techniques. With the ability to track and obtain information from miniaturized devices by electrochemistry, one can envisage an internet of things at the nanoscale with the use of networks for first-hand transmittance of data.⁹³

3.6. Conclusions and Future Outlook

Progress of nano/micro/macromotors has taken a huge leap, since the initial observation of locomotion at the experiential stage. Particularly, the fraternity's progress with surface modification and novel new materials have conferred new properties onto the motor. In this Review, we have demonstrated such self-propelling objects for the active remediation of environmental pollutants through removal and degradation, the influence that the environment exercises on the nano/micro/macromotor and their use in detection of contaminants and the tracing of chemical plumes to the source. Furthermore, we have also addressed the concern of such foreign autonomous self-powered man-made entities and their possible negative repercussions on the environment. Additionally, the use of electrochemistry and electric fields to control and detect miniaturised devices is also illustrated.

Although there are possible negative impacts from the use of such artificial self-propelled objects, the arguments we raised thus far paints a rosier picture for the future of nano/micro/macromotors. The autonomous motion of such self-propelled artificial objects

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are not to be overlooked, where efficiency and extent of reach have been amplified, in comparison to the mere diffusive mechanism in the absence of an external force. The *in situ* conversion of chemical energy into mechanical motion, raised the proposition for self-induced mixings for the environmental works of pollutant removal and contaminant degradation. Furthermore, the utilization of locomotion of these sensitized self-propelled devices to quantify and qualify chemicals, has opened a new area of work, with regards to sensors, tracers and markers for environmental monitoring. This new platform is expected to address global challenges and future needs of mankind, creating new science and technologies for a better world in the face of environmental degradation.

Fruition of these concepts and technological solutions, still needs to harness upon the innovations from scientists and engineers alike. Thus said, these applications are still at their budding stage, where scaling to the industrial level and practical employment in the field are still non-existent. The prospects of these proof-of-concept self-propelled devices are still limited by several pressing issues raised in our report: the inhibition of movement in real environments, limited lifetime, cytotoxicity, the role as a pollution vector and the constraints of synthesising these unique motors.

Design of these devices and their operations are currently at a threshold and remains a key challenge, where often additives such as surfactants and toxic hydrogen peroxide are used. A self-contained and self-sustaining device, independent from other externalities would have been the ideal scenario. The architectonics of these motors are also limited by synthetic methodologies, carried out mainly through the use of templates, with the use of template-assisted electrodeposition and roll-up technologies. More studies would have to be carried out too, to offer more predictability during applications for the collective behaviour towards a certain task, where the programmed motor can execute a definitive mode of work.

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Several recent developments have shown promising results for the fabrication of swarming numbers of such motors for the performance of specific tasks. For rolled-up technology by photolithographic methods coupled with e-beam deposition of metals, the technique itself has been shown to have a near 100 % yield.⁹⁴ Continuous advancement of metal deposition techniques such as glancing-angle deposition⁹⁵ and morphology control methodologies of direct laser lithography⁹⁶ will serve to design better and more intricate nano/micromotors.

An ensemble action by motors are especially attractive, when acting towards a common purpose for environmental clean-up. Initial work has yielded preliminary results on the collective and swarming behaviour of these miniaturised devices. This has been demonstrated in bubble-propelled motors,^{97,98} where previously these behaviours have only been observed in self-diffusiophoretic systems.⁹⁹⁻¹⁰¹ In collective emergent systems, with the mastery of energy and information control at the nano/microscale, it shall allow us to create intelligent communicating networks that are able to accomplish complex tasks as a single unit for environmental remediative measures.

Performing work with nanotechnology, particularly for environmental remediation and detection, is a sphere of work worthwhile exploration. With a saying coined by renowned scientist Richard Feynman: “There’s plenty of room at the bottom”, the coupling of nanotechnology and self-powered machines holds much promise. While several key challenges lies ahead, the progress made in the last decade has given much confidence to the scientific community. As with the early 18th century, where machines are gradually introduced by the industrial revolution, we hope that nanomachines can represent new opportunities in the 21st century, and especially so in the area of environmental remediation and monitoring. We will showcase a few advances of miniaturised motor technology in the following chapters of the thesis.

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Chapter 4

Detection of Heavy Metals by Micromotors

4.1 Introduction

4.2 Results and Discussion

4.3 Conclusion

4.4 Experimental

4.5 Supporting information

The results in this chapter were published in the following manuscripts:

Moo, J. G. S.; Wang, H.; Zhao, G.; Pumera, M. Biomimetic Artificial Inorganic Enzyme-Free Self-Propelled Microfish Robot for Selective Detection of Pb²⁺ in Water. *Chem. Eur. J.* **2014**, *20*, 4292-4296. (Cover)

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The thesis author was the primary investigator and author.

4.1 Introduction

Detection of water pollutants is an important, but challenging task. Thus to say, to be proactive, rather than being retroactive in solving problems surrounding aquatic pollution is of utmost importance.^{1,2} Through early detection, appropriate preventive measures could be taken, without the actual need of a remediation step. The response of living organisms towards aquatic contamination has been one of the frontlines of defence against the pollution of water bodies.³⁻⁵ Behavioural studies and mortality rates of fishes have been demonstrated to both identify and quantify the amount of contaminants in water.^{3,4} However, given the absence of clear guidelines, consistency and the sheer human capital and technological intensiveness required of such a set-up, the implementation is extremely difficult.⁶ Hence, there arises a need for a robust, simple and scalable system that can be easily implemented and has ease of operation.

Micromotors are new self-propelled materials at the forefront of materials science that can move autonomously to perform specific tasks.⁷⁻¹² These micro-machines or micro-robots hold great promise in numerous fields. They have been demonstrated to be able to carry out a wide spectrum of work including those in biomedical applications,¹³ cargo delivery¹⁴ and environmental remediation.^{15,16} Amongst all, the bubble ejection propulsion mechanism in a tubular catalytic engine, which utilizes hydrogen peroxide as its fuel, is one of the most intensively studied model.^{12, 17} These microjets have been shown to be able to navigate through real world environments: from the water bodies of lake water and sea water to tap water too.¹⁸ However, the surrounding chemical environment has also been demonstrated to have pronounced influence on the very behaviours of these microjets.^{19,20} Recently, Orozco *et al.* developed enzyme powered bubble-propelled micromotor, dubbed “microfish” for sensing pollutants, such as Hg^{2+} , Cu^{2+} or aminotriazole.²¹ It was argued that such artificial enzyme powered microfish can play the same role as live fish, obviating the need to use living organisms for toxicity assays. The downside of such artificial enzyme

powered microfish was that it is not selective to different pollutants and also that the enzyme layer (which is the sensitive layer) has limited shelf-time due to its biological nature. Here we wish to obviate the use of enzyme and show that artificial *inorganic* (enzyme-free) microfish can be used for selective sensing of Pb^{2+} in the waters.

The increase of heavy metals concentration in the environment has been associated with anthropogenic activities, especially that of the burning of fossil fuels and industrial activities.²² This is of particular concern, as the accumulation of such pollutants is detrimental to human health.²³ Platinum has been demonstrated to show sensitivity towards poisoning effects, as minute concentrations of heavy metals are known to retard the efficiency of catalytic convertors^{24,25} and also inhibit organic reactions during catalysis.²⁶ To evaluate the effects of poisoning by heavy metals, the decomposition of hydrogen peroxide over platinum has been used as a model of study.²⁷ In this work, we explore the possibility of using bubble-propelled Pt microfish robots to establish a correlation between their activity and the presence of heavy metal ions of Pb and Cd. A decrease in microjet activity can be attributed to the poisoning of the Pt microfish robot and the change in chemical environment. In the following sections, the use of a simple optical visualization of the microfish robots, allows for a facile inference to the type of pollutants that is present.

4.2 Results and Discussion

Here we will demonstrate that inorganic enzyme-free Pt-based microfish robots can selectively sense presence of Pb^{2+} in waters. The microfish robots are powered by the bubble propulsion caused by the catalytic decomposition of hydrogen peroxide at its inner surface of platinum to oxygen. The expulsion of the bubble at the ends, propels the microfish robots forward. The influence that heavy metals possesses over the viability of microfish robots was investigated. Two principal representative ions of Pb^{2+} and Cd^{2+} , commonly present in polluted waters,²³ were used as poisons to cripple the inorganic Pt

microfish robots. Ultrapure water was used as the control environment to which the motion of the microfish robots can be compared with. Two observable physical attributes obtained from the microfish robots were used to evaluate the propulsion of the microfish robots: the number of microfish robots which are expelling bubbles and the number of microfish robots exhibiting motion due to the bubble-propulsion. The average velocities and the corresponding standard deviation were taken. Selective introduction of the heavy metals of Pb^{2+} and Cd^{2+} , were carried out at appropriate concentrations to poison the inorganic Pt microfish robots.

Figure 4.1. Percentage of active microfish robots showing bubble ejection (Black line) and moving by microjet propulsion (Grey line). Poisoned with selected concentrations of $\text{Pb}(\text{NO}_3)_2$ (○). Poisoned with selected concentrations of $\text{Cd}(\text{NO}_3)_2$ (■). Ultrapure water was used in the control experiment, where both the running and bubbling percentages were defined as 100%. Conditions in all experiments: temperature of 23 °C, 3 wt. % of H_2O_2 and 1 wt. % SDS.

The motion of the microfish robots was first evaluated in distilled water. As illustrated in Figure 4.1, 100% of the microfish robots that ejected bubbles, demonstrated mobility in

ultrapure water. Then, selective introduction of the heavy metal salt of $\text{Pb}(\text{NO}_3)_2$ was carried out. At 0.5 mM concentration of $\text{Pb}(\text{NO}_3)_2$, only 72% of the microfish robots are bubbling and 67 % of them demonstrated bubble-propulsion motion. The gradual decrease in bubble ejection and microjet propulsion was recorded across increasing concentrations of $\text{Pb}(\text{NO}_3)_2$, until a concentration of 2 mM, where bubble ejection at 24% and bubble-propelled motion at 21% was recorded. In order to make sure that the Pt microfish robot is specifically reacting to Pb^{2+} and not to any inorganic compounds, we have looked into the literature. When the ionic strength of the KNO_3 was compared with 2 mM of $\text{Pb}(\text{NO}_3)_2$ at appropriate levels, there were lesser disabling of the microfish robots, with approximately 75% of them remaining active for bubbling and microjet propulsion.¹⁸ This was indicative that in addition to the changes in ionic strength due to the introduction of $\text{Pb}(\text{NO}_3)_2$, an additional mechanism of poisoning and disabling of the microfish robots was present, which is the poisoning of Pt surface by Pb^{2+} .

In Figure 4.1, the correlation between bubble ejection and mobility with the introduction of a Cd^{2+} poison through $\text{Cd}(\text{NO}_3)_2$ was investigated too. At 1 mM of $\text{Cd}(\text{NO}_3)_2$, 97 % of the microfish robots are bubbling and 92 % of them are exhibiting propulsion. This was in contrast to the large decrease in bubbling and propelled microfish robots in $\text{Pb}(\text{NO}_3)_2$ at the same concentration, where only 46% of the microfish robots are bubbling and 45% of them are exhibiting propulsion. The behaviour of microfish robots under influence of 2 mM $\text{Cd}(\text{NO}_3)_2$ demonstrated similar behaviour to that under KNO_3 at the same ionic strength, where approximately 75% of the microfish robots were demonstrating both bubble ejection and propulsion motion. A slowly tapering decrease in both bubble ejection and propulsion amongst the microfish robots was observed with increasing concentrations of $\text{Cd}(\text{NO}_3)_2$ and KNO_3 too. This recurring similar trend between the two salts demonstrate that the increment in ionic strength adversely affected the motion of microfish robots as observed by Zhao *et al.* in the work with chemical environments¹⁸

and Wang *et al.* with blood electrolytes.²⁰ It was only at a concentration of 9 mM of $\text{Cd}(\text{NO}_3)_2$, that a more than 50% disablement of the microfish robots (LD_{50} : the concentration of a given agent which is lethal to 50% of the artificial microfish robots)²¹ in bubble ejection and microjet propulsion could be achieved.

For the ease of comparison, the concentration of heavy metal ions which was able to poison the number of microfish robots bubbling and demonstrating mobility to less than 50% (LD_{50}), was used as a gauge. In the case of $\text{Pb}(\text{NO}_3)_2$, the amount of 1 mM of Pb^{2+} was required. However, on the other hand, 9 mM of $\text{Cd}(\text{NO}_3)_2$ was required to reduce the number of microfish robots exhibiting bubbling and microjet propulsion to below 50% (LD_{50}). This is a clear indication that platinum-based microfish robots are highly sensitive to the presence of lead ions as compared to cadmium and is able to detect the presence of lead down to micromolar concentrations, as demonstrated by the drastic drop in active microfish robots in the presence of 500 μM of $\text{Pb}(\text{NO}_3)_2$. This is in the same order of magnitude of the heavy metals found in effluents from industrial plants²⁸⁻²⁹ and sludge materials.³⁰ Notably, at these concentrations, heavy metals disrupt the ability of cellular ion channels to perform modulation, causing serious health concerns.³¹⁻³²

Figure 4.2 illustrated the velocity of the microfish robots with increasing concentrations of heavy metal ions. During the control experiment, in the absence of heavy metals, the microfish robots demonstrated an average velocity of $310 \mu\text{ms}^{-1}$. This was in line with previous velocities reported by our group, with the microfish robots fabricated via an electrochemical template procedure, achieving an average velocity of $365 \mu\text{ms}^{-1}$.¹⁸⁻²⁰ With the introduction of 1 mM of $\text{Pb}(\text{NO}_3)_2$ in Figure 4.2(a), the velocity of the microfish robots dropped to $196 \mu\text{ms}^{-1}$. This was contrasted with the case of $\text{Cd}(\text{NO}_3)_2$ at 1 mM in Figure 4.2(b), where the velocity of the microfish robots remained considerably higher as compared to the $\text{Pb}(\text{NO}_3)_2$ at $231 \mu\text{ms}^{-1}$. The Pb^{2+} poisoned microfish expressed a steady decrease in velocity from 1 mM to 2 mM as compared to Cd^{2+} poisoned microfish robots,

where a relatively steady velocity between ~ 125 to $150 \mu\text{m s}^{-1}$ was reached at concentrations above 1 mM. It was only at 9 mM of $\text{Cd}(\text{NO}_3)_2$ that the velocity of microfish dropped to $74 \mu\text{m s}^{-1}$. The phenomena observed with $\text{Cd}(\text{NO}_3)_2$ was analogous to the phenomena observed with KNO_3 , where with increasing concentrations, the velocities of the microfish robots was held relatively constant at $\sim 150 \mu\text{m s}^{-1}$.¹⁸ The similarities of the observations under influence of $\text{Cd}(\text{NO}_3)_2$ and KNO_3 for microfish robots demonstrate that the reduction in activity was principally due to changes in ionic strength. However, in the presence of Pb^{2+} , an additional poisoning mechanism of the microfish was present.

Figure 4.2. Velocity of Pt-catalysed microfish robots in electrolyte solution. **(a)** Poisoned with selected concentrations of $\text{Pb}(\text{NO}_3)_2$. **(b)** Poisoned with selected concentrations of $\text{Cd}(\text{NO}_3)_2$. Ultrapure water was used in the control experiment, where both the running and

bubbling percentages were defined as 100%. Conditions in all experiments: temperature of 23 °C, 3 wt. % of H₂O₂ and 1 wt. % SDS.

The differing behaviours of the heavy metals-poisoned microfish robots could be explained as follows. Pb²⁺ are known to be strongly adsorbed on the platinum: this is such that they cover the active sites of the platinum, resulting in the decrease in activity of the platinum's catalytic sites.²⁵ Many reports have shown that the adsorption of the lead ion on platinum is irreversible³³ and it is often termed as a permanent poison.³⁴ However, in contrast, adsorption of Cd²⁺ are noted to be weaker³⁵⁻³⁷ and the interactions are likely to be reversible. The decreased bubbling at Pb²⁺-poisoned microfish was coupled with decreased activity and velocity. Evidently, the main mechanism of bubble-propulsion has been retarded, resulting in the loss of motility.

This could largely be explained through the electronegativity of the Pb²⁺ ions. Lead, with an electronegativity of the 2.33 on the Pauling scale, is considerably more electronegative than the 1.69 in cadmium.³⁸ The adsorbed adatoms of Pb are likely to be strongly attached on the surface of platinum during adsorption, as compared to Cd, resulting in the greater degree of poisoning as demonstrated in this report. The atomic size of lead is also such that it is larger than cadmium, covering more active sites on the platinum surface, effectively disabling the microfish robots further. This blocking effect by adatoms on noble metals, leading to the decrease in catalytic activity. The term, also known as underpotential deposition of metal ions on platinum surfaces, is attributed to the higher electronegativity of the lead atoms as compared to cadmium ions, which have led to differing responses of catalytic activity due to the blocking effect. Previous reports stated that during electrocatalysis in hydrogen evolution, lower activity was found in platinum in the presence of lead, in comparison to cadmium ions. This is due to the weak adsorption of such cadmium ions in comparison to lead ions.³⁹ In another independent study of a self-

electrophoretic Pt-Au nanorod, a similar but less accentuated trend was also observed between the presence of Pb^{2+} and Cd^{2+} , illustrating the poisoning effects of Pb^{2+} .⁴⁰

However, it must be mentioned that organosulfur compounds also inhibit the activity of microfishes to similar degrees¹⁹ and the presence of red blood cells retard their movement too.⁴¹ Therefore, the contextual space in which the microfishes are applied must be taken into consideration too. More work is still required for the use of this proof-of-concept device for selective detection of compounds against a general setting.

The differing behaviour of the microfish robots towards heavy metal ions of Pb^{2+} and Cd^{2+} allows for a selective inference of the type of ions that are present in the solution. While colorimetric methods,⁴² self-electrophoretic nanomotors⁴⁰ and enzyme-powered microfishes²¹ face selectivity limitations for heavy metals at high micromolar concentrations, a Pt-based microfishes demonstrate aptitude at these ranges. This opens a simple pathway towards identifying the type of inorganics that are present in aquatic sources. The study of the activity and mobility of the inorganic Pt microfish robots allows for a facile detection of the lead ions over cadmium.

4.3 Conclusion

The poisoning of inorganic Pt microfish robots were systematically studied with the selective introduction of heavy metal salts of $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$. A greater decrease in the activity and the velocity of inorganic Pt microfish robots were observed during poisoning with $\text{Pb}(\text{NO}_3)_2$, as compared to $\text{Cd}(\text{NO}_3)_2$. The inherent properties of the lead ion's adsorption behaviour with platinum, resulted in the decreased activity of the inorganic Pt microfish robots as compared to Cd^{2+} . The differentiating behaviour of Pb^{2+} with inorganic Pt microfish robots, allows for the selective detection of Pb^{2+} over Cd^{2+} . This allows for a potential pathway for a continuous monitoring of pollutants, via an optical visualization of the activity and mobility of the inorganic Pt microfish robots.

4.4 Experimental Section

Materials. The cyclopore polycarbonate membranes with pores of 2 μm in diameter were purchased from Whatman, USA (Cat no. 7060-2511). The pores are conical in shape. Colloidal graphite (isopropanol base) was purchased from Ted Pella, Inc., USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1M KCl were purchased from CH instruments, USA. The platinum plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98+ %), sodium dodecyl sulphate (SDS), $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 $\text{M}\Omega\text{ cm}$) from a Millipore Milli-Q purification system.

Apparatus. Electrochemical deposition was carried out with a $\mu\text{Autolab}$ type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 $^\circ\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Beckman Coulter Allegra 64R centrifuge. Optical microscope videos and images were obtained with a Nikon Eclipse TE 2000-E microscope. Video sequences (100 fps) were processed with Nikon NIS-Elements software.

*Preparation of Cu/Pt concentric bimetallic microtubes.*⁴³ The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template and they are referred as ‘microfish robots’ in the following discussion. Colloidal graphite ink was applied on the one side of the

polycarbonate template with commercial cotton swabs. A piece of flattened aluminium foil was attached to the ink immediately, which serves as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl reference electrode were utilized. Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyzer connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software. The template was rinsed with 5 mL of ultrapure water (18.2 M Ω cm) for 4 times, and the Cu outer layer was deposited galvanostatically at -4 mA for 450 s. The deposition solution contained 1 M CuSO₄. Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -4 mA for 450 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled and the template was washed for 5 times with 8 mL of water each. After that, the template was ultrasonicated for 3 times in 2 mL of ultra-pure water for 3 min each time. The graphite layer was removed during the ultrasonication procedure. The template was placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly for 3 times with methylene chloride. The solution was then washed with ethanol and water for 2 times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Operation of microfish robots. The experiments for studying the influence of heavy metals on the motion of microfish robots were carried out in an aqueous solution containing 3 wt. % of hydrogen peroxide at constant surfactant concentrations (1 wt. % of SDS). A mixture of microfish robots (7 μ L), SDS (1 wt. %), H₂O₂ (3 wt. %) and appropriate

concentrations of the heavy metal salts of $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$ were applied on a glass slide freshly cleaned with nitrogen gas. Optical microscope videos and images were obtained with Nikon Eclipse TE 2000-E microscope. Video sequences (100 fps) were processed with Nikon NIS-ElementsTM software. Statistics from the number of microfishes within a reading has been included in the supporting information (Figure 4. S1).

4.5 Supporting Information

Figure 4.S1. Number of active microfish robots showing bubble ejection and moving by microjet propulsion. Poisoned with selected concentrations of $\text{Pb}(\text{NO}_3)_2$ (●). Poisoned with selected concentrations of $\text{Cd}(\text{NO}_3)_2$ (■). Ultrapure water was used in the control experiment, where both the running and bubbling percentages were defined as 100%. Conditions in all experiments: temperature of 23 °C, 3 wt. % of H_2O_2 and 1 wt. % SDS.

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Chapter 5

Graphene Oxide Adsorption of Heavy Metal Ions

5.1 Introduction

5.2 Results and Discussion

5.3 Conclusion

5.4 Experimental

5.5 Supporting information

The results in this chapter were published in the following manuscripts:

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5.1 Introduction

Graphene is an emerging material of interest, due to its two-dimensional structure, large surface area with a honeycomb lattice made of carbon.¹ The oxidised analogue, graphene oxide, has attracted an equal amount of attention due to the abundance of oxygen functionalities, which allows for easy manipulation and configuration.² To date, the bulk synthesis of graphene oxide has been accomplished through the oxidation of graphite to graphite oxide. The lamellar graphite oxide structure with increased interlayer spacing due to the oxygenated groups, allows for a facile exfoliation to graphene oxide via an ultrasonication step.

Preparation of graphite oxide has a rich history that dates back more than 150 years ago.³ Its structure has been demonstrated to contain various oxygenated groups, such as alcohols, epoxides, carbonyls and carboxylic acids.² The most accepted Lerf-Klinowski model has described graphite oxide as carbon sheets with graphitic sp^2 islands that are filled with oxygenated functional groups: with epoxyl and hydroxyl groups on the basal plane and carbonyl and carboxylic groups at the edge planes.⁴ Three principal methods for the preparation of graphite oxides have been identified in literature: Staudenmaier's method with chlorate oxidant in fuming nitric acid;⁵ Hofmann's variant with chlorate in sulphuric acid⁶ and the Hummer's method with the permanganate oxidant.⁷ Such differing preparation methods have been demonstrated to influence the composition of the oxygenated groups in the graphite oxides,⁸ which consequentially affect the chemical profile of the graphene oxides.^{9,10} Oxygenated groups in graphene oxides are demonstrated to serve quintessential roles in a wide spectrum of applications. They have been shown to be available for use in covalent chemistry,¹¹ to anchor metal nanoparticles,¹² demonstrate photoluminescence¹³ and exhibited catalytic behaviour in synthetic routes.¹⁴ Most interestingly, recent research has shown that they possessed improved adsorption rates

towards heavy metals in comparison to other common adsorbents for environmental remediation.¹⁵⁻¹⁹

Due to the recent rise in anthropogenic activities, such as industrial activities and indiscriminate disposal of refuse, environmental pollution have become a serious problem.²⁰ Water pollution caused by heavy metals is particularly of concern, due to the ready accumulation of these heavy metals in the eco-system and the health problems they pose.²¹ Remediative measures not only protect the natural habitat, but too prevent the concentration of heavy metals along the food chain, which inadvertently affects humans in the surroundings. Measures to remove these heavy metals include reduction, precipitation, membrane filtration, ion exchange and the use of adsorbents.¹⁸ Adsorption is amongst the most ideal method, as it not only removes the pollutants from the system, it also allows for proper disposal of such chemical hazards and its possible recovery for the conservation of materials.²²⁻²⁴

Carbonaceous nanomaterials have large surface area, are porous and can be decorated with oxygenated functionalities, making them fitting candidates as adsorbents.²⁵ Allotropes of carbon, such as activated carbon, carbon nanotubes and fullerene show high capacity towards the adsorbing of environmental pollutants.²⁶ Recent findings have turned the spotlight towards graphene oxide-based materials, where reports of unprecedented adsorbance of pollutants such as antibiotics,²⁷ dyes²⁸ and heavy metals¹⁵⁻¹⁹ have been recorded. Graphene oxide shows particular affinity towards the adsorption of heavy metals, due to the ready availability of oxygenated groups. The liberation from steric hindrances due to its large surface and the availability of both sides of the graphene oxide towards the binding of heavy metal ions make it an outstanding candidate. Zhao *et al.* has employed the use of Langmuir isotherm to simulate the maximum adsorption capacity of graphene oxide synthesized by the Hummer's method and calculated that the maximum adsorption capacity to be 842 mg/g for Pb (II)¹⁶ and 106.3mg /g for Cd (II).¹⁷ Recently, Sitko *et al.*, using a

chromate oxidant via the Hummer's methodology calculated a maximum adsorption capacity of 1119 mg/g for Pb (II) and 530 mg/g for Cd (II),¹⁵ for the Langmuir isotherm, surpassing the earlier work. The adsorption capacities of the graphene oxide-based materials surpass the nearest comparable sorbent by more than 30-fold, as seen in a recent compilation of sorbent adsorption capacities.¹⁶ To elucidate the reason behind this property, a structural-functional relationship must be established. Here forth, we investigate how different preparation methods for graphene oxide affect their ability in adsorption towards two important anthropogenic sources of heavy metal ions, namely, lead and cadmium in waters.

Previous works on adsorption of heavy metals focused only one type of graphene oxide that was prepared mainly by the Hummer's method. We have previously demonstrated that graphene oxides prepared by different methods contain qualitatively different oxygen containing groups and thus using graphene oxides prepared by various methods may have a strong influence upon its heavy metal adsorption properties. We will show in this report that this hypothesis is indeed true. In this report, three types of graphene oxide (GO), produced by Hummer's (HU), Hofmann's (HO) and Staudenmaier's (ST) methods were used for adsorption tests with heavy metal ions of Pb²⁺ and Cd²⁺. Fourier-transformed infrared (FTIR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy/ elemental detection X-ray spectroscopy (SEM-EDS) spectroscopy were used to characterise the GO material before and after the adsorption experiment to understand the correlation of the oxygenated carbon functionalities with heavy metal adsorption. Inductive-coupled plasma mass spectroscopy (ICP-MS) was used to detect the amount of heavy metal ions adsorbed by the GO. We illustrate that preparation methods and consequentially, the oxygenated carbon functionality composition of the GO affects the amount of heavy metals that are adsorbed by the GO material. HU GO demonstrates the best efficacy towards removal of Pb²⁺ and Cd²⁺ ions. The result gives us an improved

understanding of the functionalities present in GO and empower us to further design and customise the GO material towards heavy metal ions adsorption.

5.2 Results and Discussion

Prior the study of adsorption of heavy metals on graphene oxides prepared by different methods, the graphene oxide (GO) materials synthesised from Hummer's (HU), Hofmann's (HO) and Staudenmaier's (ST) methods were characterised in detail, to elucidate the functionalities that were present. Adsorption experiments were carried out with 0.1 mg/mL of GO. Under ambient conditions, deionised water has a pH of 6. However, in the presence of 0.1 mg/mL of GO, the pH of the solution of HU, HO and ST reached a pH of around 3, indicative of their acidic nature.^{16,17} When 5 ppm of heavy metal ions was introduced, decreases in pH were observed in GO synthesised via HU and HO, but no such drop in pH was observed in ST (See supporting information Table 5.S1). The phenomena was particularly accentuated in HU GO, where a change in pH from 3.40 to 2.62 in the presence of Pb (II) and 2.57 in the presence of Cd (II) were observed. This was indicative of the binding event between GO and heavy metal ions, due to the substitution of protons with the metal ions.¹⁶ The GO materials, after adsorption, were then characterised to elucidate the adsorption mechanism of the GO towards heavy metal ions. Here on, the Pb (II)-adsorbed GO are labelled HU Pb, HO Pb and ST Pb, whilst the Cd (II)-adsorbed GO are labelled HU Cd, HO Cd and ST Cd. Finally, ICP-MS of the filtrate was used to determine the capacity of each individual GO towards adsorption of heavy metal ion and its efficiency.

5.2.1 FTIR Characterisation of GO Materials

Figure 5.1. (ATR) FTIR absorbance spectra of (a) HU GO film (b) HO GO film (c) ST GO film before and after heavy metal ion adsorption. **1:** $\nu_{C=O}$; **2:** $\nu_{C=C}$; **3:** ν_{C-O} (carboxyl); **4:** ν_{C-O} (epoxyl/ether); **5:** ν_{C-O} (alkoxyl/alkoxide); **6:** ν_{C-O} (epoxyl/ether).

The GO materials prepared by HU, HO and ST methods were characterised via FTIR analyses to investigate the presence of the different oxygenated carbon functionalities and the mode of binding between GO and the heavy metal ions. Six characteristic peaks centred at around 1719 cm^{-1} ($\nu_{C=O}$); 1624 cm^{-1} ($\nu_{C=C}$); 1373 cm^{-1} (ν_{C-O} (carboxyl)); 1228 cm^{-1}

$^1\nu_{\text{C-O}}$ (epoxy/ether); 1050 cm^{-1} ($\nu_{\text{C-O}}$ (alkoxy/alkoxide)); 951 cm^{-1} ($\nu_{\text{C-O}}$ (epoxy/ether)) are found in the FTIR absorbance spectra in all three of the graphene oxide films^{29,30} (See Figure 5.S1 for full spectra). It was evident that the differing oxidizing conditions have resulted in the different composition of oxygenated carbon functionalities. The HU pathway resulted in the GO material with higher oxidized functionalities as compared with the chlorate methods in HO and ST method. This could be observed in the carbonyl stretching band in the GO film, where the highest absorbance was observed in HU GO film, as compared to HO or ST.^{8,9} The FTIR absorbance spectra of the heavy metals ions adsorbed GO film were also evaluated. Park *et al.* has established that the increased intensity of the C-O stretching in the carboxyl peak and its shift towards lower wave number is associated with the co-ordination of metal ions in GO.³⁰ In Figure 5.1, lead-adsorbed GO materials of HU Pb and HO Pb both show increases in intensity of the carboxyl stretching band and a shift towards lower wavenumber upon adsorption with heavy metal ions. Notably for ST Pb, the carboxyl stretching band diminished upon adsorption of heavy metals, suggesting its lower efficacy as an adsorbent for heavy metal ions. In the case of cadmium-adsorbed graphene oxide materials, HU Cd demonstrated the same intensity of the carboxyl absorbance peak as the control HU graphene oxide film. An increase in absorbance was observed in the HO Cd graphene oxide film. However, a diminished signal for the absorbance of carboxyl group in ST Cd was again observed, suggesting its lesser role as an adsorbent. A more investigative insight to the binding mode between the oxygenated functionalities present with the heavy metal ions is undertaken via XPS.

5.2.2 XPS Characterization of GO Materials

5.2.2.1 Survey Scan

Figure 5.2. X-ray photoelectron spectroscopy spectra on graphene oxide film (a) control (b) after adsorption with 5 ppm of Pb²⁺ (c) after adsorption with 5 ppm of Cd²⁺.

The XPS technique allows for the sensitive detection of surface elemental compositions. A typical survey scan enables us to have a fast overview of the elements present in the material of interest and the percentage of the elements that are present. The representative spectra was analysed, while average values of the XPS values are found in the supporting information (Table 5.S2 and S3). Based on the survey scan in Figure 5.2, HU GO has the lowest C/O ratio (Table 5.1). This is the same phenomena as reported with graphite oxides produced by permanganate and chlorate oxidants: with the permanganate routes producing GO with significantly lower C/O ratio, as reported by Chua *et al.*⁸ and Eng *et al.*⁹ The GO film produced via the HU route in this study has a C/O value of 3.3, which is close to the value in the HU GO films produced from filtration methods reported by Yang *et al.* with a value of 2.9.³¹ HO GO and ST GO demonstrated higher C/O ratio at 3.65 and 4.00. The low atomic ratios in all three GO indicated that the oxidation process is thorough.⁸ The extent of oxidation is such that it is greatest in HU, followed by HO and

then ST. During survey XPS with heavy-metal adsorbed GO, significant peaks from the doublet of Pb 4f were identified at 139.0 and 143.8 eV in HU Pb and Cd 3d doublet at 405.0 and 411.8 eV in HU Cd.¹⁵ Minor shoulders were identified in HO Pb and HO Cd. However, no peaks were identified in ST Pb and ST Cd, likely due to the amount of Pb and Cd present being lower than the detection limit of XPS in these GO.³² It was interesting to note that the introduction of heavy metal ions does not change the C/O composition of HU and HO GO significantly as seen in Table 5.1. However, significant changes in the composition of ST GO was noted, with a drop in C/O ratio from 4.00 in ST to 3.61 in ST Pb and 3.53 in ST Cd respectively. Changes in composition of the C/O ratio has previously been observed with the introduction of metal ions during the fabrication of metal ion/ graphene oxide film.³⁰ They have been associated with the ring-opening of epoxyl groups in the presence of Lewis acidic metal cations. The Lewis acid metal may coordinate with the oxygen of the epoxide, thereby activating the epoxide by increasing the polarity of the C-O bond and the ease of weak nucleophiles attacking the carbon atom. ST Cd exhibits a greater change in oxygen composition due to the greater amount of ions present in 5 ppm of heavy metal ions as comparison to ST Pb. This corroborated with the result of increased oxygen composition being due to the introduction of hydroxyl groups during nucleophilic attack via the water molecules present.

Table 5.1. C/O ratio obtained from the XPS survey scans via elemental analysis using respective atomic percentage of C 1s and O 1s peaks.

Adsorption	HU	HO	ST
GO	3.30	3.65	4.00
GO Pb	3.27	3.64	3.61
GO Cd	3.10	3.83	3.53

5.2.2.2 High Resolution XPS C1s Scan

Figure 5.3. High-resolution XPS C 1s core-level spectra for HU, HO, and ST GO film.

Figure 5.4. High-resolution XPS C1s core-level spectra for (a) HU, HU Pb, and HU Cd (b) HO, HO Pb and HO Cd (c) ST, ST Pb and ST Cd.

High resolution XPS C1s scans were carried out to investigate the type of oxygenated carbon functionalities that were present and the mode of binding between GO and the heavy metal ions. First, the XPS spectrums of HU, HO and ST were overlaid and

compared. As illustrated in Figure 5.3, the three spectrums share a similar baseline and a direct comparison of the composition and type of oxygenated carbon functionalities present are possible. It is evident that the different oxidation treatments have resulted in different oxygenated carbon compositions. It can be observed that the GO film produced by the HU method is significantly more oxidized, with increased presence of carbonyl and carboxylic groups at the region of 287.5 to 289.5 eV.^{8,9} This was followed by the HO GO film and then ST GO film, which demonstrates the lowest amount of oxidation. This is in agreement with the survey scan that the HU GO produced by permanganate oxidation method is indeed the most oxidized.

Table 5.2A. Tabulated quantitative distributions of deconvoluted peaks from the high-resolution C 1s spectra of HU, HO and ST.

Material	C 1s peak distribution [%]				
	C=C	C-C	C-O	C=O	O-C=O
HU	40.1	11.3	30.3	13.1	5.3
HO	47.1	3.1	44.9	3.5	1.3
ST	50.2	5.8	38.3	4.7	0.9

Table 5.2B. Tabulated quantitative distributions of deconvoluted peaks from the high-resolution C 1s spectra of HU, HO and ST.

Material	C 1s peak distribution [%]				
	C=C	C-C	C-O	C=O	O-C=O
HU Pb	34.2	12.3	33.9	15.6	4.1
HU Cd	41.7	6.9	35.2	11.7	4.7
HO Pb	50.1	1.7	43.2	3.4	1.7
HO Cd	50.5	3.9	40.1	4.3	1.2
ST Pb	47.7	4.5	42.6	4.0	1.3
ST Cd	44.7	4.5	46.0	3.6	1.2

The C1s core-level spectra were deconvoluted into Gaussian peaks centered at 284.4, 285.4, 286.7, 287.6 and 288.8 eV which corresponded to the binding energies of C=C, C-C, C-O, C=O, O-C=O respectively, as illustrated by Figure 5.4. Typically, the raw spectrum of the HU, HO and ST GO exhibits a strong C-O peak and a strong C=C peak. It was also noted that in HU GO film, larger proportions of carbonyl and carboxyl groups were found as described by Chua *et al.* with graphite oxides produced by permanganate methods.⁸ This was in comparison to those produced by chlorate methods in HO and ST as illustrated in Table 5.2A.

Significant shifts in binding energies observed in HU GO was, however, not observed in HO and ST GO film, after adsorption with heavy metal ions. As shown in Figure 5.4 (a), after adsorption of heavy metal ions, the peaks from the XPS spectrum of HU Pb broadened. Furthermore, there was a shift towards higher binding energies for the carbonyl and carboxyl groups. In particular, upon deconvolution, the binding energy of the O-C=O peak shifted by 0.7 eV towards higher binding energies from 288.8 to 289.5 eV. The binding energy of C=O shifted 0.3 eV from 287.6 eV to 287.9 eV too. This was indicative that the heavy metal ions are bound to the carboxylate groups via an inner sphere mechanism as demonstrated by Guan *et al.* with adsorbed carboxylate groups.³³ The formation of inner-sphere complexes between the carboxylic groups and metal ions has increased the effective nuclear charge and hence increased the binding energy of the electron.

XPS spectra of chlorate-prepared GO of HO and ST after heavy-metal ion adsorption were studied. No significant shifts in peaks or change in composition was observed in chlorate-prepared HO GO as seen in Figure 5.4(b). However, for ST GO, there is a trend of the C-O peak increasing from ST Pb to ST Cd after adsorption experiments.

Park *et al.* explained that the presence of the metal Lewis acid sites, resulted in the ring-opening of the epoxides, due to the attack by weak water nucleophiles.³⁰ As a higher amount of divalent ions is present in 5 ppm of Cd (II) as compared to Pb (II), the ring-opening of epoxides is more extensive in ST Cd as compared to ST Pb, as observed by the C/O ratios in survey scans. Consequently, the introduction of these oxygen groups increased the amount C-O percentages in ST Pb and ST Cd as shown in Table 5.2B to 42.6% and 46.0% respectively as compared to the control ST GO film at 38.3%. This phenomena corresponded well to the survey scans, where there was a decreasing C/O ratio from ST to ST Pb and then to ST Cd. This suggests that GO prepared by ST method are rich in epoxides that are easily cleaved by Lewis acidic metal cations, as compared to the variant in HO GO or permanganate oxidized HU GO.

Figure 5.5 SEM images of HU, HO and ST GO film (Magnification = 40 000 X).

5.2.3 SEM-EDS Analysis

SEM images, as illustrated in Figure 5.5, demonstrates the GO films to be irregularly stacked sheets of carbon with large surface area and a wavy morphology as previously reported.³⁰ No particles of lead and cadmium were observed in the images, indicating that the take-up of the heavy metal ions, followed an adsorptive mechanism. Using the EDS coupled to the SEM, analysis of Pb content in HU Pb indicate an elemental composition with weight percentage of 1.97% and an atomic percentage of 0.05%. The subsequent analysis of Cd content in HU Cd indicated a weight percentage of 0.4% and an atomic percentage of 0.03 %. No corresponding concentrations were found on HO Pb, HO

Cd, ST Pb and ST Cd, indicating that the adsorbed lead and cadmium are below detection limits of the SEM-EDS.³²

5.2.4 Adsorption Capability of GO Towards Heavy Metals

The capacity of HU, HO and ST GO towards adsorption of Pb (II) and Cd (II) were evaluated via ICP-MS analysis of the filtrate. Figure 5.6(a) describes the adsorption of the different GO species towards Pb (II). It was evident that HU has the best adsorptive behaviour towards Pb (II), followed by HO GO and ST GO. Similarly, in Figure 5.6(b), HU GO demonstrated the best adsorption behaviour towards Cd (II) amongst the three GO, followed by HO and ST, in decreasing order.

Figure 5.6. Adsorption of heavy metal ions using HU, HO and ST GO in (a) 5 ppm of Pb²⁺ (b) 5 ppm of Cd²⁺, after ultrasonication for 2 hours with [GO]= 0.1 mg/mL at 25 ± 3 °C.

Table 5.3. Efficiency of GO towards adsorption of heavy metal ions of Pb (II) and Cd (II) and the adsorption capacity after ultrasonication for 2 hours with initial ion concentration, $[Co] = 5$ ppm, $[GO] = 0.1$ mg/mL at 25 ± 3 °C.

Material	Pb (II) removal		Cd (II) removal	
GO	Efficiency (%)	Capacity (mg/g)	Efficiency (%)	Capacity (mg/g)
HU	74.6±14.4	37.3±7.2	34.2±14.1	18.3±7.6
HO	40.7±12.3	20.3±6.2	5.0±7.7	2.7±4.1
ST	29.9±17.0	14.9±8.5	1.5±7.9	0.79±4.2

The tabulated data for the efficiency and capacity of the three different GO towards adsorption of heavy metals are illustrated in Table 5.3. HU demonstrates the best efficacy towards adsorption of Pb (II) and Cd (II). The efficiency towards adsorption of Pb (II) is at 74.6 % and it possesses a capacity of 37.3 mg/g under the given conditions. For Cd (II), HU demonstrates an efficiency of 34.2 % and a capacity of 18.3 mg/g at the given conditions. The efficiency of the graphene oxide adsorption decreases in the order of HU, HO to ST in both heavy metals. Experimental data from the adsorption of heavy metals for Pb and Cd from previous studies demonstrate HU GO to have an adsorption capacity of 35.9 mg/g for Pb (II) and 14.9 mg/g for Cd (II).¹⁹ Our values for HU graphene oxide at 37.3 mg/g for Pb and 18.3 mg/g draws strong coherence with previously published data, indicating the strong adsorption of the HU GO towards heavy metal ions.

Characterization of the GO and ICP-MS data illustrated that the carboxyl groups play a strong role in the adsorption of heavy metal ions. This was supported by FTIR spectrum, where it was evident that carboxyl absorbance peak, which was relatively strong in HU and HO GO samples after adsorption with Pb (II) and Cd (II) ions, was substantially weaker for ST GO. High resolution C 1s XPS spectra also placed similar correlation between the amount of adsorbed heavy metal ions and the amount of carboxyl groups that

are present in GO. This was particularly accentuated in the broadening of the XPS peaks in HU GO with Pb (II). Analogous phenomena have been observed in the raw spectra of HU GO with Mg^{2+} modified graphene oxide films³⁰ and metal ion adsorption with dichromate oxidized GO, where significantly wider C 1s spectra were observed upon co-ordination with metal ions, indicative of the binding event.¹⁵ On closer scrutiny via deconvolution, in HU Pb, the deconvoluted peak of O-C=O in the C 1s spectra shifted by 0.7 eV to higher energies, consequently leading to the peak broadening.

The XPS C 1s peak distributions showed that the GO synthesized by HU, HO and ST methodologies has different amount of oxygenated carbon functionalities. Particular attention is reserved for the strong correlation between adsorption of the heavy metal ions and the amount of carboxyl groups present, which increases in concentration from ST, HO to HU GO. This was particular evident from Table 5.2 A, which depicts the oxygenated carbon functionality distribution in the three different GO. HU GO, which contains the highest amount of carboxylic groups, showed greatest affinity towards adsorption of Pb (II) and Cd (II) in our findings. On the other hand, HO GO, while having the highest amount of C-O at 44.9 % surface composition in C 1s XPS spectra as compared to 30.3 % in HU GO, does not show better adsorption affinity towards heavy metal ions. C=O groups are also demonstrated to play a lesser role, as ST GO which contains a higher percentage of carbonyl groups does not show higher efficacy for removal of ions than HO GO. Visualisation via SEM-EDS also indicated that HU, which is rich in carboxyl groups, showed the best efficacy in the removal of heavy metal ions. Hence, it can be seen that epoxyl, hydroxyl and carbonyl groups do not play an active role in the adsorption of heavy metal ions. Carboxyl groups are the principal oxygenated carbon functionality in graphene oxide that adsorbs heavy metal ions.

The Lerf-Klinowski model depicts graphene oxide as containing epoxyl and hydroxyl groups on the basal plane and carbonyl and carboxylic groups at the edge planes.⁴

Axial hydroxyl or epoxy groups on the basal plane primarily bind to heavy metal ions via a monodentate mode along the c-axis of the graphene oxide.¹⁸ However, two primary modes of co-ordination via carboxyl groups have been elucidated: it can bind either via a monodentate or a bidentate mode to the heavy metal ions. Park *et al.* has shown that these edge-bound metal with carboxyl groups are more tightly bound to the graphene oxide as compared to the axial interactions of oxygenated groups.³⁰ It must be also noted that the more acidic nature of the carboxyl groups, as compared to hydroxyl groups, allows it to lose the proton readily to form a complex with the heavy metal ions as compared to the axial hydroxyl or epoxy groups.¹⁶ XPS characterization by Sitko *et al.* with the GO produced via dichromate routes demonstrated that the increased amounts of carboxyl groups produced, have subsequently led to unprecedented adsorption with heavy metal ions.¹⁵ The same phenomena was observed with Park *et al.*, where carboxyl groups are demonstrated to bind to metal ions strongly, resulting in additional mechanical strength.³⁰

During customization of the GO towards the removal of the heavy metal ions, due consideration must be put forth on the functionalities introduced onto the GO. Hence, much thought must be given to the oxidation procedure, as it has a profound influence on the functionalities introduced. Through the arbitrary introduction of metal ions to different GO, we are able to elucidate which oxygenated carbon functional groups are responsible for adsorption. HU shows the best adsorption capability as compared to HO and ST. The presence of carboxylate groups on GO demonstrates strong correlation with adsorption ability. Additional efforts to introduce carboxylate groups on GO through the use of alternative oxidation methodology via dichromate oxidant as shown in Sitko's work¹⁵ or the Tour's methods via a modified Hummer's method¹⁰ during the synthetic stage, to improve the adsorption capability of GO towards adsorption of heavy metal ions is envisaged.

5.3. Conclusion

Herein, the adsorption capability of the graphene oxide synthesised via HU, HO and ST routes were evaluated. These GO contain a variety of oxygenated carbon functionalities, such as epoxy, alcoholic, carbonyl and carboxyl groups. Such different preparation routes have fundamentally resulted in GO with different compositions. The aforementioned three types of GO were then investigated for the removal of Pb (II) and Cd (II) ions from aqueous solutions. The adsorption of Pb (II) and Cd (II) were found to be strongly dependent on the preparation methods of GO. FTIR and XPS indicated that the carboxyl groups in GO play a crucial role in the adsorption of heavy metal ions. The trend demonstrates a direct relation between the amount of carboxyl functional groups and the adsorption of metal ions of Pb (II) and Cd (II). Adsorption ability increase in the order from ST, HO and to a maximum in HU. This is in direct correlation with the amount of carboxyl groups present in the GO synthesised via different methodologies. More work will be required to directly functionalise the graphene oxide with carboxylic functionalities during the synthetic stage, for direct usage of these new age materials towards environmental remediation. Graphene oxide-based sorbents is envisaged to play a crucial role in removing heavy metal ions from aqueous waste.

5.4. Experimental Section

Materials. Graphite (<20 μm), sulphuric acid (95–98 %), sodium nitrate, hydrochloric acid (37 %), potassium chlorate (98 %), $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{Pb}(\text{NO}_3)_2$ were purchased from Sigma–Aldrich (Singapore). Potassium permanganate and fuming nitric acid (>90 %) were obtained from J.T. Baker. Milli-Q water (resistivity: 18.2 $\text{M}\Omega\text{ cm}$) was used throughout the experiments. Supported Anopore AAO inorganic membranes (diameter=25 mm; pore size=0.22 μm) was purchased from Whatman.

Apparatus. XPS was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). A JEOL-7600F semi-in-lens FE-SEM was used to acquire SEM images. Attenuated total reflectance (ATR) FTIR measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory.

*GO preparation with Staudenmaier's method.*⁵ Graphite source was used as-received without additional treatment prior to oxidations. Sulphuric acid (17.5 mL, 95–98 %) and nitric acid (9 mL, > 90 %) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 °C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0 °C to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at RT. Upon completion of the reaction, the mixture was poured into ultrapure water (1 L) and filtered. GO was then redispersed and washed repeatedly in HCl solutions (5 %) to remove sulphate ions. GO was finally washed with ultrapure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50 °C for five days before use.

*GO preparation with Hofmann's method.*⁶ Sulphuric acid (17.5 mL, 95–98 %) and nitric acid (9 mL, 63 %) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 °C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0 °C to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was

loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at RT. Upon completion of the reaction, the mixture was poured into ultrapure water (1 L) and filtered. GO was then redispersed and washed repeatedly in HCl solutions (5 %) to remove sulphate ions. GO was finally washed with ultrapure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50 °C for five days before use.

*GO preparation with modified Hummer's method.*⁷ Graphite (0.5 g) was stirred with sulphuric acid (23.0 mL, 95–98 %) for 20 min at 0 °C prior to the addition of NaNO₃ (0.5 g) in portions. The mixture was left to stir for 1 h. KMnO₄ (3 g) was then added in portions at 0 °C. The mixture was subsequently heated to 35 °C for 1 h. Water (40 mL) was then added into the mixture, which resulted in the temperature of the mixture to rise up to 90 °C. The temperature was maintained at 90 °C for 30 min. Additional water (100 mL) was added into the mixture. This was followed by a slow addition of H₂O₂ (30 %, ca. 10 mL). The warm solution was filtered (RC membrane, 0.22 µm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in the oven at 50 °C for five days prior to usage

Adsorption experiment.^{18, 34, 35} Stock colloidal solutions of Hummer's (HU), Hofmann's (HO) and Staudenmaier's (ST) graphite oxide of concentration 1 mg/mL each was first prepared in Milli-Q water. The graphite oxide solution was then ultrasonicated for 1 hour to obtain the as desired graphene oxide (GO) colloidal solution. For the adsorption experiment, stock GO colloidal solution was diluted to a concentration of 0.1 mg/mL. Prepared stock solutions of 10 mg/mL Cd(NO₃)₂•4H₂O and 5 mg/mL Pb(NO₃)₂ were used as the sources of heavy metals. 5 ppm of the respective heavy metals was added to the corresponding colloidal GO solution via micropipettes. The mixture was ultrasonicated for 2 hours for adsorption to take place. Filtration was then carried out with Whatman 0.22 µm

AAO membrane filters. The as obtained graphene oxide film was dried for two days, before use in (ATR) FTIR, XPS and SEM-EDS characterisations. The filtrate was also collected for analysis. Triplicate runs of the adsorption of metals by GO were carried out.

The efficiency (E) of 0.1 mg/mL (100 ppm) of graphene oxide towards adsorption of Pb (II) and Cd (II) under these given conditions is given by: $E = [(C_0 - C_f)/C_0] \times 100\%$, where C_0 and C_f are the initial and final concentration of metal ions in ppm after adsorption. The capacity of the graphene oxide towards the adsorption of heavy metals was also evaluated via calculating the mass of heavy metal ion adsorbed per unit mass of the sorbent (mg/g).

Attenuated total reflectance (ATR) FTIR measurements. Attenuated total reflectance (ATR) FTIR measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory. A ZnSe crystal was used as the ATR crystal. The respective GO film was transferred on top of the crystal and analysed.

XPS analysis. Both wide survey and high-resolution spectra for C1 s were collected. Relative sensitivity factors were used for evaluation of atomic C/O ratios from XPS wide survey spectra measurements. XPS samples were prepared by transferring the GO film to the carbon tape with the materials under study.

SEM-EDS. A JEOL-7600F semi-in-lens FE-SEM was used to acquire SEM images. The respective graphene oxide films were transferred to a carbon tape held onto a SEM sample holder for analyses. The analyses of the samples were carried out at an average working distance of 6 mm. SEM-EDS experiments were carried out at an average working distance of 15 mm. Elemental composition was determined by the EDS (Oxford) coupled to the JEOL-7600F.

ICP-MS analysis. Cd and Pb concentrations in the filtrate were determined using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent 7700, Japan). ICP-MS

analysis calibration curves were carried out using standard solutions, where concentrations ranged from 0.1 to 5 ppm and 1 to 10 ppm for Pb and Cd respectively were obtained. All measurements were performed in triplicates and the average values were reported.

5.5 Supporting Information

Table 5.S1 Average pH of colloidal GO solutions before and after adsorption of 5 ppm Pb (II) and 5 ppm Cd(II) from duplicates.

GO material	pH		
	Control	Pb-adsorption	Cd-adsorption
HU	3.40±0.01	2.62±0.10	2.57±0.05
HO	2.92±0.01	2.55±0.14	2.72±0.01
ST	2.80±0.01	2.78±0.02	3.18±0.01

Table 5.S2 Average C/O ratio obtained from the XPS survey scans via elemental analysis using respective atomic percentage of C 1s and O 1s peaks from duplicate readings.

Adsorption	HU	HO	ST
GO	3.33±0.04	3.66±0.01	3.83±0.19
GO Pb	3.08±0.20	3.57±0.08	3.57±0.05
GO Cd	2.94±0.16	3.58±0.27	3.41±0.13

Table 5.S3A Average tabulated quantitative distributions of deconvoluted peaks from the high-resolution C 1s spectra of HU, HO and ST from duplicate readings.

Material	C 1s peak distribution [%]				
	C=C	C-C	C-O	C=O	O-C=O
HU	42.4±3.4	9.5±2.5	31.9±2.3	11.3±2.5	4.8±0.6
HO	47.9±1.1	4.4±1.9	43.2±2.5	3.4±0.3	1.1±0.3
ST	50.5±0.4	6.3±0.6	38.1±0.4	4.2±0.7	0.9±0.1

Table 5.S3B Average tabulated quantitative distributions of deconvoluted peaks from the high-resolution C 1s spectra of HU, HO and ST from duplicate readings.

Material	C 1s peak distribution [%]				
	C=C	C-C	C-O	C=O	O-C=O
HU Pb	34.7±0.7	11.3±1.3	35.6±2.5	14.2±2.0	4.1±0.1
HU Cd	41.3±0.6	7.7±1.1	35.1±0.2	11.2±0.3	4.8±0.1
HO Pb	49.0±1.6	1.6±0.2	44.9±2.4	3.1±0.4	1.4±0.3
HO Cd	49.1±2.0	2.6±1.8	43.2±4.3	3.8±0.7	1.4±0.3
ST Pb	48.8±1.5	3.8±1.0	42.2±0.6	4.0±0.00	1.3±0.1
ST Cd	47.1±3.4	3.1±2.0	44.6±2.0	4.1±0.6	1.2±0.0

Figure 5.S1. (ATR) FTIR transmission spectra of (a) HU (b) HO (c) ST GO film before and after heavy metal ion adsorption.

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Chapter 6

Pt-Graphene Oxide Micromotor for Removal of Heavy Metals

6.1 Introduction

6.2 Results and Discussion

6.3 Conclusion

6.4 Experimental

6.1. Introduction

On-the-fly remediation protocols have higher efficiency, shorter clean-up time and is a cheaper alternative to large water reclamation plants at big factories.¹⁻³ The sustained movement of dynamic micromotors can be used for transporting water-purification nanomaterials with extended reach and encourages fluid mixing.⁴⁻⁸

Scheme 6.1. Fabrication of a platinum (Pt) and graphene-oxide based micromotor.

Graphene oxide has been illustrated to have a large surface area and endowed with rich oxygen functional groups that are capable of removing heavy metal ions.⁹⁻¹² In order to induce self-propulsion in these materials, we chose a strategy of oxygen bubble propulsion in hydrogen peroxide for the environmental remediation of heavy metals, using platinum as the catalytic material, as shown in Scheme 6.1. Such bubble-propelled micromotors are chosen due to the high amount of momentum they produced, which was associated with detachment of oxygen microbubbles¹³ and their ability to move in a multitude of environments.¹⁴⁻¹⁶

6.2 Results and Discussion

Scheme 6.2. Synthesis of Pt-GO micromotor

Chapter 6: Pt-Graphene Oxide Micromotor for Removal of Heavy Metals

The synthesis procedure is illustrated in Scheme 6.2. The graphene oxide (GO) can be incorporated inside the polycarbonate membrane through a vacuum filtration pathway with the graphene oxide solution, which traps the few-layer GO sheets within the pores of the polycarbonate membrane.¹⁷ Following which, deposition of a platinum layer can be accomplished on pores entrapped with few-layer GO using a conductive membrane-templated method by electrochemical deposition.⁷ The GO infused polycarbonate membrane was left to dry before sputtering with a silver layer, that serves as the conductive layer for electrodeposition. The electrochemical institution of the platinum layer was finally accomplished using a galvanostatic method with a constant electrical current held at -20 mA for 1200 s. The micromotors was then released from the polycarbonate membrane method by dissolving in dichloromethane, followed by washing with ethanol and water. Conical Pt micromotors that has GO incorporated are able to eject oxygen microbubbles due to the decomposition of hydrogen peroxide by the catalytic platinum layer, pushing it forward, giving rise to the origin of the propulsion. As these Pt-functionalised graphene oxide (Pt-GO) micromotors weaved through the contaminated sample, they are able to remove the heavy metal ions.⁹ Techniques used for the future stepwise characterisation, will include Fourier-transformed infra-red spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) to evaluate the mode of binding between graphene oxide and the pollutants of heavy metal of cadmium, which is the chosen heavy metal ion of study.⁹ Scanning electron microscopy coupled to elemental detection X-ray spectroscopy (SEM-EDS) to study the Pt-GO composite's morphology and the elemental composition before and after adsorption experiments will be undertaken too, to investigate the uptake of the heavy metal ions. A more intricate study will need the inclusion of inductively-coupled plasma mass spectroscopy (ICP-MS) to detect the amount of heavy metals absorbed. Optical microscopy will also be used to evaluate the locomotion of the Pt-GO micromotors during environmental remediation of the pollutants using imaging software.

Chapter 6: Pt-Graphene Oxide Micromotor for Removal of Heavy Metals

Figure 6.1 depicts the SEM images of the micromotors and graphene oxide synthesised. The control Pt micromotors are tubular in structure as depicted in Figure 6.1 A and uniform in morphology. Graphite oxide has been successfully synthesised from graphite through Hummer's method. With the subsequent ultrasonication of a graphite oxide solution at 0.1 mg mL^{-1} and filtration through a $5 \text{ }\mu\text{m}$ membrane, nanosheets of graphene oxide can be obtained as shown in Figure 6.1 B. This solution of graphene oxide was used for a secondary filtration step for the infusion of graphene oxide inside the polycarbonate membrane. Permeation of graphene oxide within the structure of the polycarbonate membrane was followed by an electrochemical deposition in a platinum solution resulted in the (platinum-graphene oxide) Pt-GO micromotors in Figure 6.1C. The dark patches incorporated in the Pt-GO micromotors are suggestive of the graphene oxide structure that has been incorporated. A magnified image of the Pt-GO micromotor showcased the successful incorporation of graphene oxide sheets within the micromotor structure in Figure 6.1D. This composite material of Pt-GO micromotor will be used for the removal of heavy metal ions of cadmium.

Figure 6.1. Scanning electron microscope image of a Pt micromotor **B** Graphene oxide nanosheets **C** Pt-GO micromotor **D** Magnified image of Pt-GO micromotor.

Chapter 6: Pt-Graphene Oxide Micromotor for Removal of Heavy Metals

The remarkable adsorption abilities of graphene oxide coupled with the self-propulsion using chemical fuel of hydrogen peroxide's decomposition to oxygen bubbles, allows for enhanced fluid dynamics and permitting *in situ* decontamination works for pollutants^{7,18-24} and chemical threats^{5,8} alike. The steady propulsion and continuous thrust allows for the potential sustained works for the removal of pollutants remotely and untethered, depicted in Scheme 6.3.

Scheme 6.3. Environmental clean-up by Pt-GO micromotor.

The current study has just finished its preliminary investigation. Subsequent steps will include the study of adsorption and removal of heavy metals, together with the locomotive studies of the Pt-GO micromotors in the presence of the heavy metal ions.

6.3 Conclusion

We have demonstrated the capability to couple the infusion of graphene oxide within membranes and electrodeposition to construct Pt-GO composite micromotor. Such combinatorial self-propelled micromotors show promise of removing ions from aqueous solutions. On-the-fly strategy of using self-propelled micromotors to remove pollutants by the combination of self-propulsion and large-area-to-volume ratio carbonaceous nanomaterials may prove to be the next frontier of pollutant management.

6.4 Experimental Section

Materials. The cyclopore polycarbonate membranes with pores of 5 μm in diameter were purchased from Whatman, USA (Cat no. 7060-2511). The pores are conical in shape. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH instruments, USA. The platinum plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98 +%), sodium dodecyl sulphate (SDS). Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 $\text{M}\Omega \text{ cm}$) from a Millipore Milli-Q purification system. Potassium permanganate was obtained from J.T. Baker.

Apparatus. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 $^{\circ}\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The silver working electrode was sputtered on the cyclopore membrane and copper tape was used as the transducer. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Hermle Z233m-2 microcentrifuge. SEM images were taken with a JEOL-7600F semi-in-lens FE-SEM. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software.

Preparation of Graphene Oxide. Graphite oxide was prepared using graphite according to Hummer's method whereby graphite (0.5 g) was stirred with sulphuric acid (23.0 mL, 95–

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98%) for 20 min at 0 °C prior to the addition of NaNO₃ (0.5 g) in portions. The mixture was left to stir for 1 h. KMnO₄ (3 g) was then added in portions at 0 °C. The mixture was subsequently heated to 35 °C for 1 h. Water (40 mL) was then added into the mixture and resulted in the temperature of the mixture rising up to 90 °C. The temperature was maintained at 90 °C for 30 min. Additional water (100 mL) was added into the mixture. This was followed by a slow addition of 30% H₂O₂ (~10 mL). The warm solution was filtered (RC membrane, 0.22 µm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in a vacuum oven at 30 °C for 5 days prior to usage. Graphene oxide (GO) was obtained by subjecting graphite-oxide through ultrasonication [37 kHz] for 8 h in water to give a 0.1 mg mL⁻¹ dispersion.

Preparation of GO-infused conductive membrane. Cyclopore polycarbonate membranes with pores of 5 µm in diameter was used. Graphene oxide (GO) filtration with 5 µm polycarbonate membrane using 10 mL of 0.1 mg/mL GO solution was first carried out. The resulting filtrate was used for a secondary filtration step through another 5 µm polycarbonate membrane to obtain the graphene oxide incorporated cyclopore membrane template for electrochemical deposition. The membrane was left to dry for 2 days. Sputtering with a layer of conductive silver layer with approximate thickness of 100 nm was carried out. This membrane was used for the electrodeposition of Pt-GO concentric microtubes.

*Preparation of Pt-GO concentric bimetallic microtubes.*²⁵ The Pt-GO concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate infused with graphene oxide. Copper tape was attached to the silver sputtered membrane as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. Platinum

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counter electrode and Ag/AgCl /1.0 M KCl (aq.) reference electrode were utilized. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The template was rinsed with 5 mL of ultrapure water (18.2 MΩ cm) for 4 times and the platinum segment was electrodeposited subsequently at -20 mA for 1200 s, using the commercial Pt plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled and the template was washed for 5 times with 8 mL of water each. After that, the template was ultrasonicated for 3 times in 2 mL of ultra-pure water for 3 min each time. The template was placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly for 3 times with methylene chloride. The solution was then washed with ethanol and water for 2 times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Operation of micromotors. The experiments for studying the influence of heavy metals on the motion of micromotors were carried out in an aqueous solution containing 3 wt. % of hydrogen peroxide at constant surfactant concentrations (1 wt. % of SDS). A mixture of micromotors (7 μL), SDS (1 wt. %), H₂O₂ (3 wt. %) and appropriate concentrations of Cd(NO₃)₂ were applied on a glass slide freshly cleaned with nitrogen gas. Optical microscope videos and images were obtained with Nikon Eclipse TE 2000-E microscope. Video sequences (100 fps) were processed with Nikon NIS-ElementsTM software.

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Chapter 7

Acetylene Bubble-Powered Autonomous Capsule: Towards *In Situ* Fuel

7.1 Introduction

7.2 Results and Discussion

7.3 Conclusion

7.4 Experimental

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The results in this chapter were published in the following manuscripts:

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The thesis author was the primary investigator and author.

7.1. Introduction

Self-propelled devices have been proposed to be fielded in numerous scenarios:¹ both in environmental remediation² and biomedical applications.^{3,4} Thus said, the ability for the provision of fuel *in situ* is an important aspect in self-powered systems,⁵⁻⁹ obviating the need for high concentrations of toxic hydrogen peroxide for oxygen bubble-propelled devices by catalytic decomposition reactions. Such fuel-free machines grant higher degrees of freedom and autonomy during their operations. The use of water as an *in situ* fuel under ambient conditions, utilising chemical reactions of reactive metals such as magnesium⁶⁻⁸ and aluminum⁹ have been demonstrated in hydrogen bubble-propelled motors. Alternatively, the Marangoni effect, which employs interfacial tension, minimizes changes towards the surrounding environment, through a self-contained and self-sustained device.¹⁰⁻¹⁶ Pumera and co-workers illustrated the use of a slow asymmetrical release of entrapped DMF from a polysulfone capsule, being capable of moving at high velocities and has long life-spans in the order of minutes.¹⁵ Later, Orozco *et al.* exemplified the use of the Marangoni effect with an encapsulation strategy, with the controlled release of surfactants from millimetre-sized vessels.¹⁷ Both these works highlighted the need for a measured release of the reactants for propulsion to occur.

Thus far, the use of water as a fuel, has been limited to reactive metals to produce simple molecules of elemental hydrogen bubble for propulsion. The use of ionic and neutral compound substrates have not been demonstrated for use as a potential source of fuel, where complex gaseous molecules could be used as a propellant for autonomous moving objects. In this study, we demonstrated that the use of an encapsulated calcium carbide motor is capable to demonstrate motion, which is propelled by the expulsion of acetylene bubbles. The study expands the scope of bubble-propulsion beyond hydrogen and oxygen bubble-propelled motors. The acetylene bubble-powered motor, utilizing water as a co-

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reactant, is an additional step towards self-propelled devices in aqueous medium without the addition of chemical propellants in the environment.

7.2. Results and Discussion

Designing an autonomous self-propelled motor such that it is independent from the environment and using the fuel source *in situ* has been a challenging task. In this report, we illustrate the use of a calcium carbide motor, exploiting water as a co-reactant. The devised motor is able to traverse the surface of an aqueous medium via acetylene bubble-propulsion.

Scheme 7.1 Depiction of **A** Polymer encapsulation of calcium carbide granule and operation of acetylene-powered capsule motor in water **B** Reaction scheme of calcium carbide in aqueous media.

As shown in Scheme 7.1A, the calcium carbide granule was encapsulated with a polymer layer through a phase inversion strategy. Namely, it was carried out via a sequential immersion in 10 wt. % polysulfone in DMF solution (PSf/DMF) followed by ethanol. The thin layer of polysulfone encapsulates the calcium carbide granule, hereby designated as PSf/CaC₂ capsule motor. It must be noted that the 10 wt. % PSf/DMF mixture is the optimal encapsulation concentration. A 5 wt. % PSf/DMF concentration for encapsulation resulted in a rapid rupture of the PSf membrane and the consequent increase beyond, to a 15 wt. % concentration led to the sinking of the PSf/CaC₂. Previously, it has been demonstrated that such phase inversion membranes allows for the creation of

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nanoporous films.¹⁵ These pores are important for the contact of the surrounding aqueous media with the encapsulated substrate. The reaction of the calcium carbide with water, leads to the formation of acetylene and calcium hydroxide salt as shown in Scheme 7.1B. This ensuing chemical reaction that occurs at the interface led to the formation of acetylene bubbles, which escapes and propels the capsule motor forward.

Figure 7.1 Tracking image for the path of a PSf/CaC₂ capsule motor at **A** $t = 0$ s **B** $t = 16$ s on the surface of water with 0.05 wt. % SDS. Translational motion was observed. Arrow indicates initial position of capsule motor. Scale bar indicates 1 cm.

At $t = 0$ s, the encapsulated PSf/CaC₂ was introduced into the 0.05 wt. % SDS surfactant solution in Figure 7.1A. The PSf/CaC₂ capsule motor is of millimetre-sized, with approximate dimensions of 3 mm across each end. The surfactant serves to lower the surface tension for ease of bubble formation and ensures the stability of the bubbles that are formed.¹⁸ An almost instantaneous effervescence was observed. This corresponded to the water seeping past the PSf membrane to react with the core of calcium carbide. The chemical reaction between water and calcium carbide produces acetylene bubbles, via an acid-base reaction. A subsequent stream of bubbles was released from the PSf/CaC₂, where they aided the buoyancy of the PSf/CaC₂ capsule motor at the water/air interface.¹⁹ This was followed by the translational motion of the PSf/CaC₂ across the surface of the solution in Figure 7.1 B.

Figure 7.2 Average speed profiles of PSf/CaC₂ capsule motor with $\Delta t = 1$. A total path length of 37 cm was travelled and the average speed was 2.3 cm s⁻¹.

In Figure 7.2, the speeds of the PSf/CaC₂ capsule motor are described. The motor expressed an average speed of 2.3 cm s⁻¹, moving a total distance of 37 cm. This is approximately 8 body lengths per second. Two ranges of speeds were found for the PSf/CaC₂ capsule motor. From 0 to 9 s, the motor demonstrated speeds of between 2-4 cm s⁻¹. For the later part of the translational motion from 9 to 16 s, the PSf/CaC₂ motor demonstrated slower speeds of 1-2 cm s⁻¹. This is in part due to the consumption of the calcium carbide fuel and resulted in the decrease of propulsion force, leading to the reduction of speeds. Beyond the 16 s timeframe as shown in Figure 7.2, the motion of the capsule becomes limited with a retarded bubble formation.

The converse direct introduction of the PSf/CaC₂ into the aqueous medium without surfactant resulted in the motion of the capsule motor to be highly localized. The PSf/CaC₂, bobbed up and down the water/air interface. This exhibited the quintessential role of the surfactant for propulsion. The need for buoyancy of the PSf/CaC₂ capsule motor¹⁹ and the sustenance of the bubbles²⁰ are required in order for translational movement to be observed. Separately, in the absence of the PSf membrane, an uncontrolled formation of bubbles due to the chemical reaction of the calcium carbide and water in direct contact resulted. The calcium carbide was rapidly expended and was accompanied with a foaming on the surface

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of the aqueous media. No significant propulsion behaviour was observed on the calcium carbide granule. Formation of the encapsulation film surrounding the capsule governs the motion of the PSf/CaC₂ capsule motor, where in the event of rupture, it resulted in a highly localized motion of calcium carbide fragments. This highlighted the need for the controlled reaction of the calcium carbide with water, in order to allow for the biased movement of the capsule motor, as reported by Zhao *et al.*¹⁵ and Orozco *et al.*¹⁷ The role of the PSf membrane as a binder to confer integrity and asymmetry to the particulate as a single entity is crucial for the biased movement for the bubble-propelled device.

Figure 7.3 Calculated mean-squared displacement (MSD) of PSf/CaC₂ capsule motor on the surface of water with 0.05 wt. % SDS added.

In Figure 7.3, the graph of mean squared displacement (MSD) against change in time intervals has been demonstrated to be an upward parabolic curve, indicative of an additional propelling force being present.^{21,22} This resulted in the millimetre-sized PSf/CaC₂ to move at an average of ~8 body lengths per second. This corresponded well with our hypothesis, that the production of a stream of bubbles powering the PSf/CaC₂ capsule motor, has given rise to the ballistic thrust needed for motion. The motion of the PSf/CaC₂ capsule motor changed direction multiple times during the course of 16 s as

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shown in Figure 7.1, which was indicative of propulsion behaviour, eliminating any convective current effects.²²

7.3 Conclusion

In conclusion, we have demonstrated the operation of an autonomous self-propelled device based on the encapsulation of calcium carbide in polysulfone, packing the fuel within the motor and operating under a chemical fuel-free environment. The capsule motor runs on the basis of a controlled reaction with water, using acetylene bubbles as the means of propulsion. The extension of autonomous moving devices using alternative sources of *in situ* fuels is an important step towards widening the capabilities of such self-propelled machines. Alternative bubble-powered systems such as acetylene in our case and possibly other analogous complex molecules produced for bubble-propulsion may be possibly used as feedstock for further chemical manipulation in self-propelled autonomous systems.

7.4 Experimental Section

Materials. Calcium carbide (CaC_2), sodium dodecyl sulphate (SDS) and polysulfone (PSf) and *N, N'*-dimethylformamide (DMF) were purchased from Sigma-Aldrich.

Preparation of polysulfone solution. The precursor encapsulation solution was prepared by mixing polysulfone with *N, N'*-dimethylformamide and dissolved into a clear solution using an ultrasonic bath for 30 min. The concentration of polysulfone (PSf) in DMF (PSf/DMF) was 10 wt. % by weight.

Encapsulation of calcium carbide. Encapsulation of calcium carbide was carried out using a phase inversion strategy. Typically, a calcium carbide granule with an approximate dimension of 3 mm at each end was chosen. The granule was introduced into a 10 wt. % PSf/DMF solution, followed by the immersion into ethanol, where the phase inversion occurred. The encapsulated calcium carbide was left to dry for 5 minutes before being used for propulsion experiments.

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The propulsion experiments were carried out in a glass container with dimensions 20 cm x 20 cm x 5.5 cm depth. An aqueous solution containing 0.05 wt. % SDS was placed into the dish. A Casio HD video-recorder was placed over the glass container to capture the motion of the capsule motor. The video sequences were analysed using Nikon NIS-Elements software, where the average velocities were calculated.

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Chapter 8

Influence of pH on Catalytic Bubble-Propelled Micromotors

8.1 Introduction

8.2 Results and Discussion

8.3 Conclusion

8.4 Experimental

8.5 Supporting information

The results in this chapter were published in the following manuscripts:

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8.1 Introduction

The emergence of micromotors as devices to perform work and the collective efforts of the scientific community have led to the progression of the field by leaps and bounds in the past decade.¹ The miniaturized size, ultrafast motion and the capability of surface functionalization with consequent interactions throughout the solution have led to a plethora of applications. They have been demonstrated to be able to do work for both environmental remediation²⁻⁴ and that of biomedical usage.⁵⁻⁷ In view of its possible application as a nanotool to perform work in the environment and a physiological medium, much effort has been directed towards the study of the interplay between the micromotors and their environment,⁸⁻¹⁷ with possible implications for the enhancement of self-propulsion.¹⁸⁻²⁵

The search for a viable propelling agent for powering micromotors in varying environments has been ongoing for an extended period. *In situ* and *ex situ* methodologies have been demonstrated to power such micro-objects. *Ex situ* methods include that of using acoustic,²⁶ magnetic manipulation²⁷ and that of ultraviolet²⁸ and infra-red irradiation.²⁹ Even then, the use of a chemical fuel such as hydrogen peroxide cannot be completely obviated. Ultraviolet irradiation on TiO₂ single component microtubes required the presence of hydrogen peroxide in a radical decomposition reaction for oxygen-bubble propulsion.²⁸ Similarly, near-infrared activation of the microtubular platinum-based engines to perpetuate motion necessitates the occurrence of hydrogen peroxide, albeit at lower concentrations.²⁹ Remote manipulation of micromotors requires the use of external fields of magnetic and acoustic origins, which is inherently a peripheral energy source. An actuation of the micromotors *in situ* allows for the reduction of complexity surrounding their operations and to permit function independent of external fields.

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Hydrogen peroxide continues to be the fuel of choice for many micromotors. The work of Sanchez *et al.* exemplified micromotors that move at ultrafast speeds and their operation at low hydrogen peroxide concentrations in bubble-propelled rolled-up microtubes.³⁰ Thermal activation of such micromotors has also been illustrated for enhanced locomotion in physiological media.²⁹⁻³¹ Exploring possible additives, Laocharoensuk *et al.* used micromotors that are made of carbon nanotube composite materials and exhibited sublime performance with hydrogen peroxide in the presence of basic hydrazine, being powered by a self-diffusiophoretic mechanism.²² This was later revalidated by Gao *et al.*'s work with the bubble-propelled polyaniline/platinum tubular micromotors in a mixture of hydrogen peroxide and hydrazine.^{23,24} Addition of hydrazine have been known to increase the decomposition of hydrogen peroxide to form oxygen.^{22,25} However, hydrazine itself is biologically toxic, adding to the complexity of the micromotor operation.

Tubular micromotors have been deployed in media of varying pH. Orozco *et al.* reported the utility of micromotors in the presence of sodium carbonate and sodium hydroxide with hydrogen peroxide for an environmental clean-up strategy of nerve agents.¹⁸ The presence of reactive OOH^- oxidative nucleophiles in alkaline media have resulted in the breakdown of these deleterious materials during enhanced fluid mixings. The ease of hydrogen peroxide to form oxygen microbubbles for propulsion and formation to reactive species in the alkaline environment have been the principal reasons for their purported environmental remediation effect. At the other end of the spectrum, Gao *et al.* have also demonstrated hydrogen bubble-propelled zinc-packed tubular microrockets to possess enhanced motion in increasingly acidic media.³² For Fenton-reaction-based micromotors running with platinum on hydrogen peroxide, optimum performance was found to be between pH 2 and 3 for an environmental clean-up of organic contaminants.³³

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The silver nano/micromotor is another suitable model for the investigation of the influence of an alkaline environment. Silver has been demonstrated for micromotor-propulsion in many instances as hydrogen peroxide decomposes readily to form oxygen on the material.^{34,35} Kagan *et al.* also illustrated that the underpotential deposition of silver as the principal reason for the high mobility in their bimetallic diffusiophoretic nanorod motors.³⁶ Pumera and co-workers had suggested asymmetric Janus silver microparticles to be a worthy candidate to replace platinum as a material for fabricating bubble-propelled micromotors.³⁷

In a separate report, Dey *et al.* illustrated the chemotactic movement of palladium-based micromotors in alkaline medium, due to chemical gradients that are generated.³⁸ Self-contained amphoteric aluminium Janus type micromotors in acidic and alkaline media³⁹ have also been known to show varying performances with pH. For a bubble propulsion system, the prospect of wielding the micromotors with enhanced propulsion is extremely desirable.

However, no systematic study has been employed for the changes in velocities and activities of bubble-propelled catalytic micromotors with the monotonic increase in pH. Hydrogen peroxide has been known to show enhanced decomposition under alkaline media.⁴⁰⁻⁴⁴ For the better understanding of the locomotive events at the microscale, such a study on the micromotors with pH changes is essential. The investigation across a spectrum of pH will be employed for the bubble-propelled catalytic micromotors. In this report, we illustrate that the level of acidity and alkalinity show dramatic influences on the activity and velocities of both Janus-type and tubular micromotors. This property can then be possibly extrapolated for the detection of changes in pH, to complement present sensing strategies, which are sometimes complicated and prone to fouling.

8.2 Results and Discussion

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Herein, we illustrate that hydrogen peroxide fuelled bubble-propelled micromotors demonstrate locomotive differences and detection capabilities across different pH. Two representative bubble-propelled micromotors of Janus and microtubular configurations were investigated. These micromotors are powered by bubble propulsion caused by the catalytic decomposition of hydrogen peroxide over the material. Detachment of the bubbles propels the motion of these micromotors. Velocities of Janus silver micromotors were investigated with increasing pH and the comparison with increased fuel concentrations was made. Activity and velocities of microtubular Cu/Pt micromotors were also elucidated with varying pH.

Figure 8.1 Velocity of Janus Ag micromotors in selected pH adjusted with NaOH (aq.) electrolyte solution. Conditions in all experiments: 23 °C, 3 wt. % H_2O_2 and 0.25 wt. % SDS.

Locomotion of silver micromotors was investigated with increased alkalinity of the environment. The asymmetric geometry of the Janus silver micromotors coupled with bubble formation, led to a net force, which propels these particles. The Janus silver micromotors' velocity increased with elevated pH in the presence of the sodium hydroxide, as shown in Figure 8.1. Velocity of the silver micromotors in 3 wt. % hydrogen peroxide and 0.25 wt. % of SDS, increased from $14 \mu\text{m s}^{-1}$ at pH 7 to $320 \mu\text{m s}^{-1}$ in the presence of base at the pH of 12, by a multiple of almost 23 times. Such enhanced motion of the silver

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Janus microparticles is in direct correlation to the increase of pH in the solution. The upper limit for the operation of the micromotors was observed to be pH 12, as the sodium dodecyl sulphate surfactant precipitates out due to common-ion-effect in the presence of sodium hydroxide when attempts to increase the pH to 13 was carried out.

Hydrogen peroxide can be both an oxidising and a reducing agent (See supporting information). Possible means to harness the full chemical potential by the introduction of the base have been demonstrated due to the transformation of hydrogen peroxide to the negatively charged hydrogen peroxide anion, which is the activated form.¹⁸ The energetically unstable anion of OOH^- decomposes at a faster rate, to produce more oxygen, culminating in the increment of propulsion.⁴⁰⁻⁴² This was confirmed by microscopy observations that the increase in base concentration, was coupled with a greater ejection of bubbles, leading to faster velocities. The drastic change in the micromotor's velocity with pH change also insinuates of its possible usage as detector for the alkalinity of environment.

Figure 8.2 Velocity of Janus Ag micromotors with increasing hydrogen peroxide concentrations in the absence of NaOH (aq.) electrolyte solution. Conditions in all experiments: 23 °C and 0.25 wt. % SDS.

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For comparison, the velocities of the micromotors were tracked with elevated concentrations of hydrogen peroxide in Figure 8.2. The velocity of the Janus silver microparticles increases with elevated hydrogen peroxide concentrations, from $14 \mu\text{m s}^{-1}$ at 3 wt. % hydrogen peroxide to $80 \mu\text{m s}^{-1}$ at 9 wt. % hydrogen peroxide. However, in comparison, the accompanying introduction of sodium hydroxide at pH 10 with 3 wt. % hydrogen peroxide, a velocity of $123 \mu\text{m s}^{-1}$ was reached, far exceeding the equivalent of the addition of 3 times the hydrogen peroxide concentration at 9 wt. %, where a speed of only $80 \mu\text{m s}^{-1}$ was achieved in the Janus silver micromotor. This demonstrates an alternative methodology of improving the activity of the micromotors, where the full chemical potential of hydrogen peroxide can be maximized with usage of greater alkalinity during operations.

Keeping things in perspective, the pH readings are stable at the time window of observation, with no significant changes. However, it was noted that attempts for pH detection in the acidic region, leads to the dissolution of the Janus silver microparticles. This is due to the tendency of hydrogen peroxide to behave as an oxidizing agent in the acidic pH.⁴⁵ Therefore, it is essential for us to take into regard an alternative model in the form of tubular micromotors Cu/Pt, which shows stable readings from pH 0-12.

The corresponding activity of tubular micromotors at a comprehensive pH range from 0 to 12 was investigated, as shown in Figure 8.3. The dual component Cu/Pt tubular micromotors were fabricated from a two-step electrodeposition method, where copper was first deposited on the outer layer followed by the inner layer of platinum via a templated approach. The decomposition of the hydrogen peroxide to oxygen bubbles and the subsequent detachment from the inner layer of platinum in the micromotors propels them forward.¹ The active area of Cu/Pt tubular micromotors has been under confinement conditions, where localized decomposition of the hydrogen peroxide on the inner concentric platinum surface led to the formation and ejection of oxygen bubbles. Activity

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of micromotors were evaluated based on the number of micromotors demonstrating bubble ejection and propulsion. It is also noteworthy that the catalytic micromotors can be recovered by filtration and centrifugation methods (See Figure 8.S1). The activities of these self-propelled devices did not deteriorate upon recovery and resuspension in hydrogen peroxide (See Figure 8.S2).

Figure 8.3 Number of 5 μm Cu/Pt tubular micromotors showing bubble ejection (Grey) and microjet propulsion (Black) with pH range from 0-12 adjusted with HCl (aq.) and NaOH (aq.). Conditions in all experiments: 23 $^{\circ}\text{C}$, 3 wt. % of H_2O_2 and 0.25 wt. % SDS.

Across the pH range investigated, the Cu/Pt tubular micromotors were observable under microscopic observation and showed little signs of dissolution. It was noted that an initial dip of the activity of the micromotors was observed with the first introduction of sodium hydroxide and hydrochloric acid to adjust the pH to pH 6 and 8 respectively. Concentrations of salts at millimolar levels are known to reduce the activity and impede motion of the micromotors due to changes in ionic strengths.^{11, 16}

In the alkaline region from pH 8 to 12, with a monotonic increase in sodium hydroxide, the activity of micromotors doubled for bubble ejection from 16 to 33 units and microjet propulsion from 9 to 18 units. The basic environment with the introduction of

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sodium hydroxide has been instrumental in increasing the activity of the micromotors, where a greater decomposition of hydrogen peroxide results in greater bubble propulsion

Curiously, the acidic region indicated an increase in activity below the pH 4, where there were originally 8 bubbling micromotors and 6 self-propelled micromotors. As illustrated in Figure 8.3 at pH 3, the number of bubbling micromotors increased to 38 and the number of micromotors demonstrating microjet propulsion increased to 35. This was a five-fold increase of activity. The same story was seen at the pH 2, where there was 35 micromotors bubbling and 33 of them undergoing self-propelled motion. This was at odds with the earlier hypothesis that only an alkaline environment can increase the activity of the micromotors. Furthermore, at the pH of 0 and 1, the activity of the micromotors dropped dramatically. At pH 0, only 2 micromotors are bubbling and 1 of which demonstrated self-propelled motion.

Figure 8.4. Velocity of 5 μm Cu/Pt tubular micromotors with pH range from 0-12 adjusted with HCl (aq.) and NaOH (aq.). Conditions in all experiments: 23 $^{\circ}\text{C}$, 3 wt. % H_2O_2 and 0.25 wt. % SDS.

A closer inspection at the velocities of the micromotors were undertaken to gain a better picture. The subsequent introduction of sodium hydroxide and hydrochloric acid have also modulated the velocities of the Cu/Pt micromotors, as depicted in Figure 8.4. The

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evaluation of the micromotors' velocities were carried out and it was found that the addition of sodium hydroxide increased the velocities. From the pH of 7 to 12, the velocity of the Cu/Pt micromotors increased by nearly 4-fold, from the velocity of $62 \mu\text{m s}^{-1}$ to $230 \mu\text{m s}^{-1}$.

The decrease in pH from 7 to 4, reflected a slight decrease in velocity. However, as with the activity, tubular micromotors experienced a rapid increase in velocities at the pH of 2 and 3. Beyond this pH range, a dramatic decrease in the motion was observed at pH 0 and 1. As depicted in Figure 8.4, the micromotor was virtually immobile at the pH value of zero. A higher magnification microscopic imaging tool was employed to visualize the possible changes to these micromotors that might have constituted the observations.

Using scanning electron microscopy (SEM), the morphology of the micromotors was investigated in Figure 8.5. The micromotors were initially immersed in the hydrogen peroxide solutions of pH 0, 2, 7 and 12 respectively. They were then extracted by filtration by a nanoporous membrane before washing with copious amount of water and dried. The SEM images are featured in Figure 8.5, with a summary of the structural changes showed in the inset as a schematic. As seen in the SEM imaging, no morphological changes was registered at pH 7 and 12, in Figure 8.5C and 8.5D respectively. It was observed that the tubular micromotor at pH 2, was significantly corroded in the time window of 15 min, exposing the inner platinum layer partially. These exposed surfaces of the micromotors were made available for catalytic decomposition of hydrogen peroxide, leading to better activities and velocities in Figure 8.3 and 4. However, with further additions of hydrochloric acid to reach pH 0 in Figure 8.5A, the structural integrity of the tubular micromotor collapsed. The tubular micromotor's ends appeared to be closed and the length of the tube broke down onto itself in Figure 8.5A and as depicted in the enclosed schematic. This significantly impaired the microjet propulsion and the aftereffects were illustrated in

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the deteriorated activity and decreased velocities earlier in Figure 8.3 and Figure 8.4 at pH values of 0 and 1.

Figure 8.5 SEM images of 5 μm Cu/Pt tubular micromotors extracted from different pH using filtration with a nanoporous membrane at **A** pH 0 **B** pH 2 **C** pH 7 **D** pH 12 respectively. pH range was adjusted with HCl (aq.) and NaOH (aq.). Conditions in all experiments: 23 $^{\circ}\text{C}$, 3 wt. % H_2O_2 and 0.25 wt. % SDS; Immersion in solutions of differing pH was carried out for 15 min. Inset: Schematics of structural changes to tubular micromotors after immersion.

It was deduced that the acidity and presence of hydrogen peroxide compromised the structural integrity of the tubular micromotors from pH 2 to 3, exposing a larger catalytic platinum surface area for bubble propulsion. However, the accompanying decrease to pH 0 and 1, curtailed the movement of the micromotors as the structural integrity of the concentric bimetallic Cu/Pt micromotor was severely lost and the structure collapsed as seen in Figure 8.5A. However, in the alkaline region, namely from pH 8 to 12, the increased alkalinity enhanced the decomposition of hydrogen peroxide to oxygen for bubble propulsion.

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Both Janus and tubular micromotors have shown selective locomotion in aqueous media, reflecting the changes in pH. As catalytic systems, Janus silver micromotors have been demonstrated to show greater increases in velocities than Cu/Pt micromotors in the presence of alkaline conditions. The catalytic surface of the microtubes is confined in the core of the inner concentric layer, which is concave in shape,²⁸ whereas in comparison, the Janus particles possess both concave and convex surfaces co-existing in the same asymmetric solid particle.³⁷ With regards to geometry, the Janus particles, while having the same order of magnitude in size ($\sim 20\ \mu\text{m}$) at the longest dimension as the microtubes ($\sim 15\ \mu\text{m}$), the Janus particles themselves have a significantly larger catalytic surface area. These geometric differences in the two micromotors resulted in the contrasting locomotive performances, for gas nucleation, accumulation and expulsion under different pH.^{28,37,46} Thus, the larger boost in propulsion of Janus silver micromotors is due to the larger active surface area as compared to the confined regions of the concentric bimetallic Cu/Pt micromotor under well-defined conditions on the tubular area.³⁷ The manipulation of pH, with the concomitant increase of locomotion in these catalytic micromotors, serve as a strategy for detecting changes in pH and a pathway to increase locomotive events in self-propelled entities.

8.3 Conclusion

In conclusion, we have demonstrated the working range of the micromotors and that they are capable of detecting pH. The increase in pH, leads to faster decomposition of the hydrogen peroxide on the surface of the catalytic micromotors, bringing on greater activity from bubble ejection and higher velocities. The effect is epitomized by the increase in velocities of the Janus silver micromotors. The activity and velocities of the Cu/Pt tubular micromotors were also increased in the presence of alkali. However, the extent of this increase in self-propulsion for the tubular micromotors of Cu/Pt were lesser in extent than that of the Janus silver micromotors. Structural changes to the tubular micromotors through

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corrosive effects of the acid have also inevitably changed the propulsion behaviour. Through the observation of the activity and velocities of micromotors, the detection of pH can be accomplished on-the-fly, particularly in the neutral to alkaline region. The pH-dependent propulsion behaviour also serves as a possible pathway towards locomotive enhancements for catalytic bubble-propelled motor systems.

8.4 Experimental Section

Materials. The cyclopore polycarbonate membranes with pores of 5 μm (Cat. 7060-2513) and 0.2 μm (Cat. 6809-6022) in diameter were purchased from Whatman, USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH instruments, USA. The platinum plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98 +%), sodium dodecyl sulphate (SDS), sodium hydroxide, hydrochloric acid (37 %) and silver microparticles were purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 $\text{M}\Omega\text{ cm}$) from a Millipore Milli-Q purification system.

Apparatus. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 $^{\circ}\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. pH was recorded using a Satorius Docu-pH-Meter. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The silver working electrode was sputtered on the cyclopore membrane and copper tape was used as the transducer. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and

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centrifugation was carried out with a Hermle Z233m-2 microcentrifuge. SEM images were taken with a JEOL-7600F semi-in-lens FE-SEM. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software.

*Preparation of Cu/Pt concentric bimetallic microtubes.*⁴⁷ The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template. Silver (~100 nm) was sputtered on the one side of the polycarbonate template. Copper tape was attached to the silver-sputtered polycarbonate template, which serves as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The template was rinsed with 5 mL of ultrapure water (18.2 MΩ cm) for 4 times, and the Cu outer layer was deposited galvanostatically at -20 mA for 1200 s. The deposition solution contained 1 M CuSO₄ and 1 M H₂SO₄. Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -20 mA for 1200 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled. The template was polished with 0.05 μm alumina powder to remove the sputtered silver and was washed for 5 times with 8 mL of water by ultrasonication each time. It was then placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly for 2 times with methylene chloride. The solution was then washed with ethanol and water for 2 times each and centrifuged for 3 min after each

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washing step. The tubes were finally collected and stored in deionised water at room temperature.

Operation of micromotors. The experiments for studying the influence of alkalinity on the motion of micromotors were carried out in an aqueous solution containing 3 wt. % of hydrogen peroxide at constant surfactant concentrations (0.25 wt. % of SDS) with asymmetric Janus silver particle micromotors. For tubular Cu/Pt micromotors, an aqueous solution containing 3 wt. % hydrogen peroxide at constant surfactant concentrations (0.25 wt. % SDS) was maintained. A mixture of micromotors (2.5 μL), SDS, H_2O_2 and appropriate concentrations of sodium hydroxide and hydrochloric acid was pre-adjusted in an Eppendorf tube for pH calibration before 10 μL of it was applied on a glass slide freshly cleaned with nitrogen gas. Under a typical investigation, 58 ± 8 micromotors are in the sample solution. Only micromotors demonstrating activity of bubbling and microjet propulsion were taken into account. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-ElementsTM software. Average velocities were computed from 10 readings.

Extraction of micromotors. Micromotors in hydrogen peroxide solution were extracted by two separate methodologies: filtration and centrifugation. Nanoporous track-etched cyclopore membrane of pore size 200 nm were purchased from Whatman, USA. After immersion for 15 min, the solution containing micromotors were slowly dropped onto the nanoporous membrane under vacuum suction. The membrane was then flushed with copious amounts of water with the vacuum in operation. For the scanning electron microscopy measurements, the membrane was transferred to the sample stub, before being sputtered with platinum to prevent charging effects. Centrifugation involved spinning the solution of micromotors in an Eppendorf tube at 6000 rpm, before washing with deionised water. The cycle was repeated for 3 times. The pellet of micromotors remaining in the

Eppendorf tube was resuspended in deionised water by ultrasonication for 5 min and can be reused for motility studies in different pH.

8.5 Supporting Information

It is noted that hydrogen peroxide can undergo numerous redox reactions. Here we consider the thermodynamics of the hydrogen peroxide's decomposition through standard electrode potentials.

Acidic pH:

Alkaline pH:

Under acidic conditions, hydrogen peroxide is a better oxidising agent (Eq. 8.1). This is seen at the standard electrode potential of 1.77 V (Eq. 8.1) versus that of 0.87 V (Eq. 8.3) in alkaline conditions. This leads to the aggressive oxidation of the metallic micromotors and loss of structural integrity. On the contrary, in alkaline conditions, the hydrogen peroxide tends to be a reducing agent (Eq. 8.4) than in acidic conditions (Eq. 8.2) and allows the structural integrity of the micromotor to be kept.

However, notably at alkaline conditions, the hydrogen peroxide anion is prone to disproportionation to decompose to oxygen.⁴⁰⁻⁴⁴ In the presence of a catalytic metal, this leads greater amount of oxygen gases being evolved, leading to greater propulsion seen.

Figure 8.S1 Optical micrographs of 5 μm Cu/Pt tubular micromotors extracted by filtration from a 200 nm cyclopore polycarbonate membrane. Arrows indicate position of typical micromotors.

Figure 8.S2 Reusability for 3 iterations and the corresponding velocities of micromotors, after extraction by centrifugation and resuspension into hydrogen peroxide solution. Error bar indicate 1 standard deviation from 3 readings of micromotor's velocities. Conditions in all experiments: 23 $^{\circ}\text{C}$, 3 wt. % H_2O_2 and 0.25 wt. % SDS.

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Chapter 9

Photochromic Control of Bubble-Propelled Micromotors

9.1. Introduction

9.2. Results and Discussion

9.3. Conclusion

9.4. Experimental

9.5. Supporting Information

The results in this chapter were published in the following manuscripts:

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The thesis author was the primary investigator and author.

9.1 Introduction

Submicron-sized machines, which lie at the next frontier of nanotechnology, are expected to perform a plethora of tasks.¹ While they have been proposed to play important roles in biomedical delivery² and pollution management,³⁻⁵ exerting control over the motion of these nano/micro-objects is still in its rudimentary phase. Among the magnetotactic,⁶⁻⁸ acoustic⁹ and optical methods¹⁰⁻¹² that have been put forth, the latter shows the greatest potential, due to its inherent variability and the degrees of freedom it offers. Photocontrol can be described as either local or global effect, where a point or field source is used to influence the motion of single nano/micromachine or a whole ensemble respectively. For bubble-propelled nano/micromotors, methods of UV,^{10,12,13} visible¹⁴ and infra-red light¹⁵⁻¹⁷ control have been employed. The use of infra-red irradiation has only been successful in addressing individual micromotors that operate at the lower limits of self-propulsion, resulting in sluggish performance.¹¹ However, in all cases, the necessity for continuous irradiation prevents their use in photosensitive systems,¹² posing a formidable challenge in biomedical applications. Meanwhile, extensive lithographic microarrays were also required¹⁴, where changes in the whole environment were used to control the velocity of the nano/micromotors.^{3-5,18,19}

Thus, we sought an alternative optical approach, which relies on a simple molecular switch in the medium, which can be turned on and off multiple times with relatively short light pulses. While previous efforts have been directed towards the modification of the micromotors¹² or the preparation of special surfaces over which the bubble-propelled engines move,¹⁴ we targeted the micromotor environment instead, because it obviates the need for custom-built nano/micromotors¹² and for creating exquisite microarrays.¹⁴ Most importantly, it removes the bottleneck in controlling large swarms,¹¹ where every nano/microdevice has to be addressed independently. Spiropyran additives appeared most suitable for this general task, because unlike the other widely used photochromic molecules

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– azobenzenes and diarylethenes, their isomerization is accompanied by a large change in dipole moment.²⁰ The photoinduced ring opening of spiropyrans to their respective zwitterionic merocyanines has been previously used to alter ionic strength,²¹ surface tension²² and micellar concentration²³ of solutions *in situ*. Scheme 9.1 shows the ring-opening and ring-closing of spiropyrans, with different alkyl chain length at the spiropyran. Thus, we hypothesized that it can strongly affect the motion of micromotors, as these are the factors critical for bubble propulsion.²⁴⁻³²

Scheme 9.1 The reversible photoisomerization of spiropyran additive SP-C8 under UV irradiation to merocyanine MC-C8. Structural and schematic representations of SP-C8 and MC-C8 are shown.

We chose to employ bimetallic concentric Cu/Pt micromotors that run on hydrogen peroxide, due to their straightforward preparation,³³ robust nature,^{34,35} and ability to operate in a multitude of environments.^{3-5,18,19} Decomposition of H₂O₂ on the inner catalytic surface produces oxygen gas, that in the presence of sodium dodecyl sulphate (SDS) forms micro-bubbles,^{36,37} which propels the micromotor. The locomotion of the micromotors was greatly affected by the spiropyran/merocyanine ratio, which could be adjusted through the selective use of ultraviolet and visible white light. We were able to regulate the activity and velocity of the micromotors by effectively modulating their environment. In addition, we illustrated the capability to manipulate not only an ensemble, but also single micromotors with the

help of focused light from a conventional microscope. The presence of the spiropyran coupled with light allows for photochromic control on non-photoactive substrates of micromotors.

9.2 Results and Discussion

Figure 9.1 Percentage change in activity of micromotors showing bubble ejection (Red) and moving by microjet ejection (Blue) in the presence of different spiropyrans: SP-C1, SP-C8 and SP-C18, before and after UV irradiation at 365 nm for 1 min. Conditions in all experiments: 23 °C, 7 wt. % H₂O₂, 0.05 wt. % SDS, 0.02 wt. % SP-C8.

Preliminary screening of the influence by different spiropyrans with various carbon chain lengths on the micromotor's performance was investigated (Figure 9.1) in an aqueous medium. Spiropyran are labelled SP-C1, SP-C8 and SP-C18, indicating the methyl, octyl or octadecyl side chain at the spiropyran molecule respectively (refer to Scheme 9.1). The activity of the micromotors can be evaluated with two parameters. Namely, they are the number of micromotors ejecting bubbles for propulsion and the velocities of the micromotors. Here in Figure 1, the activity was measured in terms of the number of

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micromotors demonstrating bubble ejection and microjet propulsion. Hydrophobic SP-C8 and SP-C18 formed colloidal solutions with SDS in the running solution, whilst SP-C1 fully dissolved. Only SP-C8 and SP-C18 demonstrated decreases in micromotor activity, after shining of 365 nm UV light for 1 min, as shown in Figure 9.1. While SP-C1 was fully soluble in the running solution, it did not influence the micromotors with UV irradiation, illustrating a similar effect just as the presence of only SDS. The common trend between SP-C8 and SP-C18 of decreasing micromotor behaviour with UV irradiation validated our hypothesis. Due to the most accentuated changes in activity, SP-C8 was chosen to be the additive of study.

With the introduction of UV irradiation, we have been able to interconvert the photochromic additive to influence the motion of the micromotors. We utilised a spiropyran (SP-C8) that has a hydrophobic eight carbon chain on the nitrogen of the indole, which can interact with the sodium dodecyl sulphate surfactant. Upon UV irradiation, spiropyran can be converted to merocyanine (MC-C8). We undertook investigations of the interactions of photochromic additives in these two different states with the micromotors. The additives demonstrate photocontrol over the micromotors, with the type of irradiation source affecting the activity (number of micromotors bubbling and number of micromotors demonstrating microjet propulsion) (Figure 9.S1). Due to the reversibility of such a photochemical reaction and its effect on the micromotors, it was of especial interest and warranted further investigation.

Figure 9.2 UV-Vis spectra **A** Spiropyran SP-C8 after 1 min of UV irradiation and followed by 15 min of vis-light irradiation **B** Spiropyran SP-C8 conversion to MC-C8 with successive increment of UV excitation time in 20 s intervals **C** Spiropyran MC-C8 conversion to SP-C8 with successive increment of vis-light excitation time in 5 min intervals **D** Reversibility of spiropyran SP-C8 to MC-C8 and *vice-versa* with alternation of 1 min UV irradiation and 15 min visible light irradiation for three cycles. Conditions in all experiments: 0.05 wt. % SDS, 0.02 wt. % SP-C8 in water.

The molecular states of the spiropyran SP-C8 at its 2 different isomers, closed-ring form spiropyran and the open-ringed merocyanine under an aqueous system with a SDS surfactant, were investigated in Figure 9.2. We employed the use of a UV-Vis spectrophotometer in the investigation of SPC-8 in the 2 different states using 0.02 wt. % SP-C8 in 0.05 wt. % SDS in Figure 9.2A. In the ambient state, the solution of spiropyran absorbs strongly at peaks of 289, 349 nm. This was indicative that the solution of spiropyran

exists as the closed-ring isomer, the SP-C8 form.^{38,39} These two peaks correspond to the indoline and chromene moiety of the spiropyran. However, on irradiation with UV at 365 nm, there was a drastic change in absorption spectra. A new strong absorption peak at 562 nm appeared, concomitant with the depletion of the peaks at 289 and 349 nm. This was indicative of the ring-opening event of the closed-ring spiropyran (SP-C8) to the opened-ring form of merocyanine (MC-C8), with the C-O ring opening in the cyclic ring of spiropyran.⁴⁰ The extended π -conjugation in the planar structure resulted in the shift of the absorption band to 562 nm.^{41,42} The open-ringed MC-C8 form absorbs strongly at 562 nm, giving rise to a purplish colour to the solution.⁴³ A subsequent irradiation with 15 min of visible light was able to convert most of the MC-C8 to SP-C8 molecular form, where the solution turns from purple to colourless, with a corresponding diminished peak at 562 nm.

The ring-opening of the closed ring spiropyran, investigated with 365 nm UV light in accordance to time dependence, was studied in Figure 9.2B. This photochemical reaction was studied at increasing time intervals of 20 s. The maximum absorption spectra was reached at a time after 60 s. The absorption peak in the spectra at 562 nm slowly increased, before it reached saturation after 60 s. Conversely, the introduction of visible light for intervals of 5 min until a total time of 15 min was able to switch the molecular state of the MC-C8 to SP-C8 in Figure 9.2C.^{21,44} A near-total reversibility was observed in the UV-Vis spectra at the end of 15 min of visible light irradiation. The converse exposure of the micromotor towards increasing times of visible light results in the depletion of the peak at 562 nm in MC-C8, where a near-total depletion of the peak at 562 nm was observed. The subsequent restoration of the peak at 289, 349 nm to SP-C8 was observed after irradiation with visible light.

For optical cycling, the interconversion of SP-C8 to MC-C8 were studied for 3 cycles in Figure 9.2D. Successful conversions between these two molecular forms, with the exhibition of their characteristic UV-Vis spectra was observed. However, notably, there

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was a decreasing trend in the efficiencies during transformation of spiropyran to merocyanine and *vice-versa* with increased number of interconversions. Subsequent photoisomeric conversions from SP-C8 to MC-C8, was not able to reach the magnitude of absorbance at 562 nm at the end of the first optical cycle, as described in Figure 9.2D. Additionally, the restoration of the peaks at 289, 349 nm was incomplete and there was an appearance of a growing shoulder peak at 562 nm with increased number of visible light irradiation cycling. The ratio of spiropyran to merocyanine is altered with increased number of cycles. This was especially evident at the absorbance peak in 562 nm after UV and visible light irradiation, where a sustained growth of a shoulder peak was observed after each optical cycle. This phenomena, will play a critical role in the tuning of the micromotor locomotion, when we apply an *in situ* study of micromotor movement in the medium of spiropyran, as described later in the discussion.

Figure 9.3 Surface tension with and without UV irradiation at 365 nm for 1 min in the absence/presence of 0.02 wt. % spiropyran SP-C8 additive, measured using pendant drop

profile analysis from five readings. Conditions in all experiments: 23 °C, with 0.05 wt. % SDS and 7 wt. % H₂O₂.

To examine the changes in the medium experimentally, conductivity measurements allow for a direct insight of critical micelle concentrations (CMC) of the aqueous surfactant solution. The methodology is explained with more details in the supporting information of Figure 9.S2.⁴⁵ In the absence of the spiropyran, the CMC of SDS was found to be 0.21 wt. %. This was close to the experimentally reported values in the vicinity of 0.24 wt. %.⁴⁶⁻⁴⁹ Following that, the CMC of the SDS with the presence of 0.02 wt. % spiropyran was investigated. Before and after UV irradiation, both SP-C8 and MC-C8 demonstrated a CMC value of 0.20 wt. % SDS. This slight decrease from 0.21 wt. % was reflective of foreign entities in the solution.^{49,50} However, it was noteworthy to indicate that the SDS surfactant concentrations used in the solution of spiropyrans at 0.05 wt. %, was four times lesser the concentration of the SDS needed to reach critical micellar concentration in the study, at 0.20 wt. %.

Proceeding forward, we used a pendant drop methodology to investigate the surface tension measurements,⁵¹⁻⁵³ as seen in Figure 9.3. The spiropyran solutions of 0.02 wt. % SP-C8, 0.05 wt. % SDS, and 7 wt. % hydrogen peroxide were investigated experimentally before and after irradiation with an UV light source, at 365 nm. Before irradiation with UV light, the surface tension in the SP-C8 solution has a value of 40.4 mN m⁻¹. After irradiation with UV light, the surface tension increased to 45.1 mN m⁻¹. This was in contrast to the surface tension of the SDS solutions before and after UV irradiation, where values of 37.0 mN m⁻¹ and 35.8 mN m⁻¹ were recorded. This slight decrease of surface tension of SDS solutions after UV irradiation was likely due to thermal effects of heating the solution from the lamp.⁵⁴ However, on irradiation with UV, the SP-C8 solution demonstrated a large increase in surface tension. Since there was no significant changes to the CMC, the large modifications in surface tension was the contributing factor to the changes in performance

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of the micromotors in the solution. The controllable switching of the surface tension of the solution, with SP-C8 interconversion to MC-C8, tunes the propulsion of the micromotor, where a change of surface tension at 4.7 mN m^{-1} was found. This is a 12 % change in surface tension. Such changes have been known to change the locomotion of self-propelled objects.^{14,22,32,55}

Gradients in surface tension have been reported to constitute changes in self-propelled motion. This has been effected through the types and concentrations of surfactants, affecting the motion of the micromotor.^{31,32} Wang *et al.* have investigated the use of differently charged surfactants, demonstrating that various ion-bearing molecules affect the motion of the micromotors.³¹ In an extension to the study, Sanchez and co-workers found that the inherent ability to change surface tension that these surfactants confer to the solution altered the motion of the micromotor.³² Experimentally, these effects have been demonstrated at the macroscale too. A self-propelled Marangoni-effect propelled millimetre-sized capsule's locomotion was affected by gradients in surface tension of the solution it was immersed in.²² Later, a centimetre-dimensioned UV phototriggered boat was shown to be powered by a surface tension gradient, with values between 65 to 72 mN m^{-1} , demonstrating self-propelled motion.⁵⁶ At the miniaturized scale, Solovev *et al.* also demonstrated that a micro-sized object was able to demonstrate induced motion changes, with the active breakdown of the surfactant, amounting to changes in wetting of the inner surface of the micromotor.¹⁴ This eventually led to decreased frequency and size of microbubbles produced for propulsion.

In our set-up, these changes in surface tension is extended to an entire environment, which is the medium. The changes in surface tension alters the micromotor's movement in solution. With the introduction of UV light, the isomerisation of the SP-C8 to MC-C8 occurred. The hydrophobic SP-C8 has been dissolved in the presence of SDS. The photoisomerisation of the SP-C8, results in the reduced concentration of free species of

SDS in the solution, due to the ease of agglomeration in the planar MC-C8.^{20,57} The changes in surface tension of the solution, shown in Figure 9.3, from SP-C8 to MC-C8 and *vice versa*, changes the surface tension from 40.4 mN m⁻¹ to 45.1 mN m⁻¹ and back again, with the activation by UV and visible light irradiation respectively. This interswitchability between the spiropyrans and in turn changes of surface tension, results in the change in motion of the micromotor, allowing control over a non-photoactive entity using light. This is analogous to the recent report by Klajn and co-workers in using light to control the self-assembly of non-photochromic nanoparticles, with an astute tuning of hydrogen binding modes in the medium with spiropyrans.⁵⁸

Figure 9.4A Schematic of selective activation and deactivation of micromotors with controlled use of ultraviolet and visible light under isolation from a microscope objective lens. **B** Changes in activity of micromotors with the alternation of UV irradiation for 1 min and visible light irradiation for 15 min with corresponding changes in absorption band of MC-C8 at 562 nm. Conditions in all experiments: 23 °C, 7 wt. % H₂O₂, 0.05 wt. % SDS, 0.02 wt. % SP-C8. Standard deviation of data point is obtained from previous 15 measurements. (UV = 365 nm, Vis = visible light from halogen lamp)

The switching of the activity under photoexcitation, of an ensemble of micromotors in aqueous system was investigated in Figure 9.4. Through the field-irradiation of photoexcitation stimuli onto the swarm of micromotors, their activity can be altered. The micromotor was deactivated with a UV light source and can be reactivated using a visible light source shone through the objective lens as seen in Figure 9.4A. The values of moving

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entities were normalized to average values of 17 micromotors under ambient conditions. Subsequently, motion of the micromotors was halted with intervals of UV light illumination for 1 min, followed by reactivation by visible white light irradiation for 15 min. In a typical study, under ambient conditions, 100% of the micromotors were moving under microjet propulsion. Upon irradiation with UV for 1 min, the number of micromotors dropped to 40 % moving by microjet propulsion in Figure 9.4B. Due to the variability in the velocities of these self-propelled entities (See Supporting Information Figure 9.S3, S4; Table 9.S1, S2), not all micromotors are stopped by the photoirradiation under the timeframe investigated. Afterwards, we are able to reactivate the immobilized micromotors with visible light irradiation for 15 min at the second cycle, where 100% of the micromotors were again moving. Thus, the micromotors demonstrate reversibility to be deactivated and then reactivated during propulsion. The cycle can be repeated at least 3 times between a lowering and subsequent increase of activity, albeit with diminishing effect at higher number of cycles. The values from the populations of micromotors were subjected to a vigorous t-test statistical sampling, where statistically significant differences in activity were found, confirming that photochromic irradiation with spiropyrans has indeed modulated the locomotion of the micromotors (See Supporting Information Figure 9.S5, S6). In Figure 9.4B, it was illustrated that the decrease of activity corresponds to the increase in absorbance of the MC-C8 at 562 nm and the corresponding increase, was matched by the decrease in absorbance of the MC-C8. This clearly indicated the molecular transformation and the corresponding ratios of SP-C8: MC-C8, with the indicative absorption band at 562 nm in Figure 9.4B, has directly affected the motion of the micromotors. It is noteworthy that the deactivation and consequent reactivation is persistent throughout these 3 cycles. Unlike other studies, which only show a single on/off state,¹² Figure 9.4B clearly shows that the micromotor activity can be repeatedly altered.

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During the interconversion from neutral SP-C8 to zwitterionic MC-C8, there was a drastic change in micromotor environment. Photoexcitation of the molecular switch changes the ionic strength⁵⁹ and surface tension^{44,60,61} affecting the micromotor environment drastically, altering their locomotion. However, aggregation with MC-C8 results in an irreversible change on the micromotor upon extended photoisomerisation with increased iterations.⁴⁰⁻⁴⁶ This was reflected by the declining responsiveness with repetitions at higher number of cycles, as illustrated by the decreased changes in absorbance band at 562 nm in Figure 9.4B. The photocontrol effect eventually tapers off at higher number of iterations, due to side reactions⁶²⁻⁶⁴ and slow kinetics⁶⁵⁻⁶⁷ between transformations in spiropyrans, resulting in the diminished ability to alter the activity of the micromotors. At the second cycling of UV irradiation, the moving micromotors can only be depressed to 60 % with UV irradiation. On the third cycle, the number of micromotors was reactivated to 86% and the subsequent deactivation only reached 58 % of active micromotors. The agglomeration of MC-C8 has constituted to molecular transformation fatigue,⁶⁸⁻⁷¹ resulting in the diminished tuning of micromotor behaviour on the 3rd cycle of optical stimulation in Figure 9.4B. It is known that the zwitterionic merocyanine form experiences an energy barrier to revert back to the spiropyran. For example, the stacking of the planar zwitterionic merocyanine leads to the decreased reversibility to form the spiropyran molecule. This is attributed to the strong dipole-dipole interactions of merocyanine's highly charged forms and the structural π - π stacking of the aromatic rings.^{72,73} The strong stabilization of the protonated open-ring isomer of merocyanine also retarded the reciprocal ring-closing reaction,^{68,72-75} factoring in on the poor intermolecular transformation to spiropyran, due to competing reactions.⁶²⁻⁶⁴ This is in addition to the slow kinetics between the transformation of the closed-form of spiropyrans and open-form of merocyanines.⁶⁵⁻⁶⁷ All these compounded into the diminished ability of the spiropyran to alter the micromotor's motion with increased number of cycles.

Figure 9.5. Instantaneous velocities of one micromotor with alternation of UV irradiation at 365 nm for 2 min and visible light irradiation for 6 min. Insets: Screenshots (250 x 250 μm) from the points highlighted in circles. Yellow arrows point to position of observed micromotor. Each data point is the instantaneous velocity recorded over 5 s. Conditions in all experiments: 23 $^{\circ}\text{C}$, 7 wt. % H_2O_2 , 0.05 wt. % SDS, 0.02 wt. % SP-C8.

Nevertheless, encouraged by the initial findings, we wanted to investigate in depth the mechanism of these phenomena. A single micromotor is manipulated with a combination of the visible light irradiation from objective lens and ultraviolet lamp in Figure 9.5. In a typical study, after UV irradiation was used to slow the micromotor down, visible white light was used to reactivate the micromotor. Under ambient conditions in the insets of Figure 9.5, the micromotor demonstrate a steady stream of bubbles that allows for propulsion. However, upon UV irradiation, the micromotor encounters slower bubble emission with accompanied reduction in propulsion demonstrated. Upon extended irradiation, the micromotor began to move in tighter circles, before eventually stopping

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with on-site bubbling. However, on irradiation with visible white light, the micromotor can be revived, albeit reaching lower velocities. This was observed with a visible reduction in number of bubbles produced and the corresponding increase in the size of micromotor bubbles. The phenomena was also validated in another separate single micromotor, which was revived after extended irradiation with visible white light after UV immobilization (Shown in Figure 9.S7). The photochromic change in SP-C8 has resulted in a change in micromotor environment, which eventually led to a reduction in velocities and activity.

Behavior of the micromotor with different photoexcitation stimuli has been illustrated in velocity-time graph in Figure 9.5. Upon UV irradiation, the reduced velocities were tracked with an observed decrease in frequency of bubble emission and increase in bubble size. The micromotor was then reactivated with visible light irradiation to bring the motility back in micromotor for microjet propulsion. The reversibility of spiropyrans to exert control over the micromotor's self-propulsion was limited to the second cycle of reactivation, where the velocity of the micromotor did not increase appreciably. This was accompanied by an increase in the size of the bubbles and the decreased frequency. Conversion from merocyanine to spiropyran under visible light irradiation, permits the formation of a steady stream of bubbles, allowing the micromotors to move at faster velocities upon reactivation. Thus, the photochromic additive play a critical role in the tuning of the ionic strength and consequently effective surfactant concentrations.⁴⁷ The increase in the velocity of the micromotor is directly proportional to the amount of the effective surfactant concentration that is present in the solution.^{14,31,32} The micromotor can be deactivated and then reactivated just by using UV irradiation in tandem with visible white light irradiation.

Numerical representation of the micromotor's velocity against time with different photoexcitation stimuli depicts a clearer picture, as shown in the velocity-time graph in Figure 9.5, where the reversibility of such a mechanism was studied for 2 cycles. We were

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able to start, stop and change the velocities of micromotors as illustrated by the plot of velocity against time graph in Figure 9.5. Under ambient conditions, the micromotor was moving at a velocity of $57 \mu\text{m s}^{-1}$. The velocity of the micromotor dropped to $44 \mu\text{m s}^{-1}$ under UV irradiation. Upon extended irradiation at 2.5 min, the micromotor's velocity further decreased until it reached a halt. After irradiation, with a focused visible white light through the microscope optics, the motility of the micromotor was restored. The average velocity reached $37 \mu\text{m s}^{-1}$ after 4 min. The mobility of the micromotor was varied between 22 to $38 \mu\text{m s}^{-1}$ from 4 to 8 min, but after irradiation with UV light, it decreased to $11 \mu\text{m s}^{-1}$ on the 8.5 min. The micromotor's velocity was maintained in the band between 11 to $20 \mu\text{m s}^{-1}$, before finally dropping to zero after the time of the UV irradiation elapsed at 11 min.

Decreases in the effective surfactant concentration through the interconversion of the hydrophobic SP-C8 to the open ringed form zwitterionic MC-C8, influenced the surface tension for bubble formation⁴⁷ due to the aggregation of surfactant.⁷⁶ This was in association with the larger size of the microbubble detached from the system and decreased frequency of the bubble emission after UV light irradiation. The micromotor was then reactivated under visible light irradiation, reaching an average velocity of $15\text{-}29 \mu\text{m s}^{-1}$ from 11 to 12.5 min.

After successfully demonstrating that we can alter and monitor the velocity of individual micromotors, we turned our attention to selective de/activation of only one micromotor out of many. This level of control with switchable modes between two states has not been demonstrated before in an autonomous system. First, we chose 2 micromotors that are in close proximity with each other. The choice of photoirradiation with the corresponding typical activation/deactivation of the micromotors should be routinely achieved. Nonetheless, due to the limitation of the optical hardware to restrict ultraviolet light to a spatially confined zone, the ultraviolet lamp was first used to deactivate the

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micromotors, before the selective activation of the micromotor of interest with a microscope. By using an objective lens, we focused visible white light on the micromotor of interest in Figure 9.6. The modified localized environment that was created by the irradiation effects altered the propulsion of the micromotor, resulting in the increase of velocities of up to two-fold. This demonstrated a selective activation of the micromotor, creating localized micro-environments against a background of a differing macro-environment.

Figure 9.6 Instantaneous velocities of two micromotors after field deactivation with UV irradiation and reactivation with visible light irradiation only on the observed motor. Insets: Screenshots of points are highlighted in circles. Solid-squares are $400 \times 400 \mu\text{m}$ and the dashed-squares are $100 \times 100 \mu\text{m}$. Yellow arrows point to position of observed micromotor. Each data point is the instantaneous velocity recorded over 5 s. Conditions in all experiments: 23°C , 7 wt. % H_2O_2 , 0.05 wt. % SDS, 0.02 wt. % SP-C8.

First, two micromotors of two distinctive velocities were identified: the observed micromotor was identified and moving at $17 \mu\text{m s}^{-1}$, whilst the other micromotor, which

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served as our reference, was moving at $152 \mu\text{m s}^{-1}$ at 0.7 min in Figure 9.6. In order to create a different locality in the bulk environment, we switched from 100 X to 400 X, where a higher intensity of visible white light will be incident onto the observed motor (Inset of Figure 9. 6 in dashed-squares). This effectively focused the visible white light irradiation from the 400 X objective to become a point photoexcitation source.

In Figure 9.6, it was noted that the velocity of the micromotor steadily climbed to a maximum velocity of $43 \mu\text{m s}^{-1}$ at 2.0 min during visible light irradiation and reached a peak velocity of $55 \mu\text{m s}^{-1}$ at 3.2 min, even after the removal of the photoexcitation source. The velocity of the micromotor then dropped back to $26 \mu\text{m s}^{-1}$ at the 3.4 min. Removal of excitation source, allows the steady diffusion of the photochromic additive in the MC-C8 configuration, back to the region of the photoexcited point. This termination of the selective illumination has caused the velocity to return to $26 \mu\text{m s}^{-1}$. The localized illumination is 6.25 % of the total field of vision irradiated, where the MC-C8 open form of the spiropyran predominates, leading to the decrease in propulsion as observed in 3.4 min upon extended times. Spatiotemporal control of a targeted micromotor under optical confinement was thus achieved while under continuous photoexcitation bombardment. The phototrigger allows us to call up the activity in one of the multiple self-propelled objects in the mix. In another sample, where the immobilized motor is determined to be the reference motor, the observed micromotor could also be activated with optical means. These demonstrations are reproducible and the ability for an astute selection of micromotor to be switched on represents another inventory in the manipulation of self-propelled motion.

While we note the exciting opportunities ahead, key challenges remain for the widespread utility of the micromotors in a spiropyran modulated chemical fuel propelled micromotor. Stability of spiropyran remains a bottleneck, where they are susceptible to chemical changes in pH,⁷⁷⁻⁷⁹ solvent polarity,⁶¹ metal ions⁸⁰ and thermal stimuli⁸¹ in addition to changes by photochemical means. The sustained presence of chemical fuels,^{3,5,18}

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such as chemically reactive hydrogen peroxide is still needed for bubble-propelled locomotion of the micromotors. Directional control also remains a key barrier to be overcome, for specifically orientating self-propelled micromotors, during the confinement of the micromotors in specific zonal pathways. This is due to the limitations of the techniques of irradiation,⁸² for spatially separating pathways in the medium for self-propelled microdevices at the miniaturized scale.

From a broader horizon, we have shown that point irradiation or field irradiation of the micromotor has allowed for the selective activation/deactivation of the micromotors. Singular micromotor manipulation with the use of the objective lens of the microscope, in the accompaniment of a photochromic additive of SP-C8 has been achieved. Field irradiation by flooding the ensemble with photoexcitation to alter micromotors' behavior has also been demonstrated. We envision the use of photochromic additives in the solution of the micromotors to not only alter the activity, but also to guide the motion of the micromotors. For example, virtual channels can be created through optical means to induce direction and orientation of motion into the self-propelled objects towards specific targets like environmental pollutants and sites for drug delivery.

9.3 Conclusion

In conclusion, we have demonstrated the high utility of a spiropyran additive as a molecular switch in bubble-propelled micromotor systems. From an ensemble perspective, we were able to control the number of active bubble-propelled engines, while at the single micromotor level, we could manipulate velocity and stop-go motion by simply shining light of the appropriate wavelength. The subtle changes of the environment through UV and visible light irradiation represents an attractive strategy to exert control over artificial moving objects. It allows the precise manipulation of individual particles or a whole swarm of them. This is a general concept that is not exclusive to spiropyrans and it can be applied

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with any photochromic molecule that interacts with the surfactant or even other components of the running solution. The studies shown above thus bring us closer to a future when we can use optical means on nano/micro-machines to not only control their kinetics, but also guide them in all three dimensions, achieving full spatiotemporal control. Such control of non-photoactive nano/micromachines in a photochromic medium is envisaged for the future.

9.4 Experimental

Materials. The cyclopore polycarbonate membranes with pores of 2 μm in diameter were purchased from Whatman, USA (Cat no. 7060-2511). The pores are conical in shape. Colloidal graphite (isopropanol base) was purchased from Ted Pella, Inc., USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH instruments, USA. The platinum plating solution was obtained from Technic Inc., USA. Spiroyrans were purchased from Sigma-Aldrich and Vitas-M laboratory. Namely, 3',3'-dimethyl-6-nitro-1'-octyl-1',3'-dihydrospiro[chromene-2,2'-indole] spiropyran (SP-C8) and 3',3'-dimethyl-6-nitro-1'-octadecyl-1',3'-dihydrospiro[chromene-2,2'-indole] spiropyran (SP-C18) were purchased from Vitas-M laboratory. 1',3'-Dihydro-8-methoxy-1',3',3'-trimethyl-6-nitrospiro[2H-1-benzopyran-2,2'-(2H)-indole] spiropyran (SP-C1), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98+%), sodium dodecyl sulphate (SDS) and sulphuric acid (98+ %) were purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 $\text{M}\Omega \text{ cm}$) from a Millipore Milli-Q purification system. Spiroyrans are labelled according to the length of the normal alkyl chains on the nitrogen of the indole group (See supporting information Scheme 9.S1).

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Apparatus. Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyser (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software (Eco Chemie). The electrodeposition procedure was conducted at room temperature (23 °C) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl was used as the reference electrode. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Beckman Coulter Allegra 64R centrifuge. Optical microscope videos and images were obtained with Nikon Eclipse 50i microscope. Video sequences (11 fps) were processed with Nikon NIS-Elements software. UV light irradiation from UVP Blak-Ray B100AP/R High Intensity UV lamp at 100 W with 365 nm in the ultraviolet region running at 230 V and 50 Hz with 2.0 A, was used as a bench top irradiation utility. Visible white light irradiation from halogen lamp unit Nikon Model UN2-PSE100 running at 230 V and 50/60 Hz with 0.8 A was used. For the UV-vis spectra, a CARY 100 UV-VIS spectrophotometer coupled to a personal computer was used for the photochromic measurements. Conductivity measurements were carried out with a Schott LF413T electrode coupled to a Schott 960 digital meter. Surface tension was measured with a pendant drop method using CAM101 drop profile analysis device (KSV Instruments, Finland), coupled with a personal computer. A JEOL-7600F semi-in-lens field-emission SEM coupled to Oxford EDX was used to acquire SEM images.

Preparation of Cu/Pt concentric bimetallic microtubes. The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template. For the working electrode, colloidal graphite ink was applied on the one side of the polycarbonate template with commercial cotton swabs. A piece of flattened aluminium foil was attached to the ink immediately to the graphite ink electrode. The template was then assembled into a customized electrochemical deposition

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cell. Platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized. Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyser connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software. The template was rinsed with 5 mL of ultrapure water (18.2 M Ω cm) for 4 times, and the Cu outer layer was deposited galvanostatically at -2 mA for 600 s. The deposition solution contained 1 M CuSO₄ and 1 M H₂SO₄. Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -2 mA for 600 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled and the template was washed for 5 times with 8 mL of water each. After that, the template was ultrasonicated for 3 times in 2 mL of ultra-pure water for 3 min each time. The graphite layer was removed during the ultrasonication procedure. The template was then placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly for 3 times with methylene chloride. The solution was then washed with ethanol and water for 2 times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Preparation of the spiropyran solution. The stock solution of 20 mg/ mL of each respective spiropyran was first prepared in acetone. To a solution of 1.0 mg of SDS dissolved in 980 μ l of distilled water, 20 μ l of the respective spiropyran stock solution was added slowly *via* a pipette under ultrasonic conditions where the mixture was in continuous agitation. This was accomplished using an electronic micropipette that was able to continuously dispense small units of the solution at 2 μ l each under positive displacement mode. The final concentration of the spiropyran was determined to be 0.04 wt. % with 2 wt. % acetone and 0.1 wt. % SDS. The solution was then checked under microscope for the clarity of solution.

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The solution from SP-C8 forms a uniform and stable emulsion. Meanwhile, spiropyran SP-C18 is difficult to disperse even under ultrasonic conditions. Additionally, the spiropyran SP-C1 forms an unstable emulsion that rapidly breaks down on standing for 30 min to form crystalline solids (See Figure 9.S8). Only SP-C8 and SP-C18 demonstrated photochromaticity at 365 nm (See Figure 9.S9).

Operation of micromotors. For the operation of the micromotors, 50 μl of spiropyran SP-C8 solution was added to an Eppendorf tube. To the Eppendorf tube, 30 μl of the micromotors stock solution was then added. This was followed by the addition of 20 μl of hydrogen peroxide at 35 wt. %. The final concentration of the running solution is 0.02 wt. % of spiropyran, with 0.05 wt. % SDS and 7 wt. % H_2O_2 . For the immobilisation of the micromotor, UV irradiation was carried out for 1 min before measurements under optical microscope were taken. During immobilisation and activation cycling experiments, the measurements were carried out in an Eppendorf, where the micromotors can be immobilised with UV rays irradiation for 1 min and reactivated by 15 min of visible light irradiation in sequence for 3 cycles using spiropyran SP-C8. Samples of the micromotors in 10 μl batches from the same Eppendorf tube were taken at each interval and measured. Measurements of 15 repetitions were carried out on a glass slide cleaned with a stream of nitrogen. Optical microscope videos and images were obtained with Nikon Eclipse 50i microscope. Video sequences (11 fps) were processed with Nikon NIS-Elements TM software.

Determination of UV-Vis spectrum. The absorbance of the solution was measured in a cuvette by a UV-Vis spectrophotometer. For the transformation from SP-C8 to MC-C8 using UV irradiation of 365 nm, a solution of 0.02 wt. % SP-C8 in 0.05 wt. % SDS was used, in increasing intervals of 20 s exposure time up to a duration of 60 s. Conversely, the transformation of MC-C8 to SP-C8 was accomplished with visible light with increasing intervals of 5 min exposure time up to a duration of 15 min. The reversibility of the

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switching of the SP-C8 was investigated with 1 min of UV irradiation and then followed by a duration of visible light irradiation for a time of 15 min for 3 cycles.

Determination of critical micellar concentration. The conductivity of the solution of 0.02 wt. % SP-C8 was tested with increasing concentrations of the surfactant SDS (See Figure 9.S2). Measurements were taken after a stable reading for 5 seconds was achieved. Conductivity of the solution increased with increased concentrations of the electrolyte SDS. However, changes in the gradient of the conductivity of the solution was observed beyond a certain concentration, constituting to micelle formation. The intersection of these two gradient curves were then calculated graphically to obtain the critical micellar concentration of the solution.

Determination of surface tension. A solution of 0.02 wt. % SP-C8 in 0.05 wt. % SDS with 7 wt. % hydrogen peroxide was measured for changes in surface tension. The density of the solution was measured before each measurement was taken. For each measurement, a volume of 5-6 microliter of the solution was dispensed by a pendant drop method using CAM101 drop profile analysis device (KSV Instruments, Finland), coupled with a personal computer. Surface tension of SP-C8 was measured before and after irradiation of UV light at 365 nm for 1 min. Control experiments for SDS, before and after exposure to 1 min of UV irradiation, were also carried out. The final value was taken using pendant drop profile analysis from five readings

Single micromotor activation and deactivation. Selected micromotor was segregated under optical isolation using microscopic methods of 100 X magnification. The micromotor were immobilised with UV rays irradiation for 2 min and reactivated by 6 min of visible light irradiation for 2 cycles. For the creation of a localised micromotor environment, two micromotors in close proximity under 100 X magnification from the field of vision were chosen. These micromotors were irradiated for 2 min by UV light, with the region of

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interest under microscopic observation. To alter the micromotor environment, the observed micromotor was focused under high magnification of 400 X, before irradiation with visible white light to activate the motor. The non-illuminated micromotor will be termed as the reference micromotor. Optical microscope videos and images were obtained with Nikon Eclipse 50i microscope. Video sequences (11 fps) were processed with Nikon NIS-Elements TM software. Instantaneous velocities were calculated from velocities within a 5 s interval in the video, using Nikon NIS-Elements software.

9.5 Supporting Information

Scheme 9.S1 Schematics of the structure of the spiropyrans studied.

Figure 9.S1 Number of micromotors showing bubble ejection (Red) and moving by microjet ejection (Blue) in **A** Absence of SP-C8 and **B** Presence of SP-C8 before and after UV irradiation at 365 nm for 1 min. Conditions in all experiments: 23 °C, 7 wt. % H₂O₂, 0.05 wt. % SDS, 0.02 wt. % SP-C8.

In the absence of spiropyran in Figure 9.S1A, there was an average of 23 micromotors bubbling and 21 micromotors experiencing propulsion. Upon irradiation with 365 nm UV radiation for 1 min, the additional supply of energy resulted in an increase of

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13% of the number of micromotors experiencing bubbling, to about 26 micromotors and increase of 19% to 25 micromotors experiencing propulsion. This is synonymous with the UV irradiation of the micromotor, resulting in an increase in decomposition of the hydrogen peroxide in the inner catalytic lining.

The activity of the micromotors with the presence of spiropyran was measured. In the presence of SP-C8 in Figure 9.S1B, activity of the micromotors were slightly lower at 20 for the micromotors bubbling and 17 micromotors moving under microjet propulsion as compared to Figure 9. S1 A. Micromotors moving under SP-C8 environment was investigated for changes after UV light irradiation and demonstrated a converse trend as the micromotors in the absence of SP-C8 after UV irradiation. After UV irradiation for 1 min, the micromotor moving under the influence of merocyanine demonstrated depressed activity with the number of micromotors bubbling being depressed to 9, which is a 45% decrease. Similarly, the decrease in the number of micromotors demonstrating propulsion in an ensemble decreases to 8, representing a decrease of 47%. This insinuates a possible retardation of the micromotor due to the change in molecular environment after UV irradiation. There was a great variability to the velocities of the micromotor, but the velocities of an ensemble of micromotors was generally suppressed in the presence of the SP-C8 dye after UV irradiation, as seen in supporting information Figure 9.S4.

Figure 9.S2 Conductivity measurements of **A** SDS **B** SDS in 0.02 wt. % SP-C8 and **C** SDS in 0.02 wt. % MC-C8 after UV treatment against increasing concentration of the SDS. Intersection of the two trend lines indicate the critical micellar concentration of the SDS in the respective solutions.

Figure 9.S3 **A** Dimensions **B** HR-SEM image **C** EDX of Pt content and **D** EDX of Cu content in 2 μm Cu/Pt micromotors. (Magnification = 1000 X)

Table 9.S1. Dimensions of micromotors.

Reading from 50 measurements	Average length (μm)	Standard Deviation (μm)
d_1	1.3	0.2
d_2	1.9	0.3
l_1	9.7	2.2

Table 9.S2. SEM-EDX elemental atomic composition of micromotors.

EDX	Cu	Pt
Atomic Percentage	0.23	0.15

Figure 9.S4 Change in velocities of micromotors with and without UV irradiation at 365 nm for 1 min in the absence/presence of 0.02 wt. % spiropyran SP-C8 additive. Conditions in all experiments: 23 °C, 7 wt. % H_2O_2 , 0.05 wt. % SDS.

Figure 9.S5 T-tests and p-values between micromotors under conditions with and without UV irradiation at 365 nm for 1 min in the absence/presence of 0.02 wt. % spiropyran SP-C8 additive. Conditions in all experiments: 23 °C, 7 wt. % H₂O₂, 0.05 wt. % SDS.

Populations of the micromotors were used as sample arrays for the t-test in a null hypothesis and alternative hypothesis setting. The t-test of the micromotors arrays were carried out at 95 % confidence interval, with a two-tailed distribution and two-sample unequal variance in the supporting information. A p-value of less than 5 % will insinuate that one would reject the null hypothesis and accept the alternative hypothesis.

The p-values for the trends were computed with a 95 % confidence interval and the trends for all of them were below 5%, except for one. We have attached the value of these p-values in the supporting information in Figure 9.S5. Thus, in a null hypothesis scenario, we will reject the null hypothesis and accept the alternative hypothesis. It is evident that UV irradiation has changed the locomotion of the populations of micromotors.

As seen in Figure S5, the introduction of SP-C8 to the solution changed the behaviour of the micromotors, where a p value of 4.38 % was obtained between micromotors in SDS and in SPC8. The comparison of the p-value of SP-C8 before and after

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UV irradiation demonstrated a p-value of less than 0.002 %. The introduction of UV and visible light to transform between SP-C8 to MC-C8, has changed the locomotion of the micromotors. This was in contrast to the p-value of 19.3 %, a value greater than 5 %, for micromotors in SDS solution before and after UV irradiation. In this scenario, one would fail to reject the null hypothesis and would not accept the alternative hypothesis, that an additional factor has been responsible for changing the behaviour of the populations of micromotors.

Figure 9.S6 T-test and p-values between populations of micromotors in consecutive adjacent points for changes in self-propelled micromotor activity with the alternations of UV irradiation for 1 min and visible light irradiation for 15 min. Conditions in all experiments: 23 °C, 7 wt. % H₂O₂, 0.05 wt. % SDS, 0.02 wt. % SP-C8. Each data point is from 15 measurements. Error bars indicate standard deviation. (UV = 365 nm, Vis = visible light from halogen lamp)

For the on-off optical cycling of the micromotors in Figure 9.S6, continuous and adjacent points were used in the null hypothesis and t-test. We realised a value of less than 5 % between each consecutive point. Thus, we reject the null hypothesis and accept the alternative hypothesis. The introduction of UV and visible light to transform between SP-C8 to MC-C8, has changed the locomotion of the micromotors.

Figure 9.S7 Instantaneous velocities of micromotors with UV irradiation cycling at 365 nm for 2 min and extended visible light irradiation for 8 min. Each data point is the instantaneous velocity recorded over 5 s. Conditions in all experiments: 23 °C, 7 wt. % H₂O₂, 0.05 wt. % SDS, 0.02 wt. % SP-C8.

Figure 9.S8 Breakdown of SP-C1 emulsion over time **A** t = 0 min **B** t = 30 min. Conditions in all experiments: 23 °C, 0.05 wt. % SDS, 0.02 wt. % SP-C1.

Figure 9.S9 A Fluorescence of UV-irradiated spiropyran dyes: SP-C1, SP-C8 and SP-C18.

B Coloration of spiropyran dyes SP-C1, SP-C8 and SP-C18 after UV irradiation.

Conditions in all experiments: 23 °C, 0.05 wt. % SDS, 0.02 wt. % spiropyran.

In Figure 9.S9, spiopyrans SP-C8 and SP-C18 demonstrate photochromaticity at the ultraviolet region of 365 nm. This was evident in the fluorescence under ultraviolet irradiation, to glow red. SP-C8 and SP-C18 demonstrated photochromaticity at the parameters examined. The unstable spiropyran SP-C1 does not show appreciable photochromatic activity at the parameters measured. The solution of SP-C8 and SP-C18 turned purple after UV irradiation, while SP-C1 remains as a relatively translucent mixture.

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Chapter 10

Control of Bubble-Propelled Micromotors via an Ultrasonic Pathway

10.1 Introduction

10.2 Results and Discussion

10.3 Conclusion

10.4 Experimental

10.5 Supporting Information

The results in this chapter were published in the following manuscripts:

Moo, J. G. S.; Mayorga-Martinez, C. C.; Wang, H.; Teo, W. Z.; Tan, B. H.; Trung, D. L.; Gonzalez-Avila, R.; Ohl, C. D.; Pumer, M. Bjerknes Forces in Motion: Long-Range Translational Motion and Chiral Directionality Control in Bubble-Propelled Micromotors via an Ultrasonic Pathway. **2017** (Manuscript in preparation)

The thesis author was the primary investigator and author.

10.1 Introduction

Self-propelled devices represent the next frontier of nanotechnology.¹⁻³ These autonomous moving devices are able to use chemical fuel to propel themselves and perform an assortment of tasks and operations. Since their purported usage in different arenas of work, there have been an ever widening usage of these nanodevices, ranging from the applications in environmental remediation⁴⁻⁶ to applications such as biomedicine.⁷⁻⁸ To that effect, scientists and engineers have been working on different utilities to control their locomotion, so that their missions to be accomplished are done in a controlled and measured fashion.

External stimuli ranging from light, magnetism, electrical fields have been employed to control their motion and directionality.⁹ We have demonstrated that with a light induced change in the molecular switch of a spiropyran, it is possible to alter the performance of a bubble-propelled catalytic micromotor.¹⁰ Bubble-propelled micromotors based on catalytic hydrogen peroxide decomposition have been demonstrated to show augmented motion with visible light in the presence of microarrays of platinum.¹¹ Recently, phototactic micromotors, moving by self-electrophoresis in the presence of UV light have also been illustrated using hydrogen peroxide and redox shuttles.¹² An electrodeposition methodology to add nickel into the structure of the micromotor has also been illustrated to forge magnetic properties into the micromotor.¹³⁻¹⁴ Similarly, through electron beam deposition, ferromagnetic materials such as iron can be instituted, to achieve the same effect.¹⁵ This has also been demonstrated in a nickel modified micromotor by Zhao and co-workers, where they showed that by coupling a magnet with a micromotor, the directionality can be tuned, inheriting magnetotactic properties.¹³

While there have been many proposed usages of the nanomotors in a variety of environments, the control of such self-propelled devices remains ridden with challenges.

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To date, the simultaneous powering and directionality control coupled with the changes in speed of the micromotors remains elusive. Powering micromotors by the use of rotational magnetic field has been demonstrated and shows promise for dual empowerment of directionality and locomotion, but this requires the use of a changing magnetic field and magnetic elements, increasing the complexity of materials to be used.^{14, 16} The designs of these micromotors are often intricate.¹⁷

On the other hand, direct use of acoustic pressures has been used to power micromotors.¹⁸⁻²⁰ Mallouk's group has attributed the motion of nanorods under ultrasound to asymmetric shapes¹⁸ and difference in materials¹⁹ of bipolar rods, which are made readily via an electrodeposition technique. This was followed by reports of using such nanorods as biomedical nanovehicles for drug delivery²¹⁻²⁴ and possibly as mixers.²⁵ In recent work, Wang and co-workers demonstrated that these nanomachines are capable of delivering drugs²¹⁻²² to small interfering RNA for gene therapy²³ and transporting individual cells.²² Furthermore, bio-compatible iron nanoparticles-infused red blood cells can demonstrate motion under ultrasonic fields and were further guided by magnetism, due to the asymmetrical distribution of the nanoparticles.^{24, 26} While acoustic propulsion of great versatility has been demonstrated,¹⁸⁻²⁹ inducing directional changes has not been observed by using purely acoustic excitation sources. It must be added that the above examples require the wholesale imposition of an acoustic field effect coupled with a magnetic element in order to exact directional change into the micromotor and that intricately-engineered multi-component materials are needed to be introduced into the micromotor.

Recently, the use of ultrasound in bubble propulsion has also represented new opportunities. Xu *et al.* has demonstrated the use of ultrasound for the stopping and deceleration of the bubble-propelled micromotor, through the control of bubble evolution and ejection.²⁰ However, it must be noted that these micromotors have been incorporated

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with magnetic materials. A directed locomotion towards a magnetic attractant, was utilized to exhibit this stop/go motion. Notably, the change of directionality of the micromotor using ultrasound in bubble-propelled micromotors has not been demonstrated yet. Thus, the utility of such bubbles on locomotion of the micromotor may represent themselves as new frontiers of exploration in tuning propulsion of miniaturized objects.

The time varying pressure in an ultrasound field leads to oscillation of compressible bodies such as bubbles in an otherwise incompressible liquid. When an oscillating bubble is subjected to spatial pressure gradient it experiences an effective net force over its period of oscillation. These so-called Bjerknes force have been studied in the seminal work of V. Bjerknes and his father C.A. Bjerknes.³⁰ In a standing wave sound field, bubbles (depending on their phase of oscillation) may either move to the antinode or nodes.³¹ If multiple bubbles are nearby, they are not only subjected to the primary sound field but also by the sound field generated from the neighboring oscillating bubbles, which creates a Bjerknes force. Thus the primary Bjerknes force is the force caused by the primary sound field leading to the bubble oscillation while the secondary Bjerknes force is the mutual force from bubbles oscillating close by. Similar to the primary Bjerknes force, it can be attractive or repulsive. In general, if the two bubbles are oscillating in-phase then the force is attractive and becomes repulsive for out-of-phase oscillations.³²⁻³³ It is not only large compressible objects, such as bubbles, which experience a force in an acoustic field. Incompressible and weakly compressible objects scatter sound and as a result of the reaction force, become accelerated. This has been utilized to drive nanoparticles present in a solution into motion.^{18-19, 27-29, 34}

In this work, we utilize the secondary Bjerknes force to accelerate micromotors to velocities 2-3 orders of magnitude higher as compared to their averaged natural velocity. Furthermore, we also show that it's possible to change the chiral rotational motion of the

micromotor, through the use of pulsed ultrasonic excitations. A detailed account to the bubble dynamics and the resulting forces is given.

10.2 Results and Discussion

An investigation of the interactions between oscillating bubbles and a bubble-ejecting tubular micromotor in the presence of an ultrasound field is undertaken. When similar-sized bubbles undergo oscillations due to an ultrasonic acoustic field, attractive secondary Bjerknes force can manifest between each of them,³⁰⁻³³ as seen in Scheme 10.1 A. This results in an attractive force between the two bubbles. Similarly, a microbubble that is produced within the hollow concentric micromotors can undergo vibration. Thus, in the case where the surrounding microbubbles are of similar sizes, attractive force occurs between the micromotor-entrapped bubble and these microbubbles. Visibly, this results in the motion of the micromotor and bubbles towards each other as seen in Scheme 10.1 B. Separately, when differentially-sized microbubbles vibrates in an ultrasonic field in Scheme 10.1 C, repulsion occurs. This effect is illustrated in Scheme 10.1 D, where the smaller sized bubble entrapped within the micromotor undergoes repulsion away from a larger sized bubble, which has been a result of an earlier aggregation and coalescence from smaller microbubbles. Visually, this results in the repulsion of the micromotor away from the large bubble. These two scenarios will be illustrated in our experimental and theoretical study. In addition, the secondary Bjerknes force between bubbles are used to manipulate directionality in a bubble-propelled micromotor.

Scheme 10.1 Illustration of the interactions between microbubbles due to secondary Bjerknes force from the vibrations of bubbles in an ultrasound field. **A** Attractive forces between similar-sized microbubbles. **B** Attractive forces between similar-sized microbubble within a micromotor and bubbles in the surrounding environment **C** Repulsive forces between differentially-sized bubbles. **D** Repulsive forces between differentially-sized bubble within the micromotor and a large bubble in the surrounding environment.

The set-up to investigate the effect of ultrasound on the bubble-propelled micromotors is as illustrated in Scheme S10.1. Ultrasound is generated with a piezoelectric transducer (STEMINC SM 111 model) glued from below to a glass slide (Corning® Large Glass Microscope Slide, 7.5 x 5.0 x 0.1 cm). The piezoelectric transducer is driven into the fundamental thickness mode at 1.1 MHz, where the ultrasound is transmitted by the glass slide and radiated into the well as ultrasound waves^{18, 20} The droplet containing the micromotors is deposited in a well entrapped in Kapton tape on the glass slide and before the ultrasonic excitation was applied. The running solution consists of hydrogen peroxide, which is the fuel and sodium dodecyl sulphate as the surfactant to reduce surface tension. Decomposition of hydrogen peroxide on the inner catalytic platinum micromotor produces oxygen bubbles in the hollow concentric structure. Accumulation of the oxygen bubbles

and their eventual ejection pushes the micromotor forward.³⁵ With this setup, we study the interaction of the microbubbles and the micromotor under ultrasound excitation.

10.2.1 Effect of Acoustic Driving Amplitude

Figure 10.1 Selected frames from high-speed movies before and after pulsed ultrasound excitation at 1.1 MHz for 1000 cycles. The left column is taken 1.0 ms before the ultrasound (US) is turned on, and the right column 1.0 ms after the US is switched off. The voltage amplitude at the piezoelectric transducer is increased from **A** 50 V to **B** 70 V and **C** 100 V. Conditions: 23°C, 2 wt. % of H₂O₂ and 1 wt. % SDS. Arrows point to the position of the micromotors.

We start with demonstrating the effect of the driving voltage of the piezoelectric transducer. The transducer is operated for a short duration of 1000 cycles at 1.1 MHz, i.e. the ultrasound is only on for about 0.91 ms. At 50 V, from Figure 10.1A which depicts the scene consisting of the micromotor trailed by the microbubbles is mostly unaltered by the US burst. From a driving voltage of 70 V, we observe an effect of the US on the trailing

bubbles: some of the bubbles move closer to each other and thereby inducing a flow. This flow transports the micromotor towards the bubble trail and thus in the opposite direction of earlier micromotor's trajectory. Increasing the voltage to 100 V leads to a rapid coalescence of the bubbles into a compact cluster into which the micromotor is entrained.

10.2.2 Time Resolved Micromotor Trajectory

Figure 10.2 **A** Position of the micromotor as a function of time. The origin is set to its position when the US is switched on ($t = 0$). The inset depicts the position of the micromotor. **B** Speed of the micromotor during free roaming motion and when the US is switched on. The inset details the velocity of the micromotor during a bubble discharge event. Conditions: 23 °C, 2 wt. % of H_2O_2 and 1 wt. % SDS.

Next we analyze the trajectory of the micromotor with the US field by tracking its position. Using a cross correlation technique, the position of the micromotor, $\vec{x}$, can be tracked with sub-pixel accuracy.³⁶ The origin is set to the position of the micromotor at time $t = 0$, the time when the US is switched on. Figure 10.2A depicts the distance, $r = |\vec{x}|$

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of the micromotor from the origin obtained from high-speed recordings taken at 10,000 frames s^{-1} . The micromotor ejects at a rate of 70 Hz microbubbles resulting into a staircase shaped distance versus time curve. Each step is about 0.8 μm leading to an averaged velocity of about 58 $\mu\text{m s}^{-1}$. When the burst of ultrasound with 1000 cycles at frequency of 1.1 MHz (100 V) starts at $t = 0$, the micromotor moves in the opposite direction almost instantaneously. The inset of Figure 10.2A displays the total track of the micromotor over the course of time. Prior to the ultrasound, the micromotor moves along a mildly wiggly line, once the US is switched on, a straight trajectory is seen which brings the motor approximately back to the original x -position yet deviates by about 40 degree in the y -direction. Figure 10.2B analyses the speed of the micromotor, $\dot{r} = dr/dt$, as a function of time. As the motor moves towards the origin its speed deviates from zero only when a bubble is discharged. This is a pretty fast event where the velocity increases to about 5-6 mm s^{-1} and relaxes back to zero within 0.3 ms, see inset of Figure 10.2B. The acceleration during that brief moment is of the order of 30-40 m s^{-2} . The speed during the bubble discharge is negative, i.e. towards the origin, while during the time the US is acting, the speed is towards the bubble trail and away from the origin. The magnitude of the speed during US exposure is up to 5 times higher than the maximum speed during bubble expulsion. Yet comparing the speed of the micromotor during US exposure with the averaged speed in absence of US then we have an increase of nearly 400 times.

10.2.3 Dynamics of the Micromotor under Ultrasound Exposure

The motion of the micromotor during US exposure is the result of acoustic radiation forces³⁷⁻³⁸ induced by bubble volume oscillations.³¹ The amplitude of the bubble oscillations increases with pressure and therefore voltage amplitude. Above a threshold voltage, here about 70V, the forces generated overcome the fluidic resistance; then the radiation force leads to the movement of the bubbles which may result into their agglomeration³⁹ or even coalescence⁴⁰.

Generally, compressible objects in a time varying pressure field such as in an ultrasound field experience an acoustic force. There, two particular forces are observed. The primary Bjerknes force caused by pressure gradients of the primary sound field, and the secondary Bjerknes force. The latter is caused by the interaction of nearby oscillating bubbles. Next we'll show that the secondary Bjerknes force for the present study is the dominating force.

Figure 10.3 are the frames showing the bubble and micromotor motion once the US field is switched on at $t = 0$ for an interval of 0.91 ms (1000 cycles at 1.1 MHz). Within 0.3 ms, the bubbles earlier aligned along the trajectory of the micromotor to form a dense cluster. Here, we can rule out the primary Bjerknes force, as the bubbles are moving under a mutually attractive force. The secondary Bjerknes force acting here is in general attractive for bubbles of similar size, see Section 2.3. Starting from about $t = 0.5$ ms the micromotor moves towards the cluster, touches the cluster and then rotates counterclockwise by around 45°.

Figure 10.3 Selected frames from a high-speed recording of the bubble and micromotor dynamics. At time $t = 0$, the US is switched on and the formation of a cluster of bubbles results. From 0.5 ms on, the micromotor and the cluster move towards each other. Conditions: 23°C, 2 wt. % of H_2O_2 and 1 wt. % SDS. Motion of micromotors after ultrasonic excitation at 1.1 MHz, 1000 bursts, 100 V.

The events leading to the rapid acceleration of the micromotor opposite to its original trajectory are (1) the formation of a bubble cluster followed by (2) the motion of

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the micromotor towards the bubble cluster. Once the motor touches the cluster it may align by rotation, see for example in Figure 10.3 during $0.8 \text{ ms} < t < 1.0 \text{ ms}$. After the US exposure, the micromotor will continue its trajectory along this newly aligned direction.

Another possibility to change rapidly direction of a micromotor utilizes the repulsive force between bubbles of largely different sizes. An example where this control is at work is shown in the experiment in Figure 10.4. It consists of two steps: during the first step, the US exposure leads to the formation of a cluster of bubbles and the attraction of the micromotor towards this cluster. At some later time, the microbubbles coalesce into a large bubble. For the second step, a burst of US is applied again. The microbubbles generated by the micromotor are repelled by the large bubble, leading to rapid motion of the micromotor away from the bigger bubble from each other (for theoretical explanation, see Section 2.4). This rapid event re-orientates the micromotor, which are depicted in Figure 10.4 with a stacked overview in Figure 10.4A and selected frames in Figure 10.4B. Figure 10.4A shows the traces of the micromotor labeled with the respective number and an arrow pointing in the direction of motion. The micromotor with the trace labeled (0) moves at first in the North direction (as defined in the Figure 10.4A), the trace (1) is occurring after the first US exposure. Then the bigger bubble has been formed. Between trace (1) and trace (2) a second burst of US is generated. This is leading to a rapid movement of the bigger bubble to the East and the micromotor to the South. Trace (2) is now approximately opposite to the original direction (0). For clarity the three tracks are shown in the selected frames in Fig 4B, with (0) showing the micromotor moving North, (1) micromotor moving West and slowly away from the big bubble, and (2) change of orientation where the bigger bubble has moved to the East and micromotor to the South.

Figure 10.4 Utilizing repulsive forces for micromotor steering. **A** Overlay of the traces of micromotor and large bubble, color-labeled red and blue respectively. The (0), (1), (2) are traces obtained, in before, after the 1st US burst, and after the 2nd ultrasound burst, respectively, being labeled with the respective number and an arrow pointing in the direction of motion. **B** Still frames captured before (0), after the 1st ultrasound excitation (1) and after the 2nd ultrasound excitation (2) respectively. Conditions: 23 °C, 2 wt. % of H₂O₂ and 1 wt. % SDS. Motion of micromotors after ultrasonic excitation at 1.1 MHz, 1000 bursts, 70 V controlled by external function generator with 100 ms gap in between.

As illustrated, both attractive and repulsive forces are found between the microbubbles and the micromotor. We undertook simulations to investigate the magnitude and directivity of the secondary Bjerknes force and modelled the resulting trajectory as found in Figure 10.2 and Figure 10.4.

10.2.4 Secondary Bjerknes Force under Small Amplitude Driving

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It is important to note that the oxygen bubbles generated by the micromotors are compressible while the liquid surrounding the bubble is mostly incompressible. When we add a sound field, $p(t)$, to the static pressure p_∞ and into the aqueous H_2O_2 solution the bubbles undergo periodic oscillations. The sound pressure may be written as:

$$p(t) = p_a \sin(2\pi ft) \quad (10.1)$$

where p_a is the acoustic pressure amplitude, in Eq. (10.1)

During one half of the cycle, the gas is compressed while during the second half, they expand. In the limit of small acoustic pressures, bubbles undergo harmonic oscillations and respond as a harmonic oscillator with resonance frequency f_r and an equilibrium radius R^{eq} .

This resonance frequency which is also called the Minnaert frequency⁴¹ is given by:

$$f_r^2 = \frac{1}{4\pi^2 \rho (R^{eq})^2} \left(3\gamma p_\infty - \frac{2\sigma}{R^{eq}} \right) \quad (10.2)$$

where σ is the coefficient of surface tension and γ is the polytrophic gas coefficient, in Eq. (10.2). In the experiments the frequency is $f_r = 1.1$ MHz, thus the linear resonance radius is R^{eq} approximately $3.1 \mu m$ with $p_\infty = 1$ bar and liquid density, $\rho = 998 \text{ kg m}^{-3}$.

The harmonic oscillations may occur below or above the resonance frequency f_r , resulting in a phase lag between the acoustic field and the bubble's response, i.e.

$$R(t) = R^{eq} (1 - \varepsilon \sin(2\pi ft - \phi)) \quad (10.3)$$

where the relative amplitude of the bubble oscillation is ε and the ϕ is the phase of oscillation in Eq. (10.3).

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For example, a bubble oscillating quasistatically, i.e. at a very low driving frequency; it shrinks during the positive sound pressure cycle and expands during the negative sound pressure. The zero-phase can be arbitrarily chosen. We select $(\phi = 0)$ for a bubble responding to a low driving frequency. In contrast, bubbles driven by the sound field far above the resonance frequency have their largest volume during the positive pressure and their minimum during the negative pressure cycle.

The study of the mutual attraction and repulsion of oscillating objects in liquids has been undertaken.⁴² The resulting force is named after them as secondary Bjerknes force. If we apply their results to harmonic oscillating bubbles we obtain:

$$F_B = \frac{\rho}{4\pi} \frac{dV_1}{dt} \frac{dV_2}{dt} \frac{1}{d_{12}}, \quad (10.4)$$

where V is the bubble volume, and d_{12} the distance between their two centres. A positive force in Eq. (10.4) is attractive and a negative force repulsive. We can now insert the expression for the small bubble pulsation Eq. (10.3) into Eq. (10.4) and average over one oscillation cycle to obtain a time averaged force

$$\langle F_B \rangle_T = f \int_0^{1/f} F_B dt = 8\pi^3 f^2 \rho \varepsilon_1 \varepsilon_2 (R_1^{\text{eq}})^3 (R_2^{\text{eq}})^3 \cos(\phi_1 - \phi_2) \frac{1}{d_{12}} \quad (10.5)$$

Eq. (10.5) shows that the magnitude and direction is a function of the phase difference between both bubbles. If $(|\phi_1 - \phi_2| < \pi/2)$, we have an attractive force, while for phases $(|\phi_1 - \phi_2| > \pi/2)$ the bubbles repelled each other. Thus if both bubbles are below and above resonance size they attract each other, while if one is smaller and the other larger than resonance size, they repel each other. Putting the concept together, it means that for sufficiently small driving pressures for bubbles to attract each other, it must be on the conditions that both are less than $\approx 3.1 \mu\text{m}$ or that both are above $\approx 3.1 \mu\text{m}$. If one bubble

is smaller and the other is larger than $\approx 3.1 \mu m$, they move away from each other. At higher driving pressures, typically larger than about 10 % of the static pressure bubbles, they demonstrate non-linear oscillations and while Eq. (10.4) holds, the simple response function Eq. (10.3) and the resulting force Eq. (10.5) needs to be re-considered.

Next, we embark on describing the motion pathways of bubbles in the presence of the secondary Bjerknes force due to the oscillating bubbles under high-pressure amplitudes.

10.2.5 Modelling the Large Amplitude Driving and the Resulting Trajectories

Instead of a linear response we need now to utilize the full bubble dynamics equation. Here we use the Keller-Miksis model,⁴³ Eq. (10.6) for the volume oscillation of the bubble, translational motion and coupling from neighbouring bubbles. It accounts for energy dissipation through viscosity and acoustic radiation losses. Due to the stronger driving amplitude of bubble i , coupling between neighbouring bubbles $j \neq i$ occurs. This interaction is approximated with the last term in Eq. (10.6). The second last term is the Bernoulli pressure generated by the translation of the bubble.

$$\begin{aligned} & \left(1 - \frac{\dot{R}_i}{c}\right) R_i \ddot{R}_i + \left(\frac{3}{2} - \frac{\dot{R}_i}{2c}\right) \dot{R}_i^2 = \\ & \frac{1}{\rho} \left(1 - \frac{\dot{R}_i}{c}\right) \left[p(R_i) - p_l(t) \right] + \frac{R_i}{\rho c} \frac{d}{dt} [p(R_i) - p_l(t)] \\ & + \frac{1}{4} \mathbf{u}_i \cdot \mathbf{u}_i - \sum_{i \neq j} \frac{1}{r_{ij}} \left(R_j^2 \ddot{R}_j + 2 R_j \dot{R}_j^2 \right) \end{aligned} \quad (10.6)$$

The pressure in the liquid just outside the gas bubble $p(R_i)$ is related with the gas pressure by the jump condition due to normal viscous stresses and the Laplace pressure in Eq. (10.7):

$$p_l(R_i) = \left(p_\infty + \frac{2\sigma}{R_i^{\text{eq}}} \right) \left(\frac{R_i^{\text{eq}}}{R_i} \right)^{3\gamma} - \frac{2\sigma}{R_i} - \frac{4\mu\dot{R}_i}{R_i} \quad (10.7)$$

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The pressure in the liquid far away from the bubble contains the static pressure p_∞ and the time dependent acoustic pressure $p_l(t) = p_\infty + p(t)$.

Besides the radial dynamics we also want to model their translation. Mettin *et al.* have developed and tested a simple approach that abstracts the fluid mechanics into individual forces acting on the bubbles and its displaced fluid mass.⁴⁴⁻⁴⁵ This approach can be written as Newton's 2nd law for each bubble, i

$$\frac{d}{dt}(M_i(t) \vec{U}_i(t)) = \frac{d}{dt}\left(\frac{1}{2}\rho V_i(t) \vec{U}_i(t)\right) = \vec{F}_{B,i} + \vec{F}_{D,i} \quad , \quad (10.8)$$

stating the change of momentum of the bubble's momentum, L.H.S. of Eq. (10.8), is caused by the forces acting on the bubble, R.H.S of Eq. (10.8). For the present problem, we only considered secondary Bjerknes force, $F_{B,i}$ and the drag force $F_{D,i}$. Note that the mass of the bubble content is ignored and only the added mass of the liquid is accounted for.

We can rewrite Eq. (10.8) into a differential equation for the position of bubble $\vec{x}$

$$\frac{d^2 \vec{x}_i}{dt^2} = \frac{d\vec{U}_i}{dt} = \frac{1}{R_i^3} \left[\frac{3}{2} \frac{1}{\pi\rho} (\vec{F}_{B,i} + \vec{F}_{D,i}) - 3R_i^2 \dot{R} \vec{U}_i \right] \quad (10.9)$$

By integrating Eq. (10.9) together with Eq. (10.6) we obtain a full set of equations to solve for the bubble position as a function of time $\vec{x}_i(t)$.

We use a formulation for the drag force $F_{D,i}$ in Eq. (10.10) from Mettin et al.,⁴⁵

$$\mathbf{F}_{D,i} = -12\pi\mu R_i \mathbf{U} \quad (10.10)$$

The last important part is to deal with bubbles coming into contact. In experiments we see that bubbles attracting each other will remain for some time in close contact and may coalesce. Forces at close distances in the interaction term of Eq. (10.6) needs correction, typically by adding corrections of higher order in the underlying potential flow model.⁴⁶⁻⁴⁸

Yet bubbles in close contact are separated by a thin liquid film undergoing oscillatory flow. A proper description would need to account for additional viscous effects. Here, we ignore details of bubbles oscillating at close distance and to prevent coalescence, the secondary Bjerknes force is set to become repulsive once the bubbles are closer than $R_i^{\text{eq}} + R_j^{\text{eq}}$. This is achieved by multiplying a factor, α in Eq. (10.11), to the Bjerknes force $\vec{F}_{B,i}$

$$\alpha = \tanh \left[100 \left(\frac{d_{ij}}{R_i^{\text{eq}} + R_j^{\text{eq}}} - 1 \right) \right] \quad (10.11)$$

Next, we considered the input of these factors into a simulation, where we describe the forces encountered by the bubbles under ultrasound and their eventual trajectories of motion.

10.2.6 Comparison of Simulations with Experiments

We start with a study on magnitude and direction of the secondary Bjerknes force for bubbles driven at $f = 1.1$ MHz and in the size range from $1 \leq R^{\text{eq}} \leq 10 \mu\text{m}$. For this we calculate Bjerknes force coefficient, f_B , averaged over one oscillation cycle in Eq. (10.12):

$$f_B = \frac{\rho}{4\pi} \langle \dot{V}_1(t) \dot{V}_2(t) \rangle \quad (10.12)$$

Here we use water as a liquid with $\mu = 0.001 \text{ Pa} \cdot \text{s}$, $\rho = 998 \text{ kgm}^{-3}$, $\gamma = 1.4$ and $\sigma = 0.072 \text{ Nm}^{-1}$. A positive coefficient is an attractive force, while a negative coefficient is repulsive. The coefficient varies over many orders of magnitude. Therefore, we plot not the value, but the logarithm of the coefficient $\log_{10} |f_B|$ in Figure 10.5. Positive coefficients are coloured in red and negative (repulsive bubbles) in blue. The three frames of Figure 10.5 cover the radii parameter space for the acoustic pressures, p_a at 0.1, 0.7 and 1.9 bar, from Figure 10.5A to 5C respectively. Values very close to zero in $\log_{10} |f_B| < -21$

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are omitted. The linear resonance radius is indicated with a circle in Figure 10.5A for guidance. Although f_B does not explicitly depend on the distance between the bubbles d_{12} the radial dynamics does. As we are interested in bubbles oscillating nearby each other we fix the distance to $d_{12} = 2(R_1^{\text{eq}} + R_2^{\text{eq}})$ for the calculations shown in Figure 10.5.

For low driving-pressure of $p_a = 0.1$ bar, two distinct rectangular regions show attraction and repulsion, while for larger pressures these regions develop as a result of the nonlinearities of the bubble oscillations convoluted boundaries. Although the details of the boundaries change the general regions of mutual attraction, repulsion does not. An example to discuss the motion of an ensemble of bubbles due to secondary Bjerknes force is shown in Figure 10.S1 (See Supporting Information).

Figure 10.5 Radii parameter space for the acoustic pressures p_a , **A** 0.1 bar **B** 0.7 bar and **C** 1.9 bar. Regions of attraction (red) and repulsion (blue) for two bubbles R_1 and R_2 positioned at a distance $d_{12} = 2(R_1^{\text{eq}} + R_2^{\text{eq}})$.

Figure 10.6 is a simulation approach to Figure 10.4. We assume that a sub-resonance radius bubble $R_1^{\text{eq}} = 0.9 \mu\text{m}$ is within the microtube and is neighbored by a bubble larger than resonance radius $R_2^{\text{eq}} = 4.75 \mu\text{m}$ in Figure 10.6A. We observe in Figure 10.4 that most of the larger bubble moves away with a speed of approx. 25 mm s^{-1} , while

the micromotor remains stationary. We explain the larger velocity of R_2 with a higher drag of the micromotor, which then eventually leads to the observed flipping. This larger form drag is ad-hoc modelled by using a 50 times higher viscosity in the drag formulation of R_1 , see Eq. (10.10). We assume a pressure of 1 bar for 1 ms and obtain reasonable displacements of the larger bubble of approximately $35\ \mu\text{m}$, similar to the experimental results in Figure 10.6B. This showcases the repulsive effect under an acoustic field of two bubbles with inherently different sizes. The drag coefficient was adjusted to account for the measured displacement of the tube. This leads to a drag of 50 times that of the spherical bubble. A factor of approximately 2 is expected for a closed cylinder moving along the axis of symmetry,¹ this accounts for skin friction and pressure drag for Stoke's flow far from any walls. The complex geometry of the open tube, the nearby wall, and the sliding friction of the tube along the glass substrate will further increase this friction coefficient. Therefore, a factor of 50 was approximated. However, this value can only be tested with a detailed analysis of the flow using computational fluid mechanics and is beyond the scope of the present work. Thus, it was noted that the friction of the bubble powered tube is considerably higher than what would be expected for a free spherical bubble.

Figure 10.6 **A** Simulated image of bubble-ejecting micromotor and large microbubble. **B** Tracked pathway of small bubble and large bubble. Dynamics of a small microbubbles ($R_1^{\text{eq}} = 0.9 \mu\text{m}$) and a large bubble ($R_1^{\text{eq}} = 4.75 \mu\text{m}$) driven at $p_a = 1$ bar acoustic pressure for a duration of 1 ms.

As shown in Figure 10.7, we model the formation of a cluster of bubbles spun out by the micromotor as shown in Figure 10.2. The cluster consists of 6 bubbles below the linear resonance radius, as depicted in Figure 10.7A. We chose a moderate driving of $p_a = 0.5$ bar as the closeness of the bubbles shields the acoustic pressure. Coupled with the microbubble within the micromotor, they gather together into the microbubble aggregate in Figure 10.7B. The formation of the cluster occurs within 15 cycles of the driving and their motion traces are shown in Figure 10.7C.

Figure 10.7 Simulation of the cluster formation shown in Figure 10.3. **A** Initial position of microbubbles before ultrasound excitation. **B** Final position of microbubbles after ultrasound excitation. **C** Simulated trace of microbubbles' motion pathway after ultrasonic excitation.

The parameters are:

$R_1^{\text{eq}} = 2.0 \mu\text{m}$, $R_2^{\text{eq}} = 1.1 \mu\text{m}$, $R_3^{\text{eq}} = 1.1 \mu\text{m}$, $R_4^{\text{eq}} = 1.4 \mu\text{m}$, $R_5^{\text{eq}} = 1.6 \mu\text{m}$, $R_6^{\text{eq}} = 1.6 \mu\text{m}$ and 15 cycles at $p_a = 0.5$ bar.

The simulation shows strong correspondence to the observed phenomena, where emitted bubbles originate from catalytic decomposition of hydrogen peroxide by micromotors. Next, we attempt to broaden the scope of applying such secondary Bjerknes forces upon motion pathways of bubble-propelled micromotors.

10.2.7 Influence of Ultrasound upon Chirality of Motion

Figure 10.8 Frame by frame analysis of the change in chirality of motion of the micromotor: from anti-clockwise motion to clockwise motion. Red line represents the trace of the micromotor from initial to final position. Conditions: 23°C, 2 wt. % of H_2O_2 and 1 wt. % SDS. Motion of micromotors after ultrasonic excitation at 100 V, 1.1 MHz, 1000 bursts, controlled by external function generator with 100 ms gap in between.

Next, we explore a series of ultrasound excitation at higher potentials of 100 V. We embarked on an experiment demonstrating the switching of chiral motion direction, by using ultrasound at a higher voltage of 100 V, which constitutes a higher power. In two-dimensions, the circle swimming of the micromotor results in chirality of motion; meanwhile in three dimensions, when there is a swimming along a three-dimensional path, it results in helicoidal motion.⁵⁰⁻⁵¹ The origin of such circular motion can be due to the particle shape and the anisotropic propulsion mechanism.⁵⁰ Directed motion in the micromotor, has resulted in the propulsion of the micromotor forward. However, these coupling of rotational and translational motion resulted in a torque, leading to the circular routes.⁵¹ Hereby, with the provision of an out-of-plane force, we can possibly switch the chirality of the path, in other words, changing the direction from an anti-clockwise direction to a clock-wise motion or vice versa.

As depicted in Figure 10.8, the micromotors were initially moving in a circular anti-clockwise direction. The first ultrasound stimulus at 0.08 s, results in the translation of the micromotor, moving over 11.5 μm . We observed drastic changes in translational velocities of the micromotors when the micromotors began their orbital motion in smaller circles and helicoidal anticlockwise fashion, which cause bubbles to aggregate. The next ultrasound pulse that occurs in 0.18 s, translates the micromotors over large distances, with a length in excess of 100 μm . This constitutes a 1700 body lengths per second and it assumes a motion pathway of helicoidal clockwise direction. The close proximity of the small bubbles with each other constitute to a bubble aggregate, due to the high density of the bubbles that are present. Next, the repulsive effect between bubble aggregates and small bubbles is manifested again during the next ultrasound excitation.⁵² On the third ultrasound pulse, the micromotor travelled a distance of 110 μm , where a clockwise circular path was then taken. Notably, the micromotor has switched from an anti-clockwise motion to a clockwise motion. It is noted that singular small microbubbles are built up within the micromotors, prior to

ejection.⁵³ The repulsion between these small bubbles that are accumulated within the micromotor and bubble aggregates due to secondary Bjerknes forces has been demonstrated, a telling result of repulsion between bubbles of dissimilar sizes. Chiral motion of the micromotor can be changed by tuning the use of ultrasound excitation, from an anti-clockwise direction to a clockwise direction, using an out-of-plane secondary Bjerknes force due to the inherent difference in sizes of bubbles. Effectively, through the flipping of the micromotor, we have been able to change the direction of the micromotor movement.

Separately, through ultrasonic excitation, the random walk of a micromotor is also illustrated, where the chiral rotational directionality is continuously altered at regular intervals (See Supporting Information Figure 10.S2-S4).

Figure 10.9 A (i)-(iii) Case studies of large jumps in micromotors before and after a single pulse of ultrasonic bursts, superimposed into a composite image. Black arrow represent initial position of micromotor and white arrow represent final position of the micromotor

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after large jumps in 0.01 s. **B** (i)-(iii) Schematics of large jumps in micromotor before and after a single pulse of ultrasonic bursts. Repulsive forces between the micromotor in initial position (highlighted in blue) and the bubbles to the final position (highlighted in red). **C** Instantaneous velocities of micromotors at 0.01 s steps. Dashed line indicate trajectory of jump event. Conditions: 23°C, 2 wt. % of H₂O₂ and 1 wt. % SDS. Motion of micromotors after ultrasonic excitation at 1.1 MHz, 1000 bursts, controlled with an external function generator at pulses of 10 Hz.

In Figure 10.9, we illustrate the typical large-distance motion of the micromotor in the proximity of large bubbles or bubble aggregates. In Figure 10.9A (i)-(iii), superimposed images of the micromotor before and after translation over large distance are depicted in three separate case studies. A clearer schematic of the large jump is depicted in Figure 10.9 B (i)-(iii), where repulsive forces are experienced between the bubbles and the micromotors (highlighted in blue) in initial position to final position (highlighted in red). In Figure 10.9C (i)-(iii), a 3D plot of the large velocities are covered by the micromotors in the event of an ultrasound excitation are illustrated. In Figure 10.9A (i)-(iii), the distances travelled in decreasing order were, 109.7 μm , 66.8 μm and 24.6 μm respectively. The instantaneous velocities were 10966, 6677 and 2462 $\mu\text{m s}^{-1}$ respectively. With the typical lengths of the micromotors at 5.8 μm in length, large distances were covered by the micromotors, where ~ 400 -1900 body lengths of the micromotor were translated per second. Differences of the distances travelled by the micromotors were due to the interference of the differing bubble sizes, and the different locations of the microbubbles in the surrounding environment as seen in Figure 10.9(A). A pristine environment with only distinct bubble aggregates and the microbubble emitted from the micromotor allows for the occurrence of larger repulsive force to be realized. This was exemplified in Figure 10.9A (i), which produces the largest distance travelled by the micromotor. Due to lesser interferences from bubbles of different

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sizes and locations, larger repulsive forces were manifested and the micromotor experiences larger translational distance.

10.3 Conclusion

In conclusion, we have demonstrated the use of ultrasound in bubble-propelled systems, where changes in locomotion of the micromotor are illustrated. Ultrasound excitation shows capability in demonstrating stop/go motion in bubble-propelled micromotor. Such pulsed ultrasound excitations are able to produce bubble aggregates from the stream of individual microbubble plumes. Furthermore, interactions between bubble aggregates and ejected microbubbles are leveraged to induce locomotion changes in the micromotor. The interaction of the bubbles generated by the micromotor and the ultrasound field has been modeled successfully qualitatively with a reasonable quantitative results. The route of the micromotor can be altered by selective introduction of ultrasound pulses, leading to Bjerknes forces. Additionally, such repulsive forces, can result in rapid translations and move the micromotors over large distances. Furthermore, with the ultrasound pulses, directionality of the micromotor can be changed, where the chiral motion of the micromotor can be altered with pulsed ultrasound excitation. The dexterous use of ultrasound pulses shows promise to manipulate locomotion in micromotors.

10.4 Experimental

Materials. The cyclopore polycarbonate membranes with pores of 2 μm were purchased from Whatman, USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH Instruments, USA. The platinum plating solution was obtained from Technic, Inc., USA. Sodium dodecyl sulphate (SDS) were purchased from Sigma-Aldrich. Chemicals were used as received and

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the solutions were prepared using ultrapure water (18.2 M Ω cm) from a Millipore Milli-Q purification system.

Apparatus. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The deposition procedure was conducted at room temperature using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Beckman Coulter Allegra 64R centrifuge. Optical microscope videos and images were obtained using a Nikon Eclipse Ti-E microscope fitted with a SA-X2 scientific CMOS camera, with a Photron FASTCAM SA1.1 software. Video sequences were processed with Nikon NIS-Elements software. The amplifier unit was of the design AG Series Amplifier LF Amplifier/Generator from T&C Power Conversion, Inc. The primary waveform generator and secondary waveform generator was of the design 33220A 20-MHz Function/Arbitrary Waveform Generator, Agilent Technologies and 3390 50 MHz Arbitrary Waveform Generator, LXI Kiethley respectively. The oscilloscope to observe the waveform function generated is sourced from Tektronix TDS 1001B.

*Preparation of Pt Microtubes.*⁵⁴ The microtubes were synthesized with a modified electrochemical deposition procedure on cyclopore polycarbonate templates. For the microtubes prepared using membranes with 2 μ m pores, silver ($\sim$ 200 nm) was sputtered on the one side of the polycarbonate template. Copper tape was attached to the silver-sputtered polycarbonate template, which serves as the working electrode for the plating experiments. The template was then assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized.

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Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer connected to a computer and controlled by NOVA Version 1.8 software. For the microtubes electrodeposited in 2 μm pores, only platinum was deposited with the commercial plating solutions. For 2 μm microtubes, platinum was electrodeposited galvanostatically at -20 mA for 600 s. When the deposition of microtubes was finished, the electrochemical cell was disassembled. For the membranes with sputtered silver layer, the template was polished with 0.06 μm alumina powder to remove the sputtered silver and was washed 5 times with 8 mL of water by ultrasonication each time. It was then placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly 3 times with methylene chloride. The solution was then washed with ethanol and water 2 times each and centrifuged for 3 min after each washing step. Finally, all the prepared micro/nanotubes were treated with concentrated nitric acid to remove the other impurities. After washing out nitric acid, the resulting tubes were stored in water at room temperature.

Operation of Micromotors. The experiments for studying the dynamics of the motion of micro/nanomotors were carried out in an aqueous solution containing 2 wt. % H_2O_2 at constant surfactant concentrations (1 wt. % of SDS). A mixture of microtubes, SDS, H_2O_2 was applied on a glass slide freshly cleaned with nitrogen gas. Optical microscope videos and images were obtained using a Nikon Eclipse Ti-E microscope fitted with a SA-X2 camera, a Photron FASTCAM SA1.1 software. Video sequences were processed with Nikon NIS-Elements software and Tracker.³⁶

Ultrasound experiments. The experiment was performed on a glass slide platform of dimensions 75 X 50 X 1 mm. A piezoelectric transducer (PZT) of diameter 20 mm was attached to the bottom of the glass slide using acrylic glue. A Kapton tape well of diameter 6 mm was attached to the top of the glass slide where 10 μl of the running micromotor

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solution was sampled. The PZT transducer was subsequently excited by an amplifier (AG1006, LF Amplifier/Generator, T&C Power Conversion). The input signal of the amplifier is controlled by a function generator (33220A 20-MHz Function/Arbitrary Waveform Generator, Agilent Technologies). A secondary generator (3390 50 MHz Arbitrary Waveform Generator, LXI Kiethley) is used to generate the signals used to trigger the pulses of the ultrasound excitation at defined frequencies. These signals are monitored by a digital sampling oscilloscope (TDS 1001B, Tektronix). The secondary function generator is used to trigger the pulses of ultrasonic excitation in the primary function generator to generate fixed frequencies of ultrasonic excitations.

10.5 Supporting Information

Scheme S10.1 Illustration of ultrasound set-up. Bubble-propelled micromotors that are entrapped in a Kapton tape well are driven into resonant oscillations using a piezoelectric transducer glued to the glass slide. FG: Function generator, AMP: Amplifier, PIEZO: piezoelectric transducer.

Figure 10.S1 **A** Motion of bubbles arranged on a circle. The large bubble is above resonance size, while the five smaller ones are below resonance size. **B** Trajectories of small bubbles and big bubbles after ultrasound excitation.

For this case the bubbles start from the perimeter of a circle of $20 \mu\text{m}$ in diameter, see Figure 10.S1 A. Five of them have a radius of $R_1^{\text{eq}} = 1.5 \mu\text{m}$ and one bigger has a radius of $R_2^{\text{eq}} = 5 \mu\text{m}$; thus their radii are below and above the linear resonance size. We expect attraction between the small bubbles and repulsion of the small bubbles away from the large bubble (acoustic pressure is 0.1 bar). The simulation covers 6,000 cycles. Figure 10.S1B depicts the trajectory of all 6 bubbles. The bigger bubble moved to the right while the smaller move first away from the big bubble but later as the repulsive force reduces form a cluster of small bubbles due to their attractive force. A rough estimate of the average speed of the bubbles can be obtained from the balance of the drag force, $\vec{F}_d$ with the secondary Bjerknes force $\vec{F}_B$.

$$U_{\text{avg}} = \frac{f_b}{12d_{12}^2\pi\mu R} \quad (10.S1)$$

The velocity of R_2 can be estimated by picking $5 \times 10^{-3} \text{ kg m}^3 \text{ s}^{-2}$ from Figure 10.S1 A and using an averaged distance of the big bubble to the 5 smaller bubbles of $=15 \mu\text{m}$. Inserting

these values into Eq. (10.S1) we obtain a velocity of $U_{avg} = 3.5 \text{ mm s}^{-1}$ or a distance of $19 \mu\text{m}$ travelled over the 6,000 cycles, which agrees approximately with the distance seen in Figure 10.S1B.

Furthermore, directional motion of the bubble-propelled micromotor can be made into a random walk and demonstrate switchability between these modes of motion in the presence of acoustic excitation.

Figure 10.S2 A (i) Mean-squared displacement of micromotor moving by self-propelled motion (ii) Typical trajectory of micromotors in 0.2 s moving in self-propelled motion. B (i) Mean-squared displacement of micromotor moving by self-propelled motion in the presence of ultrasound excitation at 100 V. (ii) Typical trajectory of micromotors in 0.2 s moving in ultrasound excitation at 100 V. Conditions: 23°C , 2 wt. % of H_2O_2 and 1 wt. % SDS. Motion of micromotors after ultrasonic excitation at 100 V, 1.1 MHz, 1000 bursts, controlled by external function generator with 100 ms gap in between.

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To investigate if directional motion of the micromotor is sustained under a powerful ultrasound field, the mean-squared-displacements for the micromotor in the absence and presence of ultrasound are computed. In Figure 10.S2A (i), the self-propelled motion of the micromotor shows directed propulsion and the mean-squared-displacement against time has an upward parabolic curve, illustrating directed self-propulsion.⁵⁴⁻⁵⁵ It illustrates the typical path made by the micromotor in 0.2 s. However, on the introduction of ultrasound excitations, the mean squared distance increases by a factor of 10 times, where the motion was observed for 0.2 s. However, noticeably the original upward parabolic curve becomes a straight line in Figure 10.S2 B (i), which is analogous to random walker's motion.⁵⁵ However, greater distances are travelled in Figure 10.S2 B (ii) as compared to the distance travelled of the same micromotor moving under solely self-propulsion in 7 A (ii), where a path length of 257 μm is translated as compared to 101 μm in a duration of 0.2 s. While the introduction of ultrasonic pulses at 100 V at 10 Hz of 1000 bursts at 1.1 MHz moves the micromotors over greater distances, the randomization of the directional motion of the micromotor has occurred. The locomotion of the micromotor demonstrates reversibility between directed self-propulsion and random motion with the imposition of standing waves in the liquid droplet.

Figure 10.S3 Z-project of micromotor in a 4 x 5 squares. **A** Micromotor moving in the absence of ultrasound excitation for 1.0 s. **B** Micromotor moving in the presence of ultrasound for 1.0 s. Motion of micromotors after ultrasonic excitation at 100 V, 1.1 MHz, 1000 bursts, 100 V controlled by external function generator with 100 ms gap in between.

The larger distances that are covered by the micromotors recorded in a duration of 1.0 s was illustrated in the Z-projection graph in Figure 10.S3. The field of view is 1350 X 1800 μm . A Z-projection of the motion of the micromotor in the absence and presence of micromotor motion is illustrated. In the absence of any ultrasound excitation, the motion of the micromotor occupies 4 squares in the Z-projection in Figure 10.S3A. However, in the presence of ultrasound as depicted in Figure 10.S3B, the micromotor covered 10 squares. While randomizing the motion, the larger distances were travelled by the micromotor in presence of ultrasonic excitation, due to the forces of repulsion manifested from differentially sized microbubbles oscillating in the acoustic field.

Figure 10.S4 Random walk taken by micromotors due to influence of ultrasound. Figure 10.S4(ii)-(xi) show the motion of micromotor after the ultrasound pulses. Figure 10.S4 (i) and Figure 10.S4 (xii) show the motion of the micromotor before and after the ultrasound pulses. The thin and thick dashed lines illustrate the paths taken by the micromotors in the presence and absence of the ultrasound. Conditions: 23 °C, 2 wt. % of H_2O_2 and 1 wt. % SDS. Motion of micromotors at ultrasonic excitation at 1.1 MHz, 1000 bursts, 100 V controlled by external function generator with 100 ms gap in between.

The randomisation of directed motion can be distinguished from the self-propelled motion's path. In Figure 10.S4 (i), it illustrates the initial path taken by the micromotor in the absence of any ultrasound excitation. From Figure 10.S4 (ii)-(xi), it demonstrated alterations of the motion of micromotor with 10 iterations of ultrasound excitations. The ultrasound pulse was then stopped at 0.94 s and the micromotor was allowed to continue its directed propulsion in Figure 10.S4 (xii). Distinctively, in Figure 10.S4 (xii), from the track path lines taken by the micromotor, there are two distinct zones of locomotion from self-propelled micromotors that can be seen. The average distance travelled per unit time in the ultrasound-modulated motion of the micromotor in duration from 0.04 to 0.94 s was demonstrated to be 2.1 times the average velocity of the micromotor from 0.94 s to 1.24s.

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In the absence of any ultrasonic excitation, the micromotor experience directed propulsion from bubble emission. However, upon ultrasonic excitations in the proximity of larger bubble clusters, the randomisation of self-propelled motion due to secondary Bjerknes forces. The self-propelled micromotor can switch between directional motion and ultrasound-induced random walk upon acoustic excitation.

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Chapter 11

Electrochemical Monitoring of Autonomous Self-Propelled Capsule

11.1 Introduction

11.2 Results and Discussion

11.3 Conclusion

11.4 Experimental

11.5 Supporting Information

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11.1 Introduction

The remote monitoring of autonomous self-propelled objects is a very important task as it would allow for truly autonomous and independent systems. Such self-propelled materials have been envisaged to be the next step forward for smart materials.¹ Invention of novel architectures and the simultaneous monitoring of these devices are currently the two-pronged approach towards greater exertion of control and understanding in self-propelled systems. Whilst the advent over the design and manipulation of such autonomous moving objects have been remarkable, the progress in techniques over the real-time detection of such moving entities have not kept up to pace.²⁻⁴ Optical analysis of the motion through recordings remains to be the tool of choice, where often post-analysis using computation demanding movie analyses is required for meaningful information to be extracted. Such optical monitoring is impossible in real systems where optically non-transparent environment is envisioned. For the real-time monitoring of these objects and miniaturization of these technologies, there arises a need for alternative means to the detection of motion. An automated tracking of self-propelled autonomous motors shall allow us to identify and describe the position as a function of time. Hence, the challenges in monitoring locomotion of self-propelled systems in real-time and to demonstrate miniaturization capability, portends that a new measure beyond optical means is required.

Electrochemical methods have been demonstrated to be able to track and monitor the motion of particles at the micro and milli-metre scale. Pioneering works by Compton and co-workers, have demonstrated that displacement of micrometre-sized objects can be determined through triangulation of potential-transients of three individually addressable electrodes⁵ and cyclic voltammetry.⁶ These solution-based electrochemical procedures have not only showed efficacy in locating the displacement of the object of interest. Recent advances have also demonstrated capability in monitoring moving particles. Rees *et al.* used an integrated electrode system to track the motion of a micrometre-sized spherical

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ball, that was moved by magnetic forces.² The same team later demonstrated that by using photoelectrochemistry,³ the motion of a millimetre-sized organism could be monitored by confining it within capillary walls. Liu *et al.* have also demonstrated the use of electrochemistry for detection of moving droplets in the channels of a lab on a chip platform.⁴ Such channel environments have been the subject of much studies involving interactions between moving objects and the physical environment.⁷⁻¹¹ For a self-propelled autonomous entity, it is indeed complementary to consider the motion of moving objects to be constrained by the walls, as much of its projected use has been in that of a vascular system such as blood capillaries¹² and that of channels in microfluidic devices.¹³

Thus far, all the above methods have been carried out by voltammetric means, where the motion of the entity results in the perturbances in the electrochemical signal.¹⁴ The principle relies on the interaction of the particle with the surface of electrode by disrupting the diffusional field around the electrode. However, these techniques often requires the accompaniment of an electroactive species in the solution, which may complicate the set-up.² Charge transfer from the particulate to the electrode, due to particles in solution striking the electrode surface, has been demonstrated for the quantification and sizing of silver nanoparticles.¹⁵ Electrochemical signals have been generated by nanoparticles striking the electrode surface at a potential bias, resulting in a redox reaction. Separately, ultrasonication could be used to activate these particles for electrochemical detection due to impact-mediated charge transfer.^{16, 17} Such impact-mediated charge transfer is particularly suitable for monitoring the movement of self-propelled moving objects. In this context, it is intuitive that an object while moving across the surface of the electrode will be able to influence electrochemical signals, when placed in close proximity and is of sufficient magnitude. In this report, we demonstrate that this hypothesis is indeed true.

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Our group has previously demonstrated the mobility of a polymer capsule, powered by the Marangoni effect, as an autonomous self-propelled object.¹⁸⁻²⁰ The polymer capsule demonstrated versatility in traversing multiple environments: in between an organic and aqueous phase²⁰ and solutions of varying Reynolds number.²¹ In this study, the capsule will be the subject of interest for electrochemical detection of motion. We demonstrate the ability to track the capsule moving around a circular track. Trajectory of an autonomous self-propelled capsule can be determined by an electrochemical set-up linked to the potentiostat. The work illustrates the efficacy of an electrochemical method for tracking autonomous self-propelled objects with a view that analogous systems can detect micro and nano-sized objects via an electrochemical method as a function of time. Additionally, the capsule demonstrate “cognitive” capability, as they sense and navigate around obstacles.²² Electrochemical information obtained from the autonomous self-propelled capsule can be used to pin-point the position of the obstacle. Thus, electrochemical detection of motion in the capsule system shows not only efficacy in tracking the movement, but demonstrate possible applications to transmit information about the surroundings it has been subjected to.

11.2 Results and Discussion

Figure 11.1 **A** Scheme of electrochemical set-up of the capsule track for monitoring motion using a three electrode system. **B** Experimental set-up. Scale: Width of channel is 1 cm.

As shown in Figure 11.1 A, the capsule track was constructed in a manner such that constant electrical contact was kept throughout the experiment. Reference and counter electrodes were placed outside the track, while remaining in constant electrical contact with the solution through a salt bridge. An inert electrolyte of NaNO_3 was chosen, such that no reaction will occur between the polymer capsule and the electrolyte. The conventional set-up of the experiment is shown in Figure 11.1 B.

Figure 11.2 **A** Current-time plot for a capsule in motion in 10 mM NaNO_3 with the electrode at a potential bias of -1.0 V vs. Ag/AgCl (1.0 M KCl). Each peak represents the capsule crossing the electrode surface. **B** Zoomed-in electrochemical profile of capsule crossing the electrode surface. Inset: Illustration of a capsule crossing the surface of the electrode, where each peak correspond to the time it crosses the electrode surface.

The electrochemical detection of motion in a self-propelled millimetre-sized polymer capsule is illustrated in our work. Electrochemical information from the motion of the capsule not only reveals the position and speed, but too transmits information about the physical obstacles in its path. The capsule is made from a droplet of PSf/DMF (7 μl) dropcasted into the electrolyte solution. An immediate phase inversion occurred, as the polymer solidified in contact with the aqueous media.¹⁸ The volume of precursor solution used, produced a capsule of diameter 3 mm. Unique distributions of pores were found

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across the two interfaces of the capsule: $\sim 20\text{ }\mu\text{m}$ at the PSf/air interface and $\sim 130\text{ nm}$ at the PSf/water interface.^{18, 23} This resulted in the slow release of the DMF from the solidified capsule. As previously reported by our group, the capsule is propelled by the Marangoni effect, caused by the asymmetric release of DMF from the capsule to the PSf/water interface at the edges. The difference in surface tension (γ) of the aqueous solution ($\gamma_{\text{Water}} = 72.0\text{ mN m}^{-1}$) and DMF ($\gamma_{\text{DMF}} = 35.2\text{ mN m}^{-1}$) results in the Marangoni effect. Hence, the asymmetric release of DMF and differences in surface tension between the DMF/water at the interface of capsule resulted in a net force. This propels the capsule from a region of low surface tension to a region of high surface tension, leading to the facile motion of the capsule.

The sequence of events that occur at the capsule upon introduction to an aqueous solution is now described. The in-house designed circular capsule track coupled with a three-electrode electrochemical system was used for the study. The droplet of PSf/DMF mixture is introduced at a position 90° away from the glassy carbon working electrode. On dropcasting, the capsule remains relatively immobile for a few seconds as it solidifies, before exhibiting high speeds of up to 11.6 cm s^{-1} , which is nearly 40 body lengths per second, drawing consistence with previous results.¹⁸ The capsule revolved about the circular track in periodic motion. It was observed that whenever the capsule moves across the surface of the electrode, an electrochemical response is generated, as seen in Figure 11.2 A. As the capsule plies the length of the electrode surface, the phenomena gave rise to two separate signals: a peak and a trough. Here on, the peak is chosen as the point of reference for the capsule moving across the electrode surface. The reason of choice is twofold. First and foremost, the trough is less sharp in shape and a clear distinction of the valley is hard with increased number of oscillations, due to the time taken for the capsule to cross the length of the electrode surface. The choice of the peak also reduces the tendency of a time lapse between the crossing event and that of what was recorded in the electrochemical profile.

The recorded peaks correspond to the proximity event of the capsule and the dissipated DMF with the electrode. Upon the capsule's occurrence at the working electrode, there is a disturbance of the electrical double layer and rapid increase in the cathodic current, where the potential was held at -1.0 V (Refer to Figure 11.S1 for the electrochemical calibrations). Similar peaks have been observed in acoustically activated microparticles impacting the surface of the electrode in the absence of electroactive species in the solution.¹⁷ Such a phenomena has been attributed to the disturbance of the surface charge on the electrode.²⁴ As the number of oscillations increased, the DMF loading of the capsule decreased and the corresponding signal has a reduced intensity as seen in Figure 11.2 B. It is evident that the DMF loading of the polymer capsule also affects the reading of the electrochemical signal generated.

Figure 11.3. Average speed profiles of capsule obtained respectively by optical analysis (Optical) and current-time responses of electrochemical signals (Electrochemical), with increasing number of occurrences at the electrode.

In Figure 11.3, the average speeds of the polymer capsule were captured respectively through optical analysis (Optical) using a video software and through the current-time responses of electrochemical signals (Electrochemical) using the devised automated system. The average speed of the polymer capsule at the selected occurrence on

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the electrode, achieved by post-analysis of tracking using video software, was very close to the values measured by the electrochemical method. Differences between the average speeds recorded by the optical and electrochemical methods only expressed a difference in value of 3.5 % on average. This demonstrated that the electrochemical method is robust for the detection of average speeds of the polymer capsule, drawing very close values to that of the speed obtained from the optical tracking done through video software.

Figure 11.4. Decay of average speeds of capsule observed through the current-time response via electrochemical signals, with increasing number of occurrences at the electrode.

We further investigated the relationship of capsule's average speed with increasing oscillations, using the electrochemical method in Figure 11.4. A decay of the speed of the capsule with increasing oscillations could be obtained, using the normalized initial speed. The decay of the speed of the capsule in the circuit can be described by the equation $y = -0.0253x + 1.08$, where x is the number of occurrences at the electrode and y is the normalized capsule speed with respect to the initial speed recorded. With increasing number of periods, the decay of the speed of the capsule is taken to be $-0.0253x$, where x is the number of occurrences at the electrode. Hence, through an electrochemical method, we

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have been able to describe the average speeds of the capsule, utilizing the time taken to reach the electrode surface for each period as the polymer capsule revolves around the circuit. The work also illustrates the high efficiency of the capsule motor, where it follows an approximate linear loss of 2.5% from the initial speed recorded per oscillation.

Figure 11.5. Magnified current-time electrochemical profiles of capsule when roadblock is **A** 180° **B** 135° **C** 90° **D** 45° from electrode in circuit. Inset: **(i)** Scheme of position of roadblock (Black square), detection electrode (Blue rectangle) and **(ii)** Typical electrochemical signal from the paths taken by a capsule to complete the circuit back and forth from the roadblock to the electrode.

With an intentional introduction of a Teflon cube of dimension 1 cm in the circular track as a roadblock, the directionality of motion in the capsule can be reversed, generating unique electrochemical profiles in Figure 11.5 A – 11.5 D, based on the positions of the roadblocks. The pathway of the capsule is illustrated with the scheme in the inset of Figure 11.5 A (i) – 11.5 D (i). As described earlier, the polymer capsule experienced translational motion around an unhindered track. However, on reaching the end of the possible route for

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the circular track with the roadblock's presence, the capsule experienced an instantaneous speed of zero, before turning back. This reversal of motion has been highly dependent on the mechanism of motion of the capsule. Previously, we have demonstrated that the diffusion of DMF from the porous PSf structure, showed capability of movement in complicated channels.²² This is due to their inherent ability to alter the surrounding environment, through the controlled release of chemicals from the polymer capsule. On reaching the terminus of the blocked channel, the capsule demonstrated the ability to change the direction of the translational motion. DMF is dissipated continuously from the polymer capsule, until a net resultant force results due to differences in surface tension of the solution. The capsule's ability to continuously change the surface DMF/water composition, results in this propulsion behaviour at the terminus, where it reverses its motion into the unblocked pathway, by remediating the surrounding environment. Consequently, unique electrochemical profiles indicative of the positions of the roadblocks can be recorded, as illustrated in the insets of Figure 11.5 A (ii) – 11.5 D (ii). The change in directionality of the capsule and the crossing event in proximity of the electrode have been the origin of the unique signals, which were further validated by control experiments in Figure 11.S2 of the supporting information.

The roadblocks are placed at an angle of 45°, 90°, 135° and 180° from the location of the electrode as demonstrated in Figure 11.5. Apart from the peaks generated when the roadblock is central to the electrode at 180°, which gave a peak-to-peak separation ratio of close to 1, the other readings at 45°, 90° and 135° gave rise to one recurrent long time period and one recurrent short time period, which are proportional to the distance that the capsule needed to travel to and fro from the roadblock to the electrode (Refer to Figure 11.S3 for full electrochemical profiles). Ratios of these recorded times, which draws reference with the position of the roadblock to the electrode, enables the capsule to be used as a tool for surveying the location of the roadblock.

Figure 11.6 **A** Illustration of the paths taken by a capsule in order to complete the circuit back and forth from the roadblock to the electrode. **B** Electrochemical signal and ratio obtained by the time taken for capsule to complete the circuit back and forth from the roadblock to the electrode.

Two unique times taken for the capsule to travel to the roadblock and back to the electrode can be captured, when the distances from the electrode to the roadblock are different along the two paths. We define the longer time taken for the capsule to travel from the roadblock to the electrode to be τ_1 and the time taken for the capsule to travel the shorter distance to be τ_2 as seen in Figure 11.6 A. Since the time taken for the capsule to traverse the two paths are different and is dependent on the location of the roadblock, unique ratios could be obtained by taking the ratio of τ_1/τ_2 , as depicted in Figure 11.6 B. These four separate placements of roadblocks have generated four unique ratios. Given the high efficiency of the polymer capsule motor, we are able to pattern the position of the roadblock by the unique ratios generated in accordance to the distance travelled by the polymer capsule.

Figure 11.7. A graph of ratio (τ_1/τ_2) against position of roadblock from electrode, marked by the capsule via electrochemical signals. On the top of Figure 11.7 is shown the scheme: Position of roadblock (Black square), detection electrode (Blue rectangle). Values are averaged from 8 readings and the line has a R^2 value of 0.95.

Using these data, a linear relationship between the location of the roadblock and the ratio (τ_1/τ_2) can be established, as the capsule traverses back and forth to the roadblock at differing placements from the electrode, as seen in Figure 11.7. The self-propelled autonomous capsule is able to transmit this information, by travelling to the terminus of the roadblock and back, through the time taken to return to the position of the electrode, where it generates a peak in the current-time plot. The ratios are unique to the position of the roadblock. All four positions of the roadblock generated unique ratios that are proportional to the time taken to traverse to the roadblock and back to the electrode. The “cognitive” ability of the capsule to interpret the terminus of the channel has been instrumental in reconstructing the position of the roadblock from the electrochemical profiles.

The linear relationship from these four reference points suggest that the position of the roadblock could be inferred from electrochemical information, using the time taken for the capsule to traverse the distance to the roadblock and back to the electrode. However, more work is still required to precisely define the distance of the roadblock from the electrode. An array of electrodes for monitoring will possibly help to further triangulate the motion of a capsule or multiple capsules. The work is not exhaustive to detection of artificially synthesized motors, as pollutants too, are known to experience enhanced self-propulsion.²⁵ Construction of such sensors for the detection of motion shall also play a vital role for environmental monitoring.

Sliver nanoparticles, in Brownian motion, have been detected using electrochemical methods. The collisions of these particulates with an electrode at a positive potential bias,¹⁵ resulted in spikes from impact electrochemistry. These electrochemical signals find their origins from the electrochemical oxidation reactions that occur. Hence, we believe that an analogous concept can be applied to motors down to the order of nanometre scale too. A key challenge will then be for the detection of these motors under the superimposition of an electrochemical background current, due to the presence of fuel source and other additives.

11.3 Conclusion

We have demonstrated the efficacy of an electrochemical method for the detection of motion in a self-propelled polymer capsule. Electrical signals from the real-time electrochemical information generated by the polymer capsule can be used to obtain the speed of the periodic motion of the capsule motors. This methodology demonstrates efficacy when compared to other conventional methods of post-analyses. With the intentional introduction of a roadblock, the direction of the capsule was purposefully changed. The self-powered autonomous polymer capsule demonstrated capability in mitigating roadblocks, by instantaneously switching its direction on reaching the terminus.

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Electrochemical information obtained from such a Marangoni-effect powered polymer capsule is able to transmit information about the position of the roadblock. We envision a future widespread use of an electrochemical system in observing the motion of a self-propelled motor, especially in well-defined channels. This novel method is expected to wield us with the appropriate tools to better our understanding of the motion in self-propelled objects.

11.4 Experimental

Materials. Sodium nitrate and polysulfone was obtained from Sigma–Aldrich (Singapore). *N, N*-dimethylformamide (DMF) was obtained from Merck. Glassy carbon electrodes with a diameter of 3 mm and Ag/AgCl (1.0 M KCl) electrode were obtained from CH Instruments. Platinum electrode with a diameter of 2 mm was obtained from Autolab.

Apparatus. Voltammetric experiments were performed at room temperature by using a three-electrode configuration. A platinum electrode served as the counter electrode (C.E.), whilst an Ag/AgCl (1.0 M KCl) electrode served as the reference electrode (R.E.). The glassy carbon (GC) electrode was used as the working electrode (W.E.). All electrochemical potentials are stated versus the Ag/AgCl (1.0 M KCl) reference electrode. All voltammetric experiments were performed on a μ -Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

Preparation of capsule. The capsule precursor was prepared by mixing polysulfone (PSf, Sigma-Aldrich) with *N,N'*-dimethylformamide (DMF, Merck) and was dissolved into a clear solution using an ultrasonic bath for 30 minutes. 7 μ l of the precursor solution was dropcasted into the electrolyte in each experiment at an approximate distance of 2.5 cm from the surface of the electrolyte solution surface.

Electrochemical set-up. The experiments were carried out in a Teflon maze with most of its running pathways possessing the width of 1 cm and a depth of 1 cm. The dimension of

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the Teflon plate was $42\text{ cm} \times 26\text{ cm} \times 2\text{ cm}$. The distance from the centre of the circular track to the centre of the channel is 15 cm, which gave the track an approximate circumference of 47.1 cm. The circuit was constructed such that all exits are blocked with Teflon cubes of sides 1 cm. Electrical connectivity to the counter and reference electrodes was ensured by allowing a salt bridge to the bulk electrolyte, where the working electrode was located. The capsule is thus confined to the circular track of the Teflon maze. Working, counter and reference electrodes are wrapped with Parafilm to prevent leakage of the electrolyte. 45 mL of NaNO_3 solution (10 mM), which acts as the electrolyte, was placed into the maze channel. The capsule predominantly travels in the middle of the channel due to the shape of the water meniscus with the Teflon maze's wall. The channel has a width, being 10 mm. As the capsule has a diameter of approximately 3 mm, the capsule approaches very close to the electrode surface in the order of millimetre range, at an approximate distance of 3.5 mm. Slight fluctuation may occur, due to the path taken by the capsule along the track. After each individual experiment, the liquid was taken out and the channel was cleaned with a Kimwipe dabbed with ethanol, before flushing twice with 45 mL of deionized water. A Casio HD video-recorder was placed over the maze for video recordings. The video sequences were analysed using Nikon NIS-Elements™ software and the average speeds were calculated.

Renewal of glassy carbon electrode .Glassy carbon electrode surfaces were renewed by polishing with alumina particles (0.05 μm) on a cloth and washed with copious amount of water.

11.5 Supporting Information

11.5.1 Electrochemical Calibration

Figure 11.S1 **A** Current-time plot for a capsule in motion in 10 mM NaNO₃ with the electrode at a potential bias of +1.0 V vs. Ag/ AgCl (1.0 M KCl). **B** Effect of ionic strength on the current-time curve with the electrode at a potential bias of -1.0 V vs. Ag/ AgCl (1.0 M KCl).

To measure the crossing event of the polymer capsule across the electrode, the potential held at -1.0 V vs. Ag/ AgCl (1.0 M KCl) reference electrode was adopted, after the converse positive bias at +1.0 V didn't give any response, as shown in Figure 11.S1 A.

In Figure 11.S1 B, optimization of the concentration for electrolyte was also carried out. It was found that the 10 mM of NaNO₃ gave sufficient amplitude to the signal as compared to 1 mM NaNO₃, where the signal was relatively weak. Increasing the concentration of the electrolyte to 100 mM, did not garner an appreciative increase in signal. Thus, we limited the examinable parameters to be at the potential bias of -1.0 V vs. Ag/AgCl (1.0 M KCl) with a 10 mM NaNO₃ electrolyte.

11.5.2 Locomotive Events

Figure 11.S2 Electrochemical detection of locomotive events via current-time plots. **A** For a capsule in the proximity of the electrode. White arrow indicates onset of proximity event. **B** For a capsule reversing its motion. Black arrows indicate reversal of capsule's directionality.

Additionally, two other locomotive events of the self-propelled capsule can be detected: the presence of the capsule in proximity of the electrode and the reversal of motion of the capsule.

When the capsule is permanently in close proximity to the electrode, a sustained dip in current of the electrode is observed as shown in Figure 11.S2 A. This is in contrast when the capsule assumed periodic motion, where a systemic electrochemical signal can be generated. When the capsule is continuously in proximity of the electrode, the signals are flattened off. This is indicative that the periodic electrochemical signal of the capsule is derived from the cyclical motion of the capsule and the capsule's sustained presence near the electrode surface has been the origin of the dip as observed in the current-time graph.

Furthermore, in the event when the capsule re-orientates itself, during oscillation in the circular track, it is observed that the electrochemical signal generated is inherently different, as seen in Figure 11.S2 B. This is accompanied by the reduction in time period needed to generate the peak in the current-time graph. This is suggestive that the electrochemical signal shows capability to be able to detect the change in direction of motion of the capsule. This switching in direction of motion also suggests that the capsule, might be able to reorientate itself in the presence of a physical barrier.

11.5.3 Mapping the Roadblock

Figure 11.S3 Full current-time electrochemical profiles of capsule when roadblock is **A** 180° **B** 135° **C** 90° **D** 45° from electrode in circuit. Inset: Scheme of position of roadblock (Black square), detection electrode (Blue rectangle).

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Chapter 12

Monitoring Micromotors by Particle-Electrode Impact Voltammetry

12.1 Introduction

12.2 Results and Discussion

12.3 Conclusion

12.4 Experimental

12.5 Supporting Information

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The thesis author was the primary investigator and author.

12.1. Introduction

Microscopic and nano-sized self-propelled objects have been tasked with performing tasks and operations in a multitude of environments.^{1,2} The miniaturized size,³ ultrafast motion^{4,5} and capability for custom surface configuration^{6,7} for specific operations have diversified their uses in different arenas. This has included biological uses for delivery of medicine⁸ and nanosurgery,⁹ in addition to carrying out complicated missions for environment remediation, ranging from pollutants removal^{10, 11} to chemical weaponry detoxification.¹² Such is the diversity of operations, which has ranged from the use in confined spaces of vascular channels within blood vessels¹³ to extensive open bodies of water for environmental remediation.^{14, 15}

Till date, the evaluation of their performances has still been reliant on the use of an optical microscope coupled with an imaging software. Optical analysis is still the most advocated utility for observation of micromotor's motion, as it allows for kinetic events to be recorded, while allowing a live feed of possible non-locomotive events.¹⁶⁻¹⁸ However, it is laborious from the perspective of having to use manual tracking of the autonomously moving entity. Workflow of such processes often include recording of videos before post-processing using computationally intensive tracking software. Automation in the form of tracking the self-propelled object is few and far between and it is also met with limitations due to the difficulties in achieving contrasts in different mediums.¹⁹ There is also an inherent limitation of viewing particles that reach 200 nm in size, which is the limit of detection.²⁰ Furthermore, considerations that these self-propelled objects are moving in three dimensions must be put into place. Typical optical analysis will only have the motion of the micromotors being tracked in 2D. While it has been able to observe particles in a single plane, common microscopy is not ready for 3D imaging of moving objects in real-time. Evaluating objects that are moving in and out of the focal plane in the z-axis have hardly been considered. The most advanced form of scanning confocal microscopy

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demands the use of fluorescence spectroscopy coupled with additives, in the presence of complementary light excitation and detection sources.²¹⁻²³ Furthermore, there is a trade-off between resolution, scanning speed and area being imaged as the speed is inversely proportional to area.²² These techniques haven been primarily used to map biological cells, typically moving by a crawling mechanism at 5.2 $\mu\text{m}/\text{min}$. This is in addition to limitations of depth and field of view and particularly the formation of bubbles which can obscure visibility but not often remarked on by practitioners in the field. More aptly put forth, having to extract samples from the bulk solution and to be placed on a microscope platform for observation is also far from ideal. This calls for the need of innovative techniques, obviating additives, to monitor locomotion of micromotors.

Impact electrochemistry has been demonstrated to be able to detect colloidal particles.^{16, 24-34} The technique can be categorically divided into three different types: (i) redox reaction on the impacting particle (ii) redox reaction of the chemical markers co-existing in the solution with the impacting electrocatalytic particle and (iii) the impacting particle's disruption of the interfacial electrochemistry when in proximity of the electrode. The latter method of perturbances when in proximity of the electrode is well suited to different environments, and especially so in the case of fast-moving micromotors near the electrode interface. For an on-site measurement of such self-propelled objects at a miniaturized scale, an electrochemical impact voltammetry methodology is well-suited for the task.

While examples using impact electrochemistry to detect impacting colloidal particles during Brownian motion have been aplenty, the detection of self-propelled micromotors has been limited. Compton *et al.* have previously demonstrated the detection of a basalt ball moved by magnetic forces, being detected by perturbances of the diffusion layer in a interdigitated electrode, while being immersed in the electroactive species of ferrocene.³⁵ Photoelectrochemistry has also been used to position a micro-organism moving

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in a confined vascular environment, using individually addressable electrodes.³⁶ Flowing water droplets under confinement in a channel environment, was also continuously detected at an integrated electrode in the microfluidic chip.³⁷ We later devised an electrochemical set-up that was able to track millimetre-sized Marangoni-effect powered capsule, while mapping remote positions of obstacles in the circuit.³⁸ It is noteworthy that most of these demonstrations have been singular units of the self-propelled devices and under a channel environment. In another instance, changes in electrochemical signals from swarms of fragmented flagella of micro-organisms with increasing concentrations was ascribed to increased agitation of the solution near the interface of the electrode.³⁹ However, a methodology for continuous monitoring individual artificial self-propelled objects moving in solution has not been demonstrated yet.

Pioneering works by Bard *et al.* have detected electrocatalytic particles in Brownian motion, due to signal amplification of analytes by electrocatalytic particles in solution.^{34,40} This was followed up by a series of work detecting nanoparticles moving by Brownian motion, including dissolution of particles and blocking of electrodes.^{16-18,27,30,41} Particularly, the work by Kanoufi *et al.*^{16,17} in the oxidation of nanoparticles under potential bias has illustrated that while electron transfer can be measured by electrochemical nanoimpacts, events such as incomplete dissolution and chemical side reactions such as precipitations cannot be examined in detail. On this note, the report of nanoparticles that are transferred atop an electrode surface by Crooks *et al.* is particularly intriguing.¹⁸ The blocking of the electrode by the particulates has been the principal origin of such a signal, where a dip from the background current is observed. Notably, in our endeavour, we wish to demonstrate that microbubble-propelled miniaturised machines can be detected by electrochemical means and the velocities shows correlation to the concentration of the fuel (hydrogen peroxide) it is immersed in. Catalytic breakdown of hydrogen peroxide to oxygen bubbles for bubble ejection has been the origin of propulsion. Amidst a high molar concentration of

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a background current from the oxidation of electroactive hydrogen peroxide,^{5, 42, 43} any blocking of the electrode results in a dramatic decrease of the anodic current. As such, effects due to incomplete dissolution and chemical side reactions such as precipitations are minimal and avoided in such catalytically bubble-propelled systems.

Electrochemical detection distinguishes the motion of chemically self-powered micromotors apart from the fluid flow by detecting the movement of each individual microdevice. Previous efforts to eliminate the use of optics, have been limited to having the micromotors being traced passively with coloured dyes by naked eyes or a camera.^{14, 44} At the macro level, for a fast monitoring of the micromotors, Pumera *et al.* illustrated an electrochemical system for capturing the time needed for a millimetre-sized self-propelled capsule to complete a circuit.³⁸ The use of a microelectrode system adds another possibility to monitor motion of such self-powered micro-robots. For our setup, we investigated self-propelled particles that exhibit translational motion in the solution due to the bubble formation and bubble ejection, resulting in locomotion. These particulates are immersed in their own fuels, which are typically in molar concentrations,^{5, 42, 43} as compared to millimolar concentrations found in most impact electrochemistry studies,^{34, 40} representing three orders of magnitude difference. Blocking of the electrode by a micrometre-sized object and its emitted bubbles on the electrode of similar dimensions, will result in a drastic drop in current. This illustrates possibly another strategy to amplify signals in buoyant bubble-propelled micromotors. Previous efforts at detecting micro-objects have included the use of acoustic stimuli to suspend the particles, which resulted in agitation of the solution.²⁸ We have eliminated the inferences for the need of an external energy source. These micron-sized robots, which expresses autonomous motion while being immersed in its own fuel, can be monitored independently. While an array of microelectrodes can evaluate and track the precise location of single particles in a confined environment, our

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proposal seeks to understand the locomotive properties of self-propelled objects in free solution, where the surface tension is low⁴⁵ and effects from trapping are small.⁴⁶

In this report, we detail an *in situ* electrochemical monitoring of micromotors, which was able to illustrate the number of micromotors moving in solution and their velocities, paving the way of an alternative methodology in continuous evaluation of their motion in solution.

12.2. Results and Discussion

Janus silver micromotors were chosen as the target of study, as they are able to move at fast speeds when immersed in the fuel of hydrogen peroxide.⁴⁷ In the presence of alkaline conditions, these self-propelled entities were able to move at even higher speeds, due to the rapid decomposition of hydrogen peroxide in basic media.⁴⁸ The decomposition of hydrogen peroxide to oxygen bubbles and the subsequent detachment led to a resultant force due to the asymmetric shapes of these Janus silver micromotors, propelling them forward.⁴⁷ Videos of the micromotors can be recorded using a camera coupled with the microscope utilizing a tracking software, where the velocities of the micromotors were then measured.

Figure 12.1A Schematic of a micromotor impacting a carbon microfiber electrode surface and the corresponding electrochemical signal generated **B** Typical chronoamperogram with

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0.01 mg/mL silver micromotors at 33 μm carbon microelectrode. Conditions in all experiments: 23°C, 3 wt. % of H_2O_2 , 50 mM NaOH and 0.25 wt. % SDS.

Particle-electrode impact voltammetry method was utilized for the detection of micromotors, where during a typical measurement, the potential was held at 0.8 V *vs.* Ag/AgCl/1.0 M KCl via a chronoamperometric method in a time interval of 60 s. In a typical collision event as featured in Figure 12.1A, the micromotor impacts the surface of the carbon microfiber electrode, resulting in an electrochemical spike being generated. No corresponding signals were detected in the absence of the silver micromotors in the blank control, confirming that the origin of the signals are from the moving microparticles in solution (Figure 12.S1). In a typical cyclic voltammogram, oxidation of hydrogen peroxide at the carbon microelectrode occurs at 0.4 V and culminated in a peak at 1.0 V (Figure 12.S2A). For the detection of silver micromotors, potentials were kept below 1.0 V, to avoid the oxidation of water. The potentials were determined with a range of values between -0.4 V to 0.8 V, where the potential at 0.8 V gave regular signals of the Janus silver micromotors perturbing the diffusion layer (Figure 12.S2B). In a typical measurement, the potential of 0.8 V *vs.* Ag/AgCl/1.0 M KCl was used, in the presence of a sodium hydroxide electrolyte, SDS surfactant and hydrogen peroxide fuel. Figure 12.1B illustrates a typical chronoamperogram, where a concentration of 0.01 mg/mL of silver micromotors was studied.

The background current has been attributed to the electro-oxidation of hydrogen peroxide,⁴⁹ where increasing concentrations of hydrogen peroxide led to higher anodic current levels (Figure 12.S3). Even upon a 10 X magnification, no obvious background spikes or noise are found in Figure 12.S1B, in the absence of silver micromotors. These self-propelled entities move near the interface of electrode/electrolyte, leading to the impact of the micromotors with electrode, as it approaches near the electrode surface. Such motion in the proximity results in the blocking of the electrode from the diffusion layer, as observed

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by the decrease in diffusion-limited current. With the perturbation, there was a decrease in the background current in Figure 12.1B. Only upon the individual micromotor's departure from the surface, a gradual return of the oxidation current was observed. As seen in Figure 12.1, the deviations from the flat profile in a typical control experiment in Figure 12.S1, is wholly attributed to the presence of the micromotors. As such, each spike in the electrochemical signal in the presence of micromotors is treated as a collision event. The spikes are calculated typically from a 20 % threshold event from the largest spikes registered in an electrochemical signal.

The microelectrode with a diameter of 33 micrometre was chosen, as its size is in the same order of magnitude as the size of micromotors in solution (Figure 12.S4A and Table 12.S1). The time taken for the micromotors to traverse through are typically in the order of milliseconds as seen in Figure 12.1 and can be captured by electrochemical signals. This timescale is different from the diffusion timescale of the nanoparticles, which is at tens of nanoseconds.³¹ Since the crossing event is in order of milliseconds and the response time is at 3 μ s with a bandwidth of 1 MHz,⁵⁰ the crossing event can thus be recorded in our electrochemical signal. Blocking of the electrode by a micrometre-sized object on the electrode of similar dimensions, resulted in a drastic drop in current as shown in Figure 12.1. This is attributed to the generated bubbles propelling the micromotors and the micromotor itself blocking the diffusion layer, resulting in a decrease in the oxidation current. The disruption of the diffusional field around the microelectrode has led to the alteration of the current response, which can be interpreted with experimental calibration.³⁵ In proximity of an electrode in the presence of an electroanalyte, the existence of a foreign entity will affect the response of the current on the electrode.

The concentration of the hydrogen peroxide is at 3 wt. % or 0.882 M. This is the equivalent of 882 mM. It is significantly higher than the amount of electroanalyte commonly used for the diffusion-blocking mechanism^{18, 51} during particle-impact

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voltammetry as exemplified by Lemay *et al.* where the electroactive material in solution is at 0.31 mM. There is a nearly ~3000 X increase in the concentration of the electroactive species.⁵¹ The blocking of the electrode by the micromotor results in a drastic decrease of the anodic oxidation current of the hydrogen peroxide. This is validated again in the matching decrease in background current in the tens and hundreds of nA in the absence of the hydrogen peroxide (Figure 12.S1).

Each spike from the electrical signals is ascribed to one micromotor coming to the proximity of the electrode surface. At the time of 1 s, reduction in the background current was observed in Figure 12.1B, which is attributed to the perturbation of diffusion layer. Upon extended durations, at the time of 2 s, it marked the departure of the micromotor, where a return to the anodic oxidation background current of the hydrogen peroxide was observed. Due to the active motion of the silver micromotors, the background oxidation current now occurs at a higher current than that in the case of the micromotors' absence. This is the same phenomena as reported by Shitanda and co-workers, when biological units of the micro-size regime are moving in the proximity of the electrode interface, leading to better mixing and higher background current.^{39, 52-54} Particle-electrode impact voltammetry was used with varied concentrations of the silver micromotors, within a fixed time interval of 60 s. As illustrated in Figure 12.2A, the number of spikes detected by our developed electrochemical method increases with the rise in concentration of the Janus silver micromotors, from 0.002 mg/mL to 0.01 mg/mL. This corresponded to the increase in frequency of the micromotors occurring near the interfacial region of the electrode/solution. Each spike correspond to an impacting event of the silver micromotor with the plane of the microelectrode, with the consequent blocking of the diffusion layer at the interface of the electrode and the solution.

Figure 12.2A Typical electrochemical signals for silver micromotors at 33 μm carbon microelectrode with increasing motor concentration from 0.002 mg/mL to 0.010 mg/mL **B** Electrochemical spike counts for silver micromotors against increasing motor concentration **C** Optical counts for silver micromotors against increasing motor concentration. Conditions in all experiments: 23°C, 3 wt. % H_2O_2 , 50 mM NaOH and 0.25 wt. % SDS.

Figure 12.2B demonstrates a numerical approach in quantifying the number of spikes, which increases with increasing concentrations of micromotors. The number of spikes increases from 5 spikes in 0.002 mg/mL to 22 spikes to 0.010 mg/mL. It follows a linear trend that the number of spikes in the electrical signals increases with the concentration of the Janus silver micromotors. The stochastic approach to measure the number of micromotors in solution, by observing the impacts of silver micromotors with the electrode surface shows strong correlation with the concentrations of silver micromotors. Bringing a step further, we verified this result using optical observations of the number of micromotors moving by bubble propulsion in Figure 12.2C through direct microscopic measurements with the number of spikes detected electrochemically, and a

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similar trend was observed. As the concentration of micromotors by weight increases, the number of micromotors in solution increases accordingly from 93 micromotors in 0.002mg/mL to 560 micromotors in 0.010 mg/mL in a sample droplet. The optical measurements coupled with the observations of spikes measured electrochemically, serve as a calibration curve for the measurements of particle concentrations. Strong correlation between the optical measurements and the number of micromotors impacting the surface of the electrode through electrochemical measurements endorse this strategy as a possible method to physically measure the concentrations and number of micromotors that are moving in solution *in situ*.

Figure 12.3 A Typical chronoamperogram for silver micromotors at 33 μm carbon microelectrode against increasing concentration of hydrogen peroxide **B** Electrochemical spike counts for silver micromotors at 33 μm carbon microelectrode against increasing

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concentration of hydrogen peroxide **C** Optical counts for silver micromotors against increasing concentration of hydrogen peroxide **D** Velocities for silver micromotors against increasing concentration of hydrogen peroxide. Conditions in all experiments: 0.01 mg/mL silver micromotors, 23°C, 50 mM NaOH and 0.25 wt. % SDS.

We investigated the number of spikes detected electrochemically within a 60 s interval with increased fuel concentrations (Figure 12.3A). As the hydrogen peroxide concentration increases, the corresponding number of spikes increased. In contrast, the absence of any silver micromotors fails to develop any spikes even with increased hydrogen peroxide (Figure 12.S3), strongly suggesting the need for self-propelled micromotors for collision events. This is ascribed to the increased number of bombardment and collisions of the silver micromotors in the proximity of electrode surface. From Figure 12.3B, the number of spikes detected electrochemically increased steadily from 23 spikes in 3 wt. % hydrogen peroxide to 40 spikes in 6 wt. % hydrogen peroxide. This eventually tapers off to 42 spikes in 9 wt. % hydrogen peroxide. The number of spikes plateaued off, probably due to the maximum number of possible micromotors demonstrating propulsion has already been reached. This corresponded well with the observed number of micromotors through optical microscopy in Figure 12.3C, where the number of micromotors increases from 560 to 727 micromotors per mL, from 3 to 6 wt. % hydrogen peroxide fuel, before tapering off at 800 micromotors/mL in 9 wt. % hydrogen peroxide. There was also a trend of increasing velocities of the micromotors with increased concentrations of the hydrogen peroxide. Noticeably, the amplitude of the electrochemical signal intensity increases, likely due to the higher velocities that these micromotors are moving in solution as shown in Figure 12.3D, where the velocities increased from 146 to 204 $\mu\text{m s}^{-1}$, as the concentration of hydrogen peroxide fuel is increased from 3 to 9 wt. %.

At different fuel levels, where the velocities of the micromotors differs, the residence time of the spike taken by a micromotor during the perturbation event in Figure

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12.3A changes too. The average residence time from 3 representative readings of the micromotors at different fuel concentrations were calculated. At 3 wt. % hydrogen peroxide fuel, the average residence time registered by a micromotor was 2.0 s. With increasing fuel concentrations, the time decreased to 1.4 s for 6 wt. % and then 1.2 s for 9 wt. %. Through the residence time recorded, we are also able to differentiate the different velocities of the micromotors. This is an indication of how the velocities of the micromotors affect the electrochemical signals detected. Using this information, we can possibly obtain the velocities and the corresponding fuel of the hydrogen peroxide that the micromotors are immersed in.

Valuable information can be derived from the flux of the silver micromotors. Through empirical counting of the micromotors optically, we are able to calculate the number of micromotors per unit volume. The product of the concentration and average velocities of the micromotors give us the molar flux of the micromotors, as seen in supporting information, Eq. 12.S1. The data is shown in the supporting information in Table 12.S3 and Figure 12.S5. A similar trend of increased flux of the micromotors with increased concentrations of the hydrogen peroxide was also observed.

In the setup, the electrodes are placed above the solution of the silver micromotors. As a result, the perturbation of the electrodes by the rising microbubbles from the micromotors must be accounted for as they impact the electrode surface. Typically, the emission rates of the micromotors are at tens to hundreds of Hz.^{47,55} A droplet's height typically measures at 586 μm , but depth of focus using a 10 X optical lenses is 8.5 μm . This is reflective of the problem of using optics to observe micromotors. As illustrated in supporting information Table 12.S4 and supporting Figure 12.S6, it shows the relationship between concentration of micromotors with the physical counts and electrochemical counts, where differences of four orders of magnitudes was observed. The product of the correction factor for the depth of focus from the microscopic lenses and the bubble emission rate give

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rise to differences from the micromotors observed under microscopic observation, as compared to electrochemical detection of micromotor using particle-electrode impact voltammetry.

There are inherent limits of the depth of focus of the microscope and multiple signals can be detected due to bubbles emitted by the micromotors. These led to the number of spikes detected by the electrochemical measurements to be greater than the optical counts, representing an amplification of the signal for ease of detection. These two factors are needed to account for the proportionality constant. As such, the height of droplet with the corresponding depth of focus from the microscope⁵⁶ and effects from the bubbles emitted by the micromotors must be detailed for.⁵⁷

An exact value for the proportionality constant in a self-propelled micromotor differs greatly from a diffusion-based nanoparticle. An intricate insight to the exact value of the proportionality constant needs to take account of the dynamics of the shape,⁵⁸ mass⁵⁹ and Reynolds number of the solution,⁶⁰ which is beyond the scope of discussion of the paper.

The time taken to traverse across the longest dimension of the electrode is typically in the order of milliseconds and can be captured by electrochemical signals. For example, at 3 wt. % hydrogen peroxide, with micromotors moving at an average velocity of $146 \mu\text{m s}^{-1}$, the time needed to cross a $33 \mu\text{m}$ -length diameter is computed to be 226 ms. This corresponds well to the crossing event by the recorded electrochemical signal that was depicted in Figure 12.1B, which is in the order of hundreds of milliseconds. The perturbation of the diffusion layer, led to the observation in the change of current. Since the timescale of the crossing event is in the order of milliseconds, it validates this electrochemical strategy as a fitting methodology for detecting the locomotive events of the self-propelled entities in solution.

Entrance of a micromotor, the crossing event and its eventual departure was recorded in the electrochemical signals. These separate events correspond to the initial decrease in anodic current, the formation of a spike and the eventual translocation from the electrode surface leading to the restoration of the anodic current.

The large variation in signal magnitude originates from the differences in the velocities of the micromotor in the hydrogen peroxide fuel. The intrinsic non-uniformity in the micromotor's sizes as seen in supporting information Figure 12.S4, Table 12.S1 resulted in different velocities in Figure 12.3. This inherent difference in sizes of the micromotors and then the velocities, resulted in the large variations in signal magnitudes during the decrease in anodic background current. The general trend of increased number of electrochemical counts with increased concentrations persists. We foresee a more consistent reading with a further optimization of the technique, accounting for the difference in dimensions of the micromotors.

Figure 12.4 **A** Typical chronoamperogram with 3 μm Cu/Pt micromotors at 33 μm carbon microelectrode with increasing motor concentrations. **B** Electrochemical spike counts for 3 μm Cu/Pt micromotors at 33 μm carbon microelectrode against increasing motor concentration. **C** Optical counts for 3 μm Cu/Pt micromotors against increasing motor

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concentration. Conditions in all experiments: 23 °C, 9 wt. % of H₂O₂, 50 mM NaOH and 0.25 wt. % SDS.

After the investigations with Janus silver micromotors, we investigated the electrochemical response from a swarm of Cu/Pt micromotors. These tubular micromotors of micrometre dimensions moving in solution were electrodeposited from a polycarbonate membrane with a 3 µm pore (Figure 12.S4B and Table 12.S2). The decomposition of hydrogen peroxide in the inner lining of the catalytic platinum layer to oxygen bubbles and the subsequent detachment propels the tubular bimetallic concentric micromotor forward. As we increased the concentration of the micromotors from the stock solution, the number of spikes recorded within a fixed time interval of 60 s increases. This is illustrated when the concentration (in relative volume percentages from the stock solution) increases from 0.033 to 0.20 % in Figure 12.4A. This is due to increased electrode-particle collisions, as we have elucidated with the Janus type micromotors. However, from Figure 12.4B, the number of spikes appear to follow a non-linear trend and tapers off at higher concentrations of micromotors, from 9 spikes in 0.033 % to 16 spikes in 0.067 %, plateauing out at 18 spikes in 0.20 % from the stock solution. To investigate this phenomena, we undertook optical microscopy, where the observed number of micromotors moving by self-propulsion in Figure 12.4C continues to increase from 47 to 207 micromotors per mL when the concentration of micromotors taken from the stock solution increased from 0.033 to 0.20 %. It is in contrast to the electrochemical measurements from Figure 12.4A. This is possibly due to tubular micromotors tending to move along the bottom surface of the solution, while the Janus type silver micromotors are able to move near the air/solution interface, due to the greater number of bubbles that are produced. Tubular micromotors move further away from the electrode surface due to limited ability to generate bubbles in the hollow concentric centre, as compared to the silver Janus particles.

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Typically in a blank solution, a clean and flat profile was observed in the control experiments with no observation of any spikes, even at 10 X magnification (Figure 12.1B). The spikes are 3 X higher than the noise and are calculated typically from a 20 % threshold event from the largest spikes registered in an electrochemical signal. Generation of bubbles are confined to the inner catalytic layer of the tubular Cu/Pt micromotor, and escaping through the ends of the micromotors. This is in strong contrast to the Janus type micromotors, which produces bubbles on all of its outer surfaces giving such Janus type micromotors ease of detection via an electrochemical method. Further work to refine the methodology for an all-encompassing strategy for quantification and qualification of self-propagated moving objects are undergoing, to make it a more polished technique.

We have demonstrated a vast improvement to the detection of the Marangoni-effect propelled capsule motor.³⁸ Previously, detection is limited to the macro-level, with the motion of the motor to be confined in a channel environment and motion of the motor must be held in proximity of the electrode detector in an orthogonal fashion. In the current technique, we are able to detect micron-sized motors that are freely moving in solution, no limitations are required on the environments in which the micromotors are moving. The micromotors need not be confined in channels and can be in free solution. Their motion need not be limited to be perpendicular with the detector electrode. This difference between the methodologies permits the work to be extended to the motion of the micromotors which are suspended in solution. The current method can also detect beyond a single micromotor. A simple immersion of the micron-sized electrode allows the possible determination of the number of micromotors in solution and their locomotive properties.

Typically, for the blocking of the electrode surface, nanoparticles in the order of hundreds of nanometres can be detected^{18, 32, 51}. In this experiment, particles of several microns in size were investigated. Similarly, we believe that with our current technique, detection of such particles can be reduced to several hundreds of nanometres in size. Such

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scale is expected for the limit of detection using impact electrochemistry via a blocking mechanism. Furthermore, in other impact electrochemistry techniques, smaller-sized particles have been detected. Dissolution of nanoparticles during detection via impact electrochemistry can be scaled down to tens of nanometres in size.⁶¹ For electrocatalytic redox reaction of analytes in solution, nanoparticles of sizes up to a few nanometres in scale have been distinguished, where such signal amplification can be accomplished.³⁴ Simultaneous *in situ* study of electrochemical responses coupled with optical observations can serve to optimize the technique further.

12.3. Conclusion

We have demonstrated an alternative methodology of monitoring the locomotion of self-propelled micromotors. Two different types of micromotors, the Janus silver micromotor and the tubular Cu/Pt micromotor, with their corresponding optical and electrochemical responses were investigated. Particle-electrode impact voltammetry has been able to quantify and qualify the number of micromotors moving in solution on-site. In addition, we are able to detect the modulation of hydrogen peroxide propellant concentrations by electrochemical signals, where there was an increment in active micromotors and faster velocities. We foresee the widespread utilization of such electrochemical techniques to monitor locomotion of micromotors on-site. This is crucial in improving situational awareness of the task at hand, during environmental and biological applications. The obviation of optical tracing with microscopic tools and elimination of computationally demanding software can be accomplished with the advent of particle-electrode impact voltammetry for tracking and surveying self-propelled micromotors.

12.4 Experimental

Materials. The cyclopore polycarbonate membranes with pores of 3 μm in diameter were purchased from Whatman, USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH instruments, USA. Micro carbon fibre electrode with an inner diameter of 33 μm and outer diameter of 4 mm (Cat. No. 0020002) was purchased from BAS Inc., Japan. The platinum plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98+ %), sodium dodecyl sulphate (SDS), sodium hydroxide, hydrochloric acid (37 %) and silver microparticles were purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 $\text{M}\Omega\text{ cm}$) from a Millipore Milli-Q purification system.

Apparatus. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 $^{\circ}\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The silver working electrode was sputtered on the cyclopore membrane and copper tape was used as the transducer. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Hermle Z233m-2 microcentrifuge. SEM images were taken with a JEOL-7600F semi-in-lens FE-SEM. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software.

*Preparation of Cu/Pt concentric bimetallic microtubes.*⁴³ The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a

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cyclopore polycarbonate template. Silver (~100 nm) was sputtered on the one side of the polycarbonate template. Copper tape was attached to the silver-sputtered polycarbonate template, which serves as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The template was rinsed with 5 mL of ultrapure water (18.2 M Ω cm) for 4 times, and the Cu outer layer was deposited galvanostatically at -20 mA for 1200 s. The deposition solution contained 1 M CuSO₄ and 1 M H₂SO₄. Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -20 mA for 1200 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled. The template was polished with 5 μ m alumina powder to remove the sputtered silver and was washed for 5 times with 8 mL of water by ultrasonication each time. It was then placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly for 2 times with methylene chloride. The solution was then washed with ethanol and water for 2 times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Operation of micromotors. The experiments for studying the motion of micromotors were carried out in an aqueous solution containing 3-9 wt. % of hydrogen peroxide at constant surfactant concentrations (0.25 wt. % of SDS) in 50 mM NaOH with asymmetric Janus silver particle micromotors. For tubular Cu/Pt micromotors, an aqueous solution containing

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9 wt. % hydrogen peroxide at constant surfactant concentrations (0.25 wt. % SDS) was maintained in 50 mM NaOH.

Optical monitoring of micromotors. For locomotive studies, 10 μL of the micromotor solution was dropcasted on a glass slide freshly cleaned with nitrogen gas. Only moving micromotors demonstrating active microjet propulsion were taken into account. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-ElementsTM software. Average velocities and number of active moving micromotors were computed from 15 readings.

Electrochemical monitoring of micromotors. A micro carbon fibre electrode of diameter 33 μm was used as the working electrode. Glassy carbon counter electrode (3 mm) and Ag/AgCl/1.0 M KCl reference electrode were utilized. In a typical measurement, 0.25 wt. % of SDS is used as the surfactant and 50 mM NaOH is used as the electrolyte. Upon addition of the micromotors into the solution of SDS, hydrogen peroxide and sodium hydroxide, the solution is sonicated for 10 s for mixing, without the production of excessive bubbles before measurement. Corresponding blank experiments in the absence of micromotors were also carried out. The measurement was carried out by a three-electrode set-up, which was dipped into the electrolyte solution containing the micromotors, hydrogen peroxide and the surfactant. Electrochemical measurement was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). Average electrochemical spikes observed were computed from 5 readings.

12.5 Supporting Information

Figure 12.S1 **A** Typical chronoamperogram at 33 μm carbon microelectrode at 0.8 V vs. Ag/AgCl/ 1.0 M KCl, in absence of each component of 3 wt. % H_2O_2 , 0.25 wt. % SDS or 0.01 mg/mL silver micromotors respectively. **B** Typical chronoamperogram at 33 μm carbon microelectrode at 10 X magnification in the presence and absence of 0.01 mg/mL silver micromotors with 3 wt. % of H_2O_2 and 0.25 wt. % SDS. Conditions in all experiments: 23°C, 50 mM NaOH. Potential held at 0.8 V vs. Ag/AgCl/ 1.0 M KCl.

Figure 12.S2 **A** Cyclic voltammetry scan of 33 μm carbon microelectrode immersed in hydrogen peroxide at a scan rate of 100 mV s^{-1} **B** Chronoamperogram with 0.01 mg/mL silver micromotors at 33 μm carbon microelectrode at different potentials *vs.* Ag/AgCl/ 1.0 M KCl. Conditions in all experiments: 23°C , 3 wt. % of H_2O_2 , 50 mM NaOH and 0.25 wt. % SDS.

Figure 12.S3 Typical chronoamperogram at 33 μm carbon microelectrode at 0.8 V vs. Ag/AgCl/ 1.0M KCl with no silver micromotors with increasing hydrogen peroxide concentrations. Conditions in all experiments: 23°C, 50 mM NaOH and 0.25 wt. % SDS.

Figure 12.S4 A (i) SEM of silver Janus micromotors at 200 X magnification (ii) 10,000 X magnification B (i) tubular 3 μm Cu/Pt micromotors at 200 X magnification (ii) 10,000 X magnification.

Table 12.S1 Size of silver Janus micromotors

Dimensions	Size (μm)
l	9.4 ± 2.9

Table 12.S2 Size of 3 μm Cu/Pt tubular micromotors

Dimensions	Length (μm)
d_1	4.7 ± 0.5
d_2	3.3 ± 0.4
l_1	14.8 ± 1.8

Equation 12.S1.⁶² Advective flux of micromotors bombarding a surface per unit area per unit time.

$$Flux = [Concentration\ of\ micromotors] \times Velocity$$

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Table 12.S3. Flux of silver micromotors bombarding a microelectrode of diameter of 33 μm at increasing concentrations of hydrogen peroxide in a duration of 60 s. Conditions in all experiments: 23 $^{\circ}\text{C}$, 50 mM NaOH and 0.25 wt. % SDS.

[H ₂ O ₂] (wt. %)	No. of propelling micromotors (cm ⁻³)	Velocities (cm s ⁻¹)	Flux ¹ (cm ⁻² s ⁻¹)
3	560 $\pm$ 264	0.0146 $\pm$ 0.0060	8.15 $\pm$ 5.12
6	727 $\pm$ 310	0.0175 $\pm$ 0.0094	12.74 $\pm$ 8.75
9	800 $\pm$ 287	0.0204 $\pm$ 0.0066	16.32 $\pm$ 7.90

Figure 12.S5 Flux of silver micromotors against increasing concentrations of hydrogen peroxide fuel bombarding a microelectrode of diameter of 33 μm in a duration of 60 s. Conditions in all experiments: 23 $^{\circ}\text{C}$, 50 mM NaOH and 0.25 wt. % SDS.

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Table 12.S4. Correlation between physical counts obtained optically and electrochemical counts of silver micromotor bombarding a microelectrode of diameter of 33 μm in a duration of 60 s. Conditions in all experiments: 23°C, 3 wt. % of H_2O_2 , 50 mM NaOH and 0.25 wt. % SDS.

Concentration (mg/mL)	μ -motors (cm^{-3})	Velocities (cm s^{-1})	Flux ($\text{cm}^{-2} \text{s}^{-1}$)	Physical counts in 60 s	Electrochemical counts in 60 s
0.002	93 $\pm$ 59	0.0146 $\pm$ 0.0060	1.35 $\pm$ 1.03	0.0007 $\pm$ 0.0005	5.4 $\pm$ 3.9
0.004	200 $\pm$ 146	0.0146 $\pm$ 0.0060	2.91 $\pm$ 2.44	0.00149 $\pm$ 0.0013	8.6 $\pm$ 6.2
0.006	353 $\pm$ 177	0.0146 $\pm$ 0.0060	5.14 $\pm$ 3.34	0.00264 $\pm$ 0.0017	13.0 $\pm$ 9.1
0.008	447 $\pm$ 173	0.0146 $\pm$ 0.0060	6.51 $\pm$ 3.69	0.00334 $\pm$ 0.0019	15.0 $\pm$ 7.9
0.01	560 $\pm$ 264	0.0146 $\pm$ 0.0060	8.15 $\pm$ 5.12	0.00418 $\pm$ 0.0026	22.6 $\pm$ 7.5

Figure 12.S6 Physical counts obtained optically and electrochemical counts of silver micromotors against concentration (mg/mL) bombarding a microelectrode of diameter of 33 μm in a duration of 60 s. Conditions in all experiments: 23°C, 3 wt. % of H_2O_2 , 50 mM NaOH and 0.25 wt. % SDS.

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Chapter 13

Conclusion and Epilogue

13.1 Conclusion

13.1.1 Micromotors to Monitor and Remove Heavy Metal Ions

13.1.2 Influence of Environment and Optimizing Motor Performances

13.1.3 Monitoring Self-Propelled Miniaturized Systems

13.2 Epilogue

13.1 Conclusion

Motion of micromotors represents new opportunities in water monitoring and environmental remediation. These self-propelled devices are able to use chemical fuel to move autonomously. Having it as a thought experiment, it is a paradigm change from using diffusion-led forces to active agitation of the solution. The notion that we can detect changes in the environment by registering the changes in performances of the self-propelled devices is much akin to observing the motion of chemical-sensitive organisms moving in water, which finds its motility controlled by the surrounding environment. Such an artificial system obviates the need to observe locomotion of the animal's behaviour over long time windows and difficulties in calibrating their response in biological systems. Electrochemistry can be used to detect the motion of micromotor in both channel and open-water environments, permitting the introduction of independent systems. This is of especial importance as we integrate them into miniaturized motors: self-powered systems for water monitoring and remediation.

Through changes in locomotion of the micromotor, micromotor motion be calibrated to detect changes in different environments. Additionally, the coupling with functional carbon nanomaterials, allows the removal of heavy metals. New designs of the micromotor are constantly sought after. Controlling the performances of the micromotor can be exacted through reconfiguration of the micromotor. This can also be accomplished through a revamp of micromotor design and the tuning of changes in the environment. Exerting external influences by changing the chemical environment such as the pH, the use of photochromic light sources and ultrasound all represent opportunities to tune the performances of micromotors. Monitoring self-propelled devices by an electrochemical strategy allows the detection of the self-propelled devices. This represents a facile technology to detect the motion of the micromotor.

Chapter 13: Conclusion and Epilogue

In this thesis, we have explored (1) Use of micromotors for monitoring and removing heavy metal ions, (2) Influence of environment and optimising motor performances, (3) Monitoring self-propelled miniaturized systems.

13.1.1 Micromotor to Monitor and Remove Heavy Metal Ions

Micromotors demonstrate both capabilities to detect the presence of heavy metals and their subsequent removal. Due to changes of locomotion in the presence of these pollutants and their movement throughout the polluted source, they can act as effective remedies to the removal of toxicants. By integrating functional carbon nanomaterials into the micromotor's structure, these micromotors demonstrate possible dual capabilities of detecting heavy metals and removing them, showing great efficacies.

In **Chapter 4**, we demonstrate that tubular bimetallic Cu/Pt concentric micromotor shows capabilities to detect presence of heavy metals in water. Through poisoning of the active platinum surface, differences in locomotion was found. This can be identified through the changes in activity and velocities of the micromotor. Differentiation between two different heavy metal ions of lead and cadmium was accomplished.

Chapter 5 explores the use of graphene oxide from different oxidation routes to remove heavy metal ions of cadmium and lead from water. Graphene oxide synthesized from Staudenmaier's, Hoffman's and Hummer's method were used as subjects of study. Hummer's method shows the best capability to remove heavy metals of cadmium and lead. This is due to the presence of the greater amount of carboxylic groups.

Chapter 6 explores the integration of Hummer's graphene oxide into micromotor structure and their proposed subsequent use to remove heavy metals from the waters. The incorporation of graphene oxide inside the micromotor's structure is illustrated. The composite graphene oxide-platinum micromotor demonstrates promise to actively remove heavy metals from water.

13.1.2 Influence of Environment and Optimising Motor Performances

The environment exerts an influence on the performances on the micromotor. Most micromotors, to date use hydrogen peroxide as a fuel. There is a need to explore alternative designs of micromotor and to fully exploit the chemical potentials of the fuel. Limits to control of these miniaturized micromotors remain. The full manipulation of these micromotors in photochromic and acoustic fields strive to streamline the performance of these devices for directed and velocity control.

Chapter 7 explores the use of a polymer encapsulation strategy to contain a reactive species that produces gaseous products, using it as a propellant for propulsion. This demonstrates that a controlled release of acetylene bubbles for a polysulfone-encapsulated calcium carbide particle, which effervesces with water, can act as a strategy for bubble propulsion.

Chapter 8 explores the effect of pH on the performances of catalytic micromotors. The effect on the locomotion of both catalytic Janus and tubular micromotors are explored. In the alkaline region, the increased decomposition of hydrogen peroxide in alkaline medium results in increased bubble ejection and increased activity. Under acidic conditions, corrosion occurs, with a gradual exposure in the platinum surface before an eventual loss in structural integrity, which led to impaired locomotion in the extremities.

In **Chapter 9**, we examine the control of micromotors through use of photochromic additives. The interconversion between spiropyran to merocyanine in the presence of ultraviolet light and the reversion back from merocyanine to spiropyran in the presence of visible light changes the surface tension. We demonstrate the capability to tune the activity of swarms of micromotors and to selectively modulate the individual micromotor's velocity.

Following in **Chapter 10**, we examined the use of ultrasound to move and to change the rotational direction of the micromotors. The use of bubble's oscillations in an acoustic

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field to alter the locomotion through the use of secondary Bjerknes forces demonstrate capability to move them over large distances and tune the rotation pathway of the micromotor.

13.1.3 Monitoring Self-Propelled Miniaturized Systems

Electrochemistry demonstrates an ease of monitoring the movements of artificial self-propelled miniaturized devices using simple equipment. We illustrate that such a strategy is capable to detect artificial moving devices down to the micron-size. The self-propelled entity's perturbances of the electrochemical signal in the proximity of an electrode at a potential bias, is able to transmit information about the environment that it has been exposed to, bringing us closer to an internet of things by transmitting information about the self-propelled objects in the miniaturised regime.

In **Chapter 11**, we illustrate the detection of a Marangoni-effect self-propelled micromotor in a channel environment by using electrochemistry. The electric signals generated by the micromotor can be used to interpret the environment that the motor has been exposed to, such as physical obstacles within a channel environment. We further developed the work by showcasing the detection of micron-sized self-propelled objects in **Chapter 12**. We have been able to detect the concentrations of the hydrogen peroxide chemical fuels and velocities of both Janus and tubular micromotors.

13.2 Epilogue

Humans for centuries have been fascinated with locomotion in the natural world. An artificial system is a machine or device that is able to do work. As with the Wright brothers replicating the wings of the birds in an airplane and the streamlined structure of a fish being found in the designs of ships, many constructions of the conventional world has been adapted from nature. However, to replicate these performances at the miniaturized or nanoscale, things are not so trivial.

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The micro-organism of the amoeba stretches its cellular membrane to move forward. Some bacteria has developed tail-like structures to propel itself so that it can catch its prey. We have been able to produce bubble ejection in a synthetic object, which is able to reach hundreds of micrometres per second, which is two orders of magnitude faster than biological systems. It raises new questions of how these artificial systems are able to compete against environmental and natural systems in the transport of materials.

Miniaturised motors represents a next step forward for self-propelled devices for water monitoring and remediation, shown in the grand vision in Scheme 13.1. Their sensitized response to the environment makes them able to detect and remove toxic materials. These artificial systems can act as first responders towards changes in environmental and water quality.

Scheme 13.1. Grand vision of micromotors for water monitoring and remediation.

Control of the environment and making them independent of chemically polluting chemicals remains the main hurdle. We showcased a few of the possible choices to evade such chemicals to harness the full chemical potential of the micromotors for environmental

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remediation and monitoring. This is shown through using alternative propellants that use water as a co-reactant and maximising the chemical potential of the fuel utilised by catalytic microengines. Using light and ultrasound stimulation sources, we have been able to control micromotor's locomotion too.

Furthermore, we have been able to monitor their motion electrochemically, paving the way for autonomous systems, where they can interpret changes in the environments they have been exposed to.

As the father of nanotechnology Richard Feynman said, there is much space for us to explore at the bottom. Here, we wish to add that there is indeed much space for us to move at the bottom, when we break down the walls of powering miniaturized systems at the nanoscale. We believe that the next leap forward for the use of such self-propelled devices in water monitoring and remediation is now truly within reach.

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Appendices

■ Nano/Micro/Macromotors

Chemical Energy Powered Nano/Micro/Macromotors and the Environment

James Guo Sheng Moo and Martin Pumera*^[a]

Abstract: The rise of miniaturized artificial self-powered devices, demonstrating autonomous motion, has brought in new considerations from the environmental perspective. This review addresses the interplay between these nano/micro/macromotors and the environment, recent advances, and their applications in pollution management. Such self-propelled devices are able to actuate chemical energy into mechanical motion in situ, adding another powerful dimension towards solving environmental problems. Use of synthetic nano/micro/macromotors has demonstrated potential in environmental remediation, both in pollutant removal and contaminant degradation, owing to motion-induced mixing. At the same time, the chemical environment exerts

influence on the locomotion of the motors. These sensitized self-powered devices demonstrate capabilities for being deployed as sensors and their chemotactic behaviors show efficacy to act as first responders towards a chemical leakage. Thus, the notion of a self-propelling entity also entails further investigation into its inherent toxicity and possible implications as a pollutant. Future challenges and outlook of the use of these miniaturized devices are discussed, with specific regard to the fields of environmental remediation and monitoring, as we move towards their wider acceptance. We believe that these tiny machines will stand up to the task as solutions for environmental sustainability in the 21st century.

1. Introduction

Locomotion at the nano-, micro- and macroscale has been the subject of much interest of the scientific community.^[1] Biological soft matter systems, from enzymes at the nanoscale^[2] to the micrometer cilia in bacteria,^[3] have been demonstrated to be able to do work.^[4] However, they are often unstable and not resilient to changes in the environment.^[5] Replicating how Nature achieves that level of complexity, with an added element of hardness in unconventional environments, has been a constant pursuit in the community.^[6] Synthetic hard matter shows promise in overcoming these challenges, with a combination of metals being utilized as key components in miniaturized motors.^[7] With the innovations of materials scientists, chemists, and physicists alike, we have endowed these objects with specialized functions, enabling them to perform a multitude of tasks and operations.^[8] Coupling them with biological components ranging from simple biomolecules,^[9] to enzymes^[10] and small organisms^[11] has also now been made possible. The capacity of these self-propelled devices to perform tasks and operations are now many-fold, where specificity and efficiency are seeing unprecedented levels.

Environmental deterioration has been occurring at such a rapid rate that our natural resources are becoming depleted at an unsustainable rate. Water scarcity has become a pressing issue, where the pollution of water sources has drastically reduced the capacity of countries to provide for their burgeoning population.^[12] Decentralization strategies, amidst rapid urbanization, allow for the rapid deployment of resources where it is most needed.^[13] The rise of self-propelled miniaturized objects raises the promise of remotely powered devices that are able to do work untethered^[8,14] and reduce the contact time that ground workers have to spend handling toxic chemicals.^[15] These motors have demonstrated the abilities of

remote monitoring^[10,16] and remediation through rapid breakdown^[15] and removal,^[16b,17] by using the fuel in situ. This removes the logistics of having to move bulky equipment to support such operations, creating an added dimension of robustness in handling environmental issues.

The pairing of autonomous self-propelled objects and nanomaterials has provided new opportunities in the space of dynamic nanoscale motion-based environmental solutions.^[1] Optical observations of their locomotion and motion-induced stirring have offered new blueprints towards solving problems pertaining to pollution. These have reduced reaction times and extended the reach of the active reactants to throughout the solution mixture. Furthermore, the inherent properties of the nanomaterial, with its reactive chemistry and high surface area to volume ratio, presents innovative solutions that could be exploited for environmental causes.^[18] Peripheral functionalization has also endowed it with additional specificity and directionality, allowing it to home in on the site of interest with the help of an external agent, such as electromagnetic,^[19] magnetic,^[20] or acoustic stimuli.^[21]

The Mallouk/Sen groups have pioneered several micro/nano-motor devices on the basis of an asymmetrical chemical gradient that allows for propulsion of the motors.^[1b,14,22] The motors run principally on the mechanisms of self-electrophoresis and self-diffusiophoresis, where the basis of a spatially separated chemical gradient is required. The self-generation of a localized gradient through chemical free energy of the environment suggests that these motors are reactive to the compounds that are present in the solution, which garners a response from these nano/microdevices.^[22d] This would have been ideal for the operation of motors during chemical monitoring, where responsive motors can react to the changes in the solution. However, in an environmental remediation setting, the presence of a plethora of chemicals in the solution led to high ionic strengths and the increased substrate concentrations in the solution interfered with the mechanisms operating such nano/micromotors. It is noted that these motors generally move at speeds on the order of tens of micrometres per second,^[23] which might not generate the level of power used in mixings. This is in comparison to bubble-propelled nano/

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micromotors and interfacial tension-propelled devices, where they have been shown to be capable of high power.^[1c,d]

Although we advocate the use of these self-propelled devices in environmental monitoring and remediation, we must remain conscious of their limitations.^[24] The environment settings that these motors are used in are very often the obstacles that prevent them from achieving their ideal performance.^[17b,25] These conditions have not only reduced the efficiency of the motors, but may very well turn them into an active pollution factor that warrants further contemplation before utilization in the field.^[26]

In this review, we reflect on the recent advances in nano/micro/macromotors along with their possible ramifications on the environmental and water sectors. Motion-induced mixing by the motors has added an extra element of efficiency towards pollutant removal and decontamination works. The influence of the environment with regards to the performance of these devices and their corresponding applications as sensors and tracers for environmental monitoring will also be discussed. Lastly, the possible implications of environmental degradation by the self-propagated entities themselves are raised in this review. Thus, we have attempted to develop an encompassing review detailing nano/micro/macromotors and their roles in the environment, the limitations they face, and the future ahead as the community forges forward towards their wider acceptance and employment.

2. Overview of the nano/micro/macromotor and environment nexus

For a clearer understanding of this field, this review has been divided into three sections, as shown in Figure 1. First, the remediation of the environment, with regards to pollutant removal and contaminant degradation by motor-enhanced mixing is discussed. Second, the influence of the environment

Figure 1. Nano/micro/macromotors and the environment.

on the motors will be elucidated and will be extended into the possible use of their locomotion as sensors. The chemotactic behavior of relevant motors to detect chemicals will also be included for their roles as tracers and markers. Third, the role of nano/micro/macromotors as a pollutant owing to toxicity effects and their inherent self-propagating factor are considered. The examples listed in each section are arranged in increasing size order from the nano/micromotor to the macromotor, which is millimeters in size. The macromotor continues to be of importance due to the operational readiness of being able to cover long distances in a short time.^[27]

3. Nano/micro/macromotors remediating the environment

By converting chemical energy from the environment into mechanical energy, nano/micro/macromotors have given environmental specialists more degrees of freedom in the area of pollutant disposal onsite and offsite. The mechanical agitation brought about by the motion of the motors enables efficient mixing of the solution. This is particularly so for bubble-propelled micro/nanomachines, where the rising bubbles can serve as an additional mixing agent.^[15a,b] The drag on the mixture by the micro/nano/macromotor brings the active reactants to the source, enabling better contact and reach, for removal and degradation of pollutants.

Two recurrent themes are at play in the use of self-propelled devices for environmental remediation. These are the roles of size^[1b,22c,28] and speed.^[1c,d] At these scaled-down dimensions,

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the role of inertia plays a lesser role compared to that of the viscous drag of the solution.^[28] The solution-borne motors are immersed in their own fuel, which effects the continuity of the instantaneous forces that propels the motion, since inertia no longer plays an essential role in locomotion.^[1b]

Surface forces become an important factor at the miniaturized scale.^[28] This is proportional to the relative amount of exposed area per unit volume becoming larger. When an object is scaled down by a proportion, ΔL , the volume of the object reduces by a scale of ΔL^3 , whereas the object's surface only reduces by a scale of ΔL^2 . The large surface area to volume ratio allows for larger contact areas, while continuously bringing in active materials by drag forces to regions in need of environmental remediation, allowing for facile action. As we scale down, the average distance between self-propelled devices and the substrates becomes reduced in comparison with a single bulk entity with equivalent mass. This aids the chemical remediation process that is going to take place with increased reach together with increased contact area. Additionally, it is noted that physical forces, such as electrostatic interactions and Van der Waal's forces, increase in the proportion of ΔL^2 and ΔL^6 , respectively, with reduced distances, aiding the remediative actions.

The speeds of the micromotors play an important role during remediative action.^[15] Orozco et al. demonstrated that self-stirring effects due to the collective hydrodynamic movement of the micromotors can reach up to the equivalent of 200 rpm (rounds per minute), a rate that was in part due to the high speeds of up to $500 \mu\text{m s}^{-1}$ that were reached.^[15a] Soler et al. also demonstrated that the drag of the micromotors brought active materials into proximity with the contaminant, allowing active mixing at increased speeds.^[15b]

In situ and onsite decontamination protocols are possible with such a set-up as the stirring component can be brought to the source requiring remediative treatment.^[15] This decentralization strategy obviates the need for big bulky equipment in the form of conventional externally powered stirring devices. Instead of sending the pollutants to large factories for remediative action, we are able to bring the "mixers" of micromotors to the source.

3.1 Pollutant removal

Actively moving motors are able to herd and gather oil spills to curb the spread and allow for easier clean-up of chemicals. Motors that simultaneously move and leach chemicals often operate at interfaces (water/air), effecting the dissipation of chemicals that aids environmental remediation. This is especially helpful during operations for the clean-up of oil, where it remains on the water/air interface and extends over large areas. Current technology defers to the use of oil booms or chemical booms for containment, where it remains relatively stationary and is

categorically a passive mechanism.^[29] The employment of motors that move by leaching chemicals provides a new protocol for environmental clean-up.

3.1.1 Removal of oil

By using a self-assembly strategy with gold surfaces, Guix et al. modified the surface of a microtubular motor with a monolayer of alkanethiol to form the superhydrophobic layer as shown in Figure 2A.^[17a] *n*-Dodecanethiol was shown to be the optimum carbon chain length for the efficacious transport of oil droplets away from a contaminated water surface, while the micromotor was powered by the decomposition of hydrogen peroxide to oxygen bubbles in the inner platinum layer. A similar strategy was employed on a hydrogen bubble-propelled gold-coated magnesium Janus particle in water, as illustrated in Figure 2B.^[17d] The self-assembled monolayer (SAM) of octadecanethiol demonstrated a good rate for removal of oil droplets away from the surface. The Janus particle, when exposed to a real environment, was able to operate without the need of an external fuel source. The pitting mechanism of the chloride ions in seawater prevents passivation from occurring and the macrogalvanostatic corrosion of magnesium in seawater, to form hydrogen bubbles, propels the motor forward. Modifi-

Figure 2. A) Composition of a superhydrophobic layer-modified tubular micromotor for removal of oil droplets and the mechanism of a) approach, b) capture, and c) transport. Reprinted with permission from Ref. [17a]. B) Composition of a self-propelled Janus microsphere for removal of oil droplets and the mechanism of a) approach, b) capture, and c) transport. Reprinted with permission from Ref. [17d]. C) Sequential images detailing the shepherding of oil droplets by a SDS-loaded Marangoni capsule into a single droplet for easy removal. Reprinted with permission from Ref. [17b]. D) a) Fe^{III} -contaminated water in the absence of SDS-loaded Marangoni capsule. b) Flocculation of Fe^{III} -contaminated water by a SDS-loaded Marangoni capsule for easy removal. Reprinted with permission from Ref. [16b].

cation with a nickel layer enables magnetic manipulation of both the Janus and microtube motors for directed movement towards the contaminant source.

Polymer capsules propelled by the Marangoni effect have been demonstrated to move up to 64 body lengths per second and over distances of up to 24.5 m.^[17b] Polysulfone dissolved in dimethyl formamide (PSf/DMF) undergoes phase inversion and solidifies upon contact with an aqueous solution to form a polymer capsule. The porous PSf polymer capsule releases DMF asymmetrically, resulting in a difference in surface tension, which culminates in propulsion—a phenomena otherwise termed as the Marangoni effect. Additionally, these capsules can be loaded with chemicals that have the ability to remediate environmental pollutants.^[16b, 17b,c] Zhao et al. demonstrated the ability of these polysulfone/DMF capsules to load the surfactant, sodium dodecyl sulfate (SDS).^[17b] These laden self-propelled capsules, on introduction into aqueous medium, release SDS and actively spread them. As illustrated in Figure 2C, on close proximity with the capsule the oil droplets are repelled by the surfactant and gradually merge to form a large puddle, enabling an easy clean-up protocol. In follow-up research, the positively and negatively charged and the neutral surfactant-infused polymer capsules all showed capabilities for gathering of oil droplets.^[17c]

3.1.2 Removal of ions

The SDS-loaded PSf capsule also demonstrated efficacy in the removal of heavy metals, particularly Fe^{III}, through precipitation as depicted in Figure 2D.^[16b] The surfactant released by the PSf capsule converts the Fe^{III} in the aqueous phase to solid precipitates. Such a motion-induced mixing with controlled release of surfactant is another important mechanism of remediation, wherein contaminants could be flocculated and separated easily.

Later, using a similar concept based on the Marangoni effect, Orozco et al. designed tubular motors from pipette tips that are capable of releasing SDS in solution.^[30] The release of the SDS surfactant results in a change in surface tension, which exerts a force on the motor to move in a circular motion. These tubular motors can be easily loaded with coordination factors that result in precipitation for the purposes of environmental remediation. In this fashion, the authors demonstrated that heavy-metal pollutants, such as Pb^{II}, could also be removed by utilizing the coordination agent ethylenediaminetetraacetic acid (EDTA). Furthermore, the agents used in this exercise are environmentally friendly and the tubular motors could be recycled for subsequent usage. The controlled release of the active substrate with the consequent efficient mixing has allowed for the precipitation of heavy metals through chelation chemistry.

The utilization of self-propelled nanoadsorbents by Jurado-Sanchez et al. was demonstrated for the removal of both heavy metals and organic pollutants alike.^[31] Activated carbon nanoparticles were modified with a layer of platinum to confer locomotion by bubble-propulsion in hydrogen peroxide, where the microbubble tail can act as an additional mixing

agent. These Janus-type motors operate on the principles of adsorption and microfiltration through their porous structures. The autonomous self-propelled motors demonstrate efficacy in removing organics such as 2,4-dinitrotoluene, rhodamine 6G, and methyl-paraoxon, as well as heavy metals such as lead. The dynamic mixing system has extended reach, higher efficiency, and shorter clean-up times in comparison with static activated carbon nanoadsorbents, which require an external mixing force.

3.2 Contaminant degradation

Hydrogen peroxide has been used ubiquitously in the context of environmental remediation for the breakdown of organic matter.^[32] This is especially so in the context of advanced oxidation processes, where highly reactive species, hydroxyl radicals, are formed.^[33] Fenton's reaction is one of the chemical methods for producing such radicals *in situ*.^[34] We must remain mindful that hydroxyl radicals are still one of the strongest oxidizing agents known. The coupling of the self-stirring motion and highly oxidative agents are attractive for the management of aqueous waste.

3.2.1 Advanced oxidation process

Hydrogen peroxide is commonly used in self-propelled systems owing to the ease of decomposition of hydrogen peroxide to oxygen and water. However, it must also be realized that the compound itself is a strong oxidizing agent, which has been used by the environmental^[35] and defense community for the degradation of harmful organic substances.^[36] As illustrated in Figure 3A, Orozco et al. extended this research with the use of base-activated peroxy anions for the motor-induced self-stirring breakdown of organophosphate nerve agents using bubble-propelled polymer/platinum composite micromotors.^[15a] The large-scale collective motion of the micromotors led to hydrodynamic interactions for enhanced mixing. Such collective movement of the micromotors, together with the decomposition of hydrogen peroxide to oxygen bubbles, increased the yield and speed of reaction by efficient fluid mixing. UV spectroscopic observation at 400 nm of the *p*-nitrophenol byproduct indicated that the forced convection and enhanced mass transport of the peroxide with the bubble-propelled micromotor is the principal cause of oxidative decontamination. The subsequent exclusion of micromotors or surfactants essential for bubble-propulsion did not give any reduction in contaminant levels in the control experiments. The shorter timescale, lower peroxide loading, and high efficiency of such a procedure is projected to have real applications in detoxifying chemical threats.

At approximately the same time, Soler et al. explored the use of micromotors in environmental remediation by using active materials from the surface of the micromotor to participate in the degradation of organic pollutants, as illustrated in Figure 3B.^[15b] These rolled-up Fe/Pt tubular micromotors have a configuration such that platinum lines the inner surface and

Figure 3. A) Micromotor-based accelerated oxidative decontamination of chemical threats with H_2O_2 as the fuel for the in situ generation of base-activated nucleophilic oxidant, OOH^- . Reprinted with permission from Ref. [15a]. B) Self-propelled Pt-based microtube with an outer Fe layer that dissolves in catalytic amounts to participate in Fenton reactions with hydrogen peroxide, producing hydroxyl radicals for the degradation of organic pollutants with induced mixing. The motor is based on pipette tips containing a mixture of enzyme remediator (10%) and SDS propellant (90%). Reprinted with permission from Ref. [30]. C) Enzyme-releasing biocatalytic motor that degrades pollutants with induced mixing. The motor is based on pipette tips containing a mixture of enzyme remediator (10%) and SDS propellant (90%). Reprinted with permission from Ref. [30]. D) Schematic illustration of the dual-function plant (radish) motors in hydrogen peroxide. The dual-function motors combine the catalase-driven bubble propulsion and peroxidase-based decontamination capability to enable in-motion bioremediation. Reprinted with permission from Ref. [15c].

the outer layer is made of iron. Under the typical harsh condition of pH 2.5 and 15% H_2O_2 , iron gradually dissolves into the solution mixture. The catalytic amount of Fe^{II} participates in the Fenton reaction with hydrogen peroxide, which produces the highly oxidizing species, hydroxyl radicals. The hydroxyl radicals readily break down the complex organic molecule of rhodamine 6G (R6G) into inorganic byproducts. Active participation of the autonomous moving Fe/Pt microtubes also leads to the micromotors stirring the solution owing to their motion and the stream of oxygen bubbles forming on the Pt inner lining. In addition, the drag on the microtube allows the active species of Fenton's reaction to be brought to the contaminant source. The environmental strategy of using Fe/Pt microtubes completes the clean-up of the R6G in 5 h and is 12 times faster compared with the scenario in which only Fe microtubes are introduced. Only minute quantities of iron are introduced into the system; the micromotors can be readily recovered by using a magnet and used in the next batch of environmental remediation.

Photocatalytic breakdown of chemical and biological agents by using self-propelled spherical motors that operate by using water as the fuel, without the addition of chemical fuels and active agents, have been demonstrated by Wang and co-workers.^[37] In this case, the motor has a Janus structure with an inner core of a spherical magnesium scaffold as the fuel, followed by an interlayer of gold particles, which is finally encapsulated by an active titanium oxide layer on the outside. The $\text{TiO}_2/\text{Au}/\text{Mg}$ micromotor operates by macrogalvanic corro-

sion of magnesium in water to produce hydrogen bubbles for propulsion. Typically, these micromotors have a lifetime of minutes owing to the limited fuel packed into the device. On ultra-violet irradiation, the reactive oxygen radicals that are formed on the TiO_2 surface in water break down organic and biological materials alike. The continuous motion of these micromotors has been instrumental in stirring solutions of harmful agents for degradation. This continuous motion also aided in the self-renewal of the titanium dioxide surface, which would otherwise be easily passivated.

3.2.2 Biocatalytic remediation

The pipette-based tubular motors designed by Orozco et al., which were highlighted earlier for their ability to carry chelating agents, are also capable of carrying bioremediation enzymes too.^[30] In Figure 3C, the

release of the active enzyme, with the subsequent efficient mixing, allows for facile degradation of environmentally harmful compounds to benign substances. Simultaneous employment of six laccase-carrying SDS motors demonstrated the capability for 77.4% degradation of 50 mL of 250 μM 2-amino-4-chlorophenol to innocuous substances in 30 min. An analogous experiment for the eriochrome black-T (EB-T) azo-dye resulted in complete degradation of the pollutant, with the same parameters. Direct introduction of laccase at ten times the concentration in a localized position does not affect the rate of degradation significantly, confirming the importance of the motor-induced stirring of the solution. The controlled release of the active compounds, followed by the motor-induced stirring, is again highlighted as an important strategy for efficient reactions to occur.

Biocatalytic millimeter-sized plant tissue motors have also been employed for remediation of waters containing toxic phenolic compounds, as depicted in Figure 3D.^[15c] Enzymatic bioremediation of aromatic compounds, such as guaicol, catechol, and 2-amino-4-chlorophenol, represents an attractive strategy over decontaminative work by micro-organisms, due to toxicity limitations. By introducing asymmetry to the tissue compounds in the presence of hydrogen peroxide, these biomotors demonstrated propulsion—effectively increasing the induced convective fluid mixing—coupled with detoxification capabilities. The two principal enzymes of peroxidase and catalase in radish-tissue-based motors worked harmoniously together for active fluid mixing and decontamination,

respectively. Hydrogen peroxide is the fuel for propulsion for catalase, while also being the co-substrate for the detoxification reaction for peroxidase, with both enzymes being inherently present in radish tissues. These systems are projected to be suitable for wastewater decontamination works involving $1\text{--}600\text{ mg L}^{-1}$ of phenols with about 1 to 4 tissue motors per milliliter, with complete removal being reached in an hour. The motion of these tissue motors and the release of bubbles extended the reach of the enzymes across the reaction vessel, while improving mass transfer through active mixing.

3.3 Immobilized micromotors: micropumps

Self-powered pumps are analogous models of immobilized motors that actively inject fluid flow into the mixture. Micropumps impart momentum to fluid circulation through the use of chemical gradients, resulting in the mechanisms of self-electrophoresis, self-electroosmosis, or self-diffusiophoresis^[1b] effecting fluid flow. Recent developments have also included micropumps that are independent of the substrate materials, utilizing depolymerization reactions upon introduction of external stimuli, such as using chemical and ultraviolet factors.^[38] Further manipulation of the fluid-flow directions can be accomplished by changing the fuel source^[39] or the zeta potential of the particle and the micropump's surface.^[40]

Depolymerization to reactive monomers that can remove inorganics from the solution are particularly interesting owing to the amplification provided by the catalytic reactions. The proposition of in situ production of high amounts of reactive monomers onsite with portable micropumps coupled with the amplification by catalytic reactions is especially attractive for the pumping of ambient fluid and removal of deleterious materials.

Although these reactions are catalytically driven, fluid speeds have remained low, on the order of $1\text{--}10\text{ }\mu\text{m s}^{-1}$.^[38b] Furthermore, the pumping speeds vary with reaction rates and distance from the pumps themselves. More work is required in order for the micropumps to be fielded in real-world applications and to allow for a uniform hydrodynamic mixing throughout the solution.

4. Influence of the environment on nano/micro/macromotors

The environment in which the nano/micro/macromotor operates exerts a strong influence on the activity and velocity of these self-propelled entities. Previously, it was shown that a bubble-propelled micromotor can move independently of the salt effect, which can otherwise adversely affect the self-electrophoresis mechanism.^[41] However, recent research has given converse results, showing that these bubble-propelled motors and Marangoni-type millimeter-sized capsules are affected by the presence of ions, organic molecules, biomolecules, and cells, all of which influence the propulsion of these autonomous self-moving objects. In addition, this very behavior could be exploited with optical observations

for the construction of sensors and chemotaxis for tracing and marking environmental contaminants.

4.1 Nano/micro/macromotors are influenced by the chemical environment

Micromotors have been applied to do work in microfluidic chips, for environmental remediation and within the biological vascular systems. However, it is known that the fluids inside these environments contain high amounts of ions and other biomolecules. Thus, it is of utmost importance to test the robustness of micromotor systems using conditions that reflect the actual environment. For the study of chemical environment and micromotors, two principal designs of micromotor have been used. The first is the template-assisted electrodeposited micromotors (ED micromotors).^[42] These tubular micromotors are concentric and multi-component in nature, consisting of metals or polymers. The second design is from roll-up technology, which uses a sacrificial polymer template with consequent electron-beam deposition of metal layers. These rolled-up micromotors (RU micromotors) usually consist of multiple layers of metal that roll up upon removal of stress from the etching of the sacrificial polymer.^[7a]

Zhao et al. illustrated the effects of using different water matrixes from the real environment and probed their possible influence on bimetallic Cu/Pt ED micromotors.^[43] The activity and velocity of micromotors in natural water matrixes such as lake water and seawater were all negatively influenced, as shown in Figure 4A. In the presence of 9% seawater (by volume), the velocity of the micromotor reached zero. Test runs with ion chromatography confirmed that the high salt content was the principal cause of retardation in the micromotor's activity and velocity. A later report using a poly(3,4-ethylenedioxythiophene) (PEDOT)/Pt ED micromotor was also negatively influenced by the type of water matrix and it was again shown to be most adversely influenced by a seawater matrix, where the salt content was the highest.^[25f] Although the trends were similar, the magnitude of the changes were conspicuously different. It must be recognized that the design of these two configurations were inherently different, with the bimetallic Cu/Pt ED micromotor being significantly more impeded by the presence of high salt concentration than the polymer PEDOT/Pt ED micromotor. This is perhaps due to heavier metal components being used in the Cu/Pt ED micromotor. Thus, we must always bear in mind the design of the micromotor during observation of locomotive events.

In a similar way, the carrier fluid in blood contains a plethora of substances such as ions in the blood electrolyte,^[25g] biomolecules,^[25e] metabolites,^[25h] proteins in blood,^[25d] and red blood cells,^[44] which may affect the motion of the micromotor. Indeed, the presence of these entities was demonstrated to slow down the activity and velocity of the micromotors. For simple molecules and ions, four principal reasons have been proposed for the retardation of micromotors: (i) sensitivity of the active platinum layer in the micromotor to poisoning; (ii) interruption of the radical reaction during decomposition of the hydrogen peroxide through quenching; (iii) the addition of

Figure 4. A) Motion of the electrodeposited (ED) micromotor in lake water (left) and seawater (right). Reprinted with permission from Ref. [43]. B) Motion of rolled-up (RU) micromotor in the presence of sulfur-containing molecules, DMSO (left) and methionine (right). Reprinted with permission from Ref. [25e]. C) Motion of ED micromotor in blood electrolytes, NaCl (left) and phosphate buffer (right). Reprinted with permission from Ref. [25g]. D) Motion of ED micromotor in the presence of uric acid. Reprinted with permission from Ref. [25h]. Conditions for all experiments: 3 wt% H_2O_2 and 1 wt% SDS.

ions leading to aggregation of surfactants, which increases surface tension and leads to the reduction of detachment rate of the ejected microbubbles from the micromotor; (iv) increase of viscosity of the chemical environment impeding the motion of the motors. For large biological components, in addition to viscosity, chemisorption and physisorption also impede the motion of the motors. In the following, we have arranged the discussion with regards to the impediments in increasing size order: from simple ions and molecules, to large biological components, and at the cellular level. Additionally, we also

discussed the role of surfactants for bubble-propulsion and self-propulsion in unconventional environments.

4.1.1 Ions and simple molecules

The first work on such effects was represented by Zhao et al. with their work on chemical environments retarding the propulsion of micromotors.^[25e] Dimethyl sulfoxide (DMSO) exhibited strong inhibition of the movement of platinum-based RU micromotors. This is largely due to its role as a quencher of the hydrogen peroxide decomposition reaction as shown in Figure 4B, left. The research was extended to sulfur-containing simple molecules with RU micromotors, where entities such as methionine showed strong poisoning effects (Figure 4B, right). Poisoning effects of such sulfur-containing groups can be related to the branching of the alkyl groups in the sulfur-containing groups, with the thiol in cysteine exhibiting more pronounced poisoning effects than the thioether in methionine. Beyond sulfur-containing moieties, Wang et al. used ions found in blood electrolytes to exemplify the negative effects (Figure 4C).^[25g] The increasing ionic concentration of these species exhibited debilitating effects on microtubular motors. The team later extended the work to include the common metabolite, uric acid.^[25h] As a radical scavenger, uric acid also caused a dramatic decrease in the viability of micromotors (Figure 4D), an effect that was due to the reduced capacity for radical decomposition of hydrogen peroxide in the Cu/Pt ED micromotor. Hence, the presence of these substances have a strong influence on the micromotor's mobility and a spectrum of simple chemicals can affect the integrity of operation of microtubular motors in hydrogen peroxide.

4.1.2 Large biomolecules and cells

For large biomolecules and at the cellular level, it was proposed that chemisorption or physisorption led to the passivation of the active platinum surface and the increased viscosity of the solution also played a determining role in the slowdown of the micromotors.^[25d,44] The presence of proteins such as serum albumin impeded the motion of micromotors (Figure 5A).^[25d] In addition to the passivated platinum surface, the increased viscosity elevated the drag force on the micromotors, in accordance with Stoke's law, resulting in the retardation. Zhao and colleagues' work with blood components (red blood cells and blood serum) shed the same light on the increase in viscosity impeding the motion of the micromotors and the inactivation of the surface by biological components.^[44] Additionally, blockage of the opening of the microtubular motor by red blood cells through chemisorption or physisorption can occur.^[44] This cripples the micromotor as the fuel source is effectively cut off, resulting in the reduction of velocities in the micromotors (Figure 5B). Wang et al. managed to circumvent the problem of the impaired ED micromotor by increasing the hydrogen peroxide concentration in the presence of excess blood electrolyte.^[25g] However, it must be noted that high concentrations of hydrogen peroxide are toxic. Soler et al. later overcame the impediments of navigating

Figure 5. A) Influence of serum albumin concentration on the velocities of ED micromotors. Reprinted with permission from Ref. [25d]. B) Influence of red blood cell concentration on the velocity of RU micromotors. Reprinted with permission from Ref. [44]. C) Average speeds of RU micromotors in suspensions of red blood cells: average speeds during 4 temperature cycles of 5 min each between 25 and 37 $^{\circ}\text{C}$ in a 10 $\times$ diluted solution. Reprinted with permission from Ref. [45]. Conditions for all of the experiments: 3 wt% H_2O_2 and 1 wt% SDS.

RU micromotors through reconstituted blood in 10% blood serum in phosphate buffer at tenfold dilution, by raising the temperature of the serum to 37 $^{\circ}\text{C}$, that is, body temperature (Figure 5C).^[45] The team proved that the high viscosity of blood at room temperature has been one of the critical factors for the retardation of micromotors in physiological medium. The synergy of a reduction in viscosity of blood and thermal activation of the micromotors at elevated temperatures shows promise for autonomous self-propelled objects in complex biological media.

4.1.3 Viscosity of solution

Undeniably, viscosity can become a crucial factor for self-propelled objects under environmental conditions, wherein

turbidity and dissolved substances play a contributing role. At the sub-millimeter or micrometer level, in the case of Cu/Pt ED micromotors, the Reynolds number is influenced by the velocity due to fuel (H_2O_2) concentrations and the viscosity of the solution.^[25c] Reynolds number-dependent bubble formation and the damping effect of viscosity are the principal causes for instability in a micromotor's trajectory. The transition for the style of motion from a linear to non-linear motion for a micromotor is found to occur at a critical Reynolds number of 10^{-3} (Figure 6A).

Figure 6. A) The directionality of motion of the micromotor is influenced by Reynolds numbers. Reprinted with permission from Ref. [25c]. B) Directionality of motion for a Marangoni capsule is influenced by Reynolds numbers, resulting in linear translational (a), rotational (b), and non-linear translation motion (c). Scale bars indicate 2 μm ; tracking time of 1 s. Reprinted with permission from Ref. [25a].

Zhao et al. investigated the motion of a Marangoni PSf polymer capsule under different Reynolds number regimes.^[25a] Three different types of motion can be identified with the capsule: linear, rotational, and non-linear modes (Figure 6B). It was found that the trajectory of the system can be switched to a non-linear mode by increasing the viscosity or Reynolds number of the solution through the addition of viscous glycerol.

4.1.4 Presence and type of surfactant

Surfactants are essential for the formation of microbubbles and their detachment in a bubble-propelled nano/micromotor.

Wang et al.^[46] and Simmchen et al.^[47] demonstrated that the type of surfactant determines the mobility of motors owing to their relationship with bubble formation on the platinum catalytic surface, used to power the locomotion. Wang et al. advocated the stabilization of charges on the platinum surface by the charge-carrying surfactants, a modification that has led to a difference in performances, of which the negatively charged SDS demonstrated the best efficacy. However, Simmchen et al. proposed the reduction of surface tension by surfactants was the principal factor for bubble formation and thus for propulsion to occur. Further investigation is still warranted for a clearer understanding of the role of surfactants in bubble propulsion.

4.1.5 Unconventional environments

Environmental remediation is often undertaken under unconventional conditions and the differing solvent compositions may affect the velocity and activity of self-propelled objects, in addition to their trajectory patterns. At the macroscale, Marangoni PSf capsules were demonstrated to operate under ionic conditions and at high concentrations of organic solvents, such as acetonitrile, ethanol, acetic acid, and DMF.^[17b] However, due to changes in surface tension in the presence of organic solvents, the velocity of the PSf capsule will be attenuated at high concentrations of these organic solvents. The presence of surfactants and oil droplets also results in changes in the motion of the capsule owing to negative chemotactic effects that result in repulsion.^[17b,c] Such changes in behavior were also observed in an interlayer of organic and aqueous phases, where these PSf capsules demonstrate versatility in traversing between the oil and water layers, albeit at a lower velocity.^[25b]

4.2 Nano/micro/macromotors as environmental sensors

One of the first instances of nanomotors demonstrating selectivity to chemicals in the environment is represented by the work by Kagan et al. with Au–Pt nanowire-based nanomotors, as shown in Figure 7A. The nanomotors exhibited sensitivity to the presence of Ag^+ ions in the solution through a self-diffusiophoretic mechanism.^[48] The underpotential deposition of silver on Au–Pt nanowires in the presence of hydrogen peroxide allowed for increased catalytic activity. This enhanced catalytic decomposition of hydrogen peroxide increased the reaction products, leading to enhanced self-diffusiophoretic locomotion. As a result, this constitutes the motion-based sensitive chemical detection of trace silver ions at the micromolar range as higher speeds are recorded in the presence of silver.

As shown in Figure 7B, Orozco et al. used an artificial enzyme-powered microfish for the multiplex study of water quality.^[10] Exploitation of the change in propulsion behavior in the presence of pollutants in water was used as the source of stimuli input. The catalase-immobilized microtubular PEDOT/Au microfish exhibited competence in sensing the presence of inorganic and organic pollutants, such as sodium azide, mercury ions, copper ions, and aminotriazole (AT). The authors used the toxin-induced retardation of motion in the tubular micro-

Figure 7. A) Au–Pt nanowire average speed in 5 wt % H_2O_2 solution containing 100 μM metal nitrate salt solutions and the motion-based chemical sensing of trace Ag^+ . Reprinted with permission from Ref. [48]. B) Schematic depiction of pollutants impeding microfish motion and the dependence of survival time upon pollutant concentration, where survival time is the time taken for the fish to lose directional movement and reach minimal speed. Conditions: 2 wt % H_2O_2 and 5 wt % sodium cholate. Reprinted with permission from Ref. [10]. C) Selectivity of RU micromotor motion towards cysteine and serine. Reprinted with permission from Ref. [25e]. D) Selectivity of ED micromotor motion towards Pb^{2+} and Cd^{2+} . Reprinted with permission from Ref. [16a]. Conditions in C) and D): 3 wt % H_2O_2 and 1 wt % SDS.

motors and life expectancy as gauges for poison concentrations. However, it must be noted that the methodology does not offer a selective process across the compendium of toxins covered and that the concentrations were of different orders of magnitude. It must also be noted that the presence of biological components in the motor means that the device does not have long shelf-life.

The selectivity of bubble-propelled synthetic platinum-based micromotors was uncovered by Zhao et al. in their work with chemical environments.^[25e] It is well known that thiol-based groups can be chemisorbed onto surfaces by the formation of sulfur bridges on a pristine platinum surface. The work was extended to use sulfur-containing thiol in cysteine as an analogue for the oxygen-containing hydroxyl group in serine. This selective poisoning by the sulfur-containing derivative is illustrated in Figure 7C. Suppression of velocity in the RU micromotor was not visible in the presence of 10 mM of serine. However, no moving micromotors were observed in the presence of cysteine at the same concentration.

Utilizing the sensitivity of platinum surfaces in the ED micromotors towards poisoning, direct selective detection of Pb^{2+} over Cd^{2+} through optical visualization of the activity and mo-

bility of these microtubular Cu/Pt ED microfish can be realized (Figure 7D).^[16a] By examining the LD₅₀ values (lethal dose for 50% disablement of activity in micromotor), a quantitative treatment of the selectivity of microfish towards heavy metal ions can be achieved, where the presence of 0.96 mM Pb²⁺ is required to impair the mobility of the micromotor as opposed to 8.9 mM Cd²⁺. The stronger poisoning by lead ions can be attributed to the stronger electronegativity of lead versus cadmium, leading to stronger adsorption and greater decrease in activity of the active sites of platinum.

There has been much progress in the detection of pH levels with the recent advent of micromotors that run on acid–base reactions. This was led by the discovery of Gao et al. that zinc-based micromotors move in an extreme acidic environment through hydrogen bubble-propulsion.^[49] The team's later work showcased amphoteric aluminium-based motors that are able to work in both extremes of pH.^[50] With the inclusion of a palladium surface in the aluminum-based motor, coupled with the presence of hydrogen peroxide, unique velocity values could be obtained at different pH ranges. Indeed, these propulsion systems can be extended to the monitoring of industrial processes and even changes in pH in the environment. They exhibit competence to be the first indicators of industrial leakage, especially in chemical environments that use extreme pH as part of the manufacturing procedures. However, it must be noted that the lifetimes of these devices are on the order of minutes due to the limited fuel packed into the device.

4.3 Tracing and marking the chemical plume

Chemotactic behavior of Pt/Au rods towards higher concentrations of hydrogen peroxide due to diffusivity has been demonstrated.^[51] The motion towards higher concentrations of fuel is due to the higher average displacement the rods experience in regions of increased hydrogen peroxide fuel. The locomotion continues as these micromotors travel up the chemical gradient. This is the first illustration of inanimate objects demonstrating chemotactic behavior and the capability to move against concentration gradients towards the fuel source.

Tracing the chemical to its source, in the event of leakage, would take a proactive stance in averting release of toxic chemicals into the environment. Micromotors have been known to demonstrate chemotactic behavior towards fuel sources ranging from hydrogen peroxide^[52] to polymeric materials of nucleotides^[2] and simple organic molecules.^[53] Dey et al. illustrated the construction of bubble-propelled palladium-coated microspheres of polystyrene that move towards regions of higher alkali content (Figure 8A).^[52a] The biased movement of these artificial devices in an external pH gradient has been attributed to the chemotactic behavior towards hydroxide, which acts as a chemical attractant. The pH chemotaxi is able to move towards higher pH regions with increasing velocity, following the source of the alkali.

Later, Baraban et al. studied the motion of two asymmetric man-made catalytic structures in channel environments, specifically, platinum-based tubular RU micromotors and Janus-type microspheres, running on the decomposition of hydrogen per-

Figure 8. A) Schematic of a Pd microsphere moving towards an alkali-rich zone, away from the fuel-rich zone of hydrogen peroxide. Reprinted with permission from Ref. [52a]. B) Schematic of chemotactic attraction of catalytic micromotors in channels towards hydrogen peroxide-rich zones in a microfluidic flow cell with three merging inlets in Ψ -shape: Inlet i_1 contains hydrogen peroxide as the fuel; micromotors are introduced through inlet i_2 ; inlet i_3 contains water. Scale bar: 5 μm . Reprinted with permission from Ref. [52b]. C) Increasing angular deflection with increasing concentration of SDS at the reservoir: The SDS reservoir is labelled "s" and the inlet for introduction of SDS into circuit is labelled "x"; zero-velocity points give an angle shown by the red lines; paths along which the droplets moved are marked by dashed lines. Reprinted with permission from Ref. [16b].

oxide to oxygen (Figure 8B).^[52b] They showed that these artificial structures are able to move across a fuel gradient towards the source. The inherent sensing capacity to navigate in a channel environment and the self-induced directed movement towards desired locations may allow these systems to be transformed into first responders for chemicals, such as pollutants in water.

A similar chemotactic behavior of Marangoni PSf capsules towards the presence of surfactants using long-range interactions was also identified. Zhao et al. illustrated negative chemotactic behavior through the deflection and directionality change of a SDS-loaded PSf capsule in the presence of another

SDS-loaded PSf capsule, with these interactions extending over relatively long distances of several centimeters.^[17b] Under boundary conditions, optical marking of the origin for the source of chemicals can be realized in such a system by using the PSf capsule, demonstrating promise for optical checking and marking of leaks in channel and pipe environments, as seen in the confined environment in Figure 8C.^[16b] The behavior of PSf capsules was studied by using a circular track that contains increasing surfactant concentrations in a central reservoir.^[16b] Such confinement of the PSf capsule in a circular maze resulted in monotonic increase in angular deflection of the PSf with increasing concentrations of the SDS surfactant, demonstrating the capability to detect SDS concentration.

5. Nano/micro/macromotors polluting the environment

Environmental considerations of self-propelled objects and their possible implications on the surroundings are important points to note before their actualization in the field. Heavy-metal-containing nano/micromotors deployed under harsh conditions, together with the presence of corrosive hydrogen peroxide, mean that leaching is a possible concern. Furthermore, the cytotoxicity of such miniaturized materials must be established before wider employment can actually occur. Such long-range self-moving materials may in fact result in the increased transport and spread of pollutants. In this section, we explore the possible role of self-propelled objects as active spreaders of toxic pollutants.

5.1 Corrosion of micromotors

Nano/microtubular motors are often made from two or more different heavy metals, so as to introduce asymmetry into the structure of the nano/micromotors and confer propulsion.^[42b,54] Sequential layers of metals in electrical contact are known to exhibit a degradation phenomenon known as macrogalvanostatic corrosion in aqueous solutions, which is further exacerbated by the presence of an electrolyte.^[17d] The origin of such a behavior finds its basis in the different electrochemical potentials of the two metals. Such a continuous boundary between these metals results in a current flowing from the metal with a higher electrochemical potential to the metal with a lower electrochemical potential. The metal with the more positive electrochemical potential effectively dissolves, resulting in the corrosion of the metals. In the presence of the mixture of hydrogen peroxide and surfactant, concentric bimetallic tubular Cu/Pt micromotors were demonstrated to show corrosion of 1.3% Pt and 54% Cu by weight after a week (Figure 9A).^[24a] The effect is not limited to such template-assisted electrodeposited microtubes: rolled-up microtubes, which utilized a sequential deposition of multilayer sheets of metals, showed a similar behavior. In such circumstances, the differentially layered metals demonstrate corrosion at 3.9% for Pt, 11% for Ti, 29% for Cr, and 79% for Fe by weight. Elevated levels of heavy metals are especially detrimental to human health.^[55] Advocates for the use of micromotors must think about

Figure 9. A) Corrosion of concentric bimetallic Cu/Pt micromotors in various media. a) 7.76% H₂O₂ (fuel) and 1.15% SDS; b) 1.15% SDS; c) distilled water. Values stated reflect the amount of the micromotors corroded within 7 days. Reprinted with permission from Ref. [24a]. B) Cell viability of A549 cells determined from the WST-8 and MTT assays after a 24 h exposure to different dilutions of the stock solution containing the nanomotors. Reprinted with permission from Ref. [24b]. C) Calculated mean-squared displacement of 1,1-diphenylhydrazine aggregates on the surface of sea water. Reprinted with permission from Ref. [26].

whether the presence of such quantities of heavy metals will cause significant or justifiable amounts of poisoning to the biological subject or the natural environment.

5.2 Toxicity of micro/nanomotors

The toxicity of the nano/micromotors challenges the premise of whether such devices can be used in environmental and biomedical contexts. Studies of cytotoxicity in these materials are few and far between. Chng et al. recently demonstrated that an Au/Pt composite nanomotor fabricated through a template-assisted electrochemical method is cellularly benign and

can find possible applications *in vivo*.^[24b] To draw relevance for future possible *in vivo* applications, the A549 lung epithelial cell line was used. Assays of WST-8 and MTT of the A549 cell line of lung epithelial cells demonstrated >90% viability after 24 h exposure (Figure 9B). A lengthened exposure of 48 h in the presence of Au/Pt nanomotors showed little change. Additionally, the nanomotors show no distinguishable dose-dependent toxicity effects on cellular numbers.

5.3 Propagation of pollutants by self-propulsion

Modelling the transport of pollutants in the environmental system has often been undertaken by using a passive model based on diffusion. However, it has been shown that millimeter-sized aggregates of organic molecules are able to demonstrate self-induced motion, actively interacting with the surrounding environment. Zhao et al. illustrated this phenomena with the use of an aromatic hydrazine molecule, 1,1-diphenylhydrazine, in different natural aqueous media. The agglomerate demonstrated transport of up to 5 orders of magnitude larger than the standard molecular diffusion co-efficient, as illustrated by the mean-squared displacement values of the particle in seawater (Figure 9C).^[26] The asymmetric dissolution of these particles led to the build-up of differences in surface tension, thereby propelling them, a phenomena otherwise termed as the Marangoni effect. This alternative mechanism for pollutants to actively move through a chemically induced effect provides us with valuable input for consideration with regards to the spread of environmental pollutants. Self-propelled objects are able to travel long distances, at an order multiple times of their body length. Hence, these pollution factors are not localized and are only limited in accordance to the chemical energy provided. The possible ramifications of self-propelled objects include the dispersal of toxic materials due to corrosion effects, inherent toxicity due to nanoscale dimensions, and alterations to pollutant transport modelling. These are new environmental considerations due to the self-powered movement.

6. Conclusion and future outlook

Progress in nano/micro/macromotors has taken a huge leap since the initial observation of locomotion at the experimental stage. Particularly, the community's progress with surface modification and novel new materials has conferred new properties onto the motor. In this review, we have demonstrated the potential of such self-propelling objects for the active remediation of environmental pollutants through removal and degradation, the influence that the environment exercises on the nano/micro/macromotor, and their use in the detection of contaminants and the tracing of chemical plumes to the source. Furthermore, we have also addressed some of the concerns about such foreign autonomous self-powered man-made entities and their possible negative repercussions on the environment.

Although there are possible negative impacts from the use of such artificial self-propelled objects, the arguments we

raised paint a rosy picture for the future of nano/micro/macromotors. The autonomous motion of such self-propelled artificial objects are not to be overlooked as the efficiency and extent of reach are significantly amplified compared with the mere diffusive mechanism in the absence of an external force. The *in situ* conversion of chemical energy into mechanical motion raises the possibility for self-induced mixings for pollutant removal and contaminant degradation. Furthermore, the utilization of locomotion of these sensitized self-propelled devices to quantify and qualify chemicals has opened a new area of work with regards to sensors, tracers, and markers for environmental monitoring. This new platform is expected to address global challenges and future needs of mankind, creating new science and technologies for a better world in the face of environmental degradation.

Bringing these concepts and technological solutions to fruition still requires innovations from scientists and engineers alike. That said, these applications are still at the budding stage, where scaling to the industrial level and practical employment in the field have still to be realised. The prospects of these proof-of-concept self-propelled devices are still limited by several pressing issues raised in our report: movement is inhibited in real environments, limited lifetime, cytotoxicity, the role as a pollution vector, and the constraints of synthesizing these unique motors.

Design of these devices and their operations are currently at a threshold and key challenges remain, especially where additives such as surfactants and toxic hydrogen peroxide are used. A self-contained and self-sustaining device, independent from other external factors would be the ideal scenario. The architectonics of these motors is also limited by current synthetic methodologies, which are mainly carried out through the use of templates, template-assisted electrodeposition, or roll-up technologies. More studies will have to be carried out to offer more predictability for the collective behavior towards a certain task during applications, where the programmed motor can execute a definitive mode of work.

Several recent developments have shown promising results for the fabrication of swarming numbers of such motors for the performance of specific tasks. For example, application of roll-up technology by photolithographic methods coupled with electron-beam deposition of metals has been shown to have near 100% yield.^[56] Continuous advancement of metal deposition techniques such as glancing-angle deposition^[57] and morphology control methodologies by direct laser lithography^[58] will serve to design better and more intricate nano/micromotors.

When acting towards a common purpose, for example, for environmental clean-up, an ensemble action by multiple motors is especially attractive. Initial work has yielded preliminary results on the collective and swarming behavior of these miniaturized devices. This has recently been demonstrated in bubble-propelled motors,^[59] where previously these behaviors had only been observed in self-diffusiophoretic systems.^[22d,60] In collective emergent systems, with the mastery of energy and information control at the nano/microscale, it shall allow us to create intelligent communicating networks that are able

to accomplish complex tasks as a single unit for environmental remediative measures.

Performing work with nanotechnology, particularly for environmental remediation and detection, is a sphere of work worth exploration. With a saying coined by renowned scientist Richard Feynman: "There's plenty of room at the bottom," the coupling of nanotechnology and self-powered machines holds much promise. Although several key challenges lie ahead, the progress made in the last decade has given much confidence to the scientific community. As with the early 18th century, where the well-being of human civilization was improved by the industrial revolution, we hope that nanomachines can create new opportunities in the 21st century, especially in the areas of environmental remediation and monitoring.

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Nano/Microrobots Meet Electrochemistry

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Artificial autonomous self-propelled nano and microrobots are an important part of contemporary technology. They are typically self-powered, taking chemical energy from their environment and converting it to motion. They can move in complex environments and channels, deliver cargo, perform nanosurgery, act as chemotaxis and perform sense-and-act actions. The electrochemistry is closely interwoven within this field. In the case of self-electrophoretically driven nano/microrobots, electrochemical mechanism has been the basis of power, which translates chemical energy to motion. Electrochemistry is also a major tool for the fabrication of these micro and nanodevices. Electrochemistry and electric fields can be used for the directing of nanorobots and for detection of their positions. Ultimately, nano and microrobots can dramatically improve performances of electrochemical sensors and biosensors, as well as of the energy generating devices. Here, all aspects in the fundamentals and applications of electrochemistry in the realm of nano- and microrobots are reviewed.

the flow of electrons in such a “fuel cell”, leading to self-electrophoretic motion. In the same frame, electrochemical techniques such as electrodeposition, offers a precise and low cost option for the fabrication of these multi-component micro- and nanostructured objects. Additionally, electrochemistry and electric fields can be used for controlling the motion of these nano/micro or macrorobots. These self-propelling nano- and microrobots can enhance electrochemical sensing, which in turn can be used to detect motion of the robots. Furthermore, the microrobots themselves can be utilized as active parts of electrochemical and electrical energy generation devices. While a general coverage of the fabrication of nano/micro-motors has been accomplished,^[2,15,16] an in-depth discussion of the central role of electrochemistry from the electrosynthesis

1. Introduction

Nano and microrobots, also called nanomotors or nanosubmarines, are tiny devices capable of autonomous motion in the environment, exhibiting in many ways, motion similar to the motion of microorganisms.^[1–3] They can convert chemical energy from the environment (fuel, food) to kinetic motion. They can perform, similarly to microorganisms, actions of chemotaxis^[4,5] or magnetotaxis.^[6] Additionally, they are able to capture and release a target object, ranging from cells,^[7] DNA^[8] to proteins^[9,10] and release them at the given place. These devices can perform useful actions, from environmental decontamination^[11–13] to penetrating cell membranes and delivering small interfering RNA (siRNA) for treatment.^[14]

Electrochemistry is also an inseparable part in the majority of these devices. They can be powered by electrochemically generated energy, where the robot's body acts as a short-circuited fuel cell. The robot, itself is propelled by the flow of hydrated protons at the surface of nano/microrobot, which compensate for

of these nano/microdevices, electrochemical principles behind their operations, to the application stage is found wanting. The flow of electrons vis-à-vis the locomotion of nano/micro-particles is an integral component in order for movement in the miniaturized scale to occur. Here, we provide a review of this intimate relationship between electrochemistry and nano/microrobots.

In this article, we highlight the role of electrochemistry in synthesizing materials for self-powered nano/microdevices, the aspect of charge transfer and changes in electrochemical potentials for locomotion, control of self-propelled motion using electrochemistry and electric fields, possible applications in electrochemical sensing and energy generation using nano/microscale motion. The arrangement of the review is built from a bottom-up approach. Namely, they will be divided into: (i) Fabrication of nano/microrobot by electrochemical methods, (ii) Electrochemically powered nano/microrobots based on self-electrophoresis, (iii) Control of nano/microrobot motion through electrochemistry and electric fields and (iv) Applications of nano/microrobots in electrochemical sensing, mixing and energy generation.

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2. Fabrication of Nano/Microrobot by Electrochemical Methods

The electrochemical methods represent a versatile way for producing nano/microscale robots using simple experimental procedures, low-cost equipment and reagents, while being easy

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to scale as well. Here, we describe in detail the technology used to produce nano/microrobots at different sizes and shapes by electrochemical methods. For the better understanding, we classify the techniques into two groups: template-based (Figure 1A) and template-less (Figure 1B).

2.1. Fabrication of Template-Based Nano/Microrobots

The template-based method uses a template of porous membrane and electrodeposition techniques for sequential electrodeposition of different material into the holes of the membrane template, followed by nano/micromotors' release. For this aim, the three-electrode system is used. This composed typically of silver/silver chloride reference electrode (RE), platinum counter electrode (CE) and the working electrode (WE), that is a porous membrane with a conductive layer deposited on one side (see Figure 1A). Using membranes of different size and shapes, it is possible to obtain rigid,^[17,18] flexible,^[19,20] tubular^[21,22] and spiral^[23,24] nano/microrobots, while through the application of an external electric field (electrodeposition), a variety of materials such as metals,^[17,25,26] polymers^[17,27] and 2D materials^[11,28] can be deposited.

The first nanomotors produced by electrodeposition and using porous membrane as a template (Figure 2A) were bimetallic nanowires of gold (anodic end) and platinum^[17,18] or nickel^[25] (cathodic end). The non-Brownian movement was propelled in aqueous hydrogen peroxide solutions (H₂O₂) and two different mechanisms were proposed for its motion: by establishment of a concentration gradient involving self-phoretic mechanisms^[17,29] and by growth and recoil of oxygen bubbles in the Pt or nickel end.^[25] Subsequently, to improve the nanomotors' speed, the electrochemical deposition of alloyed Ag-Au replaced the gold segment^[30] and carbon nanotubes (CNT) were incorporated in the Pt segment.^[31] Furthermore, Zacharia et al. demonstrated that the addition of silica nanoparticles into the nickel or gold solution for co-deposition with the metal, resulted in the enhanced catalytic efficiency of the bimetallic nanowires by increasing the surface area, which in turn leads to enhancement in the speed of the nanowire motors.^[32] Looking for an alternative fuel, a bubble-free nanomotor based on asymmetrical bimetallic Cu/Pt nanowire was proposed, this system was propelled in dilute aqueous solutions of Br₂ or I₂. The motion mechanism proposed was self-electrophoresis induced by the redox reaction which occurs at both terminals of the nanomotors. Moreover, they demonstrated that the velocity of nanomotors depends on the fuel concentration and the rod length.^[33]

All these nanomotors displayed autonomous movement and the direction of movement was subject to random fluctuations. The directed motion of these catalytic nanomotors was the next step to improve the behavior of motors. This was done by the electrochemical incorporation of nickel segment of nanomotors between the platinum and gold segments, allowing the guidance by magnetic field (Figure 2B).^[34] This development gave rise to the implementation of the rigid and flexible nanowire motors that move in presence of rotary magnetic field. Zhang et al. prepared Ni nanowires by electrodeposition through nanoporous templates, where these nanowires can be driven by a

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uniform rotating magnetic field in water. These nanowires can be propelled and precisely steered near horizontal, vertical, or curved walls by a tumbling motion. Moreover, cargo transport of 6 μm diameter microbeads by such nanowires was implemented successfully.^[38] Ferromagnetic Janus nanowire was operated via rotation in a plane nearly parallel to the horizontal plane.^[39] Gold nanowires were obtained by template-based and electrodeposition techniques; then Janus layer formation carried out via the nickel thermal evaporation process. In addition, the magnetic Janus nanomotors were incubated with human bronchial epithelial cells (HBE-16) to manipulate these cells via rotational maneuvering using individual nanomotors.^[39]

The Ozin team introduced a new class of flexible nanomotors based on Ni/Au/Pt nanowires with hinges made of polyelectrolyte multilayers (PEMs), prepared by electrochemical deposition, as illustrated in Figure 2C (i). The flexible nanowire is synthesized using template-based electrochemical growth of rigid nanowires of different metals segments, with the layer by layer self-assembly of PEMs, which was used as a hinge and the eventual segment-selective chemical etching of gold using KI/I₂ solution. The resulted Ni-Pt PEM1 nanomotor (PEM1 = one bilayer of polyelectrolytes), showed bending in the presence of magnetic field. The Pt end of the nanomotor was fixed and only the Ni end responded to the external magnetic field.^[19]

Magnetically driven fuel-free flexible metallic nanowires were also developed by electrodeposition. This flexible nanomotor was prepared by sequential electrodeposition of the Au, Ag, and Ni segments into the alumina membrane followed by partial chemical etching of the silver segment using hydrogen peroxide (Figure 2C (ii)).^[20] This approach, in comparison to its predecessor is easier to produce and the Au segment can be functionalized. Moreover, the locomotion of the nanomotors (forward or backward motion) was controlled by the modification of the length of the Au segment (head) and Ni segment (tail); and the nanomotors' speed can be controlled by varying the frequency of the applied field.^[20] Following that, the same group proposed a new simplified design of flexible nanomotors based on Ni (head) and Ag (flexible tail) segments prepared by electrodeposition.^[40] These flexible nanomotors were propelled

Figure 1. Schematic drawing for A) template-based vs B) template-less electrochemical fabrication methods of nano/microrobot. A) Three electrode system for template-based nano/microrobots fabrication (WE: working electrode, which is a porous membrane coated with conductive layer; RE: reference electrode; CE: counter electrode). B) Template-less fabrication via bipolar electrochemistry, illustrating a cell for template-less nano/microrobots fabrication.

at high speeds using external magnetic field. The ions or other chemical species present in the real biological settings, did not affect the performance of the nanomotors.^[40] For this reason, the authors were able to perform a directed delivery of drug-loaded magnetic polymeric particles to HeLa cancer cells using magnetically driven flexible nanomotors through a micro-channel in biological media.^[41] Recently, Jang et al. reported an undulatory movement under oscillating magnetic field of flexible nanomotor composed of a flexible tail of polypyrrole and the head of two segments of nickel connected by hinges,^[42] as they adapted the fabrication concept reported by Ozin and co-workers.^[19] The undulating motion observed mainly occurs along the flexible tails. The authors observed that the efficiency in terms of velocity increases when they used two nickel segments instead of a single-segmented design in the head of nanomotors.^[42]

In recent years, the attention of scientists has been focused on fuel-free acoustic nanomotors based on rigid nanowires due to its great potential in biomedical applications. These kinds of nanomotors are propelled by ultrasound and are prepared using template-based electrochemistry methods. Mallouk's team demonstrated that Au and Au/Ru nanowires, in the presence of ultrasonic standing waves at high frequency range (MHz), are able to propel, rotate, align, assemble and have an autonomous motion in water and in high ionic strength solutions.^[18] Moreover, they demonstrated by a contrasting control experiment (using spherical particles and polymeric wires) that the directional motion mechanism (self-acoustophoresis) of these nanomotors in an acoustic field is due to the concave or convex ends of the nanowires prepared using template-based electrodeposition method.^[18] When the acoustic waves are scattered, the energy is concentrated at the curvature of concave end. While at the convex end, the acoustical scattering is radially weakened. The energy density distribution generates a localized

pressure gradient that affects only the individual nanowire. The shape of the concave end, is generated upon the deposition of the sacrificial silver layer, during the electrodeposition of the gold nanowires in the alumina membranes.^[18] Wang and co-workers explored possible pathways for an enhanced performance of such electrodeposited motors by including a nickel segment for magnetic guidance and improved its velocity via the template-based electrodeposition method.^[35] Moreover, they evaluated the dependence of nanomotor velocity with ultrasound transducer power in deionized water, seawater, serum, and saliva. Finally, they performed the cargo transport of magnetic bead, *E. coli* and *S. aureus* using unmodified, lectin-modified and Concanavalin A-modified Au/Ni/Au nanowires respectively.^[35] To extend the application of these nanomotors for drug delivery capability, an electropolymerized polypyrrole-polystyrene sulfonate (PPy-PSS) segment is added to Au/Ni/Au nanowire motor (see Figure 2D (i)).^[35] PPy-PSS serves as a carrier for the brilliant green (BG) (used as model antiseptic drug) via electrostatic forces (between the positive charge of the drug and the negatively charged PPy-PSS polymeric backbone).^[35] Extending their work described above, Wang's team developed approaches for environmental control and biomedical application. Making use of the advantageous functionalization of these gold nanomotors and their compatibility with biological systems, a highly efficient and rapid bacteria-killing strategy based on the coupling of antibacterial activity of the lysozyme with the continuous movement of fuel-free nanowire motors was reported.^[36] In this case, the nanomotor is composed of two segments, one of solid gold and the other of porous gold (Figure 2D (ii)). This porous segment was obtained from the electrochemical deposition of alloyed gold and silver followed by etching of silver component. Moreover, the porous gold allows the immobilization of lysozyme by using the cysteamine method.^[36]

Figure 2. Electrochemical deposition of wire-based nanomotors. A) Rigid and flexible nanomotors produced by sequential electrodeposition of different materials into the membrane holes. B) Au/Ni/Au/Ni/Pt multi-segmented nanowire. Reproduced with permission.^[34] Copyright 2005, Wiley-VCH. C) (i) Flexible nanomotor propelled by magnetic field. Reproduced with permission.^[19] Copyright 2007, Nature Publishing Group. (ii) Au-Ag/Ni nanowire which has a partial dissolution of the central silver segment. Reproduced with permission.^[20] Copyright 2010, American Chemical Society. D) (i) Three or four-segments of Au/Ni/Au and polypyrrole-polystyrene sulfonate (PPy-PSS)/Au/Ni/Au nanowires. Reproduced with permission.^[35] Copyright 2013, American Chemical Society. (ii) Porous gold (p-Au)/solid Au (s-Au) nanowire with concave end in the Au segment. Reproduced with permission.^[36] Copyright 2015, American Chemical Society. E) SEM images of PPy/Cd and CdSe/Au/CdSe nanomotors that can be propelled under external AC electric field. Reprinted with permission.^[37] Copyright 2010, Royal Society of Chemistry.

The same group combined these synthetic nanomotors with DNA technology for rapid monitoring of miRNA-21 expression in individual intact cancer cells by “OFF-ON” fluorescent switching using gold nanomotors modified with graphene oxide,^[43] and as a powerful therapeutic option for siRNA (small interfering RNA’s) delivery that greatly enhances intracellular gene delivery compared to existing methods.^[14] In addition to the ultrasound-driven nanomotors described previously, red blood cells^[44] were coated to gold nanowires for obtaining biomimetic nanomotor sponges. These nanomotor sponges present an efficient movement in blood and rapidly neutralize the membrane-damaging toxins, melittin in red blood cells.^[45]

On the other hand, during the search for other alternative fuel-free nanomotors, researchers have developed nanowires prepared by membrane template-assisted electrodeposition whose motion was powered by electric field. In this way, the first studies were reported by Chien and co-workers. They showed a method to control gold nanowires by AC electric field distribution in terms of assembly, chaining, acceleration in a direction parallel or perpendicular to its orientation, concentrating populations of these devices onto designated places.^[46] This group also reported other methods for assembling gold nanowires in suspension into scaffolds using strategically designed electrodes and AC electric field, demonstrating that the assembling efficiency and the morphology are strongly dependent on the applied AC voltage as well as the frequency.^[47] Following the same line of research, the Chien group implemented a controllable high-speed rotary Au, Pt and Ni electrochemically prepared nanowires using a rotating electric field provided by AC voltages applied to multiple electrodes. Moreover, the rotation of the nanowires can be instantly switched on or off with precisely controlled chirality, rotation speeds and total angle of rotation.^[48] Finally, Calvo-Marzal and co-workers, demonstrated that polypyrrole/cadmium (PPy-Cd) and CdSe-Au-CdSe Schottky barrier nanodiodes fabricated by electrodeposition through porous alumina membrane template (Figure 2E) can be propelled under a spatially uniform AC electrical field.^[37]

The tubular micromotors produced by electrodeposition and template-based methods, appear as a cost-efficient alternative means in comparison to rolled-up micromotors produced by photolithography. The approach for this type of micromotors was reported by the Wang team. The conical microtube engine is based on the sequential electrochemical deposition of platinum and gold layers onto a silver wire template, followed by etching of the silver segment.^[49] After that, they developed catalytic tubular micromotors based on simplified conical membrane-template (Figure 3A). These micromotors are composed of polyaniline (PANI) and platinum, electrodeposited in a layer by layer fashion, gradually from the outer layer to the inner layer. The outer layer (PANI) defines the shape, while the inner layer (platinum) is the catalyst material capable of decomposing the fuel and producing the bubbles.^[21] A commercially available cyclopore polycarbonate membrane, with a symmetrical pore structure of double-cone, is used for the preparation of this bilayer microtubular structure. First, the aniline monomer polymerizes on the inner wall of the membrane, by solvophobic and electrostatic interactions.^[21] After which, the platinum layer is electrodeposited inside the polyaniline tube (Figure 3B (i)). This approach allows mass production and created smaller-sized micromotors, as compared with the rolled-up microengines, requiring low fuel concentrations and moving at very high speeds.^[21]

Later, PANI/Pt microtubular engines are modified with layers of gold, nickel and titanium by electron beam deposition. The Au layer of the engines allow the easy functionalization with the Concanavalin A (ConA) lectin bioreceptor for the fast and real-time separation of *Escherichia coli* bacteria from diverse real samples.^[9] Moreover, the PANI/Pt tubular micromotors were used as non-invasive micro mixers in conventional immunoassay.^[53] Searching for other fuels, Wang team proposed acid-driven hydrogen-bubble-propelled micromotors

Figure 3. A) Tubular nano/micromotors fabricated by electrodeposition and template-based methods. B) SEM images of polymer/metallic tubular micromotor. (i) Polyaniline (PANI)/Pt. Reproduced with permission.^[21] Copyright 2011, American Chemical Society. (ii) Poly(3, 4-ethylenedioxythiophene) (PEDOT)/Pt. Reproduced with permission.^[27] Copyright 2012, Royal Society of Chemistry. (iii) PANI/Ni/Zinc. Reprinted with permission.^[26] Copyright 2012, American Chemical Society. C) Metallic tubular nano/micromotors. (i) Au/Pt nanomotors. Reproduced with permission.^[22] Copyright 2013, Royal Society of Chemistry. (ii) Cu/Pt micromotors. Reproduced with permission.^[50] Copyright 2013, Royal Society of Chemistry. (iii) Cu/Ag micromotors. Reproduced with permission.^[51] Copyright 2016, Royal Society of Chemistry. D) Aptamer modified-rGO/Pt micromotors. Reproduced with permission.^[52] Copyright 2016, American Chemical Society.

based on PANI/zinc electrodeposited microtube with autonomous motion in highly acidic media, by generating hydrogen bubbles by the inner Zn surface (Figure 3B (iii)).^[26]

Different polymers for the outer layers and inner catalytic metal surfaces were evaluated and Gao et al. determined that poly (3, 4-ethylenedioxythiophene) PEDOT/Pt tubular micromotor (Figure 3B (ii)) is faster than other micromotor produced by other materials.^[27] The incorporation of nickel layer between PEDOT and Pt allow the magnetic guidance, making this micromotor an autonomous engine for several applications for environmental control^[54] to biomedical applications.^[10,55] Moreover, taking advantage of the carboxyl groups of the PEDOT surface, the immobilization of antigen for cargo application was demonstrated.^[56] Other interesting implementation for capture and cargo transport of specific target was done through complementary nanocavities in the outer polymeric layer of the micromotors. The polymeric layer was electrodeposited in the presence of the target analyte, which was used as imprinting the mould into the micromotor's structure.^[10] The electropolymerization

of poly (3-aminophenylboronic acid) (PABA) was used instead of PEDOT and PANI in the tubular micromotors to provide the autonomous loading, directional transport, and triggered unloading ("catch and release") of target analytes such as specific monosaccharides and yeast cells.^[56]

Wang's team prepared microtubes based on the PEDOT/gold framework electrochemically, where the inner gold layer was modified with catalase. The resulting micromotor was powered by H₂O₂ decomposition by enzyme immobilized in the inner gold layer. The water quality testing was performed by the micromotor as its speed decreased when catalase was inhibited in the presence of pollutants.^[57]

Pumera's group studied template-assisted electrodeposited nano/microtubes of different sizes under bubble propulsion.^[22,50,51,58] The gold/platinum (Figure 3C (i))^[22] and platinum nanotubes^[58] reported, represented the smallest bubble propelled nanomotors fabricated by template-based method. These nanomotors have similar dimensions to bimetallic nanowires described before.^[17,25] To achieve this tubular structure, instead of sputtering a gold layer, a film of colloidal graphite ink or silver conductive ink was applied on one side of the polycarbonate or AAO template for the electrosynthesis of Pt or Pt/Au nanotubes respectively. Afterwards, the sacrificial copper layer was applied at higher currents (−10 and −2 mA for Pt or Pt/Au nanotubes respectively). These modifications allowed metal depositions exclusively onto the membrane walls, creating the nanotubes, not the nanowires. The fabrication process of copper/platinum^[50] (Figure 3C (ii)) and copper/silver^[51] (Figure 3C (iii)) micromotors were also reported, being similar to those used to fabricate Pt/Au nanotubes.^[22]

Wang et al. developed tubular micromotors based on electrodeposition of graphene and metal (Pt, Au).^[28] The electrodeposition of graphene oxide by cyclic voltammetry in a negative potential window, reduced the oxygen moieties in the graphene surface,^[59] restoring the sp² carbon network and its hydrophobicity. Consequently, the aggregated graphene layers on the inner wall of the membrane are produced by hydrophobic interactions.^[28] This graphene film is conductive and favored the electroplating of platinum layer. The resulting tubular micromotors shows higher speed in very low concentration of H₂O₂ due to the rough morphology observed in the inner Pt.^[28] The toxin (ricin B) sensing for the biodefence application was demonstrated; this system took advantage of the quenching capabilities of the graphene for "off-on" real-time fluorescence for ricin B detection (Figure 3D).^[52] These micromotors were used for capture, transfer, and removal of Pb. The abundant amount of carbonyl and carboxyl groups in graphene component of the motor surface, allowed the adsorption of Pb.^[13,60] Later, a modified ZrO₂-graphene/Pt micromotors is proposed for selective capture of nerve agents, in this case graphene and ZrO₂ NPs are co-deposited in a one step. The selective capture of chemical warfare agents (CWAs) is done by acid–base Lewis interaction between phosphate groups (Lewis base) of the CWAs and the electron-deficient zirconium cation (Lewis acid) of the zirconia present in the outer layer of the micromotor.^[61]

Transition metal dichalcogenides represent another group of interesting two-dimensional materials that recently attracted the attention of the research community due to the promise of better energy storage, catalysis and biosensing.^[62] With this

aim, our group developed a novel tubular microrobot based on the co-electrodeposition of polyaniline and tungsten disulphide nanoparticles layer as an active material for the charge storage application^[62] and Pt layer as a catalyst component for the microrobots propulsion. The application as a self-propelled microcapacitor was also demonstrated. Besides, Singh et al. also prepared a tubular microrobot based on an amorphous thin layer of molybdenum disulphide, for load/release drug and fluorescent detection of nucleic acids and protein by the quencher capabilities of the MoS₂.^[63,64] These are demonstrated here for efficient loading and release of the drug doxorubicin, and rapid and sensitive “OFF–ON” fluorescent detection of important nucleic acids (miRNA-21) and proteins (thrombin).

Recently, template electrodeposition method for the production of small helical magnetic nanomotors was reported.^[23] Such approach consists of the growth of Pd/Cu nanowire by electrodeposition into the pores of the membrane, followed by chemical etching of the Cu and Ni layer coated by electrobeam, producing small helices that are magnetically driven, as illustrated in Figure 4A.^[23] The formation of the nanosprings is based on the localized hydrogen evolution reaction through

the hydroxyl-terminated surfaces of alumina nanochannels that allow the peripheral growth of the Pd atoms in the acid plating solutions containing Pd and Cu ions by applying a fixed potential.^[65] For this reason, Li et al. modified the polycarbonate membrane with alumina by atomic layer deposition.^[23] Following along the same line of research, Li et al. developed a template-based bi-segment hybrid nanomotors, which is propelled in the presence of magnetic or acoustic fields (see Figure 4B).^[24] These hybrid nanomotors, are composed of a gold segment and Pd-coated Ni nanospring segment. The propulsion in biological media and the control of the speed, directionality and collective behaviors by changing the field inputs were demonstrated.^[24]

2.2. Fabrication of Template-Less Nano/Micromotors

Bipolar electrochemistry is a simple electrochemical technique based on the polarization of electrically conducting materials (bipolar electrode) in electric fields by two driving electrodes (Figure 1B). In this way, numerous objects (the bipolar electrodes) can be dispersed in the solution and modified simultaneously without a direct electrical contact. This technique is able to produce gradients of electrodeposited materials as well as to provide a means for site-selective deposition.^[66,67] Taking advantage of these qualities, Kuhn's team used bipolar electrochemistry as an alternative method to generate dissymmetric micro- and nano-objects, that can be powered by bubble propulsion^[68] and magnetic field^[69] for locomotion. They produced asymmetric carbon nanotubes decorated with nickel (Figure 5A (ii)) and propelled them in a magnetic field. In this case, the electrochemical reduction of nickel ions to the metallic state during deposition is produced in the opposite terminal where water oxidation occurs.^[69] In the same way, they also prepared asymmetric carbon microtubes with platinum ends (Figure 5A (iii)). These Pt-carbon microtubes are bubble-propelled in presence of H₂O₂.^[68] The micromotors are fabricated using capillary assisted bipolar electrodeposition, as they incorporated a capillary electrophoresis setup to apply the electric field (Figure 5A (i)).^[66,68,69] Later, they synthesized Janus nano/microobjects from carbon tubes and glassy carbon beads modified with platinum and gold respectively (Figures 5B (ii) and 5B (iii)).^[70] For this aim, a new methodology was implemented that consists of a three-compartment cell (Figure 5B (i)), one for the electrochemical reactions (containing the substrates and the reagents in a highly viscous solution) and the other two compartments where gold and platinum electrodes are immersed in solvent containing supporting electrolyte connected to a high-voltage power supply. In this way, they limited the bubble formation, while avoiding solvent evaporation and convection due to Ohmic heating.^[70]

The same group produced a partial oxidation of the electrically conductive hybrid polyaniline-alginate beads (Figure 5B (iv)) by bipolar electrochemistry; these beads display pulsating and continuous motion that depends on the oxidation time and the ethanol loaded inside the beads, before the bipolar electrochemical treatment. The hydrogel beads are propelled on the water by Marangoni effect. When the ethanol is released from oxidized side of the conductive hydrogel beads it produces low

Figure 4. A) Helical magnetic nanomotors. (i) Template-based fabrication of helical magnetic nanomotors. Reproduced with permission.^[23] Copyright 2014, Royal Society of Chemistry. (ii) SEM images of Pd/Cu nanowire, the Pd helical nanospring and the Ni-coated Pd helical nanospring. Reproduced with permission.^[23] Copyright 2014, Royal Society of Chemistry. B) Magneto–acoustic hybrid nanomotors. (i) Template-assisted fabrication of the bisegment magneto–acoustic hybrid nanomotors. Reproduced with permission.^[24] Copyright 2015, American Chemical Society. (ii) SEM image of a magneto–acoustic hybrid nanomotor. Scale bar: 500 nm. Reproduced with permission.^[24] Copyright 2015, American Chemical Society.

Figure 5. A) (i) Capillary assisted bipolar electrodeposition set-up. Reproduced with permission.^[69] Copyright 2010, Elsevier. (ii) Selective bipolar electrodeposition of nickel at one end of a carbon tube. Reproduced with permission.^[69] Copyright 2010, Elsevier. (iii) Selective bipolar electrodeposition of platinum at one end of carbon microtube. Reproduced with permission.^[68] Copyright 2011, Elsevier. B) (i) Bipolar electrodeposition cell composed of three compartments. Reproduced with permission.^[70] Copyright 2012, Wiley-VCH. (ii) Carbon tube modified with platinum through bipolar electrodeposition. Reproduced with permission.^[70] Copyright 2012, Wiley-VCH. (iii) Glassy carbon beads of 20 μm modified with gold through bipolar electrodeposition respectively. Reproduced with permission.^[70] Copyright 2012, Wiley-VCH. (iv) Asymmetrically modified PANI-alginate electroconductive hydrogel bead, obtained by partial oxidation using bipolar electrochemistry. Reproduced with permission.^[71] Copyright 2015, Royal Society of Chemistry.

surface tension locally and the beads were driven into the direction of higher surface tension.^[71]

In the **Table 1**, the template-based and template-less electrochemical fabrication methods for producing nano/microrobot of different sizes, shapes and materials are summarized. **Table 2** describes the comparison of the template-based against the template-less electrodeposition method. While template-based methods are time-consuming, due to the numerous washing steps involved to release the micromotors from the template, they offer wider possibilities of dimensions and materials to be used. The inherent need for template-less method to achieve bipolar potential differences in particles limits the size of the particles to be micrometer-sized, but it makes up ground by using a facile single step to introduce asymmetry into the microrobots.

3. Electrochemically Powered Nano/Microrobots Based on Self-Electrophoresis

One of the most common propulsion mechanisms is based on electrochemically generated self-electrophoresis.^[72] The particle acts as a short-circuited fuel cell. As illustrated in **Figure 6A**, the redox reactions (electrochemical reactions) at the two ends of the particle generate chemical gradients which will induce self-generated local electric field. Ions in the double layer at the surface of the particle migrate in response to the electron flow inside the particle, leading to fluid flow adjacent to the particle and propelling

it in the opposite direction.^[1] Based on the self-electrophoresis mechanism, a variety of electrochemical devices including motors, rotors, transport vehicles, pumps and sensors have been developed. These will be described in the sections below.

3.1. Motors

One typical example of self-electrophoresis is the motion of bimetallic nanorods in H_2O_2 solution.^[17] As an important type of self-propelled nano/micromotors, bimetallic nanorods have been extensively studied by researchers. Oxidation and reduction reactions occur at the opposite ends of the nanorod:^[78]

The four-electron reduction of O_2 to H_2O is suggested to be the predominant cathodic reaction. The half-cell reactions result in a proton concentration gradient, which generates an electric field. The redox reactions at the two ends of the nanorod set up

Table 1. Fabrication of nano/microrobots by different electrochemical methods.

Fabrication Methods	Type/Shape	Material	Size
Template-based	Rigid	Au/Pt ^[17]	370 nm × 2 μm
		Au/Pt-CNT ^[31]	250 nm × 3.1–6 μm
		Cu/Pt ^[33]	200 nm × 1–4 μm
		Au/Ni/Au/Ni/Pt ^[34]	400 nm × 1.5 μm
		Au/Ni/Au ^[36]	250 nm × 1.8 μm
		Ag-Au/Au ^[20]	200 nm × 2 μm
		Au ^[47]	300 nm × 10 μm
		PPy/Cd ^[37]	200 nm
	Flexible	{Ni...Pt}PEM ^{*(19)}	200 nm × 5 μm
		Au/Ag/Ni ^[20]	200 nm × 6.5 μm
		Ni/Ag ^[41]	200 nm × 5.8 μm
		PPy/Hinge ^{**} /Ni/Hinge/Ni ^[42]	200 nm × 15.5 μm
	Tubular	Au/Pt ^[22]	300 nm × 4.5 μm
		PANI/Pt ^[21]	2 μm × 8 μm
		PEDOT/Pt ^[27]	0.8 μm × 4 μm
		PANI/Ni/Zn ^[26]	5 μm × 20 μm
		Cu/Pt ^[50]	2 μm × 7 μm
		Cu/Ag ^[51]	3.5 μm × 15 μm
		rGO/Pt ^[28]	5 μm × 10 μm
		GO/Pt ^[11]	5 μm × 20 μm
		MoS ₂ /Pt ^[63]	5 μm × 20 μm
		PANI-WS ₂ NPs/Pt ^[62]	3.5 μm × 10 μm
	Nanospring	Ni-coated Pd ^[23]	2–3 μm × 30–50 μm
		Au/Ni-coated Pd ^[24]	200 nm × 3 μm
Template-less	Wires	Carbon tube/Pt ^[68]	5 to 20 μm
		Carbon tube/Ni ^[69]	20 μm
	Janus	PANI-alginate ^[71]	2 mm

an internal electron flow and simultaneous proton migration in the electrical double layer at the metal-solution interface. The resultant fluid flow causes the propulsion of the nanorod in the other direction. According to the Helmholtz-Smolucchowski equation, the velocity due to self-electrophoresis can be calculated from, $U = \mu_e J/k$, where μ_e is the electrophoretic mobility of the motor, J is the current density due to the electrochemical reaction and k is the conductivity of the solution. As a result, the velocity of the self-electrophoretic micromotors decreases linearly with the conductivity of the solution.^[29]

In addition to bimetallic nanorods, a series of bimetallic Janus particle motors relying on self-electrophoresis have been

developed. Wheat et al. reported a facile method of fabricating bimetallic spherical motors that are able to exhibit motion in the presence of H₂O₂.^[79] Polystyrene spheres are first fully coated with Au by repeated processes of sputter coating and resuspension to ensure the entire surface is covered, followed by the sputter coating of one hemisphere with Pt. Gibbs et al. provided another way to prepare asymmetric Pt/Au coated silica spherical motors with various Au exposure areas (A) using dynamic shadowing growth method.^[80] The rotation of the substrate with a polar angle after Au deposition by electron beam evaporator allows the subsequent Pt deposition to leave part of the Au layer exposed. By tuning the rotating angle, the exposed Au area can be changed systematically. In agreement with the electrochemically induced self-electrophoresis mechanism, the average moving speed, u of the motor in H₂O₂ is found to scale as $u \propto A^{3/2}$. The comparison between the fabricated Pt/Au micromotor and Pt-only micromotor which has the same Pt-coating area demonstrates that self-electrophoresis mechanism generated greater active motion. Additionally, the bimetallic Janus nanoparticles reported by Lee et al.^[73] revealed that self-electrophoresis can also be effective at the nanoscale, comparable with some enzymes (Figure 6B). The 30 nm Pt-Au Janus nanoparticles prepared by glancing angle deposition of Au on Pt nanoseeds are capable of displaying enhanced diffusion at the scale where no actively propelled living organisms can be deployed.

The fuel for electrochemically driven self-electrophoretic motion of nano/micromotors is not limited to H₂O₂. Researchers have devoted considerable efforts to develop systems where other fuel can be used. A light-driven TiO₂-Au self-electrophoretic micromotor powered in pure water under an extremely low ultraviolet light intensity has been demonstrated by Dong et al.^[74] Due to the photocatalytic activity of the TiO₂-Au system, oxidation of water occurs at TiO₂ upon exposure to UV light and the resulting electrons are consumed by the reduction of protons at Au (Figure 6C). The movement of protons generates a fluid flow toward Au near the micromotor surface, propelling the micromotors with the TiO₂ hemisphere facing the front. The light induced self-electrophoresis mechanism allows the propulsion of the micromotors to be switched on and off by controlling light. Speed acceleration of the micromotors can be accomplished by increasing the light intensity or by adding low concentrations of H₂O₂ to enhance self-electrophoretic effects. The insertion of a deposited paramagnetic Ni layer between Au and TiO₂ enables magnetic control of the directionality of the motion. It has also been demonstrated that dilute aqueous Br₂ or I₂ solutions can be used as fuel to propel Cu-Pt nanorods on the basis of self-electrophoresis.^[33] As shown in Figure 6D, in aqueous Br₂ or I₂ solution, the Cu end acting as the anode is oxidized while the Pt end serves as the cathode where the reduction of halogen occurs. The redox reactions at the two ends will induce self-electrophoresis to

Table 2. Comparison of the electrochemical methods used for nano/microrobots fabrication.

Fabrications method	Procedure	Size	Materials
Template-based	Multi-steps, high consuming time.	From nanometer to micrometer scale	Polymer, metal, 2D-material and mixture.
Template-less	One-step, low consuming time.	Micrometer scale	Metal and polymer.

Figure 6. A) Schematic illustration of self-electrophoresis mechanism. B) The self-electrophoretic motion of Pt-Au Janus nanoparticle in H_2O_2 . Reproduced with permission.^[73] Copyright 2014, American Chemical Society. C) UV light induced self-electrophoresis of TiO_2 -Au Janus micromotors in water. Reproduced with permission.^[74] Copyright 2016, American Chemical Society. D) Self-electrophoresis mechanism of Cu-Pt nanorods in (i) aqueous Br_2 and (ii) aqueous I_2 . Reproduced with permission.^[33] Copyright 2011, American Chemical Society. E) Biocatalytically induced self-electrophoresis of (HRP)PPy-Au(Cyt c) nanorods in O_2 and H_2O_2 solutions. Reproduced with permission.^[75] Copyright 2014, Wiley-VCH. F) Schematic image of the design of fast microscale rotors. Reproduced with permission.^[76] Copyright 2009, American Chemical Society. G) The structure of Au gear-like rotor with Pt deposited in the tooth regions. Reproduced with permission.^[77] Copyright 2005, Wiley-VCH.

propel the nanorod. By measuring the open-circuit potential, the redox couples were determined. In aqueous Br_2 , the Cu segment is oxidized:

Dissolved in water, Br_2 partially disproportionates into HBrO and HBr . HBrO was preferentially reduced:

In aqueous I_2 , the Cu segment is oxidized to CuI and the standard potential is calculated based of the solubility of CuI , whereas I_2 is reduced.

However, the Cu-Pt micromotors have the disadvantage of limited lifetime due to the reaction of the Cu segment. Corrosion is a general problem of non-noble catalytic micromotors.^[81]

Attributable to the great potential of nano/micromotors to be employed in biomedical field, development of biocompatible fuel has attracted considerable interest. The propulsion of a carbon fiber modified with glucose oxidase and bilirubin oxidase at the water- O_2 interface, in a similar way how electrochemical biofuel cells work, was demonstrated by Mano et al.^[82] The redox centers of the two enzymes at the opposite ends were electrically connected to the fiber by redox polymer. The oxidation of glucose on the glucose oxidase (GOx) end and O_2 reduction on the bilirubin oxidase (BOD) end will generate an electron flow through the carbon fiber, which is accompanied by the transport of ions in the electrical double layer of the fiber and propels the fiber in the opposite direction. Another example of biocatalytically induced self-electrophoresis motion is the propulsion of a nanorod with a polypyrrole (PPy) segment and a

gold segment, which are modified with horseradish peroxidase (HRP) and Cytochrome C (Cyt c) respectively, in mixtures of xanthine oxidase (XOD) and hypoxanthine (Figure 6E).^[75] Xanthine oxidase is known to generate $O_2^{\cdot-}$ and H_2O_2 in the presence of its substrate, hypoxanthine, both in vivo and in vitro. The immobilized Cytochrome C is reduced by $O_2^{\cdot-}$, while the immobilized horseradish peroxidase is oxidized by H_2O_2 . As both hemeproteins are capable of direct electron transfer to and from solid substrates, the reactions at the two ends will cause an electron flow through the nanorod and generate self-electrophoretic motion.

The self-electrophoretic motors can also be involved in dual propulsion systems employing both chemical energy and other forms of energy, such as magnetic field and ultrasound. The combination of chemically powered propulsion and magnetically driven motion has been demonstrated on a multi-segment nanowire, which sequentially contains Pt, Au, flexible Ag and Ni segments.^[83] The Pt-Au portion is responsible for self-electrophoretic propulsion in the presence of H_2O_2 fuel while the Au-Ag_{flex}-Ni portion is used for magnetic actuation under rotating magnetic field. The motion induced by the two modes of propulsion is in opposite direction, thus facilitating on-demand change in the motion direction. Additionally, the simultaneous chemical and acoustic propulsion can be realized on bimetallic nanorods without further modification on the structure.^[84] The nanorods will experience fast transition between organized assembly and random motion upon switching ultrasound on and off. The different propulsion forces also enable motion in reverse directions.

While nano/microrobots based on self-electrophoresis has been purported to carry out complicated biomedical tasks, it remains limited on the biocompatibility of the fuel of choice. As seen in Table 3, most of these self-propelled devices are based on hydrogen peroxide chemistry. In recent times, new self-electrophoretic mechanisms using photochemistry^[74] and biologically electroactive molecules^[75,82] have led to prospective biocompatible fuels that show promise for in vivo operations. Coupling alternative propulsion systems to a biocompatible fuel in a self-electrophoretic mechanism may present new opportunities for powering nano/microrobots for drug delivery.

3.2. Rotors

In addition to self-electrophoretic motors displaying linear motion, self-propelled rotors relying on electrochemically induced self-electrophoresis which are capable of exhibiting rotational behavior have also been demonstrated by several groups. Ozin and co-workers have described two kinds of rotational behavior.^[25] The prepared Au-Ni nanorods can exhibit both tethered rotational motion around an impurity anchor site and circular motion in orbit around a center. The asymmetry in the long axis contributes to directional axial motion of the nanorods, while induced asymmetry in the direction perpendicular to the long axis will lead to rotational motion. Mirkin and co-workers coated one face of electrodeposited Au-Pt-Au three-segment nanorods with an Au/Cr bilayer using a thermal evaporator, resulting in nanostructures with only one side of the Pt segment exposed.^[85] The fabricated structure is able to

display both clockwise and counterclockwise rotation in the presence of H_2O_2 . The rotational speed of the nanorotor can be adjusted by varying the H_2O_2 concentration or the ratio of Pt to Au segment lengths. Above 3% H_2O_2 , the rotational speed is limited by the Pt area instead of H_2O_2 concentration. When the Pt/Au ratio is near 2.0, the nanorotor reached the maximum rotation speed. Wang et al. designed a nanorotor which exhibits a rotational speed ~10 times faster than previously reported. In order to create asymmetry in the short axis, Cr, SiO_2 , Cr, Au and Pt layers were sequentially vapor-deposited on one side of the electrosynthesized Au-Ru nanorods (Figure 6F).^[76] The additional Au/Pt bimetallic layer adds a perpendicular self-electrophoretic force which moves the nanorod toward the center of the orbit and therefore leads to rotational motion. Catchmark et al. fabricated a gold gear-like structure with platinum deposited in the tooth regions using photolithography (Figure 6G).^[77] The asymmetric structure can exhibit high speed rotational motion in H_2O_2 . The torque generated by the electrophoretic forces lead to the rotation of the structure towards the platinum-coated side of the gear tooth, which agrees with the electrochemically induced electrophoresis mechanism.

3.3. Microrobots as Transport Vehicles

Microrobots have the potential to be developed into transport vehicles for desired cargoes, enabling applications such as drug delivery, target isolation and bioassays. The feasibility of using electrochemically powered self-electrophoretic micromotors for transport of colloidal cargoes has been demonstrated.^[86] Two strategies have been employed to attach cargoes to the bimetallic nanorods. The attachment of positively charged polystyrene-amidine microspheres to the nanorods can be achieved by electrostatic interaction with an additional negatively charged polypyrrole segment in the nanorod. The linkage of streptavidin-coated cargoes and nanorods functionalized with biotin-terminated disulfides on the Au end was achieved by specific biotin-streptavidin interaction. Unloading of cargoes is another important aspect that needs to be taken into consideration for cargo delivery. The development of photochemically assisted cargo release routes fill in this gap for self-electrophoretic micromotors.^[87] One unloading method is based on the rapid dissolution of an added Ag segment in the presence of chloride ions, H_2O_2 and UV light (Figure 7A (i)). In another method of cargo drop-off, a photocleavable linker (PCL) between the nanorod and the microsphere cargo will undergo photolysis upon UV illumination to release the cargo (Figure 7A (ii)). The addition of ferromagnetic nickel segment in the nanorods allows the transport of magnetic cargoes as well as control of the nanorods by external magnetic field. The loading and transport of magnetic cargoes in microchannel network has been demonstrated. The unloading of the magnetic cargoes can be easily accomplished by a fast change in the motion direction of the nanorod.

3.4. Pumps

The bimetallic electrochemical system has also been developed as micropumps which are capable of pumping fluid and

Table 3. Summary of electrophoretic mechanisms in nano/micromotors.

Type	Composition	Fuel	Propulsion	Dimension	Velocity (fuel concentration)	Biocompatibility of fuel
Metallic nanorods	Two metals ^[78]	H ₂ O ₂	Self-electrophoresis	Diameter: 370 nm Length: 2 μ m	4–30 μ m s ⁻¹ (5%)	Toxic
	Cu-Pt ^[33]	Br ₂ , I ₂		Diameter: 370 nm Length: 1–4 μ m	7–20 μ m s ⁻¹ (0.2 mM Br ₂)	Toxic
Janus particles	Silica-Au-Pt ^[80]	H ₂ O ₂	Light-induced self-electrophoresis	Diameter: 2 μ m	1–3 μ m/s (5%)	Toxic
	Polystyrene-Au-Pt ^[79]	H ₂ O ₂		Diameter: 2, 3, 5 μ m	7–15 μ m s ⁻¹ (1.5%)	Toxic
	Au-Pt ^[73]	H ₂ O ₂		Diameter: 30 nm	660 μ m s ⁻¹ (1.5%)	Toxic
	TiO ₂ -Au ^[74]	H ₂ O		Diameter: 1 μ m	25 μ m s ⁻¹	Biocompatible
Biohybrid Micromotors	GOx-Carbon fiber-BOD ^[82]	Glucose, O ₂	Self-electrophoresis	Diameter: 7 μ m Length: 0.5–1 cm	1 cm s ⁻¹ for 20 s (10 mM)	Biocompatible
	HRP-PPy-Au-Cyt c ^[75]	XOD, hypoxanthine		Diameter: 260 nm Length: 1.7 μ m	N.A.	Biocompatible

particles. While micropumps do not belong to the class of micromotors, they are important actuators. The bipolar electrocatalytic decomposition of fuel over the bimetallic surface creates an asymmetric distribution of ions and thus generates a local electric field. The electric field will induce electroosmotic flow of surrounding fluid as well as electrophoresis of tracer particles. The velocity of particles undergoing electrophoresis in an electroosmotic flow is the combined effect of the electroosmotic component and the electrophoretic component. According to the Smoluchowsky equation, in the electric fields produced by ion gradients, the observed velocity of the particle (U) can be expressed as $U = \epsilon(\zeta_p - \zeta_w)E/\eta$, where ϵ is the permittivity of the solution, ζ_p and ζ_w are the zeta potentials of the particle and the substrate wall, and η is the solution viscosity.^[29] For the particles whose direction of electrophoretic migration is the same with electroosmotic flow, there is no doubt about the direction of the particle motion. However, for the particles which have zeta potential (ζ_p) of the same sign with the substrate (ζ_w), electrophoresis and electroosmosis compete with each other, driving motion in opposite directions. Whether electrophoresis or electroosmosis dominates depends on the magnitude of the zeta potential of the particle (ζ_p) relative to that of the substrate wall (ζ_w).

Kline et al. reported a micropump system consisting of a silver disk immobilized on a gold underlayer, as shown in Figure 7B.^[88] The electrochemical decomposition of H₂O₂ involving both the silver and the gold components creates a proton gradient in the solution above the metal. Protons generated by oxidation of H₂O₂ at the anode (gold) migrate to cathode (silver), resulting in the electroosmotic fluid flow towards the silver center along the gold surface. At the disk, fluid continuity requires convective return flow above the electrode plane. Positively charged particles like amidine-functionalized spheres experience an additional electrophoretic force in the same direction with the electroosmotic flow, leading to a faster migration towards the silver island. Negatively charged particles experience an electrophoretic force away from the silver cathode, opposite to the electroosmotic flow. For carboxylated polystyrene particles with a more negative zeta potential ($\zeta_p = -60$ mV), dominant electrophoresis transport them outward, resulting in circular patterns around the silver islands. For gold rods with a less negative zeta potential ($\zeta_p = -7$ mV),

dominant electroosmotic flow transport them inward and circulate in a tight convection roll near the silver island.

According to the electrokinetic mechanism, an electrical connection between anode and cathode is essential to pump the motion of fluid and particles. Paxton et al. demonstrated that electroosmotic fluid flow induced by electric field in the solution near Pt/Au interdigitated microelectrodes (IMEs) in the presence of H₂O₂ can be switched on and off externally.^[29] A switch was wired in series with the IME electrochemical setup so that the pump can be controlled by switching the electrochemical decomposition pathway on and off. Jun et al. developed a self-pumping membrane by sputtering platinum and gold films on the opposite sides of track-etched polycarbonate membranes.^[89] An external switch is electrically connected with the platinum and gold electrode to control the fluid flow. In an aqueous solution of 0.01 wt.% H₂O₂, the electroosmotic pumping membrane is activated. As illustrated in Figure 7C, the decomposition of H₂O₂ on opposite surfaces of the membrane generates a transmembrane potential, under which protons flow through the pores of the membrane and create a flow.

Beyond the H₂O₂ micropumping system, Ibele et al. developed a system using hydrazine (N₂H₄) or *asym-N*, *N*-dimethylhydrazine (N₂Me₂H₂) as fuels and they also demonstrated the possibility of controlling the direction of fluid flow via the choice of fuel.^[90] The electrochemical setup consists of a spatially defined Pd disk immobilized on a gold surface. In aqueous N₂H₄ solution, Pd component with a more negative mixed potential act as anode. Silica colloids ($\zeta_p = -124$ mV) and amidine functionalized latex colloids ($\zeta_p = -44$ mV) all experience electrophoresis toward the Pd disk and electroosmosis away from the Pd disk. Due to the different magnitude of the zeta potential of the colloids relative to that of the gold surface, the dominant force is different for the two kinds of colloids. For silica colloids, dominant electrophoresis drives them toward the Pd disk while for amidine functionalized latex colloids, dominant electroosmosis propels them away from the Pd disk. The pumping direction can be switched by replacing N₂H₄ with N₂Me₂H₂. In the presence of N₂Me₂H₂, the Au component with a more negative potential acts as the anode. As a result, the directions of electrophoresis and electroosmosis exerted on the colloids are all switched, thus changing the migration direction of both the two kinds of colloids.

Figure 7. A) Schematic drawing of motor design for (i) Silver-assisted and (ii) PCL-assisted cargo drop-off. Reproduced with permission.^[87] Copyright 2010, Wiley-VCH. B) Illustration of electroosmotic flow and electrophoretic force that different particles experience in the setup of Au-Ag micropumps. Reproduced with permission.^[88] Copyright 2005, American Chemical Society. C) Working principle of the self-pumping membrane. Reproduced with permission.^[89] Copyright 2010, Wiley-VCH. D) Motion-based nucleic acid detection using self-electrophoretic nanorods. Reproduced with permission.^[8] Copyright 2010, Nature Publishing Group. E) The experimental setup of chemotaxis study. (i) Agar gel experiment (ii) Capillary experiment. Reproduced with permission.^[4] Copyright 2007, American Physical Society.

3.5. Sensors

Self-electrophoretic nanorod motors have demonstrated great potential to be employed in motion-based chemical sensing. The self-electrophoresis mechanism decides that the axial velocity of nanorods in H_2O_2 decreases dramatically with increasing the solution conductivity. Different from other ions, the addition of silver ions will bring about unusual speed acceleration instead of reduction.^[91] Based on these findings,

various motion-based sensing strategies have been developed by researchers. Wu et al. described a DNA sensing using self-electrophoretic nanorod motors, which relies on silver concentration-dependent speed and distance signals of bimetallic nanorod motors (Figure 7D).^[8] Thiolated capture probes immobilized on a gold electrode interact with complementary nucleic acid target and leads to duplex formation which will capture silver nanoparticle tagged detector probes subsequently. After removing the excess unbound detector probes

with washing step, the addition of H_2O_2 lead to dissolution of the captured silver nanoparticles tags into ions. The silver containing H_2O_2 is then separated from the gold electrode and added to nanorod solution, which will induce changes in their speed and generate concentration-dependent nanomotor distance signal. Ezhilan et al. demonstrated motion-based detection of silver ion source, which allows for the determination of both the source location and silver ion concentration.^[92] This detection approach relies on the diffusiophoretic motion of Au passive nanorods and Au-Pt active nanorods in a silver gradient as well as on the silver ions induced speed acceleration of the bimetallic nanorods. The addition of H_2O_2 on a planar silver source placed in the center lead to the dissolution of the source and the diffusion of silver ions generate silver ion gradients in the domain. For Au passive nanorods, they move away from the source gradually due to diffusiophoresis, resulting in the complete depletion of the passive nanorods near the source. For Au-Pt active nanorods capable of exhibiting self-electrophoretic motion in H_2O_2 , the mobility and the speed acceleration near the silver source weakens the diffusiophoresis-induced depletion, allowing them to move closer to the source and form more diffuse interface between the depletion region and rest of the domain. Given all the described effects, a Langevin model is proposed and key model parameters are extracted from the experimental data, providing an estimate for the full spatiotemporal distribution of the silver ions near the source.

The chemotaxis of bimetallic nanorods described by Hong et al. provides possibilities for sensing of a signaling molecule which can be a promoter or an inhibitor for the motion of the nanorods.^[4] Two types of experiments were conducted to examine the chemotactic behavior of nanorods towards H_2O_2 . In one experimental setup, an H_2O_2 -soaked agarose gel was placed in the center of a chamber filled with aqueous suspension of Pt-Au nanorods to generate H_2O_2 concentration gradient (Figure 7E (i)). The bimetallic nanorods displayed motion toward the gel and accumulated at the gel with time, indicating the transport of nanorods up the H_2O_2 concentration gradient. The other type of experiment was carried out using long rectangular glass capillaries which were filled with different concentrations of H_2O_2 to diffuse out and capped at one end with wax in Figure 7E (ii). The capillaries were placed into a Petri dish containing a suspension of Pt-Au nanorods in deionized water. After 1 hour, the capillary containing the highest initial concentration of H_2O_2 was found to have the highest concentration of bimetallic nanorods, which again demonstrates the chemotactic behavior of bimetallic nanorods in the presence of H_2O_2 concentration gradient.

4. Control of Nano/Microrobot Motion through Electrochemistry and Electric Fields

Nano/microrobots convert energy from the surrounding environment into locomotion. Electrical fields are able to modulate energetics within such micromotors or the immediate environment that they are enveloped in. Through the astute tuning of the surface charges or realizing electrochemical reactions on the interface between the media and the solid entity, propelling forces can be manifested.^[4,66,67,93–98] The utility of an electrical

field affects locomotive events, in which it has been demonstrated to not only change the propulsion, but illustrate guidance on the self-propelled micromotor.

Principally, both dielectric materials and conducting materials can all be subjects of interest to be manipulated. This can be accomplished through the modification of charges on the entity itself or the interfacial polarity at the electrochemical double layer at the particle, where polarity-based attractive or repulsive forces can be realized by the external driving electrodes.

The type of electric fields, define the mechanism of propulsive forces that can be realized on the object. External electrical fields can result in a redistribution of charges across the materials, leading to physical or chemical events, which have been known to control the motion of nano/micromotors and their properties, ranging from pure electrostatic forces, fluid flow to physical forces actualized by electrochemical reactions. These include the use of direct current and alternating current as external electrical fields that are used to exact autonomous motion on the object. Two types of electrical fields are of interest: DC (Direct current) electric field and AC (Alternating current) electric field. Electrophoresis (Figure 8A) and bipolar electrochemistry (Figure 8B) operates on the use of DC electric field. Separately, dielectrophoresis (Figure 8C), induced charge electrophoresis (Figure 8D), diode rectification to localized electrophoresis (Figure 8E) are based on the usage of an AC electric field.

Common parameters include the potentials applied across the electrodes, electrolytes, conductivity of the material, geometry and symmetry. Here, the discussions are divided into the type of electric fields used: DC or AC electrical fields. In addition to motion control, we will also delve into the electrochemical luminescence, their deployment as sensors, the applications of electrochemistry-modulated propulsive power to do work and remote electrical powering of electrochemical devices. Table 4 summarizes the properties of the electrical field applied and characteristics of the particle to be maneuvered.

4.1. Direct Current (DC) Electric Field

Direct current electric field is able to exert a uniform electric field across a fluid. It grants the user much ease to control the magnitude of the potential difference across the field. Exchanging the polarity of the electrodes also enables the converse reversal in motion. Furthermore, the locomotion can be easily started and stopped by turning the electric field on and off. Here, we investigate two electrochemical phenomena that can be realized in a DC field: Electrophoresis and bipolar electrochemistry.

4.1.1. Electrophoresis

Under the influence of a uniform electric field, electrophoresis causes the motion of the charged particles to move towards electrodes of the opposite charge (Figure 8A). The principal driving force for electrophoretic force is the charge on the particle and the electrophoretic mobility of the particles in the

solvent under the influence of an applied electric field.^[99] The charges can be borne on the particle itself or the enveloping electrochemical double layer surrounding the particulate. Two principal factors that affect electrophoresis are the properties of the colloidal solution and that of the electrical settings.^[99,100] This has become an established technique and protocols have been developed regarding the choice of electrolyte, pH, particle sizes, potential and current, due to the advent of electrophoretic deposition of nanomaterials.^[99]

Here, we list two examples that make use of electrophoretic phenomena to reversibly alter the locomotion of particles. Takinoue et al. demonstrated that micrometer-sized water-in-oil emulsion, prepared in the presence of surfactants, are capable of continuous motion.^[101] The dielectric water droplet experiences an electrophoretic attraction towards the tungsten electrode and electrostatic repulsion upon contact. The two tungsten electrodes are staggered diagonally to allow continuous rotary motion, with exertion of potential differences at 40 V. Upon contact, charge transfer occurs and the droplet encounters repulsion due to the identical charges of the droplet with the electrode. The droplet then was attracted to the oppositely charged electrode due to electrophoretic forces. Upon contact, charge transfer occurs at the oppositely charged electrode and repulsion occurs again. The cycle can be continuous and the rotary cycle repeats itself.

Liquid metal droplets have been demonstrated to show locomotion due to electrophoretic phenomena. This was demonstrated by Liu and co-workers with a graphite electrode that is immersed into the liquid gallium amalgam, where the composite shows capability of shape deformation under voltage, due to the electrochemical reactions that take place on the metal surface.^[102,103] As the electrode is punctured into the liquid metals, the liquid amalgam and the electrode act as a single unit of electrode. When a pair of oppositely charged electrodes is used, the liquid metal is able to demonstrate shape deformation and locomotion towards the other pole of the graphite electrode, as illustrated in Figure 9A. The prey-predator relationship is mimicked between the composite electrode and the electrode of the opposite polarity.^[103] Furthermore, when a liquid bulk gallium amalgam is introduced as an cathode with a small capillary outlet, small spherical spheres are dispensed from the capillary tube under an electric field towards the anode.^[102] It has been demonstrated to achieve micrometer-sized units when a fast ejection of bulk liquid gallium amalgam is executed.^[104] With an electric field of 61 V m^{-1} , the liquid metal droplet reaches a velocity of 3 cm s^{-1} . The electric field generated across the different poles has been the origin of the liquid amalgam droplet showing locomotion towards the electrode of opposite polarity, in an electrophoretic phenomena.^[102,103]

4.1.2. Bipolar Electrochemistry for Motion

Bipolar electrochemistry allows for the powering of a conducting element, hereby termed as the bipolar electrode that is within an electrical field generated by a D.C. current (Figure 8B).^[66] It functions in solutions of low ionic strength as the conducting element must be more conductive than the medium itself.^[108] Length of the particle defines the potential difference across

the two terminus of the particle, which inadvertently affects the driving force and motion, where physical reactions such as bubble emissions^[105] and dissolution/deposition of a material^[109] can be realized. In addition, this relationship of the maximum polarization voltage between two terminuses of the particle confined between the bipolar electrodes, is also defined by the electric field exerted by the driving electrodes.

Figure 8B illustrates the setup of a conducting element that occurs between 2 driving electrodes during bipolar electrochemistry. If a substantial potential is conferred in an exerted electric field, two differing electrochemical reactions can occur across the conducting element. In an aqueous solution, hydrogen evolution reaction (HER) occurs at the cathode of the bipolar electrode, while oxygen evolution reaction (OER) occurs at the anode, as illustrated in Figure 8B. The production of the hydrogen gas (Equation (1)) at one end and the oxygen gas (Equation (2)) at the other is not equal due to stoichiometric amount of electrons that passes through the bipolar electrode. The greater production of hydrogen bubbles on the bipolar electrode results in locomotion. This resulting formation and ejection of the bubbles propels the particle in a recoil mechanism. It moves in the direction opposite to where the more vigorous hydrogen bubble production was located, with the oxygen-bubbles producing hemisphere facing the front. However, to increase the efficiency of the bipolar electrode during propulsion, an additive of hydroquinone, $\text{C}_6\text{H}_6\text{O}_2$ (HQ) can be added. The oxidation of HQ (Equation (3)) precedes that of the oxidation of water to oxygen, where the oxidation results in quinone, $\text{C}_6\text{H}_4\text{O}_2$ (Q) formation. The elimination of the oxygen bubbles retarding propulsion during electrochemical reaction in a bipolar electrode increases the efficiency of the unit, where the locomotion is wholly dependent on hydrogen bubble ejection.^[105]

The electric field (E) applied on the object is equal to the potential applied (ΔV_1) divided by the distance (l) between the two poles of the driving electrodes, $E = \Delta V_1/l$. Potential difference (ΔV_2) is thus exacted across the conducting element, termed as the bipolar electrode, in the electric field. The potential difference (ΔV_2) can be calculated from the product of electric field and the dimension of the bipolar electrode across its cathode and anode: $\Delta V_2 = E \times d$. If it exceeds the electrochemical potential of $\Delta V_2 = E_2^0 - E_1^0 = 1.23 \text{ V}$, the redox reactions of water occurs and the bipolar electrode commences its locomotive behavior. In the presence of hydroquinone, the potential can be depressed to $\Delta V_3 = E_3^0 - E_1^0 = 0.70 \text{ V}$

4.1.2.1. Motion in 1 Dimension: Micrometer-sized objects such as $275\text{-}\mu\text{m}$ sized glassy carbon sphere can be moved under a channel environment with an electric field of 43 V cm^{-1} , as illustrated in Figure 9B.^[105] Directionality can be altered by switching

Figure 8. Schematics of electrochemical control of micromotors. A) Electrophoresis of a particle moving towards an oppositely charged electrode. Reproduced with permission.^[95] Copyright 2009, Royal Society of Chemistry. B) Bipolar electrochemistry of a conducting particle moving by bubble emission.^[66] C) Dielectrophoresis of a particle experiencing either attraction or repulsion in an AC/DC electric field: Particles more polarizable than the media moves into the gradient of higher electric field intensities, positive dielectrophoresis (pDEP); Less polarizable particles move away from these regions, negative dielectrophoresis (nDEP). Reproduced with permission.^[96] Copyright 2010, Royal Society of Chemistry. D) Induced-charge electrophoresis in an anisotropic Janus particle, resulting in unequal electroosmotic flows, culminating in the particle motion orthogonal to the applied electric field. Reproduced with permission.^[97] Copyright 2008, American Physical Society. E) Diode rectification of an AC field converted to localized DC electrophoresis, with the diode propelling with the direction of the cathode(−) facing front. Reproduced with permission.^[98] Copyright 2007, Nature Publishing Group.

the polarity of driving electrodes, while changes in magnitude of the potential difference alter the velocity of the micromotor.^[107] The dimensions of the micromotors and the external electric field strength defines the difference in polarity across the bipolar electrode in the unit of self-propelled micromotor. Roche and co-workers illustrated this through the manipulation of the magnitude of potential difference, affecting the velocities of the micromotor and oriented the direction by changing the polarity of the driving electrodes.^[107] The external electric field was

controlled between 11 to 44 V cm^{−1} and changes in polarity was actualized to control the directional velocities of the micromotor via a bipolar electrochemistry methodology.

Physical factors such as the confinement of the conductive particle in the capillary walls define the motion of the particle itself. This is to prevent the particle from encountering tumbling forces, which reduces the efficiency of the unit.^[108] Surfactants can be added to aid the formation and release of the bubbles evolved.^[105] Proton concentration,^[105] OER

Table 4. Summary of electrochemical and electric field manipulations of particles for locomotion.

Particle-localized manipulation	E-Field	Homogeneous E-field	Directionality Control of Particle	Remote Electric Powering	Particle Conductivity	Particle Size Limit
Electrophoresis	D.C.	Yes	Yes	No	Dielectric and conductor	nm
Bipolar electrochemistry	D.C.	Yes	Yes	Yes	Conductor	μm
Dielectrophoresis	A.C./D.C.	No	Dependent on polarizability of particle	No	Dielectric and conductor	nm
Induced charge electrophoresis	A.C.	Yes	Dependent on Janus particle orientation	No	Anisotropic conducting and dielectric surfaces	nm
Diode Rectification to Localized Electrophoresis	A.C.	Yes	Dependent on diode polarity orientation	Yes	Semiconducting	nm

Figure 9. A) (i) Deformation and (ii) Locomotion following the movement of the cathode in 1.0 M NaOH with 0.3 mL of gallium and graphite electrode rods of diameter, 2 mm with a potential difference of 8 V. Reproduced with permission.^[103] Copyright 2014, Nature Publishing Group. B) Translational motion generated with a 275 μm glassy carbon sphere in a microchannel with a 43 V cm^{-1} electric field in an aqueous solution of 7 mM HCl and 14 mM hydroquinone. Scale bar: 100 μm . Reproduced with permission.^[105] Copyright 2011, Nature Publishing Group. C) Levitation of a light-emitting glassy carbon bead. Series of optical images showing a glassy carbon bead emitting electrochemiluminescence at different times during its motion. The bead was placed in a U-shaped cell, filled with 100 mM PBS buffer containing 0.5 mM $[\text{Ru}(\text{bpy})_3]^{2+}$, 100 mM TPA, and surfactants in an external electric field of 25.5 V cm^{-1} . The left image was taken under visible light and the other images were taken in the dark. Inset: Plot showing the change in height, h , of the bead as a function of time, t . Reproduced with permission.^[106] Copyright 2012, Wiley-VCH. D) (i) Scheme of a vertical rotor powered by the reduction of protons coupled to hydroquinone oxidation. (ii) Rotational motion of a vertical bipolar rotor, made out of an asymmetrical conductive polycarbonate sheets, in an electric field of 5 V cm^{-1} in 50 mM HCl and 100 mM hydroquinone. Scale bar: 0.5 cm. Reproduced with permission.^[105] Copyright 2011, Nature Publishing Group. E) Still frames showing the LED-swimmer while changing the polarity of the horizontal electric field (44 V cm^{-1} in x -direction); inversion of the polarity at 4 s leads to a change in color and of the direction of the horizontal motion. Scale bar: 5 mm. Reproduced with permission.^[107] Copyright 2014, Nature Publishing Group.

suppressant hydroquinone,^[110] dimensions of particulates^[108] and the inherent overpotential of the material during electrochemical reactions^[105] in concern should also be accounted for. The material considerations for electrocatalytic effects is a key factor, when the choice of a substrate such as carbon or metal, is deliberated for a bipolar electrode.^[105]

4.1.2.2. Vertical Motion: Under confinement in a capillary tube, it allows for the accumulation of the hydrogen bubbles underneath the microparticle endowing buoyancy to the object, as shown in Figure 9C.^[108] The continuous accumulation of the bubbles allows an upward force to be built for upward motion. This has been extensively illustrated by the Kuhn group, where hydrogen bubbles acts as buoyant forces for ascension.^[106–108,111] The forces work against gravity and enable the object travel in the z -axis.

This was later exemplified by Loget et al. who used a 1 mm conductive glassy carbon bead to move a polymer cargo up a glass capillary tube in an electric field of 23 V cm^{-1} .^[108] The defined spaces of the capillary tube entrap the hydrogen bubbles beneath the glassy carbon bead for a buoyant force to uplift the bead and moving the cargo.

To overcome the problem of requiring the physical environment to trap gases, Roche et al. designed micromotors that can trap gas bubbles independently of the surrounding.^[107] A rocket-shaped motor with an escape corridor that allow the bubble to leak out, leads to an equilibrium between bubble production and escaping bubbles. This permits translational movement in the z -axis with constant acceleration with a defined amount of entrapped gases. Meanwhile, a cube-shaped motor with no escape catch accumulates gas bubbles underneath the miniaturized motor, which experiences increasing driving force with time and therefore an increasing acceleration.

In addition, Roche et al. designed the cube shape swimmer to have two sets of metal wires that are orthogonal to each other. This allows controllable upward and horizontal motion of the device.^[107] When coupled with two sets of bipolar electrodes, the x - z motion of the micromotors can be controlled. It will not be unimaginable that with a third set of bipolar electrodes that the device can achieve motor motion in all three dimensions.

4.1.2.3. Cyclical Motion: Horizontal and vertical asymmetry can be factored into rotors, resulting in complicated and elaborate movements.^[105] Changing the shapes and materials of the

object, has also enabled increased modes of action from the micromotors. Loget et al. designed rotors that are capable of revolving, by placing the conductive element asymmetrically on the fan-shaped rotor, pivoted by a glass stick.^[105]

The flaps of the fan-shaped rotor are made of conductive carbon-doped polycarbonate membrane that was covered with an insulating tape, with only the terminus exposed asymmetrically, as shown in Figure 9D.^[105] For the horizontal rotor, four flap-units of the exposed terminus follows a rotational symmetry, allowing electrochemical OER to occur on the flap near the cathode driving electrode and HER on the flap in proximity of the anode driving electrode. This creates momentum in electric field of 0.5 kV m^{-1} , achieving rotating speeds of 1.9° s^{-1} or 0.32 r.p.m. During rotation, when passing the central point at the axis parallel to the external driving electrodes, the electroactive parts of the rotor exchange their polarization. The formation and ejection of the bubbles along all four individual flaps result in the motion of the rotor in an anti-clockwise fashion.

For the vertical rotor, buoyancy of the bubbles produced has also been utilized as a driving force.^[105] However, the rotational symmetry of the rotor, may result in competing forces at opposite ends of the flap in the rotor, as illustrated in Figure 9D (i), due to bubble buoyancy. The introduction of hydroquinone to suppress oxygen evolution reaction to form oxygen bubbles, increases the efficiency of the vertical rotor. Consequently, it also increases the amount of hydrogen bubbles produced due to increased HER with the lower ΔV that is required to drive hydrogen bubble evolution across the bipolar electrode in the rotor for hydrogen bubble production. The motion increases by two-fold, at a rotational speed of 4.2° s^{-1} or 0.70 r.p.m. , as illustrated in Figure 9D (ii). An additional buoyant upward force and expansion of bubbles with increased hydrogen bubble production have been the principal origin in the vertical rotor's revolution.

A valve based on the bipolar chemistry was also created with a carbon membrane attached to a graphite in an enclosed compartment.^[110] Formation of hydrogen gas on a single side, with the suppression of oxygen evolution with hydroquinone allows the buoyant bubbles to gradually collect in the compartment and push up the flap of the valve, when the upward-acting forces overcome the weight of the load. However upon escaping, the buoyant forces reduced dramatically as the bubbles escape, resulting in the subsequent drop of the cap. The valve is thus closed again, repeating itself in a cyclical fashion as long as the electric field is kept on.

4.1.2.4. Electrochemical Devices: When Faradaic currents run through the bipolar electrode, with opposite electrochemical reactions occurring along the two different poles, it demonstrates capability of powering a small device. This demonstrates a remote powering of the unit through electric fields passing through the bipolar electrode. Such remote powering, allows far-ranged powering of a thermometer sensor and transmitting the data to a receiver.^[107]

Light emitting diodes (L.E.D.), which only allows the flow of current in one direction, allows the micromotors to be directionally labelled.^[107] Since the diodes only allow a unidirectional flow current, it represents a method of signaling the

direction of motion. In this above listed example in Figure 9E, while moving in the horizontal direction from left to right, the LED is lighted red and the converse direction will be lighted green, as the polarity of the electric field is being reversed. This illustrated a micromotor with directional signaling, while also demonstrating the capability of remote sensing in the electric field direction.

4.1.2.5. Control of Multiple Micromotors: Field effects of changing the polarity and magnitude of the motion of the micromotors can be achieved easily by altering the voltage across the two external electrodes in bipolar electrochemistry. This will culminate in the changes in magnitude and directionality of the micromotors. With the switching off of the electrochemical system, the motion of the micromotors can be terminated. However, the use of bipolar electrochemistry with a resulting global effect on the motion of multiple units has been limited, due to the need of entrapping bubble gases for locomotion.^[107,108]

However, the converse selective localized modulation of bipolar self-electrophoretic micromotors be accomplished through a selective usage of the electrochemical potential. Calvo-Marzai et al. demonstrated that with a micrometer-sized electrode, through the adjustment of oxygen concentration via a potentiostatic method, locomotive properties in populations of Au-Pt micromotors moving by self-electrophoresis can be altered, due to changes in surface tensions and suppression of oxygen concentrations during self-electrophoresis.^[112] The global effect of controlling multiple micromotors by bipolar electrochemistry can be realized with a more intricate look at bipolar electrode and channel designs in the foreseeable future.

4.1.2.6. Electrochemical Self-Regeneration: Bipolar electrochemistry has been demonstrated to manipulate the chemical composition of a solid metal. This was demonstrated with the use of a zinc dendrite that was immersed in the solution of zinc ions in a capillary tube.^[109] The simultaneous deposition of zinc at cathode and dissolution of zinc at the other pole of the anode, results in locomotion, much alike chemical wave propagation. A micrometer-sized zinc dendrite is able to reach a velocity of $80 \mu\text{m s}^{-1}$ in an electric field of 70 V cm^{-1} .

4.1.2.7. Sensing Chemicals by Bipolar Electrochemistry: Bipolar electrochemistry coupled with electrochemical luminescence allows the self-propelled object to be monitored remotely, indicating the location of the device using light, as illustrated in the vertically rising motor in Figure 9C.^[106] This was first illustrated with $[\text{Ru}(\text{bpy})_3]^{2+}$ and co-reactant tri-*n*-propylamine (TPA) with a glassy carbon bead in a vertical capillary channel. On anodic oxidation of the above two substrates, their intermediate states react with each other to regenerate an excited molecular state of $[\text{Ru}(\text{bpy})_3]^{2+}$ which relaxes and releases a photon simultaneously. The light from this emission results in the orange glow of the micromotor device. Hence, this electrochemiluminescence allows the tracking of micromotor in a dark environment. Meanwhile, the cathodic end of the bipolar electrode produces hydrogen bubbles for propulsion and giving rise to buoyant forces.

The orange glow is seen as moving towards the cathode of the external driving electrode. Later in another study, Sentic et al. used $[\text{Ru}(\text{bpy})_3]^{2+}$ in bipolar electrochemistry, but replaces the TPA with enzyme co-factor NADH. Upon expenditure of NADH to NAD^+ , it can be regenerated by glucose dehydrogenases in the presence of glucose.^[113] Glucose dehydrogenase oxidizes glucose to produce NADH from NAD^+ . Thus, the bipolar electrode with the presence of increased concentrations of glucose, was able to give off an increased orange glow, demonstrating capabilities to detect glucose concentrations in the medium.^[114]

This effect was later extrapolated to the molecule luminol.^[111] However, this reaction now takes place in an alkaline environment in the presence of sodium hydroxide, hydrogen peroxide and luminol. The electrochemical oxidation of hydrogen peroxide to oxygen propels the micromotor, with a concomitant reaction of oxygen with luminol to give off blue electrochemiluminescence. In this case, the bipolar electrode is seen moving towards the anode of the external driving electrode in the setup. Thus, these two methodologies give the user an array of choices of tracking self-propelled micromotor in a dark environment using bipolar electrochemistry.

4.2. AC Electrokinetics

Use of AC fields to affect motion of micromotors has the benefit of avoiding electrolysis of water and competing forces in water such as DC electrophoresis across the two poles of the electrodes due to the high frequencies of the changing polarities.^[95] There are in principal, direct or indirect pathways in which one can manipulate matter using an alternating electric field. A direct pathway includes dielectrophoresis of a particle experiencing either attraction or repulsion in an electric field (Figure 8C): In positive dielectrophoresis (pDEP), particles more polarizable than the media moves into the gradient of higher field intensities; in negative dielectrophoresis (nDEP), less polarizable particles move away from these regions. Indirect pathways using AC electric fields include induced-charge electrophoresis and diode rectification of an AC field leading to localized DC electrophoresis. Induced-charge electrophoresis occurs in an anisotropic Janus particle, resulting in unequal electroosmotic flows (Figure 8D). This culminates in the particle motion orthogonal to the applied electric field. Diode rectification of an AC electric field converted to localized DC electrophoresis, by inducing an electric field within the object (Figure 8E), with the diode propelling in the direction of the cathode(−) in front.

4.2.1. Direct Pathway

In a non-uniform AC field, dielectrophoretic forces can manifest.^[95,115] This is a development of the particle being more polarizable than the media. The particles are being attracted to or repelled from the higher electric field intensity region due to interaction of induced dipole with the gradient of an AC electric field.^[46–48] In positive dielectrophoresis (pDEP), particles that are more polarizable than the media are pulled along the gradient into the areas of the highest field intensities. In

contrast, in negative dielectrophoresis (nDEP), particles that are less polarizable than the media are pushed away from these areas. This is also especially prominent in DC fields, but can be overwhelmed by electrophoretic effects and water electrolysis at high potentials. Dielectrophoretic forces can therefore guide the motion of the particles.

Altering trajectories with positive DEP has been used to guide the direction of micromotor motion that is moving by self-electroosmotic effects.^[116] This was illustrated in Au/Pt Janus particles that have been capped with gold and platinum layers, before immersion in hydrogen peroxide solutions. These particles move by self-electroosmosis with the Pt side facing front. Under an AC field, instead of random motion, the direction of the micromotor can be manipulated unidirectionally. Flow from the alternating current electroosmosis (ACEO) generated by the electrodes' alternating current electric field are able to uplift the micromotor and guidance from pDEP directs the micromotor's navigation. Coupled with another perpendicular set of electrodes sandwiching the micromotors in Figure 10A (i), the set-up allows for the two dimensional control of the micromotors along 2 axes, in Figure 10A (ii). The frequencies were controlled at 10–1000 kHz and voltages of 8 and 12 V_{p-p}.

4.2.2. Indirect Pathway

Janus particles are particulates that have asymmetric surfaces. They form different induced electrochemical double layers due to the differing polarization, in the presence of an AC electric field. At frequencies of lower than 10 kHz, the Janus particles are able to move. This is due to the difference in polarizability across two different sides of the surface and sufficient time is allowed for fluid flow to occur. This was exemplified in the gold coated polystyrene particle.^[97] The electric field acts tangentially on the diffuse counter ions on the induced electrochemical double layer in the Janus particle, resulting in an electroosmotic flow from the boundary of the gold/polystyrene modification towards the midpoint in the Janus particle. The flow across the metallic component is greater than the flow in the dielectric counterpart in the uncapped polystyrene component. This results in the direction of motion in the particle to be normal to the electric field.

Diode rectification of an alternating current (AC) field has been realized by Velev et al.^[98] The localized DC voltage induced into the diodes from the AC field resulted in an electrophoretic motion on the diode. The particle-localized rectification of the AC electric field to direct voltage will lead to an electroosmotic ionic flux at the interface of the particle, which leads to a propelling force on the diode to be exerted in the opposite direction, as depicted in Figure 10B.

The polarized current direction of the diode can only flow in a specific direction under the AC electric field, depending on the alignment of the diode polarity (Figure 10B (i)). The corresponding current rectification phenomena can occur in a diode when it is exposed to an alternating current electric field. This results in a current flow between the anode(+) and cathode(−) of the diode. The current flow then induces an electro-osmotic flow of the ions at the interface of the diode and the electrolyte.

Figure 10. A) (i) Scheme of 2D controlled motion of self-propelled micromotors moving by self-electroosmosis sandwiched between electrodes that are perpendicular to each other in a grid immersed in hydrogen peroxide solutions. When AC voltage is applied on a bottom electrode, the micromotor moves along the bottom electrode. When the AC voltage is then switched onto a top electrode, the micromotor is uplifted by ACEO (alternating current electroosmosis) and guided by pDEP to the top electrode, before moving along the electrode pathway by self-electroosmosis. (ii) Trajectories of micromotors for 20 s. AC electric field (1 kHz, 8 V_{p-p}) was applied to the electrodes B1, T1, B2, T2, and B3 in sequence. Reproduced with permission.^[116] Copyright 2015, Wiley-VCH. B) Optical micrographs of diode micromotor. (i) Overlay of image sequence with directional pathway of a self-propelling diode for a total duration of 43 s. (ii) Two LEDs light up and move towards the top or bottom depending on the orientation of their anodes (marked with white paint). All experiments in (i)–(ii) were carried out at electric field of $E = 120 \text{ V cm}^{-1}$, frequency 1 kHz and 10^{-6} M NaCl . The scale bars represent 5 mm. Reproduced with permission.^[98] Copyright 2007, Nature Publishing Group.

With the diode held at the interface between air and water by surface tension, the electroosmotic flow powers the propulsion of the micromotor in the opposite direction. Typically, the external field of 120 V cm^{-1} and frequency of 1 kHz is used. Speeds can reach up to 8 cm^{-1} for a millimeter-sized diode. An arranged assembly of diodes can also permit for the design for rotational motion in the diodes. An encirclement of the diodes in a circular shape, with the polarity of the diodes in an anti-clockwise fashion, results in a rotary motion in an anti-clockwise manner. LEDs can also be utilized in the above scenario, resulting in light emitting devices that were powered by the AC electric fields, as illustrated in Figure 10B (ii). Depending on the relative orientation of the anode, the diodes can be manipulated to travel in different directions.

Miniaturization of the diodes was also realized by Wang and co-workers through the use of electrodeposition protocols, where different segments of the conductive polymers and metals were being electrodeposited.^[37] 200 nm diameter segmented Schottky barrier diode nanowires such as polypyrrole-cadmium and CdSe-Au-CdSe nanowires can be achieved. In the presence of a uniform AC field, the polypyrrole-cadmium semiconductor diodes experience the rectified field-induced electroosmotic flow, demonstrating locomotion at 17.2 μm s^{-1} , at 7 body lengths per second. These diodes pave the way for

use in microfluidics, where immobilized diodes can be used as pumps to introduce flow into the entrapped system.

The utilization of electric fields enable particle localized manipulation for control of multiple^[98] or single microrobots,^[105] when the electric field applied interacts with the particulate. In contrast with chemotactic^[4] and phototactic motion^[117] in aqueous environments, where it requires large concentrations of fuels to be added to the surroundings, the use of an electric field for particle locomotion remains fuel-free. This is in addition to the option of altering the magnitude of the velocities, through changes in voltages of the driving electrodes and in turn customization of the electric fields applied.^[107] The utilization of electromagnetic waves for tuning the decomposition of fuel using visible^[118] and UV light^[119] has led to large area illumination, where side reactions often occur. Furthermore, such movement has been limited to only start/stop motion and directionality changes remain elusive.^[118,119] Point sources in lasers to power microrobots are often needed for directionality to be conferred to the object.^[120] In comparison to magnetic fields, where targets are limited to certain ferromagnetic materials,^[41] electric fields demonstrate ability to manipulate a range of materials. The particle localized electric field allows the customization of each individual nano/microrobots within an electric field, through an astute tuning of the polarity,

voltages and on/off modes of the driving electrodes, permitting directionality, magnitude of velocities and stop/go motion to be simultaneously programmed to the nano/microrobot.

5. Applications of Nano/Microrobots in Electrochemical Sensing, Mixing and Energy

Micromachines can be used for enhanced mixing due to their motion and the motion of exhaust microbubbles, which can lead to dramatically enhanced electrochemical sensing and energy generation. In addition, the electrochemical sensors themselves can sense motion of these tiny robots, which allows their detection in occluded environments.

5.1. Microrobots Assisted Mixing and Electrochemical Sensing

With advent of bipolar electrochemistry (BPE),^[67] modern fabrication technologies such as 3D printing^[121] and screen printing,^[122] electrochemistry are making great strides towards the replacement of the conventional electrodes and cells to simplify and speed up both analysis and synthesis in small scales. Recent developments in the fabrication, architecture, motion and operation of a wide range of small-scale motors can assist and make significant changes in modern electrochemistry in small scales. The ability of self-propelled motors to accelerate the decontamination process, through mechanical mixing due to their continuous motion in the solution has been reported to assist electrochemical measurements.^[123,124] Jurado-Sánchez et al. showed that their activated carbon/Pt micromotors were capable of removing pollutants like dinitrotoulene (DNT) and lead ions (Pb^{2+}) from aqueous solution, and the efficient removal of the pollutants was illustrated by conducting cyclic voltammetry (for DNT) and stripping voltammetry (for Pb^{2+}) experiments on the contaminated aqueous solutions after five minutes treatment with the activated carbon/Pt micromotors.^[123] In another study Orozco et al. used chronoamperometry to measure the current signal of a phenolic pollutant (2-amino-4-chlorophenol; 2A-4CP) solution at fixed intervals (10, 20 and 30 minutes) from the point of self-propelled tubular motors' addition, to prove that the motors' movement in the solution resulted in a faster decontamination process.^[124]

Recent studies show that the application of small-scale motors in electrochemistry is not limited to mixing activity due to the fluid convection.^[113,125–127] They are able to move within the sample fluid to find and isolate the analyte of interest, obviating the need for tedious sample preparation procedures, altering environments and converting non-detectable target analytes to electroactive species, that are easily detectable with an electrochemical strip platform.^[125,126,128,129] Several sizes and shapes of the small scale motors involving different propulsion mechanisms such as Marangoni-effect, fuel powered and BPE-powered motors have been used in electrochemical sensing application. Below, a discussion of using such miniaturized self-propelled devices during detection will be described.

Magnesium microparticles coated with a gold layer (Janus Mg/Au) can be employed as a novel disposable electrochemical

sensing strategy.^[126,127] Mg/Au Janus particle are able to degrade/convert non-electroactive compounds to electroactive compounds quickly due to generation of hydroxide ions and hydrogen bubbles (Equation (4)). Since the formation of $\text{Mg}(\text{OH})_2$ layer may passivate the Mg surface, the additional coating with gold is required to achieve efficient production of hydrogen, due to galvanic and pitting corrosion effects in presence of Au layer.

Wang's group has reported electrochemical measurement (chronoamperometry) of paraoxon, a nerve agent, in presence of bubble-generating Au/Ni/Mg Janus micromotors. The incorporation of the magnesium Janus micromotors on the printable sensor strips have two effects: (i) generation of hydroxide ions (OH^-) to promote the degradation of methyl paraoxon into p-nitrophenol; (ii) vigorous production of hydrogen bubbles (H_2) over a prolonged duration at an optimal and fixed position from the working electrode, through the use of a magnet, to generate a strong mixing effect for the dispersion of the electroactive p-nitrophenol by-product and obtain a homogenous solution for electroanalysis of the latter. The successful integration of built-in mixing feature on screen-printed electrodes with the use of bubble-generating microengines will enhance the mass transport of the electroanalyte, which was previously limited to diffusion transport. Therefore, this increases the sensitivity of using cost-effective, simple and disposable printable electrodes (Figure 11A).^[127]

Recently Escarpa's group used Mg/Au micromotor-based platform for electroanalysis DPP (diphenyl phthalate), a non-electroactive compound, found in biological and food samples. Due to the pH increase from the presence of micromotors, DPP was degraded into phenol (Figure 11B). Consequently, the micromotor coupled with electrochemistry methodology was used for direct analysis of phthalate in viscous biological (human plasma) and food (milk and whisky) samples without any sample handling and obtained excellent accuracy. The result shows that micromotors play critical role to improve sensitivity, through mixing. In addition, selectivity is improved in the electrochemical potential window by shifting the detection peak from 0.7 to 0.4 V during electroanalysis.^[126] Both studies represent that the integration of micromotors and screen printed electrodes as an attractive approach with advantages such as direct analysis without any sample preparation, the possibility of simultaneous detection of multiple analytes, disposability, portability and cost-effectiveness.

In another study, Escarpa and co-workers reported the ability of Marangoni effect powered millimetre-scaled motors (millimotor) for mobile biosensing of H_2O_2 in environmental and clinical samples. Marangoni-effect powered millimotors prepared by loading pipette tips with sodium dodecyl sulphate (SDS) and horseradish peroxidase (HRP). Once millimotors are placed in solution, asymmetric release of SDS and HRP induced changes in the surface tension, resulting in a frontward force which drags the tip through the solution (Figure 11C). The simultaneous release of HRP into the sample catalysed the oxidation of the mediator 3, 3', 5, 5'-Tetramethylbenzidine (TMB). The oxidized form of TMB was measured by chronoamperometry through

Figure 11. Micromotor assisted mixing and electrochemically sensing application. A Mechanism and reactions involved in paraoxon degradation to *p*-nitrophenol accelerated by micromotors. Reproduced with permission.^[127] Copyright 2015, Royal Society of Chemistry. B Schematic of the Mg/Au Janus micromotors-based strategy for (i) the simultaneous degradation/detection of DPP and (ii) the reactions involved in the degradation of DPP by Mg/Au Janus micromotors. Reproduced with permission.^[126] Copyright 2016, American Chemical Society. C Scheme of self-propelled enzyme-based motor for hydrogen peroxide detection. Reproduced with permission.^[125] Copyright 2015, American Chemical Society. D Mechanism involved in glucose detection. Reproduced with permission.^[113] Copyright 2014, Royal Society of Chemistry.

its reduction at -0.2 V in a carbon screen-printed electrode, possessing good limits of detection ($2.4\text{ }\mu\text{M}$).^[125] This enzyme-based motor preserved biocatalytic activity, provide continuous release of fresh enzyme and accelerate enzymatic reaction without the need for homogeneous sample or physical and chemical attachment of enzyme.

On the other hand, controlling and tracking the motion of small motors in real time is highly demanding for many potential applications. In recent years, lots of efforts have been made to design motor with specific optical or magnetic property.^[15,106] In addition to the application of BPE for fabricating a new generation of nano/micromotors as discussed earlier in the section, BPE has been able to propel the motors instead of conventional mechanisms such as chemical, thermal, magnetic and acoustic control. Coupling electrochemiluminescence (ECL) and BPE opened new windows in application of small scale motors in modern electrochemistry measurements. A conductive motor can be placed into an electric field, promoting localized oxygen or hydrogen evolution, resulting in control of motion and trigger a simultaneous light emission by mean of electrogenerated ECL due to the asymmetric electroactivity induced by BPE.^[113]

Sentic et al. have successfully designed a self-propelled red-light-emitting motor and employed for enzymatic glucose detection dynamically. As illustrated in Figure 11D, glucose is oxidized to gluconolactone by glucose oxidase, while driving the proportional conversion of NAD^+ to NADH , which is then used as a sacrificial co-reactant for ECL emission by $[\text{Ru}(\text{bpy})_3]^{2+}$. Once ECL swimmer moves towards higher concentration of glucose, light emission is increased and a concentration gradient of glucose was revealed. The simultaneous H_2 evolution from water at the other pole allows the propulsion of the beads. On the foundation of this strategy, local detection and information of enzymatic substrates will be achievable in non-uniform samples with concentration gradients.^[113]

5.2. Electrochemistry for Detecting Nano/Microrobot Motion

The converse use of electrochemistry to detect the motion of artificial moving miniaturized devices has recently been demonstrated. Previously, attempts to use electrochemistry to quantify the motion of moving objects have mostly been limited to organisms such as *daphnia magna*^[130] and fragmented biological

units.^[131] In one of the first instances of using electrochemical methods to track a moving man-made miniaturized object, Compton et al. demonstrated the use of an interdigitated electrode to survey positions of a millimeter-sized basalt ball moved by magnetic forces.^[132] Later, motion of water droplets in a microfluidic chip were detected by Fisher and co-workers using an on-chip electrode set-up.^[133] However, it is only until recent times that chemically powered units of autonomously moving objects were monitored using electrochemical techniques. The disturbances from the motion of Marangoni-effect self-propelled polymer capsule across a glassy carbon electrode was used to monitor the velocity in a well-defined channel environment.^[134] They even extended this work to detect the position of blockages in the channel environment. Later, the same team demonstrated the efficacy of using particle-electrode impact voltammetry to quantify the concentrations and velocities of micrometer-sized autonomously moving devices of silver Janus particles and tubular Cu/Pt micromotors.^[135] Motion of micro-robots was later monitored under a microchannel environment using a similar electrochemical technique.^[136] With the ability to track and obtain information from miniaturized devices by electrochemistry, one can envisage an internet of things at the nanoscale with the use of networks for first-hand transmittance of data.^[137]

5.3. Converting Chemical Energy to Electricity for Energy Applications

The production of electrical energy in the electrochemical fuel cells has been an important topic of interest. Here, we review these applications and put them in the context of integration with locomotion from micromotors.^[138–141] Osada et al.'s work was the pioneer study in energy application in year 2000, where they developed an amphiphilic polymer gel which secretes an organic solvent (such as alcohol or THF) on the surface of water. This event resulted in surface tension gradients and culminated in locomotion of the object. The motion of the polymer gel was able to induce an electromotive energy and drive a generator.^[138] Similarly Matsui and co-workers (2015) incorporated diphenylalanine peptides (DPAs) into metal-organic frameworks (MOF) to make a self-propelling micromotor which is capable to deliver DPA peptides without damage of the framework through water exchange. They demonstrated micromotors can generate electric power by the integration with a plastic rotor and a magnet.^[141] The motion of these above mentioned mini-generators were driven by the Marangoni-effect due to the differences of surface tensions from the compounds released.^[138,141] The Marangoni-effect systems are unsustainable due to the eventual surface saturation of the chemicals released, which removed the graduation of the local surface tension differences and consequently reduced the motor's movement to a stop.^[142] To overcome this challenge, Chattopadhyay et al. proposed to replace horizontal motion with vertical motion by performing diving/surfacing cycled movements on a surface using a composite magnetic particle and Pd nanoparticles by decomposing hydrogen peroxide. The magnetic component induced electromotive forces, due to the autonomous motion of micro-robot into a Faraday coil linked to an electrical circuit was then

illustrated.^[139] However, the finding showed that self-propelled chemical-energy powered entities has an inherent limit, due to the constraints of the size of devices used, as it will not be able to drive large devices. The magnet loaded in this small device maintains only a weak magnetic field intensity and in practical situations, a larger magnet is often required. Shi's group introduced a new and mild strategy that cut the magnetic line with a conductive line in the vertical direction.^[143–145] They designed a functionally co-operating device to convert energy for reutilization through diving-surfacing cycles propelled by H₂,^[143] CO₂^[144] or O₂^[145] bubbles produced from the reaction between Mg/CaCO₃ and HCl or the decomposition of H₂O₂ on Pt catalyst. Due to superhydrophobicity of the pH-reactionary surface, these smart devices are able to be buoyant on water surface in acidic media. By means of shifting the pH of the solution from acidic to alkaline conditions, the pH-responsive surface is altered to be superhydrophilic and the consequent diving event occurs. Tuning the pH of aqueous solution into the acidic region, resulted in these smart devices coming back up to the surface, propelled by the H₂, CO₂ or O₂ bubbles produced from the reaction between Mg/CaCO₃ and HCl or the decomposition of H₂O₂ on Pt catalyst. A magnetic field maintained parallel to these smart devices, will generate an induced current based on Faraday's law, by cutting the magnetic lines through a metallic conductive element, serving as mini-generators. These mini-generators, can act potentially as a power supply for small scale sensors and robots through the conversion of chemical energy to electricity on-site. On the other hand, all these mini-generators were fabricated with commercially accessible copper foam coated with silver, which is easy to prepare and doesn't require special and complex chemicals compared to the current small-scale generators which produce electricity through the conversion of thermal or kinetic energy using specialty materials (piezoelectric or pyroelectric).^[146,147]

Recently, the same group has made a three-jaw mini-generator which is able to do a step-wise conversion from chemical energy into the mechanical energy, followed by conversion to electricity.^[148] This mini-generator was driven by O₂ bubbles resulting from the system of decomposing H₂O₂ using platinum. The self-powered generator exhibits turning motion, due to oxygen bubbling, when it is located under a solenoid coil in a solution of H₂O₂ (10%). This Pt-H₂O₂ system is used as a model to demonstrate the possibility of collecting energy from the surroundings for practical energy production, based on Faraday's law. It was proposed to solve the non-sustainability issue of energy generation in a Marangoni-effect motion powered systems.

Hydrogen has been proposed to be used as an environment-friendly fuel with high energy density and in light-weight systems. With recent advances in fuel cells, providing a solution for the practical and portable energy generator is highly demanding and remains a challenge. Wang's group demonstrated the capability of micromotors to generate on-site H₂ efficiently and overcome the challenges associated with H₂ transportation and storage.^[140] They explained the use of catalytic self-propelled Pt-black/Ti Janus micromotors for controlled gas generation in a hydrogen fuel cell model car by producing hydrogen and oxygen in situ, with NaBH₄ and H₂O₂ respectively. This work presents the significance of using self-propelling micromotors

as a tool for fast and efficient in situ hydrogen production in the presence of NaBH_4 compared to the traditional method of using a static catalyst.

Recently, our group proposed a self-propelled supercapacitor for on-demand circuit configuration based on microrobots electrodeposited from WS_2 NPs / polyaniline(PANI) and platinum layers. The energy storage capability of these microrobots was measured by the evaluation of their capacitive behavior using gold microelectrode integrated in a microfluidic channel. The self-propelled supercapacitor reported shows high capacitive behavior in terms of specific capacitance ($1400 \mu\text{F cm}^{-2}$), energy density (0.07 mW h m^{-2}) and power density (120 mW m^{-2}). This new concept represents a new generation of smart and miniaturized supercapacitor devices based on microrobots that are capable of carrying electric charges to specific destinations.^[62]

6. Nano/Microrobots in Drug Delivery and their Electrochemistry

Nano/microrobots have shown promising abilities to deliver drugs and their capabilities have been strongly intertwined with electrochemistry. Electrochemically deposited micromotors have been the more prevalent models of study in biomedicine, where proposed functions such as cell transport,^[35,149] drug delivery,^[41,150] genetic therapy^[14] and intricate operations such as manipulating intracellular organelles have been demonstrated.^[151] Additionally, electrochemical processes such as self-electrophoresis-based nanomotors have been shown to transport the anti-cancer drugs of doxorubicin.^[41] Through electrodeposition, incorporation of model drugs of silica and gold nanoparticles have been demonstrated in zinc based micromotors, where degradation in acidic environments trigger the release.^[152,153] Electrosynthesis also shows capability of introducing unique additives into the microstructure, where molecular imprinting allows moulds of proteins to be sculpted into the structure, where specific transport of proteins can be achieved.^[10] On the side note, the electrostatic nature of the surface charges to hold onto the drug particles have been demonstrated,^[154,155] where reversible changes in environment such as pH change can lead to the release in the cargo.^[154] This is especially true for drugs loaded in liposomes, where on-demand release can be achieved after mechanical or chemical breakdown.^[150]

While chemotactic^[4] and phototactic behavior^[117] has been expressed with nano/microrobots, this is difficult to achieve under in vivo environments without the use of toxic reagents such as hydrogen peroxide. However, it is duly noted that nanomotors themselves show biocompatibility.^[156] With the toolbox made available by recent research, biocompatible fuels such as zinc running on acidic environments,^[152,153] or magnesium in neutral or alkaline conditions have been developed.^[157] Encapsulation using enteric coating found in oral drugs in electrochemically fabricated micromotors shows the ability to distribute the devices to different regions of the gastrointestinal tract when the coating thickness is altered.^[157] By designing unique degradation zones, drugs can be specifically released, such as onset of an acidic environment.^[153] Alternatively, a

photothermal release of drugs at intended zones can also be achieved using a near infra-red source.^[154] Magnetic spatiotemporal manipulation coupled with controlled cargo release can also be realized at targeted drop zones with electrodeposited nickel modification at the nano/microrobot.^[35,41] An inventory of the above utilities is needed to achieve the ultimate aim of targeted drug therapy. Continuous work is needed to design micromotors which can release drugs on the prompting of a specific chemical or physical signature. Electrochemistry to synthesize, power and control nano/microrobots will prove to be an important field of research in drug delivery.

7. Conclusions

We have discussed various electrochemical techniques, which allows for the fabrication of large amounts of nano/microrobots from diverse materials, with and without the use of template. Furthermore, we also have discussed the role of the electrochemistry in the motion of self-electrophoretically driven micromotors and the general fluid flow generated, driven by electrochemical reactions. To illustrate guidance and control over these artificially fabricated objects, we showcased that electrochemistry and electric fields can aid the navigation of these devices. For an intrinsic look at locomotion, we have also described how the electrochemistry can be used as a tool for the detection of the motion of these tiny devices, which is especially useful in occluded solutions. Last but not least, we described how the micro and nanorobots can dramatically increase efficiencies of electrochemical sensors and energy generation devices. An insight to how electrochemistry influences the use of nano/microrobot in biomedical usage is also provided. The impact of electrochemistry on nano/microrobot design and their utility is significant. Coupling this understanding with the application of these self-propelled devices will serve to better our craft at using them to accomplishing complex missions.

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Sensors

Biomimetic Artificial Inorganic Enzyme-Free Self-Propelled Microfish Robot for Selective Detection of Pb^{2+} in WaterJames Guo Sheng Moo, Hong Wang, Guanxia Zhao, and Martin Pumera^{*[a]}

Abstract: The availability of drinking water is of utmost importance for the world population. Anthropogenic pollutants of water, such as heavy-metal ions, are major problems in water contamination. The toxicity assays used range from cell assays to animal tests. Herein, we replace biological toxicity assays, which use higher organisms, with artificial inorganic self-propelled microtubular robots. The viability and activity of these robots are negatively influenced by heavy

metals, such as Pb^{2+} , in a similar manner to that of live fish models. This allows the establishment of a lethal dose (LD_{50}) of heavy metal for artificial inorganic microfish robots. The self-propelled microfish robots show specific response to Pb^{2+} compared to other heavy metals, such as Cd^{2+} , and can be used for selective determination of Pb^{2+} in water. It is a first step towards replacing the biological toxicity assays with biomimetic inorganic autonomous robotic systems.

Introduction

Detection of water pollutants is an important but challenging task. Thus to say, to be proactive rather than being retroactive in solving problems surrounding aquatic pollution is of utmost importance.^[1,2] Through early detection, appropriate preventive measures could be taken without the actual need of a remediation step. The response of living organisms towards aquatic contamination has been one of the frontlines of defense against the pollution of water bodies.^[3–5] Behavioral studies and mortality rates of fish have been demonstrated to both identify and quantify the amount of contaminants in water.^[3,4] However, given the absence of clear guidelines, consistency and the sheer human capital and technological intensiveness required of such a set-up, the implementation is extremely difficult.^[6] Hence, this arises a need for a robust, simple, and scalable system that can be easily implemented and has ease of operation.

Micromotors are new self-propelled materials at the forefront of materials science that can move autonomously to perform specific tasks.^[7–12] These micromachines or microrobots hold great promise in numerous fields. They have been demonstrated to be able to carry out a wide spectrum of work including those in biomedical applications,^[13] cargo delivery,^[14] and environmental remediation.^[15,16] Amongst all, the bubble-ejection propulsion mechanism in a tubular catalytic engine, which utilizes hydrogen peroxide as its fuel, is one of the most

intensively studied model.^[12,17] These microjets have been shown to be able to navigate through real-world environments: from the water bodies of lake water and sea water to tap water.^[18] However, the surrounding chemical environment has also been demonstrated to have pronounced influence on the very behaviors of these microjets.^[19,20] Recently, Orozco et al. developed enzyme-powered bubble-propelled micromotor, dubbed “microfish” for sensing pollutants, such as Hg^{2+} , Cu^{2+} , or aminotriazole.^[21] It was argued that such artificial enzyme-powered microfish can play the same role as live fish, obviating the need to use living organisms for toxicity assays. The downside of such artificial enzyme-powered microfish is that it is not selective to different pollutants and also that the enzyme layer (which is the sensitive layer) has limited shelf-time due to its biological nature. Herein, we obviate the use of enzyme and show that artificial inorganic (enzyme-free) microfish can be used for selective sensing of Pb^{2+} in water.

The increase of heavy-metals concentration in the environment has been associated with anthropogenic activities, especially that of the burning of fossil fuels and industrial activities.^[22] This is of particular concern, because the accumulation of such pollutants is detrimental to human health.^[23] Platinum has been demonstrated to show sensitivity towards poisoning effects, because minute concentrations of heavy metals are known to retard the efficiency of catalytic convertors^[24,25] and also inhibit organic reactions during catalysis.^[26] To evaluate the effects of poisoning by heavy metals, the decomposition of hydrogen peroxide over platinum has been used as a model of study.^[27] Herein, we explored the possibility of using bubble-propelled Pt microfish robots to establish a correlation between their activity and the presence of heavy-metal ions of Pb and Cd. A decrease in microjet activity can be attributed to the poisoning of the Pt microfish robot and the change in chemical environment. In the following sections, the use of

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a simple optical visualization of the microfish robots, allows for a facile inference to the type of pollutants that is present.

Results and Discussion

Herein, we demonstrate that inorganic enzyme-free Pt-based microfish robots can selectively sense presence of Pb^{2+} in water. The microfish robots are powered by the bubble propulsion caused by the catalytic decomposition of hydrogen peroxide at its inner surface of platinum to oxygen. The expulsion of the bubble at the ends propels the microfish robots forward. The influence of heavy metals over the viability of microfish robots was investigated. Two principal representative ions of Pb^{2+} and Cd^{2+} , commonly present in polluted water, were used as poisons to cripple the inorganic Pt microfish robots.^[23] Ultrapure water was used as the control environment to which the motion of the microfish robots can be compared. Two observable physical attributes obtained from the microfish robots were used to evaluate the propulsion of the microfish robots: the number of microfish robots, which are expelling bubbles and the number of microfish robots exhibiting motion due to the bubble propulsion. The average velocities and the corresponding standard deviation were taken. Selective introduction of the heavy metals of Pb^{2+} and Cd^{2+} were carried out at appropriate concentrations to poison the inorganic Pt microfish robots.

The motion of the microfish robots was first evaluated in ultrapure water. As illustrated in Figure 1, 100% of the microfish robots that ejected bubbles demonstrated mobility in ultrapure water. Then, selective introduction of the heavy-metal salt of $\text{Pb}(\text{NO}_3)_2$ was carried out. At 0.48 mM concentration of $\text{Pb}(\text{NO}_3)_2$ (100 ppm of lead), only 72% of the microfish robots were bubbling, and 67% of them demonstrated bubble-propulsion motion. The gradual decrease in bubble ejection and microjet propulsion was recorded across increasing concentrations of $\text{Pb}(\text{NO}_3)_2$, until a concentration of 1.92 mM at which

bubble ejection at 24% and bubble-propelled motion at 21% were recorded. To make sure that the Pt microfish robot is specifically reacting to Pb^{2+} and not to any inorganic compounds, we looked into the literature. When the ionic strength of the KNO_3 was compared to 1.92 mM of $\text{Pb}(\text{NO}_3)_2$ at appropriate levels, there was little disabling of the microfish robots with approximately 75% of them remaining active for bubbling and microjet propulsion.^[18] This was indicative that in addition to the changes in ionic strength due to the introduction of $\text{Pb}(\text{NO}_3)_2$, an additional mechanism of poisoning and disabling of the microfish robots was present, namely, the poisoning of Pt surface by Pb^{2+} .

In Figure 1, the correlation between bubble ejection and mobility with the introduction of a Cd^{2+} poison through $\text{Cd}(\text{NO}_3)_2$ was investigated. At 0.89 mM of $\text{Cd}(\text{NO}_3)_2$ (100 ppm of cadmium), 97% of the microfish robots were bubbling, and 92% of them were exhibiting propulsion. This was in contrast to the large decrease in bubbling and propelled microfish robots in $\text{Pb}(\text{NO}_3)_2$ at the similar molar concentrations at which only 46% of the microfish robots were bubbling, and 45% of them were exhibiting propulsion. The behavior of microfish robots under influence of 1.78 mM $\text{Cd}(\text{NO}_3)_2$ demonstrated similar behavior to that of under influence of KNO_3 at comparable concentrations at which about 75% of the microfish robots were demonstrating both bubble ejection and propulsion motion. A slowly tapering decrease in both bubble ejection and propulsion amongst the microfish robots was observed with increasing concentrations of $\text{Cd}(\text{NO}_3)_2$ and KNO_3 . This recurring similar trend between the two salts demonstrates that the increment in ionic strength adversely affected the motion of microfish robots as was observed by Zhao et al. in their work on chemical environments^[18] and Wang et al. with blood electrolytes.^[20] Only at a concentration of 8.9 mM of $\text{Cd}(\text{NO}_3)_2$ was observed that more than 50% disablement of the microfish robots (LD_{50} : the concentration of a given agent, which is lethal to 50% of the artificial microfish robots) in bubble ejection and microjet propulsion could be achieved.^[21]

For the ease of comparison, the concentration of heavy-metal ions, which was able to poison the number of microfish robots bubbling and demonstrating mobility to less than 50% (LD_{50}), was used as a gauge. In the case of $\text{Pb}(\text{NO}_3)_2$, the amount of 0.96 mM of Pb^{2+} was required. On the other hand, 8.9 mM of $\text{Cd}(\text{NO}_3)_2$ was required to reduce the number of microfish robots exhibiting bubbling and microjet propulsion below 50% (LD_{50}). This is a clear indication that platinum-based microfish robots are highly sensitive to the presence of lead ions compared to cadmium, and that they are able to detect the presence of lead down to micromolar concentrations, as was demonstrated by the drastic drop in active microfish robots in the presence of 0.48 mM of Pb^{2+} . This is in the same order of magnitude as the heavy metals found in effluents from industrial plants^[28–29] and sludge materials.^[30] Notably, at these concentrations, heavy metals disrupt the ability of cellular ion channels to perform modulation, causing serious health concerns.^[31–32]

Figure 2 illustrates the velocity of the microfish robots with increasing concentrations of heavy metal ions. During the con-

Figure 1. Percentage of active microfish robots showing bubble ejection (black line) and moving by microjet propulsion (grey line). Poisoned with selected concentrations of $\text{Pb}(\text{NO}_3)_2$ (○). Poisoned with selected concentrations of $\text{Cd}(\text{NO}_3)_2$ (■). Ultrapure water was used in the control experiment, in which both the running and bubbling percentages were defined as 100%. Conditions in all experiments: 23 °C, 3 wt% of H_2O_2 , and 1 wt% SDS.

Figure 2. Velocity of Pt-catalyzed microfish robots in electrolyte solution. a) Poisoned with selected concentrations of $\text{Pb}(\text{NO}_3)_2$. b) Poisoned with selected concentrations of $\text{Cd}(\text{NO}_3)_2$. Conditions in all experiments: 23 °C, 3 wt % of H_2O_2 , and 1 wt % SDS.

trol experiment in the absence of heavy metals, the microfish robots demonstrated an average velocity of $310 \mu\text{m s}^{-1}$. This is in line with previous velocities reported by our group, with the microfish robots fabricated by an electrochemical template procedure, achieving an average velocity of $365 \mu\text{m s}^{-1}$.^[18–20] With the introduction of 0.96 mM of $\text{Pb}(\text{NO}_3)_2$ shown in Figure 2a, the velocity of the microfish robots dropped to $196 \mu\text{m s}^{-1}$. This is in contrast to the case of $\text{Cd}(\text{NO}_3)_2$ at 0.89 mM shown in Figure 2b, in which the velocity of the microfish robots remained considerably higher compared to the $\text{Pb}(\text{NO}_3)_2$ at $231 \mu\text{m s}^{-1}$. The Pb^{2+} poisoned microfish expressed a steady decrease in velocity to about $110 \mu\text{m s}^{-1}$ at a concentration of 1.92 mM compared to Cd^{2+} poisoned microfish robots, in which a relatively steady velocity between approximately 125 to $150 \mu\text{m s}^{-1}$ was reached at concentrations above approximately 1 mM. It was only observed at 8.9 mM of $\text{Cd}(\text{NO}_3)_2$ that the velocity of microfish dropped to $74 \mu\text{m s}^{-1}$. The phenomena observed with $\text{Cd}(\text{NO}_3)_2$ was analogous to the phenomena observed with KNO_3 , in which during concentration increase, the velocities of the microfish robots was held relatively constant at approximately $150 \mu\text{m s}^{-1}$.^[18] The similarities of the observations under influence of $\text{Cd}(\text{NO}_3)_2$ and KNO_3 for microfish robots demonstrate that the reduction in activity was mostly due to changes in ionic strength. However, in the

presence of Pb^{2+} , an additional poisoning mechanism of the microfish took place.

The differing behaviors of the heavy-metals-poisoned microfish robots could be explained as follows. Pb^{2+} is known to be strongly adsorbed on the platinum: these ions cover the active sites of the platinum, resulting in the decrease in activity of the platinum catalytic sites.^[25] Many reports have shown that the adsorption of the lead ion on platinum is irreversible,^[33] and it is often termed as a permanent poison.^[34] But in contrast, adsorption of Cd^{2+} was noted to be weaker, and the interactions are likely to be reversible.^[35–37] The decreased bubbling at Pb^{2+} -poisoned microfish was coupled with decreased activity and velocity. Evidently, the main mechanism of bubble propulsion has been retarded, resulting in the loss of motility.

This could be explained by electronegativity of the Pb^{2+} ions. Lead, with an electronegativity of the 2.33 on the Pauling scale, is considerably more electronegative than the value of 1.69 for cadmium.^[38] The adsorbed atoms of Pb are likely to be strongly attached on the surface of platinum during adsorption compared with Cd, resulting in the greater degree of poisoning, as demonstrated herein. The atomic size of lead is also larger than cadmium, covering more active sites on the platinum surface, effectively disabling the microfish robots further. In another independent study of a self-electrophoretic Pt/Au nanorod, a similar but less accentuated trend was also observed between the presence of Pb^{2+} and Cd^{2+} , illustrating the poisoning effects of Pb^{2+} .^[39]

However, organosulfur compounds also inhibit the activity of microfish to similar degrees^[19] and the presence of red blood cells retard their movement.^[40] Therefore, the contextual space, in which the microfish are applied, must be considered. More work is still required for the use of this proof-of-concept device for selective detection of compounds against a general setting.

The differing behavior of the microfish robots towards heavy-metal ions of Pb^{2+} and Cd^{2+} allowed a selective inference of the type of ions that are present in the solution. Although colorimetric methods,^[41] self-electrophoretic nanomotors,^[39] and enzyme-powered microfish^[21] face selectivity limitations for heavy metals at high micromolar concentrations, these Pt-based microfish demonstrated aptitude at these ranges. This opens a simple pathway towards identifying the type of inorganic substances present in aquatic sources. The study of the activity and mobility of the inorganic Pt microfish robots allows a facile detection of the lead ions over cadmium.

Conclusion

The effects of poisoning of inorganic Pt microfish robots were systematically studied with the selective introduction of the heavy-metal salts $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$. A greater decrease in the activity and the velocity of inorganic Pt microfish robots were observed during poisoning with $\text{Pb}(\text{NO}_3)_2$ compared to $\text{Cd}(\text{NO}_3)_2$. The inherent properties of the lead ion-adsorption behavior with platinum resulted in the decreased activity of the inorganic Pt microfish robots compared to Cd^{2+} . The differentiating behavior of Pb^{2+} with inorganic Pt microfish robots

allowed the selective detection of Pb^{2+} over Cd^{2+} . This allowed a potential pathway for a continuous monitoring of pollutants by an optical visualization of the activity and mobility of the inorganic Pt microfish robots.

Experimental Section

Materials

The cyclopore polycarbonate membranes with pores of 2 μm in diameter were purchased from Whatman, USA (cat. no. 7060–2511). The pores are conical in shape. Colloidal graphite (isopropanol base) was purchased from Ted Pella, Inc., USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1 M KCl were purchased from CH instruments, USA. The platinum-plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98 + %), sodium dodecyl sulfate (SDS), $\text{Pb}(\text{NO}_3)_2$, and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ were purchased from Sigma–Aldrich. Chemicals were used as received, and the solutions were prepared by using ultrapure water (18.2 M Ω cm) from a Milli-pore Milli-Q purification system.

Apparatus

Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 °C) by using a three-electrode arrangement. A platinum electrode was utilized as a counter-electrode, and Ag/AgCl was used as the reference electrode. The ultrasonication process was carried out with a Fisher-brand FB 11203 ultrasonicator, and centrifugation was carried out with a Beckman Coulter Allegra 64R centrifuge. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software.

Preparation of Cu/Pt concentric bimetallic microtubes^[42]

The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template, and they were referred as “microfish robots” in the following discussion. Colloidal graphite ink was applied on the one side of the polycarbonate template with commercial cotton swabs. A piece of flattened aluminium foil was attached to the ink immediately, which serves as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl reference electrode were utilized. Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyzer connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software. The template was rinsed with ultrapure water (5 mL; 18.2 M Ω cm) for four times, and the Cu outer layer was deposited galvanostatically at –4 mA for 450 s. The deposition solution contained 1 M CuSO_4 . Consequently, after removing the deposition solution, the template was rinsed five times with water (8 mL). The platinum segment was electrodeposited subsequently at –4 mA for 450 s, by using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled, and the template was washed five times with water (8 mL). After that,

the template was ultrasonicated for three times in ultra-pure water (2 mL) for 3 min each time. The graphite layer was removed during the ultrasonication procedure. The template was placed in an Eppendorf tube with methylene chloride (2 mL) and ultrasonicated, until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly three times with methylene chloride. The solution was then washed with ethanol and water two times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Operation of microfish robots

The experiments for studying the influence of heavy metals on the motion of microfish robots were carried out in an aqueous solution containing hydrogen peroxide (3 wt%) at constant surfactant concentrations (1 wt% of SDS). A mixture of microfish robots (7 μL), SDS (1 wt%), H_2O_2 (3 wt%), and appropriate concentrations of the heavy-metal salts of $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2$ were applied on a glass slide that was freshly cleaned with nitrogen gas. Statistics from the number of microfish within a reading is given in the Supporting Information.

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Graphene Oxides Prepared by Hummers', Hofmann's, and Staudenmaier's Methods: Dramatic Influences on Heavy-Metal-Ion Adsorption

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Graphene oxide (GO), an up-and-coming material rich in oxygenated groups, shows much promise in pollution management. GO is synthesised using several synthetic routes, and the adsorption behaviour of GO is investigated to establish its ability to remove the heavy-metal pollutants of lead and cadmium ions. The GO is synthesised by Hummers' (HU), Hofmann's (HO) and Staudenmaier's (ST) methodologies. Characterisation of GO is performed before and after adsorption experiments to investigate the structure–function relationship by using Fourier-transform infrared spectroscopy and X-ray photoelectron spectroscopy. Scanning electron microscopy coupled with elemental detection spectroscopy is used to investigate morphological changes and heavy-metal content in the adsorbed GO. The filtrate, collected after adsorption, is analysed by inductively coupled plasma mass spectrometry, through which the efficiency and adsorption capacity of each GO for heavy-metal-ion

removal is obtained. Spectroscopic analysis and characterisation reveal that the three types of GO have different compositions of oxygenated carbon functionalities. The trend in the affinity towards both Pb^{II} and Cd^{II} is HU GO > HO GO > ST GO. A direct correlation between the number of carboxyl groups present and the amount of heavy-metal ions adsorbed is established. The highest efficiency and highest adsorption capacity of heavy-metal ions is achieved with HU, in which the relative abundance of carboxyl groups is highest. The embedded systematic study reveals that carboxyl groups are the principal functionality responsible for heavy-metal-ion removal in GO. The choice of synthesis methodology for GO has a profound influence on heavy-metal-ion adsorption. A further enrichment of the carboxyl groups in GO will serve to enhance the role of GO as an adsorbent for environmental clean-up.

1. Introduction

Graphene is an emerging material of interest, owing to its two-dimensional structure and large surface area with a honeycomb lattice made of carbon.^[1] The oxidised analogue, graphene oxide (GO), has attracted an equal amount of attention because of the abundance of oxygen functionalities, which allows its easy manipulation and configuration.^[2] To date, the bulk synthesis of GO has been accomplished through the oxidation of graphite to graphite oxide. The lamellar graphite-oxide structure has increased interlayer spacing of the oxygenated groups, and this allows for facile exfoliation to GO by means of an ultra-sonication step.

The preparation of graphite oxide has a rich history that can be dated back more than 150 years.^[3] Its structure has been demonstrated to contain various oxygenated groups, such as alcohols, epoxides, carbonyls and carboxylic acids.^[2] The most accepted Lerf–Klinowski model describes graphite oxide as carbon sheets with graphitic sp² islands that are filled with oxygenated functional groups, that is, epoxy and hydroxyl

groups on the basal plane and carbonyl and carboxyl groups at the edge planes.^[4] Three principal methods for the preparation of graphite oxides have been identified in the literature: Staudenmaier's (ST) method, which uses chlorate oxidant in fuming nitric acid,^[5] Hofmann's (HO) variant, which uses chlorate in sulfuric acid^[6] and Hummers' (HU) method, which uses a permanganate oxidant.^[7] The different methods of preparation have been demonstrated to influence the composition of the oxygenated groups in the graphite oxide,^[8] which consequently affects the chemical profile of the GO.^[9] The oxygenated groups in GO have been demonstrated to serve essential roles in a wide spectrum of applications. They have been shown to be available for use in covalent chemistry,^[10] anchor metal nanoparticles,^[11] demonstrate photoluminescence^[12] and exhibit catalytic behaviour in synthetic routes.^[13] Most interestingly, recent research has shown that they possess improved adsorption rates towards heavy metals in comparison to other common adsorbents for environmental remediation.^[14]

Owing to the recent rise in anthropogenic activities such as industrial activities and indiscriminate disposal of refuse, environmental pollution has become a serious problem.^[15] Water pollution caused by heavy metals is of particular concern, owing to the ready accumulation of these heavy metals in the ecosystem and the health problems they pose.^[16] Remedial measures not only protect the natural habitat, but also prevent the heavy metals from accumulating in the food chain and in-

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advertently affecting humans. Measures to remove these heavy metals include reduction, precipitation, membrane filtration, ion exchange and the use of adsorbents.^[14d] Adsorption is one of the most ideal methods, as it not only removes the pollutants from the system, but also allows for the proper disposal of such chemical hazards and their possible recovery for the conservation of materials.^[17]

Carbonaceous nanomaterials have a large surface area, are porous and can be decorated with oxygenated functionalities, which make them fitting candidates for adsorbents.^[18] Allotropes of carbon, such as activated carbon, carbon nanotubes and fullerene show high capacity towards the absorption of environmental pollutants.^[19] Recent findings have turned the spotlight towards GO-based materials, for which reports of unprecedented adsorption of pollutants such as antibiotics,^[20] dyes^[21] and heavy metals^[14] have been recorded. GO shows particular affinity towards the adsorption of heavy metals, because of the ready availability of oxygenated groups. The absence of steric hindrance as a result of its large surface area and the availability of both sides of the GO for the binding of heavy-metal ions make it an outstanding candidate. Zhao et al. employed the Langmuir isotherm to simulate the maximum adsorption capacity of GO synthesised by the HU method, and calculated the maximum adsorption capacity to be 842 mg g^{-1} for Pb^{II} ^[14b] and 106.3 mg g^{-1} for Cd^{II} .^[14c] Recently, Sitko et al., using a chromate oxidant and HU methodology, calculated a maximum adsorption capacity of 1119 mg g^{-1} for Pb^{II} and 530 mg g^{-1} for Cd^{II} ,^[14a] from the Langmuir isotherm, which surpassed the results of earlier work.^[14c] The adsorption capacities of the GO-based materials surpass the nearest comparable sorbent by more than 30-fold, as shown in a recent compilation of sorbent adsorption capacities.^[14b] To elucidate the reason behind this property, a structure–function relationship must be established. Herein, we investigate how different preparation methods for GO affect the ability of the resultant GO to adsorb two important anthropogenic sources of heavy-metal ions, namely, lead and cadmium in water.

Previous work on the adsorption of heavy metals focused on only one type of GO that was prepared mainly by the HU method. We have previously demonstrated that GO prepared by different methods contains different amounts of oxygen-containing groups,^[9a] and we, thus, expect the method of preparation of the GO to have a strong influence upon the heavy-metal adsorption properties of the GO. In this report, we show that this hypothesis is indeed true. Three types of GO, that is, GO produced by HU, HO and ST methods, were used for adsorption tests with the heavy-metal-ions Pb^{II} and Cd^{II} . Fourier-transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy/elemental detection spectroscopy (SEM/EDS) were used to characterise the GO material before and after the adsorption experiment to understand the correlation of the oxygenated carbon functionalities with heavy-metal adsorption. Inductively coupled plasma mass spectrometry (ICP–MS) was used to detect the amount of heavy-metal ions adsorbed by the GO. We illustrate that the preparation method, and consequently the oxygenated carbon functionality composition, of the GO affects

the amount of heavy-metal ions that are adsorbed by the GO material. HU GO demonstrates the best efficiency towards the removal of Pb^{II} and Cd^{II} ions. The results give us an improved understanding of the functionalities present in GO, and empower us to further design and customise the GO material towards heavy-metal-ion adsorption.

2. Results and Discussion

Prior to the study of the adsorption of heavy-metals onto GO prepared by different methods, the GO materials synthesised from the HU, HO and ST methods were characterised in detail to elucidate the functionalities that were present. Adsorption experiments were carried out with 0.1 mg mL^{-1} of GO. Under ambient conditions, deionised water has a pH of six; however, in the presence of 0.1 mg mL^{-1} of HU, HO or ST GO, the pH of the solution was around three, which indicates their acidic nature.^[14b,c] When 5 ppm of heavy-metal ions were introduced, a decrease in pH was observed in the solutions containing GO synthesised using the HU and HO methods, but no such drop in pH was observed for the ST GO (see Table S1 in the Supporting Information). This phenomenon was particularly accentuated in HU GO, for which a change in pH from 3.40 to 2.62 in the presence of Pb^{II} and to 2.57 in the presence of Cd^{II} was observed. This is indicative of binding between GO and heavy-metal ions, owing to the substitution of protons with the metal ions.^[14b] After adsorption, the GO materials were characterised to elucidate the adsorption mechanism of GO towards heavy-metal ions. Hereafter, the Pb^{II} -adsorbed GOs are labelled HU Pb, HO Pb and ST Pb, and the Cd^{II} -adsorbed GOs are labelled HU Cd, HO Cd and ST Cd. Finally, ICP–MS of the filtrate was used to determine the capacity and efficiency of each individual GO towards the adsorption of heavy-metal ions.

2.1. FTIR Characterisation of GO Materials

The GO materials prepared by HU, HO and ST methods were characterised by FTIR analyses to investigate the presence of the different oxygenated carbon functionalities and the mode of binding between GO and the heavy-metal ions. Figure 1 shows six characteristic peaks centred at around 1719 cm^{-1} ($\nu_{\text{C=O}}$); 1624 cm^{-1} ($\nu_{\text{C=C}}$), 1373 cm^{-1} ($\nu_{\text{C-O}}$; carboxyl), 1228 cm^{-1} ($\nu_{\text{C-O}}$; epoxy/ether), 1050 cm^{-1} ($\nu_{\text{C-O}}$; alkoxy/alkoxide) and 951 cm^{-1} ($\nu_{\text{C-O}}$; epoxy/ether) were found in the FTIR absorbance spectra in all three of the GO films (see Figure S1 for full spectra).^[22] The differing IR stretching bands of GO suggest that the differing oxidising conditions resulted in different compositions of oxygenated carbon functionalities. The HU pathway resulted in a GO material with increased amounts of more highly oxidised functionalities compared to the chlorate oxidation in the HO and ST methods. This could be observed in the carbonyl stretching band of the GO film; comparatively higher absorbance was observed for the HU GO film than for the HO or ST films.^[8,9] The FTIR absorbance spectra of the heavy-metal ions adsorbed on the GO film were also evaluated. Park et al. have established that the increased intensity of the carboxyl C–O stretching peak and its shift towards lower

Figure 1. (ATR) FTIR absorbance spectra of a) HU GO film, b) HO GO film and c) ST GO film before and after heavy-metal-ion adsorption indicating the $\nu_{\text{C}=\text{O}}$ (1), $\nu_{\text{C}-\text{O}}$ (2), $\nu_{\text{C}-\text{O}}$ (carboxyl; 3), $\nu_{\text{C}-\text{O}}$ (epoxy/ether; 4), $\nu_{\text{C}-\text{O}}$ (alkoxyl/alkoxide; 5), $\nu_{\text{C}-\text{O}}$ (epoxy/ether; 6) signals.

wavenumber is associated with the co-ordination of metal ions in GO.^[22b] As shown in Figure 1, the lead-adsorbed GO materials HU Pb and HO Pb both show an increase in the intensity of the carboxyl stretching band and a shift towards a lower wavenumber upon adsorption of heavy-metal ions. Notably for ST Pb, the carboxyl stretching band diminished upon adsorption of heavy metals, suggesting its lower efficiency as an adsorbent for heavy-metal ions. For the cadmium-adsorbed GO materials, a similar trend was observed across the three different GO

samples. The reasons underpinning the above observations warrant further analysis, in which definitive identification of elemental species is plausible. Further investigations into the binding mode between the oxygenated functionalities and the heavy-metal ions was undertaken by using XPS.

2.2. XPS Characterisation of GO Materials

2.2.1. Survey Scan

The XPS technique allows the sensitive detection of surface elemental compositions. A typical survey scan gives a fast overview of the elements present in the material of interest and their associated percentage abundance. The carbon-to-oxygen (C/O) ratio could be obtained by comparing the C 1s to O 1s peaks in the GO. Based on the survey scan in Figure 2, HU GO has the lowest C/O ratio (see also Table 1). The same phenom-

Table 1. C/O ratio obtained from XPS survey scans by elemental analysis using respective atomic percentages of C 1s and O 1s peaks.

Adsorption	HU	HO	ST
GO	3.30	3.65	4.00
GO Pb	3.27	3.64	3.61
GO Cd	3.10	3.83	3.53

enon has been reported for graphite oxides produced by permanganate and chlorate oxidants; the permanganate routes produce GO with a significantly lower C/O ratio, as reported by Chua et al.^[8] and Eng et al.^[9a] The GO film produced by using the HU route in this study has a C/O value of 3.3, which is close to the value for the HU GO films produced from filtration methods reported by Yang et al. (2.9).^[23] HO GO and ST GO demonstrated higher C/O ratios of 3.65 and 4.00. The low atomic ratios in all three GO samples indicate that the oxidation process is thorough.^[8] The extent of oxidation is greatest

Figure 2. XPS spectra on GO film: a) control, b) after adsorption with 5 ppm Pb^{II} and c) after adsorption with 5 ppm Cd^{II}.

in HU, followed by HO and then ST. During survey XPS with heavy-metal-adsorbed GO, significant peaks from the Pb 4f doublet were identified at 139.0 and 143.8 eV in HU Pb, and from the Cd 3d doublet at 405.0 and 411.8 eV in HU Cd.^[14a] Comparatively, only minor peaks were identified in HO Pb and HO Cd. However, no peaks were identified in ST Pb and ST Cd; this is likely because the amount of Pb and Cd is lower than the detection limit of XPS in these GO samples.^[24] Notably, the introduction of heavy-metal ions does not significantly change the C/O composition of HU or HO GO, as seen in Table 1. However, a significant change in the composition of ST GO was noted, with a drop in C/O ratio from 4.00 in ST, to 3.61 in ST Pb and to 3.5 in ST Cd. Changes in the C/O ratio have previously been observed with the introduction of metal ions during the fabrication of metal ion/GO films.^[22b] These changes are associated with the ring-opening of epoxy groups in the presence of Lewis acidic metal cations. The co-ordination of the epoxy oxygen atoms to the Lewis acidic metal cation activates it for attack by weak nucleophiles such as water. ST Cd exhibited a greater change in oxygen composition, because of the greater number of ions present in 5 ppm of Cd^{II} solution than in 5 ppm Pb^{II} solution. This corroborates with the increased oxygen composition that is due to the introduction of hydroxyl groups during nucleophilic attack by the water molecules present.

2.2.2. High-Resolution XPS C 1s Scan

High-resolution XPS C 1s scans were carried out to investigate the types of oxygenated carbon functionalities that were present and the mode of binding between GO and the heavy-metal ions. First, the XPS spectra of HU, HO and ST were overlaid and compared. As illustrated in Figure 3, the three spectra

Figure 3. High-resolution XPS C 1s core-level spectra for HU, HO and ST GO films.

share a similar baseline. Hence, direct comparison of the composition and the types of oxygenated carbon functionalities present is possible. It is evident that the different oxidation treatments result in different oxygenated carbon compositions. The GO film produced by the HU method is significantly more

oxidised, with an increased presence of carbonyl and carboxyl groups in the region between 287.5 and 289.5 eV.^[8–9] This is followed by the HO GO film and then the ST GO film, which demonstrates the lowest amount of oxidation. This is in agreement with the survey scan, which also showed that the HU GO produced by the permanganate oxidation method was the most oxidised.

The C 1s core-level spectra were deconvoluted into Gaussian peaks centred at 284.4, 285.4, 286.7, 287.6 and 288.8 eV, which correspond to the binding energies of C=C, C–C, C–O, C=O and O–C=O respectively, as illustrated in Figure 4. Typically, the raw spectra of HU, HO and ST GO each exhibit a strong C–O peak and a strong C=C peak. Larger proportions of carbonyl and carboxyl groups were found in the HU GO film as described by Chua et al. for graphite oxides produced by permanganate methods.^[8] This is in comparison to GO produced by chlorate oxidation in the HO and ST methods as illustrated in Table 2.

Table 2. Tabulated quantitative distributions of deconvoluted peaks from the high-resolution C 1s spectra of HU, HO and ST GO.

Material	C 1s peak distribution [%]				
	C=C	C–C	C–O	C=O	O–C=O
HU	40.1	11.3	30.3	13.1	5.3
HO	47.1	3.1	44.9	3.5	1.3
ST	50.2	5.8	38.3	4.7	0.9
HU Pb	34.2	12.3	33.9	15.6	4.1
HU Cd	41.7	6.9	35.2	11.7	4.7
HO Pb	50.1	1.7	43.2	3.4	1.7
HO Cd	50.5	3.9	40.1	4.3	1.2
ST Pb	47.7	4.5	42.6	4.0	1.3
ST Cd	44.7	4.5	46.0	3.6	1.2

Significant shifts in binding energies were observed in HU GO after adsorption of heavy-metal ions. However, this was not seen in the HO and ST GO films. As shown in Figure 4a, the peaks from the XPS spectrum of HU Pb broadened after adsorption of heavy-metal ions. Furthermore, there was a shift towards higher binding energies for the carbonyl and carboxyl groups. In particular, upon deconvolution, the binding energy of the O–C=O peak shifted by 0.7 eV towards higher binding energies from 288.8 to 289.5 eV. The binding energy of C=O also shifted 0.3 eV from 287.6 to 287.9 eV. This is indicative that the heavy-metal ions are bound to the carboxylate groups through an inner-sphere mechanism as demonstrated by Guan et al. with adsorbed carboxylate groups.^[25] The formation of inner-sphere complexes between the carboxyl groups and metal ions increases the effective nuclear charge and hence increases the binding energy of the electron.

XPS spectra of chlorate-prepared HO and ST GO films after heavy-metal-ion adsorption were studied. No significant shifts in the peaks or changes in the composition were observed in chlorate-prepared HO GO, as shown in Figure 4b. However, for ST GO (Figure 4c), there is a trend for the C–O peak to increase on going from ST Pb to ST Cd after adsorption experiments. Park et al. explained that the presence of the metal

Figure 4. High-resolution XPS C 1s core-level spectra for a) HU, HU Pb and HU Cd, b) HO, HO Pb and HO Cd and c) ST, ST Pb and ST Cd.

Lewis acid sites results in ring-opening of epoxides due to attack by weak water nucleophiles.^[22b] As a larger amount of divalent ions is present in 5 ppm of Cd^{II} solution than in 5 ppm Pb^{II} solution, the ring-opening of epoxides is more extensive in ST Cd than in ST Pb; this is observed in the C/O ratios from the survey scans. The introduction of these oxygen groups increases the percentages of C–O in ST Pb and ST Cd to 42.6% and 46.0%, respectively, compared to the control ST GO film at 38.3% (see Table 2). This phenomenon corresponds well with the survey scans, in which the C/O ratio decreased on going from ST to ST Pb and then to ST Cd. This suggests that GO films prepared by the ST method are richer in epoxides, which are easily cleaved by Lewis acidic metal cations, than the HO GO variant or permanganate oxidised HU GO.

2.3. SEM/EDS Analysis

SEM images, as illustrated in Figure 5, show the GO films are irregularly stacked sheets of carbon with large surface areas and

Figure 5. SEM images of HU, HO and ST GO films (magnification = 40 000 ×).

wavy morphologies, as previously reported.^[22b] No particles of lead or cadmium were observed in the images, which indicates that the take-up of heavy-metal ions follows an adsorptive mechanism. Analysis of HU Pb using SEM/EDS indicates an elemental composition with a weight percentage of 1.97% Pb and an atomic percentage of 0.05%. Subsequent analysis of the Cd content in HU Cd indicated a weight percentage of 0.4% Cd and an atomic percentage of 0.03%. No corresponding concentrations were found for HO Pb, HO Cd, ST Pb or ST Cd, which indicates that the amounts of adsorbed lead and cadmium are below the detection limits of the SEM/EDS instrument.^[24]

2.4. Adsorption Capability of GO Towards Heavy Metals

The capacity of HU, HO and ST GO towards the adsorption of Pb^{II} and Cd^{II} were evaluated by ICP–MS analysis of the filtrate. Figure 6a describes the capability of the different GO species

Figure 6. Adsorption of heavy-metal ions by using HU, HO and ST GO in a) 5 ppm of Pb^{II} and b) 5 ppm of Cd^{II}, after ultra-sonication for 2 h with [GO] = 0.1 mg mL⁻¹ at 25 ± 3 °C.

to adsorb Pb^{II}. It is evident that HU GO has the best adsorptive behaviour towards Pb^{II}, followed by HO GO and ST GO. Similarly, as shown in Figure 6b, HU GO demonstrates the best adsorption behaviour towards Cd^{II} amongst the three GO films, followed by HO GO and ST GO, in decreasing order.

Tabulated data for the efficiency and capacity of the three different GO films towards adsorption of heavy metals are given in Table 3. HU GO demonstrates the best efficiency towards adsorption of Pb^{II} and Cd^{II}. The efficiency of HU GO towards adsorption of Pb^{II} is 74.6% and it has a capacity of 37.3 mg g⁻¹ under the given conditions. For Cd^{II}, HU GO demonstrates an efficiency of 34.2% and a capacity of 18.3 mg g⁻¹ at the given conditions. The efficiency of the GO adsorption decreases in the order of HU GO, HO GO to ST GO for both heavy metals. Experimental data for the adsorption of the heavy-metals Pb and Cd from previous studies demonstrate that HU GO has an adsorption capacity of 35.9 mg g⁻¹ for Pb^{II} and 14.9 mg g⁻¹ for Cd^{II}.^[14e] Our values for HU GO of

Table 3. Efficiency and adsorption capacity of GO towards adsorption of heavy-metal ions Pb^{II} and Cd^{II}.^[a]

Material GO	Pb ^{II} removal efficiency [%]	Pb ^{II} capacity [mg g ⁻¹]	Cd ^{II} removal efficiency [%]	Cd ^{II} capacity [mg g ⁻¹]
HU	74.6	37.3	34.2	18.3
HO	40.7	20.3	5.0	2.7
ST	29.9	14.9	1.5	0.79

[a] After ultra-sonication for 2 h with initial ion concentration [C₀] = 5 ppm and [GO] = 0.1 mg mL⁻¹ at 25 ± 3 °C.

37.3 mg g⁻¹ for Pb and 18.3 mg g⁻¹ for Cd strongly agree with previously published data and indicate the strong adsorption of heavy-metal ions onto HU GO.

Characterisation and ICP–MS data have illustrated that the carboxyl groups play a strong role in the adsorption of heavy-metal ions. This is particularly accentuated in the broadening of the XPS peaks for HU Pb. Analogous phenomena have been observed in the raw spectra of HU GO with Mg^{II}-modified GO films,^[22b] and for metal-ion adsorption with dichromate-oxidised GO, for which significantly wider C 1s spectra were observed upon co-ordination with metal ions. The wider spectra indicate the binding event.^[14a] The deconvoluted high-resolution XPS C 1s peak distributions show that GO synthesised by the HU, HO and ST methodologies have different amounts of oxygenated carbon functionalities. Particular attention is reserved for the strong correlation between the adsorption of heavy-metal ions and the amount of carboxyl groups present, which increases in concentration from ST GO to HO GO to HU GO. This is evident from Table 2, which shows the oxygenated carbon functionality distribution in the three different GO samples. HU GO, which contains the highest amount of carboxyl groups, showed the greatest affinity towards the adsorption of Pb^{II} and Cd^{II} in our findings. However, although HO GO has the highest amount of C–O functionalities (44.9% surface composition in the C 1s XPS spectra compared to 30.3% in HU GO), it does not show better adsorption affinity towards heavy-metal ions. C=O groups are also demonstrated to play a lesser role. ST GO, which contains a higher percentage of carbonyl groups, does not show a higher efficiency for the removal of ions than HO GO. Hence, it can be seen that epoxy, hydroxyl and carbonyl groups do not play an active role in the adsorption of heavy-metal ions. Carboxyl groups are the principal oxygenated carbon functionality in GO that adsorbs heavy-metal ions.

The Lerf–Klinowski model depicts GO as containing epoxy and hydroxyl groups on the basal plane and carbonyl and carboxyl groups at the edge planes.^[4] Axial hydroxyl or epoxy groups on the basal plane primarily bind to heavy-metal ions in a monodentate mode along the *c* axis of the GO.^[14d] However, two primary modes of co-ordination by carboxyl groups have been elucidated; these can bind either through a monodentate or a bidentate mode to the heavy-metal ions. Park et al. have shown that these edge-bound metals co-ordinated to carboxyl groups are more tightly bound to the GO than those that interact with the axial oxygenated groups.^[22b] The

more acidic nature of the carboxyl groups allows the proton to be more easily lost than the proton in the axial hydroxyl or epoxy groups to form a complex with heavy-metal ions.^[14b] XPS characterisation of the GO produced using the dichromate routes by Sitko et al. demonstrated that the increased amount of carboxyl groups led to unprecedented adsorption of heavy-metal ions.^[14a] The same phenomenon was observed by Park et al., who demonstrated that carboxyl groups bind strongly to metal ions, which results in additional mechanical strength.^[22b]

In customisation of GO for removal of the heavy-metal ions, due attention must be paid to the preparatory steps. The size of the GO^[26,27] can be controlled by the graphite parent material.^[27] For the functionalities introduced onto the GO, much thought must be given to the oxidation procedure, as it has a profound influence on the chemical groups introduced. Through the arbitrary introduction of metal ions to different GO, we were able to elucidate the oxygenated carbon functional groups that are responsible for adsorption. HU GO shows the best adsorption capability compared to HO GO and ST GO. The presence of carboxylate groups on GO is strongly correlated with adsorption ability. Additional efforts to introduce carboxylate groups onto GO through the use of alternative oxidation methodology during the synthetic stage, for example, by using a dichromate oxidant as shown in Sitko's work,^[14a] or by Tour's method,^[9b] to improve the adsorption capability of GO towards heavy-metal ions is envisaged.

3. Conclusion

Herein, the adsorption capability of GO synthesised by using the HU, HO and ST routes was evaluated. These GO samples contain a variety of oxygenated carbon functionalities, such as epoxy, alcohol, carbonyl and carboxyl groups. The different preparation routes fundamentally result in GO films with different compositions. The three aforementioned types of GO were then investigated for the removal of Pb^{II} and Cd^{II} ions from aqueous solutions. The adsorption of Pb^{II} and Cd^{II} were found to be strongly dependent on the method used to prepare the GO. FTIR spectroscopy and XPS indicated that the carboxyl groups in GO play a crucial role in the adsorption of heavy-metal ions. Adsorption ability increases in the order ST GO < HO GO < HU GO for both Pb^{II} and Cd^{II}. This is in direct correlation with the amount of carboxyl groups present in the GO, with the highest concentration found in HU GO. More work is required to directly functionalise GO with carboxyl functionalities during the synthesis in order to use these new-age materials for environmental remediation. GO-based sorbents are envisaged to play a crucial role in removing heavy-metal ions from aqueous waste.

Experimental Section

Materials

Graphite (< 20 µm), sulfuric acid (95–98%), sodium nitrate, hydrochloric acid (37%), potassium chlorate (98%), Cd(NO₃)₂·4H₂O and

Pb(NO₃)₂ were purchased from Sigma–Aldrich (Singapore). Potassium permanganate and fuming nitric acid (> 90%) were obtained from J. T. Baker. Milli-Q water (resistivity: 18.2 MΩ cm) was used throughout the experiments. Supported Anopore AAO inorganic membranes (*d* = 25 mm; pore size = 0.22 µm) were purchased from Whatman.

Instruments

XPS was performed with a Phoibos 100 spectrometer and a monochromatic Mg X-ray radiation source (SPECS, Germany). A JEOL-7600F semi-in-lens field-emission SEM was used to acquire SEM images. Attenuated total reflectance (ATR) FTIR measurements were carried out on a PerkinElmer Spectrum 100 system coupled with a universal ATR accessory containing a ZnSe crystal. An Agilent 7700 ICP–MS equipped with a third generation He collision/reaction cell was used to determine the metal concentrations.

Synthesis of GO

GO Preparation with ST Method:^[5] The graphite source was used as-received without additional treatment prior to oxidation. Sulfuric acid (17.5 mL, 95–98%) and nitric acid (9 mL, > 90%) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 °C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and to obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0 °C to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at RT. Upon completion of the reaction, the mixture was poured into ultra-pure water (1 L) and filtered. The GO was then re-dispersed and washed repeatedly with HCl solutions (5%) to remove sulfate ions. The GO was finally washed with ultra-pure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50 °C for five days before use.

GO Preparation with HO Method:^[6] Sulfuric acid (17.5 mL, 95–98%) and nitric acid (9 mL, 63%) were added to a reaction flask containing a magnetic stir bar. The mixture was cooled at 0 °C for 15 min. Graphite (1 g) was then added to the mixture under vigorous stirring to avoid agglomeration and to obtain a homogeneous dispersion. Potassium chlorate (11 g) was slowly added to the mixture (over 15 min) at 0 °C to avoid sudden increases in temperature and the formation of chlorine dioxide gas, which is explosive at high concentrations. After the complete dissolution of potassium chlorate, the reaction flask was loosely capped to allow evolution of gas, and the mixture was stirred vigorously for 96 h at RT. Upon completion of the reaction, the mixture was poured into ultra-pure water (1 L) and filtered. The GO was then re-dispersed and washed repeatedly with HCl solutions (5%) to remove sulfate ions. The GO was finally washed with ultra-pure water until a neutral pH of the filtrate was obtained. The GO slurry was then dried in a vacuum oven at 50 °C for five days before use.

GO Preparation with a Modified HU Method:^[7] Graphite (0.5 g) was stirred with sulfuric acid (23.0 mL, 95–98%) for 20 min at 0 °C prior to the addition of NaNO₃ (0.5 g) in portions. The mixture was left to stir for 1 h. KMnO₄ (3 g) was then added in portions at 0 °C. The mixture was subsequently heated to 35 °C for 1 h. Water (40 mL) was then added to the mixture, which resulted in the temperature of the mixture rising to 90 °C. The temperature was maintained at 90 °C for 30 min. Additional water (100 mL) was added to

the mixture. This was followed by a slow addition of H₂O₂ (30%, ≈ 10 mL). The warm solution was filtered (RC membrane, 0.22 μm) and washed with warm water (100 mL). The solid was subsequently washed with a copious amount of water until a neutral pH was obtained. The materials were kept in the oven at 50 °C for five days prior to usage.

Adsorption Experiment

Stock colloidal solutions of HU, HO and ST graphite oxide of concentration 1 mg mL⁻¹ were first prepared in Milli-Q water. The graphite oxide solution was then ultra-sonicated for 1 h to obtain the desired GO colloidal solution. For the adsorption experiment, the stock GO colloidal solution was diluted to a concentration of 0.1 mg mL⁻¹. Prepared stock solutions of 10 mg mL⁻¹ Cd(NO₃)₂·4H₂O and 5 mg mL⁻¹ Pb(NO₃)₂ were used as the sources of heavy metals. The respective heavy-metal solution (5 ppm) was added to the colloidal GO solution using micropipettes. The mixture was ultra-sonicated for 2 h for adsorption to take place.^[14d,28] Filtration was then carried out with Whatman 0.22 μm AAO membrane filters. The obtained GO film was dried for two days before characterisation by (ATR) FTIR, XPS and SEM/EDS methods. The filtrate was also collected for ICP-MS analysis. Triplicate runs of the adsorption of metals by GO were carried out.

The efficiency (*E*) of GO (0.1 mg mL⁻¹, 100 ppm) towards the adsorption of Pb^{II} and Cd^{II} under the given conditions is given by: $E = [(C_0 - C_f)/C_0] \times 100\%$, in which *C*₀ and *C*_f are the initial and final concentrations of metal ions in ppm after adsorption. The capacity of the GO towards the adsorption of heavy metals was also evaluated by calculating the mass of heavy-metal ion adsorbed per unit mass of the sorbent (mg g⁻¹).

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Acetylene bubble-powered autonomous capsules: towards *in situ* fuel†

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A fuel-free autonomous self-propelled motor is illustrated. The motor is powered by the chemistry of calcium carbide and utilising water as a co-reactant, through a polymer encapsulation strategy. Expulsion of acetylene bubbles powers the capsule motor. This is an important step, going beyond the toxic hydrogen peroxide fuel used normally, to find alternative propellants for self-propelled machines.

Self-propelled devices have been proposed to be fielded in numerous scenarios:¹ both in environmental remediation^{2,3} and biomedical applications.^{4,5} Thus said, the ability for the provision of an *in situ* fuel is an important aspect in self-powered systems,^{6–10} obviating the need for high concentrations of toxic hydrogen peroxide for oxygen bubble-propelled devices by catalytic decomposition reactions. Such fuel-free machines grant higher degrees of freedom and autonomy during their operation. The use of water as an *in situ* fuel under ambient conditions, utilising chemical reactions of reactive metals such as magnesium^{7–9} and aluminum¹⁰ have been demonstrated in hydrogen bubble-propelled motors. Alternatively, the Marangoni effect, which employs interfacial tension, minimizes changes towards the surrounding environment, through a self-contained and self-sustained device.^{11–17} Pumera and co-workers illustrated the use of a slow asymmetrical release of entrapped DMF from a polysulfone capsule, being capable of moving at high velocities and has long life-spans in the order of minutes.¹⁶ Later, Orozco *et al.* exemplified the use of the Marangoni effect with an encapsulation strategy, with the controlled release of surfactants from millimeter-sized vessels.¹⁸ Both these studies highlighted the need for a measured release of the reactants for propulsion to occur.

Thus far, the use of water as a fuel has been limited to reactive metals to produce simple molecules of elemental

hydrogen bubbles for propulsion. The use of ionic and compound substrates has not been demonstrated for use as a potential source of fuel, where complex gaseous molecules could be used as a propellant for autonomous moving objects. In this study, we demonstrated that the use of an encapsulated calcium carbide motor is capable to demonstrate motion, which is propelled by the expulsion of acetylene bubbles. The study expands the scope of bubble-propulsion beyond hydrogen and oxygen bubble-propelled motors. The acetylene bubble-powered motor, utilizing water as a co-reactant, is an additional step towards fuel-free self-propelled devices in aqueous medium.

Designing an autonomous self-propelled motor such that it is independent of the environment and using the fuel source *in situ* has been a challenging task. In this report, we illustrate the use of a calcium carbide motor, exploiting water as a co-reactant. The devised motor is able to traverse the surface of an aqueous medium *via* acetylene bubble-propulsion.

As shown in Scheme 1A, the calcium carbide granule was encapsulated with a polymer layer through a phase inversion strategy. Namely, it was carried out *via* a sequential immersion in 10 wt% polysulfone in DMF solution (PSf-DMF) followed by ethanol. The thin layer of polysulfone encapsulates the calcium carbide granule, herein designated as the PSf-CaC₂ capsule motor. It must be noted that the 10 wt% PSf-DMF mixture is the optimal encapsulation concentration. A 5 wt% PSf-DMF concentration for encapsulation resulted in a rapid rupture of

Scheme 1 Depiction of (A) polymer encapsulation of the calcium carbide granule and operation of the acetylene-powered capsule motor in water and (B) the reaction scheme of calcium carbide in aqueous media.

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the PSf membrane and the consequent increase beyond, to a 15 wt% concentration led to the sinking of PSf-CaC₂. Previously, it has been demonstrated that such phase inversion membranes allows for the creation of nanoporous films.¹⁶ These pores are important for the contact of the surrounding aqueous media with the encapsulated substrate. The reaction of calcium carbide with water leads to the formation of acetylene and calcium hydroxide as shown in Scheme 1B. This ensuing chemical reaction that occurs at the interface led to the formation of acetylene bubbles, which escape and propel the capsule motor forward.

At $t = 0$ s, the encapsulated PSf-CaC₂ was introduced into the 0.05% SDS surfactant solution in Fig. 1A. The PSf-CaC₂ capsule motor is millimeter-sized, with approximate dimensions of 3 mm across each end. The surfactant serves to lower the surface tension for ease of bubble formation and ensures the stability of the bubbles that are formed.¹⁹ An almost instantaneous effervescence was observed. This corresponded to the water seeping past the PSf membrane to react with the core of calcium carbide. The chemical reaction between water and calcium carbide produces acetylene bubbles, *via* an acid-base reaction. A subsequent stream of bubbles was released from PSf-CaC₂, which aided the buoyancy of the PSf-CaC₂ capsule motor at the water/air interface.²⁰ This was followed by the translational motion of PSf-CaC₂ across the surface of the solution as shown in Fig. 1B (see Video S1, ESI†).

In Fig. 2, the speeds of the PSf-CaC₂ capsule motor are described. The motor expressed an average speed of 2.3 cm s⁻¹, moving a total distance of 37 cm. This is approximately 8 body lengths per second. Two ranges of speeds were found for the PSf-CaC₂ capsule motor. From 0 to 9 s, the motor demonstrated average speeds between 2 and 4 cm s⁻¹. For the later part of the translational motion from 9 to 16 s, the PSf-CaC₂ motor demonstrated slower average speeds of 1–2 cm s⁻¹. This is in part due to the consumption of the calcium carbide fuel and resulted in the decrease of propulsion force, leading to the reduction of speeds. Beyond the 16 s timeframe as shown in Fig. 2, the motion of the capsule becomes limited with retarded bubble formation.

The converse direct introduction of PSf-CaC₂ into the aqueous medium without a surfactant resulted in the motion of the capsule motor to be highly localized (see Video S2, ESI†). The PSf-CaC₂ motor bobbed up and down the water/air interface. This exhibited the quintessential role of the surfactant for propulsion. The need for buoyancy of the PSf-CaC₂ capsule motor²⁰ and the sustenance

Fig. 2 Average speed profiles of the PSf-CaC₂ capsule motor with $\Delta t = 1$ s. A total path length of 37 cm was travelled and the average speed was 2.3 cm s⁻¹.

of the bubbles²¹ are required in order to observe the translational movement. Separately, in the absence of the PSf membrane, an uncontrolled formation of bubbles resulted due to the chemical reaction of calcium carbide and water in direct contact. Calcium carbide was rapidly expended and was accompanied by foaming on the surface of the aqueous media. No significant propulsion behaviour was observed on the calcium carbide granule. Formation of the encapsulation film surrounding the capsule governs the motion of the PSf-CaC₂ capsule motor, where in the event of rupture, it resulted in a highly localized motion of calcium carbide fragments. This highlighted the need for the controlled reaction of calcium carbide with water, in order to allow for the biased movement of the capsule motor, as reported by Zhao *et al.*¹⁶ and Orozco *et al.*¹⁸ The role of the PSf membrane as a binder to confer integrity and asymmetry to the particulate as a single entity is crucial for the biased movement for the bubble-propelled device.

In Fig. 3, the graph of mean squared displacement (MSD) against change in time intervals has been demonstrated to be an upward parabolic curve, indicative of an additional propelling force being present.^{22,23} This resulted in the millimeter-sized PSf-CaC₂ to move at an average of ~ 8 body lengths per second. This corresponded well with our hypothesis that the production of a stream of bubbles powering the PSf-CaC₂ capsule motor has given rise to the ballistic thrust needed for motion. The motion of the PSf-CaC₂ capsule motor changed direction multiple times during the course of 16 s as shown in Fig. 1, which was indicative of propulsion behaviour, eliminating any convective current effects.²³

In conclusion, we have demonstrated the operation of a fuel-free autonomous self-propelled device based on the encapsulation of calcium carbide in polysulfone. The capsule motor runs on the basis of a controlled reaction with water, using acetylene bubbles as the means of propulsion. The extension of autonomous moving devices using alternative sources of *in situ* fuels is an important step towards widening the capabilities of such self-propelled machines. Alternative bubble-powered systems such as acetylene in our case and possibly other analogous complex molecules

Fig. 1 Tracking image for the path of a PSf-CaC₂ capsule motor at (A) $t = 0$ s and (B) $t = 16$ s on the surface of water with 0.05% SDS. Translational motion was observed. Arrow indicates the initial position of the capsule motor. Scale bar indicates 1 cm.

Fig. 3 Calculated mean-squared displacement (MSD) of the PSf-CaC₂ capsule motor on the surface of water with 0.05% SDS added.

produced for bubble-propulsion may be possibly used as feed-stock for further chemical manipulation in self-propelled autonomous systems.

Calcium carbide (CaC₂), sodium dodecyl sulfate (SDS), polysulfone (PSf) and *N,N'*-dimethylformamide (DMF) were purchased from Sigma-Aldrich.

The precursor encapsulation solution was prepared by mixing polysulfone with *N,N'*-dimethylformamide and dissolved into a clear solution using an ultrasonic bath for 30 min. The concentration of polysulfone (PSf) in DMF (PSf-DMF) was 10% by weight.

Encapsulation of calcium carbide was carried out using a phase inversion strategy. Typically, a calcium carbide granule with an approximate dimension of 3 mm at each end was chosen. The granule was introduced into a 10 wt% PSf-DMF solution, followed by immersion in ethanol, where the phase inversion occurred. The encapsulated calcium carbide was left to dry for 5 minutes before being used for propulsion experiments.

The propulsion experiments were carried out in a glass container with dimensions 20 cm × 20 cm × 5.5 cm. An aqueous solution containing 0.05% SDS was placed on the dish. A Casio HD video-recorder was placed over the glass container to capture the motion of the capsule motor. The video sequences were

analysed using Nikon NIS-Elements software, where the average velocities were calculated.

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Micromotors

Influence of pH on the Motion of Catalytic Janus Particles and Tubular Bubble-Propelled Micromotors

James Guo Sheng Moo, Hong Wang, and Martin Pumera^{*[a]}

Abstract: Self-propelled miniaturized machines harness the chemical potential of their environment for movement. Locomotion of chemically powered micromotors have been hugely dependent on the surroundings. The use of pH to alter the mobility of micromotors is demonstrated in this work through the manipulation of hydrogen peroxide chemistry in different acidity/alkalinity. The sequential addition of sodium hydroxide to increase the pH of the solution led to a consequent increase in activity of micromotors.

Meanwhile, addition of hydrochloric acid compromised the structural integrity of the microstructures, culminating in locomotive changes. Such dramatic changes in activity and velocities of the micromotors allow the usage of this behavior for pH detection. This concept was illustrated with Janus silver micromotors and tubular bimetallic Cu/Pt micromotors. Alteration of pH serves as a useful general strategy for increasing hydrogen peroxide decomposition for enhanced oxygen-bubble propulsion in catalytic micromotors.

Introduction

The emergence of micromotors as devices to perform work and the collective efforts of the scientific community have led to the progression of the field by leaps and bounds in the past decade.^[1] The miniaturized size, ultrafast motion, and the capability of surface functionalization with consequent interactions throughout the solution have led to a plethora of applications. They have been demonstrated to be able to do work for both environmental remediation^[2] and that of biomedical usage.^[3] In view of its possible application as a nanotool to perform work in the environment and a physiological medium, much effort has been directed towards the study of the interplay between the micromotors and their environment,^[4] with possible implications for the enhancement of self-propulsion.^[5]

The search for a viable propelling agent for powering micromotors in varying environments has been ongoing for an extended period. In situ and ex situ methodologies have been demonstrated to power such micro-objects. Ex situ methods include that of using acoustics,^[6] magnetic manipulation,^[7] and that of UV^[8] and IR irradiation.^[9] Even then, the use of a chemical fuel such as hydrogen peroxide cannot be completely obviated. Ultraviolet irradiation on TiO₂ single-component microtubes required the presence of hydrogen peroxide in a radical decomposition reaction for oxygen-bubble propulsion.^[8] Similarly, near-IR activation of the microtubular platinum-based engines to perpetuate motion necessitates the occurrence of hydrogen peroxide, albeit at lower concentrations.^[9] Remote ma-

nipulation of micromotors requires the use of external fields of magnetic and acoustic origins, which is inherently a peripheral energy source. An actuation of the micromotors in situ allows for the reduction of complexity surrounding their operations and to permit function independent of external fields.

Hydrogen peroxide continues to be the fuel of choice for many micromotors. The work of Sanchez et al. demonstrated micromotors that move at ultrafast speeds and their operation at low hydrogen peroxide concentrations in bubble-propelled rolled-up microtubes.^[10] Thermal activation of such micromotors has also been illustrated for enhanced locomotion in physiological media.^[9–11] Exploring possible additives, Laocharoen-suk et al. used micromotors that are made of carbon nanotube composite materials and exhibited sublime performance with hydrogen peroxide in the presence of basic hydrazine, being powered by a self-diffusiophoretic mechanism.^[5e] This was later revalidated by the work of Gao et al. with bubble-propelled polyaniline/platinum tubular micromotors in a mixture of hydrogen peroxide and hydrazine.^[5f,g] Addition of hydrazine has been known to increase the decomposition of hydrogen peroxide to form oxygen.^[5e,h] However, hydrazine itself is biologically toxic, adding to the complexity of the micromotor operation.

Tubular micromotors have been deployed in media of varying pH. Orozco et al. reported the utility of micromotors in the presence of sodium carbonate and sodium hydroxide with hydrogen peroxide for an environmental clean-up strategy of nerve agents.^[5a] The presence of reactive OOH[−] oxidative nucleophiles in alkaline media have resulted in the breakdown of these deleterious materials during enhanced fluid mixings. The ease of hydrogen peroxide to form oxygen microbubbles for propulsion and formation to reactive species in the alkaline environment have been the principal reasons for their purported environmental remediation effect. At the other end of the

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spectrum, Gao et al. have also demonstrated hydrogen bubble-propelled zinc-packed tubular microrockets to possess enhanced motion in increasingly acidic media.^[12] For Fenton-reaction-based micromotors running with platinum on hydrogen peroxide, optimum performance was found to be between pH 2 and 3 for an environmental clean-up of organic contaminants.^[13]

The silver nano/micromotor is another suitable model for the investigation of the influence of an alkaline environment. Silver has been demonstrated for micromotor-propulsion in many instances as hydrogen peroxide decomposes readily to form oxygen on the material.^[14] Kagan et al. also illustrated that the underpotential deposition of silver as the principal reason for the high mobility in their bimetallic diffusiophoretic nanorod motors.^[15] Pumera and co-workers had suggested asymmetric Janus silver microparticles to be a worthy candidate to replace platinum as a material for fabricating bubble-propelled micromotors.^[16]

In a separate report, Dey et al. illustrated the chemotactic movement of a palladium-based micromotors in alkaline medium, which is due to chemical gradients that are generated.^[17] Self-contained amphoteric aluminum Janus-type micromotors in acidic and alkaline media^[18] have also been known to show varying performances with pH. For a bubble propulsion system, the prospect of wielding the micromotors with enhanced propulsion is extremely desirable.

However, no systematic study has been employed for the changes in velocities and activities of bubble-propelled catalytic micromotors with the monotonic increase in pH. Hydrogen peroxide has been known to show enhanced decomposition under alkaline media.^[19] For the better understanding of the locomotive events at the microscale, such a study on the micromotors with pH changes is essential. The investigation across a spectrum of pH will be employed for the bubble-propelled catalytic micromotors. Herein, we illustrate that the level of acidity and alkalinity show dramatic influences on the activity and velocities of both Janus-type and tubular micromotors. This property can then be possibly extrapolated for the detection of changes in pH.

Results and Discussion

Herein, we illustrate that hydrogen peroxide fueled bubble-propelled micromotors demonstrate locomotive differences and detection capabilities across different pH values. Two representative bubble-propelled micromotors of Janus and microtubular configurations were investigated. These micromotors are powered by bubble propulsion caused by the catalytic decomposition of hydrogen peroxide over the material. Detachment of the bubbles propels the motion of these micromotors. Velocities of Janus silver micromotors were investigated with increasing pH and the comparison with increased fuel concentrations was made. Activity and velocities of microtubular Cu/Pt micromotors were also elucidated with varying pH.

Locomotion of silver micromotors was investigated with increased alkalinity of the environment. The asymmetric geometry of the Janus silver micromotors coupled with bubble for-

mation, led to a net force, which propels these particles. The velocity of the Janus silver micromotors increased with elevated pH in the presence of the sodium hydroxide (Figure 1). Velocity of the silver micromotors in 3% hydrogen peroxide and 0.25% of SDS increased from $14 \mu\text{m s}^{-1}$ at pH 7 to $320 \mu\text{m s}^{-1}$ in the presence of base at the pH of 12 by almost 23 times.

Figure 1. Velocity of Janus Ag micromotors in selected pH adjusted with NaOH (aq) electrolyte solution. Conditions in all experiments: 23 °C, 3 wt. % H_2O_2 and 0.25 wt. % SDS.

Such enhanced motion of the silver Janus microparticles is in direct correlation to the increase of pH in the solution. The upper limit for the operation of the micromotors was observed to be pH 12, as the sodium dodecyl sulfate surfactant precipitates out owing to the common-ion effect in the presence of sodium hydroxide when attempts to increase the pH to 13 was carried out.

Possible means to harness the full chemical potential by the introduction of the base have been demonstrated by the transformation of hydrogen peroxide to the negatively charged hydrogen peroxide anion, which is the activated form.^[5a] The energetically unstable anion of ^-OOH decomposes at a faster rate, to produce more oxygen, culminating in the increment of propulsion.^[19a-c] This was confirmed by microscopy observations that the increase in base concentration, was coupled with a greater ejection of bubbles, leading to faster velocities. The drastic change in the micromotor velocity with pH change also suggests its possible usage as detector for the alkalinity of environment.

For comparison, the velocities of the micromotors were tracked with elevated concentrations of hydrogen peroxide (Figure 2). The velocity of the Janus silver microparticles increases with elevated hydrogen peroxide concentrations, from $14 \mu\text{m s}^{-1}$ at 3% hydrogen peroxide to $80 \mu\text{m s}^{-1}$ at 9% hydrogen peroxide. However, in comparison, the accompanying introduction of sodium hydroxide at pH 10 with 3% hydrogen peroxide, a velocity of $123 \mu\text{m s}^{-1}$ was reached, far exceeding the equivalent of the addition of three times the hydrogen peroxide concentration at 9%, where a speed of only $80 \mu\text{m s}^{-1}$ was achieved in the Janus silver micromotor. This demonstrates an alternative method of improving the activity of the micromotors, where the full chemical potential of hydro-

Figure 2. Velocity of Janus Ag micromotors with increasing hydrogen peroxide concentrations in the absence of NaOH (aq) electrolyte solution. Conditions in all experiments: 23 °C and 0.25 wt. % SDS.

gen peroxide can be maximized with usage of greater alkalinity during operations.

Keeping things in perspective, the pH readings are stable at the time window of observation, with no significant changes. However, it was noted that attempts for pH detection in the acidic region, leads to the dissolution of the Janus silver microparticles. This is due to the tendency of hydrogen peroxide to behave as an oxidizing agent in the acidic pH.^[20] Therefore, it is essential for us to take into regard an alternative model in the form of tubular Cu/Pt micromotors, which shows stable readings from pH 0–12.

The corresponding activity of tubular micromotors at a comprehensive pH range from 0 to 12 was investigated (Figure 3). The dual-component Cu/Pt tubular micromotors were fabricated from a two-step electrodeposition method, where copper was first deposited on the outer layer followed by the inner layer of platinum by a templated approach. The decomposition of the hydrogen peroxide to oxygen bubbles and the subsequent detachment from the inner layer of platinum in the micromotors propels them forward.^[20] The active area of Cu/Pt tubular micromotors has been under confinement conditions, where localized decomposition of the hydrogen peroxide on the inner concentric platinum surface led to the formation and ejection of oxygen bubbles. Activity of micromotors were evaluated based on the number of micromotors demonstrating

Figure 3. Number of 5 μm Cu/Pt tubular micromotors showing bubble ejection (gray) and microjet propulsion (black) with pH range from 0–12 adjusted with HCl (aq.) and NaOH (aq.). Conditions in all experiments: 23 °C, 3 wt. % of H_2O_2 and 0.25 wt. % SDS.

bubble ejection and propulsion. It is also noteworthy that the catalytic micromotors can be recovered by filtration and centrifugation methods (Supporting Information, Figure S1). The activities of these self-propelled devices did not deteriorate upon recovery and resuspension in hydrogen peroxide (Supporting Information, Figure S2).

Across the pH range investigated, the Cu/Pt tubular micromotors were observable under microscopic observation and showed little signs of dissolution. It was noted that an initial dip of the activity of the micromotors was observed with the first introduction of sodium hydroxide and hydrochloric acid to adjust the pH to 6 and 8, respectively. Concentrations of salts at millimolar levels are known to reduce the activity and impede motion of the micromotors owing to changes in ionic strengths.^[4d,i]

In the alkaline region from pH 8 to 12, with a monotonic increase in sodium hydroxide, the activity of micromotors doubled for bubble ejection from 16 to 33 units and microjet propulsion from 9 to 18 units. The basic environment with the introduction of sodium hydroxide has been instrumental in increasing the activity of the micromotors, where a greater decomposition of hydrogen peroxide results in greater bubble propulsion.

Curiously, the acidic region indicated an increase in activity below the pH 4, where there were originally 8 bubbling micromotors and 6 self-propelled micromotors. As illustrated in Figure 3 at pH 3, the number of bubbling micromotors increased to 38 and the number of micromotors demonstrating microjet propulsion increased to 35. This was a five-fold increase of activity. The same story was seen at the pH 2, where there was 35 micromotors bubbling, with 33 of them undergoing self-propelled motion. This was at odds with the earlier hypothesis that only an alkaline environment can increase the activity of the micromotors. Furthermore, at the pH of 0 and 1, the activity of the micromotors dropped dramatically. At pH 0, only 2 micromotors are bubbling, and 1 of these demonstrated self-propelled motion.

A closer inspection at the velocities of the micromotors were undertaken to gain a better picture. The subsequent introduction of sodium hydroxide and hydrochloric acid have also modulated the velocities of the Cu/Pt micromotors, as depicted in Figure 4. The evaluation of the velocities of the micromotors were carried out and it was found that the addition of sodium hydroxide increased the velocities. From the pH of 7 to 12, the velocity of the Cu/Pt micromotors increased by nearly 4-fold, from the velocity of $62 \mu\text{m s}^{-1}$ to $230 \mu\text{m s}^{-1}$.

The decrease in pH from 7 to 4 reflected a slight decrease in velocity. However, as with the activity, tubular micromotors experienced a rapid increase in velocities at the pH of 2 and 3. Beyond this pH range, a dramatic decrease in the motion was observed at pH 0 and 1. As depicted in Figure 4, the micromotor was virtually immobile at the pH value of zero. A higher magnification microscopic imaging tool was employed to visualize the possible changes to these micromotors that might have constituted the observations.

Using scanning electron microscopy (SEM), the morphology of the micromotors was investigated (Figure 5). The micromo-

Figure 4. Velocity of 5 μm Cu/Pt tubular micromotors with pH range from 0–12 adjusted with HCl (aq.) and NaOH (aq.). Conditions in all experiments: 23 °C, 3 wt.% H_2O_2 and 0.25 wt.% SDS.

tors were initially immersed in the hydrogen peroxide solutions of pH 0, 2, 7, and 12, respectively. They were then extracted by filtration by a nanoporous membrane before washing with copious amount of water and dried. The SEM images are featured in Figure 5, with a summary of the structural changes showed in the inset. As seen in the SEM imaging, no morphological changes was registered at pH 7 and 12, in Figure 5C and 5D, respectively. It was observed that the tubular micromotor at pH 2, was significantly corroded in the time window of 15 min, exposing the inner platinum layer partially. These exposed surfaces of the micromotors were made available for catalytic decomposition of hydrogen peroxide, leading to better activities and velocities (Figure 3 and 4). However, with further additions of hydrochloric acid to reach pH 0 (Figure 5A), the structural integrity of the tubular micromotor collapsed. The ends of the tubular micromotor appeared to be closed and the length of the tube broke down onto itself (Figure 5A, inset). This significantly impaired the microjet propulsion and the aftereffects are illustrated in the deteriorated activity and decreased velocities earlier (Figures 3 and 4) at pH values of 0 and 1.

It was deduced that the acidity and presence of hydrogen peroxide compromised the structural integrity of the tubular micromotors from pH 2 to 3, exposing a larger catalytic platinum surface area for bubble propulsion. However, the accompanying decrease to pH 0 and 1 curtailed the movement of the micromotors as the structural integrity of the concentric bimetallic Cu/Pt micromotor was severely lost and the structure collapsed, as seen in Figure 5A. However, in the alkaline region, namely from pH 8 to 12, the increased alkalinity enhanced the decomposition of hydrogen peroxide to oxygen for bubble propulsion.

Both Janus and tubular micromotors have shown selective locomotion in aqueous media, reflecting the changes in pH. As catalytic systems, Janus silver micromotors have been demonstrated to show greater increases in velocities than Cu/Pt micromotors in the presence of alkaline conditions. The catalytic surface of the microtubes is confined in the core of the inner concentric layer, which is concave in shape,^[8] whereas in comparison, the Janus particles possess both concave and convex surfaces co-existing in the same asymmetric solid particle.^[16]

Figure 5. SEM images of 5 μm Cu/Pt tubular micromotors extracted from different pH using filtration with a nanoporous membrane at A) pH 0, B) pH 2, C) pH 7, and D) pH 12, respectively. Scale bars: 5 μm . The pH range was adjusted with HCl (aq.) and NaOH (aq.). Conditions in all experiments: 23 °C, 3 wt.% H_2O_2 , and 0.25 wt.% SDS; immersion in solutions of differing pH was carried out for 15 min. Inset: structural changes to tubular micromotors after immersion.

With regards to geometry, the Janus particles, while having the same order of magnitude in size (ca. 20 μm) at the longest dimension as the microtubes (ca. 15 μm), the Janus particles themselves have a significantly larger catalytic surface area. These geometric differences in the two micromotors resulted in the contrasting locomotive performances, for gas nucleation, accumulation, and expulsion under different pH.^[8,16,21] Thus, the larger boost in propulsion of Janus silver micromotors is due to the larger active surface area as compared to the confined regions of the concentric bimetallic Cu/Pt micromotor under well-defined conditions on the tubular area.^[16] The manipulation of pH, with the concomitant increase of locomotion in these catalytic micromotors, serve as a strategy for detecting changes in pH and a pathway to increase locomotive events in self-propelled entities.

Conclusion

In conclusion, we have demonstrated the working range of the micromotors and that they are capable of detecting pH. The increase in pH leads to faster decomposition of the hydrogen peroxide on the surface of the catalytic micromotors, bringing on greater activity from bubble ejection and higher velocities. The effect is epitomized by the increase in velocities of the Janus silver micromotors. The activity and velocities of the Cu/Pt tubular micromotors were also increased in the presence of alkali. However, the extent of this increase in self-propulsion for the tubular micromotors of Cu/Pt were lesser in extent than that of the Janus silver micromotors. Structural changes to the tubular micromotors through corrosive effects of the acid have also inevitably changed the propulsion behavior. Through the observation of the activity and velocities of micromotors, the detection of pH can be accomplished on-the-fly, particularly in the neutral to alkaline region. The pH-dependent

propulsion behavior also serves as a possible pathway towards locomotive enhancements for catalytic bubble-propelled motor systems.

Experimental Section

Materials: The cyclopore polycarbonate membranes with pores of 5 μm (Cat. 7060-2513) and 0.2 μm (Cat. 6809-6022) in diameter were purchased from Whatman, USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Dichloromethane and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH instruments, USA. The platinum plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98+%), sodium dodecyl sulfate (SDS), sodium hydroxide, hydrochloric acid (37%) and silver microparticles were purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 M Ω cm) from a Millipore Milli-Q purification system.

Apparatus: Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 $^{\circ}\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. pH was recorded using a Satorius Docu-pH-Meter. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The silver working electrode was sputtered on the cyclopore membrane and copper tape was used as the transducer. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Hermle Z233m-2 microcentrifuge. SEM images were taken with a JEOL-7600F semi-in-lens FE-SEM. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software.

Preparation of Cu/Pt concentric bimetallic microtubes:^[22] The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template. Silver (ca. 100 nm) was sputtered on the one side of the polycarbonate template. Copper tape was attached to the silver-sputtered polycarbonate template, which serves as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. A platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA Version 1.8 software (Eco Chemie). The template was rinsed with 5 mL of ultrapure water (18.2 M Ω cm) 4 times, and the Cu outer layer was deposited galvanostatically at -20 mA for 1200 s. The deposition solution contained 1 M CuSO_4 and 1 M H_2SO_4 . Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -20 mA for 1200 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled. The template was polished with 0.05 μm alumina powder to remove the sputtered silver and was washed for 5 times with 8 mL of water by ultrasonication each time. It was then placed in an Eppendorf tube with 2 mL of dichloromethane and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm

for 3 min and washed repeatedly 2 times with dichloromethane. The solution was then washed with ethanol and water 2 times each and centrifuged for 3 min after each washing step. The tubes were finally collected and stored in deionized water at room temperature.

Operation of micromotors: The experiments for studying the influence of alkalinity on the motion of micromotors were carried out in an aqueous solution containing 3 wt.% of hydrogen peroxide at constant surfactant concentrations (0.25 wt.% of SDS) with asymmetric Janus silver particle micromotors. For tubular Cu/Pt micromotors, an aqueous solution containing 3 wt.% hydrogen peroxide at constant surfactant concentrations (0.25 wt.% SDS) was maintained. A mixture of micromotors (2.5 μL), SDS, H_2O_2 , and appropriate concentrations of sodium hydroxide and hydrochloric acid was pre-adjusted in an Eppendorf tube for pH calibration before 10 μL was applied on a glass slide freshly cleaned with nitrogen gas. Under a typical investigation, an average of 58 ± 8 micromotors are found in the sample solution for 15 repetitions. Only micromotors demonstrating activity of bubbling and microjet propulsion were taken into account for measurements. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software. Average velocities were computed from 10 readings.

Extraction of micromotors: Micromotors in hydrogen peroxide solution were extracted by two separate methods: filtration and centrifugation. Nanoporous track-etched cyclopore membrane of pore size 200 nm were purchased from Whatman, USA. After immersion for 15 min, the solution containing micromotors were slowly dropped onto the nanoporous membrane under vacuum suction. The membrane was then flushed with copious amounts of water with the vacuum in operation. For the scanning electron microscopy measurements, the membrane was transferred to the sample stub, before being sputtered with platinum to prevent charging effects. Centrifugation involved spinning the solution of micromotors in a Eppendorf tube at 6000 rpm, before washing with deionized water. The cycle was repeated 3 times. The pellet of micromotors remaining in the Eppendorf tube was resuspended in deionized water by ultrasonication for 5 min and can be reused for motility studies in different pH.

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Keywords: hydrogen peroxide • Janus particles • micromotors • pH • sensors

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Photochromic Spatiotemporal Control of Bubble-Propelled Micromotors by a Spiropyran Molecular Switch

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Supporting Information

ABSTRACT: Controlling the environment in which bubble-propelled micromotors operate represents an attractive strategy to influence their motion, especially when the trigger is as simple as light. We demonstrate that spiropyrans, which isomerize to amphiphilic merocyanines under UV irradiation, can act as molecular switches that drastically affect the locomotion of the micrometer-sized engines. The phototrigger could be either a point or a field source, thus allowing different modes of control to be executed. A whole ensemble of micromotors was repeatedly activated and deactivated by just altering the spiropyran–merocyanine ratio with light. Moreover, the velocity of individual micromotors was altered using a point irradiation source that caused only localized changes in the environment. Such selective manipulation, achieved here with an optical microscope and a photochromic additive in the medium, reveals the ease of the methodology, which can allow micro- and nanomotors to reach their full potential of not just stochastic, but directional controlled motion.

KEYWORDS: photochromic, spiropyrans, micromotors, self-propulsion, control

Submicrometer-sized machines, which lie at the next frontier of nanotechnology, are expected to perform a plethora of tasks.¹ While they have been proposed to play important roles in biomedical delivery² and pollution management,^{3–5} exerting control over the motion of these nano/micro-objects is still in its rudimentary phase. Among the magnetotactic,^{6–8} acoustic⁹ and optical methods^{10–12} that have been put forth, the latter shows the greatest potential, due to its inherent variability and the degrees of freedom it offers. Photocontrol can be described as having either local or global effect, where a point or a field source is used to influence the motion of single nano/micromachine or a whole ensemble, respectively. For bubble-propelled nano/micromotors, methods of UV,^{10,12,13} visible¹⁴ and infrared light^{15–17} field illumination control have been employed. And while infrared irradiation has been successful in addressing individual micromotors, those operated only at the lower limits of self-propulsion, resulting in sluggish performance.¹¹ However, in all cases, the necessity for continuous irradiation prevents their use in photosensitive systems,¹² posing a formidable challenge in biomedical applications. Meanwhile, extensive lithographic microarrays were also required,¹⁴ where changes in the whole environment were used to control the velocity of the nano/micromotors.^{3–5,18,19}

Thus, we sought an alternative optical approach, which relies on a simple molecular switch in the medium, which can be turned on and off multiple times with relatively short light pulses. While previous efforts have been directed toward the modification of the micromotors¹² or the preparation of special surfaces over which the bubble-propelled engines move,¹⁴ we targeted the micromotor environment instead, because it obviates the need for custom-built nano/micromotors¹² and for creating exquisite microarrays.¹⁴ Most importantly, it removes the bottleneck in controlling large swarms,¹¹ where every nano/microdevice has to be addressed independently. Spiropyran additives appeared most suitable for this general task, because unlike the other widely used photochromic molecules—azobenzenes and diarylethenes, their isomerization is accompanied by a large change in dipole moment.²⁰ The photoinduced ring opening of spiropyrans to their respective zwitterionic merocyanines has been previously used to alter ionic strength,²¹ surface tension²² and micellar concentration²³ of solutions *in situ*. Thus, we hypothesized that it can strongly affect the motion of micromotors, as these are the factors

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Scheme 1. Reversible Photoisomerization of Spiropyran Additive SP-C8 under UV Irradiation to Merocyanine MC-C8^a

^aStructural and schematic representations of SP-C8 and MC-C8 are shown, where C8 refers to R = C₈H₁₇.

critical for bubble propulsion.^{24–32} Scheme 1 shows the ring-opening and ring-closing of spiropyrans, with different length alkyl chain attached to the spiropyran.

We chose to employ bimetallic concentric Cu/Pt micromotors that run on hydrogen peroxide, due to their straightforward preparation,³³ robust nature,^{34,35} and ability to operate in a multitude of environments.^{3–5,18,19} Decomposition of H₂O₂ on the inner catalytic surface produces oxygen gas, that in the presence of sodium dodecyl sulfate (SDS) forms microbubbles,^{36,37} which propel the micromotor. The locomotion of the micromotors was greatly affected by the spiropyran/merocyanine ratio, which could be adjusted through the selective use of ultraviolet and visible white light. We were able to regulate the activity and velocity of the micromotors by effectively modulating their environment. In addition, we illustrated the capability to manipulate not only an ensemble, but also single micromotors with the help of focused light from a conventional microscope. The presence of the spiropyran in the solution allowed for photochromic control of non-photoactive micromotors.

RESULTS AND DISCUSSION

Preliminary screening of the influence of different spiropyrans with various carbon chain lengths on the micromotor's performance was investigated in an aqueous medium (Figure 1). Spiropyrans are labeled SP-C1, SP-C8, and SP-C18, indicating the methyl, octyl, or octadecyl side chain at the spiropyran molecule, respectively (refer to Scheme 1). The activity of the micromotors can be evaluated with two parameters. Namely, they are the number of micromotors ejecting bubbles for propulsion and the velocities of the micromotors. Here in Figure 1, the activity was measured in terms of the number of micromotors demonstrating bubble ejection and microjet propulsion. The more hydrophobic SP-C8 and SP-C18 formed colloidal solutions with SDS in the running solution, while SP-C1 fully dissolved. Only SP-C8 and SP-C18 demonstrated decreases in micromotor activity, after shining of 365 nm UV light for 1 min, as shown in Figure 1. While SP-C1 was fully soluble in the running solution, it did not influence the micromotors with UV irradiation, illustrating a similar effect just as the presence of only SDS. The common trend between SP-C8 and SP-C18 of decreasing micromotor behavior with UV irradiation validated our hypothesis. Due to the most accentuated changes in activity, SP-C8 was chosen to be the additive of study.

With the introduction of UV irradiation, we have been able to interconvert the photochromic additive to influence the motion of the micromotors. We utilized a spiropyran (SP-C8)

Figure 1. Percentage change in activity of micromotors showing bubble ejection (red) and movement by microjet propulsion (blue) in the presence of different spiropyrans: SP-C1, SP-C8, and SP-C18, before and after UV irradiation at 365 nm for 1 min. Error bars show the 1 standard deviation of 15 measurements. Conditions in all experiments: 23 °C, 7 wt % H₂O₂, 0.05 wt % SDS, 0.02 wt % SP-C8.

that has a hydrophobic eight carbon chain on the nitrogen of the indole, which can interact with the sodium dodecyl sulfate surfactant. Upon UV irradiation, spiropyran can be converted to merocyanine (MC-C8). We undertook investigations of the interactions of photochromic additives in these two different states with the micromotors. The additives demonstrate photocontrol over the micromotors, with the type of irradiation source affecting the activity (number of micromotors bubbling and number of micromotors demonstrating microjet propulsion) (Figure S1). Due to the reversibility of such a photochemical reaction and its effect on the micromotors, it was of special interest and warranted further investigation.

The molecular states of the spiropyran SP-C8 and its opening isomer merocyanine were investigated in aqueous solution with SDS surfactant (Figure 2). UV-vis spectra of SP-C8 in the 2 different states using 0.02 wt % SP-C8 in 0.05 wt % SDS are shown in Figure 2A. In the ambient state, the solution of spiropyran absorbs strongly at 289 and 349 nm. This was indicative of the spiropyran solution existing as the closed-ring isomer, the SP-C8 form.^{38,39} The two peaks correspond to the

Figure 2. UV–vis spectra. (A) Spiropyran SP-C8 after 1 min of UV irradiation and followed by 15 min of vis-light irradiation. (B) Spiropyran SP-C8 conversion to MC-C8 with successive increment of UV excitation time in 20 s intervals. (C) Spiropyran MC-C8 conversion to SP-C8 with successive increment of vis-light excitation time in 5 min intervals. (D) Reversibility of spiropyran SP-C8 to MC-C8 and *vice versa* with alternation of 1 min UV irradiation and 15 min visible light irradiation for three cycles. Conditions in all experiments: 0.05 wt % SDS, 0.02 wt % SP-C8 in water.

indoline and chromene moiety of the spiropyran. However, on irradiation with UV at 365 nm, there was a drastic change in the absorption spectrum. A new broad peak at 562 nm appeared, concomitant with the depletion of the peaks at 289 and 349 nm. This was indicative of the closed-ring spiropyran (SP-C8) isomerizing to the open-ring merocyanine (MC-C8).⁴⁰ The extended π -conjugation in the planar structure resulted in a characteristic red shift of the absorption.^{41,42} The MC-C8 absorbs strongly at 562 nm, giving rise to a purplish color of the solution.⁴³ A subsequent irradiation with 15 min of visible light was able to convert most of the MC-C8 to SP-C8 molecular form, where the solution turns from purple to colorless, with a corresponding diminished peak at 562 nm.

The ring-opening of the spiropyran, irradiated with 365 nm UV light, was studied as a function of time in Figure 2B. This photochemical reaction was studied at increasing time intervals of 20 s. The absorption peak in the spectra at 562 nm slowly increased, before reaching saturation at 60 s. Conversely, the introduction of visible light for intervals of 5 min until a total time of 15 min was able to switch the molecular state of the MC-C8 to SP-C8 in Figure 2C.^{21,44} A near-total reversibility was observed in the UV–vis spectra at the end of 15 min of visible light irradiation. The exposure of the solution to visible light resulted in the depletion of the peak at 562 nm in MC-C8, and the restoration of the 289 and 349 nm peaks to SP-C8 was observed after irradiation with visible light.

For optical cycling, the interconversion between SP-C8 and MC-C8 was studied for 3 cycles in Figure 2D. Successful

conversions between these two molecular forms, with the exhibition of their characteristic UV–vis spectra, were observed. However, notably, there was a decreasing trend in the efficiencies during transformation of spiropyran to merocyanine and *vice versa* with the increased number of interconversions. Subsequent photoisomeric conversions from SP-C8 to MC-C8 were not able to reach the magnitude of absorbance at 562 nm at the end of the first optical cycle, as shown in Figure 2D. Additionally, the restoration of the peaks at 289 and 349 nm was incomplete and there was an appearance of a growing shoulder peak at 562 nm with increased number of visible light irradiation cycling. The ratio of spiropyran to merocyanine is altered with increased number of cycles. This was especially evident in the absorption peak at 562 nm where a sustained growth of a shoulder peak was observed after each optical cycle. This incomplete conversion will play a critical role in the understanding of micromotor locomotion, in a spiropyran medium, as described later in the discussion.

To examine the changes in the running solution experimentally, we performed conductivity measurements for a direct insight of critical micelle concentrations (CMC). The methodology is explained with more details in the Supporting Information of Figure S2.⁴⁵ In the absence of the spiropyran, the CMC of SDS was found to be 0.21 wt %. This was close to the reported values in the vicinity of 0.24 wt %.^{46–49} Following that, the CMC of the SDS in the presence of 0.02 wt % spiropyran was investigated. Before and after UV irradiation, both SP-C8 and MC-C8 demonstrated a CMC value of 0.20 wt

% SDS. This slight decrease from 0.21 wt % was reflective of foreign entities in the solution.^{49,50} However, it was noteworthy to indicate that the SDS surfactant concentrations used in the micromotor running solution was 0.05 wt %, i.e., four times lower than the concentration of the SDS needed to reach critical micellar concentration.

In order to understand the influence of spiropyran on the micromotor environment, we used a pendant drop methodology to investigate the surface tension changes^{51–53} (Figure 3)

Figure 3. Surface tension with and without UV irradiation at 365 nm for 1 min in the absence/presence of 0.02 wt % spiropyran SP-C8 additive, measured using pendant drop profile analysis from five readings. Conditions in all experiments: 23 °C, with 0.05 wt % SDS and 7 wt % H₂O₂.

of spiropyran solutions containing 0.02 wt % SP-C8, 0.05 wt % SDS, 7 wt % hydrogen peroxide before and after irradiation with 365 nm UV light. The surface tension in the SP-C8 solution increased from 40.4 mN m⁻¹ to 45.1 mN m⁻¹ after irradiation with UV light. This was in contrast to the surface tension of the SDS solutions before and after UV irradiation, where values of 37.0 and 35.8 mN m⁻¹ were recorded, respectively. This slight decrease was likely due to thermal effects of heating the solution from the lamp.⁵⁴ However, on irradiation with UV, the SP-C8 solution demonstrated a large increase in surface tension, which given that there were no significant changes to the CMC, must be the main contributing factor to the changes in performance of the micromotors in the solution. The controllable switching of the surface tension of the solution, with SP-C8 interconversion to MC-C8, tunes the propulsion of the micromotor. Modulating the surface tension by 4.7 mN m⁻¹ (12% difference) has been shown to change the locomotion of self-propelled objects.^{14,22,32,55}

Surface tension gradients have been reported to alter self-propelled motion by affecting the types and concentrations of surfactants.^{31,32} Wang *et al.* have investigated the use of differently charged surfactants, demonstrating that various ion-bearing molecules affect the motion of the micromotors.³¹ In an extension to the study, Sanchez and co-workers found that the inherent ability to change surface tension that these surfactants confer to the solution altered the motion of the micromotors.³² Experimentally, these effects have been demonstrated at the macroscale too. A self-propelled Marangoni-effect propelled millimeter-sized capsule's locomotion was affected by gradients in surface tension of the solution in which it was immersed.²² Later, a centimeter-dimensioned

UV phototriggered boat was shown to be powered by a surface tension gradient, with values between 65 and 72 mN m⁻¹, demonstrating self-propelled motion.⁵⁶ At the miniaturized scale, Solovev *et al.* also demonstrated that a micro-sized object was able to demonstrate induced motion changes, with the active breakdown of the surfactant, amounting to changes in wetting of the inner surface of the micromotor.¹⁴ This eventually led to decreased frequency and size of microbubbles produced for propulsion.

In our setup, these changes in surface tension are extended to the whole environment, altering the micromotor's movement in the medium. With the introduction of UV light, the isomerization of the SP-C8 to MC-C8 occurred. The hydrophobic SP-C8 has been dissolved in the presence of SDS. The photoisomerization of the SP-C8 results in the reduced concentration of free species of SDS in the solution, due to the ease of agglomeration in the planar MC-C8.^{20,57} The changes in surface tension of the solution, shown in Figure 3, from SP-C8 to MC-C8 and *vice versa*, change the surface tension from 40.4 to 45.1 mN m⁻¹ and back again, with the activation by UV and visible light irradiation, respectively. This interswitchability between the spiropyrans and, in turn, changes of surface tension result in the change in motion of the micromotors, allowing control over a nonphotoactive entity using light. This is analogous to the recent report by Klajn and co-workers in using light to control the self-assembly of nonphotochromic nanoparticles, with an astute tuning of hydrogen bonding modes in the medium with spiropyrans.⁵⁸

The switching of the activity under photoexcitation of an ensemble of micromotors in aqueous system is shown in Figure 4. The micromotors were deactivated with UV light and reactivated using a visible light source shone through the objective lens as depicted in Figure 4A. The values of moving entities were normalized to the average value of 17 micromotors under ambient conditions. Subsequently, motion of the micromotors was halted with intervals of UV light illumination for 1 min, followed by reactivation by visible white light irradiation for 15 min. In a typical study, under ambient conditions, 100% of the micromotors were moving under microjet propulsion. Upon irradiation with UV for 1 min, the number of micromotors dropped to 40% moving by microjet propulsion in Figure 4B. Due to the variability in the velocities of these self-propelled entities (see Supporting Information Figures S3 and S4), not all micromotors were stopped by the photoirradiation under the time frame investigated. Afterward, we were able to reactivate the immobilized micromotors with visible light irradiation for 15 min at the second cycle, where 100% of the micromotors were again moving. Thus, the micromotors demonstrate reversibility to be deactivated and then reactivated during propulsion. The cycle can be repeated at least 3 times between a lowering and subsequent increase of activity, albeit with diminishing effect at higher number of cycles. The values from the populations of micromotors were subjected to a rigorous *t*-test statistical sampling, where statistically significant differences in activity were found, confirming that photochromic irradiation with spiropyrans has indeed modulated the locomotion of the micromotors (see Supporting Information Figures S5 and S6). Figure 4B illustrates that the decrease of activity corresponds to the increase in absorbance of MC-C8, and the subsequent increase was matched by the decrease in absorbance at 562 nm. This clearly indicates that the molecular transformation and the corresponding ratios of SP-C8/MC-C8, have directly affected

Figure 4. (A) Schematic of selective activation and deactivation of micromotors with controlled use of ultraviolet and visible light under isolation from a microscope objective lens. (B) Changes in activity of micromotors with the alternation of UV irradiation for 1 min and visible light irradiation for 15 min with corresponding changes in absorption band of MC-C8 at 562 nm. Conditions in all experiments: 23 °C, 7 wt % H₂O₂, 0.05 wt % SDS, 0.02 wt % SP-C8. Error bars represent the 95% confidence intervals derived from 9 separate measurements of the number of active micromotors under ambient conditions for “Ambient” and “Vis” data points, and 8 measurements after one UV irradiation period for the “UV” data points, respectively. (UV = 365 nm, Vis = visible light from halogen lamp).

the motion of the micromotors. It is noteworthy that the deactivation and consequent reactivation is persistent throughout these 3 cycles. Unlike other studies, which only show a single on/off state,¹² Figure 4B clearly shows that the micromotor activity can be repeatedly altered.

Photoexcitation of the molecular switch changes the ionic strength⁵⁹ and surface tension^{44,60,61} affecting the micromotor environment drastically, altering their locomotion. However, aggregation of MC-C8 results in an irreversible change in micromotor behavior upon extended photoisomerisation with increased iterations.^{40–46} This was reflected by the declined responsiveness with repetitions at higher number of cycles, seen in the decreased changes in the absorption band at 562 nm (Figure 4B). The photocontrol effect eventually tapers off at higher number of iterations, due to side reactions^{62–64} and slow kinetics^{65–67} of photoactive transformations, resulting in the diminished ability to alter the activity of the micromotors. At the second cycling of UV irradiation, the moving micromotors can only be depressed to 60% with UV irradiation. On the third cycle, the number of micromotors was reactivated to 86% and the subsequent deactivation only reached 58% of active micromotors. The agglomeration of MC-C8, also referred to

as molecular transformation fatigue,^{68–71} resulted in the diminished tuning of micromotor behavior on the third cycle of optical stimulation (Figure 4B). It is known that the zwitterionic merocyanine form experiences an energy barrier to revert back to the spiropyran. For example, the stacking of the planar zwitterionic merocyanine leads to the decreased reversibility to form the spiropyran molecule. This is attributed to the strong dipole–dipole interactions of merocyanine’s highly charged forms and the structural π – π stacking of the aromatic rings.^{72,73} The strong stabilization of the protonated open-ring isomer of merocyanine also retards the reciprocal ring-closing reaction,^{68,72–75} factoring in on the poor intermolecular transformation to spiropyran, due to competing reactions.^{62–64} This is in addition to the slow kinetics between the transformation of the closed-form of spiropyrans and open-form of merocyanines.^{65–67} All these compounded into the diminished ability of the spiropyran to alter the micromotor’s motion with increased number of cycles.

Nevertheless, encouraged by the initial findings, we wanted to explore further the mechanism of these phenomena. A single micromotor was manipulated with a combination of the visible light irradiation from the objective lens and ultraviolet lamp (see Figure 5). In a typical study, after UV irradiation was used

Figure 5. Instantaneous velocities of one micromotor with alternation of UV irradiation at 365 nm for 2 min and visible light irradiation for 6 min. Insets: screenshots (250 × 250 μm) from the points highlighted in circles. Yellow arrows point to position of observed micromotor. Each data point is the instantaneous velocity recorded over 5 s. Conditions in all experiments: 23 °C, 7 wt % H₂O₂, 0.05 wt % SDS, 0.02 wt % SP-C8.

to slow the micromotors down, visible white light was used to reactivate them. Under ambient conditions in the insets of Figure 5, a micromotor demonstrated a steady stream of bubbles that allowed for propulsion. However, upon UV irradiation, the micromotor switched to slower bubble emission with accompanied reduction in propulsion (Video S1). Upon extended irradiation, the micromotor began to move in tighter circles, before eventually stopping with on-site bubbling. Upon irradiation with visible white light, the micromotor was revived, albeit reaching lower velocities. This was observed with a visible reduction in number of bubbles produced and the corresponding increase in the size of micromotor bubbles. The phenomena was also validated in another separate single micromotor, which was revived after extended irradiation with visible white light after UV immobilization (shown in Figure S7 and Video S2).

The photochromic change in SP-C8 resulted in a change in micromotor environment, which eventually led both to reduction in velocities and activity.

Behavior of the micromotor with different photoexcitation is illustrated in a velocity–time graph in Figure 5. Upon UV irradiation, the reduced velocities correlate with an observed decrease in frequency of bubble emission and increase in bubble size. Conversion from merocyanine to spiropyran under visible light irradiation led to the formation of a steady stream of bubbles, allowing the micromotors to move at higher velocities upon reactivation. The micromotor can be deactivated and then reactivated just by using UV irradiation in tandem with visible white light irradiation.

Numerical representation of the micromotor's velocity against time with different photoexcitation stimuli depicts a clearer picture, as shown in the velocity–time graph in Figure 5, where the reversibility of such a mechanism was studied for 2 cycles. We were able to twice stop and restart an individual micromotor as illustrated by the plot of velocity against time graph in Figure 5. Under ambient conditions, the micromotor was moving at a velocity of $57 \mu\text{m s}^{-1}$. The velocity of the micromotor dropped to $44 \mu\text{m s}^{-1}$ under UV irradiation. Upon extended irradiation at 2.5 min, the micromotor's velocity further decreased until it came to a halt. After irradiation, with a focused visible white light through the microscope optics, the motility of the micromotor was restored. The average velocity reached $37 \mu\text{m s}^{-1}$ after 4 min. The mobility of the micromotor varied between 22 and $38 \mu\text{m s}^{-1}$ from 4 to 8 min, but after irradiation with UV light, it immediately decreased to $11 \mu\text{m s}^{-1}$. The micromotor's velocity remained between 11 and $20 \mu\text{m s}^{-1}$, before finally dropping to zero just after the UV irradiation cycle was over.

Decreases in the effective surfactant concentration through the interconversion of the hydrophobic SP-C8 to the open ringed form zwitterionic MC-C8 influenced the surface tension for bubble formation⁴⁷ due to the aggregation of surfactant.⁷⁶ This was in association with the larger size of the microbubble detached from the system and decreased frequency of the bubble emission after UV light irradiation (see Video S1 in Supporting Information). The micromotor was then reactivated under visible light irradiation, reaching an average velocity of $15\text{--}29 \mu\text{m s}^{-1}$ from 11 to 12.5 min.

After successfully demonstrating that we can alter and monitor the velocity of an individual micromotor within a swarm, we turned our attention to selective de/activation of only one micromotor out of many. This level of control with switchable modes between two states has not been demonstrated before in an autonomous system. First, we chose 2 micromotors that are in close proximity with each other. The choice of photoirradiation with the corresponding typical activation/deactivation of the micromotors should be routinely achieved. Nonetheless, due to the limitation of the optical hardware to restrict ultraviolet light to a spatially confined zone, the ultraviolet lamp was first used to deactivate the micromotors, before the selective activation of the micromotor of interest with a microscope. By using an objective lens, we focused visible white light on the micromotor of interest in Figure 6 (see Video S3). The modified localized environment that was created by the irradiation altered the propulsion of the micromotor, resulting in the increase of velocities of up to 2-fold. This demonstrated a selective activation of the micromotor, creating localized microenvironments against a background of a differing macro-environment.

Figure 6. Instantaneous velocities of two micromotors after field deactivation with UV irradiation and reactivation with visible light irradiation only on the observed motor. Insets: screenshots of points are highlighted in circles. Solid-lined squares are $400 \times 400 \mu\text{m}$ and the dashed ones are $100 \times 100 \mu\text{m}$. Yellow arrows point to position of observed micromotor. Each data point is the instantaneous velocity recorded over 5 s. Conditions in all experiments: 23°C , 7% H_2O_2 , 0.05% SDS, 0.02% SP-C8.

First, two micromotors of two distinctive velocities were identified: the observed micromotor was identified and moving at $17 \mu\text{m s}^{-1}$, while the other micromotor, which served as our reference, was moving at $152 \mu\text{m s}^{-1}$ at 0.7 min in Figure 6. To create a different locality in the bulk environment, we switched the magnification from $100\times$ to $400\times$, and in doing so greatly increased the intensity of visible white light incident upon the area surrounding the observed motor (inset of Figure 6 in dashed-squares). This effectively focused the visible white light irradiation from the $400\times$ objective lens to become a point photoexcitation source.

In Figure 6, it was noted that the velocity of the micromotor steadily climbed to a maximum velocity of $43 \mu\text{m s}^{-1}$ at 2.0 min during visible light irradiation and reached a peak velocity of $55 \mu\text{m s}^{-1}$ at 3.2 min, even after the removal of the focused photoexcitation source. The velocity of the micromotor then dropped back to $26 \mu\text{m s}^{-1}$ at the 3.4 min. Removal of excitation source allowed the steady diffusion of the photochromic additive in the MC-C8 configuration, back to the region of the photoexcited point. This effectively terminated the selective activation causing the velocity to return to $26 \mu\text{m s}^{-1}$. The localized illumination was 6.25% of the total field of vision irradiated, where the MC-C8 open form of the spiropyran predominated, leading to the decrease in propulsion as observed in 3.4 min upon extended times. Spatiotemporal control of a targeted micromotor under optical confinement was thus achieved while under continuous photoexcitation. The phototrigger allowed us to call up the activity in one of the multiple self-propelled objects in the mix. In another sample, where we chose an immobilized motor to act as reference, we achieved the same type of control over the observed micromotor purely through optical means (see Video S4). These demonstrations are reproducible and the ability for an astute selection of micromotor to be switched on represents another inventory in the manipulation of self-propelled motion.

While we note the exciting opportunities ahead, key challenges remain for the widespread utility of the chemical fuel propelled micromotors in a spiropyran-modulated environ-

ment. Stability of spiropyran remains a bottleneck, because they are susceptible to changes in pH,^{77–79} solvent polarity,⁶¹ metal ions⁸⁰ and thermal stimuli,⁸¹ in addition to irreversible photochemical reactions. The sustained presence of chemical fuels,^{3,5,18} such as chemically reactive hydrogen peroxide, is still needed for bubble-propelled locomotion of the micromotors. Directional control also remains a key barrier to be overcome, for specifically orientating self-propelled micromotors during the confinement of the micromotors in specific zonal pathways. This is in part due to the limitations of the irradiation techniques,⁸² at the miniaturized scale.

From a broader horizon, however, we have shown that point irradiation or field illumination of the micromotor running solution allows for the selective activation/deactivation of bubble-propelled engines. Single micromotor manipulation with the use of the objective lens of the microscope, in the presence of a photochromic additive SP-C8, has been achieved. Field irradiation by flooding the ensemble with photoexcitation to alter micromotors' behavior has also been demonstrated. We envision the use of photochromic additives in the solution of the micromotors to not only alter the activity, but also guide the motion of the micromotors. For example, virtual channels can be created through optical means to induce direction and orientation of motion into the self-propelled objects toward specific targets like environmental pollutants and sites for drug delivery.

CONCLUSION

In conclusion, we have demonstrated the high utility of a spiropyran additive as a molecular switch in bubble-propelled micromotor systems. From an ensemble perspective, we were able to control the number of active bubble-propelled engines, while at the single micromotor level, we could manipulate velocity and stop–go motion by simply shining light of the appropriate wavelength. The subtle changes of the environment through UV and visible light irradiation represents an attractive strategy to exert control over artificial moving objects. It allows the precise manipulation of individual particles or a whole swarm of them. This is a general concept that is not exclusive to spiropyran and it can be applied with any photochromic molecule that interacts with the surfactant or even other components of the running solution. The studies shown above thus bring us closer to a future when we can use optical means on nano/micromachines to not only control their kinetics, but also guide them in all three dimensions, achieving full spatiotemporal control.

EXPERIMENTAL SECTION

Materials. The cyclopore polycarbonate membranes with pores of 2 μm in diameter were purchased from Whatman, USA (Cat no. 7060-2511). The pores are conical in shape. Colloidal graphite (2-propanol base) was purchased from Ted Pella, Inc., USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH Instruments, USA. The platinum plating solution was obtained from Technic, Inc., USA. Spiropyran were purchased from Sigma-Aldrich and Vitas-M laboratory. Namely, 3',3'-dimethyl-6-nitro-1'-octyl-1',3'-dihydrospiro[chromene-2,2'-indole]spiropyran (SP-C8) and 3',3'-dimethyl-6-nitro-1'-octadecyl-1',3'-dihydrospiro[chromene-2,2'-indole]spiropyran (SP-C18) were purchased from Vitas-M laboratory. 1',3'-Dihydro-8-methoxy-1',3',3'-trimethyl-6-nitrospiro[2H-1-benzopyran-2,2'-(2H)-indole]spiropyran (SP-C1), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98+ %), sodium dodecyl sulfate (SDS) and sulfuric acid (98+ %) were

purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 M Ω cm) from a Millipore Milli-Q purification system. Spiropyran are labeled according to the length of the alkyl chains on the nitrogen of the indole group (see Supporting Information Scheme S1).

Apparatus. Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software (Eco Chemie). The electrodeposition procedure was conducted at room temperature (23 $^{\circ}\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Beckman Coulter Allegra 64R centrifuge. Optical microscope videos and images were obtained with Nikon Eclipse 50i microscope. Video sequences (11 fps) were processed with Nikon NIS-Elements software. UV light irradiation from UVP Blak-Ray B100AP/R High Intensity UV lamp at 100 W with 365 nm in the ultraviolet region running at 230 V and 50 Hz with 2.0 A was used as a benchtop irradiation utility. Visible white light irradiation from halogen lamp unit Nikon Model UN2-PSE100 running at 230 V and 50/60 Hz with 0.8 A was used. For the UV–vis spectra, a CARY 100 UV–vis spectrophotometer coupled to a personal computer was used for the photochromic measurements. Conductivity measurements were carried out with a Schott LF413T electrode coupled to a Schott 960 digital meter. Surface tension was measured with a pendant drop method using CAM101 drop profile analysis device (KSV Instruments, Finland), coupled with a personal computer. A JEOL-7600F semi-in-lens field-emission SEM coupled to Oxford EDX was used to acquire SEM images.

Preparation of Cu/Pt Concentric Bimetallic Microtubes. The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template. For the working electrode, colloidal graphite ink was applied on the one side of the polycarbonate template with commercial cotton swabs. A piece of flattened aluminum foil was attached to the ink immediately to the graphite ink electrode. The template was then assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized. Electrochemical deposition was carried out with a μ Autolab type III electrochemical analyzer connected to a computer and controlled by General Purpose Electrochemical Systems version 4.9 software. The template was rinsed with 5 mL of ultrapure water (18.2 M Ω cm) for 4 times, and the Cu outer layer was deposited galvanostatically at -2 mA for 600 s. The deposition solution contained 1 M CuSO_4 and 1 M H_2SO_4 . Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -2 mA for 600 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled and the template was washed 5 times with 8 mL of water each. After that, the template was ultrasonicated 3 times in 2 mL of ultrapure water for 3 min each time. The graphite layer was removed during the ultrasonication procedure. The template was then placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly 3 times with methylene chloride. The solution was then washed with ethanol and water 2 times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Preparation of the Spiropyran Solution. The stock solution of 20 mg/mL of each respective spiropyran was first prepared in acetone. To a solution of 1.0 mg of SDS dissolved in 980 μL of distilled water was slowly added 20 μL of the respective spiropyran stock solution *via* a pipet under ultrasonic conditions where the mixture was in continuous agitation. This was accomplished using an electronic

micropipette that was able to continuously dispense small portions of the solution at 2 μL each under positive displacement mode. The final concentration of the spiropyran was determined to be 0.04 wt % with 2 wt % acetone and 0.1 wt % SDS. The solution was then checked under microscope for the clarity of solution. The solution from SP-C8 forms a uniform and stable emulsion. Meanwhile, spiropyran SP-C18 is difficult to disperse even under ultrasonic conditions. Additionally, the spiropyran SP-C1 forms an unstable emulsion that rapidly breaks down on standing for 30 min to form crystalline solids (see Figure S8). Only SP-C8 and SP-C18 demonstrated photochromaticity at 365 nm (see Figure S9).

Operation of Micromotors. For the operation of the micromotors, 50 μL of spiropyran SP-C8 solution was added to an Eppendorf tube. To the Eppendorf tube was added 30 μL of the micromotors stock solution. This was followed by the addition of 20 μL of hydrogen peroxide at 35 wt %. The final concentration of the running solution was 0.02 wt % of spiropyran, with 0.05 wt % SDS and 7 wt % H_2O_2 . For the immobilization of the micromotor, UV irradiation was carried out for 1 min before measurements under optical microscope were taken. Measurements of 15 repetitions were carried out. During immobilization and activation cycling experiments, the measurements were carried out in an Eppendorf tube, where the micromotors can be immobilized with UV rays irradiation for 1 min and reactivated by 15 min of visible light irradiation in sequence for 3 cycles using spiropyran SP-C8. Samples of the micromotors in 10 μL batches from the same Eppendorf tube were taken at each interval and measured. Measurements of 9 separate recordings of the number of active micromotors under ambient conditions for "Ambient" and "Vis" data points, and 8 measurements after one UV irradiation period for the "UV" data points were carried out. All measurements were performed on a glass slide cleaned with a stream of nitrogen. Optical microscope videos and images were obtained with Nikon Eclipse 50i microscope. Video sequences (11 fps) were processed with Nikon NIS-Elements software.

Determination of UV–Vis Spectrum. The absorbance of the solution was measured in a cuvette by a UV–vis spectrophotometer. For the transformation from SP-C8 to MC-C8 using UV irradiation of 365 nm, a solution of 0.02 wt % SP-C8 in 0.05 wt % SDS was used, in increasing intervals of 20 s exposure time up to a duration of 60 s. Conversely, the transformation of MC-C8 to SP-C8 was accomplished with visible light with increasing intervals of 5 min exposure time up to a duration of 15 min. The reversibility of the switching of the SP-C8 was investigated with 1 min of UV irradiation and then followed by a duration of visible light irradiation for a time of 15 min for 3 cycles.

Determination of Critical Micellar Concentration. The conductivity of the solution of 0.02 wt % SP-C8 was tested with increasing concentrations of the surfactant SDS (see Figure S2). Measurements were taken after a stable reading for 5 s was achieved. Conductivity of the solution increased with increased concentrations of the electrolyte SDS. However, changes in the gradient of the conductivity of the solution were observed beyond a certain concentration, constituting to micelle formation. The intersection of these two gradient curves were then calculated graphically to obtain the critical micellar concentration of the solution.

Determination of Surface Tension. A solution of 0.02 wt % SP-C8 in 0.05 wt % SDS with 7 wt % hydrogen peroxide was measured for changes in surface tension. The density of the solution was measured before each measurement was taken. For each measurement, a volume of 5–6 μL of the solution was dispensed by a pendant drop method using CAM101 drop profile analysis device (KSV Instruments, Finland), coupled with a personal computer. Surface tension of SP-C8 was measured before and after irradiation of UV light at 365 nm for 1 min. Control experiments for SDS, before and after exposure to 1 min of UV irradiation, were also carried out. The final value was taken using pendant drop profile analysis from five readings.

Single Micromotor Activation and Deactivation. Selected micromotor was segregated under optical isolation using microscopic methods of 100 $\times$ magnification. The micromotors were immobilized with UV rays irradiation for 2 min and reactivated by 6 min of visible light irradiation for 2 cycles. For the creation of a localized micromotor

environment, two micromotors in close proximity under 100 $\times$ magnification from the field of vision were chosen. These micromotors were irradiated for 2 min by UV light, with the region of interest under microscopic observation. To alter the micromotor environment, the observed micromotor was focused under high magnification of 400 $\times$ /1000 $\times$, before irradiation with visible white light to activate the motor. The nonilluminated micromotor will be termed as the reference micromotor. Optical microscope videos and images were obtained with Nikon Eclipse 50i microscope. Video sequences (11 fps) were processed with Nikon NIS-Elements software. Instantaneous velocities were calculated from velocities within a 5 s interval in the video, using Nikon NIS-Elements software.

ASSOCIATED CONTENT

Supporting Information

Supporting videos are available online. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nano.5b07847.

Additional experimental data (PDF)

Video S1 (AVI)

Video S2 (AVI)

Video S3 (AVI)

Video S4 (AVI)

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Remote Electrochemical Monitoring of an Autonomous Self-Propelled Capsule

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S Supporting Information

ABSTRACT: While self-propelled autonomous devices have witnessed dramatic developments in recent years, the systems monitoring their movement have not kept up and continue to rely on optical tracking, which is difficult to automate and requires large processing power for analysis of recorded movies. Optical systems are also limited by transparency of the media/system and are constrained by the depth and field of view. A system that obviates these inherent limitations of optical tracking is urgently needed. Here, the remote monitoring by an electrochemical method is presented. It demonstrates efficacy in the determination of the location of an autonomous self-propelled capsule in continuous motion regardless of transparency or optical limitations. The “cognitive” ability of the capsule to survey positions of obstacles is also illustrated. This novel method allows for the mapping of self-propelled motors and their surroundings, via the transmission of real-time information, in a channel environment. Such a system provides a break-through of monitoring limitations and allows for truly autonomous systems.

INTRODUCTION

The remote monitoring of autonomous self-propelled objects is a very important task, as it would allow for truly autonomous and independent systems. Such self-propelled materials have been envisaged to be the next step forward for smart materials.¹ Invention of novel architectures and the simultaneous monitoring of these devices are currently the two-pronged approach toward greater exertion of control and understanding in self-propelled systems. While the advent over the design and manipulation of such autonomous moving objects have been remarkable, the progress in techniques over the real-time detection of such moving entities have not kept pace.^{2–4} Optical analysis of the motion through recordings remains to be the tool of choice, where often postanalysis using computation demanding movie analyses is required for meaningful information to be extracted. Such optical monitoring is impossible in real systems where optically nontransparent environment is envisioned. For the real-time monitoring of these objects and miniaturization of these technologies, there arises a need for alternative means to the detection of motion. An automated tracking of self-propelled autonomous motors shall allow us to identify and describe the position as a function of time. Hence, the challenges in monitoring locomotion of self-propelled systems in real-time and to demonstrate miniaturization capability, portends that a new measure beyond optical means is required.

Electrochemical methods have been demonstrated to be able to track and monitor the motion of particles at the micro and milli-meter scale. Pioneering works by Compton and co-workers, have demonstrated that displacement of micrometer-sized objects can be determined through triangulation of

potential-transients of three individually addressable electrodes⁵ and cyclic voltammetry.⁶ These solution-based electrochemical procedures have shown efficacy in locating the displacement of the object of interest. Recent advances have also demonstrated capability in monitoring moving particles. Rees et al. used an integrated electrode system to track the motion of a micrometer-sized spherical ball, that was moved by magnetic forces.² The same team later demonstrated that by using photoelectrochemistry,³ the motion of a millimeter-sized organism could be monitored by confining it within capillary walls. Liu et al. have also demonstrated the use of electrochemistry for detection of moving droplets in the channels of a lab on a chip platform.⁴ Such channel environments have been the subject of many studies involving interactions between moving objects and the physical environment.^{7–11} For a self-propelled autonomous entity, it is indeed complementary to consider the motion of moving objects to be constrained by the walls, as much of its projected use has been in that of a vascular system such as blood capillaries¹² and that of channels in microfluidic devices.¹³

Thus far, all the above methods have been carried out by voltammetric means, where the motion of the entity results in the perturbances in the electrochemical signal.¹⁴ The principle relies on the interaction of the particle with the surface of electrode by disrupting the diffusional field around the electrode. However, these techniques often requires the accompaniment of an electroactive species in the solution,

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which may complicate the setup.² Charge transfer from the particulate to the electrode, due to particles in solution striking the electrode surface, has been demonstrated for the quantification and sizing of silver nanoparticles.¹⁵ Electrochemical signals have been generated by nanoparticles striking the electrode surface at a potential bias, resulting in a redox reaction. Separately, ultrasonication could be used to activate these particles for electrochemical detection due to impact-mediated charge transfer.^{16,17} Such impact-mediated charge transfer is particularly suitable for monitoring the movement of self-propelled moving objects. In this context, it is intuitive that an object while moving across the surface of the electrode will be able to influence electrochemical signals, when placed in close proximity and is of sufficient magnitude. In this report, we demonstrate that this hypothesis is indeed true.

Our group has previously demonstrated the mobility of a polymer capsule, powered by the Marangoni effect, as an autonomous self-propelled object.^{18–20} The polymer capsule demonstrated versatility in traversing multiple environments: in between an organic and aqueous phase²⁰ and solutions of varying Reynolds number.²¹ In this study, the capsule will be the subject of interest for electrochemical detection of motion. We demonstrate the ability to track the capsule moving around a circular track. Trajectory of an autonomous self-propelled capsule can be determined by an electrochemical setup linked to the potentiostat. The work illustrates the efficacy of an electrochemical method for tracking autonomous self-propelled objects with a view that analogous systems can detect micro and nanosized objects via an electrochemical method as a function of time. Additionally, the capsule demonstrates “cognitive” ability, as it senses and navigates around obstacles.²² Electrochemical information obtained from the autonomous self-propelled capsule can be used to pinpoint the position of the obstacle. Thus, electrochemical detection of motion in the capsule system shows not only efficacy in tracking the movement, but demonstrate possible applications to transmit information about the surroundings to which it has been subjected.

■ EXPERIMENTAL SECTION

Materials. Sodium nitrate and polysulfone was obtained from Sigma–Aldrich (Singapore). *N,N'*-dimethylformamide (DMF) was obtained from Merck. Glassy carbon electrodes with a diameter of 3 mm and Ag/AgCl (1.0 M KCl) electrode were obtained from CH Instruments. Platinum electrode with a diameter of 2 mm was obtained from Autolab.

Apparatus. Voltammetric experiments were performed at room temperature using a three-electrode configuration. A platinum electrode served as the counter electrode (C.E.), while an Ag/AgCl (1.0 M KCl) electrode served as the reference electrode (R.E.). The glassy carbon (GC) electrode was used as the working electrode (W.E.). All electrochemical potentials are stated versus the Ag/AgCl (1.0 M KCl) reference electrode. The voltammetric experiments were performed on a μ -Autolab type III electrochemical analyzer (Eco Chemie, The Netherlands) controlled by General Purpose Electrochemical Systems Version 4.9 software (Eco Chemie).

Preparation of Capsule. The capsule precursor was prepared by mixing polysulfone (PSf, Sigma-Aldrich) with *N,N'*-dimethylformamide (DMF, Merck) and was dissolved into a clear solution using an ultrasonic bath for 30 min. Seven μ L of the precursor solution was dropcasted into the electrolyte

in each experiment at an approximate distance of 2.5 cm from the surface of the electrolyte solution surface.

Electrochemical Setup. The experiments were carried out in a Teflon maze with most of its running pathways possessing the width of 1 cm and a depth of 1 cm. The dimension of the Teflon plate was $42 \times 26 \times 2$ cm³. The distance from the center of the circular track to the center of the channel is 7.5 cm, which gave the track an approximate circumference of 47.1 cm. The circuit was constructed such that all exits are blocked with Teflon cubes of sides 1 cm. Electrical connectivity to the counter and reference electrodes was ensured by allowing a salt bridge to the bulk electrolyte, where the working electrode was located. The capsule is thus confined to the circular track of the Teflon maze. Working, counter and reference electrodes are wrapped with Parafilm to prevent leakage of the electrolyte. 45 mL of NaNO₃ solution (10 mM), which acts as the electrolyte, was placed into the maze channel. The capsule predominantly travels in the middle of the channel due to the shape of the water meniscus with the Teflon maze's wall. The channel has a width, being 10 mm. As the capsule has a diameter of approximately 3 mm, the capsule approaches very close to the electrode surface in the order of millimeter range, at an approximate distance of 3.5 mm. Slight fluctuation may occur, due to the path taken by the capsule along the track. After each individual experiment, the liquid was taken out and the channel was cleaned with a Kimwipe dabbed with ethanol, before flushing twice with 45 mL of deionized water. A Casio HD video-recorder was placed over the maze for video recordings. The video sequences were analyzed using Nikon NIS-Elements software, and the average speeds were calculated.

Renewal of Glassy Carbon Electrode. Glassy carbon electrode surfaces were renewed by polishing with alumina particles (0.05 μ m) on a cloth and washed with copious amount of water.

■ RESULTS AND DISCUSSION

As shown in Figure 1A, the capsule track was constructed in a manner such that constant electrical contact was kept throughout the experiment. Reference and counter electrodes were placed outside the track, while remaining in constant electrical contact with the solution through a salt bridge. An inert electrolyte of NaNO₃ was chosen, such that no reaction will occur between the polymer capsule and the electrolyte. The conventional setup of the experiment is shown in Figure 1B.

The electrochemical detection of motion in a self-propelled millimeter-sized polymer capsule is illustrated in this work. Electrochemical information from the motion of the capsule not only reveals the position and speed, but also transmits information about the physical obstacles in its path. The capsule is made from a droplet of PSf/DMF (7 μ L) dropcasted into the electrolyte solution. An immediate phase inversion occurred, as the polymer solidified in contact with the aqueous media.¹⁸ The volume of precursor solution used, produced a capsule of diameter 3 mm. Unique distributions of pores were found across the two interfaces of the capsule: ~ 20 μ m at the PSf/air interface and ~ 130 nm at the PSf/water interface.^{18,23} This resulted in the slow release of the DMF from the solidified capsule. As previously reported by our group, the capsule is propelled by the Marangoni effect, caused by the asymmetric release of DMF from the capsule to the PSf/water interface at the edges. The difference in surface tension (γ) of the aqueous solution ($\gamma_{\text{Water}} = 72.0$ mN m⁻¹) and DMF ($\gamma_{\text{DMF}} = 35.2$ mN m⁻¹) results in the Marangoni effect. Hence, the asymmetric

Figure 1. (A) Scheme of electrochemical setup of the capsule track for monitoring motion using a three electrode system. (B) Experimental setup. Scale: Width of channel is 1 cm.

release of DMF and differences in surface tension between the DMF/water at the interface of capsule resulted in a net force. This propels the capsule from a region of low surface tension to a region of high surface tension, leading to the facile motion of the capsule.

The sequence of events that occur at the capsule upon introduction to an aqueous solution is now described. The in-house designed circular capsule track coupled with a three-electrode electrochemical system was used for the study. The droplet of PSf/DMF mixture is introduced at a position 90° away from the glassy carbon working electrode. On dropcasting, the capsule remains relatively immobile for a few seconds as it solidifies, before exhibiting high speeds of up to 11.6 cm s^{-1} , which is nearly 40 body lengths per second, drawing consistence with previous results.¹⁸ The capsule revolved about the circular track in periodic motion (see SI Video S1). It was observed that whenever the capsule moves across the surface of the electrode, an electrochemical response is generated, as seen in Figure 2A. As the capsule plies the length of the electrode surface, the phenomena gave rise to two separate signals: a peak and a trough. Here on, the peak is chosen as the point of reference for the capsule moving across the electrode surface. The reason for choice is 2-fold. First and foremost, the trough is less sharp in shape and a clear distinction of the valley is hard with increased number of oscillations, due to the time taken for the capsule to cross the length of the electrode surface. The choice of the peak also reduces the tendency of a time lapse between the crossing event and that of what was recorded in the electrochemical profile.

Figure 2. (A) Current–time plot for a capsule in motion in 10 mM NaNO_3 with the electrode at a potential bias of -1.0 V vs Ag/AgCl (1.0 M KCl). Each peak represents the capsule crossing the electrode surface. (B) Zoomed-in electrochemical profile of capsule crossing the electrode surface. Inset: Illustration of a capsule crossing the surface of the electrode, where each peak corresponds to the time it crosses the electrode surface.

The recorded peaks correspond to the proximity event of the capsule and the dissipated DMF with the electrode. Upon the capsule's occurrence at the working electrode, there is a disturbance of the electrical double layer and rapid increase in the cathodic current, where the potential was held at -1.0 V (refer to SI Figure S1 for the electrochemical calibrations). Similar peaks have been observed in acoustically activated microparticles impacting the surface of the electrode in the absence of electroactive species in the solution.¹⁷ Such a phenomenon has been attributed to the disturbance of the surface charge on the electrode.²⁴ As the number of oscillations increased, the DMF loading of the capsule decreased and the corresponding signal has a reduced intensity as seen in Figure 2B. It is evident that the DMF loading of the polymer capsule also affects the reading of the electrochemical signal generated.

In Figure 3, the average speeds of the polymer capsule were captured respectively through optical analysis (optical) using a video software and through the current–time responses of electrochemical signals (electrochemical) using the devised automated system. The average speed of the polymer capsule at the selected occurrence on the electrode, achieved by postanalysis of tracking using video software, was very close to the values measured by the electrochemical method. Differences between the average speeds recorded by the optical and electrochemical methods only expressed a difference in

Figure 3. Average speed profiles of capsule obtained respectively by optical analysis (optical) and current–time responses of electrochemical signals (electrochemical), with increasing number of occurrences at the electrode.

value of 3.5% on average. This demonstrated that the electrochemical method is robust for the detection of average speeds of the polymer capsule, drawing very close values to that of the speed obtained from the optical tracking done through video software.

We further investigated the relationship of capsule's average speed with increasing oscillations, using the electrochemical method in Figure 4. A decay of the speed of the capsule with

Figure 4. Decay of average speeds of capsule observed through the current–time response via electrochemical signals, with increasing number of occurrences at the electrode.

increasing oscillations could be obtained, using the normalized initial speed. The decay of the speed of the capsule in the circuit can be described by the equation $y = -0.0253x + 1.08$, where x is the number of occurrences at the electrode, and y is the normalized capsule speed with respect to the initial speed recorded. With increasing number of periods, the decay of the speed of the capsule is taken to be $-0.0253x$, where x is the number of occurrences at the electrode. Hence, through an electrochemical method, we have been able to describe the average speeds of the capsule, utilizing the time taken to reach the electrode surface for each period as the polymer capsule revolves around the circuit. The work also illustrates the high efficiency of the capsule motor, where it follows an approximate

linear loss of 2.5% per oscillation from the initial speed recorded.

With an intentional introduction of a Teflon cube of 1 cm³ dimension in the circular track as a roadblock, the directionality of motion in the capsule can be reversed, generating unique electrochemical profiles in Figure 5A–D, based on the positions of the roadblocks. The pathway of the capsule is illustrated with the scheme in the inset of Figure 5A(i)–D(i). As described earlier, the polymer capsule experienced translational motion around an unhindered track. However, on reaching the end of the possible route for the circular track with the roadblock's presence, the capsule experienced an instantaneous speed of zero, before turning back. This reversal of motion has been highly dependent on the mechanism of motion of the capsule. Previously, we have demonstrated that the diffusion of DMF from the porous PSf structure, showed capability of movement in complicated channels.²² This is due to their inherent ability to alter the surrounding environment, through the controlled release of chemicals from the polymer capsule. On reaching the terminus of the blocked channel, the capsule demonstrated the ability to change the direction of the translational motion. DMF is dissipated continuously from the polymer capsule, until a net resultant force results due to differences in surface tension of the solution. The capsule's ability to continuously change the surface DMF/water composition, results in this propulsion behavior at the terminus, where it reverses its motion into the unblocked pathway, by remediating the surrounding environment. Consequently, unique electrochemical profiles indicative of the positions of the roadblocks can be recorded, as illustrated in the insets of Figure 5A(ii)–D(ii). The change in directionality of the capsule and the crossing event in proximity of the electrode have been the origin of the unique signals, which were further validated by control experiments in Figure S2 of the SI.

The roadblocks are placed at an angle of 45°, 90°, 135°, and 180° from the location of the electrode as demonstrated in Figure 5. Apart from the peaks generated when the roadblock is central to the electrode at 180°, which gave a peak-to-peak separation ratio of close to 1, the other readings at 45°, 90°, and 135° gave rise to one recurrent long time period and one recurrent short time period, which are proportional to the distance that the capsule needed to travel to and fro from the roadblock to the electrode (refer to SI Figure S3 for full electrochemical profiles). Ratios of these recorded times, which draw reference with the position of the roadblock to the electrode, enable the capsule to be used as a tool for surveying the location of the roadblock.

Two unique times taken for the capsule to travel to the roadblock and back to the electrode can be captured, when the distances from the electrode to the roadblock are different along the two paths. We define the longer time taken for the capsule to travel from the roadblock to the electrode to be τ_1 and the time taken for the capsule to travel the shorter distance to be τ_2 as seen in Figure 6A. Since the time taken for the capsule to traverse the two paths are different and is dependent on the location of the roadblock, unique ratios could be obtained by taking the ratio of τ_1/τ_2 , as depicted in Figure 6B. These four separate placements of roadblocks have generated four unique ratios. Given the high efficiency of the polymer capsule motor, we are able to pattern the position of the roadblock by the unique ratios generated in accordance to the distance traveled by the polymer capsule.

Figure 5. Magnified current–time electrochemical profiles of capsule when roadblock is (A) 180°, (B) 135°, (C) 90°, and (D) 45° from electrode in circuit. Inset: (i) Scheme of position of roadblock (black square), detection electrode (blue rectangle) and (ii) typical electrochemical signal from the paths taken by a capsule to complete the circuit back and forth from the roadblock to the electrode.

Figure 6. (A) Illustration of the paths and corresponding time taken by a capsule in order to complete the circuit back and forth from the roadblock to the electrode. (B) Electrochemical signal and ratio obtained by the time taken for capsule to complete the circuit back and forth from the roadblock to the electrode.

Using these data, a linear relationship between the location of the roadblock and the ratio (τ_1/τ_2) can be established, as the capsule traverses back and forth to the roadblock at differing placements from the electrode, as seen in Figure 7. The self-propelled autonomous capsule is able to transmit this

Figure 7. A graph of ratio (τ_1/τ_2) against position of roadblock from electrode, marked by the capsule via electrochemical signals. On the top is shown the scheme: Position of roadblock (black square), detection electrode (blue rectangle).

information by traveling to the terminus of the roadblock and back, through the time taken to return to the position of the electrode, where it generates a peak in the current–time plot. The ratios are unique to the position of the roadblock. All four positions of the roadblock generated unique ratios that are proportional to the time taken to traverse to the roadblock and back to the electrode. The “cognitive” ability of the capsule to interpret the terminus of the channel has been instrumental in reconstructing the position of the roadblock from the electrochemical profiles.

The linear relationship from these four reference points suggest that the position of the roadblock could be inferred from electrochemical information, using the time taken for the capsule to traverse the distance to the roadblock and back to the electrode. However, more work is still required to precisely define the distance of the roadblock from the electrode. An array of electrodes for monitoring will possibly help to further triangulate the motion of a capsule or multiple capsules. The work is not exhaustive to detection of artificially synthesized motors, as pollutants too, are known to experience enhanced self-propulsion.²⁵ Construction of such sensors for the detection of motion shall also play a vital role for environmental monitoring.

From the nanoscale perspective, silver nanoparticles in Brownian motion have been detected using electrochemical methods. The collisions of these particulates with an electrode at a positive potential bias¹⁵ have resulted in spikes from impact electrochemistry. These electrochemical signals find their origins from the electrochemical oxidation reactions that occur. Hence, we believe that an analogous concept can be applied to motors down to the order of nanometer scale too. A key challenge will then be for the detection of these motors under the superimposition of an electrochemical background current, due to the presence of fuel source and other additives.

CONCLUSIONS

We have demonstrated the efficacy of an electrochemical method for the detection of motion in a self-propelled polymer capsule. Electrical signals from the real-time electrochemical information generated by the polymer capsule can be used to obtain the speed of the periodic motion of the capsule motors. This methodology demonstrates efficacy when compared to other conventional methods of postanalyses. With the intentional introduction of a roadblock, the direction of the capsule was purposefully changed. The self-powered autonomous polymer capsule demonstrated capability in mitigating roadblocks, by instantaneously switching its direction on reaching the terminus. Electrochemical information obtained from such a Marangoni-effect powered polymer capsule is able to transmit information about the position of the roadblock. We envision a future widespread use of an electrochemical system in observing the motion of a self-propelled motor, especially in well-defined channels. This novel method is expected to wield us with the appropriate tools to better our understanding of the motion in self-propelled objects.

ASSOCIATED CONTENT

Supporting Information

Electrochemical calibrations of the peaks, locomotive events detected electrochemically and full electrochemical profiles of capsules with various roadblocks are available. Video of the capsule crossing the electrode surface is provided as Video S1.

This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Self-Propelled Micromotors Monitored by Particle-Electrode Impact Voltammetry

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S Supporting Information

ABSTRACT: Self-propelled micromotors, as the next frontier of smart miniaturized machines, are able to carry out a plethora of tasks and operations. To date, the evaluation of their capabilities and behavior has been carried out by optical microscopy coupled with computationally intensive imaging software. The use of particle-electrode impact voltammetry serves as an alternative means of quantifying and qualifying their locomotion. Perturbances of the diffusion layer at the electrode interface from the locomotion of the micromotor in solution resulted in spikes that were recorded by electrochemical signals. The number of micromotors and velocities of these self-propelled objects could be evaluated and examined in real-time in situ. This phenomenon was illustrated with Janus silver micromotors and tubular Cu/Pt micromotors. An on-site tracking of self-powered miniaturized entities propagating in solution serves as an alternative means for monitoring during their operations in environmental and biological applications.

KEYWORDS: micromotor, impact electrochemistry, voltammetry, self-propulsion, detection, aqueous environment

Microscopic and nanosized self-propelled objects have been tasked with performing tasks and operations in a multitude of environments.^{1,2} The miniaturized size,³ ultrafast motion,^{4,5} and capability for custom surface configuration^{6,7} for specific operations have diversified their uses in different arenas. This has included biological uses for delivery of medicine⁸ and nanosurgery,⁹ in addition to carrying out complicated missions for environment remediation, ranging from pollutant removal^{10,11} to chemical weaponry detoxification.¹² Such is the diversity of operations, which has ranged from the use in confined spaces of vascular channels within blood vessels¹³ to extensive open bodies of water for environmental remediation.^{14,15}

Up to the present day, the evaluation of their performance has been reliant on the use of an optical microscope coupled with an imaging software. Optical analysis is still the most advocated utility for observation of micromotor's motion, as it allows for kinetic events to be recorded, while allowing a live feed of possible nonlocomotive events.^{16–18} However, it is laborious from the perspective of having to use manual tracking of the autonomously moving entity. Workflow of such processes often include recording of videos before postprocessing using computationally intensive tracking software. Automation in the form of tracking the self-propelled object is scarce, and it has also been met with limitations due to the difficulties in achieving contrast in different media.¹⁹ There is also an inherent limitation of viewing particles that reach 200 nm in size, which is the limit of resolution.²⁰ Furthermore, considerations that these self-propelled objects are moving in three dimensions must be put into place. Typical optical

analysis will only have the motion of the micromotors being tracked in 2D. While one has been able to observe particles in a single plane, common microscopy is not ready for 3D imaging of moving objects in real time. Evaluating objects that are moving in and out of the focal plane on the *z*-axis has hardly been considered. The most advanced form of scanning confocal microscopy demands the use of fluorescence spectroscopy coupled with additives, in the presence of complementary light excitation and detection sources.^{21–23} Furthermore, there is a trade-off between resolution, scanning speed, and area being imaged as the speed is inversely proportional to area.²² These techniques have been primarily used to map biological cells, typically moving by a crawling mechanism at 5.2 $\mu\text{m}/\text{min}$. This is in addition to limitations of depth and field of view, and particularly the formation of bubbles which can obscure visibility, but is not often remarked on by practitioners in the field. More aptly put forth, having to extract samples from the bulk solution and place on a microscope platform for observation is also far from ideal. This calls for the need of innovative techniques, obviating additives, to monitor locomotion of micromotors.

Impact electrochemistry has been demonstrated to be able to detect colloidal particles.^{16,24–34} The technique can be categorically divided into three different types: (i) redox reaction on the impacting particle; (ii) redox reaction of the chemical markers coexisting in the solution with the impacting

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Figure 1. (A) Schematic of a micromotor impacting a carbon microfiber electrode surface and the corresponding electrochemical signal generated. (B) Typical chronoamperogram with 0.01 mg/mL silver micromotors at 33 μm carbon microelectrode. Conditions in all experiments: 23 $^{\circ}\text{C}$, 3 wt % of H_2O_2 , 50 mM NaOH, and 0.25 wt % SDS.

electrocatalytic particle; and (iii) the impacting particle's disruption of the interfacial electrochemistry when in proximity to the electrode. The latter method of perturbances when in proximity to the electrode is well suited to different environments, and especially so in the case of fast-moving micromotors near the electrode interface. For an on-site measurement of such self-propelled objects at a miniaturized scale, an electrochemical impact voltammetry methodology is well-suited for the task.

While examples using impact electrochemistry to detect impacting colloidal particles during Brownian motion have been aplenty, the detection of self-propelled micromotors has been limited. Compton et al. have previously demonstrated the detection of a basalt ball moved by magnetic forces, being detected by perturbances of the diffusion layer in a interdigitated electrode, while being immersed in the electroactive species of ferrocene.³⁵ Photoelectrochemistry has also been used to position a micro-organism moving in a confined vascular environment, using individually addressable electrodes.³⁶ Flowing water droplets under confinement in a channel environment were also continuously detected at an integrated electrode in the microfluidic chip.³⁷ We later devised an electrochemical setup that was able to track millimeter-sized Marangoni-effect powered capsules, while mapping remote positions of obstacles in the circuit.³⁸ It is noteworthy that most of these demonstrations have been singular units of the self-propelled devices and under a channel environment. In another instance, changes in electrochemical signals from swarms of fragmented flagella of micro-organisms with increasing concentrations was ascribed to increased agitation of the solution near the interface of the electrode.³⁹ However, a methodology for continuously monitoring individual artificial self-propelled objects moving in solution has not been demonstrated yet.

Pioneering works by Bard et al. have detected electrocatalytic particles in Brownian motion, due to signal amplification of analytes by electrocatalytic particles in solution.^{34,40} This was followed up by a series of work detecting nanoparticles moving by Brownian motion, including dissolution of particles and blocking of electrodes.^{16–18,27,30,41} Particularly, the work by Kanoufi et al.^{16,17} in the oxidation of nanoparticles under potential bias has illustrated that while electron transfer can be measured by electrochemical nanoimpacts, events such as incomplete dissolution and chemical side reactions such as precipitations cannot be examined in detail. On this note, the report of nanoparticles that are transferred atop an electrode

surface by Crooks et al. is particularly intriguing.¹⁸ The blocking of the electrode by the particulates has been the principal origin of such a signal, where a dip from the background current is observed. Notably, in our endeavor, we wish to demonstrate that microbubble-propelled miniaturized machines can be detected by electrochemical means and the velocities shows correlation to the concentration of the fuel (hydrogen peroxide) it is immersed in. Catalytic breakdown of hydrogen peroxide to oxygen bubbles for bubble ejection has been the origin of propulsion. Amidst a high molar concentration of a background current from the oxidation of electroactive hydrogen peroxide,^{5,42,43} any blocking of the electrode results in a dramatic decrease of the anodic current. As such, effects due to incomplete dissolution and chemical side reactions such as precipitations are minimal and avoided in such catalytically bubble-propelled systems.

Electrochemical detection distinguishes the motion of chemically self-powered micromotors apart from the fluid flow by detecting the movement of each individual microdevice. Previous efforts to eliminate the use of optics have been limited to having the micromotors traced passively with colored dyes by the naked eye or a camera.^{14,44} At the macrolevel, for a fast monitoring of the micromotors, Pumera et al. illustrated an electrochemical system for capturing the time needed for a millimeter-sized self-propelled capsule to complete a circuit.³⁸ The use of a microelectrode system adds another possibility to monitor motion of such self-powered microrobots. For our setup, we investigated self-propelled particles that exhibit translational motion in the solution due to the bubble formation and bubble ejection, resulting in locomotion. These particulates are immersed in their own fuels, which are typically in molar concentrations,^{5,42,43} as compared to millimolar concentrations found in most impact electrochemistry studies,^{34,40} representing 3 orders of magnitude difference. Blocking of the electrode by a micrometer-sized object and its emitted bubbles on the electrode of similar dimensions will result in a drastic drop in current. This illustrates possibly another strategy to amplify signals in buoyant bubble-propelled micromotors. Previous efforts at detecting micro-objects have included the use of acoustic stimuli to suspend the particles, which resulted in agitation of the solution.²⁸ We have eliminated the need for an external energy source. These micron-sized robots, which express autonomous motion while being immersed in their own fuel, can be monitored independently. While an array of micro-electrodes can evaluate and track the precise location of single

Figure 2. (A) Typical electrochemical signals for silver micromotors at 33 μm carbon microelectrode with increasing motor concentration from 0.002 mg/mL to 0.010 mg/mL. (B) Electrochemical spike counts for silver micromotors against increasing motor concentration. (C) Optical counts for silver micromotors against increasing motor concentration. Conditions in all experiments: 23 $^{\circ}\text{C}$, 3 wt % H_2O_2 , 50 mM NaOH, and 0.25 wt % SDS.

particles in a confined environment, our proposal seeks to understand the locomotive properties of self-propelled objects in free solution, where the surface tension is low⁴⁵ and effects from trapping are small.⁴⁶

In this report, we detail an in situ electrochemical monitoring of micromotors, which was able to illustrate the number of micromotors moving in solution and their velocities, paving the way for an alternative methodology in continuous evaluation of their motion in solution.

RESULTS AND DISCUSSION

Janus silver micromotors were chosen as the target of study, as they are able to move at fast speeds when immersed in the fuel of hydrogen peroxide.⁴⁷ In the presence of alkaline conditions, these self-propelled entities were able to move at even higher speeds, due to the rapid decomposition of hydrogen peroxide in basic media.⁴⁸ The decomposition of hydrogen peroxide to oxygen bubbles and the subsequent detachment led to a resultant force due to the asymmetric shapes of these Janus silver micromotors, propelling them forward.⁴⁷ Videos of the micromotors can be recorded using a camera coupled with the microscope utilizing a tracking software, where the velocities of the micromotors were then measured.

The particle-electrode impact voltammetry method was utilized for the detection of micromotors, where during a typical measurement, the potential was held at 0.8 V vs Ag/AgCl/1.0 M KCl via a chronoamperometric method in a time interval of 60 s. In a typical collision event as featured in Figure 1A, the micromotor impacts the surface of the carbon microfiber electrode, resulting in an electrochemical spike being generated. No corresponding signals were detected in the absence of the silver micromotors in the blank control, confirming that the origin of the signals are from the moving microparticles in solution (Figure S1). In a typical cyclic voltammogram, oxidation of hydrogen peroxide at the carbon microelectrode occurs at 0.4 V and culminated in a peak at 1.0

V (Figure S2A). For the detection of silver micromotors, potentials were kept below 1.0 V, to avoid the oxidation of water. The potentials were determined with a range of values between -0.4 and 0.8 V, where the potential at 0.8 V gave regular signals of the Janus silver micromotors perturbing the diffusion layer (Figure S2B). In a typical measurement, the potential of 0.8 V vs Ag/AgCl/1.0 M KCl was used, in the presence of a sodium hydroxide electrolyte, SDS surfactant, and hydrogen peroxide fuel. Figure 1B illustrates a typical chronoamperogram, where a concentration of 0.01 mg/mL of silver micromotors was studied.

The background current has been attributed to the electro-oxidation of hydrogen peroxide,⁴⁹ where increasing concentrations of hydrogen peroxide led to higher anodic current levels (Figure S3). Even upon 10 $\times$ magnification, no obvious background spikes or noise are found in Figure S1B, in the absence of silver micromotors. These self-propelled entities move near the interface of electrode/electrolyte, leading to the impact of the micromotors with electrode, as it approaches the electrode surface. Such motion in the proximity results in the blocking of the electrode from the diffusion layer, as observed by the decrease in diffusion-limited current. With the perturbation, there was a decrease in the background current in Figure 1B. Only upon the individual micromotor's departure from the surface, was a gradual return of the oxidation current observed. As seen in Figure 1, the deviations from the flat profile in a typical control experiment in Figure S1 are wholly attributed to the presence of the micromotors. As such, each spike in the electrochemical signal in the presence of micromotors is treated as a collision event. The spikes are calculated typically from a 20% threshold event from the largest spikes registered in an electrochemical signal.

The microelectrode with a diameter of 33 μm was chosen, as its size is along the same order of magnitude as the size of micromotors in solution (Figure S4A and Table S1). The time taken for the micromotors to traverse through is typically on

Figure 3. (A) Typical chronoamperogram for silver micromotors at 33 μm carbon microelectrode against increasing concentration of hydrogen peroxide. (B) Electrochemical spike counts for silver micromotors at 33 μm carbon microelectrode against increasing concentration of hydrogen peroxide. (C) Optical counts for silver micromotors against increasing concentration of hydrogen peroxide. (D) Velocities for silver micromotors against increasing concentration of hydrogen peroxide. Conditions in all experiments: 0.01 mg/mL silver micromotors, 23 $^{\circ}\text{C}$, 50 mM NaOH, and 0.25 wt % SDS.

the order of milliseconds as seen in Figure 1 and can be captured by electrochemical signals. This time scale is different from the diffusion time scale of the nanoparticles, which is at tens of nanoseconds.³¹ Since the crossing event is on the order of milliseconds and the response time is at 3 μs with a bandwidth of 1 MHz,⁵⁰ the crossing event can thus be recorded in our electrochemical signal. Blocking of the electrode by a micrometer-sized object on the electrode of similar dimensions resulted in a drastic drop in current as shown in Figure 1. This is attributed to the generated bubbles propelling the micromotors and the micromotor itself blocking the diffusion layer, resulting in a decrease in the oxidation current. The disruption of the diffusional field around the microelectrode has led to the alteration of the current response, which can be interpreted with experimental calibration.⁵⁵ In proximity of an electrode in the presence of an electroanalyte, the existence of a foreign entity will affect the response of the current on the electrode.

The concentration of the hydrogen peroxide is 3 wt % or 882 mM. It is significantly higher than the amount of electroanalyte commonly used for the diffusion-blocking mechanism^{18,51} during particle-impact voltammetry as exemplified by Lemay et al. where the electroactive material in solution is at 0.31 mM. There is a nearly $\sim 3000\times$ increase in the concentration of the electroactive species.⁵¹ The blocking of the electrode by the

micromotor results in a drastic decrease of the anodic oxidation current of the hydrogen peroxide. This is validated again in the matching decrease in background current in the tens and hundreds of nA in the absence of the hydrogen peroxide (Figure S1).

Each spike from the electrical signals is ascribed to one micromotor coming in proximity of the electrode surface. At the time of 1 s, reduction in the background current was observed in Figure 1B, which is attributed to the perturbation of diffusion layer. Upon extended durations, at the time of 2 s, it marked the departure of the micromotor, where a return to the anodic oxidation background current of the hydrogen peroxide was observed. Due to the active motion of the silver micromotors, the background oxidation current now occurs at a higher current than in the case of the micromotors' absence. This is the same phenomena reported by Shitanda and co-workers, when biological units in the microsize regime are moving in the proximity of the electrode interface, leading to better mixing and higher background current.^{39,52–54} Particle-electrode impact voltammetry was used with varied concentrations of the silver micromotors, within a fixed time interval of 60 s. As illustrated in Figure 2A, the number of spikes detected by our developed electrochemical method increases with the rise in concentration of the Janus silver micromotors, from

0.002 to 0.01 mg/mL. This corresponded to the increase in frequency of the micromotors occurring near the interfacial region of the electrode/solution. Each spike corresponds to an impacting event of the silver micromotor with the plane of the microelectrode, with the consequent blocking of the diffusion layer at the interface of the electrode and the solution.

Figure 2B demonstrates a numerical approach in quantifying the number of spikes, which increases with increasing concentrations of micromotors. The number of spikes increases from 5 spikes in 0.002 mg/mL to 22 spikes to 0.010 mg/mL. It follows a linear trend that the number of spikes in the electrical signals increases with the concentration of the Janus silver micromotors. The stochastic approach to measure the number of micromotors in solution, by observing the impacts of silver micromotors with the electrode surface shows strong correlation with the concentrations of silver micromotors. Bringing a step further, we verified this result using optical observations of the number of micromotors moving by bubble propulsion in Figure 2C through direct microscopic measurements with the number of spikes detected electrochemically, and a similar trend was observed. As the concentration of micromotors by weight increases, the number of micromotors in solution increases accordingly from 93 micromotors in 0.002 mg/mL to 560 micromotors in 0.010 mg/mL in a sample droplet. The optical measurements coupled with the observations of spikes measured electrochemically, serve as a calibration curve for the measurements of particle concentrations. Strong correlation between the optical measurements and the number of micromotors impacting the surface of the electrode through electrochemical measurements endorse this strategy as a possible method to physically measure the concentrations and number of micromotors that are moving in solution in situ.

We investigated the number of spikes detected electrochemically within a 60 s interval with increased fuel concentrations (Figure 3A). As the hydrogen peroxide concentration increases, the corresponding number of spikes increased. In contrast, the absence of any silver micromotors fails to develop any spikes even with increased hydrogen peroxide (Figure S3), strongly suggesting the need for self-propelled micromotors for collision events. This is ascribed to the increased number of bombardment and collisions of the silver micromotors in the proximity of electrode surface. From Figure 3B, the number of spikes detected electrochemically increased steadily from 23 spikes in 3 wt % hydrogen peroxide to 40 spikes in 6 wt % hydrogen peroxide. This eventually tapers off to 42 spikes in 9 wt % hydrogen peroxide. The number of spikes plateaued off, probably due to the maximum number of possible micromotors demonstrating propulsion has already been reached. This corresponded well with the observed number of micromotors through optical microscopy in Figure 3C, where the number of micromotors increases from 560 to 727 micromotors per mL, from 3 to 6 wt % hydrogen peroxide fuel, before tapering off at 800 micromotors/mL in 9 wt % hydrogen peroxide. There was also a trend of increasing velocities of the micromotors with increased concentrations of the hydrogen peroxide. Noticeably, the amplitude of the electrochemical signal intensity increases, likely due to the higher velocities that these micromotors are moving in solution as shown in Figure 3D, where the velocities increased from 146 to 204 $\mu\text{m s}^{-1}$, as the concentration of hydrogen peroxide fuel is increased from 3 to 9 wt %.

At different fuel levels, where the velocities of the micromotors differs, the residence time of the spike taken by

a micromotor during the perturbation event in Figure 3A changes too. The average residence time from 3 representative readings of the micromotors at different fuel concentrations were calculated. At 3 wt % hydrogen peroxide fuel, the average residence time registered by a micromotor was 2.0 s. With increasing fuel concentrations, the time decreased to 1.4 s for 6 wt % and then 1.2 s for 9 wt %. Through the residence time recorded, we are also able to differentiate the different velocities of the micromotors. This is an indication of how the velocities of the micromotors affect the electrochemical signals detected. Using this information, we can possibly obtain the velocities and the corresponding fuel of the hydrogen peroxide that the micromotors are immersed in.

Valuable information can also be derived from the flux of the silver micromotors. Through empirical counting of the micromotors optically, we are able to calculate the number of micromotors per unit volume. The product of the concentration and average velocities of the micromotors gives us the molar flux of the micromotors, as seen in Supporting Information, eq S1. The data is shown in the Supporting Information in Table S3 and Figure S5. A similar trend of increased flux of the micromotors with increased concentrations of the hydrogen peroxide was also observed.

In the setup, the electrodes are placed above the solution of the silver micromotors. As a result, the perturbation of the electrodes by the rising microbubbles from the micromotors must be accounted for as they impact the electrode surface. Typically, the emission rates of the micromotors are at tens to hundreds of Hz.^{47,55} A droplet's height typically measures at 586 μm , but depth of focus using a 10 $\times$ optical lenses is 8.5 μm . This is reflective of the problem of using optics to observe micromotors. As illustrated in Supporting Information Table S4 and supporting Figure S6, it shows the relationship between concentration of micromotors with the physical counts obtained optically and electrochemical counts, where differences of 4 orders of magnitude were observed. The product of the correction factor for the depth of focus from the microscopic lenses and the bubble emission rate give rise to differences from the micromotors observed under microscopic observation, as compared to electrochemical detection of micromotor using particle-electrode impact voltammetry.

There are inherent limits of the depth of focus of the microscope and multiple electrochemical signals can be detected due to bubbles emitted by the micromotors. These led to the number of spikes detected by the electrochemical measurements to be greater than the optical counts, representing an amplification of the signal for ease of detection. These two factors are needed to account for the proportionality constant. As such, the height of droplet with the corresponding depth of focus from the microscope⁵⁶ and effects from the bubbles emitted by the micromotors must be detailed.⁵⁷

An exact value for the proportionality constant in a self-propelled micromotor differs greatly from that for a diffusion-based nanoparticle. An intricate insight into the exact value of the proportionality constant needs to take into account the dynamics of the shape,⁵⁸ mass,⁵⁹ and Reynolds number of the solution,⁶⁰ which is beyond the scope of discussion of the paper.

The time taken to traverse across the longest dimension of the electrode is typically in the order of milliseconds and can be captured by electrochemical signals. For example, at 3 wt % hydrogen peroxide, with micromotors moving at an average velocity of 146 $\mu\text{m s}^{-1}$, the time needed to cross a 33- μm -

Figure 4. (A) Typical chronoamperogram with 3 μm Cu/Pt micromotors at 33 μm carbon microelectrode with increasing motor concentrations. (B) Electrochemical spike counts for 3 μm Cu/Pt micromotors at 33 μm carbon microelectrode against increasing motor concentration. (C) Optical counts for 3 μm Cu/Pt micromotors against increasing motor concentration. Conditions in all experiments: 23 $^{\circ}\text{C}$, 9 wt % of H_2O_2 , 50 mM NaOH, and 0.25 wt % SDS.

length diameter is computed to be 226 ms. This corresponds well to the crossing event by the recorded electrochemical signal that was depicted in Figure 1B, which is on the order of hundreds of milliseconds. The perturbation of the diffusion layer led to the observation in the change of current. Since the time scale of the crossing event is on the order of milliseconds, it validates this electrochemical strategy as a fitting methodology for detecting the locomotive events of the self-propelled entities in solution.

Entrance of a micromotor, the crossing event, and its eventual departure was recorded in the electrochemical signals. These separate events correspond to the initial decrease in anodic current, the formation of a spike, and the eventual translocation from the electrode surface leading to the restoration of the anodic current.

The large variation in signal magnitude originates from the differences in the velocities of the micromotor in the hydrogen peroxide fuel. The intrinsic nonuniformity in the micromotor's sizes as seen in Supporting Information Figure S4 and Table S1 resulted in different velocities in Figure 3. This inherent difference in sizes of the micromotors, and then the velocities, resulted in the large variations in signal magnitudes during the decrease in anodic background current. The general trend of increased number of electrochemical counts with increased concentrations persists. We foresee a more consistent reading with a further optimization of the technique, accounting for the difference in dimensions of the micromotors.

After the investigations with Janus silver micromotors, we investigated the electrochemical response from a swarm of Cu/Pt micromotors. These tubular micromotors of micrometer dimensions moving in solution were electrodeposited from a

polycarbonate membrane with a 3 μm pore (Figure S4B and Table S2). The decomposition of hydrogen peroxide in the inner lining of the catalytic platinum layer to oxygen bubbles and the subsequent detachment propels the tubular bimetallic concentric micromotor forward. As we increased the concentration of the micromotors from the stock solution, the number of spikes recorded within a fixed time interval of 60 s increases. This is illustrated when the concentration (in relative volume percentages from the stock solution) increases from 0.033% to 0.20% in Figure 4A. This is due to increased electrode–particle collisions, as we have elucidated with the Janus-type micromotors. However, from Figure 4B, the number of spikes appear to follow a nonlinear trend and tapers off at higher concentrations of micromotors, from 9 spikes in 0.033% to 16 spikes in 0.067%, plateauing out at 18 spikes in 0.20% from the stock solution. To investigate this phenomena, we undertook optical microscopy, where the observed number of micromotors moving by self-propulsion in Figure 4C continues to increase from 47 to 207 micromotors per mL when the concentration of micromotors taken from the stock solution increased from 0.033% to 0.20%. This is in contrast to the electrochemical measurements from Figure 4A, which is possibly due to tubular micromotors tending to move along the bottom surface of the solution, while the Janus-type silver micromotors are able to move near the air/solution interface, due to the greater number of bubbles that are produced. Tubular micromotors move further away from the electrode surface due to limited ability to generate bubbles in the hollow concentric center, as compared to the silver Janus particles.

Typically in a blank solution, a clean and flat profile was observed in the control experiments with no observation of any

spikes, even at 10 $\times$ magnification (Figure 1B). The spikes are 3 $\times$ higher than the noise and are calculated typically from a 20% threshold event from the largest spikes registered in an electrochemical signal. Generation of bubbles is confined to the inner catalytic layer of the tubular Cu/Pt micromotor, and escaping through the ends of the micromotors. This is in strong contrast to the Janus-type micromotors, which produces bubbles on all of its outer surfaces giving such Janus-type micromotors ease of detection via an electrochemical method. Further work to refine the methodology for an all-encompassing strategy for quantification and qualification of self-propagated moving objects is underway, to make it a more polished technique.

We have demonstrated a vast improvement here to the detection capabilities, in comparison to the previous report on the monitoring of Marangoni-effect propelled capsule motor.³⁸ Previously, detection is limited to the macrolevel, with the motion of the motor to be confined in a channel environment and held in proximity to the electrode detector in an orthogonal fashion. In the current technique, we are able to detect micron-sized motors that are freely moving in solution; no limitations are required to the environments in which the micromotors are moving. The micromotors need not be confined in channels and can be in free solution. Their motion need not be limited to being perpendicular with the detector electrode. This difference between the methodologies permits the work to be extended to the motion of the micromotors which are suspended in solution. The current method can also detect beyond a single micromotor. A simple immersion of the micron-sized electrode allows the possible determination of the number of micromotors in solution and their locomotive properties.

Typically, for the blocking of the electrode surface, nanoparticles on the order of hundreds of nanometers can be detected.^{18,32,51} In this experiment, particles of several microns in size were investigated. Similarly, we believe that with our current technique, detection of such particles can be reduced to several hundreds of nanometers in size. Such a scale is expected for the limit of detection using impact electrochemistry via a blocking mechanism. Furthermore, in other impact electrochemistry techniques, smaller-sized particles have been detected. Dissolution of nanoparticles during detection via impact electrochemistry can be scaled down to tens of nanometers in size.⁶¹ For electrocatalytic redox reaction of analytes in solution, nanoparticles of sizes up to a few nanometers in scale have been distinguished, where such signal amplification can be accomplished.³⁴ Simultaneous in situ study of electrochemical responses coupled with optical observations can serve to optimize the technique further.

CONCLUSION

We have demonstrated an alternative methodology for monitoring the locomotion of self-propelled micromotors. Two different types of micromotors, the Janus silver micromotor and the tubular Cu/Pt micromotor, with their corresponding optical and electrochemical responses were investigated. Particle-electrode impact voltammetry has been able to quantify and qualify the number of micromotors moving in solution on-site. In addition, we are able to detect the modulation of hydrogen peroxide propellant concentrations by electrochemical signals, where there was an increment in active micromotors and faster velocities. We foresee the widespread utilization of such electrochemical techniques to monitor

locomotion of micromotors on-site. This is crucial in improving situational awareness of the task at hand, during environmental and biological applications. The obviation of optical tracing with microscopic tools and elimination of computationally demanding software can be accomplished with the advent of particle-electrode impact voltammetry for tracking and surveying self-propelled micromotors.

EXPERIMENTAL SECTION

Materials. The cyclopore polycarbonate membranes with pores of 3 μm in diameter were purchased from Whatman, USA. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Methylene chloride and ethanol were purchased from Tedia, USA. Pt electrodes with 1 mm diameter and Ag/AgCl/1.0 M KCl were purchased from CH instruments, USA. Microcarbon fiber electrode with an inner diameter of 33 μm and outer diameter of 4 mm (Cat. No. 0020002) was purchased from BAS Inc., Japan. The platinum plating solution was obtained from Technic Inc., USA. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (98+%), sodium dodecyl sulfate (SDS), sodium hydroxide, sulfuric acid (95–98%), and silver microparticles were purchased from Sigma-Aldrich. Chemicals were used as received and the solutions were prepared using ultrapure water (18.2 $\text{M}\Omega \text{ cm}$) from a Millipore Milli-Q purification system.

Apparatus. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA v 1.8 software (Eco Chemie). The deposition procedure was conducted at room temperature (23 $^\circ\text{C}$) using a three-electrode arrangement. A platinum electrode was utilized as a counter electrode, and Ag/AgCl/1.0 M KCl was used as the reference electrode. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The silver working electrode was sputtered on the cyclopore membrane and copper tape was used as the transducer. The ultrasonication process was carried out with a Fisherbrand FB 11203 ultrasonicator, and centrifugation was carried out with a Hermle Z233m-2 microcentrifuge. SEM images were taken with a JEOL-7600F semi-in-lens FE-SEM. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software.

Preparation of Cu/Pt Concentric Bimetallic Microtubes.⁴³

The Cu/Pt concentric bimetallic microtubes were synthesized with a modified electrochemical deposition procedure on a cyclopore polycarbonate template. Silver ($\sim 100 \text{ nm}$) was sputtered onto one side of the polycarbonate template. Copper tape was attached to the silver-sputtered polycarbonate template, which serves as the working electrode for the plating experiments. The template was assembled into a customized electrochemical deposition cell. Platinum counter electrode and Ag/AgCl/1.0 M KCl reference electrode were utilized. Electrochemical deposition was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA v 1.8 software (Eco Chemie). The template was rinsed with 5 mL of ultrapure water (18.2 $\text{M}\Omega \text{ cm}$) for 4 times, and the Cu outer layer was deposited galvanostatically at -20 mA for 1200 s. The deposition solution contained 1 M CuSO_4 and 1 M H_2SO_4 . Consequently, after removing the deposition solution, the template was rinsed 5 times with 8 mL of water. The platinum segment was electrodeposited subsequently at -20 mA for 1200 s, using the commercial plating solutions. When the deposition of microtubes was finished, the electrochemical cell was disassembled. The template was polished with 5 μm alumina powder to remove the sputtered silver and was washed 5 times with 8 mL of water by ultrasonication each time. It was then placed in an Eppendorf tube with 2 mL of methylene chloride and ultrasonicated until the whole template was dissolved. The electrochemically deposited microtubes were collected by centrifugation at 6000 rpm for 3 min and washed repeatedly 2 times with methylene chloride. The solution was then washed with ethanol and water for 2 times each and centrifuged for 3 min after each washing step. The tubes were stored in water at room temperature.

Operation of Micromotors. The experiments for studying the motion of micromotors were carried out in an aqueous solution containing 3–9 wt % of hydrogen peroxide at constant surfactant concentrations (0.25 wt % of SDS) in 50 mM NaOH with asymmetric Janus silver particle micromotors. For tubular Cu/Pt micromotors, an aqueous solution containing 9 wt % hydrogen peroxide at constant surfactant concentrations (0.25 wt % SDS) was maintained in 50 mM NaOH.

Optical Monitoring of Micromotors. For locomotive studies, 10 μ L of the micromotor solution was dropcasted on a glass slide freshly cleaned with nitrogen gas. Only moving micromotors demonstrating active microjet propulsion were taken into account. Optical microscope videos and images were obtained with a Nikon Eclipse 50i microscope. Video sequences were processed with Nikon NIS-Elements software. Average velocities and number of active moving micromotors were computed from 15 readings.

Electrochemical Monitoring of Micromotors. A microcarbon fiber electrode of diameter 33 μ m was used as the working electrode. Glassy carbon counter electrode (3 mm) and Ag/AgCl/1.0 M KCl reference electrode were utilized. In a typical measurement, 0.25 wt % of SDS is used as the surfactant and 50 mM NaOH is used as the electrolyte. Upon addition of the micromotors into the solution of SDS, hydrogen peroxide, and sodium hydroxide, the solution is sonicated for 10 s for mixing, without the production of excessive bubbles before measurement. Corresponding blank experiments in the absence of micromotors were also carried out. The measurement was carried out by a three-electrode setup, which was dipped into the electrolyte solution containing the micromotors, hydrogen peroxide, and the surfactant. Electrochemical measurement was carried out with an Autolab PGSTAT 101 electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands) connected to a computer and controlled by NOVA v 1.8 software (Eco Chemie). Average electrochemical spikes observed were computed from 5 readings.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssensors.6b00314.

Optimization of experimental electrochemical conditions, SEM characterization of micromotors, and flux of micromotors bombarding the surface of electrode (PDF)

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Notes

The authors declare no competing financial interest.

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