Preparation, motion study and application of autonomous micro and nano devices

Maric, Tijana

2020

Maric, T. (2020). Preparation, motion study and application of autonomous micro and nano devices. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/140957>

<https://doi.org/10.32657/10356/140957>

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**NANYANG
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SINGAPORE

**Preparation, Motion Study and Application of Autonomous
Micro and Nano Devices**

Tijana Marić

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2020

**Preparation, Motion Study and Application of Autonomous
Micro and Nano devices**

Tijana Marić

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research done by me except where otherwise stated in this thesis. The thesis work has not been submitted for a degree or professional qualification to any other university or institution. I declare that this thesis is written by myself and is free of plagiarism and of sufficient grammatical clarity to be examined. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

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Richard David Webster

Authorship Attribution Statement

This thesis contains material from 7 papers published in the following peer-reviewed journals and from 2 papers that are submitted.

Chapter 2 is published as:

1) Maric, T., Moo, J.G.S., Khezri, B., Sofer, Z. and Pumera, M., **2017**. Black-phosphorus-enhanced bubble-propelled autonomous catalytic micromotors. *Appl. Mater. Today*, 9, pp.289-291. <http://dx.doi.org/10.1016/j.apmt.2017.08.007>;

The contributions of the co-authors are as follows:

- Prof. Pumera proposed the concept and supervised the project.
- Dr. Moo gave project direction and helped with data analysis.
- Dr. Khezri performed Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) experiments.
- Prof. Sofer prepared black phosphorus and engaged in scientific discussions on the project.
- I performed all experiments, data analysis and wrote the manuscript.

2) Maric, T., Beladi-Mousavi, S.M., Khezri, B., Sturala, J., Nasir, M.Z.M., Webster, R.D., Sofer, Z. and Pumera, M., **2019**. Functional 2D Germanene Fluorescent Coating of Micromotors for Micromachines Multiplexing. *Small*, p.1902365. <https://doi.org/10.1002/sml.201902365>;

The contributions of the co-authors are as follows:

- Prof. Pumera directed and supervised the work.
- Dr. Beladi-Mousavi and Dr. Khezri gave project direction and helped with data analysis.

- Dr. Sturala contributed to the synthesis of 4-fluorophenylgermanane and methylgermanene.
- Dr. Nasir worked on manuscript preparation and revision.
- Prof. Webster engaged in scientific discussions on the project.
- Prof. Sofer provided guidance and interpretation of the experimental data.
- I performed all experiments, data analysis and wrote the manuscript.

Chapter 3 is published as:

1) Maric, T., Mayorga-Martinez, C.C., Khezri, B., Nasir, M.Z.M., Chia, X. and Pumera, M., **2018**. Nanorobots Constructed from Nanoclays: Using Nature to Create Self-Propelled Autonomous Nanomachines. *Advanced Functional Materials*, 28(40), p.1802762. <https://doi.org/10.1002/adfm.201802762>;

The contributions of the co-authors are as follows:

- Prof. Pumera conceived the idea and advised on improvements of the project.
- Dr. Mayorga-Martinez gave project direction and designed the study.
- Dr. Khezri performed Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) experiments.
- Dr. Nasir worked on manuscript preparation and revision.
- Dr. Chia performed the material characterizations.
- I co-designed the project with Dr. Mayorga-Martinez, performed all experiments, data analysis and wrote the manuscript.

2) Maric, T., Mayorga-Martinez, C.C., Nasir, M.Z.M. and Pumera, M., **2019**. Platinum–Halloysite Nanoclays Nanojets as Sensitive and Selective Mobile Nanosensors for Mercury Detection. *Advanced Materials Technologies*, 4(2), p.1800502.

<https://doi.org/10.1002/admt.201800502>;

The contributions of the co-authors are as follows:

- Prof. Pumera supervised the research.
- Dr. Mayorga-Martinez advised on improvements of the project and contributed to scientific discussion of the article.
- Dr. Nasir conceived the idea, worked on manuscript preparation and revision.
- I designed the experiment, analysed data and wrote the manuscript.

Chapter 4 is published as:

Maric, T., Nasir, M.Z.M., Mayorga-Martinez, C.C., Rosli, N.F., Budanović, M., Szőkölóvá, K., Webster, R.D., Sofer, Z. and Pumera, M., **2019**. Cloisite Micromotors as Self-Propelling Cleaners for Fast and Efficient Removal of Improvised Organophosphate Nerve Agents. ACS applied materials & interfaces. <https://doi.org/10.1021/acsami.9b08332>;

The contributions of the co-authors are as follows:

- Prof. Pumera supervised the research.
- Dr. Nasir conceived the idea, provided guidance and edited the manuscript drafts.
- Dr. Mayorga-Martinez contributed to scientific discussion of the article.
- Rosli assisted with cytotoxicity experiments.
- Dr. Budanovic performed quantification study of fenitrothion adsorbed.
- Dr. Szőkölóvá performed the material characterizations.
- Prof. Webster and Prof Sofer proposed the concept and advised on improvements of the project.
- I conducted the experiments, analysed data and wrote the manuscript.

Chapter 5 is published as:

Maric, T., Nasir, M.Z.M., Rosli, N.F., Budanović, M., Webster, R.D., Cho, N.J. Pumera M., **2019**. Micromotors Derived from Variety Plant Pollen Grains for Efficient Environmental

Clean-Up and Anti-Cancer Drug Delivery, *Advanced Functional Materials*, 2000112.

<https://doi.org/10.1002/adfm.202000112>;

The contributions of the co-authors are as follows:

- Prof. Pumera conceived the idea and designed the study.
- Dr. Nasir conceived the idea and supervised the research.
- Rosli assisted with cytotoxicity experiments.
- Dr. Budanovic performed Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) experiments.
- Prof. Cho prepared and analyzed pollen grains.
- Prof. Webster advised on improvements of the project.
- I conducted the experiments, analysed data and wrote the manuscript.

Chapter 6 is published as:

Maric, T., Nasir, M.Z.M., Webster, R.D., Pumera, M., 2019. Tailoring Metal/TiO₂ Interface to Influence Motion of Light-Activated Janus Micromotors. *Advanced Functional Materials*, 30, p.1908614. <https://doi.org/10.1002/adfm.201908614>;

The contributions of the co-authors are as follows:

- Prof. Pumera directed and supervised the work.
- Dr. Nasir gave project direction, guidance and interpretation of the experimental data.
- Prof Webster worked on manuscript preparation and advised on improvements of the project.
- I conducted the experiments, analysed data and wrote the manuscript.

Chapter 7 is published as:

1) Maric, T., Nasir, M.Z.M., Wang, Y., Khezri, B. and Pumera, M., **2018**. Corrosion due to ageing influences the performance of tubular platinum micromotors. *Nanoscale*, 10(3), pp.1322-1325. DOI: 10.1039/c7nr05775j;

The contributions of the co-authors are as follows:

- Prof. Pumera directed and supervised the work.
- Dr. Nasir gave project direction, guidance and interpretation of the experimental data.
- Dr. Yong helped with measurement velocity of micromotors.
- Dr. Khezri worked on manuscript preparation and revision.
- I co-designed the project with Prof. Pumera, performed all experiments, data analysis and wrote the manuscript.

2) Maric, T., Nasir, M.Z.M., Budanovic, M., Webster, R.D., Pumera. M., **2019**. Corrosion of Light Powered Pt/TiO₂ Micromotors, accepted.

The contributions of the co-authors are as follows:

- Prof. Pumera conceived the idea and designed the study.
- Dr. Nasir gave project direction, guidance and interpretation of the experimental data.
- Dr. Budanovic performed Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) experiments.
- Prof Webster worked on manuscript preparation and advised on improvements of the project.
- I co-designed the project with Prof. Pumera, performed all experiments, data analysis and wrote the manuscript.

**List of other International Refereed Journal Publications not inserted as
thesis chapters**

- Wang, H., Potroz, M.G., Jackman, J.A., Khezri, B., Marić, T., Cho, N.J. and Pumera, M., **2017**. Bioinspired spiky micromotors based on sporopollenin exine capsules. *Advanced Functional Materials*, 27(32), p.1702338.
<https://doi.org/10.1002/adfm.201702338>;

23/12/2019

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Abstract

Micro- and nanomachines are a new generation of nanotechnology, allowing nano- and micro-objects to propel themselves, perform autonomous motion and other on demand actions, such as drug delivery or environmental remediation. Micromotors are microscale devices which are made to perform various types of movements. They are an upcoming topic of today's research due to their interesting size and multiple potential uses. In the last decade, scientists have become increasingly interested in these devices. Autonomous self-powered miniaturized machines are the next step for nanotechnology. They consist of different types of micro- and nanoscale compounds, and can be propelled using various sources of energy inputs.

The versatility of new nanomaterials coupled with emerging catalysts, has enabled fabrication of different micro- and nanomotors suitable for detection of water pollutants but also for cargo delivery in biomedicine. In this thesis, black phosphorus, 2D germanene and TiO₂ nanoparticles were studied as promising materials for micro- and nanomotors preparation and they exhibited very efficient motion properties in aqueous media. Natural nanoclays and pollen micromotors were shown as excellent and cheap adsorbents which were used to reduce concentration of various pollutants in water. Henceforth, this research is focused on the use of natural micro and nanoparticles for preparation of micromotors that have suitable properties of adsorbents.

Furthermore, our study was based on the stability and durability of micromotors over prolonged periods of time with a focus on the influence of ageing in “storage” conditions- the difference in performance of ‘freshly prepared’ and ‘old’ micromotors could have a significant impact on the reliability and reproducibility of the micromotors. From our findings, it was discovered that the performance and morphologies of ‘new’ and ‘old’ differ greatly over the

period of study. As such, it would be of importance to prepare new batches of micromotors for enhanced efficiency and reproducibility of experimental analysis.

Acknowledgements

The final outcome of this thesis includes a lot of assistance and mentoring from a lot of people and I am extremely grateful to had all of you around during my PhD journey.

First, I would like to thank Professor Martin Pumera for providing me opportunity to be part of this amazing group. Thank you for continuous support, encouragement and patient guidance over the past 3 and a half years. My deepest appreciation also goes to my current advisor, Associate Professor Richard D. Webster for accepting me in his group, for his advice and suggestion during development of research projects. I would also like to thank Associate Professor Zdenek Sofer for collaboration, practical advice and preparation of materials.

In particular, I would like to offer my special thankful to Dr. Zafir for the willingness to give his time generously whenever needed, for his motivation, selfless support and understanding. Now, I would like to express appreciation to Dr. James Moo which introduced me to the micromotors world and taught me the first steps of electrochemistry and the value of hard work. I would not forget to remember Dr. Carmen for sharing her micromotors experience and more over for her support that meant a lot to me. I wish to thank Maja for her contribution to my projects but also for friendship. Special thanks goes to Nasuha and Farhanah for being great batch mates and company through all university courses and seminars.

I would also like to extend my thanks to the current team members: Hui Ling, Wei Li Dr. Adriano, Dr. Alessandra, Raymond, Arnold, Si Man, Xian Liang, Shafique as well as to senior members: Dr. Shu Min, Dr. Xinyi, Dr. Bahareh, Prof Hong, Dr. Rui, Dr. Yong, Dr. Lei, Dr. Rou Jin, Dr. Naziah, Yi Heng and Xing Juan. Thank you, guys, for all support, hours spent together in the lab, fun and laugh we have had in the last 3 and a half years.

Besides my lab mates, I would like to thanks Dzeneta and Nevena for being always there so I didn't feel lonely and far from home. I thank my best Serbian friends Selena, Dusica, Deba, Nevena, Visnja and my godmother Mina for being always there for me and providing me a lot of supports for any of my decision. I could not have imagined having a better friends in my life.

Last but not the least, I would like to thank my mum and brother for their love and support from the first day I was born. I owe a huge debt of gratitude to my uncle Boban and Peđa and my aunt Sneza for being here at the crucial moment of my career. Special thanks goes to Zoran for his support and many helpful tips he has given me in the past.

OVAJ DOKTORAT POSVEĆUJEM MOJOJ MAMI **DANI**, NJOJ KOJA JE JEDINA ZNALA MOJE BOLI KAO I BABI **MACI** KOJA JE MI JE PRUŽILA MNOGO LJUBAVI.

VECNO CU BITI ZAHVALNA!

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Chapter 1

Introduction

1.1 Objectives and goals of Thesis

1.2 Literature review

1.3 References

1.1 Objectives and goals of Thesis

The thesis consists of four main parts combined into one whole micro- nanomotors story. Two motion mechanisms: self-propulsion and light propulsion mechanisms are studied to improve faster motion properties and develop more efficient micro- nano size devices for water remediation and biomedical applications.

Objective 1: Fabrication and motion study of micro- nanomotors constructed from novel two-dimensional layered materials.

Developments in nanotechnology have enabled the use of new nanomaterials for preparation of micromotors, with graphene rapidly becoming a favoured material of choice. Recently, scientists have shown great interest in another two-dimensional material, transition metal chalcogenides (TMDs) due to their excellent chemical, optical and mechanical properties.

Herein we studied black phosphorus and 2D germanene materials for the preparation of micro- and nanomotors. Black phosphorus (BP) is suitable to be used as a composite material for the fabrication of nanodevices that can be extended for the detection of toxic chemicals. We explored the possibility of incorporating black phosphorus in catalytic bubble-ejecting autonomous micromotors and showed that the power output and velocities were greatly enhanced when compared to bare platinum micromotors.

Furthermore, we fabricated micromotors labelled with two different fluorescent derivatives of 2D germanene (4-fluorophenylgermanene (2D-Ph-Ge) and methylgermanene (2D-Me-Ge)). While there are reports of fluorescent tagging of micromotors, we wanted to highlight the use of two different fluorescent derivatives concurrently, in order to distinguish both 2D-Me-Ge and 2D-Ph-Ge labelled micromotors based on their fluorescence emission upon UV light irradiation.

Objective 2: Exploring the usage of widely accessible natural materials for micro- and nanomotors preparation and studying their potential applications.

The creation of micro- nanomotors which would be suitable for future applications in a simple and low-cost way is the main aim of this research objective. We found inexpensive, naturally available nanoparticles that are suitable for the preparation of nanomotors that also have excellent adsorbent properties. The small dimensions of nanoclays materials allow for a small particle size with large specific surface area as well as high number of active places for ion exchange and adsorption of contaminants. To utilize these nanomachines in industrial-scale operations, one would need very inexpensive fabrication route. Here we show a previously unexplored route, using existing natural nanotubes based on the Halloysite nanoclays, to fabricate functional nanomotors. These clay nanotubes can be mined in ton quantities and can be used as the base for the nanomachines. It is well known that the surface groups of these clays bind strongly with heavy metals, therefore, one can utilize them for environmental remediation as well as sensitive and selective mobile nanosensors for mercury detection.

Furthermore, we utilized naturally available Cloisite nanoclays to fabricate autonomous self-propelled nanomotors and demonstrated their excellent performances in pesticide removal due to their excellent adsorption capability. The fabricated Cloisite nanomotors exhibited low cytotoxicity even at high concentrations when incubated with human lung carcinoma epithelial cells which make them safe for human handling.

Pollen is one of the most stable structures in the world, capable of surviving harsh treatments and millions of years. Pollen grains are available in large variety of shapes and sizes. Herein, we demonstrated the use of a wide variety of naturally abundant, non-toxic pollen grains for the efficient fabrication of platinum-pollen (Pt-pollen) micromotors capable of propelling with fast velocities for environmental and biomedical applications. Dandelion, Pine,

Lotus, Sunflower, Poppy, Camellia, Lycopodium, Cattail and Galla pollen grains were selected and investigated. Their mobilities rendered by their fabrication as micromotors enhanced their performance in the removal of heavy metals in aqueous medium, and as drug carriers of doxorubicin.

Objective 3: Fabrication and motion study of catalytic light-powered micromotors

Catalytic light-powered micromotors have become a major focus in current autonomous self-propelled micromotors research. The attractiveness of such machines stems from the fact that these motors are liquid “fuel-free”, with their motion modulated by irradiation. It is often assumed that as catalytic micro/nanoparticles, they can function eternally. In order to study how different metal types affect the velocities of metal/TiO₂ micromachines in the presence of UV irradiation in pure water, we prepared Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ Janus micromotors. These metals have different chemical potentials and behave with different catalytic properties with respect to the water splitting reaction, with both properties expected to affect the photoelectrochemically induced reaction and propulsion rates, which indeed they were found to do. Tafel analysis was performed for various metal/TiO₂ micromotors and it was concluded that the effective velocity is a result of synergistic (couple) effects of chemical potential and catalysis.

Objective 4: Influence of ageing on the performance of micromotors

We studied the corrosion of self-propelled and catalytic light-powered micromotors. Storage of platinum tubular catalytic micromotors in water causes their corrosion which results in the lower mobility and performance. This has important implications for construction and storage of these autonomous micromotors. The reusability and reproducibility in performance of fabricated Pt/TiO₂ micromotors stored over a duration of weeks was studied. It was found that despite retaining the Janus structure, the surface morphology of the fabricated micromotors

greatly changed with a decrease in composition of titanium dioxide and platinum and a corresponding increase in Ti and Pt in the storage solution. The motion of the fabricated micromotors decreased drastically under ultraviolet (UV) irradiation (in fuel-free mode) after just 5 days of storage and these machines eventually stopped moving after 15 days. Similar observations were obtained when the platinum tubular micromotors were stored over an experimental duration of 28 days. An additional characterisation study was done to explain the chemical reactions that occur on the micromotors surfaces.

1.2 Literature review

1.2.1 Natural biomotors

Macromachines are very common in society. Cars, airplanes, motor bikes and boats have changed our daily transportation, but we can also find very efficient devices in the nature. The presence of biomotors in the environment has changed and facilitated human life and autonomous locomotion is not unique in living organisms. If we look into the biological world, we can encounter many complex biomotors that have existed since before human life began. Most bacteria are triggered by helical force generators called flagella using ATP as an energy source.^[1] On the other hand, T4 phage viral engines exhibit twice the power of conventional car engines when scaled up.^[2] This class of biomotors include bacterial FtsK motor proteins, which play the role of copying genetic material in prokaryotic cells and chromosome segregation.^[3] FoF₁-ATP synthase is also a well-known membrane motor protein which is called an ATP generator as it plays a role of converting proton gradients into ATP by two rotary motors Fo and F₁.^[4]

A typical example of a motor protein is kinesin. Kinesins move along microtubule (MT) filaments, and can be powered by the hydrolysis of adenosine triphosphate.^[5] Similarly, myosin and dynein are linear motors which carry out many different cellular functions such as transport in neurons. Dynein is responsible for the bending of cilia and flagella and myosin for

muscle contraction and exocytosis.^[6,7] All of these three type of motors consists of head, neck and tail, which allow them to move directionally and convert chemical energy into mechanical motion.

DNA helicases are genome enzymes which have an important role in cell biology. They are responsible for breaking the bond between double helix bases pairing of DNA and can move along DNA by chemical energy that comes from DNA polymerization.^[8]

These natural examples of biomotors provide inspiration for further work to synthesize artificial motors in nano and microscales. However, this task of locomotion relocation from bio to artificial object opens new challenge for scientists. Biomimic systems can be replicated by using working principles and concepts applying to artificial objects.

1.2.2 History of nano/micromotors

In 1959, the well-known Nobel Prize winner and physicist, Richard Feynman, held a speech at the annual American Physical meeting where he predicted the possibility of fabricating machines in nanoscale which could do chemical conversion by mechanical manipulation. His speech called “There’s Plenty of Room at the Bottom” represents the starting point in nanotechnology and Mr Feynman is named the father of nanotechnology.

In 1966, Richard Fleischer directed the science-fiction movie, *Fantastic Voyage*. The story depicts navigation of a submarine crew into the scientist body to repair a disease in his brain. The film demonstrated the possibility of using technology and scientific knowledge to transform matter from micro to miniaturize scale, albeit in a fantasy setting.

The first work on self-propelled catalytic nano and micromotors was reported 15 years ago by Crespi *et al.* at the Pennsylvania State University.^[9] They fabricated Pt-Au rod nanomotors which moved autonomously in water solution after the addition of hydrogen peroxide as a fuel. Interestingly, their lengths and average velocities were comparable to

multiflagellar bacteria (4 μm and 8 $\mu\text{m/s}$, respectively).^[10] Subsequently, micro and nanomotors became the subject of numerous studies and progressed very quickly. Autonomous self-powered miniaturized machines are the next step for nanotechnology. Inspired by biomotors, nowadays it has become normal to expect that nano and micromotors perform a variety of complex tasks. They consist of different types of nanoscale compounds, and can be propelled using various sources of energy inputs.^[11] Studies by scientists and researchers have illustrated the various functions of these devices.^[12–14]

Micromotors can be powered by two different methodologies. The first method includes the self-propelled motion of micromotors (*in situ* method) while the second method propels the micromotors using an external energy source (*ex situ* method) such as electricity, magnetic force,^[15] ultraviolet (UV) irradiation^[16] and infrared light.^[17] There are several mechanisms which explain the movement of micromotors (Figure 1): self-electrophoresis,^[18,19] bubble propulsion,^[20–22] Marangoni effect^[23] and self-diffusiophoresis.^[24–26]

1.2.3 Self-propelled motion of micromotors

The **Marangoni effect** is observed when there is a difference in surface tension in the environment (Figure 1A).^[27] Surface tension is a phenomenon whereby liquids try to form the smallest possible surface area. The direction of motion of molecules in the liquid depends on the surface tension. Molecules tend to move from an area with a lower surface tension to an area with a higher surface tension. This is due to the greater attraction between molecules of the liquid with a larger gradient of surface tension than molecules with a smaller gradient of surface tension.^[28] Camphor boats are frequently presented in the literature as an appropriate example of the Marangoni effect.^[29–31] Self-motion of camphor boats is a result of scraping at two interfaces (air-water) and the driving force depends on the difference in the surface tension. Surface tension gradients could be formed as a result of asymmetric dissolution of the camphor boats and push the boats towards areas with lower concentration. The Marangoni effect could

be induced at a liquid-gas phase by a temperature gradient and in that case it is termed as thermocapillarity. Different groups used various heaters to generate Marangoni flow and thus activate micromotors.^[32,33] Very fast velocities (mm/s) can be generated by establishing very small temperature gradients in the liquid. Hendarto and Gianchandani reported a good relation between motors velocity, heater intensity and liquid viscosity.^[33] They achieved higher velocity by higher heater intensity in the liquid with lower viscosity.

Figure 1. A) Marangoni effect propelled micromotors. The surface tension of the γ_2 is lower than the surface tension of γ_1 so it pulls micromotors from region γ_2 to region γ_1 . Reprinted from ref. 27. B) Self-electrophoretic propelled Pt/Au micromotor. Protons move from anode to cathode, which results in the movement of micromotors in the opposite direction. Reprinted from ref. 39. C) Self-diffusiophoresis propelled Ir/SiO₂ micromotor with hydrazine addition. Fluids flow from low product concentration (around SiO₂ surface) to high flow concentration (around Ir surface). Ir/SiO₂ micromotor moves in opposite direction. Reprinted from ref. 48. D) Bubble propelled Cu/Ag tubular micromotors with hydrogen peroxide addition. Bubbles are generated and released at the end of the silver part of the micromotors. The red arrow shows the movement direction of the micromotor. Reprinted from ref. 54.

It is not rare that the Marangoni effect occurs after some of the other mechanisms (bubble propulsion or self diffusiophoresis). Zhao *et al.* reported diffusiophoretic motion of Janus catalytic micromotors in a high concentrated hydrogen peroxide medium.^[34] However, after some time the micromotors start to nucleate as a result of the micromotors accumulating in a small region. After single nucleation, the micromotors start collective motion while they grow and form ringlike structures. Driving force pushes those towards the ring center until the bubble collapses. This is spontaneously a non-bubble propelled process which could be explained as evaporation-induced Marangoni flow. Additionally, biodegradable polycaprolactone spindle micromotors can exhibit motion for a long period of time due to surface tension gradient generated by the release of anionic surfactants under different pH values.^[35]

Taking account that micromotors that are propelled by the Marangoni effect can move for a long period of time and do not require any additional energy sources, this mechanism is very suitable and attractive for environmental remediation and sensing. Our group previously reported the ability of Marangoni effect powered inorganic polysulfone capsules to sense the chemicals and remove pollutants from the environment.^[36] Another example include Marangoni flow propulsion as a consequence of a depolymerisation reaction of poly(2-ethyl cyanoacrylate).^[37]

The self-electrophoretic method occurs in a system where micromotors are fabricated from two permeable conductive materials. In this case, two electrochemical half reactions appear on both asymmetric ends of the micromotor. One end of the micromotor acts as an anode while the other end behaves like a cathode. There is an electric field generated that causes the movement of electrons through the micromotor. This is followed by the migration of protons which propels the micromotors in the opposite direction.^[38]

Bimetallic catalytic Janus nano/microparticles and nano/microtubes are typically induced by a self-electrophoresis mechanism. Malouk *et al.* developed Pt/Au nanorods propelled by decomposition of hydrogen peroxide at the two metals where the cathode reaction occurred at the Au end and the anode reaction at the Pt end of the nanorod (Figure 1B).^[39] Protons move from the Pt (anode) to the Au (cathode) and propel nanorods in the opposite direction. Same material combinations and mechanisms have been used to sense chemicals in water. Wang *et al.* reported the changes in the Pt-Au nanomotors velocity in the presence of 11 different cations but only selective acceleration in the presence of Ag^+ ions.^[40]

The Au segment of the nanomotor can be replaced with other cathodes to increase mobility of nanomotors. It was shown that a combination of Ag-Au alloys drastically increased velocity of self-electrophoretic nanomotors.^[41] The same group reported that carbon nanotubes incorporated into the Pt end of the nanowire greatly increased the nanomotors velocity and furthermore, higher velocities have been achieved upon addition of hydrazine to the H_2O_2 fuel.^[42] On the other hand, Ozin and co-workers enhanced nanomotors velocity by increasing the surface roughening of the catalytic segment which results in increasing the surface area and therefore boosting the rate of disproportionation of H_2O_2 on the surface of nanomotors.^[43] Sen and Liu fabricated Cu-Pt nanowires which can move in dilute aqueous solutions (Br_2 or I_2) by the electrophoresis mechanism.^[44] Apart from nanorods, catalytic Janus nano and micromotors could be propelled by the self-electrophoretic mechanism. Zhao *et al.* synthesised asymmetric spherical Pt/Au micromotors by a dynamic shadowing growth technique. Interestingly, they compared the self-electrophoresis motion of prepared micromotors with other mechanisms for micromotors with similar shape and size and concluded that the self-electrophoresis mechanism activates more micromotors.^[45]

The self-diffusiophoresis method. Motion of the micromotors driven by self-diffusiophoresis occurs as a consequence of a concentration gradient which is formed around

the micromotor. The Sanchez group reported that Pt-catalysed Janus particles produce an oxygen gradient around the micromotor when they are in contact with hydrogen peroxide. The final outcome results in fluid flow around the particle and propulsion of Janus particles occurs in the opposite direction.^[26] Qiao and co-workers studied Janus catalytic micromotors driven by forces induced by self-diffusiophoresis.^[46] They used different mathematical simulations and numerical implementation to explain their locomotion. Howse *et al.* demonstrated diffusiophoretic propulsion of fabricated Janus micromotors.^[47] They used a deposition method to coat a Pt layer on one side of polystyrene beads as Pt is a great catalyst for H₂O₂ decomposition into O₂ and H₂O and thus generate local concentration gradients.

Hydrazine is also commonly used as a monopropellant for micromotors motion. Wang *et al.* replaced hydrogen peroxide with hydrazine in order to achieve better mobility of Janus micromotors (Figure 1C).^[48] They used bare silica particles as a micromotor template and iridium as a catalyst to decompose hydrazine to form the following products N₂, H₂, NH₃. A concentration gradient is formed around the metal which causes water to flow from the SiO₂ side to the Ir side and propel the Janus micromotors towards the SiO₂ side. Interestingly, this system achieved very high average velocities even in low concentration hydrazine solutions (0.001 %).

Furthermore, Sanchez and co-workers designed biocompatible hybrid Janus micromotors that exhibit motion in urea environments.^[49] They used inhibitors to control the micromotors velocity by reactivating the urease enzyme. Self-propelled diffusiophoresis propulsion lasts around 10 min and motion could be prolonged by magnetic guidance, which requires coating of the magnetic component such as Fe on one half of the Janus particle.

Bubble-propulsion mechanism. The three mechanisms mentioned above do not generate bubbles, and the motion of micromotors are relatively slow. Nowadays, bubble-

propulsion mechanism is the most popular type of micromotor motion because of very high power outputs.^[50,51] Platinum is usually used for fabrication of such micromotors as it can catalyse the decomposition of fuels used with the generation of bubbles. The greatest advantage of this mechanism is its relatively high velocity of motion.

Unlike the self-electrophoretic mechanism, the bubble-propulsion mechanism is not very sensitive to high ionic strength environments and the mobility of micromotors does not depend on the environment conductivity.^[52] Schmidt *et al.* used the enzyme catalase as a catalyst for tubular micromotors preparation.^[53] Ti-Au micromotors have been prepared with a rolled up technique with an inner Au layer that was modified with an enzyme which could generate front-side bubbles by decomposition of hydrogen peroxide. Beyond platinum, our group previously designed tubular micromotors by using a cheaper catalyst (silver) for self-propulsion (Figure 1D).^[54] Similarly, Ag and MnO₂ have been used as widely available and low-cost catalysts to power micromotors in the presence of 0.1 % of H₂O₂.^[55]

Apart from H₂O₂, the first reported water-driven micromotors based on bubble propulsion mechanism were designed from Wang's group.^[56] They used stable Al-Ga alloy microparticles asymmetrically coated with a Ti layer on one side to produce hydrogen by water reduction in the presence of aluminium. The same group designed Pd-Al Janus micromotors powered in alkaline and acid environments but also in a hydrogen peroxide medium.^[57]

1.2.4 Brownian motion and Reynolds number

It is important to note two factors which affect the movement of the micromotors which are viscosity and Brownian motion.^[58] The differences between the micro and macro world can be represented using the Reynolds number, which correlates the relation between inertia force and viscosity. This phenomenon can be explained by the following equation:

$$Re = \rho u d / \eta \quad (\text{Eq. 4})$$

where Re is Reynolds number, η and ρ are the viscosity and density of fluid, respectively, u is the fluid velocity through the tube, and d is the dimension of the tube.

In a Newtonian fluid with dominant viscous forces and a low value of the Reynolds number ($Re \ll 1$),^[59] a strong pulling force is required to propel a micromotor through the system. This force must be greater than the viscous force. A low Reynolds number in combination with Brownian motion is a big problem in a small-scale system. Brownian motion is the random motion of particles in the fluid when the particle has a less specific weight than the environment in which they are located. Under such conditions, particles collide with molecules present which cause chaotic movement in the fluid. Recently, Jones's group explained a way to distinguish movement of the micromotors from the ordinary Brownian motion (Figure 2).^[60] They fabricated Pt/polystyrene spherical micromotors with hydrogen peroxide as the fuel. The trajectories of the micromotors were tracked (Figure 2A) and average values of the squared displacement were calculated (Figure 2B). The micromotors that showed only Brownian motion had linear squared displacement in time (Figure 2B, 0 % H_2O_2).

Figure 2. A) Motion of Control and Pt micromotors in water and different percentage of H_2O_2 . B) Graph of mean squared displacement of Pt/polystyrene spheres micromotors as a function of time. Reprinted from ref. 47.

1.2.5 Light as external energy source for propulsion of micromotors

It was highlighted before that micro/nanomotors are designed to perform different tasks and they are great candidates for potential applications in biomedicine as drug capturers and for targeting drug delivery. Most of fuel driven micromotors require toxic fuel for their motion which obviously limits their medical applications. As a consequence, many research groups became interested in finding new energy sources to avoid this problem. It became critically important to construct easily driven micromotors which could move directionally with low external energy requirements.

The most commonly used external energy source for powering micro/nanomotors is light.^[61,62] Light as a renewable and powerful energy source is considered as a great micromotor trigger that can be easily controlled.^[63] Sometimes these systems do not require any addition of internal fuel and do not require any physical contact with the micromotors solution.

Motion of light-induced micromotors is based on photocatalysts.^[64,65] The propulsion mechanism of photocatalytic micromotors includes transfer of photoexcited electrons from valence bands to conduction bands. This is possible only if the energy between conduction and valence bands is smaller than the photonic energy from the light sources.^[66] Different chemical reactions then occur on the surface of micromotors. Reactions are possible between electron-hole pairs, generated by photon, and water, hydrogen peroxide or dissolved oxygen. Reaction products could be bubbles and electrolytic/nonelectrolytic gradients. Photocatalytic micromotors could be propelled through the same mechanisms as chemically-propelled micromotors (self-electrophoresis, self-diffusiophoresis and bubble propulsion).

Titanium dioxide is the most frequently used photocatalyst for light-driven micromotor fabrication which can decompose hydrogen peroxide in the presence of light and also can generate bubbles. Nontoxic properties, low-cost and high stability make this material suitable

for the preparation of micromotors. The first reported photocatalytic micro/nanomotors were bare TiO_2 Janus microparticles propelled in water medium by self-diffusiophoresis.^[67] Later, various photocatalysts have been used to obtain more efficient motion properties of photocatalytic micro/nanomotors. The second important requirement for the preparation of micromotors is their ability to be induced in low harmless light intensities and fuel-free conditions or very low fuel concentrations.

Photocatalytic efficiency is different for different materials and depends on the quantum efficiency (QE). The QE can be calculated as the number ratio of product molecules and incident photons times 100. Au- TiO_2 and bare TiO_2 have different QE values irradiated by the same light intensity (4.14 % and 0.04%, respectively).^[68,69] The higher QE of Au- TiO_2 microparticles means a larger local concentration gradient around the microparticles and stronger photocatalytic efficiency (propulsion) compared to bare TiO_2 microparticles.^[70] Similarly, efficient propulsion has been obtained by Pt- TiO_2 micromotors governed by light-induced self-electrophoresis.^[70] These micromotors were remotely controlled by switching “on/off” the UV light.

Apart from photocatalytic micromotors, thermophoretic micromotors have been frequently studied due to their strong propulsion properties.^[71] Photoinduced thermophoretic micromotors are powered by generated local thermal gradients. Janus micromotors have ideal shapes for the creation of thermal gradients on their surface as they can absorb light in different amounts. This leads to fluid circulation around the Janus particles and propels them forward.^[72] Sano and co-workers have studied Janus micromotors propelled by self-thermophoresis.^[73] They half coated SiO_2 particles with an Au layer to obtain two micromotors faces, resulting in motion under laser illumination. In this case, gold plays a special role of absorbing laser energy and as a consequence creates a local temperature gradient. Similarly, near-IR lasers have been used to induce self-thermophoresis of the same micromotors.^[71] Other examples of

micromotors induced by thermophoretic effects include polymeric microcapsules sputtered by a thin external layer of Au.^[74] Polymeric tubular micromotors modified by Au nanoshells exhibited superfast velocities (153 $\mu\text{m/s}$) under NIR irradiation.^[75] However, two major disadvantages of this mechanism are: 1) high light energy is required, and 2) it could damage the cell due to high thermal radiation.^[76]

1.2.6 Fabrication of self-propelled micro/nanomotors

Electrochemical deposition is a well-known and largely developed technique in nanotechnology which includes coating (depositing) of metals or alloys onto a conducting surface from an electrolyte solution.^[77] Electrodeposition and electroless deposition are totally different terms in electrochemistry. Electroless deposition uses chemical reactions for deposition unlike electrochemical deposition that utilizes an external current or voltage.

Various type of templates have been used as bases for electrochemical deposition of micro/nanomotors, which opens the possibility of fabricating micro/nanomotors in different dimensions. Template-assisted electrochemical deposition includes membranes with different pore sizes so that tubes or wires can be synthesized.^[78] Commercial templates contain uniform and cylindrical pores. The length of the desired materials could be carefully controlled by choosing a membrane which has the same pore length as the desired micromotor.^[79] Template-assisted techniques for the preparation of self-propelled micromotors have been successfully used in the last decades owing to great potential applications of micromotors in environmental remediation and biomedicine.^[80]

Typical protocols for template-assisted electrodeposition technique begins with the membrane choice (alumina or track-etched polycarbonate) and pore diameters (0.03 to 10 μm). Subsequently, a thin layer of metal has to be sputtered on one side of the membrane to make electrical contact and the working electrode. Thereafter, the membrane has to be assembled in

an electrochemical cell so that the pore of the membrane faces a plating solution. The electrochemical system usually consists of three electrodes: working electrode (sputtered template attached to the copper tape), auxiliary electrode (platinum) and reference electrode (Ag/AgCl/1 M KCl). The potential has to be applied in the presence of an electrolyte solution containing the desired metal ions. Bottom-up growth of nanotubes/wires happens in the pores of the template. After deposition, the template has to be clean from sputtered metals by polishing with alumina powder. At the end, the membrane should be dissolved and the obtained micromotors can be collected by centrifugation and washed by methylene chloride, ethanol and water.

Paxton and co-workers have used the electrodeposition technique for the synthesis of (Pt-Au) bisegment rod shaped nanomotors.^[81] They sequentially deposited first platinum and then gold to obtain asymmetric nanomotors essential for movement through the self-electrophoretic mechanism. Wang and co-workers demonstrated the ability to drastically increase the velocity of nanomotors by incorporation of carbon nanotubes into the platinum segment using the same technique.^[42] An interesting study investigated the effect of different outer layer and various inner layer of metals (Pt, Au, Ag, and Ni-Pt alloy).^[82] Conductive polymer nanotubes have been used as the outer micromotor layer (polypyrrole, polyaniline and poly (3, 4-ethylenedioxythiophene)).^[82]

Physical vapor deposition is also widely used as a coating technique for the preparation of micro/nanomotors. It produces coatings of very thin metal layers or even alloys (1 – 10 μm thickness). The technique involves vaporization from a solid target, coating metals on the substrate in a high temperature vacuum or gaseous plasma conditions. There are a few types of physical vapor deposition but sputtering and electron beam evaporation are the most frequently used for micro/nanomotors synthesis. The sputtering method involves ejection of atoms which have a wide energy distribution ($< \text{tens of eV}$) from the target to a substrate. The

sputtering process takes place in a vacuum chamber where the substrate is placed. Argon is frequently used as the inert gas to dislodge metals atoms from the target. The kinetic energy of bombarding atoms have to be very high to make the sputtering happen. On the other hand, an electron beam can be used for target vaporization in the case of electron beam evaporation methods. Physical vapor deposition is an ideal technique for the creation asymmetric micro/nanomotors as the target could be deposited only on one side of the substrate.

Physical vapor deposition breaks the symmetry in the micro and nanomotors world and allows rotational motion. Qin and co-workers presented rotational motion of Au-Pt-Au nanomotors, prepared by Au sputtering on one side of the Au-Pt nanorods.^[83] The Wang group used the same method to fabricate trimetallic layers on the Au-Ru rods which were previously electrodeposited. They used sequential vapour deposition for three metal layers coating Cr/SiO₂/Cr, Au and Pt.^[84]

Janus micro/nanomotors are usually prepared by physical vapor deposition as the motion key of Janus micro/nanomotors is an asymmetric structure. Catalytic materials are frequently sputtered on half of the hemisphere. Gao and co-workers utilised physical vapour deposition to coat one side of Al microparticles with a Pd layer.^[85] Interestingly, it was the first reported study of micromotors which are able to be propelled in alkaline media, pH > 11. The sputtering technique has been used to coat Au layers (40 nm thickness) on TiO₂ microparticles which could be efficiently triggered by electrophoretic propulsion.^[86] These systems can self-generate an electric field in pure water and compared to most fabricated micromotors, they do not require additional fuel for self-electrophoresis. Nelson and Pane reported multiwavelength light-responsive (UV, blue, cyan, green, and red light) micromotors prepared by Au coating on black TiO₂ Janus microparticles.^[87] They observed a correlation between the micromotors velocity and the wavelength where the velocity increases with decreasing wavelength.

Platinum is still the metal of choice for the preparation of most Janus micromotors. Our group previously reported light-powered Pt-TiO₂ micromotors for efficiently degrading nitroaromatic explosives (2,4,6-trinitrotoluene (2,4,6-TNT) and 2,4-dinitrotoluene (2,4-DNT)).^[88]

The **Strain engineering** technique allows the nanomembranes to be arranged into a 3D structure. Tubular shaped micromotors could be synthesized by rolling-up the deposited membranes.^[89] Firstly, an inorganic membrane was deposited onto a photoresist layer which was subsequently removed with acetone to leave the inorganic layer in a rolled-up tube form. They used different materials and their combination for micromotor fabrication (diamond-like carbon, Pt, Pd/Fe/Pd, TiO₂, ZnO, Al₂O₃,g)Si_xN_y, Si_xN_y/Ag,). Some parameters could be controlled such as micro/nanotube diameter by adjusting the thickness of nanomembranes and built-in strain. They can be obtained in various ranges of tube diameters from 1 to 30 μm.^[90] The desired strain gradients could be achieved through manipulation of substrate temperature and deposition rate. The rolled-up micromotors usually have a bigger size compared to electrodeposited micro/nanotubes.

Schmidt *et al.* constructed multilayer rolled-up micromotors which consist of Pt, Au, Fe and Ti layers.^[91] Pt inner layer play a catalytic role for H₂O₂ decomposition and bubble generation inside a tubular micromotor while the Fe layer is used for magnetic motion control. Production of micro/nanomotors based on the rolled-up technique usually takes a long time and requires a high cost. These two disadvantages motivated scientists to work on simplifying the technique. Zhao and co-workers used a combination of graphene oxide nanosheets and Ti-Pt bilayers for the preparation of micromotors.^[92] This combination of materials allows the spontaneous formation of tubular micromotors with a Pt inner layer and a graphene oxide outer layer due to weak bonding between them and material strain. Our group also reported spontaneously formed microtubes consisting of banana and apple tissues cells and Pt coated by

physical vapor deposition.^[93] Magdanz *et al.* synthesized temperature responsive Pt-polymeric tubular micromotors.^[94] The prepared micromotors exhibit self-folding/unfolding formation by changing the temperature due to the presence of a thermoresponsive polymeric layer.

The **assembly of materials** approach allows the formation of multilayers of micro and nanomotors by deposition of oppositely charged materials layers. The layer-by-layer technique involves incorporation of various templates and different organic and inorganic molecules or colloids. He *et al.* reported chemical self-propelled multilayer nanomotors prepared by a template-assisted layer-by-layer technique.^[95] Two oppositely charged polysaccharides (chitosan and sodium alginate) were firstly absorbed into the membrane and subsequently, Pt nanoparticles were assembled on the inner wall. These multilayer micromotor structures makes them suitable for drug loading and delivery. The layer-by-layer technique could be used for fabrication of Janus micromotors. He and Li reported the first example of a multilayer hollow Janus capsule by using a template-assisted layer-by-layer-assembled technique together with microcontact printing which could behave as efficient machines but also as smart cargo.^[96]

1.2.7 Application of micro/nanomotors

Environmental remediation. Water is the most important natural resource on Earth which is necessary for the lives of plants, animals and humans. Water covers about three quarters of the Earth's surface, and most of it is saltwater in the seas and oceans. Only about 2.5 % of the total water volume belongs to fresh waters distributed on the surface, underground and in the atmosphere. Literary data indicates that only about one percent of water is available for human consumption, and that about 1.2 billion people have problems with dirty water.^[97]

Population growth and technological development has enabled the use of fresh water, but at the same time release contaminated wastewater into the environment. Self-purification aqueous systems naturally reduce the concentration of the present pollutants in water.

However, due to increasing human activity and unreasonable release of various pollutants into water, natural self-purification processes have become insufficient to maintain satisfactory quality of water.

Water pollution can be explained as any change in the physical, chemical and biological parameters of water quality that have a negative impact on biological systems. Water pollution usually occurs as a result of the accumulation of various liquid, solid and gaseous pollutants from industry, households and agriculture. The most common pollutants found in contaminated waters are heavy metals such as lead (Pb^{2+}), copper (Cu^{2+}), mercury (Hg^{2+}), arsenic (As^{3+} , As^{5+}), chromium (Cr^{3+} , Cr^{6+}) as well as organic compounds, microorganisms (bacteria, parasites,) and other chemical by-products. Heavy metals such as Zn, Cd, Pb, Hg, and As are commonly present in water due to high use in the manufacturing industry, production of batteries, paint, pigments, and pesticides. The maximum permissible concentrations of the above mentioned heavy metals in drinking water are shown in Table 1.^[98]

Table 1. Allowed concentrations of heavy metals in drinking water.

Heavy metals	Standards for Drinking Water (mg L^{-1})		
	EU ¹	WHO ²	US EPA ³
Zinc	0.1 – 5	1	5
Cadmium	0.005	0.003	0.005
Lead	0.05	0.01	0.015
Mercury	0.001	0.001	0.002
Arsenic	0.01	0.01	0.05

EU¹ – *European Commission, drinking water directive;*

WHO² – *World Health Organization;*

US EPA³ – *United States Environmental Protection Agency.*

Heavy metals are elements with a relatively high density approximately more than 5 g cm^{-3} .^[99] The term "heavy metals" includes transition metals, some metalloids, lanthanides and actinides. Heavy metals are classified in the group of the most dangerous inorganic pollutants of the environment due to non-degradability, aspiration to bioaccumulation and manifestation

of a toxic effect, even at low concentration. Some heavy metals such as Cu, Zn, Se and Fe are classified as microelements where they are toxic only at high concentrations.^[99]

Heavy metals come into water in two ways: direct and indirect. Direct contamination occurs when waste containing heavy metals are disposed directly into water bodies such as rivers and the sea. Water bodies are indirectly contaminated from run-off upon disposal of the waste on land. Consequently, the wastewaters seep into groundwater and eventually reach larger water bodies such as seas, rivers and lakes. When heavy metals get into the environment, a series of chain reactions are triggered that change the quality of the water, soil and atmosphere. For example, the presence of these pollutants in water bodies leads to changes in the physical and chemical parameters of water such as the colour, smell, taste, transparency, pH and hardness. Heavy metals can be found in natural aqueous system in the form of free ions, complexes with organic and inorganic ligands and dispersed colloids.

Regardless of the form in which they are present, increases in the concentration of these pollutants in water bodies pose negative impacts to all biological systems. For example, the increase in the concentration of heavy metals in rivers leads to an increase in their concentrations in cereals and plants growing on or near polluted areas.^[100] Upon consumption of these contaminated crops, negative effects of heavy metals are manifested both on animals and humans. When heavy metals come into the human body either through the skin, respiratory and gastrointestinal tract (food and drinking water), heavy metals are susceptible to biochemical transformations that further affect the ability of their retention and elimination from the body. Accumulation of heavy metals in fatty tissue, bones, glands with internal secretion, central nervous system, leads to serious negative consequences and harmful diseases.^[101–103]

In order to solve the existing problem of water pollution with heavy metals, efforts are being made to develop economical and environmentally friendly micromotors by which the concentration of these pollutants can be reduced to a minimum.^[104,105]

Water polluted in any of these ways is considered wastewater and represents a global problem for humanity. The lack of potable water, coupled with the increasing pollution of such water bodies has generated an increasing global demand for clean water. This need has attracted the attention of many scientific institutions and industries with aims to develop efficient and economical wastewater treatment technology to meet the increasing demand. Industrial, agricultural and atmospheric wastewaters are being purified and re-used upon purification.

The selection of purification method depends on the type and concentration of pollutants present in the water. Different methods are used which hold certain advantages and disadvantages. Chemical deposition,^[106] ion exchange,^[107] membrane filtration,^[108,109] electrochemical methods^[110] and adsorption^[111] are the most commonly used methods.^[112]

Adsorption can be described as a phenomenon where the concentration of adsorbate increases or decreases on the surface of adsorbents followed by a decrease in the surface free energy.^[113] The adsorption process is based on the selective transfer of adsorbate particles from the liquid phase to the surface of the insoluble adsorbent.^[114] The adsorption factors include the value of the specific adsorbent surface, the temperature at which the adsorption process takes place, the pH of the solution, the nature of adsorbents and adsorbate, and so on. However, high costs of instruments and materials, low efficiency, poor selectivity and the problem of handling the waste material that occurs in the process of water purification are some of the disadvantages of the above mentioned methodologies. Among the most cost effective methods, adsorption is widely adopted due to its simplicity, flexibility and easy performance, as well as the possibility of using cheap and alternative materials.^[115]

Different materials have been used for the removal of heavy metals in water by adsorption process. The various types of adsorbents used today to remove heavy metal ions from water can be classified as:

- 1) Natural (clay, zeolites, ores, agricultural, industrial bio-waste);^[116,117]

2) Synthetic (active coal, polymers, synthetic zeolites, industrial waste)^[118]

The basic parameters considered in the selection of adsorbents for purifying contaminated water are: selectivity, adsorption capacity, availability, simplicity of synthesis and modification methods, price and regeneration possibility.^[119] For example, activated coal is considered to be a particularly competitive and efficient adsorbent for the removal of heavy metals in trace amounts due to the microporous and mesoporous structure, large specific surface and high adsorption capacity.^[120-122] However, the ratio of the efficiency of the removal of pollutants to cost of obtaining and regenerating the active coal, is extremely unfavourable.^[123]

Therefore, this research will focus on finding alternative materials for fabrication of micromotors which can minimize the cost of the wastewater treatment process. Presently, alternative natural materials such as clays, zeolites and waste biomaterials (coconut shell, rice, walnut, hazelnut) are used prevalently due to high adsorption capacity and local availability.^[124] Natural adsorbents, in addition to being cheap and easily accessible, can also be modified in different ways to improve adsorption properties. Research has shown that the efficiency of removing heavy metals from water using natural unmodified or modified adsorbents is comparable to the efficiency of removal heavy metals using activated coal, while the price is significantly lower than the price of activated coal.^[125]

Recently, clays are gaining significant attention as cheap, alternative adsorbents which are used to reduce concentration of various pollutants in water. Henceforth, this thesis will focus on the use of natural microparticles for preparation of micromotors that have suitable properties of adsorbents.

The physicochemical properties of clay minerals are very complex. They are related to the structure of the clay used as well as the interaction between clay and surrounding matter which depends on many factors such as acid - base properties (pH, ionic strength), pressure

and temperature. Commercial use of clay as adsorbents is determined by their surface properties. The adsorption of ions on clay minerals is determined by the excess of the charges on the clay structure. Small dimensions of nanoclays indicate a small particle size with large specific surface area as well as high number of active places for ion exchange and adsorption of contaminants.

Clay minerals are hydrated aluminium silicates which have a layer structure. The ions or atoms in the structure are arranged in a series of parallel sheets that are interconnected to form layers. The structure of all layered silicates or clay minerals is based on tetrahedral and octahedral plates which can be combined in different ways. It has also been reported that micromotors can detect the presence of water contaminants and remove them.^[126,127] Recently, Orozco et al. modified micromotors with the enzyme catalase. These micromotors were able to detect the presence of contaminants (aminotriazole, Hg^{2+} , Cu^{2+}) in water.^[127] However, these micromotors have numerous limitations due to short lifespan of enzymes and selective detection of water pollutants. As such, work has been carried out to find non-enzymatic materials for preparation of micromotors. James and co-workers reported bubble-propelled Pt micromotors for the detection of Pb^{2+} and Cd^{2+} in the water.^[128] Graphene oxide micromotors have also been prepared for the purpose of adsorption of heavy metals.^[129] Our group prepared graphene oxide in three different ways using Hofmann's, Hummer's and Staudenmaier's methods for the adsorption of heavy metals such as Pb^{2+} and Cd^{2+} . Besides that, Jurado-Sanchez et al. prepared platinum micromotors modified with activated-carbon as nanoadsorbents for the removal of not only heavy metals but also organic materials.^[130]

Bubble propelled micromotors are not the only example of using micromotors for environmental remediation. Orozco et al. demonstrated motion of micromotors using the Marangoni effect^[131] due the presence of the laccase enzyme and SDS surfactant. The micromotors exhibited circular motion and were used for the removal of Pb^{2+} from

contaminated water. Wang and co-workers demonstrated excellent interaction of Au/Ni/PEDOT/Pt micromotors with oil droplets due to hydrophobic layer on the surface of micromotors.^[132] The same group prepared alkanethiol/Mg Janus micromotors with strong surface hydrophobicity to remove oil contaminants from seawater.^[133] Schmidt *et al.* reported excellent organic pollutants degradation by self-propelled Pt-Fe micromotors fabricated by the rolled-up technique.^[134] Organic pollutants degradation happens in the presence of H₂O₂ by the Fenton oxidation reaction. They found that the thickness of the iron layer and pH plays an important role for the efficiency of the reaction. Srivastava *et al.* prepared rolled-up hybrid micromotors composed of Pd and thin-metal films Ti/Fe/Cr for degradation of 4-nitrophenol water pollutants.^[135] Biocatalytic natural plant tissue motors have been employed to improve contamination efficiency in the polluted water.^[136] These millimetre-size motors consist of peroxidase and catalase enzymes responsible for their movement and decontamination, respectively. Self-propelled Cu/Pt conical micromotors designed by our group are capable of selectively responding to the presence of heavy metals in aqueous solutions.^[137] A sharp drop in the activity and velocity of micromotors was observed in the presence of lead ions compared to cadmium ions as Pb²⁺ ions could be strongly adsorbed on the active sites of platinum.

Biomedical applications. Recent advances in nanotechnology in terms of improvement of novel nontoxic materials and methods have led to potential usage of micro and nanomotors in biomedical applications. Significant progress has been achieved by *in vitro* studies of micromotors drug transportation to the targeted sites, but strong efforts are required for *in vivo* proof-of-concept studies.

In vivo capabilities of self-propelled micromotors usually have been demonstrated using a mouse model.^[138] It has been reported that PEDOT/Zn micromotors are able to be self-propelled in the acid environment of the mouse stomach without causing noticeable toxicity. These micromotors could be efficiently bound to the stomach wall and thereafter dissolve in

the gastric environment by releasing their cargoes (gold nanoparticles as they are frequently used as imaging agents). Enteric polymer coated/Mg micromotors were synthesised to safely move through the mouse gastric system, travel to the desired part of gastrointestinal (GI) tract and deliver a payload to the particular site of GI.^[139] The enteric coating of micromotors has a very specific function to delay bubble propulsion by dissolving the polymer layers before the micromotors reach the desired GI part. The same group demonstrated Mg-based micromotors as an attractive replacement for proton pump inhibitors.^[140] Acid has been used as the fuel and micromotors are coated with a pH responsive polymer so that they enable gastric-acid neutralization by rapidly depleting the protons. Additionally, these micromotors found application in drug transportation and release.

Kagan and co-workers have presented a new approach towards *in vitro* nanomotors cargo transportation.^[141] Magnetic iron oxide/poly(D,L-lactic-co-glycolic acid) nanoparticles were applied as cargo for transportation and release using catalytic nanomotors. Electrostatic interaction between the nanomotors and cargos is responsible for efficient cargo transportation. Similarly, electropolymerisation reactions have been used to attach nanomotors to the oppositely charged cargo (polystyrene-amidine).^[142] Sen and co-workers have demonstrated Pt/Au nanomotors cargo drop-off strategy using UV light as a trigger.^[143] They showed that the silver part of nanorods plays an important role for cargo delivery as it would be dissolved very fast under the UV irradiation (wavelength: 365 nm) and in the presence of hydrogen peroxide and Cl⁻ ions. Bilayer polymer-MnO₂ tubular micromotors have reached a super-fast velocity of 318 μm/s in the presence of 10% of H₂O₂, but also demonstrated good movement in bovine serum albumin and bovine serum mediums.^[144] After modification with poly(sodium 4-styrenesulfonate) and poly(diallyldimethylammonium chloride) they became positively charged and easily bind to the negatively charged camptothecin anticancer drug. Another important method for drug loading includes micro and nanomotors modification and

functionalisation with materials which have good interacting properties with targeting drugs. Micromotors could be attached to the incorporated materials by a self-assembled monolayer technique (SAM) which is widely used in the micromotors world. This technique usually uses gold as a material for functionalization due to strong interactions with the thiol group. Wang's group have used horseradish peroxidase for functionalization of Pt/Ni/Au–Ag nanomotors to catalyse the decomposition of hydrogen peroxide and aniline local polymerization.^[145] Additionally, the molecular imprinting concept have been employed as a capture and isolation layer for a fluorescent-tagged protein drug. Self-propelled poly(3,4-ethylenedioxythiophene)/Pt–Ni micromotors have been tailored using molecular imprinting concepts with resulting cavity binding areas for sites for Av-FITC (fluorescein isothiocyanate - labelled avidin).^[146]

On the other hand, Guan *et al.* have introduced a new approach for micromotor drug delivery by controlling the temperature.^[147] Thermoresponsive poly(*N*-isopropylacrylamide) was attached on the surface of Mg-Pt Janus micromotors in blood plasma and simulated body fluids. The motion mechanism included the reaction between magnesium and water accelerated by pit corrosion by Cl⁻ ions and the buffering effects of the two mediums. The fluorescein isocyanate (FITC) drug was loaded onto the nanomotors and while the temperature increased from 20 °C to 37 °C, the FITC was released due to a squeezing mechanism and increasing of the kinetic energy of the whole system.

Biomedical applications of micro and nanomotors could be achieved by using biocompatible materials for their preparation. Our group performed *in vitro* studies on Pt-Au tubular nanomotors to investigate the toxicity profile of the motors.^[148] It has been proven that prepared nanomotors do not have any influence on the human lung epithelial cells viability for different tested concentrations, which supports their potential real-world biomedical application.

Solovev *et al.* have worked to remedy another big problem of tissue penetration for micro and nanomotors biomedical applications. They have demonstrated that asymmetrically InGaAs/GaAs/Pt rolled-up nanomotors are able to drill and be incorporated into biomaterials.^[149] He and co-workers fabricated nano-sized mesoporous silica - chromium-platinum Janus motors which are able to enter cells and release the doxorubicin hydrochloride anticancer drug.^[149]

Schmidt and co-workers introduced new micromotors platforms for potential medical applications in cancer treatment and general gynaecological healthcare.^[150] It was a first time to use sperm for micromotor drug delivery to the tumor cells. Doxorubicin hydrochloride have been loaded into sperm cells successfully and incubated with HeLa spheroids (from cervical cancer cells) to check tumor cell-dying efficiency. They showed very high tumor cell killing efficiency (87 %) compared to bare doxorubicin hydrochloride (55%). Nelson and co-workers have developed artificial bacterial flagella micromotors modified by liposomes which are able to swim in liquids similar to natural *Escherichia coli*.^[151] Fabricated helical micromotors showed excellent efficiency for calcein delivery to C₂C₁₂ mouse myoblasts cells performed *in vitro*. He *et al.* synthesized neutrophil hybrid micromotors by drug-loaded mesoporous silica nanoparticles and bacteria membranes (*Escherichia coli*).^[152] They made this system for targeted drug transport by neutrophils chemotaxis capability and motion capability along the chemoattractant gradients generated by bacteria.

1.3 Summary

The first chapter consists of two main parts: the objectives of thesis and a literature review. This thesis has four main objectives that aim to study autonomous micro- nanomotors. The first objective is to fabricate and study the motion of micro- nanomotors constructed from novel two-dimensional layered materials. The second objective is to explore the usage of

widely accessible natural materials for the preparation of micro- and nanomotors and to study their potential applications. The third objective is to fabricate catalytic light-powered micromotors and to study their motion performance. The fourth objective is to study the influence of ageing on the performance of the micromotors.

The second part of the first chapter describes natural examples of biomotors, which provides inspiration for future work to study artificial motors in nano and micro scales. Thereafter, two different methodologies for micro-nanomotors propulsion have been explained: self-propelled motion or the propulsion of the micromotors using an external energy source (electricity, magnetic force, ultraviolet (UV) irradiation and infrared light). Also, four different mechanisms which explain the movement of micro- nanomotors (self-electrophoresis, bubble propulsion, Marangoni effect and self-diffusiophoresis) have been described with examples. Few techniques for fabrication of self-propelled micromotors (electrochemical deposition, physical vapor deposition and strain engineering) have also been explained. Utilising micro- nanomotors for environmental remediation and biomedical applications have been described in the last part of first chapter.

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Chapter 2

2D materials for fabrication of micromotors

2.1 Introduction

2.2 Black phosphorus enhanced bubble-propelled autonomous catalytic micromotors

*2.3 Functional 2D germanene fluorescent coating of micromotors for micromachines
multiplexing*

2.4 Conclusion

2.5 References

2.1 Introduction

Micro and nanomachines are a new generation of nanotechnology,^{1–11} allowing nano and micro-objects to propel themselves, to perform autonomous motion and on demand action, such as drug delivery^{12–16} or environmental remediation.^{17–20} The properties and functionality of the micro and nanomachines can be enhanced by incorporation of nanomaterials, such as carbon nanotubes,^{21–23} graphene,^{24–26} C₆₀,²⁷ or transition metal dichalcogenides.^{28,29} These nanomaterials can enhance velocity of the micromachines due to faster electron transfer or enhanced catalytic effect; it can also add functionality in terms of carboxy groups which allows i.e. heavy metals remediation.²⁴ Our group previously reported the fabrication of graphene micromotors with very high surface area which allows for the minimal amount of catalysts to be loaded.³⁰ This characteristic is important as previously used catalysts occupied most of the surface of the micromotor which allows for less possibility for surface functionalization.³¹ The graphene material is loaded with iridium at the minimum optimum amount. The used method of fabrication is simple while the presence of iridium allows the generation of bubbles with very high velocities of micromotors.

Transition metal dichalcogenides (TMDs) recently received a lot of attention from the science community due to their excellent chemical, optical and mechanical properties.^{32,33} These materials have an MX₂ formula, where M represents transition metal element (V, Mo, W) while X represents chalcogen elements (Te, S, Se). A single layer sheet of TMD is usually found as an alternating stacked structure X-M-X.³⁴ Molybdenum disulphide (MoS₂) is a commonly studied TMD material. Each layer of MoS₂ includes one atom of molybdenum which is coordinated with six atoms of sulphur in octahedral or trigonal arrangement. Molybdenum is bonded to sulphur by a covalent bond while the layers are bonded by weak van der Waals forces of attraction.³⁵ Molybdenum disulphide has great potential in biomedicine, environmental remediation and cargo delivery due to its high surface area.³⁶ Molybdenum

disulphide has been successfully incorporated into biosensors to detect different biomolecules, and can be used in clinical trials and biochemistry.³⁷ Recently, MoS₂ micromotors were fabricated which can move without fuel addition.³⁸ The micromotors were propelled by Maragoni effect due to surface tension gradient generated in aqueous solution by naphthalene added into the micromotor. Other two-dimensional materials can be explored from the promising results obtained with the used of graphene and MoS₂ which will be further elaborated in the proceeding sections.

Black phosphorus (BP) is the most thermodynamically stable allotrope of phosphorus. Due to ease of preparation, two other allotropes, red phosphorus (RP) and white phosphorus (WP) are often experimented on in the past century.³⁹ However, due to anisotropic structures, black phosphorus has unique optical properties,^{40,41} electronic conductivity,⁴² mechanical strength,^{43,44} and other thermodynamic characteristics.⁴⁵ Previous studies have shown many applications of BP nanoparticles such as: diodes,⁴⁶ lithium-ion batteries,⁴⁷ transistors,⁴⁸ and photodetectors.⁴⁹ BP nanoparticle has also found uses in biomedical applications such as photothermal cancer therapy and photoacoustic imaging.⁵⁰

Black phosphorus (BP) has generated great interest lately due to its low toxicity,⁵¹ and interesting semiconducting and mechanical properties. BP is a multi-layer material with elemental phosphorus which makes it similar to graphite.⁵² It represents immense opportunities for sensing of chemicals in both physiological and environmental samples. Due to all of these applications and excellent properties, we studied BP-Pt micromotors, their fabrication, motion studies and comparison studies with bare Pt micromotors.

On the other hand, one major challenge faced is the real time tracking of individual micro/nanomachines within the swarms of the machines which have pertinent importance. The use of fluorescence has been viewed as a viable option for rapid, *in situ* visual observation of

micromotors.⁵³ Researchers have focused their attention on addressing this limitation with increased exploration performed in developing novel fluorescent labels and improving fluorescence detection techniques. Recently, magnetically guided micromotors have been fabricated with the incorporation of fluorescent agents for *in vivo* analysis and imaging.^{54,55} Nelson *et al.* successfully tracked and monitored the motion of a swarm of artificial bacterial flagella functionalized by a NIR fluorophore.⁵⁵ Wang *et al.* fabricated tuneable ‘on-off’ fluoresceinamine functionalised micromotors for rapid and improved detection of sarin and soman simulants caused by enhanced fluid flow in the system.⁵⁶ They also reported the detection of toxins using fluorescent-labelled micromotors within a few minutes.⁵⁷ In the field of biomedicine, Mg-based fluorescent labelled micromotor pills have been successfully used for targeted delivery of drugs to specific locations within the mouse by an *in vivo* technique.⁵⁸ Conversely, TiO₂/Au/Mg micromotors were used for the destruction of *Bacillus Anthracis* spores and fluorescent imaging was performed to monitor the performance using syto-9 (green) and propidium iodide dyes (red) as labels.⁵⁹ However, these studies focused on single labelling of micromachines that perform similar functions. It would be difficult to monitor multiple micromachines with differing functions if present in the same mixture. As such, we extended and explored the concept of labelling micromachines with fluorophores of different emission wavelengths, allowing us to tag and locate micromachines *in situ* simultaneously according to their functions. A novel and stable material, 2D germanene,⁶⁰ was used as the fluorescent label. One of the main advantages of such a system using 2D germanene, is the high surface area provided by the 2D structure which decreases the minimum amount necessary to cover the micromachines.⁶¹ Also, the low solubility of germanene and its derivative in aqueous solution allows for long term detection of fluorescent-coated micromotors in different media for biological or environmental applications.⁶² Finally, functionalised 2D germanene displays high

chemical stability to 250°C which is significantly higher than that of GeH (75°C). This is a critical issue with regards to “real-world” applications.⁶²

Different emission wavelengths were achieved by functionalising 2D germanene with two different organic ligands. We studied bubble-powered catalytic micromotors consisting of an inner platinum (Pt) layer and external graphene oxide layer modified with functionalised 2D germanene, methylgermanene (2D-Me-Ge) or 4-fluorophenylgermanane (2D-Ph-Ge), which contributes to the fluorescence capability. Notably, under UV irradiation, two different micromotors with different functionalization can be distinguished by the emitted colours. We also demonstrated that loading of these micromotors with a chemotherapeutic drug (oxaliplatin) retained the fluorescent properties and could be distinguished from their unloaded counterparts (Scheme 1).

Scheme 1. Tubular micromotors with outer layers functionalised with 2D germanene derivatives (A) 2D-Ph-Ge and (B) 2D-Me-Ge respectively. The micromotors were indistinguishable under visible light irradiation (top, grey colour). They exhibited distinct colours depending on the 2D germanene derivatives immobilised when irradiated under UV light (bottom). The micromotors can be loaded with drugs and remain traceable under UV light irradiation.

2.2 Black phosphorus enhanced bubble-propelled autonomous catalytic micromotors

In this study we explored the possibility of incorporating black phosphorus onto platinum catalytic bubble propelled micromotors. Furthermore, we compared their power output and motion performance with bare platinum micromotors.

2.2.1 Experimental section

Materials: Red phosphorus was obtained from Sigma-Aldrich, Czech Republic. The cyclopore polycarbonate membranes with pores diameter of 5 μm (Whatman, USA), hydrogen peroxide (35% Alfa Aesar) and sodium dodecyl sulphate (Sigma-Aldrich) were used in the experiment. The platinum plating solution (Technic, Inc., USA), Pt electrodes and Ag/AgCl/1.0 M KCl (CH Instruments, USA) were used for electrodeposition. Deionized water (18.2 M Ω cm) from a Millipore Milli-Q purification system was used in this study.

Apparatus: SEM/EDX images were obtained via a JEOL JSM 7600F (JEOL, Japan) coupled with Oxford EDX. Nikon Eclipse optical microscope and Nikon NIS-Elements software were used during the experiment for images and video sequences, respectively. Sputtering was carried out with a JEOL JFC-1600 Auto Fine Coater. The computer was connected to Autolab PGSTAT 101 electrochemical analyser (Eco Chemie, Utrecht, The Netherlands) and used for electrochemical deposition. The micromotors electrodeposition experiments were carried out at 25°C. The three-electrode system was implemented for deposition. A platinum electrode and Ag/AgCl/1M KCl electrode were utilized as an auxiliary electrode and reference electrode, respectively. The centrifugation was executed with a Beckman Coulter Allegra 64R centrifuge and the ultrasonication process was performed with a Beckman Coulter Allegra 64R.

Preparation of BP-Pt concentric bimetallic microtubes: For the synthesis of black phosphorus, 10 g of red phosphorus has been wrapped in graphite foil. Then, the sample was subjected to a high pressure of 6 GPa and a temperature of 600 °C for 30 min using high

pressure/high temperature uniaxial pressing apparatus of 1" size. Thereafter, the sample is cooled at a rate of 100 °C/min and the graphite foil is removed.

The BP-Pt microtubes were synthesized via a modified electrochemical deposition process on a cyclopore polycarbonate template. Microtubes were prepared using membranes with 5 μm pores. Silver sputtered polycarbonate template attached to the copper tape was used as a working electrode. Platinum auxiliary electrode and Ag/AgCl/1 M KCl reference electrode were used for electrodeposition. The template was assembled into a customized deposition cell. BP suspension was prepared via ultrasonication of the BP sample of 1 mg ml⁻¹ for 8 h. BP outer layer was deposited into the 5 μm pores membranes using BP suspension with a concentration of 1.0 mg ml⁻¹ by a filtration step. Galvanostatic electrodeposition of BP layer was carried out at - 20 mA for 1200s. For the control experiments, the platinum layer was electrodeposited at the same conditions, without the BP infused membrane. After deposition, the template was polished with 5 μm alumina powder to remove the sputtered silver layer. The membrane was dissolved in methylene chloride. The electrochemically deposited microtubes were collected by centrifugation at 6,000 rpm for 3 min and washed three times via methylene chloride, ethanol, and water each. The final microtubes were retained in water at 25°C.

Preparation of tubular platinum micromotors: Cyclopore polycarbonate membrane with 5 μm pores diameter was used as a template during the electrochemical deposition process. Sputtering process was carried out with a silver target. Electrochemical deposition experiment was carried out using a three-electrode system with Ag/AgCl as reference electrode and platinum as counter electrode. The working electrode was made by connecting copper strips to the sputtered membrane. The membrane was placed into a customized electrochemical deposition cell. Platinum was galvanostatically electrodeposited at -20 mA for a duration of 40 min. Subsequently, the membrane was washed with deionized water eight times (8 ml per wash). The washed membrane was next polished with alumina powder (5 μm) to remove the

previously sputtered silver. The polished membrane was dissolved in methylene chloride. The tubular micromotors were collected by centrifugation at 8,000 rpm for 3 min and cleaned three times with methylene chloride. The cleaning process is continued with ethanol and water, three times each using the centrifuge (3 min) after each washing step. The tubular micromotors were stored in deionized water at 25 °C.

Operation of micromotors: The experiments for analyzing the motion of micromotors were performed in a water solution containing different concentrations of hydrogen peroxide in 0.25 wt % of SDS. Running solution mixture of SDS (0.25 wt %), microtubes, H₂O₂, and water were performed on a clean glass slide. Images and videos were recorded, for each of the data points in figures, 30 measurements were taken into account.

2.2.2 Results and discussion

We have prepared black phosphorus (BP) nanoparticle doped Pt micromotors by incorporation of black phosphorus nanoparticles into Pt plating solution and depositing to the cyclopore polycarbonate membrane template. Scheme 2 illustrates all processes that were used when creating micromotors.

This was the first time that the black phosphorus outer layer and the platinum inner layer were deposited on a template of polycarbonate. Micromotors are tested using scanning electron microscopy (SEM). SEM images of Figure 1 present two types of fabricated microtubes electrodeposited at -20 mA for duration of 1200 s where Figure 1A represents the black phosphorus modified platinum micromotor (BP-Pt), and Figure 1B represent bare platinum micromotor. The images were taken at the same magnification of 10 000 X to present dissimilarity of BP-Pt and bare Pt microtubes. As can be seen from Figure 1A, BP-Pt micromotors have tubular shape and multi-layered structure (platinum as the inner layer and black phosphorus as the outer layer). It is noted that the presence of the black phosphorus in

the micropores does not disturb the Pt microtube electrochemical formation. Also, upon comparing both images, the micromotors retains their conical shapes despite introducing BP.

Scheme 2. Fabrication process for the BP-Pt micromotors

Figure 1. Scanning electron microscope (SEM) images of two types of prepared microtubes. Micromotors electrodeposited at -20 mA for the 1200s in 5 μm. (A) Platinum + black phosphorus (outside) micromotor and (B) Platinum micromotors. Images were taken at the same magnification of 10 000 X.

The size of the BP-Pt micromotors and the Pt micromotors was obtained from SEM data (Table 1). The BP-Pt microtube is 9.8 μm long with the average at opposite ends around 3.3 μm and 4.2 μm . Fabricated Pt micromotors have a larger size. The length of the Pt microtube is 14.7 μm with diameters 3.5 μm and 4.7 μm . These two types of micromotors are fabricated using the same methods and the same electrodeposition times. They have uniform shapes, diameters sizes and different lengths. Using the mentioned data enables the conclusion that Pt micromotors have a longer size than BP-Pt micromotors. For this experiment, one hundred different microtubes were measured for the both types of micromotors.

Table 1. Parameters of the BP-Pt nanomotors (A), and Pt nanomotors (B).

<div style="display: flex; align-items: center; justify-content: space-between;"> <div style="margin-right: 20px;">A</div> <div> </div> </div>		
BP-Pt	Length (μm)	STD (μm)
d_1 (μm)	3.3	0.7
d_2 (μm)	4.2	0.7
r (μm)	9.8	1.7

B		
Pt	Length (μm)	STD (μm)
d_1 (μm)	3.5	0.7
d_2 (μm)	4.7	1.1
r (μm)	14.7	2.0

Energy dispersive X-ray spectroscopic (EDX) technique has been used to clearly indicate the presence of P and Pt layers on the prepared BP-Pt micromotor and Pt micromotor (Figure 2). Analysing the EDX data, the presence of phosphorus on the BP-Pt micromotor is

7.45 %, while the Pt micromotor does not contain phosphorus. The presence of platinum on the BP-Pt micromotor is 89.75 %, and for the Pt micromotor is 97.53 %. Control experiments to prove the presence of black phosphorus on the BP-Pt micromotors was carried out. The ICP-OES technique confirmed the presence of black phosphorus on the BP-Pt micromotor.

Figure 2. Energy-dispersive spectroscopy (EDS) elemental mappings of (A) Platinum + black phosphorus micromotor and (B) Platinum micromotors.

Consequently, we turned our attention to comparing the performances of BP-Pt and bare Pt micromotors at different fuel (hydrogen peroxide) concentrations. BP-Pt and bare Pt micromotors were found to move with different speeds in different H_2O_2 solutions. In Figure 3, it shows the dependence of BP-Pt micromotors on the 1%, 3% and 5% hydrogen peroxide concentration in 0.25 % SDS. This experiment is measured with optical microscopy.

Figure 3. Influence of BP/Pt and Pt micromotors velocities on the H₂O₂ concentration in the 0.25 wt % sodium dodecyl sulphate (SDS). Data were collected from 30 independent measurements to obtain the average velocity. Conditions in all experiments: 0.25 wt % SDS and temperature 23 °C. Error bars are standard deviations.

The average velocity of BP-Pt micromotors increases from 52.83 $\mu\text{m s}^{-1}$ in 1% of H₂O₂ to 171.5 $\mu\text{m s}^{-1}$ in 5 % of H₂O₂. We found that BP-Pt microtubes show higher velocities than bare Pt microtubes at all concentrations of H₂O₂ studied (Figure 3). At 5% H₂O₂ concentration, Pt micromotors were found to have an average velocity of approximately 68 $\mu\text{m s}^{-1}$, and they were moving very slow ($\sim 33 \mu\text{m s}^{-1}$) at 3 % H₂O₂ concentration. However, the velocity of BP functionalised micromotors with 5% of H₂O₂ is about three times higher than that of bare Pt micromotors. The velocity corresponds to ~ 20 bodylength s^{-1} . This difference between BP-Pt and Pt micromotors is likely due to different surface characteristics of the former, in a similar fashion as to the CNT/Pt enhanced micromotors.²¹ Wang *et al.* showed that Pt combined with carbon nanotubes drastically improved the velocity of the nanomotors compared to Au-Pt nanomotors due to enhanced electrochemical reactivity of the carbon nanotubes. BP-Pt

electrode has probably lower mixed potential compare to bare Pt electrode which lead to easier H_2O_2 oxidation and faster reaction rate.

2.3 Functional 2D Germanene Fluorescent Coating of Micromotors for Micromachines Multiplexing

Micromachines are at the forefront of materials research as they are self-propelled, smart autonomous systems capable of acting as an intelligent matter. One of the obstacles the field faces is tracking individual micromachines carrying molecular cargo from the rest of the micromachines. We have developed highly stable fluorescent markers based on chemically modified 2D germanene compounds. Two different 2D germanene derivatives, 4-fluorophenylgermanane (2D-Ph-Ge) and methylgermanene (2D-Me-Ge), exhibited different fluorescence under UV light irradiation (wavelength of 365 nm) which allows one particular micromotor to be easily distinguished in a mixture of micromotors. This offers a paradigm shift towards a new approach of multiplex detection of self-propelled micromachines. The utility is demonstrated on a drug delivery system, where micromachines carrying drugs are labelled with 2D-Ph-Ge with blue emission while bare micromachines are labelled by 2D-Me-Ge with red emission. This approach of functional fluorescent labelling would pave the way to multiple simultaneous functionalised micromachines identification in complex environments.

2.3.1 Experimental section

Synthesis of 4-fluorophenylgermanane (2D-Ph-Ge): CaGe_2 (200 mg, 1.08 mmol) was added into flame dried flask filled with an argon inert atmosphere and dry acetonitrile (20 mL) was added. Iodine monochloride (357 mg, 2.2 mmol) was added drop-wise during 10 min. The mixture was stirred overnight at room temperature. The solid was separated by centrifuge, washed with dry acetonitrile (2×5 mL) and dry THF (2×5 mL). Dry THF (10 mL) was added and the mixture was cooled in an ice bath under an argon atmosphere. 1 M THF solution of 4-

fluorophenyl magnesium bromide (5 mL, 5 mmol) was added drop-wise and the mixture was allowed to stir at room temperature for 72 h. Water (2 mL) in THF (10 mL) was added and the solid was separated by filtration through a nylon filter (0.45 μm pore size). The solid was washed with 0.1 M aqueous hydrochloric acid, water, and acetone several times and finally dried in vacuum. In the end, 2.5 mg of previously described MGe material was diluted in 8 mL of deionized water and sonicated for 1 h at room temperature

Synthesis of methylgermanane (2D-Me-Ge)⁶²: CaGe_2 (200 mg, 1.08 mmol) was placed in a sintered glass funnel. The funnel was placed into a beaker with a magnetic stirrer and methyl iodide (5 mL, 80.3 mmol) was added to the funnel. The beaker was filled with water, flushed with argon and sealed with parafilm. The mixture was stirred for 1 week at room temperature. The solid was collected by filtration, washed with water, acetone, and ethanol several times and dried in vacuum. In the end, 2.5 mg of previously described MGe material was diluted in 8 mL of deionized water and sonicated for 1 h at room temperature.

Synthesis of nano-graphene oxide (n-GO): Graphite nanofibers were dispersed in a mixture of concentrated sulfuric (90 mL) and nitric acid (30 mL) and subsequently cooled in an ice bath. Next, the mixture was oxidized by potassium chlorate (40 g) for 1 h. The resulting mixture was left to stir for two days. The reaction mixture was decanted in 3 L of deionized water and re-dispersed in the same volume of 5 % aqueous hydrochloric acid. Thereafter, the solution was centrifuged and the re-dispersion process in distilled water was repeated. The procedure was repeated all the way to establish a negative reaction on sulphate and chloride ions. Finally, the graphene oxide was left to dry in a vacuum oven at 50 $^{\circ}\text{C}$ for two days.

Micromotors preparation procedure: GO-Pt micromotors were synthesized via electrochemical deposition techniques on a cyclopore polycarbonate membranes template with 5 μm pores diameter (Whatman, catalog number: 7060-2513). Nano graphene oxide solution

was prepared by mixing nano graphene oxide (20 mg) with deionized water (20 mL). After few minutes stirring, lithium perchlorate (425 mg) purchased from Penta was added inside the solution and sonicated for 1 h. Silver was evaporated (thickness: 70 nm) on the top of the cyclopore polycarbonate membrane and used as working electrode, previously attached to the copper tape. A three-electrode system was used with platinum (Pt) as the counter electrode and an Ag/AgCl reference electrode (CH Instruments, USA). The Autolab PGSTAT204 (Metrohm Autolab, The Netherlands) electrochemical analyser connected to the NOVA 2.1.2 software was used. The outer layer was electrodeposited with n-GO suspension by chronoamperometry (applied potential: -1.5 V for 800 s). After that, n-GO deposition solution was removed, and the cell was rinsed 3 times with water. Subsequently, commercial platinum solution (Technic Asia-Pacific Pte Ltd) was galvanostatically electrodeposited at -10 mA for 1200 s. The template was polished with 5 μm alumina powder, rinsed and dried. Then, the membrane was placed in an Eppendorf tube and dissolved in dichloromethane. In the end, fabricated micromotors were collected and washed with dichloromethane, ethanol, and water. Micromotors were stored in water at ambient temperature. Fluorescent loading of micromotors was made by mixing of 2 mL of 2D-Me-Ge or 2D-Ph-Ge with 0.5 mL of micromotors solution by centrifugation (Beckman Coulter Allegra 64R, USA) at 6000 rpm for 30 min. Labelled micromotors solution was cleaned from unbounded fluorescent agents by washing in water three times.

Micromotors drug loading: One tablet of phosphate buffered saline (PBS, VWR) was dissolved in deionized water (100 mL, pH 7.4). Thereafter, 2D-Ph-Ge labelled micromotors solution (250 μL), previously prepared PBS solution (10 mL) and oxaliplatin (108 μL , 5 mg mL^{-1} , Sigma Aldrich) were homogeneously mixed and shaken for 10 min to achieve adsorption equilibrium. Cyclic voltammetry measurements were used to confirm drug adsorption onto the micromotors. CV experiments were performed at scan rate 20 mV s^{-1} and potential window from -1.5 to 1.5 V.

Operation of micromotors: Motion studies of GO-Pt micromotors were performed in pure water by adding a specific concentration of hydrogen peroxide (Penta) and 1 % of SDS (Sigma Aldrich) as a surfactant. All experiments were performed on a glass slide freshly cleaned and dried before use. Videos and images were obtained by an optical microscope (Olympus IX73) coupled with NIST software. Morphological analysis was performed using scanning electron microscope (SEM) (JEOL 7600F, Japan) and elemental mappings using energy dispersive spectroscopy (EDS) (X-Max, Oxford Instruments). Fluorescence was generated with fluorescent filter (excitation wavelength: 360-370 nm) coupled to the optical microscope, for red and blue light excitation.

2.3.2 Results and discussion

The micromotors were prepared by an electrochemical deposition technique, with the outer layer comprising of nano reduced graphene oxide of high porosity, and the inner layer consisting of platinum (Scheme 3).^{63,64} Briefly, a cathodic potential was first applied which resulted in the transformation of graphene oxide to reduced graphene oxide and their deposition on the walls of the membrane.^{65,66} Subsequently, platinum was deposited galvanostatically at -10 mA for 1200 s, which formed the inner layer of micromotors. The cyclopore polycarbonate membrane was then dissolved and the micromotors were released and washed. The fabricated micromotors were incubated with different fluorescent agents (derivatives of 2D germanene) to eventually obtain fluorescent labelled micromotors. The both 2D germanene derivatives were characterised by Raman spectrometry and showed peak at 300 cm^{-1} , a typical for E_2 Ge-Ge mode (Figure 4).

Figure 4. Details of the Raman spectra for 2D-Ph-Ge and 2D-Me-Ge.

Physical adsorption of the fluorescent 2D germanene derivatives was evident by the different colours of the decorated micromotors under UV irradiation; bright red for methylgermanene (2D-Me-Ge) and bright blue for 4-fluorophenylgermanane (2D-Ph-Ge) modified micromotors (fluorescence curves in Figure 5). Finally, 2D-Ph-Ge functionalised micromotors were loaded with oxaliplatin drug to demonstrate proof of principle (Scheme 3).

Scheme 3. Schematic representation of micromotors preparation, immobilization of functionalised 2D germanene derivatives (2D-Me-Ge, 2D-Ph-Ge), and loading with oxaliplatin drug.

Heterostructure preparations based on 2D materials are of great interest for modern electronic and optoelectronic applications of novel 2D germanene materials.^[67–70] Characterisation was first performed to determine the elemental composition and morphology of the fabricated micromotors. Scanning electron microscopy (SEM) images showed tubular structures of micromotors (Figures 6Aa, 6Ba, 6Ca) with a length of $\sim 12\ \mu\text{m}$. Energy dispersive spectroscopy (EDS) elemental analysis was performed to analyse the elemental composition and confirm the decoration of fluorescent 2D germanene derivatives onto the surface of the fabricated micromotors.

Figure 5. Fluorescence of 2D-Me-Ge and 2D-Ph-Ge under UV irradiation.

Figure 6. (a-b) Scanning electron microscope (SEM) images, (c-e) energy-dispersive spectroscopy (EDS) elemental mappings of (A) bare GO-Pt, (B) 2D-Me-Ge modified and (C) 2D-Ph-Ge modified micromotors respectively.

Figure 6A shows the results for the GO-Pt micromotor while Figures 6B and 6C represent images of micromotors immobilised with 2D-Me-Ge and 2D-Ph-Ge respectively.

The carbon mappings obtained from all three types of micromotors would suggest the successful incorporation of graphene oxide during the fabrication process. The surface 2D germanene layer is observed in Figures 5Bd and 5Cd, which suggests the successful incorporation of 2D germanene derivatives onto the outer surface of the respective micromotors.

Having ascertained the tubular structure and successful binding of 2D germanene derivatives (2D-Me-Ge and 2D-Ph-Ge) onto the outer surface of the micromotors, we next set forth to analyse the performance and motion of the micromotors. The fabricated micromotors adopt a simple propulsion mechanism. Pt catalyst deposited in the inner surface of the micromotors would catalyse the rapid decomposition of hydrogen peroxide fuel to produce oxygen gas. The bubbles produced get ejected which caused the motion of micromotors in the opposite direction, reaching very high velocities. The average velocities of GO-Pt micromotors were measured in the presence of different concentrations of hydrogen peroxide (0.5, 1, and 3 %) in 1 % SDS (Figure 7). The motion of bubble-propelled micromotors were recorded with the aid of optical microscope and analysed by NIST software. The average velocities saw an increasing trend with a corresponding increase in H_2O_2 concentration used. As predicted, the fastest motion (approximately $588 \mu\text{m s}^{-1}$) was observed in the highest H_2O_2 concentration (3 %). It was interesting to note that the fabricated micromotors displayed enviable performance (average velocity = $245 \mu\text{m s}^{-1}$) even at very low H_2O_2 concentration (0.5 %). The fabricated micromotors were able to propel at high velocities even in the presence of low levels of fuel (0.5 % H_2O_2) which outperformed that of other reported tubular micromachines.⁷¹

Subsequently, we studied the fluorescent capabilities of synthesised 2D germanene coated onto the micromachines. Figures 8A and 8B show the different colours emitted upon irradiation of only 2D-Me-Ge and 2D-Ph-Ge compounds under UV light. 2D-Me-Ge solution emitted a bright red colour and 2D-Ph-Ge emitted a bright blue colour upon irradiation. GO-Pt

micromotors were then decorated with the 2D germanene fluorescence agents and distinguished by the emitted colours. The micromotors solutions were cleaned to remove suspended 2D germanene derivatives molecules that were not attached onto the surface of the micromotors.

Figure 7. Average velocities of GO-Pt micromotors at different concentrations of H₂O₂.

The fluorescence images of labelled micromotors were captured under an optical microscope coupled with a UV filter to observe the successful attachment of fluorescent 2D germanene derivatives onto the outer surface of micromotors. Figure 8Ca shows the optical image obtained for a 2D-Me-Ge micromotor, which appears tubular in shape. Upon activation of UV light on the same micromotor, bright red fluorescence was emitted (Figure 8Cb). Similarly, a tubular 2D-Ph-Ge micromotor was observed under the optical microscope (Figure 8Cc). The micromotor emitted bright blue light under UV light as observed in Figure 8Cd. The two sets of micromotors were then mixed and captured under an optical microscope with the activation of UV light. As observed in Figure 8D, both red and blue lights were emitted

distinctly, which makes it possible to simultaneously distinguish the two differently labelled micromotors in the mixed solution; 2D-Me-Ge labelled micromotor appeared bright red, while 2D-Ph-Ge labelled micromotor appeared bright blue. The fluorescent 2D germanene derivatives (2D-Me-Ge and 2D-Ph-Ge) used possess excellent optical properties as semiconductors with strong photoluminescence emission (red and blue respectively).⁶²

Figure 8. Photoluminescence of (A) 2D-Me-Ge and (B) 2D-Ph-Ge molecules in water upon illumination at 365 nm light. (Ca, Cc) Optical microscopy, and (Cb, Cd) fluorescence images of 2D-Me-Ge and 2D-Ph-Ge modified micromotors respectively. (D) Fluorescence microscopy image of 2D-Me-Ge (red) and 2D-Ph-Ge (blue) micromotors mixture.

Cyclic voltammetry was carried out to confirm the loading of the drug (oxaliplatin) onto 2D-Ph-Ge labelled micromotors (Figure 9). Oxaliplatin dissolved in PBS solution showed a cathodic peak at ~ -0.6 V. The solution was mixed with 2D-Ph-Ge labelled micromotors and left for 20 min. Subsequently, CV measurements were performed and no apparent voltammetric signals were observed. The cathodic peak at -0.6 V disappeared which indicated the absence of oxaliplatin in the solution. The results obtained suggest possible binding of oxaliplatin onto the 2D-Ph-Ge labelled micromotors by physical adsorption. Notably, micromotors covered with oxaliplatin were also distinguishable from their unloaded counterpart (2D-Me-Ge micromotors) from their fluorescent emission. 2D-Ph-Ge micromotors covered with oxaliplatin emitted a bright blue colour while 2D-Me-Ge micromotors emitted a

bright red colour upon irradiation. The non-fluorescent oxaliplatin drug was successfully loaded onto 2D-Ph-Ge labelled micromotors and the fluorescent properties were retained. As such, the delivery of different non-fluorescent cargos to pre-defined targets can potentially be monitored in a complex mixture of differently labelled micromotors based on their fluorescence emissions.

Figure 9. Cyclic voltammograms of oxaliplatin solution (red line) and mixture of 2D-Ph-Ge labelled micromotors with oxaliplatin (blue line). Conditions: free oxaliplatin in 0.1 M KCl (red line), 0.05 M KCl aqueous solution containing oxaliplatin-loaded 2D-Ph-Ge micromotors (blue line). Scan rate = 20 mV s^{-1} and potential window from -1.5 to 1.5 V.

2.4 Conclusion

In summary, this chapter demonstrated using the 2D materials black phosphorus and germanene for micromotors preparation.

In the first part of research, black phosphorus was used in the preparation of functional micromotors using electrodeposition techniques. We demonstrated that the performance of catalytic bubble propelled micromotors can be increased by the incorporation of black phosphorus during the preparation procedure. A very important fact is that these micromotors can be prepared using a very simple methodology. These self-propelled micromotors promise good applications for the preparation of diodes, transistors and photodetectors.

In the second part, we have demonstrated the possibility of detecting micromotors by fluorescence with different emissions. The fabricated tubular reduced nanographene-platinum micromotors exhibit rapid motion and excellent efficiency even at low concentrations of hydrogen peroxide (0.5 %, velocity $\sim 245 \mu\text{m s}^{-1}$). Two different types of fluorescent 2D germanene derivatives, 2D-Me-Ge (methylgermanene) and 2D-Ph-Ge (4-fluorophenylgermanene), were used for labelling of the micromachines. These self-propelled micromotors not only exhibit fast movement in aqueous solutions but also emit strong fluorescence (bright red for 2D-Me-Ge, bright blue for 2D-Ph-Ge), which qualifies them as potential devices in bio-imaging and biomedical applications. The 2D-Ph-Ge labelled micromotors were capable of loading cargo for delivery, which can be tracked using fluorescence. Based on this approach, the scope of the use of fluorescently labelled micromotors has been expanded. Future research could be directed to targeted cargo delivery methods by loading multiple cargos onto different modified micromotors which can be monitored via fluorescence and studied *in vivo*.

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Chapter 3

Halloysite nanoclays for nanomotors fabrication and environmental application

3.1 Introduction

3.2 Experimental part

***3.3 Natural Halloysite nanoclays nanomotors as bubble propelled nanomachines for heavy
metal remediation***

***3.4 Platinum-Halloysite nanoclays nanomotors as sensitive mobile nanosensors for mercury
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3.5 Conclusion

3.6 References

3.1 Introduction

Micro/nanomotors are a new generation of nanotechnology, allowing objects to propel by themselves and perform autonomous motion.^[1–7] They have a great potential in applications such as drug delivery^[8–11] and environmental remediation.^[12–16] Micro/nanomotors can be propelled by several mechanisms such as self-electrophoresis,^[17] Marangoni effect,^[18] self-diffusiophoresis.^[19] These processes do not generate bubbles and their motion are relatively slow as compared to bubble-propulsion mechanism.^[20] Nowadays, bubble-propulsion is the most commonly used type of propulsion mechanism attributed to their high power output and velocity.^[21–22] Platinum is commonly used as the basic material for fabricating bubble propelled micro/nanomotors^[23] due to its strong catalytic ability towards fuel used which promotes spontaneous and vigorous bubble formation upon decomposition of H_2O_2 .

Pollution of water bodies with heavy metals is one of the biggest problems that humanity encounter in recent decades.^[24] Heavy metals are a group of stable pollutants that exhibit harmful effects on biological systems, even at very low concentrations.^[25] Currently, research have been aimed at improving methodologies and materials for fabrication of micro/nanomotors that can adsorb heavy metals in water. Adsorption, among the many methods used today for the purification of heavy metals, is widely adopted due to its high efficiency, economy and availability of a wide variety of natural and synthetic adsorbents.^[26,27] Among the natural adsorbents used to treat contaminated water, nanoclays has attracted great attention due to its layered structure, high capacity of ions exchange, abundance and regeneration capabilities.^[28–31] The physicochemical properties of nanoclays minerals are very complex. They are related to the structure of the clay used as well as the interaction between clay and surrounding matter which depends on many factors such as acid - base properties (pH, ionic strength), pressure and temperature. Commercial use of clay as adsorbents is determined by their surface properties. The adsorption of ions on clay minerals is determined by the excess

of the charges on the clay structure. Halloysite nanoclays has previously been used as good adsorbent material for removal of toxic compounds in aqueous solution with high efficiency.^{32–}

³⁴ For any practical environmental application one must be able to create nanomotors in large quantities – in kilogram and ton scale. Current clean room-based methods and electrochemical based methods create limited number of micro and nanomachines and these methods are unable to produce even gram quantities of micromotors. Recently, there were efforts to use iron zero nanoparticles as dissolution-based motors^[12] and pollen microparticles as templates for kilogram-scale nano/micromotor fabrication.^[35]

Here we have used naturally occurring Halloysite nanoclays nanotubes as a template for nanomotors fabrication. These clays are mined in ton quantities but have not yet been considered as the basis of nanomachines. Halloysite nanoclays structure consists of two sheets: tetrahedral and octahedral.^[36] Tetrahedral (silicate) sheets are made of silicate tetrahedrons that represent the basic building units. Oxygen atoms are located at the sides of the tetrahedron with the silicon atom in the centre. Octahedral sheets consist of individual octahedral where each aluminium atom is coordinated with six hydroxyl groups. The inner surface of the nanoclays comprise of Al_2O_3 while the outer layer consists of SiO_2 .^[37] Since Halloysite nanoclays is a naturally occurring material, this would imply that the cost of fabricating Pt-Halloysite nanoclays nanomotors would be low due to its abundant supply. Large surface area, ionic selectivity, chemical and mechanical stability, abundance and low cost are some of the factors that make Halloysite nanoclays an excellent adsorbent.^[32,38] Additionally, the intrinsic tubular structure of the Halloysite nanoclays makes it ideal for fabrication of tubular nanomotors with shorter preparation steps required where the use of additional templates and membranes are avoided. We set forth to study the possibilities of removing zinc and cadmium ions from aqueous solution using the fabricated Pt-Halloysite nanoclays nanomotors. Furthermore, we

tested Pt-Halloysite nanoclays nanomotors selectivity towards the detection of zinc, mercury, cadmium and lead.

3.2 Experimental part

Materials: Halloysite nanoclays, sodium dodecyl sulphate, cadmium nitrate tetrahydrate ($\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 98%) and zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \times 6\text{H}_2\text{O}$, 98%) were obtained from Sigma-Aldrich, Singapore. Hydrogen peroxide (35%) was purchased from Alfa Aesar, Singapore. Milli-Q purification system (Millipore, MA, United States) with resistivity of 18.2 M Ω was used in all experiments.

Apparatus: Sputtering machine (JEOL JFC-1600 Auto Fine Coater) was used. Scanning electron microscope (JEOL JSM7600F, Japan) was used for morphological analysis. All videos and images of nanomotors were captured by optical microscope (Nikon Eclipse TE 2000-E). Video sequences were obtained by Nikon NIS-Elements software connected to optical microscope. Beckman Coulter Allegra 64R centrifuge and Fisherbrand FB 11203 ultrasonicator were used during experiments. Concentrations of Zn^{2+} and Cd^{2+} were obtained with Inductively Coupled Plasma-Mass Spectrometer (Agilent 7700 Series, Japan).

Fabrication of Pt-Halloysite nanoclays nanomotors: Halloysite nanoclays (40 mg) was dispersed in Milli-Q water (20 ml) without sonication. Thereafter, 10 μm of solution was put on a glass cover slip which was previously cleaned with water. The cover slip was left to dry for 30 min under UV lamp. Subsequently, platinum was sputtered 3 times on the cover slip ($I = 40 \text{ mA}$, $t = 200 \text{ s}$ each time). The cover slip was ultrasonicated for 30 s in water to detach the nanomotors. The fabricated nanomotors were subsequently stored in water at room temperature.

Operation of nanomotors: Nanomotors were propelled in aqueous solution with the addition of hydrogen peroxide and sodium dodecyl sulphate. Velocity of nanomotors was

measured at different hydrogen peroxide concentration and constant SDS concentration (1 %). Average velocity and standard deviation of nanomotors was obtained by measuring velocities of 40 nanomotors at each concentration of hydrogen peroxide, 20 nanomotors for each time interval while 20 nanomotors at each concentration of heavy metals ions.

Removal of Zn^{2+} and Cd^{2+} : Zinc and cadmium ions adsorption studies were carried out in 400 μL of final solution. Final concentration of Halloysite nanoclays, hydrogen peroxide and SDS were 0.5 mg/mL, 4% and 1% respectively. 10 ppm of zinc/cadmium ions was added into the solution. Removal of Zn^{2+} and Cd^{2+} were measured at different remediation time. Three experiments were repeated for the same remediation time and conditions. Thereafter, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis was performed.

3.3 Natural Halloysite nanoclays nanomotors as bubble propelled nanomachines for heavy metal remediation

Self-propelled autonomous artificial micro and nanomachines are on the forefront of the current technology. These micro and nanodevices are not passive as most of the current state-of-art micro and nanotechnology, but they move and actively perform the desired tasks, usually using chemical energy from the environment. Typically these structures are fabricated via clean-room or template based electrodepositions, which produce relatively low numbers of these devices. To utilize these machines in industrial-scale operations, one would need very inexpensive fabrication routes. We showed a previously unexplored route, using existing natural nanotubes based on Halloysite clay, to fabricate functional nanomotors. These clay nanotubes can be mined in ton quantities and can be used as the base for the nanomachines. In addition, it is well known that the surface groups of these clays bind strongly with heavy metals, therefore, one can utilize them for environmental remediation, as is demonstrated here.

Naturally occurring nanoclays were used for the fabrication of Pt-Halloysite nanoclays nanomotors. The use of templates were not required during the preparation procedure which

reduces the preparation time and cost. In summary, aqueous dispersions of Halloysite nanoclays were first prepared in water. The dispersion was subsequently dropped onto a glass cover slip and left to dry. Thereafter, Pt was sputtered on the coverslip and the Pt-coated Halloysite nanoclays was then detached from the coverslip through an ultrasonication process. The prepared nanomotors are powered by a bubble-propulsion mechanism based on decomposition by the Pt catalyst. Scheme 1 provides an illustration on the fabrication procedure of the Pt-Halloysite nanoclays nanomotors described above.

Scheme 1. Schematic representation on the fabrication of platinum-Halloysite nanoclays nanomotors preparation procedure.

Figure 1A and 1B represent SEM/EDX images for Halloysite nanoclays and Pt-Halloysite nanoclays nanomotors respectively. SEM and EDX mapping analysis have been used to characterize the morphology and structure of both the Halloysite nanoclays and Pt-Halloysite nanotubes. From the SEM images obtained, the tubular structure for both type of nanotubes (Halloysite nanoclays and Pt-Halloysite nanoclays nanotube) were clearly visible. It was observed that Pt-Halloysite nanoclays nanotubes had a uniform length, of about 700 nm

while the diameters at both ends of nanotubes were ~ 100 nm and ~ 80 nm, respectively. This method of preparation of uniformly-sized nanotubes is comparable with the rolled-up technology or membrane template electrodeposition methods that have been most frequently used for nanomotors fabrication thus far.^[39] EDX images of Pt-Halloysite nanotubes show the abundant presence of platinum, aluminum, carbon, oxygen and silicon on the nanotube. Obviously, the platinum layer is absent on Halloysite nanoclays. From the EDX images, it shows the successful deposition of platinum onto the fabricated nanomotors which will enable the decomposition of H_2O_2 for propulsion.

The motion of Pt-Halloysite nanoclays nanomotors was first investigated at different concentrations of hydrogen peroxide (Figure 2). The velocities were calculated from the average of 40 different nanomotors motion. From our analysis, there is a positive correlation between the velocities of the fabricated nanomotors with increasing concentration of H_2O_2 . It was observed that the motion of the nanomotors increased with a corresponding increase in hydrogen peroxide concentration. Pt-Halloysite nanoclays nanomotors have an average velocity of $(125 \pm 38) \mu\text{m s}^{-1}$ at 4 % H_2O_2 and the motion of nanomotors reach a velocity of $(238 \pm 64) \mu\text{m s}^{-1}$ in 8% H_2O_2 . As expected, efficiency of the nanomotors depends on the morphology and hydrogen peroxide concentration.

Figure 1. SEM/EDX images of (A) Halloysite nanoclays (B) Pt-Halloysite nanoclays nanotubes with corresponding elements. Scale bars are 500 nm.

Furthermore, very high velocities of some nanomotors have been observed under the optical microscope for each concentration of H_2O_2 . These velocities were not taken into consideration when average velocities were calculated due to the limitations of the processing software used. High speeds generated were attributed to the efficient catalytic properties of platinum towards H_2O_2 which generates O_2 bubbles vigorously at one end of the nanomotors.^[40-41] Besides that, the slight differences in velocities observed at the same

hydrogen peroxide concentration originate from the difference in size and geometry of nanomotors.^[21]

Figure 2. Dependence of velocities of Pt-Halloysite nanoclays nanomotors with variable H₂O₂ concentration. Experimental conditions: 23 °C and 1 wt % SDS.

Herein, we utilized Pt-Halloysite nanoclays nanomotors and examined their potential for removal of heavy metals (Zn²⁺ and Cd²⁺) in aqueous environment. These two ions are the most common pollutants found in wastewater. The consequences of zinc overt toxicity (fatigue, nausea, lethargy and vomiting)^[42] occur with very high zinc intake (see Chapter 1, Table 1), therefore, it is of high importance to study a nano system which would be able to remove high concentrations of zinc in aqueous solution. On the other hand, very small concentrations of Cd²⁺ are considered as toxic (Chapter 1, Table 1). Small amounts of cadmium consumed in the body over a long period of time can cause bones to become weaker but large amounts of Cd²⁺ can damage the liver, kidney and heart^[43] (see Chapter 1 to find more information about heavy metals pollutants). As such, adsorption on the proposed nanomotors would be of great

significance. Adsorption is a widely adopted method for removal of heavy metals due to its simplicity, flexibility and fast performance, as well as the possibility of using cheap and alternative materials. Recently, nanoclays are gaining significant attention as cheap, alternative adsorbents which are used to reduce concentration of various pollutants in water.^[44] Halloysite nanoclays as natural adsorbents have promising performance for the removal of heavy metals from aqueous solution.^[44] Experiments were carried out to demonstrate the potential application of Pt-Halloysite nanoclays nanomotors for the removal of Zn^{2+} and Cd^{2+} (Figure 3).

Figure 3. Influence of exposure time on the velocity of Pt- Halloysite nanoclays nanomotors poisoned with 10 ppm of (A) Cd^{2+} and (B) Zn^{2+} . Error bars are standard deviations. Experimental conditions: 0.25 mg ml^{-1} of Halloysite nanoclays, 4 % H_2O_2 , 1 % SDS, 23°C .

Heavy metal ions were absent at $t = 0$ min and contains only fabricated nanomotors in the working solution. Upon the introduction of heavy metal (10 ppm), the velocity of nanomotors decreased significantly. The average velocities were calculated from 20 independent running experiments. The introduction of Cd^{2+} caused the average velocity of the fabricated nanomotors to decrease from $\sim 286 \mu\text{m}$ to $\sim 119 \mu\text{m}$ (Figure 3A). Similar observations were noted in presence of 10 ppm of Zn^{2+} where the average velocity decreased to $\sim 133 \mu\text{m}$ (Figure 3B). The decrease in the velocity of nanomotors in the presence of Zn^{2+} and Cd^{2+} is a result of the adsorption of the heavy metal ions onto both the Halloysite nanoclays part and platinum section of nanomotor.^[45] More precisely, these ions are attached onto the active sites of the nanoclays which consequently block the platinum on the surface of the Halloysite nanoclays. As such, the platinum activity is reduced, thus decreasing the ability of platinum to catalyze hydrogen peroxide decomposition and bubble generation gets affected. The decreased intensity of bubble productions of the Pt-Halloysite nanoclays nanomotors poisoned by Zn^{2+} and Cd^{2+} is correlated with the decrease in the velocity of the nanomotors.

The efficiency of the fabricated nanomotors towards the removal of heavy metal ions were next investigated and compared to that of unmodified Halloysite nanoclays. The experiments were repeated three times under the same experimental condition and the average result was obtained.

From Figure 4Aa, Pt-Halloysite nanoclays nanomotors were more efficient in removing Zn^{2+} as compared to Halloysite nanoclays 2 hours after addition of the heavy metal ion. Pt-Halloysite nanoclays nanomotors have removal efficiency of $\sim 86 \%$ of Zn^{2+} while Halloysite nanoclays has removal efficiency of about $\sim 83 \%$. The same experiment was repeated in the presence of 10 ppm of Cd^{2+} . Figure 4Ba shows comparable efficiency removal of Cd^{2+} for both Pt-Halloysite nanoclays nanomotors and Halloysite nanoclays ($\sim 86 \%$ and $\sim 85 \%$ retrospectively). The removal efficiency of zinc and cadmium ions in the presence of Pt-

Halloysite nanoclays nanomotors were similar. This could be attributed to both Zn^{2+} and Cd^{2+} having similar electronegativity values. From Pauling electronegativity scale, cadmium has electronegativity of the 1.69 which is very close to that of Zn^{2+} which has electronegativity of 1.6. As such, both Zn^{2+} and Cd^{2+} would have similar probability to get attached to the active sites of Halloysite nanoclays.

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After demonstrating the good removal efficiency of zinc and cadmium ions on Pt-Halloysite nanoclays nanomotors, we next analyzed the effect of contact time on the adsorption of the both ions. Adsorption was discontinued after 5 hours to ensure that equilibrium was established in the system.^[46] The effect of contact time on the adsorption rate of zinc and cadmium ions with Pt-Halloysite nanoclays nanomotors are represented in Figure 4Ab and

4Bb. The amount of Zn^{2+} adsorbed reached maximum in the fifth minute after addition. Similar result was also obtained for the cadmium ions. Within the first 5 min, adsorption happens very quickly where removal of Zn^{2+} and Cd^{2+} is rapidly increasing with increasing contact time. Thereafter, adsorption is found to have reached equilibrium. The rapid increase in the adsorption at the beginning process is high availability of active sites of Halloysite nanoclays where the vacant become occupied with the heavy metal ions. The removal efficiency reaches a plateau when the active sites get fully occupied. The high removal percentages within the short time interval observed can also be attributed to the propulsion effect of the nanomotors which increases the efficiency. As a result of the mobility conferred by the modified nanomotors, the adsorption of the heavy metal ions is not limited by diffusion as compared to unmodified Halloysite nanoclays.

Figure 4. Removal of (Aa) Zn^{2+} (Ba) and Cd^{2+} with Pt-Halloysite nanoclays nanomotors and plain Halloysite clay, 2h after addition of 10 ppm Zn^{2+} (Cd^{2+}). Influence of contact time on the adsorption of (Ab) Zn^{2+} and (Bb) Cd^{2+} with Pt-Halloysite nanoclays nanomotors. Experimental conditions in all experiments: 0.5 mg ml^{-1} of Halloysite nanoclays, 4 % H_2O_2 , 1 % SDS, 23°C .

3.4. Platinum-Halloysite nanoclays nanomotors as sensitive mobile nanosensors for mercury detection

As we previously proved, Pt-Halloysite nanoclays nanomotors moved with high velocities and were preliminarily shown to efficiently remove small heavy metal ions. With this understanding, we wanted to explore and analyse the performance of Pt-Halloysite nanoclays nanomotors with heavy metals of variable sizes. It would be fundamental in providing a comprehensive understanding and outlook on the effectiveness of the fabricated nanomotors towards variable heavy metal ions. In this part, we have demonstrated the different influences exerted by different heavy metal ions at low concentrations by utilising the large adsorption capacity of Halloysite nanoclays with highly mobile nanomotors. We also demonstrated its capacity as sensitive nanosensors towards the detection of Hg^{2+} at low concentrations.

Additional characterisation was performed to affirm the tubular structure and morphology of the fabricated Pt-Halloysite nanoclays with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). From Figure 5, it is evident that the fabricated nanomotors showed prominent tubular structure. From Figure 5B, the integrity of the hollow tube-like characteristic of the fabricated nanomotors remained after modification with platinum.

Having ascertained the integrity of the morphology of the fabricated nanomotors, we set forth to investigate the differences in mobility and detection capabilities of Pt-Halloysite nanomotors in the presence of various heavy metal ions (Cd^{2+} , Pb^{2+} , Zn^{2+} and Hg^{2+}). The inherent negatively charge on the outer layer of Halloysite nanoclays would interact with cations via electrostatic interactions.⁴⁷

Figure 5. (A) Scanning electron microscopy (SEM) and (B) transmission electron microscopy (TEM) images of fabricated Pt-Halloysite nanoclays. Scale bar represents 1 μm .

Scheme 2. Schematic representation of Pt-Halloysite nanomotors as sensitive nanosensors for Hg^{2+} detection, in the presence of Cd^{2+} , Zn^{2+} , Pb^{2+} . The performance of the nanomotors were evaluated based on their movement and motion.

Moreover, different sizes of heavy metal ions would potentially affect the performance of Pt-Halloysite nanomotors because of the differing adsorption capabilities. Scheme 2 shows a schematic of Pt-Halloysite nanomotors immersed inside water poisoned with various heavy metal ions. The motions were compared with Milli-Q water that serves as the control environment.

The velocities of the fabricated nanomotors were investigated with increasing concentrations of the target heavy metal ions (1 to 20 ppm) at the same hydrogen peroxide concentration (4%). From our previous findings, we have demonstrated the efficient adsorption of Cd^{2+} and Zn^{2+} with the fabricated nanomotors.⁴⁸ The fabricated nanomotors displayed enhanced removal efficiency in comparison to bare Halloysite nanoclays in 1 ppm Zn^{2+} (Figure 6).

Figure 6. Time-lapse tracking image of Pt-Halloysite nanomotors in 4 wt% of H_2O_2 and 1 wt% SDS solution.

Rapid motion of the fabricated nanomotors increased mass transfer of ions in the solution that concurrently increased rates of adsorption. Thus, adsorption of heavy metal ions is not limited by the diffusion rates of the ions and Brownian motion of Halloysite nanoclays

in the solution. We proceed to study the motion of the Pt-Halloysite nanomotors at varying concentrations of different heavy metal ions (Figure 7). In the absence of heavy metal ions (0 ppm), Pt-Halloysite nanomotors showed an average velocity of $227 \mu\text{m s}^{-1}$. The velocities of the fabricated nanomotors decreased with increasing concentrations of Cd^{2+} and Zn^{2+} ions. Upon the introduction of Cd^{2+} at 1 ppm (Figure 7A), the average velocity of nanomotors decreased to $186 \mu\text{m s}^{-1}$. A similar observation was made after addition of 1 ppm Zn^{2+} where the average velocity of nanomotors dropped to $187 \mu\text{m s}^{-1}$ (Figure 7B). The activities of the nanomotors were observed to decrease to 82.25% and 82.71% from initial value respectively at the highest concentration of respective ions added (Figure 8A). It was noted that the velocities of the nanomotors remained relatively constant with small depreciations in average velocities with increasing ions concentration. These observations were also similar to that made by Moo and co-workers with the use of enzyme-free Pt microfish.⁴⁹

The small size of the Halloysite nanoclays in the sub-nano scale provides for a larger specific surface area that increases the amount of active sites available.⁴⁷ This allows a greater amount of heavy metal ions to get adsorbed onto the Pt-Halloysite nanomotors. Additionally, the small size of adsorbent particles in the system would confer greater mass transfer in the reaction solution due to smaller diffusion resistance. The size of the adsorbent particles is an important parameter in this process. Previous studies have also determined that the presence of extracellular thiols (glutathione, cysteine and methionine) hampered the movement of nanomotors, thus reducing their velocities.³¹ The ions are able to adsorb onto the Pt surface and cover its active sites thus decreasing the velocity of the nanomotors. In cases of smaller sized ions Cd^{2+} and Zn^{2+} , adsorption occurred at a smaller extent that did not greatly compromise the activity of the sputtered platinum layer of the fabricated nanomotors. Majority of the ions adsorbed onto the Halloysite nanoclays surface while retaining the overall exposed platinum surface for catalysis of H_2O_2 fuel.

Figure 7. Velocities of Pt-Halloysite nanomotors in electrolyte solution poisoned with different concentrations of (A) Cd^{2+} , (B) Zn^{2+} , (C) Pb^{2+} and (D) Hg^{2+} . Experimental conditions: 25 °C, 4 wt% of H_2O_2 , 1 wt% SDS. Error bars for 20 independent velocities for each point.

Figure 8. (A) Activities of Pt-Halloysite nanomotors in poisoned solutions after the introduction of different heavy metal ions. (B) Time-lapse tracking images of Pt-Halloysite nanomotors in the presence of respective heavy metal ions. Experimental conditions: 25 °C, 4 wt% of H_2O_2 , 1 wt% SDS, heavy metal concentration 1 ppm.

Subsequently, we analysed the performance and poisoning effects of larger sized heavy metal ions (Pb^{2+} and Hg^{2+}) towards Pt-Halloysite nanomotors (Figure 7C and 7D). Similar to the observations made with Cd^{2+} and Zn^{2+} , the mobility and performance of nanomotors showed a decreasing trend with a corresponding increase in the concentration of heavy metal ions used. However, in comparison to the observations made in Figure 7A and 7B, there was a significant decrease in the average velocities of the nanomotors with the increase in concentration of the bigger sized heavy metal ions. The average velocities of the fabricated nanomotors were observed to decrease to $162.3 \mu\text{m s}^{-1}$ and $43 \mu\text{m s}^{-1}$ in the presence of 1 ppm Pb^{2+} and Hg^{2+} respectively, with a corresponding decrease in activities to 72% and 19% for Pb^{2+} and Hg^{2+} (Figure 8A).

One distinct observation made upon comparing Figures 7C and 7D is the profound difference in the average velocities of the nanomotors at 1 ppm of respective ions introduced. Higher average velocities of nanomotors were obtained in the presence of 1 ppm of Pb^{2+} in comparison to the same concentration of Hg^{2+} . The addition of 1 ppm Hg^{2+} caused a sharp drop in average velocity ($43 \mu\text{m s}^{-1}$) which corresponds to a decrease in nanomotors activity to 19%. In comparison, the average velocity at 1 ppm Pb^{2+} dropped to $162 \mu\text{m s}^{-1}$ which represents a decrease of 28% in the fabricated nanomotors activity (Figure 8A). It was also observed that the fabricated nanomotors ceased to move with a slight increase in Hg^{2+} concentration to 2 ppm (Figure 7D) unlike nanomotors poisoned at the same concentration of Pb^{2+} whose average velocity was $150 \mu\text{m s}^{-1}$ (Figure 7C). This would suggest that the nanomotors were unable to produce bubbles to propel brought about by the passivation of platinum which hindered its catalysis. The average velocity of nanomotors contaminated with Pb^{2+} depreciated by 63% of the initial value at the highest concentration poisoned. Upon comparing the performances of target ions, it can be inferred that the Pt-Halloysite nanomotors could have higher selectivity and sensitivity towards the adsorption of Hg^{2+} . These results were confirmed by video tracking

analysis (Figure 8B). Since velocity of the nanomotors is proportional to their moving distance, video frames show that Pt-Halloysite nanomotors in Cd^{2+} , Zn^{2+} and Pb^{2+} environments travelled longer distances. However, in the presence of Hg^{2+} , the Pt-Halloysite nanomotors travelled significantly shorter distances. Moreover, the adsorption of the heavy metal ions onto platinum is irreversible⁵⁰ It thus passivates the sputtered platinum on the outer layer of nanomotors which reduces the platinum surface area available for the decomposition of fuel necessary for the motion of the fabricated nanomotors.

Numerous studies have reported that the physical and chemical properties of heavy metal ions have strong influence on the adsorption effect, which can explain the different behaviours of Pt-Halloysite nanomotors in the presence of the respective target heavy metal ions. Electronegativity and atomic radius are very important parameters in predicting which of the heavy metal ions investigated is adsorbed with highest efficiency. Based on the Pauling scale,²⁸ Pb^{2+} and Hg^{2+} are found to have higher electronegativity values than Cd^{2+} and Zn^{2+} . A higher electronegativity value would correlate to more electrons attracted towards the ion that results in greater electron density around the ion. As such, adsorbed Pb^{2+} and Hg^{2+} could have higher tendencies to attach strongly onto the platinum surface of the nanomotors as compared to Cd^{2+} and Zn^{2+} . The atomic radius of Hg^{2+} is also the greatest among all heavy metals ions analysed. This could lead to a greater portion of active sites covered upon the adsorption of Hg^{2+} onto the platinum surface and almost instantly inhibit the motion of the fabricated nanomotors.

Based on these observations, Pt-Halloysite nanomotors are proposed as efficient mobile nanosensors for the sensitive detection of Hg^{2+} . Figure 9 shows the plot of Pt-Halloysite nanomotors velocity in mercury-poisoned solution at different concentrations. The calibration plot was obtained using the inverse of the velocity as a function of Hg^{2+} concentration (inset Figure 9). This reported system showed a wide detection range (from 0.25 to 1000 ppb) with r

= 0.94 and very low limit of detection (LOD) of 3.24 ppb. The LOD obtained for this system for the detection of Hg^{2+} lies within the permissible range of mercury in drinking water (2 to 5.5 ppb).⁵¹

Figure 9. Velocity of Pt-Halloysite nanomotors in solution poisoned with low concentration of Hg^{2+} . (Insert) Calibration plot represents reciprocal velocity of nanomotors vs. Hg^{2+} concentration.

3.5 Conclusion

In this chapter, we have demonstrated the fabrication of tubular Halloysite nanoclays nanomotors with the incorporation of Pt catalyst and their potential application in the removal of Zn^{2+} and Cd^{2+} in aqueous solution. By combining naturally occurring clay nanotubes and platinum as a catalyst, excellent efficiency was achieved for the motion of nanomotors. It was also found that the presence of heavy metals in aqueous solution adsorbs on the nanoclays based micromotors. The equilibrium adsorption of the both heavy metals was achieved within 5 min. Maximum efficiency of 86 % were obtained for removal of both Zn^{2+} and Cd^{2+} . These results show that Pt-Halloysite nanoclays nanomotors have a suitable adsorption ability for the removal of metals ions (Zn^{2+} and Cd^{2+}). Special benefits of using this type of self-propelled nanomotors include low cost and abundant availability of Halloysite nanoclays nanotubes which constitute their main part.

Second, we have studied the different behaviours of Pt-Halloysite nanoclays nanomotors after independent introduction of Cd^{2+} , Zn^{2+} , Pb^{2+} and Hg^{2+} . The presence of Hg^{2+} and Pb^{2+} has shown to have a major influence on the movement of Pt-Halloysite nanomotors as compared to that of Cd^{2+} and Zn^{2+} . Due to the superior adsorption properties of lead and mercury ions onto platinum, this caused the drastic drop in average velocities observed. Conversely, the motion behaviour of the fabricated nanomotors enables for the selective detection of ions in the following order $\text{Hg}^{2+} > \text{Pb}^{2+} > \text{Zn}^{2+} > \text{Cd}^{2+}$. Moreover, the Pt-Halloysite nanomotors were applied as mobile nanosensors for sensitive Hg^{2+} detection with low LOD obtained in the allowable range in drinking water established by the World Health Organization. The results obtained and observations made open up numerous possibilities for the application of naturally occurring materials towards the remediation and detection of environmental pollutants for future practical applications.

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Chapter 4

Cloisite micromotors as self-propelling cleaners for fast and efficient removal improvised organophosphate nerve agents

4.1 Introduction

4.2 Experimental part

4.3 Results and discussion

4.4 Conclusion

4.5 References

Cloisite micromotors as self-propelling cleaners for fast and efficient removal improvised organophosphate nerve agents

Naturally available nanoclays are well known materials with great adsorption capabilities which are available in nature. On the contrary, man-made nanostructures are often available at high-cost via precision manufacturing. Such precision nanomanufacturing is also typically used for the fabrication of self-propelled micromotors and nanomachines. Herein, we utilized naturally available Cloisite nanoclays to fabricate autonomous self-propelled micromotors and demonstrated their excellent performances in pesticide removal due to their excellent adsorption capability. Six different modified Cloisite micromotors were investigated by sputtering their nanoclays with platinum (Pt) for the fabrication of platinum-Cloisite (Pt-C) micromotors. The obtained micromotors displayed fast velocities ($v > 110 \mu\text{m/s}$) with fast and efficient enhanced removal of the pesticide fenitrothion, which is also considered as an improvised nerve agent. The fabricated Pt-C micromotors exhibited low cytotoxicity even at high concentrations when incubated with human lung carcinoma epithelial cells which make them safe for human handling.

4.1 Introduction

The ecological setbacks associated with the use of pesticides are a cause of concern as a huge proportion leech into the environment and water bodies, which affects the quality of potable water. In recent years, researchers have devoted special attention in investigating the possibility of isolating, monitoring and removing pesticides to mitigate the effects of contamination.^[1–4] Fenitrothion, a widely used organophosphate pesticide, presents a serious health and environmental problem which must be controlled to minimize harmful consequences.^[5,6] These potent pesticides are often considered as highly dangerous improvised nerve agents^[7–11] – poor man’s nerve agents.^[12–15] One novel way of safeguarding water bodies

from hazardous compounds involves the use of autonomously moving ‘cleaners’, better known as self-propelling micromotors.^[16–19]

Recent advances in the field of micromotors research have expanded their applications^[20,21] which includes removal of toxic materials in waste water^[22–27] and biomedical application.^[28–33] Cobalt ferrite micromotors were reported for the oxidative removal of tetracycline antibiotic from water via a Fenton-like reaction.^[34] Wang *et al.* illustrated chemically propelled Janus micromotors that can be powered in contaminated seawater for the removal of oil.^[35] High efficiency removal of Rhodamine 6G and methyl-paraoxon were also obtained using multi-layered self-propelled micromotors.^[36] Ren *et al.* developed light-driven Janus micromachines (Au-WO₃@C) which propel by the diffusiophoresis effect for removal of organic dye pollutants in water.^[22] However, these applications heavily rely on synthetically fabricated micromotors which may intrinsically be harmful to the environment. It also raises another potential problem on the removal and disposal of the fabricated micromotors upon the completion of their tasks. As such, there is a growing interest towards exploring and utilising environmentally-friendly alternatives.^[37] One does not have to look far with the abundance of naturally occurring nanomaterials available, namely nanoclays. The use of nanoclays eliminates the need for membranes or templates usually used as the first step of micromotors fabrication thus potentially reducing production cost and simplifying preparation procedures. There is also renewed interest in the use of natural nanoclays as adsorbents which doubles up the usability of such micromotors as self-propelling adsorbents. Recent studies have reported the fabrication of Zeolite-based micromotors for the detoxification of chemical warfare agents (CWA)^[38] as well as Halloysite nanoclays nanomachines which act as nanosensors for heavy metals detection and removal.^[39,40] While the nanoclays reported were used in their natural state, chemical modifications may be explored to tailor their performances and improve their capabilities.

Montmorillonite is a versatile naturally available nanoclays mineral which can be modified with different ammonium hydrogenated tallow modifiers resulting in the formation of clay minerals known as Cloisite nanoclays.^[41,42] The modifiers replace sodium ions present in the montmorillonite structure thus enhancing the hydrophobicity of the nanoclays which alter their performance and properties. Scheme 1 illustrates the detailed composition and structures of the respective Cloisite nanoclays. The respective Cloisite nanoclays are labelled according to the organic modifiers present. Cloisite 15A (C15A) and Cloisite 20A (C20A) consist of the same methyl substituted hydrogenated organic modifier present in differing concentrations 125 and 95 meq/100g clay, respectively. Cloisite 10A (C10A) is the only Cloisite nanoclays with a benzyl-substituted modifier (modifier concentration: 125 meq/100g clay). Cloisite 30B (C30B) differs from the remaining Cloisite nanoclays with the presence of hydroxyl organic modifiers (modifier concentration: 90 meq/100g clay). Cloisite 25A and Cloisite 93A are modified with organic quaternary ammonium cations in the following concentrations: 95 and 90 meq/100g clay respectively.

The presence of different organic modifiers in the Cloisite nanoclays would thus be expected to affect their properties and performance as adsorbents.

Where:

T is Tallow (C18 – 65 %, C16 – 5 %, C14 – 5 %)

HT is Hydrogenated Tallow (C18 – 65 %, C16 – 5 %, C14 – 5 %)

Scheme 1. Respective organic modifiers used for the synthesis of C10A, C15A, C20A, C25A, C30B and C93A. (A) Chemical structure of montmorillonite clay.

In this study, the fabrication of six different Cloisite nanoclays micromotors were demonstrated and compared towards the removal of improvised nerve agent/potent pesticide (fenitrothion) in water (Scheme 2). 6 different modified Cloisite nanoclays were used which are Cloisite 10A (C10A), Cloisite 15A (C15A), Cloisite 20A (C20A), Cloisite 25A (C25A), Cloisite 30B (C30B) and Cloisite 93A (C93A). Platinum was sputtered onto the different Cloisite nanoclays and the micromotors were propelled by the catalysis of hydrogen peroxide which serves as the fuel. The Cloisite micromotors doubled up as adsorbents for organophosphate pesticide/improvised nerve agent (fenitrothion) removal. Herein, we compared and demonstrated an effective strategy for fenitrothion removal using six different platinum-Cloisite (Pt-C) nanoclays micromotors. Moreover, their environmental friendly nature was probed.

Scheme 2. Schematic of various self-propelling Pt-C micromotors decontamination process by adsorbing fenitrothion, a potent organophosphate pesticide and improvised nerve agent. The fabricated micromotors exhibited great adsorption properties for fenitrothion removal.

4.2 Experimental part

Materials: Six different Cloisite nanoclays (Cloisite 10A, Cloisite 15A, Cloisite 20A, Cloisite 25A, Cloisite 30B, and Cloisite 93A) were purchased from BYK Additives & Instruments, Germany. Sodium dodecyl sulphate (SDS) and fenitrothion were obtained from Sigma-Aldrich (Singapore). Hydrogen peroxide (35 wt. %) was purchased from Alfa Aesar (Singapore). Phosphate buffer solution (PBS) of pH = 7.2 and trypsin were obtained from Life Technologies (Singapore). A549 human lung carcinoma epithelial cells were obtained from Bio-REV (Singapore). Water-soluble tetrazolium salt (WST-8) assay reagent was purchased from Dojindo Molecular Technologies. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum and penicillin-streptomycin liquid (1 %) were obtained from Gibco. Purified Milli-Q high quality water with resistivity of 18 M Ω cm (Millipore, MA, USA) was used. All commercially available chemicals were used as received.

Apparatus: Scanning electron microscopy was performed on JEOL scanning electron microscope (JEOL JSM7600F, Japan) with an accelerating voltage of 5.00 kV. Elemental composition and mappings were imaged with JEOL 7600F energy-dispersive X-ray spectroscopy (Japan) at an accelerating voltage of 15 kV. X-ray photoelectron spectroscopy measurements were performed on Phoibos 100 spectrometer (SPECS, Germany) using a monochromatic Mg X-Ray irradiation source. CasaXPS software was used to process XPS data files. All video sequences of micromotors motion were captured with Nikon Eclipse TE 2000-E optical microscope connected to Nikon NIS-Elements software. Other equipment used includes centrifuge (Beckman Coulter Allegra 64R), ultrasonicator (Fisherbrand FB 11203) and sputtering machine (JEOL JFC-1600 Auto Fine Coater). Qualification and quantification of fenitrothion were performed using a Waters Q-ToF Premier high-resolution mass spectrometer equipped with Waters Acquity UPLC, employing a BEH C18 (1.7 μ m, 2.1 $\times$ 100 mm) column. Absorbance readings for toxicity were determined by Thermo Multiskan GO

microplate reader (Thermo Fisher Scientific). Fourier transform infrared (FTIR) spectra were measured by an iS50R FTIR spectrometer (Thermo Scientific, USA) using diamond ATR and DLaTGS detector. Bruker D8 Discoverer powder diffractometer (Bruker, Germany) was used to collect X-ray diffractograms using Cu K α radiation ($\lambda = 0.15418$ nm) over the angular range of 1-80° (2 θ). The generation power was 40 kV and 40 mA. For the powder X-ray measurements, the clay dispersion was drop-cast over off-axes silicon holder to form a homogeneous coverage. The height position was adjusted before each measurement.

Fabrication of platinum-Cloisite micromotors: The respective Cloisite nanoclays (20 mg) were first suspended in ultrapure water (10 mL). 1 mL of suspension was dropped onto a previously washed glass slide and left to dry for 24 h in ambient conditions. Subsequently, a platinum layer was sputtered at 10 mA current and a time of 200 s. The glass slides were then ultrasonicated for 30 s to detach the micromotors. The formed platinum-Cloisite (Pt-C) micromotors were stored in water at 20 °C.

Operation of platinum-Cloisite micromotors: The micromotors were propelled autonomously in a solution containing 5 wt. % hydrogen peroxide in the presence of 1 wt. % sodium dodecyl sulphate (SDS) which serves as a surfactant. Average speeds and standard deviations were calculated from the motion tracking of 25 different micromotors.

Quantification of fenitrothion adsorbed: All prepared calibration and cleaning solutions used ultrapure water generated by a Pure Lab ELGA water-purification system (PURELAB Option-Q, UK) and high purity HPLC grade solvents including methanol (VWR) and formic acid (VWR). A total of six fenitrothion calibration solutions were prepared with concentrations of 1, 2, 5, 10, 20 and 50 $\mu\text{g/mL}$ using a certified fenitrothion standard. All blank solutions introduced no detectable interferences into the analysis with no fenitrothion present ($c < \text{detection limit}$) and all relative standard deviations (RSDs) of 3 replicate measurements

were in the range of 0.38-3.48 %. The MS system was operated in SIM mode to enhance sensitivity and selectivity of the developed method using positive electrospray ionization mode. Experimental conditions in all experiments: 0.25 mg/mL of Cloisite nanoclays, 5 wt. % H₂O₂, 1 wt. % SDS.

Equilibrium study: Adsorption isotherms experiments were performed with the following initial concentrations of fenitrothion: 2.5, 3.75, 5, 10 and 25 ppm. All experiments were carried out in the presence of 5 wt. % H₂O₂ and 1 wt. % SDS. Subsequently, the solution was filtered after 1 h to ensure that adsorption equilibrium was reached. The concentration of fenitrothion retained in the solution after filtration was calculated by the following equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad \text{Eq. 3}$$

where C₀ (mg/L) is initial concentration, C_e (mg/L) is equilibrium concentration of fenitrothion, V (L) is volume of the solution, and m (g) is mass of Cloisite micromotors used in the experiment.

Cell Culture: DMEM, 10 % fetal bovine serum and 1 % penicillin-streptomycin liquid were used for the culturing of human lung carcinoma epithelial cells (A549). The cells were incubated with 5 % CO₂ at 37 °C.

WST-8 Cell Viability Assay: Cells were seeded in 96-wells plates with each well containing 100 µL of cell suspension (~ 5000 cells). The cells were first incubated for 48 h before being introduced to the respective Pt-C micromotors. Different concentrations of micromotors were prepared (5, 20, 50, 100, 150 µg/mL) and diluted in cell culture media (100 µL final volume per well). Cells which were not exposed to Pt-C micromotors were used as negative control. After 24 h incubation of cells and Pt-C micromotors dispersion, 10 µL of stock WST-8 reagent was added to cells for each well. Subsequently, 96-well plates were

incubated for 1 h under the same incubation conditions given previously. Absorbance was measured at 450 nm together with a spectrum reading for each prepared concentration.

4.3 Results and discussion

Six different commercially available Cloisite nanoclays were sputtered with platinum 3 times ($I = 40$ mA, 200 s each time) to fabricate their respective micromotors: platinum-Cloisite 10A (Pt-C10A), platinum-Cloisite 15A (Pt-C15A), platinum-Cloisite 20A (Pt-C20A), platinum-Cloisite 25A (Pt-C25A), platinum-Cloisite 30B (Pt-C30B) and platinum-Cloisite 93A (Pt-C93A). The proposed Pt-C micromotors did not require any additional template-assisted synthesis methods which dramatically simplifies large scale preparation processes. As such, it overcomes the limitations of the present micromotors fabrication techniques which may be costly and require long tedious processes.^[43]

Characterization studies were first performed to provide better insights on the morphologies and chemical compositions of the different fabricated Pt-C micromotors. In order to understand the chemical bonding of all Cloisite nanoclays used, FTIR spectroscopy was performed (Figure 1A). FTIR spectra of all modified Cloisite samples appeared almost identical owing to the montmorillonite backbone of all Cloisite nanoclays used. The hydroxyl vibration band at 3630 cm^{-1} originated from the OH group in Cloisite.^[44] In C30B, a weak OH band can be assigned to the functionalized alkyl. The C-H functionalities from CH, CH₂ and CH₃ groups are visible at 2960 cm^{-1} , 2920 cm^{-1} and 2850 cm^{-1} respectively.^[45] However, the C-H vibration band appeared very weak and visible only on C25A and C10A. Corresponding overtones are visible around 1460 cm^{-1} . The dominant vibration band at 1000 cm^{-1} originated from Si-O-Si bonds.^[46,47] The bands between 430 to 520 cm^{-1} are attributed to vibrations of Si-O-Al and Si-O-Mg bonds.^[48] The other weaker vibration bands in the range of 500 to 1000 cm^{-1} originated from OH group translations and vibrations as well as overtones of C-H bonds. X-Ray diffraction (XRD) spectra show dominant (001) diffraction pattern at very low angles

corresponding to the presence of interlayer organic modifiers on most of the samples with the exception of C15A, where the second order (002) reflections were observed as well. The interlayer spacing were 2.09 nm for C10A, 2.59 nm for C15A, 2.48 nm for C20A, 2.03 nm for C25A, 1.90 nm for C30B and 2.66 nm for C93A. The corresponding X-ray diffractograms of the modified nanoclays samples with insert showing detail for low diffraction angles is shown on Figure 1B.

Figure 1. (A) FTIR and (B) XRD spectra of bare C10A, C15A, C20A, C25A, C30B and C93A nanoclays.

X-ray photoelectron spectroscopy (XPS) was then performed to understand the surface chemical compositions and bonding arrangements of the fabricated Pt-C micromotors (Figure 2). From wide survey scans obtained (Figure 6A), it was noted that the respective survey scans appeared identical with the presence of peaks attributed to the elements Al, C, O and Si from the montmorillonite nanoclays and Pt from sputtering process. The high-resolution Si 2p spectra of all fabricated micromotors appeared similar where two peaks were deconvoluted. A major peak corresponding to aluminosilicate was present together with a minor peak corresponding to SiO₂ (Figure 3). High resolution O 1s spectra were also deconvoluted and showed a significant presence of Al₂O₃ and small presences of metal oxides for all 6 fabricated micromotors (Figure 4). Similarly, deconvoluted high resolution Al 2s spectra consisted of a major peak corresponding to Al₂O_x (Figure 5). The high resolution XPS spectra affirm the integrity of the montmorillonite backbone of each Pt-C micromotor with the large presence of silicate sheets sandwiched between aluminium oxide sheets. High resolution C 1s XPS spectra were compared to ascertain the differences between the Pt-C micromotors attributed to the different organic modifiers present (Figures 6B to 6F). The high-resolution C 1s spectra of Pt-C10A (Figure 2B) and Pt-C93A (Figure 6F) were deconvoluted into 4 distinct peaks while the spectra for the remaining Pt-Cloisite micromotors gave 3 distinct peaks. The presence of sharp C-C peaks for all XPS spectra deconvoluted can be attributed to the tallow/hydrogenated tallow groups which confirms the presence of respective organic modifiers. From Figure 6D, Pt-C25A showed the highest intensity for C-C group which corresponds to the long alkyl chain of the organic modifier present. Pt-C15A and Pt-C20A micromotors shared the same XPS spectra profile resulting from the same organic modifier present (Figure 6C).

Figure 2. XPS wide survey scans of (A) Pt-C10A, (B) Pt-C15A, (C) Pt-C20A, (D) Pt-C25A, (E) Pt-C30B and (F) Pt-C93A micromotors.

Figure 3. XPS high-resolution Si 2p spectra for (A) Pt-C10A, (B) Pt-C15A/Pt-C20A, (C) Pt-C25A, (D) Pt-C30B and (E) Pt-C93A micromotors.

Figure 4. XPS high-resolution O 1s spectra for (A) Pt-C10A, (B) Pt-C15A/Pt-C20A, (C) Pt-C25A, (D) Pt-C30B and (E) Pt-C93A micromotors.

Figure 5. XPS high-resolution Al 2p spectra for (A) Pt-C10A, (B) Pt-C15A/ Pt-C20A, (C) Pt-C25A, (D) Pt-C30B and (E) Pt-C93A micromotors.

Figure 6. (A) XPS wide scan survey for all six Cloisite micromotors. High resolution C 1s spectra for (B) Pt-C10A, (C) Pt-C15A and Pt-C20A, (D) Pt-C25A, (E) Pt-C30B and (F) Pt-C93A micromotors respectively.

We next set forth to analyse the morphologies of the fabricated Pt-C micromotors using scanning electron microscopy (SEM). From Figure 7, it was observed that the fabricated micromotors did not have distinct and regular shapes. Most of the fabricated micromotors appeared to have wrinkled and rough surfaces with the exception on Pt-C25A which appeared to have a needle-like surface morphology. Additionally, from Figure 7, the sizes of the different micromotors do not appear distinctly different. The average dimensions of each Pt-C micromotor were found based on the average of 100 micromotors captured with SEM (Figure

8). Pt-C15A and Pt-C10A micromotors were found to have the shortest average lengths ($\sim 12 \mu\text{m}$) while Pt-C30B was found to have the longest average length ($\sim 18 \mu\text{m}$). The remaining micromotors were found to be of similar average lengths ($\sim 15 \mu\text{m}$). The micromotors were found to have almost similar overall dimensions with small differences in size distributions.

Figure 7. SEM images of Pt-C10A, Pt-C15A, Pt-C20A, Pt-C25A, Pt-C30B and Pt-C93A micromotors. Scale as indicated in images.

Figure 8. (A) Average lengths of respective Pt-Cloisite micromotors calculated from SEM images of 100 different micromotors. (B) Size distribution of respective Pt-Cloisite micromotors obtained from SEM images.

Energy-dispersive X-Ray (EDX) spectroscopy elemental mapping analysis was next performed to understand the elemental distribution within the micromotors (Figure 9). All micromotors showed the presence of elements Al, Si and O which could be attributed to the alumina and silicate sheets present in the montmorillonite nanoclays structure. From Table 1, Si to Al ratios obtained were almost 2:1 in all Cloisite micromotors. This confirms the integrity of the structure of montmorillonite clay where one alumina sheet is sandwiched between two

silica sheets. The presence of element Pt in each micromotor verified the successful sputtering of elemental platinum during the fabrication process. Elemental compositions of each element (Al, Si, O and Pt) for the respective Pt-C micromotors were summarized in Table 1 and presented in Figure 10.

Figure 9. EDX spectroscopy images of Pt-C10A, Pt-C15A, Pt-C20A, Pt-C25A, Pt-C30B and Pt-C93A micromotors. Images illustrate distribution of Al, Si, O and Pt, respectively. Scale bars represent 5 μm.

Table 1. EDX elemental compositions of respective Pt-Cloisite micromotors.

Atomic %						
Elements	10A	15A	20A	25A	30B	93A
Al K	4.7	2.93	5.61	3.5	5.4	3.99
Si K	8.7	5.35	10.73	6.76	9.32	7.06
Cu K	28.09	35.4	25.94	34.48	28.75	32.86
Pt M	1.99	2.18	0.63	0.79	0.34	1.04
O	56.52	54.5	57.09	54.61	56.18	55.05

Figure 10. EDX spectra of Pt-C10A, Pt-C15A, Pt-C20A, Pt-C25A, Pt-C30B and Pt-C93A micromotors.

Having confirmed the morphologies and chemical compositions of the different Pt-C micromotors, we next set forth to investigate their performances and motion analysis. Velocity studies were carried out to confirm motion properties of micromotors. This was accomplished by mixing Pt-C micromotors with sodium dodecyl sulphate (SDS, 1 wt. %) and hydrogen peroxide (H_2O_2 , 5 wt. %). The micromotors were able to propel as a result of the generation of oxygen gas due to the decomposition of hydrogen peroxide by platinum on the micromotor surface.^[49,50] The average velocities of the respective micromotors were obtained from an average of 25 independent measurements analysed (Figure 11A). The fabricated Pt-C micromotors were found to move with fast average velocities ($v > 100 \mu\text{m/s}$). Pt-C30B micromotors propelled with the fastest average velocity of $\sim 231 \mu\text{m/s}$ while Pt-C93A micromotors propelled with the slowest average velocity of $\sim 110 \mu\text{m/s}$. The organic modifiers present appeared to influence the motion of the Cloisite and in turn, the velocities of the fabricated micromotors. In general, the Cloisite nanoclays with quaternary ammonium modifiers propelled faster than the tertiary ammonium modifier (Pt-C93A). The faster moving micromotors, Pt-C30B ($231 \mu\text{m/s}$) and Pt-C25A ($187 \mu\text{m/s}$), had quaternary ammonium modifiers substituted with single substituted tallow/hydrogenated tallow group with long organic chains in comparison to the other micromotors. Conversely, the slower moving micromotors, Pt-93A ($110 \mu\text{m/s}$) and Pt-20A ($121 \mu\text{m/s}$), were composed of ammonium modifiers with disubstituted hydrogenated tallow groups and simple methyl groups. It was also noted that the presence of benzene in Pt-C10A micromotor as compared to a linear carbon chain in Pt-C25A retarded the motion of the micromotors. Comparing between Pt-C15A and Pt-C20A, it seemed to suggest that increasing the concentration of organic modifiers would improve the velocity of the micromotors at the same hydrogen peroxide concentration. Two key parameters are known to regulate micromotors' velocities which are surface chemical activity and surface phoretic mobility.^[51] The surface chemical activity shows how fast local

chemical gradients are established around the micromotors and how well the outer layers of micromotors generate fluid flow when interacting with the mentioned chemical gradient. Cloisite nanoclays with similar chemical modifiers would have similar surface properties and as such the fabricated Pt-Cloisite micromotors which were sputtered with Pt would have identical surface coverage of Pt and local chemical gradients. However, six different types of Cloisite nanoclays with different organic modifiers were used in this instance. Owing to the different shapes and morphologies (Figure 7), the surface area of Pt sputtered would differ despite same sputtering conditions. As such, the extent of H_2O_2 catalysis would differ for the different Pt-Cloisite micromotors. The presence of different organic modifiers would also affect the local chemical gradient generated around the fabricated micromotors. Additionally, the different Pt-Cloisite micromotors exhibited different thickness, topography and surface roughness which would influence the local chemical gradient generated.^[52] Previous studies reported that the velocities of micromotors increase with catalytic roughness on the micromotors' surfaces.^[53] Formation of fluid flow from the chemical gradient also determines micromotors' surface phoretic mobility. With respect to the mentioned property, the different surface roughness, lengths and irregular shapes of all fabricated micromotors (Figure 7) would indicate a broad range of velocities. Only Pt-C25A micromotors appeared to have spiky morphology and exhibit higher drag force according to Stokes' Law. On the other hand, surface roughness also resulted in very high drag force on the other micromotors.

Figure 11B displays motion tracking images which trace the direction of movement of micromotors taken from videos over a duration of 5 s. All fabricated micromotors displayed and propelled in similar circular motion trajectories of differing circumferences. As a consequence of the asymmetric sputtering of platinum onto the fabricated micromotors, bubbles generation occurred on one side of the micromotors. Additionally, from SEM images obtained (Figure 7), the rough surface morphology of the respective Cloisite nanoclays did

form a uniform layer of platinum sputtered which resulted in the uneven rate of bubbles formation on the surface. As such, the circular motion trajectories were observed. For the same time interval, faster moving micromotors make bigger trajectories in their motion which correspond to greater displacements and hence faster velocities. The rate of bubble formations of each micromotor differ greatly with vigorous bubbling observed for Pt-C30B and Pt-C25A while weak bubbling observed for Pt-C93A. Motion of micromotors were not observed in the absence of hydrogen peroxide fuel. Nanoclays surfaces are inherently catalytic to induced Fenton reaction due to the presence of impurities in working solutions. However, from the SEM images obtained, the fabricated micromotors were found to retain their structural integrities despite the presence of 5 *wt. %* H₂O₂ after a duration of 5 h (Figure 12) when compared to Figure 7. The fabricated micromotors remained relatively stable where they retained their structural integrity and organic modifiers in the presence of the H₂O₂ fuel.

Figure 11. (A) Average velocities of different Pt-C micromotors obtained from measurements of respective 25 independent micromotors. (B) Time-lapse tracking images of respective modified Cloisite micromotors over a duration of 5 s. Experimental conditions: 5 wt. % H_2O_2 , 23 $^\circ\text{C}$, 1 wt. % SDS.

Figure 12. SEM images of Pt-C10A, Pt-C15A, Pt-C20A, Pt-C25A, Pt-C30B and Pt-C93A micromotors 5 h after addition of 5 *wt. %* H₂O₂. Scales as indicated in images.

Due to the absence of organic functionalities, montmorillonite nanoclays could not adsorb organic compounds effectively. It was reported that modifying nanoclays with organic cation modifiers could enhance adsorption of organic compounds through hydrophobic interactions between them.^[54,55] Multiple studies also reported the applications of micromotors for removal of organic and inorganic pollutants in water.^[40,48,56–58] As such, it was postulated that the fabricated Pt-C micromotors would function effectively as mobile adsorbents for the

rapid removal of organophosphate pesticide improvised nerve agent fenitrothion in water. Having determined the fast motion of the fabricated micromotors, the removal efficiency of fenitrothion would be expected to improve in the presence of the mobile ‘cleaners’.

Initial preliminary studies were performed to analyse the Pt-C micromotors capabilities for fenitrothion removal in water samples. Scheme 3 provides an illustration on the procedure for fenitrothion removal analysis. In brief, the respective Pt-C micromotors were exposed to fenitrothion (10 ppm) for 10 min in a solution containing hydrogen peroxide (5 wt. %) and sodium dodecyl sulphate (1 wt. %). Subsequently, the Pt-C micromotors were removed and separated from the solution by filtration to analyse the resultant fenitrothion concentrations. Removal of fenitrothion was calculated from the difference between the initial fenitrothion concentration (10 ppm) and the obtained concentrations after 10 min exposure. Qualification and quantification measurements of fenitrothion removal were performed using liquid chromatography-mass spectrometry (LC-MS).

Scheme 3. Sample preparation procedure for LC-MS fenitrothion removal measurements.

A comparison of fenitrothion removal by different Pt-C micromotors after 10 min exposure is presented in Figure 13. The removal efficiency of fenitrothion in the presence of Pt-C micromotors was in the range of 69-83 %. The best percentage removal of pesticide was achieved by Pt-C10A micromotors (83 %) while the lowest removal performance was observed for Pt-C30B micromotors. Despite propelling with the fastest velocity, Pt-C30B displayed the lowest fenitrothion removal performance.

Figure 13. Percentage removal of fenitrothion with Pt-C10A, Pt-C15A, Pt-C20A, Pt-C25A, Pt-C30B and Pt-C93A micromotors after 10 min exposure to fenitrothion (10 ppm). Experimental conditions in all experiments: 0.25 mg/mL of Cloisite nanoclays, 5 wt. % H₂O₂, 1 wt. % SDS.

In order to ascertain the enhanced removal of organophosphate fenitrothion by the fabricated micromotors, comparative analysis between Pt-C micromotors and bare Cloisite nanoclays were then performed. The respective nanomotors were exposed to fenitrothion (10 ppm) for 1 min before analysis (Figure 14). It is very apparent from Figure 14 that fenitrothion adsorption efficiency significantly increased using micromotors instead of bare nanoclays. Almost all fabricated Pt-C micromotors showed improved fenitrothion removal performances as compared to their respective bare Cloisite nanoclays at a short exposure time (1 min).

However, Pt-C15A micromotors showed identical performance to its corresponding C15A nanoclays (71 %). Pt-C93A micromotors displayed the most significant enhancement in fenitrothion removal with a 3 fold increase in fenitrothion removal observed. The introduction of self-propelled Pt-C micromotors improved the adsorption performance in comparison to bare nanoclays due to enhanced fluid flow generated as a consequence of their rapid motion.^[59] From Table 1, Pt is found to be present in small percentage in the Pt-C micromotors which retained relatively large available surface area on the respective Cloisite nanoclays for adsorption of fenitrothion.

Figure 14. Comparison of fenitrothion removal by Pt-C micromotors (red) and bare Cloisite nanoclays (blue) after 1 min exposure to fenitrothion (10 ppm). Experimental conditions in all experiments: 0.25 mg/mL Cloisite nanoclays, 5 wt. % H₂O₂, 1 wt. % SDS.

Comparing the results obtained in Figures 13 and 14 indicate that adsorption continued to take place throughout the experimental duration (10 min) with higher fenitrothion removal percentages removed with the exception of Pt-C30B micromotors which remained unchanged. As an example, the amount of fenitrothion removed by Pt-C10A increased from 61 % after 1 min exposure time to 83 % after 10 min exposure time. Similar observations were noted with the remaining Pt-C micromotors. These results are in agreement with previous studies where

micromotors displayed improved removal performance with increasing reaction time.^[60] Pt-C30B micromotors did not display any apparent change in fenitrothion removal probably resulting from all available active site being occupied after 1 min exposure time. Additionally, the experimental duration of 10 min might not be sufficient for adsorption equilibrium to reach which resulted in almost insignificant changes observed.

From Figure 14, the efficient removal of fenitrothion by Pt-C93A, Pt-C20A and Pt-C10A micromotors as compared to their respective bare Cloisite nanoclays can be attributed to the presence of hydrogenated tallow groups which provide large available surface area for adsorption to occur. In addition, zeta-potential measurements were carried to obtain the surface charges of the Cloisite nanoclays (Figure 15). The addition of organic cations to Montmorillonite changes zeta potential values measured. Montmorillonite clay was modified with six different quaternary ammonium organic cations as presented in Scheme 1. Two parameters influence the zeta potential values which are cation chain length and percentage of organic cation present. Cloisite 15A and Cloisite 20A consist of the same methyl substituted hydrogenated organic modifier in differing concentrations 125 and 95 meq/100g clay respectively. As such, Cloisite 15A was found to have a positive zeta potential (42.4 mV) while Cloisite 20A had negative zeta potential value (-10.9 mV). It is noted that the zeta potential measured increased as the modifier concentration increases.^[61] Cloisite 93A was found to possess the most negative zeta potential value (-45.6 mV) while Cloisite 10A had the most positive value (42.4 mV). These values correlate closely with the structures of the organic modifiers present. In Cloisite 10A, the quaternary cation has a bulky benzene structure unlike that of the simple quaternary cation in Cloisite 93A. As such, the hydrophobicity of Cloisite 10A increased which in turn causes the hydrophobic lateral interactions between cations to increase.^[62]

Figure 15. Zeta potential values of Cloisite 10A, Cloisite 15A, Cloisite 20A, Cloisite 25A, Cloisite 30B and Cloisite 93A nanoclays.

With regards to its capability to adsorb fenitrothion, bare C10A nanoclays has the most positive surface charge (43.3 ± 5.49 mV). As such, adsorption of negatively-charged fenitrothion would be the strongest and more fenitrothion molecules would be removed. This would thus correlate to Pt-C10A having the best fenitrothion removal performance (Figure 13). However, Pt-C30A showed the worst performance attributed to the almost neutral surface charge of C30A (4.4 ± 2.07 mV). Thus, the electrostatic interaction between the nanoclays and fenitrothion molecules would be weak and less molecules would become adsorbed onto the surface of C30A nanoclays. C93A has the most negative zeta potential (-45.6 ± 2.11 mV) which would generate repulsion with the negatively-charged fenitrothion molecules and thus less organophosphate molecules can get adsorbed.

The adsorption properties of Pt-C micromotors modified with small organic cations are quite different from the adsorption properties of Pt-C micromotors modified with large organic cations. It was reported that the presence of long-chain organic cations in the Cloisite nanoclays (C25A) would form a pseudo organic phase medium for the organic extraction of contaminants.^[63] Adsorption occurs in a manner similar to the dissolution of organic pollutants in an organic solvent, where in this instance, the organic phase is fixed on the surface of the fabricated micromotors. Adsorption of fenitrothion onto the unmodified montmorillonite nanoclays is minimal in aqueous solution due to strong adsorption of water molecules onto the surface. However, the presence of organic modifiers in Cloisite nanoclays generates an organic phase as a result of the long carbon chain groups (tallow and hydrogenated tallow groups). Adsorption of fenitrothion onto Pt-C25A and Pt-C30B micromotors occur at the pseudo organic phase present on the unspattered Cloisite micromotors surfaces. Increasing the concentrations of micromotors would enhance the organic phase due to the shorter distances between the long carbon chain groups, thus leading to greater adsorption of fenitrothion. On the other hand, the characteristics of the adsorption of fenitrothion onto Pt-C10A, Pt-C15A, Pt-

C20A and Pt-C93A micromotors differ. The surfaces of these short chain modified nanoclays contain isolated quaternary ammonium cations, which are quite far separated on the aluminosilicate surface of the Cloisite nanoclays.^[64] As such, the organic modifiers present may be too small to cover the whole nanoclays surface and would behave as isolated entities on the Cloisite surface of these micromotors. For Pt-C10A, the interactions between the benzyl functionality of the modifier and the clay surface in the interlayer alter the dimensions of the adsorption regions. Fenitrothion adsorption onto Pt-C micromotors include both Van der Waals interactions and cation exchange.^[65]

The adsorption of fenitrothion with Pt-Cloisite micromotors can be described by the Freundlich and Langmuir classical models of adsorption isotherms.^[66] The linear form of Freundlich's equation is as follows:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad \text{Eq. 1}$$

where q_e (mg/g) is the adsorption capacity at equilibrium, K_f and n are Freundlich dimensionless constants which represent adsorption capacity and adsorption intensity respectively, C_e (mg/dm³) is the equilibrium concentration.

Similarly, Langmuir's classical model is expressed as:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_l} + \frac{1}{q_m} C_e \quad \text{Eq. 2}$$

where q_m (mg/g) is the maximum adsorption capacity for the monolayer when saturation is reached, and K_l (dm³/mg) is Langmuir constant related to the energy.

The experimental data obtained were fitted for both the Freundlich and Langmuir models. Figure 16 depicts the Freundlich adsorption isotherms for fenitrothion on all six fabricated Pt-Cloisite micromotors. The linear plots of $\ln C_e$ vs. $\ln q_e$ indicate that all

experimental data fit well with Freundlich's equation for all Pt-Cloisite micromotors. As seen from the Figure 16, obtained correlation coefficients (R^2) were > 0.98 .

Figure 16. Freundlich isotherm plots for fenitrothion adsorption onto Pt-Cloisite micromotors. Experimental conditions: 0.25 mg mL^{-1} Cloisite nanoclays, fenitrothion concentration: 2.5-25 ppm, 5 wt. % H_2O_2 , 1 wt. % SDS, 1 h reaction time.

Furthermore, two additional parameters ($\frac{1}{n}$ and K_f) were obtained from each plot and were determined as the intercept values in Eq. 1, respectively (Table 2). The n values obtained are greater than 1 for each micromotor used as adsorbent which indicates that fenitrothion adsorption is a physical process. Figure 17 represents plots of $\frac{C_e}{q_e}$ vs. C_e with the corresponding

R^2 . It can be concluded from the low R^2 obtained that adsorption cannot be explained by the Langmuir isotherm model except for Pt-C30B micromotors ($R^2 = 0.999$). This data indicates the heterogeneity of the surface adsorption sites on Pt-C30B where various adsorption sites with different energies are involved. The Freundlich isotherm model is based on the assumption that adsorbate binding occurs on the heterogeneous surface of micromotors and include the interactions between adsorbed species.

Table 2. Freundlich isotherm model constants and correlation coefficients for fenitrothion adsorption onto Pt-Cloisite micromotors.

Freundlich isotherm			
Adsorbent	k_f	n	R^2
Pt-C10A	138.835	1.265	0.996
Pt-C15A	172.068	1.117	0.998
Pt-C20A	369.318	1.612	0.986
Pt-C25A	246.661	1.2	0.999
Pt-C30B	309.888	1.336	0.992
Pt-C93A	183.4	1.279	0.997

Figure 17. Langmuir isotherm plots for fenitrothion adsorption onto Pt-Cloisite micromotors. Experimental conditions: 0.25 mg mL⁻¹ Cloisite nanoclays, fenitrothion concentration: 2.5-25 ppm, 5 wt. % H₂O₂, 1 % wt. SDS, 1 h reaction time.

Having demonstrated the successful fabrication and application of Pt-C micromotors for environmental remediation, it would be essential to investigate the toxicity of these nanoclays in view of the possibility of future commercial applications. Pt-C micromotors have the highest likelihood of entering into the human body via inhalation and ingestion which increases the possibility of interaction with bodily molecules.^[67] Toxicity of nanomaterials depend on their size, chemical compositions, surface area and morphology.^[68,69] As such, small differences in toxicity between the fabricated Pt-C micromotors would be postulated. *In vitro*

cytotoxic studies of all six fabricated Pt-C micromotors were analysed in comparison to bare Pt particles, which are known to be highly toxic and serves as a control for the study.

The water-soluble tetrazolium salt (WST-8) cell viability assay was used to conduct cytotoxicity experiments. In the presence of metabolically active cells, tetrazolium reagents are reduced to generate the formazan dye colour whose intensity would indicate the amount of viable cells present.^[70] Human lung carcinoma epithelial cells (A549) were chosen, taking into account that they are the most commonly exposed cells. The cytotoxicity of Pt-C micromotors were investigated by incubating A549 cells with different micromotors concentrations (0, 5, 20, 50, 100, 150 $\mu\text{g/mL}$). Control experiments were also performed with cells in the absence of any micromotors. As observed from the toxicological profile (Figure 18A), the cell viability did not change adversely with increasing concentrations of most Pt-C micromotors from 0-150 $\mu\text{g/mL}$ with the exception of Pt-C20A micromotors. The cell viability with most micromotors stayed above 80 % even at the highest concentration of micromotors introduced (150 $\mu\text{g/mL}$) with the exception of Pt-C20A micromotors which dropped to 53 % at the highest concentration. On the other hand, the cell viability dropped to 14 % upon introduction of 150 $\mu\text{g/mL}$ bare Pt particles (diameter: 3 μm) which affirms the high toxicity of Pt on human cells. These results imply that most of the fabricated micromotors are not toxic even at high concentrations and are generally safe for human handling. Cell-free control experiments were subsequently done in similar conditions to that presented in Figure 18A to analyse the effects of external interferences from the micromotors and assay used.^[71]

Figure 18. (A) Cell viability percentage of different concentrations of Pt-C micromotors and bare Pt particles (0-150 $\mu\text{g/mL}$) incubated with A549 cells for 48 h measured using WST-8 assay. (B) WST-8 control experiment where formazan formed upon 1 h of WST-8 incubation with different concentrations of Pt-C micromotors.

From Figure 18B, the absorbance was found to increase with increasing Pt particles concentration. The absorbance results obtained indicates the presence of more formazan in the assay possibly from the reduction of tetrazolium to formazan crystals by Pt particles. Conversely, such observation was not observed in the presence of all Pt-C micromotors. The control experiments performed showed that all of the Pt-C micromotors displayed low interferences with WST-8 assay reagents even in high concentration. Pt-C micromotors toxicological profiles confirm their similarly non-toxic properties and furthermore raise possibility for potential applications.

4.4 Conclusion

In this chapter, we demonstrated excellent motion capabilities of self-propelled Pt-C10A, Pt-C15A, Pt-C20A, Pt-C25A, Pt-C30B and Pt-C93A micromotors in the range of 110-231 $\mu\text{m/s}$. The fabricated Pt-C micromotors were found to be non-toxic from cytotoxicity studies even at high concentrations (150 $\mu\text{g/mL}$) which render them safe for human handling. The enhanced performance of the fabricated micromotors as mobile adsorbents or cleaners towards fenitrothion removal were also analysed. Comparison studies with respective bare Cloisite nanoclays revealed improved efficiencies of fenitrothion removal even after 1 min exposure to contaminated samples due to enhanced fluid dynamic by motion of micromotors. The Pt-C micromotors were capable of removing 69-83 % of fenitrothion after 10 min exposure to contaminated water sample. The different organic modifiers present and the zeta potentials of the Cloisite nanoclays influenced the binding affinities of the fabricated micromotors with fenitrothion. This opens up possibilities for rapid fabrication and tuning of specialised micromotors by the selective modification of naturally available nanomaterials.

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Chapter 5

Micromotors Derived from Variety Plant Pollen Grains for Efficient Environmental Clean-Up and Anti-Cancer Drug Delivery

5.1 Introduction

5.2 Experimental part

5.3 Results and discussion

5.4 Conclusion

5.5 References

Micromotors Derived from Variety Plant Pollen Grains for Efficient Environmental Clean-Up and Anti-Cancer Drug Delivery

The production of large quantities of micromachines and micromotors has been limited by fabrication methods and the use of synthetic templates. Pollen is one of the most stable structure in the world, that is capable of surviving harsh treatments and millions of years. Pollen grains are available in a large variety of shapes and sizes. Herein, we demonstrated the use of a wide variety of naturally abundant, non-toxic pollen grains for the efficient fabrication of platinum-pollen (Pt-pollen) hybrid micromotors that are capable to be propelled with fast velocities for environmental and biomedical applications. Nine different pollen grains were selected and modified (Dandelion, Pine, Lotus, Sunflower, Poppy, Camellia, Lycopodium, Cattail and Galla) to demonstrate the robustness of different types of pollen grains for potential applications in environmental remediation. The efficient mobility rendered by the fabricated micromotors enhanced their performance in the removal of heavy metals in aqueous medium. Furthermore it can act as drug carriers of doxorubicin.

5.1 Introduction

The main goal of nanotechnology is successful replacement of macroscopic vehicles with miniaturised devices - micro/nanorobots that can perform similar or improved varied functions in environmental science and biomedical applications.^[1,2] Self-propelling autonomous nano/micromotors have garnered significant interest not only in nanotechnology but other spectra of science such as medicine.^[3-7] These self-propelling miniaturised devices are able to drive by the following mechanisms: self-electrophoresis, bubbles propulsion, self-diffusiophoresis and Marangoni effect.^[8-10] The small size and fast velocities of micromotors have been favourable properties serving as highly efficient platforms for water clean-up in the removal of heavy metals,^[11,12] oils,^[13,14] dyes,^[15] chemical warfare agents,^[16] phenolic pollutants,^[17] pollutant degradation^[1,18] and pathogens.^[19] Recent efforts have also

demonstrated a wide range of biomedical application of micromotors as drug carriers,^[20] cargo delivery,^[21,22] nanosurgery^[23] and cancer therapy.^[24–26]

Previously, pollen grains have gained increasing attention due to their environmentally-friendly properties, structural stability, biocompatibility and monodispersity.^[27–34] The exine (outer shell wall) is composed of biologically and chemically resistant biopolymers called sporopollenin.^[35] Intine (inner shell wall) is made up of pectin, cellulose, proteins and polysaccharides. Additionally, the hollow internal shells of pollen grains make them suitable candidates as microencapsulant to store and deliver cargo. Cho *et al.* recently reported extraction methods of dandelion sporopollenin exine capsules (SECs) which potentially can find applications in drug delivery as well as food industry.^[36,37] Additionally, pine pollen grains were loaded with BSA for targeted oral delivery. The triple cavity structure of the pollen grains allowed for excellent loading.^[38] Similarly, *Lycopodium clavatum* spores were reported to display efficient encapsulation of 5-fluorouracil and release in gastric environment.^[39]

Herein, we demonstrate a template free approach by fabricating micromotors based on pollen grains which exhibit self-propulsion movement by bubbles formation due to the decomposition of hydrogen peroxide (H₂O₂) by platinum (Pt). Recently, our group published the exact same propulsion technique for Pt-Sunflower pollen grain micromotors,^[40] but herein due to the large number of comparing experiments, new study might still be of high importance for the development in the field. Herein, we have fabricated micromotors from nine different types of pollen grains (Dandelion, Pine, Lotus, Sunflower, Poppy, Camellia, Lycopodium, Cattail and Galla) asymmetrically sputtered with a thin layer of platinum. The fabricated platinum-pollen (Pt-pollen) micromotors were studied by investigating their applications for heavy metals removal (Hg²⁺) in aqueous medium and anti-cancer drug attachment.

5.2 Experimental section

Materials: Defatted pollen grains from Sunflower (*Helianthus annuus* L.), Pine (*Pinus taeda*) and Dandelion (*Taraxacum officinale*) families were purchased from Greer Laboratories, Inc. (Lenoir, NC). Lycopodium (*Lycopodium clavatum*) spores (S-type) and Cattail (*Typhae angustifolia*) pollen grains were purchased from Sigma-Aldrich (St. Louis, MO). Camellia (*Camellia Sinensis* L.) bee pollen granules were purchased from Yuensun Biological Technology Co., Ltd. (Xi'an, Shaanxi, China). Lotus (*Nelumbo nucifera*) and Poppy (*Papaver rhoeas*) pollens were purchased from Pepper Industrial Co., Ltd. (Zhengzhou, Henan, China). Hydrogen peroxide (H₂O₂, 35 wt. %) was obtained from Alfa Aesar (Singapore). Sodium dodecyl sulphate (SDS), MTT assay reagent and mercuric nitrate monohydrate (Hg(NO₃)₂ x H₂O) were purchased from Sigma-Aldrich (Singapore). Water-soluble tetrazolium salt (WST-8) assay reagent was supplied by Dojindo. Phosphate buffer solution (PBS), fetal bovine serum (FBS), Dulbecco's Modified Eagle medium (DMEM) and penicillin-streptomycin liquid (1 %) were purchased from Gibco. Trypsin was obtained from Life Technologies (Singapore).

Instruments: Micromotors structure characterisation were obtained by JEOL 7600F (Jeol, Japan) at an accelerating voltage of 5 kV for scanning electron microscopy (SEM) and accelerating voltage of 15 kV for energy-dispersive X-ray spectroscopy (EDS) respectively. Absorbance readings were performed using Thermo Multiskan GO (Thermo Fisher Scientific). Dynamic imaging particle analysis (DIPA) was performed by FlowCam (Fluid Imaging Technologies, Maine, USA). Sputtering machine (JEOL JFC-1600 Auto Fine Coater) was used for Pt coating onto the respective plant pollens. Optical microscope (Nikon Eclipse TE 2000-E) connected to Nikon NIS-Elements software were employed to perform velocity measurements. Concentrations of Hg²⁺ were obtained with inductively coupled plasma mass spectrometry (Agilent 7700 Series, Japan). Drug Drug loaded platinum-pollen (Pt-pollen)

micromotors fluorescence images were captured by an optical microscope coupled with filter (excitation wavelength: 560-570 nm).

Pollen Grains Preparation: 250 g of bee pollen granules were firstly refluxed in 0.5 L of acetone in a round bottom flask under magnetic stirring (50 °C, 220 rpm) for 3 h. Thereafter, the acetone was decanted and the sample was mixed with 1 L of deionized water for 1 h. Subsequently, the pollen suspension were filtered through a 300 µm diameter pore size nylon mesh and 6 µm diameter pore size filter paper. 1 L deionized water was used to hydrate the pollen sample for 30 min under magnetic stirring which was subsequently filtered. The first procedure was repeated, and the resulting sample was refluxed again in 500 mL of acetone under magnetic stirring (3 h, 400 rpm). Thereafter, acetone was removed with vacuum filtration and sample was stored in glass petri dish in a fume hood for one day. 250 mL of diethyl ether was mixed with 20 g of dried sample and stirred for 120 min (400 rpm, 25 °C). After repetition of last step twice, diethyl ether was removed by vacuum filtration. Next, freshly used diethyl ether was mixed with the pollen sample and left to stir overnight. Lastly, the pollen sample was separated from diethyl ether, transferred to petri dish and left to dry for 12 h in a fume hood.

Fabrication and Operation of Platinum-Pollen Micromotors: All nine different types of platinum-pollen (Pt-pollen) micromotors were prepared by the following procedures. 1 mg of respective plant pollen was suspended in 1 mL Milli-Q water and sonicated for 1 h. The solution was then dropped onto a glass slide (previously cleaned with ethanol) and left to dry overnight. Pt was then asymmetrically sputtered onto one side ($t = 200$ s, $I = 40$ mA). Subsequently, the glass slide was ultrasonicated for about 30 s to detach the fabricated micromotors from the glass slide. The released micromotors were stored in distilled water. Motion studies of micromotors were performed in solution which contains water, hydrogen peroxide (fuel) and sodium dodecyl sulphate (surfactant). The average velocities of

micromotors were calculated by measuring 20 individual velocities of the micromotors using an optical microscope.

Quantification of the Hg²⁺ Removed: Mercury ions adsorption studies were carried out in 500 µL of the final solution. The concentration of pollen grains, SDS and hydrogen peroxide were 0.5 mg mL⁻¹, 1 % and 4 %, respectively. 1 ppm of mercury ions was added into the solution for different durations of 1, 5, 30 and 120 min. At the end of the respective exposure times, filtration (filter membrane 3 µm pore size) was performed to remove the micromotors from solution and the filtrate was obtained. Concentrations of Hg²⁺ remaining in filtrate were obtained using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Percentage removal of Hg²⁺ by micromotors/bare pollen grains were calculated from the difference between initial concentration (1 ppm) and measured concentrations of filtrate at different exposure times. Three experiments were repeated for the same remediation time and conditions.

Equilibrium studies: Equilibrium studies were carried out in solution of Pt-pollen grains (0.25 mg/mL), H₂O₂ (3 wt. %) and SDS (1 wt. %) by varying the Hg²⁺ concentration between 0.25 and 2 ppm for 2h contact time. The solution was filtered after 2 h and the Hg²⁺ adsorbed at the equilibrium was calculated by the following equation:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (\text{Eq. 1})$$

where C₀ (mg/L) and C_e (mg/L) are initial and equilibrium concentrations of Hg²⁺, V (L) is volume of the solution, and m (g) is mass of Pt-pollen grains used in the experiment.

Cytotoxicity Assessments: MCF-7 breast cancer cell line (Bio-REV Singapore) was used to determine cytotoxicity of the Pt-pollen micromotors. The cells were prepared in a medium consisting of Dulbecco's modified eagle medium (DMEM), 10 % FBS and antibiotics (1% penicillin-streptomycin) in an incubator (conditions: 37 °C, 5 % CO₂). The cells were

seeded and grown in 96-well plates (5000 cells per well in 100 μ L) for 24 h. Thereafter, medium was replaced with 100 μ L of different dilutions of Pt-pollen micromotors suspensions in each well. Negative control experiments were performed with cells in the absence of fabricated micromotors while positive control experiments were performed in 10 % DMSO. Subsequently, cells and micromotors were incubated for 24 h (conditions: 37 $^{\circ}$ C, 5 % CO_2). 10 μ L of WST-8 reagent was introduced into each well and incubated for 1 h in wrapped aluminium foil. Lastly, absorbance was measured at excitation wavelength of 450 nm.

Loading of Doxorubicin (DOX) onto Pt-pollen micromotors: 1 mL suspension of Pt-pollen micromotors was dispersed in 28 μ M Doxorubicin solution (DOX) and left for 24 h. The mixture was centrifuged (12 000 rpm, 5 min) to remove free and unloaded DOX molecules in the solution. Cleaning procedure was repeated twice more. DOX-loaded Pt-pollen micromotors were finally resuspended in distilled water and used directly for fluorescence imaging.

5.3 Results and discussion

Nine different types of the pollen grains were studied (Dandelion, Pine, Lotus, Sunflower, Poppy, Camellia, Lycopodium, Cattail and Galla) for the fabrication of autonomously moving platinum-pollen (Pt-pollen) micromotors. These pollen grains were chosen due to their natural abundancy, great efficiency and reproducibility for micromotors preparation.^[40] Scheme 1 provides an illustration on the procedures for Pt-pollen micromotors preparation as well as proof-of-concept applications in Hg^{2+} ions removal in aqueous solution and anti-cancer drug loading. In summary, pollen grains serve as structural templates for micromotors fabrication. A thin layer of platinum was asymmetrically sputtered onto one side which functions as catalyst for the decomposition of hydrogen peroxide (H_2O_2) and formation of bubbles for propulsion of the fabricated micromotors. Although there are other low cost

catalytic materials such as MnO₂ and Ag, Pt was chosen due to its stronger catalytic ability towards fuel used and thus stronger power output and high micromotors velocities. [41]

Scheme 1. Schematic representation on the fabrication of Pt-pollen micromotors. The fabricated micromotors were subsequently used for removal of Hg²⁺ ions in aqueous solution and loaded with anti-cancer drug (Doxorubicin).

The comprehensive procedure for Pt-pollen micromotors preparation are further elaborated in experimental section. Characterisation studies were first performed to understand the unique morphological structural features of the selected pollen grains and their corresponding fabricated micromotors counterparts. To examine the differences in particle sizes, shapes and features with their respective micromotors counterparts, scanning electron microscopy (SEM), dynamic imaging particle analysis (DIPA) and energy-dispersive X-ray spectroscopy (EDS) were carried out. SEM was first performed to understand and analyse the contrasting microscopic surface features of the selected pollen grains (Figure 1). It is postulated that specific surface morphologies and characteristics would affect potential applications of the

fabricated Pt-pollen micromotors. From Figure 1, it is apparent that each type of plant pollen has uniform size distribution and porous surfaces.

Figure 1. Scanning electron microscope images of nine different types of bare plant pollens. Scales as indicated in respective images.

Based on the SEM images obtained, the different pollen grains can be broadly categorised based on their common shapes or structural features. Lotus, Sunflower and Camellia have spherical shape; Pine, Poppy, Galla, Cattail and Dandelion can be classified into a group of pollen grains which has oblate shape as they have a ratio between the lengths of the polar and equatorial axis less than 1. In contrast, Lycopodium has ration higher than 1 and it has defined prolate shape. Depending on the apertures, Lotus, Camellia, Poppy and Galla

exhibit colpus structure with elliptical or rectangular aperture with length at least two times longer than their width. Dandelion, Sunflower, Lycopodium and Cattail have pore structure with small apertures on their surface. In particular, Pine pollen grains have the distinctive “Mickey Mouse” leptomate structure where the middle portion resembles the head attached to two ears on both sides (air sacs). In the case of Lycopodium pollen, it is very well defined by many open chambers.^[42] These apparent differences in structural features could play prominent roles in the mobility and performances in potential applications of the fabricated micromotors which will be further discussed.

Figure 2. Diameter analysis performed by dynamic imaging particle analysis (DIPA) obtained from three independent measurements. Each graph presents a function of diameter vs. frequency. Scales as indicated in respective images.

Following SEM morphological structure characterisation, we studied dynamic imaging particle analysis (DIPA) to ascertain the respective pollens sizes and shapes (Figure 2). High-resolution camera (FlowCam) was used to obtain size distribution for each type of pollen by analysing 500 individual pollen grains. From Figure 2, each graph consists of histogram data and the respective fitted Gaussian curves of each pollen grain's diameter size as a function of frequency. The selected pollen grains were found to exist within a broad size range distribution with Pine pollen grains having the biggest diameter ($\sim 62 \mu\text{m}$) while Cattail pollen grains having the smallest diameters ($\sim 22 \mu\text{m}$). Presently, extensive studies have been directed towards exploring and analysis of self-propulsion of micromotors. In this part, owing to their different morphologies, we set forth to investigate the mobility's of the different fabricated Pt-pollen micromotors by performing comparative velocity experiments. The experiments were conducted in solutions containing 1 wt. % sodium dodecyl sulphate (SDS) at two different concentrations of H_2O_2 (3 and 5 wt. %). All prepared micromotors [Pt-Dandelion (Pt-Dan), Pt-Pine, Pt-Lotus (Pt-Lot), Pt-Sunflower (Pt-Sun), Pt-Poppy (Pt-Pop), Pt-Camellia (Pt-Cam), Pt-Lycopodium (Pt-Lyc), Pt-Cattail (Pt-Cat) and Pt-Galla (Pt-Gal)] exhibited higher average velocities in 5 wt. % H_2O_2 compared to 3 wt. % H_2O_2 as predicted (Figure 3A). Higher concentrations of H_2O_2 increases O_2 saturation levels which promotes nucleation rates thus resulting in faster velocities.^[43]

Comparing the velocities, the fabricated micromotors were found to move with average velocities of $>50 \mu\text{m/s}$ in 3 wt. % H_2O_2 which significantly increased ($>140 \mu\text{m/s}$) in 5 wt. % H_2O_2 . All presented average values with their respective standard deviations were calculated from 20 individual micromotors velocities. Pt-Cat micromotors were found to exhibit the slowest average velocity ($49.9 \pm 9.5 \mu\text{m/s}$ for 3 wt. % H_2O_2 and $137.2 \pm 25.8 \mu\text{m/s}$ in 5 wt. % H_2O_2) while Pt-Lot micromotors have the fastest average velocity ($217.0 \pm 36.7 \mu\text{m/s}$ in 3 wt. % of H_2O_2 and $349.9 \pm 44.2 \mu\text{m/s}$ in 5 wt. % of H_2O_2). Additionally, the motion trajectories of

the respective fabricated Pt-pollen micromotors in 3 wt. % H_2O_2 for duration of 2 s were monitored (Figure 3B).

Figure 3. (A) Dependencies of average velocities of Pt-pollen micromotors in respective H_2O_2 concentrations. Average velocities and standard deviations at respective H_2O_2 concentrations were obtained from 20 independent measurements. (B) Motion trajectories of respective Pt-pollen micromotors in 3 wt. % H_2O_2 over duration of 2 s. Scale bars represent 50 μm for each image.

It was noted that most of the Pt-pollen micromotors propelled in circular motion trajectories with the exception of Pt-Lot and Pt-Gal micromotors which moved around their respective rotation axes. Micromotors which had faster average velocities made bigger circumferences hence they generated greater displacements for the same time interval. It is important to note that Pt-pollen micromotors cannot move in the absence of both SDS and H_2O_2 .

The micromotors were able to propel by bubbles generation followed by bubbles detachment at the sputtered Pt surface. Taking into account the fact that bubbles generation is dependent on the micromotors' geometries and shapes, the average velocities obtained are in accordance with theory. Zhao *et al.* previously performed kinetics studies of Janus particles and discovered that particles with diameters $<10\ \mu m$ could not easily propel by bubbles formation.^[44] Bubbles nucleation can occur when micromotors in aqueous solution accumulate oxygen gas which reach heterogeneous nucleation energy (critical energy).^[45] It depends on the irregularity on the surface of micromotors as well as O_2 saturation concentration. From the SEM images captured (Figure 1), the different shapes and geometries of pollen micromotors were presented which are different from Janus and tubular micromotors reported. As such, the surface area and coverage of Pt sputtered surface changes accordingly. Pt-Lot and Pt-Dan micromotors have concave-like surfaces which require lesser energy to form bubbles. Thus, they display faster velocities. On the other hand, Pt-Cat and Pt-Gal micromotors have convex-like shapes. Hence, more energy would be required to generate bubbles.^[46] These can be explained from the fact that the free energy of bubbles generation for convex and concave surfaces are different (concave surface ~ 0). Additionally, any contamination or defects during fabrication of micromotors can change contact angles which reduces the efficiency of nucleation. Previously, it was shown that Pt-Sun micromotors generate bubbles between the spikes and that nucleation depends on the gaps between the spikes.^[40] Velocity of Pt-pollen

micromotors can be explained by Stokes's Law extended to non-spherical particles.^[47]

Micromotors propulsion depends on both driving force and drag force in the aqueous medium.

Drag forces on Pt-pollen micromotors can be obtained from the following equation

$$F_D = 3\pi\eta Vd_vK \quad (\text{Eq. 2})$$

where η is dynamic viscosity, V is velocity of nonspherical particle, d_v is diameter of the spherical object, K is corrected shape coefficient (can be defined for any shape).

$$K = \frac{V_s}{V} \quad (\text{Eq. 3})$$

where V_s is velocity of the sphere with the same diameter to the diameter of nonspherical particle.

The shape coefficient is larger than 1 for irregular shape micromotors, compared to spherical Janus micromotors (shape coefficient = 1). It can be interpreted as the velocity of the Pt-pollen micromotors being less than the velocity of the equivalent sphere. Camellia and Sunflower can be good example for this explanation as Sunflower pollen grain has very similar diameter to the diameter of spherical particle (Camellia pollen grain). Therefore, Camellia pollen grains have shape coefficient $K \sim 1$, and shape coefficient of Sunflower pollen grains, due to the spikes presented on the surface, is approximately 1.07. Taking into account this assumption it is expected that Pt-Cam move with higher velocity than Pt-Sun micromotors which is in correlation with experimental results. From Eq. 1, micromotors with irregular shape have larger value for drag force compared to spherical micromotors. Escarpa *et al.* previously reported that outer surface micromotors roughness increases drag force on them and therefore reduces their velocities.^[48]

Scrutinizing further, we evaluated the potential application of fabricated Pt-pollen micromotors for Hg^{2+} removal in aqueous medium. Neustadt and Pieczenik reported that Hg^{2+}

overdose could cause serious health problem such as autoimmune diseases, memory loss, insomnia and anxiety.^[49] Water polluted with heavy metals is considered wastewater and presents a global problem for humanity even in very small concentrations.^[50] The lack of potable water, coupled with the increasing pollution of water bodies generates an increasing global demand for clean water. This need has attracted the attention of many scientific institutions and industries with aims to develop efficient and economical wastewater treatment technologies to meet the increasing demand. Adsorption technique is widely adopted due to its simplicity, easy performance, and the potential use of cheaper alternative adsorbent materials. Previously, naturally available Halloysite^[51,52] were used as micromotors template and displayed efficient performance in the removal of heavy metals in wastewater. The basic parameters and properties considered in the selection of adsorbents for purifying contaminated water includes selectivity, high adsorption capacity, abundancy, simplicity of synthesis and modification, low cost and regeneration possibility. Natural pollen grains, in addition to being cheap and abundant, can also be promising micromaterials for removing Hg^{2+} ions in contaminated water. It was hypothesized that Pt-pollen micromotors are more efficient mobile adsorbents for fast Hg^{2+} ions removal compared to bare pollen grains due to enhanced assisted fluid mixing attributed to micromotors' mobility.^[40] For this purpose, Pt-pollen micromotors were exposed to aqueous solutions containing 1 ppm Hg^{2+}

Figure 4A compares the Hg^{2+} removal performance of Pt-pollen micromotors (black bars) and their corresponding bare pollen grains (red bars). Firstly, the respective micromotors were exposed to 1 ppm Hg^{2+} for different durations of 1, 5, 30 and 120 min. All graphs are displayed as a percentage of Hg^{2+} removal over exposure time durations (0 - 120 min). From Figure 4A, most prepared micromotors showed more efficient removal of Hg^{2+} compared to their respective bare pollen grains except Pt-Lot, Pt-Pine and Pt-Gal micromotors. These micromotors did not show much difference in adsorption performance compared to their

corresponding pollen grains. Pt-Lot micromotors have very large surface area and propelled with the fastest velocities.

Figure 4. (A) Influence of exposure time on Hg²⁺ removal by Pt-pollen micromotors (black bars) and corresponding bare pollen grains (red bars). (B) Comparative analysis of Hg²⁺ adsorption of all fabricated Pt-pollen micromotors at different exposure times over duration of 120 min. Error bars represent standard deviations from 3 independent measurements.

These factors could have attributed to the highly efficient removal of Hg^{2+} by Pt-Lot micromotors. Despite the fact, Pt-Lot micromotors did not show much difference in adsorption performance compared to their corresponding pollen grains which could be justified by very good adsorption capability of bare material (Lotus pollen grain) so that additional propulsion does not have much influence on adsorption performance.

Pt-Gal micromotors exhibit the slowest velocities and they exist as small particles. As such, the slow propelling Pt-Gal micromotors do not show enhanced adsorption performance compared to bare Galla pollen grains. Comparing the performances at exposure time of 1 min to their respective bare pollen grains, it was observed that removal of Hg^{2+} by Pt-Dan micromotors (37 %) showed greatest enhancement than to bare Dandelion pollen grains (12 %). The dynamic and robust propulsion motion, coupled with the fast velocity and good adsorption capabilities, could have resulted in such improved performance.

Figure 4B provides a comparison on the removal efficiency of Hg^{2+} by the fabricated micromotors over duration of 120 min. All graphs depict increasing trend for the removal for Hg^{2+} over the experimental duration and reached maximum removal percentage after 120 min. Pt-Lyc and Pt-Cat micromotors reached the highest Hg^{2+} removal efficiency of $\sim 90\%$. The high adsorption efficiency 120 min after exposure to Hg^{2+} could be attributed to the availability of high surface area on the unsputtered pollen surface for adsorption to occur. The most efficient micromotors for the removal of Hg^{2+} in aqueous medium was found to be Pt-Lyc which displayed high removal percentages throughout all analysis durations while the least efficient micromotors was Pt-Pin which displayed the lowest percentages throughout the experimental duration. It is important to note that adsorption performance is not only governed by size of micromotors. Adsorption capacity depends on surface functionalities such as surface charge, amount of active sites and available surface area on the micromotors as adsorbents. Pt-Pin micromotors have the largest surface area but it is possible that they do not have sufficient

charged sites and available adsorption areas which can result in lower adsorption capacity. Pine pollen grain is only studied pollen grain which contain from corpus and two saccus (bi-saccate), the rest are mono-compartmental as they consist of only one corpus. This structural unique of Pine pollen grains open possibility that Hg^{2+} ions load only into the exine of the porous central cavity which is water permeable, but not into the non-porous air-sac.^[53] Nonetheless, all fabricated micromotors displayed good performances with high removal percentages.

Energy-dispersive X-Ray spectroscopy (EDS) elemental mapping characterisation was performed (Figure 5) to confirm the adsorption of Hg^{2+} onto the micromotors and verify the results obtained. It can be deduced that Pt layer sputtered and Hg^{2+} present did not perturb the natural structures of the pollen grains for each fabricated micromotors (SEM images, Figure 5). Additionally, the elemental mappings obtained displayed the presence of Hg on all fabricated micromotors which strongly suggests the adsorption of the ions onto the surface of the micromotors after 120 min.

Figure 5. SEM images and corresponding EDS elemental mappings of respective fabricated Pt-pollen micromotors after adsorption of Hg^{2+} illustrating the distribution of C, O, Pt, and Hg. Scales as indicated in respective images.

Next, average velocity measurements were performed in the presence of 1 ppm Hg^{2+} for exposure duration of 30 min (Figure 6). Time $t = 0$ for each graph represents the average velocities of each fabricated Pt-pollen micromotors in the absence of Hg^{2+} . The mobility of the Pt-Dan micromotors were found to significantly decrease even after only 1 min exposure time. The presence of Hg^{2+} had the least impact on Pt-pollen micromotors after 1 min exposure time (velocity dropped by 58 % from initial value). In contrast, velocities of Pt-Gal, Pt-Lyc, Pt-Cam and Pt-pollen micromotors decreased significantly (>85 %) for the same exposure time. It was also interesting to note that Pt-Cat and Pt-Gal micromotors ceased motion after 30 min exposure time. Pt-Cat micromotors stop moving even after only 5 min exposure to Hg^{2+} . Hg^{2+} ions could attach onto the active sites of sputtered Pt layer which passivated the Pt layer and reduced the surface area of available active sites for the decomposition of H_2O_2 . Furthermore, Hg^{2+} ions can also be attached onto the unsputtered part of micromotors which affects the overall adsorption capacity of the micromotors but not their velocity as it does not block platinum on the surface of the micromotors. In the case of smaller sized micromotors (Pt-Cat and Pt-Gal), Hg^{2+} ions would have passivated the sputtered Pt layer faster due to the smaller surface area available which greatly compromised their activities and mobility. On the other hand, Pt-Cat micromotors showed efficient removal of Hg^{2+} ions after 5 and 30 min which indicates the ability of micromotors to adsorb Hg^{2+} ions not only on the platinum surface but also onto the Pt free micromotors part.

Figure 6. Average velocities of fabricated Pt-pollen micromotors at different time intervals over duration of 30 min in the presence of 1 ppm Hg^{2+} .

Furthermore, Langmuir and Freundlich models have been used in order to describe adsorption parameters. The linear form of the Langmuir model describes the relation between adsorbed Hg^{2+} and equilibrium concentration of micromotors solution:

$$\frac{1}{q_e} = \frac{1}{Q_o} + \frac{1}{Q_o K_l C_e} \quad (\text{Eq. 4})$$

where Q_o (mg/g) is the maximum adsorption coverage capacity for the monolayer when saturation is reached, and K_l (dm^3/mg) is the Langmuir constant which quantifies affinity between Pt-pollen micromotors and Hg^{2+} ions.

The Freundlich exponential equation is given by following equation:

$$\ln q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (\text{Eq. 5})$$

where q_e (mg/g) is the adsorption capacity at equilibrium, K_f and n are Freundlich dimensionless isotherm constants which represent adsorption capacity and adsorption intensity respectively, C_e (mg/L) is the equilibrium time concentration of Hg^{2+} in micromotors solution.

Experimental data obtained for the Langmuir and Freundlich models are presented in Table 1. Adsorption and correlation coefficients presented in Table 1 were calculated from the Langmuir (Figure 7) and Freundlich (Figure 8) isotherm plots for Hg^{2+} adsorption onto Pt-pollen micromotors. As it can be seen from Table 1, better approximation to the experimental values was obtained with the Langmuir equation for all the prepared micromotors except for the Pt-Pop micromotors where the experimental data fit better with the Freundlich equation. Particularly, the correlation coefficient for Pt-Pop micromotors shows a better mathematical fit with the Freundlich equation ($R^2 \sim 0.993$) than the Langmuir equation ($R^2 \sim 0.989$). This data indicates the heterogeneous adsorption surface of Pt-Pop micromotors and adsorption is followed by interactions between adsorbed species.^[54] As the $1/n > 1$ ($1/n \sim 1.223$ for Pt-Pop micromotors) indicates that the bonding energy decreases with surface density.

Taking account that the Langmuir model is based on the fact that adsorption takes place on homogeneous sites of the micromotors,^[55] adsorption of Hg^{2+} ions occurs on the homogeneous surface of the Pt-pollen micromotors (except for Pt-Pop micromotors) by forming a monolayer.

Table 1: Langmuir and Freundlich isotherm parameters for the adsorption of Hg^{2+} by Pt-pollen micromotors. Contact time 2h.

A

Langmuir isotherm			
Adsorbent	Kl (L/mg)	Qo (mg/g)	R²
Pt-Dan microrobots	-0.249	-14.144	0.999
Pt-Sun microrobots	-0.038	-102.041	0.997
Pt-Lot microrobots	-0.884	-3.190	0.992
Pt-Pop microrobots	-0.476	-8.104	0.989
Pt-Gal microrobots	0.376	15.723	0.994
Pt-Pin microrobots	0.281	16.000	0.942
Pt-Cat microrobots	0.321	20.661	0.984
Pt-Cam microrobots	-0.073	-44.248	0.995
Pt-Lyc microrobots	0.021	147.059	0.986

B

Freundlich isotherm			
Adsorbent	K_f	n	R²
Pt-Dan microrobots	34.372	0.863	0.998
Pt-Sun microrobots	24.952	0.982	0.995
Pt-Lot microrobots	276.058	0.535	0.942
Pt-Pop microrobots	68.140	0.818	0.993
Pt-Gal microrobots	32.674	1.111	0.989
Pt-Pin microrobots	31.304	0.881	0.928
Pt-Cat microrobots	55.886	1.004	0.970
Pt-Cam microrobots	16.229	1.011	0.993
Pt-Lyc microrobots	21.115	0.817	0.952

Figure 7: Langmuir isotherm plots for Hg^{2+} adsorption onto Pt-pollen micromotors. Experimental conditions: 0.25 mg/mL pollen grains, Hg^{2+} concentration: 0.25-2 ppm, 3 wt. % H_2O_2 , 1 wt. % SDS, 2 h contact time.

Figure 8: Freundlich isotherm plots for Hg^{2+} adsorption onto Pt-pollen micromotors. Experimental conditions: 0.25 mg/mL pollen grains, Hg^{2+} concentration: 0.25-2 ppm, 3 wt. % H_2O_2 , 1 wt. % SDS, 2 h contact time.

Additionally, we made an effort to investigate the potential application of the Pt-pollen micromotors in biomedicine. It would thus be of fundamental importance to perform cytotoxicity assessments of the fabricated micromotors. Bianco *et al.* previously reported that the shape, size, generation of oxygen reactive particles and morphological properties of the nanomaterials influenced their toxicological profiles.^[56] As such, it was postulated that there would be distinct differences in toxicities between the fabricated micromotors. The fabricated micromotors were also expected to display low toxicities due to the natural availability and intrinsic nature of the pollen grains. Human breast cancer cells (MCF-7) were used in the cytotoxicity assessments of all nine fabricated Pt-pollen micromotors as breast cancer is the most commonly diagnosed cancer among females and accounts for 14 % of total death from cancer.^[57] MCF-7 cell line is widely used in research and represent an essential model for molecular analysis in breast cancer research.^[58] *In vitro* cytotoxicity experiments were carried out by investigating cell viability of MCF-7 cells after 24 hours exposure to different concentrations of the respective fabricated micromotors and the toxicological profiles are presented in Figure 9. Negative and positive control experiments were performed in the absence of micromotors and presence of 10 % dimethyl sulfoxide (DMSO), respectively. The water-soluble tetrazolium salt (WST-8) cell viability assay was used to measure cell viabilities. Bars labelled as ‘0’ correspond to the highest (initial) concentration of micromotors without dilution while bars presented with a dilution factor of ‘16’ represent the smallest concentration of micromotors. All data presented in Figure 9 do not indicate the concentration-dependence toxicity on MCF-7 cells viability. The results obtained show that more than 70 % of MCF-7 cells remained viable after introduction of the highest concentration of each fabricated micromotor. Cell viability did not change drastically upon introduction of the most diluted micromotors solution. In contrast, cell viability dropped by 88 % upon addition of positive control (10 % DMSO). These results affirm the non-toxic profile of each fabricated micromotor.

All external interferences derived from WST-8 assay and micromotors were removed by subtracting from the control experiments. However, the plot shows strong variations in cell viability between different pollen grains (consistent throughout all concentrations) which is understandable taking away from the control different shape, size, morphology and structure. Different pollen grains possess different types of pollen proteins which may destruct intercellular contact proteins by proteolytic actions.^[59,60]

Figure 9. Cell viability percentages of different Pt-pollen micromotors at different dilution factors incubated with MCF-7 breast cancer cells for 24 h measured using WST-8 assay. Negative and positive experiments are displayed for comparison. The concentrations that were tested ranges from $100 \mu\text{g mL}^{-1}$ to $6.25 \mu\text{g mL}^{-1}$.

Having verified the non-toxic nature of the fabricated Pt-pollen micromotors, we further explored and evaluated their potential application as drug carriers. Previous studies showed that pollen grains have great potential to be delivery agent for different drugs.^[61-63] Loading of cargo onto the mobile Pt-pollen micromotors would potentially allow for targeted delivery and release to specified target locations in the human body. With this in mind, we decided to investigate the possibility of loading anti-cancer drug doxorubicin (DOX) onto the fabricated Pt-Lot micromotors. Pt-Lot micromotors were chosen as the representative

micromotors due to their desirable large size and great mobility. Their high surface area makes them more suitable for large quantities drug loading than smaller Pt-pollen micromotors. According to the results presented in Figure 6 where velocity of Pt-pollen micromotors is drastically reduced in the presence of Hg^{2+} ions, it can be expected that velocity of Pt-Lot micromotors reduce after drug loading as well. Taking into account that micromotors should meet biological safety requirements, only small concentration (10×10^{-6} M) of H_2O_2 can be used.^[64,65] Figure 3A exhibits very high average velocity of the Pt-Lot micromotors in both 3 % and 5 % H_2O_2 , so those micromotors have more chance to be propelled in the presence of 10×10^{-6} M of H_2O_2 (even with small velocity) compared to other prepared micromotors. DOX is known to be fluorescently active in the red light spectrum (excitation wavelength: 560-570 nm). Bare Pt-Lot micromotors did not exhibit fluorescence at the specific excitation wavelength as predicted (Figure 10B). Pt-Lot micromotors with bounded DOX (DOX-Pt-Lot) (Figure 10D) gave apparent fluorescence which suggested the successful attachment of DOX onto the surface of Pt-Lot micromotors. Hydroquinone group on DOX molecules could bind onto the sputtered Pt surface of Pt-Lot micromotors.^[66] The Pt-Lot micromotors were thoroughly washed prior to fluorescence measurements to remove interferences from the unbounded DOX molecules to avoid negative results. Additionally, upon comparison of the optical images between bare and DOX-Pt-Lot micromotors (Figures 10A and 10C), there did appear to be any noticeable differences in the structures of the micromotors which would suggest that DOX molecules did not drastically affect the structural integrity of the micromotors. Having determined the successful loading of DOX onto the fabricated micromotors, it is imperative to investigate the contribution of Pt-Lot micromotors towards the delivery of drugs to target cancer cells *via in vitro* cytotoxicity assessments using the MCF-7 cell line. From Figure 10E, both bare Lotus pollen grains and Pt-Lot micromotors did not exhibit any toxicity towards MCF-7 cells. However, introduction of DOX onto Pt-Lot micromotors in presence of 1 % SDS

and 3 % H_2O_2 resulted in the significant decrease in cell viability to 41 %. This is almost comparable to free-moving DOX (positive control) which caused cell viability to drop to ~23 %. The results obtained indicate enhanced efficiency of the Pt-Lot micromotors for DOX delivery to cancerous cells. Pollen grains can potentially be functionalised and fabricated into micromotors to be used in medical applications for non-invasive transport and delivery of drugs to targeted locations effectively.

Figure 10. (A) Optical and (B) fluorescence images of Pt-pollen micromotors. (C) Optical and (D) fluorescence images of DOX loaded Pt-pollen micromotors. (E) Cell viability results of MCF-7 cells for negative control (WST-8 assay), bare Lotus pollen, Pt-pollen micromotors, DOX loaded Pt-pollen micromotors and positive control (10 % DOX). Incubation time of 120 min. Error bars represent standard deviations for each parameter.

5.4 Conclusion

We have successfully fabricated micromotors (micromotors) derived from natural pollen grains for environmental remediation and biomedical applications. Pollen grains (Cattail, Lotus, Galla, Dandelion, Poppy, Sunflower, Camellia, Lycopodium and Pine) incorporated with Pt exhibited an excellent approach towards fabrication of micromotors in large quantities. The results demonstrated excellent micromotors propulsion by bubbles formation from the decomposition of hydrogen peroxide fuel. It was also found that Pt-pollen micromotors exhibit great adsorption towards Hg^{2+} ions in aqueous solution. Maximum adsorption efficiency of all fabricated micromotors were achieved 120 min after exposure to 1 ppm Hg^{2+} (>80 %). The velocities of the fabricated micromotors decreased significantly in the presence of 1 ppm Hg^{2+} during the experimental duration. The fabricated micromotors were found to be non-toxic from cytotoxicity assessments which expand their potential use in biomedicine and drug loading. Additionally, Pt-pollen micromotors were found to be efficient carriers of doxorubicin (DOX) drug which open potentially to be transported to target sites throughout the body.

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Chapter 6

Effect of different tailoring metals on the motion of light-activated TiO₂ Janus micromotors

6.1 *Introduction*

6.2 *Experimental part*

6.3 *Results and discussion*

6.4 *Conclusion*

6.5 *References*

6.1 Introduction

The artificial motion and control of microscale devices powered by light sources has stimulated significant research in this area in the last decade.^[1–3] Special attention is focused on studying fuel-free micromotors, where propulsion can be regulated and monitored only by light stimuli.^[4–6] Making micromotors that do not require extra fuel (H_2O_2 , Br_2 solution, acidic media etc.) for their propulsion is highly desired and recommended.^[7] Unlike self-propelled micromotors,^[8–13] light-driven micromotors face a very challenging issue of achieving high velocities when moving, but this can be accomplished using various combinations of materials with excellent catalytic performances.^[14–17] According to earlier research by Jang *et al.*, Au/B- TiO_2 Janus micromotors show multi-wavelength responses from UV to visible light.^[16] Similar observation of micromotors propulsion by visible light was made when utilising BiOI and Cu_2O materials.^[5,18] Ren *et al.* successfully demonstrated that the motion of Au- TiO_2 Janus micromotors is strongly dependent on light intensity. In other words, their velocity can be controlled not only by adding H_2O_2 fuel but also by increasing light intensity.^[2] In addition to TiO_2 , hematite (Fe_2O_3)^[19] and BiVO_4 ^[20] have been used as bases to fabricate earlier generations of light-powered micromotors, due to their wide band gap semiconducting properties. Nowadays, photoactive quantum dots are attracting a great amount of attention amongst scientists for the preparation of visible-light-driven micromotors.^[21,22]

ZnO/Pt micromotors,^[23,24] ZnO/Pt microrods^[25] and ZnO microrockets with coated Ni layer^[1] have also been demonstrated as excellent photoactive materials, exhibiting autonomous motion under UV light as well as steerable motion.

Herein we study how different catalytic metals affect the velocities of TiO_2 micromotors in the presence of UV light in pure water under fuel free conditions. Pt has traditionally been the metal of choice for the fabrication of micromotors although it is expensive, not readily available nor abundant.^[26] Nowadays, researchers have looked at

different metals to substitute Pt and have found that Fe and Au demonstrate comparable performance. Cai *et al.* reported that Fe is an efficient photocatalytic metal for micromotor fabrication.^[27] The study proved excellent directional Fe-TiO₂ micromotors movement due to thin magnetic layers of Fe on the surface of TiO₂ microspheres. Ren *et al.* reported higher velocity of Au-TiO₂ Janus micromotors in a mixture of water and dye solution than in clean water solutions. They additionally inserted Ni layers between the TiO₂ and Au layers to achieve directional magnetic guidance of the micromotors.^[28]

However, to date there have not been a comparative study conducted to find the best performance of light-driven micromotors. In this paper, we analyse the structure, motion patterns, velocities and overall behaviour of Janus micromachines made of different catalytic metals (Pt, Au, Ag, Fe, Cu) and TiO₂. The light driven asymmetrically designed Janus micromotors were constructed of highly stable, bare TiO₂ microparticles covered by metals (Pt, Cu, Fe, Ag and Au) on one half hemisphere. These materials absorb light energy and thereafter undergo a photocatalytic reaction when exposed to UV irradiance. Janus micromotors are able to propel by the generated electrolyte concentration gradient in the aqueous media. Ions can be produced in photochemical reactions to form electrolyte gradients and therefore their movement can be explained by a self-electrophoresis mechanism.^[29,30]

6.2 Experimental part

Materials: 99.5 % ethanol was obtained from Fischer (Singapore). 99 % sodium chloride, 97 % tetrabutyl titanate and sodium dodecyl sulphate (SDS) were obtained from Sigma Aldrich (Singapore). Iron, platinum, nickel, silver, gold and copper sputtering targets (57 × 0.1 mm) were purchased from Ted Pella, Inc (USA).

Apparatus: Centrifugation, sonication and sputtering procedures were performed by a Beckman Coulter Allegra 64R centrifuge, a Fisherbrand FB 11203 ultrasonicator and a JEOL

JFC-1600 Auto Fine Coater sputtering machine, respectively. Videos were captured by a Nikon Eclipse TE 2000-E optical microscope and their analyses were performed by the Nikon NIS-Elements software connected to the optical microscope. UV light for micromotors activation was generated by a mercury lamp. SEM patterns were captured with a JEOL JSM7600F scanning electron microscopy (Japan). An EDX detector was equipped with SEM for elemental analysis. μ Autolab III electrochemical analyser (Eco Chemie B.V., Utrecht, The Netherlands) connected with the software NOVA version 1.8 (Eco Chemie) was used for the Tafel based experiments.

Synthesis of Janus micromotors: Briefly, 0.2 mL of sodium chloride was dissolved in 50 mL ethanol and stirred for 5 min. Thereafter, 0.85 mL of tetrabutyl titanate that had been used as precursor for the synthesis of TiO_2 microspheres, was added into the solution. When emulsion was reached, it was incubated in the dark for 1 day. Subsequently, TiO_2 microspheres were centrifuged at 10 000 rpm for 15 min and washed with 10 mL of ethanol and water three times each. At the end of this preparation procedure, TiO_2 microspheres were sintered in the oven (2 h, 450 °C). TiO_2 microspheres with 2.27 μm average diameter were used as the template for fabrication of light-driven Janus micromotors: Pt/ TiO_2 , Cu/ TiO_2 , Fe/ TiO_2 , Ag/ TiO_2 and Au/ TiO_2 . 300 μm of TiO_2 microspheres dispersed in ethanol ($C = 1 \text{ mg mL}^{-1}$) were spread onto a glass slide and dried. The samples were sputtered with a thin layer of platinum, copper, iron, silver and gold, for 200 s and 40 mA, separately. The fabricated micromotors were released from the glass slide immersing in 10 mL of ethanol, ultrasonicated for 30 s and dispersed into 2 mL of Milli-Q water.

Motion experiments: To determine the relationship between the velocity of the Pt/ TiO_2 , Cu/ TiO_2 , Fe/ TiO_2 , Ag/ TiO_2 , Au/ TiO_2 micromotors and the sputtered target on one hemisphere of TiO_2 particles, all fabricated micromotors were propelled under the UV light (light emission: 360–370 nm) without adding any other fuel.

Electrochemical Potential Measurement: Mixed potentials of metal electrodes (Pt, Cu, Fe, Ag and Au) and illuminated TiO₂ electrode were measured and presented by Tafel plots. Electrochemical potential experiments were performed in a 2 mL electrochemical cell in a three-electrode system. Ag/AgCl, platinum and GC electrodes functioned as reference, auxiliary and working electrodes, respectively.

6.3 Results and discussion

Metal/TiO₂ spherical Janus micromotors are fabricated by a simple coating mechanism which includes sputtering of different metals (Pt, Cu, Fe, Ag and Au) onto the surface of one hemisphere of TiO₂ microparticles. More preparation details are provided in the experimental section. Upon exposure to UV irradiation, electrons are generated on the TiO₂ side of the micromotors and subsequently transferred to the metal part of the micromotors (Scheme 1). During this procedure, two half reactions are occurring: (1) water oxidation on the TiO₂ side, which produces protons and (2) proton reduction on the metal side. The resulting H⁺ gradient generates local electric field towards the TiO₂ part. Consequently, metal/TiO₂ Janus micromotors show autonomous propulsion with the TiO₂ side forward by a self-electrophoresis mechanism. Although it is well known that bare TiO₂ has high photocatalytic activity, further coating with of Pt, Cu, Fe, Ag and Au metals drastically enhances their catalytic performance in stationary systems.^[31]

Scheme 1: Schematic of tailoring metal/TiO₂ micromotors exposed to UV light

We firstly discuss the characterisation and elemental composition of all the prepared Janus micromotors. Figure 1 shows the micromotors surface morphology and size that was measured by scanning electron microscopy (SEM). It can be seen from Figure 1 that all of the prepared micromotors have a unique shape (average diameter of 2.2 μm) and that upon coating with different metals, the Janus micromotors structure is not disturbed. Additionally, energy dispersive X-ray spectroscopy (EDX) data confirms the presence of Pt, Cu, Fe, Ag and Au metals on one of the hemispheres (Figure 1).

The scope of this study is to show that TiO₂ Janus micromotors have different motion velocities depending on the sputtered metals used for their fabrication. Correlated to this, we turned our attention to the mobility of TiO₂ Janus micromotors sputtered with thin layers of Pt, Cu, Fe, Ag and Au metals. First, it was observed that bare TiO₂ microparticles show Brownian behaviour under UV exposure to 40 mW/cm². In contrast, prepared Pt/TiO₂, Cu/TiO₂, Fe/TiO₂,

Ag/TiO₂ and Au/TiO₂ spherical Janus micromotors display various velocities from 2.4 – 8.9 $\mu\text{m/s}$ under the same UV intensity (Figure 2). Control experiments in condition when UV radiation was ceased and with bare TiO₂ microparticles exposed to UV irradiation showed the same non-moving behaviour (Figure 2). Under 40 mW/cm² light intensity, Pt/TiO₂ Janus micromotors exhibit two times faster velocities than Fe/TiO₂ Janus micromotors (average $v \sim 4.27 \mu\text{m/s}$), and about four times faster movement compare to Au/TiO₂ Janus micromotors (average $v \sim 2.35 \mu\text{m/s}$). Cu/TiO₂ and Ag/TiO₂ Janus micromotors move with following average velocities: 5.03 $\mu\text{m/s}$ and 3.62 $\mu\text{m/s}$, respectively.

Figure 1. Scanning electron microscope (SEM) images and corresponding energy dispersive X-ray (EDX) images of Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ micromotors.

Figure 2. Velocity of Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂, Au/TiO₂ Janus micromotors and TiO₂ microparticles under UV light (red bars), non-moving behaviour of Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ Janus micromotors when the UV light is off (blue bars). Data were collected from 25 independent measurements to obtain the average velocity of micromotors. Error bars are standard deviations.

To validate which is the dominant mechanism resulting in the propulsion of the TiO₂–metal micromotors, control experiments were conducted using polystyrene (PS) particles instead of TiO₂ particles. Under UV light, no obvious directional movement was observed for PS–metal Janus particles, while the TiO₂–metal micromotors were moved rapidly. Another control experiment can be performed in NaCl solution or any other salt environment. TiO₂–metal Janus micromotors stop moving in the presence of 10 mM salt which further confirms the electrophoresis mechanism.^[2]

Next, the underlying reason for the difference in the velocities when different metals are deposited on TiO₂ was investigated. ^{[30],[2]} There are two possible reasons given the reactions which are powering these micromotors. The potential difference between two halves of the Janus particles and turnover on the surface due to the surface roughness and catalysis of

the reaction. The potential differences between metal electrode (Pt, Cu, Fe, Ag and Au) and illuminated TiO₂ electrode were measured (Figure 3). When comparing velocities, it can be noted that the over potential difference grows from Fe to Cu (via Ag and Au) to Pt (in line with standard chemical potentials) and in line with increasing velocities of Janus metal/TiO₂ micromotors from Fe to Cu and to Pt. However, there is an exception of Ag and Au, which show the lowest velocities. This is likely due to their poor catalysis of the hydrogen evolution reaction. [32-34]

Figure 3. A Tafel plot of the Fe, Cu, Ag, Au, Pt and TiO₂ with 40 mW/cm² light illumination

Figure 4 illustrates the motion of individual micromotors. As expected from the obtained results, Pt/TiO₂ micromotors travelled the longest distance compared to other prepared micromotors over the same time observation and in all formed shapes. Figure 6 demonstrates the time-lapse images of doublet and triplet Janus micromotors to support the velocity data presented in Figure 5.

A very interesting observation was noted. The Janus micromotors form dimers and trimers. Additionally, the formation of dimer and trimer micromotors chains were noted when

the UV radiation was turned on. Figure 5A and 5B show the average velocities of Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ dimer and trimer micromotors formed by individual single micromotors. This coupling was due to interaction between the sized-matched UV active micromotors and passive TiO₂ microparticles which stayed unsputtered in the solution.^[35] Simmchen et.al previously reported the emergence of clusters based on the pumping interaction between active Cu/TiO₂ and passive SiO₂ associated with fluid flow.^[36] Under UV irradiation, these assemblies exhibit directional motion and the highest velocity was reached by Pt/TiO₂ dimer and trimer micromotors compared to the rest of the prepared motors.

Figure 4. Time-lapse images of (A) Pt/TiO₂, (B) Cu/TiO₂, (C) Fe/TiO₂, (D) Ag/TiO₂ and (E) Au/TiO₂ Janus singe micromotor over 2 s. All measurements were done in pure water.

Chain formation decreased the Pt/TiO₂ and Cu/TiO₂ micromotors' velocities to 6.67 $\mu\text{m/s}$ and 4.89 $\mu\text{m/s}$ for double chain micromotors and 6.67 $\mu\text{m/s}$ and 2.91 $\mu\text{m/s}$ for triple chain micromotors. Interestingly, Ag/TiO₂ and Au/TiO₂ assembled chain micromotor formations exhibited the same or even higher velocities compared to the corresponding single micromotors. Additionally, the high surface area makes them more suitable for large quantities of drug loading and even co-delivery of two or more medicines than single micromotors. This notation

is very important for future applications of light powered micromotors for potential applications as capture agents for toxic compounds.^[37]

Figure 5. Velocity of Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ (A) dimer (B) trimer micromotors chains.

Figure 6. Time-lapse images of Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ dimer and trimer micromotors chains over 2 s.

6.4 Conclusion

Pt, Cu, Fe, Ag and Au metals were investigated for the preparation of UV-activated micromotors with a TiO₂ microsphere as the micromotor support. Velocities of micromotors were investigated and thus compared under the same experimental conditions. Pt/TiO₂, Cu/TiO₂, Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ Janus micromotors exhibited UV light-induced motion through a self-electrophoresis mechanism in pure water with decreasing average velocities. It can be concluded that the velocity of the micromotors strongly depends on the metal type sputtered on the microsphere surface. In addition, it was found that all types of prepared individual Janus micromotors are able to form dimer and trimer chain assemblies under UV irradiation. Fe/TiO₂, Ag/TiO₂ and Au/TiO₂ dimer and trimer micromotors exhibit similar or even higher velocities than their corresponding single micromotors under UV irradiation, which indicate their potential application for environmental remediation.

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Chapter 7

Ageing Influences the Performance of Self-Propelled and Light-Driven Micromotors

7.1 Ageing Influences the Performance of Tubular Platinum Micromotors

7.2 Corrosion of Light Powered Pt/TiO₂ Micromotors

7.3 Conclusion

7.4 Experimental part

7.5 References

7.1 Ageing Influences the Performance of Tubular Platinum Micromotors

Autonomous self-propelled nano and micromotors are in the forefront of materials research. The micromachines are typically prepared in batches, stored and subsequently used. We show here that storage of platinum tubular catalytic micromotors in water causes their corrosion which results into the lower mobility and performance. This has important implications for the construction and storage of these autonomous micromotors.

7.1.1 Introduction

Self-propelled nano and micromachines have garnered great interest among researchers and they are an emerging paradigm in nanotechnology.^{1,2} Nano/micromotors have attracted immense research attributed to their ability to travel and propel over considerable distances. In recent years, there are many efforts towards the modification and functionalization of tubular micromotors for practical on-site applications especially in medicinal applications such as drug delivery,^{3–7} environmental remediation,^{8–11} sensing applications^{12–14} and microfluidic devices.^{13,15} Presently, researchers have fabricated different types and shapes of tubular self-propelled micromotors with great control on their physical characteristics.^{16–19} However, despite the progress in the applications of micromotors, there is insufficient knowledge available on their stability and durability over prolonged periods of time. It was shown previously that Cu/Pt micromotors undergo corrosion in the running H_2O_2 solution in the presence of surfactant.²⁰ Corrosion effects may pose to be a major obstacle, especially so in biological studies with harsh experimental conditions, where it reduces and inhibits the functions of the fabricated micromotors.^{21–23} To counter this problem, micromotors have been fabricated with modified outer surfaces to prolong their working capabilities and efficiency. Various physical properties such as the lengths and diameters at different parts of the tubular micromotors, as well as the surface morphology, play important roles in the nonlinear dynamics outcome of micromotors and are known to greatly affect and influence their motion.^{24,25} The

influence of ageing of the micromotors in ‘storage’ conditions, the difference between the performance of ‘fresh’ and ‘old’ micromotors shall have a significant impact on the reliability and reproducibility of the micromotors. Despite that, the fundamental understanding on the stability of platinum micromotors over a prolonged period of time is still unexplored and not well documented. Herein, we study the influence of ageing of micromotors on their structural stability and motion.

7.1.2 Results and discussion

We set forth to study the effect of ageing of Pt tubular micromotors on their viability and mobility. We will show that extended storage of Pt catalytic tubular motors in water solution decreases their mobility. From our previous findings, the presence of surfactant was found to promote corrosion of micromotors.²⁰ With this information in hand, surfactant (SDS) was not added into the storage solution to eliminate additional external factors which promote corrosion of micromotors.

Velocities of freshly prepared and 28 days old platinum micromotors were measured for comparison purposes. The average velocities measured will provide quantitative information to better compare the efficiency of freshly made and ‘old’ micromotors. The average velocities of micromotors were found to decrease over the duration of 28 days. The average velocity of micromotors in 3% hydrogen peroxide at day 1 was 91.1 $\mu\text{m/s}$ and it decreased to 40.1 $\mu\text{m/s}$ after 28 days of storage (day 28), as shown in Figure 1. This decrease in velocity correlates to a decrease in efficiency of the tubular micromotors by 46% over the 28 days period. The experiment was repeated with a higher hydrogen peroxide concentration to reaffirm our findings. A similar trend was observed with 5% H_2O_2 concentration over 28 days (Figure 2). The average velocity of micromotors decreased from 117.5 $\mu\text{m/s}$ (day 1) to 45.9 $\mu\text{m/s}$ (day 28). A similar decrease in efficiency of the micromotors can be inferred with a decrease in average velocity by 61%. The standard error of mean obtained is the average of 10

different days during the experimental duration where 30 different micromotors were analysed per day of analysis. The error bars obtained were thus a result of same day repeatability of measurements. When applying the one-way ANOVA statistical analysis with a confidence interval of 0.95, $p < 0.05$ was obtained for both concentrations which suggests that the mean values of the average velocities obtained at the different days of analysis were different.

Upon comparison of both hydrogen peroxide concentrations, one noticeable trend was the higher average velocities obtained with 5% H_2O_2 . This result is in accordance with the fact that the velocities of micromotors increase with H_2O_2 concentration because of the increase rate of decomposition of H_2O_2 by platinum.^{19,26} The differences in morphologies between the two sets of tubular micromotors (day 1 and day 28) affect the efficiency and play a significant role towards the reproducibility of experimental analysis. It is worth to note that the significant decrease in average velocity of the tubular micromotors was observed 4 days post fabrication in both hydrogen peroxide concentrations. This suggests an affordable shelf-life of about 4 days post fabrication of micromotors for reproducible results with high efficient micromotors.

Figure 1. Change in average velocities of Pt tubular micromotors in 3% hydrogen peroxide over a period of 28 days. Data was collected from 30 independent measurements to obtain the average velocity. Experimental conditions: 0.25 wt% SDS at room temperature (25 °C).

Figure 2. Change in velocities of Pt tubular micromotors in 5% hydrogen peroxide over a period of 28 days. Data was collected from 30 independent measurements to obtain the average velocity. Conditions in all experiments: 0.25 wt% SDS at room temperature (25 °C).

We next investigated the morphology changes of the platinum tubular micromotors upon fabrication (day 1) and 28 days after fabrication (day 28). This will provide visual insights to the possible reasons for the decreased mobility of the micromotors. The dimensions and surface morphology of the different micromotors were analysed and compared using scanning electron microscopy (SEM). Figure 3(A) and (B) shows the SEM images of micromotors for the different time periods. Initial visual comparisons of the SEM images showed that the micromotors retained their tubular configuration over the duration of study. However, upon comparing Figures 3B(i) and B(ii), there are apparent visual differences to the surface morphologies of the two sets of micromotors. As compared to the freshly-prepared micromotors (Figures 3A(i) and 3B(i)), it was observed that the outer surface of ‘old’ micromotors (Figure 3A(ii) and 3B(ii)) appear highly corroded which would imply that the interior is corroded as well. Upon analysis of the dimensions, it was noted that the average length of micromotors decreased from $(16.1 \pm 1.7) \mu\text{m}$ at day 1 to $(13.9 \pm 0.7) \mu\text{m}$ at day 28. It was interesting to note that the diameters at the opposite ends of the tubular micromotors

remained almost unchanged over the 28 days duration; $(5.7 \pm 1) \mu\text{m}$ and $(4.3 \pm 0.8) \mu\text{m}$ at day 1 vs. $(5.5 \pm 0.7) \mu\text{m}$ and $(4.3 \pm 0.6) \mu\text{m}$ at day 28. The data was acquired by measuring the three different parameters (length and diameters on opposite end) of 30 samples where the average was then obtained. Whilst shortening of the motors is small, the corrosion of their surface is significant.

From the energy dispersive X-ray spectroscopy (EDS) images obtained (Figure 3C) for that of platinum, the percentage of platinum decreases by 55.9 wt% over the period of storage. The decrease in the size of the micromotors over the time period can be attributed to the oxidation of platinum by oxygen gas dissolved in deionized water resulting in the formation of platinum salt crystals. The crystals formed could have dissolved or flaked off the micromotors thus contributing to the loss of elemental platinum. This could have accounted for the corrosion effects observed in the SEM images obtained (Figures 3A and 3B). As a result, the functionalization of platinum micromotors is performed to not only enhance motion, it also aids in reducing the rate of corrosion which in turn increases the life span of micromotors.²⁷

Figure 3. Scanning electron microscopy (SEM) images of (i) freshly-prepared and (ii) old platinum tubular micromotors at magnification of (A) 10 000 X and (B) 5 000 X. (C) Energy dispersive X-ray (EDX) spectroscopy maps showing distribution of platinum of (i) freshly-prepared and (ii) old platinum tubular micromotors. Scale as indicated in the respective images.

7.2 Corrosion of Light Powered Pt/TiO₂ Micromotors

Catalytic light-powered micromotors have become a major focus in the current autonomous self-propelled micromotors research. The attractiveness of such machines stems from the fact that these motors are liquid “fuel-free”, with their motion modulated by irradiation. It is assumed that as catalytic micro/nanoparticles, they can function eternally. Herein, we study corrosion of these micromotors. In particular, the reusability and reproducibility in performance of fabricated Pt/TiO₂ micromotors stored over duration of weeks was studied. It was found that despite retaining the Janus structure, the surface morphology of the fabricated micromotors greatly changed with a decrease in composition of titanium dioxide and platinum and a corresponding increase in Ti and Pt in the storage solution. The motion of the fabricated micromotors decreased drastically under ultraviolet (UV) irradiation (in fuel-free mode) after just 5 days of storage and these machines eventually stopped moving after 15 days. However, in the presence of 5 wt. % hydrogen peroxide (H₂O₂), the average velocities remained almost constant during the 15 days. The propulsion in H₂O₂ is governed by its decomposition at Pt, whilst the fuel-free motion is based on Pt/TiO₂ heterojunction. Henceforth, it is strongly recommended to prepare fresh Pt/TiO₂ micromotors and avoid using micromotors stored more than 5 days for consistent performance and reproducible results in fuel-free mode.

7.2.1 Introduction

Autonomous self-propelled and self-powered micro and nanomachines are in the forefront of materials research. Light-driven micromotors^{28–30} are an upcoming topic of research where most photocatalytic propelled micromotors consist of TiO₂ nanoparticles^{31–34} and nanotubes.^{35,36} A photocatalytic propulsion mechanism of micromotors is highly favourable in comparison to chemically-powered micromotors^{37–44} as they exhibit autonomous motion with response to an external light source without the need for additional fuel sources

(such as H_2O_2)⁴⁵ The motion of such micromotors can be controlled by adjusting the light intensities and wavelengths used.⁴⁶

Pt/TiO₂ micromotors have been prepared that are propelled by self-electrophoresis.^{47–49} In the presence of ultraviolet (UV) irradiation, electron transfer is generated between TiO₂ and the Pt heterojunction. There is an electric field generated that causes the movement of electrons through the micromotors. Consequently, catalysis takes place on the respective Pt and TiO₂ surfaces, which either produces or utilises hydrogen ions, thus generating a diffusion gradient between the two surfaces. This is followed by the migration of protons which propel the micromotors in the opposite direction (Scheme 1).³⁴ It is assumed in the literature that these micromotors are virtually eternal (in lab experiments timescales).

Despite the advancement and research progress on light-driven micromotors in the last decade, fundamental studies on morphological stability and shelf-life have not been widely investigated. With regards to tubular Pt based bubble-recoil powered micromotors, Zhao *et al.* demonstrated that Cu/Pt micromotors undergo different rates of corrosion when stored in three different media: pure water, sodium dodecyl sulphate (SDS) and H_2O_2 solutions.⁵⁰ Similar observations were also noted and reported with Pt tubular micromotors stored in aqueous solution.⁵¹ It is known that metals are subjected to corrosion and oxidation where atoms release electrons to form ions. Corrosion processes can be very complex and highly dependent on the properties of the metals (melting point, hardness, corrosion resistance). Therefore, this study is devoted to investigating the stability and potential corrosion of Janus micromotors, particularly Pt/TiO₂ micromotors. There is the possibility of corrosion of both TiO₂ and Pt, one of them or neither one. This study is aimed at determining the stability and degradation of Pt/TiO₂ Janus micromotors stored in water over prolonged periods (15 days). Surface morphological analysis was performed to monitor any apparent surface changes to Pt/TiO₂ micromotors during the 15 days experimental duration. The storage water was also analysed to determine the extent of

corrosion from the formation of ions from the components of the fabricated micromotors. Average velocities under UV irradiation were measured to provide visual perspectives on the reusability and stability of fabricated micromotors for reproducible results. It is shown that indeed the Pt/TiO₂ micromotors are not indefinitely long-lived and instead undergo significant corrosion.

7.2.2 Results and discussion

The Pt/TiO₂ Janus micromotors were fabricated by sputtering a Pt layer on one half of the TiO₂ microparticles. These micromotors are photocatalytic and propel in water under UV irradiation by self-electrophoresis (Scheme 1A).^{52–54}

Scheme 1. (A) Schematic representation of Pt/TiO₂ Janus micromotors propelled in water by self-electrophoresis in the presence of ultraviolet (UV) irradiation. (B) Hypothetical degradation of Pt/TiO₂ Janus micromotors and deterioration in motion performance during an experimental duration of 15 days.

Studies on shelf-life of the fabricated micromotors were performed to analyse any apparent differences in their velocities and performances over prolonged storage. It is postulated that longer storage duration of Pt/TiO₂ Janus micromotors in water would influence

their surface morphology and motion performance (Scheme 1B). Herein, we set forth to investigate the influence of ageing and shelf storage of Pt/TiO₂ Janus micromotors over a duration of 15 days. The Pt/TiO₂ Janus micromotors were stored in Milli-Q deionised water at room temperature (23 °C) in plastic Falcon tubes.

Characterization was first performed to understand the surface morphology of the Janus micromotors immediately after fabrication (Day 1) and at subsequent 5 days intervals of the experimental duration (Day 5, Day 10, Day 15) by scanning electron microscopy (SEM). The surface morphologies and diameters of the Pt/TiO₂ micromotors were compared and analysed (Figure 4). Figure 4A depicts a freshly prepared micromotor while Figures 4B-D represents 5, 10 and 15 days old Pt/TiO₂ Janus micromotor, respectively. Comparing all micrographs obtained, the spherical shapes of Janus micromotors appear unchanged and unperturbed over the experimental duration of 15 days storage in water. Consistent diameters of $\sim 2.27\ \mu\text{m}$ were also obtained for all micromotors. However, closer inspection and comparison of the micromotors surface morphologies revealed significant apparent morphological differences. Stored micromotors analysed 5, 10 and 15 days post fabrication (Day 5, Day 10 and Day 15 micromotors) had rougher surface morphologies (Figures 4B to 4D) as compared to freshly prepared (Day 1) micromotors which possessed a smoother surface (Figure 4A). Day 10 and Day 15 micromotors have dimple-like features on the surface morphologies which would suggest significant chemical reactions taking place on the surfaces of the micromotors over prolonged period of storage in deionised water. The differences in morphological structure can be explained by dramatic corrosion and oxidation of both TiO₂ and Pt over the duration of 15 days stored in water.

Furthermore, energy dispersive X-ray spectroscopy (EDS) was performed to confirm the elemental differences of the micromotors over the experimental duration (Figure 5). Based on the EDS elemental mappings obtained, it was determined that the atomic percentages

dropped from 57.18 % to 22.04 % for Ti and 3.97 % to 2.47 % for Pt after an experimental duration of 15 days (Table 1). The decrease in percentages of Ti and Pt on the surfaces of the fabricated micromotors 15 days after fabrication indicated the loss of these elements that were released into the storage water due to the formation of oxidised species from dissolved oxygen gas in water. This is corroborated with the hike in percentage of surface oxygen present which increased from 38.85 % for Day 1 micromotors, 52.61 % Day 5 micromotors, 61.67 % Day 10 micromotors to 75.49 % for Day 15 micromotors.

Figure 4. Scanning electron microscopy (SEM) images of (A) Day 1, (B) Day 5, (C) Day 10 and (D) Day 15 Janus Pt/TiO₂ micromotors. Scale bars, 1 μ m.

Figure 5. Energy dispersive X-ray spectroscopy (EDS) images of (A) Day 1, (B) Day 5, (C) Day 10, and (D) Day 15 Janus Pt/TiO₂ micromotors with corresponding elemental mappings of Ti, O and Pt. Scale bars represent 2.5 μm.

Table 1. Atomic percentages of Ti, Pt and O of Day 1, Day 5, Day 10 and Day 15 micromotors obtained from energy dispersive X-ray spectroscopy (EDS).

Elements	Day 1 (at. %)	Day 5 (at. %)	Day 10 (at. %)	Day 15 (at. %)
O	38.85	52.61	61.67	75.49
Ti	57.18	43.90	35.77	22.04
Pt	3.97	3.49	2.56	2.47
Total:	100.00	100.00	100.00	100.00

In addition, inductively coupled plasma-mass spectrometry (ICP-MS) was performed to analyse Ti and Pt concentrations in the storage water of Day 1 and Day 15 micromotors. The storage solution was first filtered through 0.3 μm pore membranes (much smaller in diameter than the micromotors, which are on average 2.27 μm) to separate the micromotors and the filtrate was analysed by ICP-MS. Taking into account that the diameters of the micromotors are about 10 times larger than the membrane pores, all micromotors would be retained in the filter. Comparing the ICP-MS results on Day 1, Day 5, Day 10 and Day 15 of the storage solutions revealed an increase in concentrations of Pt and Ti in water 15 days post-fabrication (Table 2). The amount of Ti increased by almost twice (from 3.16 to 6.81 ppb) while Pt increased from 0.24 to 0.60 ppb after 15 days. The significant increase of Pt in storage solution could be attributed to the formation of platinum salt crystals from oxidation, which flaked off the surface of the micromotors. It is thus evident that Ti and Pt corroded from the fabricated Janus micromotors into water thus causing the rough morphology obtained (Figure 4C and 4D).

Table 2. Concentrations of Ti and Pt in Day 1, Day 5, Day 10 and Day 15 micromotors storage water obtained from inductively coupled plasma-mass spectrometry (ICP-MS).

Elements	Day 1 (ppb)	Day 5 (ppb)	Day 10 (ppb)	Day 15 (ppb)
Ti	3.16	5.25	5.59	6.81
Pt	0.24	0.36	0.45	0.60

Having understood the changes in the surface morphology and elemental compositions of the micromotors, we next analysed the changes in mobility and performances of the fabricated Janus micromotors to ascertain their reproducibility and reliability of results obtained over the duration of 15 days. It was previously reported that H_2O_2 and surfactants promote corrosion on the surfaces of micromotors.²⁰ The absence of these variables in the

storage solution would eliminate external influences thus providing a fair comparison on the unperturbed ageing of micromotors.

The average velocities of Pt/TiO₂ micromotors were investigated at 5 days intervals throughout the 15 days experimental duration (Figure 5). Freshly fabricated micromotors (Day 1) had an average velocity of 8.89 $\mu\text{m s}^{-1}$ (corresponds to velocity of 100 %) that dropped significantly at corresponding 5 days interval measurements. The average velocity of Day 5 micromotors saw a significant decrease by 52 % to 4.26 $\mu\text{m s}^{-1}$. The average velocity further decreased after 10 days by 57 % to 3.82 $\mu\text{m s}^{-1}$ and the micromotors eventually stopped moving after 15 days. Therefore, the efficiency and mobility of Pt/TiO₂ Janus micromotors drastically reduced during the 15 days. Control experiments were performed by storing the micromotors in light sensitive bottles, an oxygen-free environment and light sensitive bottles in an oxygen-free environment. Micromotors stored in light sensitive bottles, an oxygen-free environment and in light sensitive bottles in an oxygen-free environment showed smaller changes in average velocities at Day 5 (35 %, 37 % and 32 %), respectively.

Interestingly, micromotors stored in light sensitive bottles under oxygen-free environment displayed a similar decrease in average velocities (56 %) as micromotors stored in standard conditions at Day 10. Micromotors stored in light sensitive bottles displayed the highest average velocity over the 15 days experimental duration (4.53 $\mu\text{m s}^{-1}$).

This data obtained would indicate better reproducibility of results for micromotors 15 days after preparation when stored in control conditions in contrast to standard conditions. Interestingly, micromotors stored in light sensitive bottles in an oxygen-free environment displayed very small translational motion with a drastic increase in rotational motion at Day 15.

Figure 6. Average velocities of Pt/TiO₂ Janus micromotors at different analysis days (Day 1, Day 5, Day 10, and Day 15) under UV irradiation in water (violet bars). Control experiments were measured in light sensitive bottles (red bars), oxygen-free environment (green bars) and light sensitive bottles in oxygen-free environment (blue bars). Standard deviations were obtained from 30 independent measurements.

Additional morphological characterisation and elemental composition analysis for all control experiments were performed and presented in Figure 7 and Table 3. The obtained EDX data (Table 3) have high variances that could possibly be attributed to the fact that micromotors themselves are not uniform in composition. We monitored the leach rates under different storage conditions with ICP-MS and the results of these experiments fit very well with the motion study (Table 4).

Figure 7. Scanning electron microscopy (SEM) and corresponding energy dispersive X-ray spectroscopy (EDS) images of (A) Day 1, (B) Day 5, (C) Day 10 and (D) Day 15 Janus Pt/TiO₂ micromotors stored in (a) light sensitive bottles, (b) an oxygen-free environment and (c) light sensitive bottles in an oxygen-free environment. SEM and EDS images of Day 1 which corresponds to Figure 7A are common to all three studied cases. Scale bars, 2.5 μm

Table 3. Atomic percentages of Ti, Pt and O of Day 1, Day 5, Day 10 and Day 15 micromotors stored in different conditions and obtained from energy dispersive X-ray spectroscopy (EDS). (A) Light sensitive bottles, (B) an oxygen-free environment and (C) light sensitive bottles in an oxygen-free environment.

A

Elements	Day 1 (at. %)	Day 5 (at. %)	Day 10 (at. %)	Day 15 (at. %)
O	38.85	75.62	74.99	79.13
Ti	57.18	19.01	24.17	18.62
Pt	3.97	5.37	0.84	2.25
Total:	100.00	100.00	100.00	100.00

B

Elements	Day 1 (at. %)	Day 5 (at. %)	Day 10 (at. %)	Day 15 (at. %)
O	38.85	73.76	74.51	73.99
Ti	57.18	22.27	23.74	22.31
Pt	3.97	3.96	1.75	3.70
Total:	100.00	100.00	100.00	100.00

C

Elements	Day 1 (at. %)	Day 5 (at. %)	Day 10 (at. %)	Day 15 (at. %)
O	38.85	74.49	73.57	80.72
Ti	57.18	22.47	23.50	16.26
Pt	3.97	3.04	2.92	3.02
Total:	100.00	100.00	100.00	100.00

Table 4. Concentrations of Ti and Pt in Day 1, Day 5, Day 10 and Day 15 micromotors stored in different conditions and obtained from inductively coupled plasma-mass spectrometry (ICP-MS). (A) Light sensitive bottles, (B) an oxygen-free environment and (C) light sensitive bottles in an oxygen-free environment.

A

Elements	Day 1 (ppb)	Day 5 (ppb)	Day 10 (ppb)	Day 15 (ppb)
Ti	3.16	2.91	8.56	11.27
Pt	0.24	0.20	0.22	0.29

B

Elements	Day 1 (ppb)	Day 5 (ppb)	Day 10 (ppb)	Day 15 (ppb)
Ti	3.16	6.90	9.22	10.23
Pt	0.24	0.22	0.27	0.34

C

Elements	Day 1 (ppb)	Day 5 (ppb)	Day 10 (ppb)	Day 15 (ppb)
Ti	3.16	6.61	8.21	13.78
Pt	0.24	0.28	0.25	0.27

The decrease in velocities under the light sensitive conditions, in oxygen-free control conditions and in an oxygen-free environment and light sensitive bottles in an oxygen-free environment were significantly lesser than that under standard experimental conditions. This strongly suggests the significant role of dissolved oxygen in deionised water, which contributed to the enhanced corrosion of the micromotors and thus affected the velocities.

Additionally, comparison of the velocities of micromotors stored in light sensitive bottles to that in plastic Falcon tubes suggests the micromotors might have inherently lost their efficiency in motion due to exposure to ambient UV light, which may have caused the difference in velocities recorded under standard experimental condition to be smaller. Despite that, similar decreasing trends in velocities were still observed with no significant deceleration of corrosion effects noted.

Additional control experiments by storing TiO₂ Janus microparticles throughout the 15 days experimental duration showed no increase in the oxygen atomic percentage and a small decrease in the atomic percentages for Ti (Table 5A). Figure 8 shows SEM and EDS images of the TiO₂ Janus microparticles over the 15 days experimental duration. Table 5B revealed an increase in concentrations of Ti in water 15 days post-fabrication by almost five times (from 0.22 to 1.18 ppb). The data obtained suggests that the bare TiO₂ microparticles lead to high degradation in terms of Ti losses so it is expected that the combination of Pt and TiO₂ would lead to the same or even worse degradation.

Table 5: Atomic percentages of (A) Ti and O obtained from energy dispersive X-ray spectroscopy (EDS) and (B) Ti concentration obtained from inductively coupled plasma-mass spectrometry (ICP-MS) of Day 1, Day 5, Day 10 and Day 15 TiO₂ microparticles. Conditions: stored in Milli-Q deionized water at room temperature and plastic Falcon tubes.

A

Elements	Day 1 (at. %)	Day 5 (at. %)	Day 10 (at. %)	Day 15 (at. %)
O	82.15	80.65	75.27	77.45
Ti	17.85	19.35	24.73	22.55
Total:	100.00	100.00	100.00	100.00

B

Elements	Day 1 (ppb)	Day 5 (ppb)	Day 10 (ppb)	Day 15 (ppb)
Ti	0.22	0.46	0.83	1.18

Figure 8. Scanning electron microscopy (SEM) images and corresponding energy dispersive X-ray spectroscopy (EDS) images of TiO_2 microparticles for (a) Day 1, (b) Day 5, (c) Day 10 and (d) Day 15. Conditions: stored in Milli-Q deionized water at room temperature and plastic Falcon tubes.

The effect of corrosion and oxidation of Pt and TiO_2 on the Pt/ TiO_2 micromotors might have affected the energy gap between the two surfaces, with hindered electron transfer thus affecting the catalytic processes on both surfaces of the micromotors, which reduced the effect of self-electrophoresis for motion (Scheme 1B).

Consequently, we turned our attention to repeat similar experiment in the presence of 5 wt. % H_2O_2 and the absence of UV light. Having understood the degradation of TiO_2 from the surface of the micromotors as observed from the decreased velocities in water under UV irradiation, it would be of interest to analyse the other component of the micromotors which is Pt. This could potentially provide a visual analysis and holistic comparison on the effect of

platinum degradation on the mobility of the fabricated Pt/TiO₂ micromotors. The average velocities were measured on Day 1 and subsequent 5 days intervals (Day 5, Day 10 and Day 15) (Figure 9). Freshly fabricated (Day 1) micromotors moved at an average velocity of 3.86 $\mu\text{m s}^{-1}$. This velocity is significantly smaller as compared to micromotors propelled by UV irradiation (9.03 $\mu\text{m s}^{-1}$). The average velocity remained relatively constant at Day 5, Day 10 and Day 15, unlike that observed with UV irradiation. It was interesting to note that the motion of the micromotors in the presence of 5 wt. % H₂O₂ did not appear to be affected by long storage duration. This is due to the fact that in 5 wt. % H₂O₂, the propulsion depends only on the decomposition of H₂O₂ on the Pt surface and it does not depend on the quality of the Pt/TiO₂ heterojunction, as this is a key parameter of a fuel-free regime. It is thus possible to salvage application of Pt/TiO₂ micromotors to extend their use after 15 days in the presence of H₂O₂. However, the use of toxic fuels might not be favourable in environmental and biomedical applications.

Figure 9. Average velocities of Day 1, Day 5, Day 10 and Day 15 Pt/TiO₂ Janus micromotors in 5 wt. % H₂O₂ fuel. Standard deviations were obtained from 40 independent measurements.

7.3 Conclusion

First we investigated the changes in physiological and physical properties of Pt tubular micromotors which were stored in deionized water in the absence of surfactant (SDS) for different period of time. Throughout the experiment duration of 28 days, the micromotors decrease in length and the surface undergoes significant corrosion. The storage and corrosion had a significant influence on the motion of micromotors. The average velocities of the tubular micromotors dropped by 46% and 61% of the initial values in 3% and 5% hydrogen peroxide respectively. The micromotors appear to be stable for 4 days post fabrication for reproducible and efficient measurements. Henceforth, it would be of importance to prepare new sets of micromotors for enhanced efficiency and reproducibility of experimental analysis.

Second, morphological and motion analysis of Pt/TiO₂ Janus micromotors revealed that long duration storage of the fabricated micromotors led to the corrosion and oxidation of TiO₂ and Pt from dissolved oxygen present in the water. As such, the fabricated Janus micromotors may not be effective in generating reproducible results if used more than 5 days from their preparation in a fuel-free light-powered regime, since their motion ceased after 15 days. However, the micromotors could still be used in an H₂O₂ fuelled regime as they displayed consistent velocity in the presence of 5 wt. % H₂O₂ even after 15 days. This is due to the fact that the Pt catalytic surface is the key element for fuel-powered micromotors, whilst in fuel-free mode, the Pt/TiO₂ heterojunction quality is of high importance. These findings are significant for any further operations of Pt/TiO₂ micromotors.

7.4 Experimental part

Materials: The cyclopore polycarbonate membranes (Whatman, USA) with 5 μ m pores diameter were used. SDS was purchased from Sigma-Aldrich (Czech Republic). Hydrogen peroxide of 35% was from Alfa Aesar (Singapore). Ethanol (99.5 %) was purchased from Fischer (Singapore) while tetrabutyl titanate (97 %) and sodium chloride (99 %) were

purchased from Sigma-Aldrich (Singapore). Electrodeposition was carried out using by Ag/AgCl and platinum (Pt) electrodes (CH Instruments, USA). The platinum (Pt) solution was purchased from Technic Inc., USA. Deionized water with resistivity of 18.2 M Ω cm was obtained from Milli-Q purification system (Millipore, MA, USA) and used to prepare all solutions.

Apparatus: Scanning electron microscopy (SEM) was performed using JEOL JSM 7600F (JEOL, Japan) to obtained SEM images. All pictures and videos were captured by optical microscope (Nikon Eclipse) and software (Nikon NIS-Elements). The same optical microscope connected to a mercury lamp was used for UV light activation of the Pt/TiO₂ micromotors. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis was carried out on an Agilent 7700 Series (Japan) for the determination of Pt and Ti. NOVA software version 1.8 connected to the Autolab PGSTAT 101 electrochemical analyser (Eco Chemie, Netherlands) was used for electrochemical deposition. The deposition was carried out in the system connected with three electrodes. Platinum (Pt) electrode was used as a counter electrode and Ag/AgCl as reference electrode. Sputtering and ultrasonication processes were performed using by JEOL JFC-1600 Auto Fine Coater and Fisherbrand FB 11203 ultrasonicator. All experiments were carried out at room temperature (25 °C). Beckman Coulter Allegra 64R centrifuge was used for centrifugation process.

Preparation of tubular platinum micromotors: A cyclopore polycarbonate membrane with a 5 μ m pore diameter was used as a template during the electrochemical deposition process. Sputtering process was carried out with a silver target. Electrochemical deposition experiment was carried out using a three-electrode system with Ag/AgCl as reference electrode and platinum as counter electrode. The working electrode was made by connecting copper strips to the sputtered membrane. The membrane was placed into a customized electrochemical deposition cell. Platinum was galvanostatically electrodeposited at

-20 mA for a duration of 40 min. Subsequently, the membrane was washed with deionized water eight times (8 mL per wash). The washed membrane was next polished with alumina powder (5 μm) to remove the previously sputtered silver. The polished membrane was dissolved in methylene chloride. The tubular micromotors were collected by centrifugation at 8,000 rpm for 3 min and cleaned three times with methylene chloride. The cleaning process is continued with ethanol and water, three times each using the centrifuge (3 min) after each washing step. The tubular micromotors were stored in deionized water at 25 °C.

TiO₂ microspheres preparation: The synthesis of TiO₂ microspheres was based on the following procedure. 0.2 mL of sodium chloride was dispersed in 50 mL ethanol and stirred for 5 min. Subsequently, 0.85 mL of tetrabutyl titanate was added into the solution. When the solution changed to an emulsion, stirring was stopped. The emulsion was left to incubate in the dark for 24 h. Subsequently, it was centrifuged (10 000 rpm, 15 min) and washed repeatedly with ethanol and deionized water three times each to obtain TiO₂ particles. TiO₂ microspheres were then sintered in the oven for 2 h at 450 °C.

Synthesis of Pt/TiO₂ micromotors: 150 μL of TiO₂ microspheres suspended in ethanol was dropped onto a glass slide previously cleaned and dried. The suspension was left to dry at ambient conditions overnight. TiO₂ microspheres were subsequently coated with a Pt layer by sputtering (sputtering time: 200 s, current: 40 mA). The fabricated Pt/TiO₂ micromotors were released from the glass slide by immersing in 20 mL of ethanol, ultrasonication (20 s) and rinsing with Milli-Q water. Pt/TiO₂ Janus micromotors were store in Milli-Q deionised water at room temperature (23 °C) in plastic Falcon tubes.

Motion control of platinum micromotors: The micromotors were moved in this experiment using the aqueous solution and changing different concentrations of hydrogen peroxide in 0.25 wt% of SDS. Hydrogen peroxide (concentration of 3% or 5%), SDS and water

are mixed, and 10 μL of the mixture was dropped on a clean glass slide. The average value of velocities and standard deviation were measured for every point of the figures. 30 measurements were taken into account.

Motion control of Pt/TiO₂ micromotors: UV light was generated by an optical microscope connected to a mercury lamp (light emission: 360–370 nm). The motion of Pt/TiO₂ micromotors were investigated in water under UV light irradiation at every 5 days interval for a total duration of 15 days. Additionally, motion was also observed under normal light in 5 wt. % H₂O₂. Average velocities and corresponding standard deviations of the fabricated micromotors were calculated from 40 independent micromotors at each day of measuring.

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Chapter 8

Conclusion and Future Work

8.1 Thesis summary

8.2 Future directions

8.1 Summary

Autonomously self-propelled micro/nanomotors are bright spots in material science due to their ease of operation, fast autonomous motion and high-power output. These micro/nanomachines or micro/nanomotors have vast promising applications. In the field of nano-biomedicine, nanomotors have been used in drug delivery, medical diagnosis and nanosurgery. They are also able to accurately, rapidly and cheaply detect the presence of toxic components in the environment. They have exhibited high efficiency in remediation and decontamination processes. The most commonly employed model of motion is the bubble self-propulsion mechanism where catalytically active platinum is used for decomposition of hydrogen peroxide to generate the motion of the nanomotors.

Chapter 2 explores the use of 2D materials, black phosphorus and germanene, to fabricate micro/nanomotors. BP/Pt micromotors were prepared by the electrodeposition method with a black phosphorus outer layer and the platinum inner layer. The velocity of BP/Pt functionalised micromotors is higher than that of bare Pt micromotors in different fuel concentrations. This is due to the different surface characteristics of the former which lead to easier H_2O_2 oxidation and faster reaction rates. In the same Chapter, we explored the multiplex detection of self-propelled micromotors by labelling them with two different 2D germanene derivatives, 4-fluorophenylgermanene (2D-Ph-Ge) and methylgermanene (2D-Me-Ge). These two fluorescent markers are able to exhibit different fluorescence under UV light irradiation. We showed that the micromotors carrying drug can be easily distinguished in a mixture of micromotors only by fluorescent emission.

In **Chapter 3**, we demonstrate that tubular Pt-Halloysite nanomotors show excellent capabilities to detect and remove heavy metals present in solution. Naturally available tubular Halloysite nanoclays was modified with platinum for the fabrication of autonomous self-moving nanomotors. The heavy metal ions are readily adsorbed onto the large surface area

available on the Halloysite nanoclays. The fabricated nanomotors were observed to have a greater efficiency with sensing of larger sized metal ions (Hg^{2+} and Pb^{2+}) as compared to smaller sized ions (Zn^{2+} and Cd^{2+}) and especially sensitive towards the ‘detection’ of mercury ions. Moreover, the system displayed high sensitivity with low a limit of detection (3.24 ppb) achieved which falls in the permissible range for mercury in drinking water (2-5.5 ppb) as set by World Health Organisation (WHO).

In **Chapter 4** we present the use of naturally available materials (Cloisite nanoclays) towards addressing the high cost preparation problem of micromotors. Similar to Halloysite nanoclays, Cloisite nanoclays material is very suitable to produce micromotors in large scale. The composite platinum-Cloisite (Pt-C) micromotors demonstrated fast and efficient enhanced removal of the pesticide fenitrothion considered as an improvised nerve agent. Cytotoxicity experiments were investigated by incubating micromotors with human lung carcinoma epithelial cells (A549). It was found that Pt-C micromotors are not toxic even at high concentrations which make them safe for human handling.

Subsequently, we extended the scope of naturally abundant materials to one of the most stable structures in the world – pollen. Nine different pollen grains which were different in shape and in size (Dandelion, Pine, Lotus, Sunflower, Poppy, Camellia, Lycopodium, Cattail and Galla) were selected and modified to be micromotor templates (**Chapter 5**). Pollen grains covered with low amounts of platinum, exhibited efficient mobility and great performance in the removal of the Hg^{2+} heavy metal ions in aqueous medium. Furthermore, their non-toxic profile expands their potential use in biomedicine as drug carriers. Pt-pollen micromotors were also found to be efficient carriers of the doxorubicin (DOX) drug.

In **Chapter 6**, we studied how different metals affect the velocities of metal/ TiO_2 micromachines in the presence of UV irradiation in pure water. Pt/ TiO_2 , Cu/ TiO_2 , Fe/ TiO_2 ,

Ag/TiO₂ and Au/TiO₂ Janus micromotors were prepared in this study. The metals have different chemical potentials and different catalytic effects towards the water splitting reaction, with both effects expected to alter the photoelectrochemically induced reaction and propulsion rates. Electrochemical Tafel analysis were performed for the metal/TiO₂ structures and it was concluded that the effective velocity is a result of the synergistic effect of chemical potential and catalysis. It was found that the Pt/TiO₂ Janus micromotors exhibit the fastest motion compared to the rest of the prepared materials. Furthermore, after exposure to UV light, every fabricated micromotor showed high possibility of forming assembled chains which influence their velocity.

In **Chapter 7** we investigated the changes in physiological and physical properties of Pt-tubular micromotors and Pt/TiO₂ Janus micromotors over a period of 28 and 15 days, respectively. Throughout the experimental duration, the Pt-tubular micromotors appear to decrease in length and the outer layer becomes coarser. It was also found that the increasing number of days after preparation of micromotors had an influence on their motion for the both prepared micromotors. The average velocities of the tubular micromotors dropped by 46% and 61% of the initial values in 3% and 5% hydrogen peroxide, respectively. On the other hand, Pt/TiO₂ Janus micromotors retained the Janus structure but their surface morphology greatly changed with a decrease in composition of titanium dioxide and platinum and a corresponding increase in Ti and Pt in the storage solution. The motion of the Pt/TiO₂ Janus micromotors decreased drastically under ultraviolet irradiation after just 5 days of storage and these machines eventually stopped moving after 15 days. Pt-tubular micromotors and Pt/TiO₂ Janus micromotors appear to be relatively stable for 4 days and 5 days post fabrication for reproducible and efficient measurements, respectively. Henceforth, it would be of importance to prepare new sets of micromotors for enhanced efficiency and reproducibility of experimental analysis.

8.2 Future work

Starting from the results presented in this thesis, the research topic can be expanded in both directions: exploring new materials, techniques and methods for micromotors drug delivery application or environmental remediation. More studies should be done in order to investigate micromotors behavior as drug delivery systems and therapeutic carriers.

Previous scientific studies showed the micromotor's suitability for *in vivo* drug delivery. In the future, more experimental conditions should be set up to improve the efficiency of drug loading and drug release in the specified tissues. For instance, analysis which involves animal testing includes very high ethical standards that are still challenging because of their further applications on human being. Research can be expanded to find biocompatible materials which are able to capture different therapeutic drugs. More investigation on the protein corona on the particle surface is crucial as it plays a very important role in the interaction with environment matter.

Another fundamental research area will be to investigate external triggers for micromotors guidance and drug release (activation). Anticancer drug carriers require new motion approaches which can be independent on the local blood gradient flow and sensitive to targeted drug realise. Exogenous micromotors stimuli includes three types of triggers: light, magnetic field and ultrasound. For biomedical application, the radiation beam must penetrate deep into the tissue, interact with micromotors and realise the drug from there. From this perspective, only ultrasound and magnetic waves can successfully satisfy biomedical requirements. Fundamental studies in this area include research about the ability of micromotors to quickly realise drugs to targeted sites and decrease the toxicity of infected tissue by controlling parameters such as time and duration of exposure.

Photo-activation studies are still very popular as the drug release rate can be controlled by changing only the light intensity. However, light driver micromotors are limited for *in vivo* studies as light with wavelength < 650 nm can only penetrate a maximum of 1 cm deep into tissue due to high scattering of compounds present in the tissue such as hemoglobin and water. On the other hand, near-infrared (NIR) light driven micromotors, have higher penetration into the tissue than UV/visible light driven micromotors (< 10 cm) with minimal tissue damage. It would be interesting to combine different exogenous elements, as all single elements have a set of advantages and disadvantages and their combination should enable a better drug loading system.