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The Assemblies and Application of Silica and Iron Oxide Nanostructures

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ABSTRACT

My work has been largely focused on the synthesis of magnetically controlled multifunctional nanostructures for applications, through the assembly of iron oxide nanostructures with subsequent structure preservation using silica via simple Stöber process. Several novel structures has been discovered and prepared. For example, silica encapsulation of hydrophobic liquid droplet consists of iron oxide for storing and releasing large amount of hydrophobic molecules. Due to the stable and porous shell, no rupturing or leakage of the hydrophobic liquid can be observed even after the release. Nanometer size magnetic stirring bars were prepared. They can be easily inserted into extremely small water droplets (4 pL) for parallel stirring and mixing. For those with thick silica coating, a contracted structure can be observed and the mechanism was investigated. Lastly, fluorescent silica shell was synthesized onto the nano stir bars using cheap dye molecules. The mechanism of formation was studied and the method was modified to prevent the massive aggregations.

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LIST OF ABBREVIATIONS

4-MBA	4-mercaptobenzoic acid
APTES:	(3-Aminopropyl) triethoxysilane
BSA	Bovine Serum Albumin,
CF:	5(6)- Carboxyfluorescein
DCB	1.2-dichlorobenzene
DDV:	Drug delivery vehicles
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
Dye-APTES:	Dye conjugated with APTES via amide bond
FITC:	Fluorescein isothiocyanate
NPs:	Nanoparticles
NRs:	Nanorods
NW:	Nanowires
OL:	Oily Liquid
OV:	Oily vesicles
PDMS	Polydimethylsiloxane
PSPAA:	Poly(Styrene)-b-poly(Acrylic acid)
PVP	Polyvinylpyrrolidone
RITC:	Rhodamine-B-isothiocyanate
ROX-SE:	Carboxy-X-Rhodamine, Succinimidyl Ester
SERS:	Surface Enhance Raman Spectrum
TAMRA-SE	Carboxytetramethylrhodamine, Succinimidyl Ester
TEOS:	Tetraethyl orthosilicate
THF	Tetrahydrofuran

CHAPTER 1: INTRODUCTION

1.1 DESIGNS OF MULTI-FUNCTIONAL NANOSTRUCTURES:

Until the last decade, most of the work has been focused on developing novel synthesis of beautiful and useful nanocrystals, exploring their potential applications and to improve the stability of these nanocrystals.^[1-6] In the recent years, the field are now focused more towards the development of more advance nanostructure; nanostructures that possessed more functionality by combining different materials to achieve multifunctional nano-systems.^[7-8] These multi-component nanostructure are highly desired because most single component nanostructures simply could not satisfied all needs of a specific application, such as in catalysis,^[7] drug delivery,^[9-11] multimodal imaging^[12] and diagnosis^[9, 13] Among many properties reported, magnetic properties (for movement control) and silica (for structure stability) are commonly seen in the design of multi-functional nanostructures, because these two properties works well together in many applications, for example, magnetic property with catalytic active material and stabilized with porous and stable silica shell,^[14] magnetic property with molecular recognition on silica surface for controlled drug delivery,^[10-11] and magnetic NP with embedded fluorescent agents on silica for fluorescent imaging with selective target.^[12]

1.1.1 CORE SHELL STRUCTURE

There are only a limited number of ways to combine different materials in nanoscience. One popular example is the core-shell structure in which one component is grown as a shell (commonly polymer shell or inorganic shell) on a core NP.^[14-24] often abbreviated as core@shell (e.g. Au@SiO₂; gold NP as core encapsulated by silica as the shell).^[25] The main goal of this core shell architecture is normally to enhance the colloidal stability of the core by improving solubility, thermal stability and to prevent aggregations between the NPs.^[15] The shell can also be used to prevent lost of ligand layer on a Au core to preserve its SERS properties.^[26] In most cases, these shells are optically transparent and thin, and thus, will not obliterate fluorescent or magnetic properties of the core NPs.^[27]

The key in designing and synthesizing core shell structure is to establish a strong interaction between the shell and the core, in other words, having small interfacial energy between the two. If both materials are of the same type (such as metal@metal or oxide@oxide), their interfacial energy are usually small. For domains that have an epitaxial relationship, the trend of interfacial energies can be predicted according to their lattice mismatch. Larger lattice mismatched will result in larger strains and more defects, thus higher interfacial energy.^[28] Basically, core shell structure can be easily achieved if both materials have a small lattice mismatch, such as, CdSe@CdS,^[29-32] Pd@Pt^[28] and Au@Pt structures.^[33]

Figure 1.1: Core shell structure with multi shell-single core structure. Schematics (a) showing the formation of PSPAA polymer coating onto silica shell, while (b) showing the vice versa process. TEM image in (c) showing (Au@Silica)@PSPAA (product in schematic (a)). The darkest contrast display the AuNP, the grey middle layer is silica and the outermost shell is PSPAA. TEM (d), (e) and (f) showing subsequent growth of different shells in alternating fashion in which the lighter contrast is PSPAA while the darker contrast is silica shell. Reprinted with permission from ref.^[34], copyright © 2012 WILEY-VCH Verlag Gm bH & Co. KGaA, Weinheim.

To synthesis core shell structure with different materials, such as with oxide and metal, surface modifications are used to reduce their interfacial energy. For example, in the case of silica encapsulating gold NPs, without ligands, silica will prefer to form homogeneous nucleation rather than a shell. By introducing ligands that contain suitable functional groups, (for AuNP, such as thiol group – SH or amine group^[35-37] and for silica, carboxylate group – COOH or silane group –Si(OH)₃.^[25, 38-39] their interfacial energy will be reduced, and silica will form a shell on the Au NP. Similar with polymer shells formed via assembly of block copolymers, the hydrophobic sections normally do not form strong bonding with the core NP but by introducing ligands that contained a hydrophobic alkyl functional group with strong bonding to the NP, a polymer shell can be assembled.^[40] The hydrophobic ligands will render the NPs to be slightly hydrophobic to interact with the hydrophobic block of the polymers, forming polymer shell.

In addition to the simple one core one shell structure, there are several designs such as a single core multi shell structure, multi core single shell structure and a combination of both. With similar strategy, additional shells can be grown on the first layer, combining different components in a form of multi layer shells. Similarly, by slightly aggregating the NPs before encapsulation, core shell structure with more than one NP core can be prepared.^[41-43]

Figure 1.2: Multi core multi shell structures in which the darkest contrast is AuNPs, lightest contrast if PSPAA while the grey sections are the silica shell. Reprinted with permission from ref. ^[34], Copyright © 2012 WILEY-VCH Verlag Gm bH & Co. KGaA, Weinheim.

For example, Zhu et al.^[34] have reported on a versatile method to encapsulate silica shell on PSPAA polymer shell and vice versa (Shown in Figure 1.1 and 1.2). To form silica shell on block copolymer, no additional modification was required as they have low interfacial energy (due to the carboxylate group on the PAA (polyacrylic acid) section of the polymer). However, encapsulation of PSPAA polymer onto the silica shell requires the silica surface to be modified with APTES to introduce amine group which increases interaction with the PAA section of the polymer to form a shell.

1.1.2 JANUS STRUCTURE

In contrast to core shell structures, the shell material can also be grown eccentrically to form janus structures in which both components are exposed to the solvent (figure 1.3).^[44]

Figure 1.3: Different types of Janus NPs

To form janus NP using polymer shells, one can tune the fluidity, which is dependent on the glass transition temperature (T_g). For example, by increasing the temperature to T_g or introducing a good solvent (without heating) such as DMF to swell the polymer shell.^[15] Water can then be added to de-swell the polymer shell by extracting the DMF from the PS section of the polymer shell,^[15] locking the polymer shell in its a glassy state, encapsulating the NP core at its kinetically stable state.^[45] Another way to lock the kinetic product is through cross-linking of the polymer shell.^[36, 46]

For other types of janus NPs (besides polymer shells),^[47-48] most cases involved controlling the interfacial energy between the core and shell during shell formation. For example, in Au@ZnO (Figure 1.4),^[49] growth of ZnO (shell) on AuNPs is only possible when the heterogeneous nucleation (path β , growth of ZnO on Au NP) are more favourable than the homogeneous nucleation (path α , ZnO on ZnO). However, thermodynamically, path α is always more favourable because there is no interfacial energy for the same material.

Figure 1.4: Schematics showing the effects of interfacial energy on the nucleation and growth of ZnO domains onto the surface of AuNP; the different pathways for nucleation and growth of ZnO domains when interfacial energy is (a) high or (b) low, which result in the formation janus structure or concentric core shell structure. Reprinted with permission from ref. ^[49], copyright 2013 American Chemical Society,

We can grow a janus structure by controlling the kinetic aspect of the reaction. Simply, ZnO must reach a certain level of oversaturation during growth to form heterogeneous nucleation on the AuNP. During the process, oversaturation of ZnO would first form a domain (nucleation) on the AuNP and subsequence growth will prefer to occur on the ZnO nucleation. The growth will continue because deposition on the existing nucleation is thermodynamically more favourable than to create another fresh domain, hence, leading to a single ZnO growth in each AuNP, forming a janus structure. In addition, the interfacial energy can be further tuned by modifying the surface with ligands. By further lowering the interfacial energy, multiple nucleation in a single NP can be formed and may even result in a merged shell.^[50-52]

1.1.3 TEMPLATED NP ASSEMBLIES

Another form of combining NPs is through assembling NP on the surface of a larger structure or template; many smaller NPs can be adhered to a sticky surface of larger template such as micelles, DNA,^[53-56] hard nanostructures^[57] or on large bulk substrates.^[58] forming a raspberry-like morphology. The advantage of such design is that, the interfacial energy does not matter (as much) because it doesn't required shell growth. But at the same time, there is only limited structure control in this system; it will be much more challenging to form asymmetric structures (such as janus structure).

Generally, in a colloidal suspension, NPs are stable from aggregations and will not adhere to other colloidal surface due to charge-charge repulsion; if they do, large random aggregation will occur. In order for such NP assemblies to occur (stick) on the template, the interactions between NPs and template has to be much higher than both the interaction between the template and solvent as well as the NP and solvent. Hence, the template are usually modified with functional groups like thiol or amino groups that can form strong bonding with most NPs such as noble metals, metal oxides, and semiconductor NPs (figure 1.5).^[57]

Figure 1.5: (a) Schematics showing the synthetic process of NPs adhered onto surface of thiol group modified silica shell followed by an additional layer of silica shell. TEM images in (b) showing different types of NPs can be adhered onto the modified silica shell. Adapted with permission from ref.^[57], copyright 2013 American Chemical Society,

1.2 MAGNETIC PROPERTIES AND SILICA BASED NANOSTRUCTURES

Here, my main research is in the rational synthesis and assembly of multi-component systems focusing on magnetic nanostructures and silica encapsulation. As such, I will briefly review on magnetic NPs and silica before discussing the different designs of multi-functional systems in the later section, with focus on magnetic nanostructures and silica, as well as their combined synergy.

1.2.1 MAGNETIC NPs

In this section, I will focus on the self assembly of magnetic NPs into multifunctional nanostructure. Magnetic properties have always been a popular choice for multifunctional nanostructure because it is the most convenient way to control the mobility of any nanostructures in the billions. Generally, as all electronic and optical properties changes drastically with size, magnetic properties changes as well. When their dimensions are reduced to the nano-scale, separate domains of magnetic moment with different directions will no longer exist; there will only be one ‘superspin’ (direction) throughout the whole NP. Their orientation of magnetic moment can be easily saturated with external magnetic field and will completely demagnetize when the field is switched off. In short, magnetic NPs are superparamagnetic.^[11] As such, their behaviours with external magnetic fields are different in comparison from those in bulk, which allows many unique applications as well as the formation of beautiful self assembly structures (figure 1.6).

When magnetic NPs (in solution) are placed close to a magnet, they will align into short clustered chains.^[59] These magnetic NPs will be fully attracted towards the magnet when given enough time. When the magnetic NPs are used in high concentration, they will interact and undergo self assembly before being attracted towards the magnet. Hence, there is a window of opportunity to control and capture magnetic self assembled structure before they are 'pack' closely together near the magnet.^[59] For example, there are many self assemble structures reported in literatures which include 1D chains^[59] and rings^[60-64] as well as 2D nanorrafts and labyrinth (shown in figure 1.6).^[65] However, without any means of preserving or locking the structure, they will easily collapse or aggregate especially when the solvent dries for microscopy characterizations.^[59, 66] Hence, in many recent publications, the design of multi-functional NS often combine magnetic NPs with other types of materials such as silica, to improve stability as well as increase functionality.^[7, 14, 67]

Figure 1.6: The different structures form from magnetic assembly. TEM images in (a) showing rings structure from NiCo NPs, (g) is chain form from Fe_3O_4 NPs, (h) and (i) depicting raft structures while (j) depicting labyrinth structure from Fe_3O_4 NPs formed via magnetic assembly. Adapted with permission from ref. ^[60], ^[59], ^[66], and ^[65] copyright 2008 American Chemical Society. 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2012 American Chemical Society and 2013 American Chemical Society

1.2.2 SILICA COATING OF COLLOIDAL PARTICLES

Like magnetic NPs, silica is one of the popular materials for multi-functional nanostructures and has been widely studied in material science to enhance the stability of colloidal systems, such as polymers, surfactant aggregations and more often, colloidal particles.^[14, 25, 59] The use of silica as shell materials is mainly due to its high stability and chemical inertness (particularly in aqueous conditions), versatile synthesis with easy modifications, controlled porosity as well as optical transparency. The reason for its high stability is because of the low Van de Waals interactions in comparison to most particles, due to its low Hamaker constant.^[14] In addition, small molecules with a slightly positive charge such as water and cations will tend to attach to the polymeric silicate layer on the silica surface under basic conditions, hence, rendering electrostatic charges on the surface of the shell, providing both steric and electrostatic stability to any coated colloidal particles. More importantly, silica can be used for further functionalizations with modification using silane molecules of different functional groups (such as thiols, amine, and even alkyl chains) to combine with other different NPs for additional properties.^[57] The modified surface can also allow polymer encapsulation (i.e. PEG) to increase biocompatibility as well as functionalization with targeting peptides for applications in the biomedical field. These advantages allowed silica to be the ideal, low cost material for tailoring surface properties of colloidal particles to create lab-on-particles with tunable dimensions and novel properties.

1.3.1 STÖBER METHOD

The two main approaches to synthesis silica particles and coatings are the Stöber method ^[25, 68] and the reversed microemulsion method. Stöber method has been regarded as an easy and efficient method for coating aqueous colloidal particles (figure 1.7) because (1) the dimension of the NP or shell structures can be easily controlled from several nanometer up to a few hundred micrometer in size, (2) mild synthesis conditions and (3) produces highly monodispersed NPs.

The mechanism of formation involved hydrolysis of silica precursor (alkoxysilanes molecules, TEOS are commonly used) forming silicic acid which will then condense to form silica.^[68] The chemical equation can be briefly written as follows.

The mechanism of silica formation through Stöber method has been widely studied. It has been suggested that formation of small silica particles are slightly different from common nanocrystal growth. Briefly, after the hydrolysis of alkoxysilanes molecules, condensation will occur to connect silane molecules forming silica bond; several silica bonds formation will then form polysilicic acid molecules. These polysilicic acid molecules will then aggregate together, forming small nucleation sites for further growth of silica. These small nucleation sites will continue to aggregate to form larger silica particles with uneven sizes and rough surfaces. In the final stage of silica growth, the remaining smaller polysilicic acid molecules will grow on these aggregated

particles forming a larger silica particle with a smoothen morphology. During the silica growth, cross-linking will take place within these aggregations to form a shell.

The recipe used for Stöber method consist of alcohol solvent mixed with water, Tetraethyl orthosilicate (TEOS) and ammonia, followed with a few hours of incubation.^[68] Unlike most reactions where the particle size is mainly controlled by one or two precursors, all four reagents in the Stöber method can tune the size of silica particles or thickness of the silica shell. Being the precursor, higher amount of TEOS will results in larger particle size. However, following the nucleation and growth theory, more precursor molecules (exceeding the supersaturation) will also lead to more nucleation sites, thus forming more particles. Ammonia has been considered as the catalyst of this reaction in which higher amount of ammonia will speed up the silica growth, forming larger silica structures. Next, lesser water added will result in larger particles, due to the decreased solubility of the polysilic acid species in the solution, leading to faster and larger aggregations.

For doping of molecules in silica, positively charged molecules (such as metal ions or dyes) or small polar molecules (water or ammonia) can be added during or after silica growth. They can be easily doped into the silica matrix, thanks to the negatively charged hydroxyl groups (dangling bonds) in the silica matrix. For negatively charged molecules, they can also be loaded into the silica shell via simple silane chemistry; modification using different functional groups such as amine and thiol groups can be easily achieve allowing molecules to form covalent bonding with the silica shell.

Figure 1.7: TEM images showing core shell structure of (a) Au@SiO₂ and Fe₂O₃@SiO₂ formed from Stöber method. Smoothen shells can be seen even in encapsulations of clusters structure. Reprinted with permission from ref. ^[25], copyright 2011 American Chemical Society

1.3.2 REVERSED MICROEMULSION METHOD

As widely used as the Stöber method, the reversed microemulsion method is also an effective way in synthesizing silica and is more catered towards silica coating of hydrophobic NPs.^[69-75] Similarly, the reversed microemulsion method also utilizes hydrolysis and condensation process of alkoxysilanes molecules in basic conditions to synthesis silica particles or coating. The procedure consist of using high organic solvent (oil) to water ratio with different nonionic surfactant molecules (such as Igepal CO-520, Trion x-100 and Brij 52 or 56),^[76] TEOS and a base (i.e. Ammonia or NaOH), followed by several hours of incubations.^[77] The large volume of organic solvent is to ensure stable suspension of the hydrophobic colloidal particles, but since hydrolysis required basic aqueous condition, small amount of base is added. The surfactant molecules have 2 purposes in the synthesis. (i) stabilizing the water droplets (base) in

microemulsion,^[76] ii) to bring NPs into the aqueous phase (droplets) by undergo ligand exchange on the hydrophobic NPs.^[76] Many hydrophobic NPs have long alkyl chains as their ligand which has low affinity for silica shell growth. Through ligand exchange, silica can grow on carboxylate group of the surfactants. Unlike the Stöber method, only the amount of TEOS and Base can be used for controlling the silica shell, but it has been reported that the reversed microemulsion method has a higher control over silica nucleation, because nucleation are limited within the small water droplets. Additionally, reversed microemulsion method can also be used for coating *in situ* synthesized NPs.^[78]

1.3 SYNERGY AND APPLICATIONS OF SILICA COATED MAGNETIC NANOSTRUCTURES

While both silica and magnetic NPs are highly useful materials, they are limited by their shortcomings. Magnetic NPs lacked stability for many applications while silica (by itself) lacked functionalities.^[8] As such, silica and magnetic NPs are commonly used together; with silica coating on magnetic NP core (core shell structure) to create a stable structure with mobility control. In addition, silica material can be easily incorporated with different components. Hence, a variety of useful multifunctional nanostructures can be designed.

In this section, application of silica-magnetic nanostructures will be discussed, such as in the biomedical field, catalysis, and sensing as well as for novel self-assemblies. However, due to the overwhelming reports on silica coated magnetic NP; only some representative examples will be discussed, focusing more describing on the designs based on silica coated magnetic nanostructures.

1.3.1 BIOMEDICAL APPLICATIONS

In the biomedical field, silica-coated magnetic NPs are often used as the basic platform for designing efficient nanostructures in many biomedical applications.^[79-83] This is because magnetic NPs can be used in guided drug delivery (controlled locomotion), imaging (MRI contrast agents), hyperthermia treatment and trigger release of incorporated molecules (both via generating heat in local regions). While for silica, besides improving stability of the structure, it allowed surface modification of different molecules or polymers to improve biocompatibility as well as targeted delivery. In some cases, mesoporous silica is used to store drug molecules, and protect from the surrounding conditions during the delivery process.

1.3.1.1 Drug Delivery

In targeted drug delivery, the key is to guide the loaded drug delivery vehicles to the particular site with high precision while able to selectively attach, hold and accumulated on the desired location via surface recognition bio-molecules until the completion of the therapy.^[11, 67] The main importance is to carry high load of drug specifically to the desired target for better treatment and at the same time prevent high toxicity due to high drug dosage in other unrelated healthy parts of the body. As described, silica coated magnetic NP is an excellent candidate because of the controlled movement by magnetic NPs for guiding towards the desired target and easy surface functionalization of the silica shell to attach peptides, drug molecules and fluorescent imaging probes.

One of the successful examples was reported almost a decade ago, Dong et al.^[83] had reported the synthesis of silica coating magnetic NPs and quantum dots NPs. The structure was stable and both magnetic and fluorescent properties were well preserved even after encapsulation. Because of the silica shell, drug molecules can be anchored on the surface of the NP.

In a recent publication, Liong et. al.^[84] has reported on using mesoporous silica coated superparamagnetic NPs for drug delivery (Figure 1.8). Fluorescent dyes (FITC) were incorporated into silica matrix for imaging while the surface of the silica was further modified with folic acid (for targeting cancer cells) and phosphonate molecules with silane group (3-(trihydroxysilyl)propyl methylphosphonate) to improve dispersion of the structure in aqueous solution. In their case, hydrophobic drug molecules (camptothecin, or Paclitaxel) were inserted in the pores of the silica, selectively killing the cancer cells.

To improve drug loading, a different design of silica coated magnetic NPs were reported as well. A cavity was formed in between the silica shell and magnetic NP core to form yolk shell structure via etching.^[85] The rationale was to increase storage of drug molecules in common mesoporous silica; the loading of molecules in mesoporous silica is limited to the pores in the silica matrix, hence by having a cavity in the shell, more can be loaded while they are still protected by the silica shell.

Figure 1.8: SEM and TEM images of mesoporous silica coated magnetic NPs loaded with anticancer drug for drug delivery. Fluorescent dyes and targeting ligands were covalently bound to the modified silica surface. Reprinted with permission from ref. ^[84], copyright 2008 American Chemical Society.

In another case, similar strategy has been reported using common Stöber silica shell by tethering the drug molecules onto the surface of the silica (Figure 1.9). Yang et al.^[86] has prepared 50 nm uniform silica coated iron oxide NPs to deliver doxorubicin, DOX into cancer cells (selective protease enzyme expression). The DOX was tethered to the PEG-N₃ functionalized silica surface by click chemistry in which the molecule can be selectively released into the cancer cell where a specific enzyme (protease) was highly expressed. As superparamagnetic NP was used, beside controlled motion, the structure can be used for diagnosis by MRI techniques as well.

Figure 1.9: Schematics illustrating DOX (anticancer drug) were tethered onto the silica surface using click chemistry for drug delivery applications. Reprinted with permission from ref. ^[86], Copyright © 2008 WILEY-VCH Verlag Gm bH & Co. KGaA, Weinheim.

1.4.1.2 Trigger release using magnetic NPs.

In addition to control locomotion, magnetic NPs can be used to trigger the release of loaded drug molecules stored in mesoporous silica.^[87] Two different designs were reported both utilizing localized heat generated when magnetic NPs were subjected to alternating current (more discussion in next section 1.4.1.3). Reported by Eduardo et. al.^[88] (figures 1.10a), magnetic NPs were used as the gate keeper for the pores in the mesoporous silica, bound by DNA strands, trapping the drug molecules within. To allow better magnetic respond, and to generate heat more efficiently, additional smaller magnetic NPs were also incorporated inside the mesoporous silica. When the structures were delivered to the cancer cells, an alternating magnetic field was applied and magnetic NPs both inside and on the surface of the silica will generate heat to melt the DNA strand, hence release the drug molecules within to kill the cancer cells.^[88]

The second design was quite similar but instead of using magnetic NPs as gate keeper, nanovalves were used while magnetic NPs were only embedded within the structure (figure 1.10b).^[89] When an alternating magnetic field were applied, the heat generated will disassemble the nanovalves, hence releasing drug molecules within.^[90]

Figure 1.10: Both schemes (a) and (b) showing different designs of using magnetic NPs to generate heat to trigger the release of cargo molecules. Reprinted with permission from ref. ^[89] and ^[88], Copyright 2010 American Chemical Society and 2011 American Chemical Society.

1.4.1.3 Hyperthermia Treatment

Hyperthermia is another application of magnetic NPs in the field of biomedical. Hyperthermia is described as an abnormal increase in body temperature. As such, hyperthermia therapy works by damaging or inducing cancer cell death by increasing body temperature. In nanoscience, heat can be generated inside cells through uptake of superparamagnetic NPs, which is termed as magnetic hyperthermia.^[91] Principally, magnetic hyperthermia works by generation of heat through magnetic loss; heat converted from the magnetic

field during magnetic reversal. Hyperthermia is effective because cancer cells are more sensitive to temperature increase (around 41°C) as compared to healthy cells.^[91-92]

The main requirement for hyperthermia is the magnetic NPs, thus a simple core shell architecture design would be sufficient^[92]; core magnetic NPs between size 10-20 nm are preferred, with coating (such as silica) for better stability. Application using hyperthermia has been very successful in both in vitro and in vivo, it has also been made to the pre-clinical trials.^[91] Despite its success, currently, the major challenge is low accumulation of NPs in the specific site (in tumour cells) to generate enough heat for hyperthermia treatment.^[91] To overcome this challenge, many groups are working in improving the targeting and selectivity of NPs via surface functionalization using targeting molecules and peptides.

1.4.1.4 Magnetic Resonance Imaging, MRI

Molecular imaging is one of the highly accepted non-invasive methods commonly used in real-time visualization of cellular functions as well as to study the related molecular interactions in living organisms.^[93-94] One method of molecular imaging is magnetic resonance imaging (MRI).^[95-96] MRI has been widely used because of its ability to provide 3-D tomographical imaging of whole tissue samples (including human soft tissues) in high spatial resolutions. However, while MRI is capable of revealing anatomic details in organs, it is challenging to differentiate between normal and dead cells. Thus, contrast agents are often used to enhance the imaging sensitivity. There are 2 types of contrast agent. T1 and T2 contrast agent.^[93] T1 contrast agent increases the T1 relaxation time which results in brighter contrast of T1-weighted images while T2 contrast

agent reduce T2 relaxation times, giving dark contrast T2-weighted images.^[91] Small superparamagnetic NPs fall in the category of T2 contrast agent. In many reports, silica coated superparamagnetic iron oxide NPs has been long studied and modified to be an efficient MRI T2-contrast agent; forming stable and biocompatible structure for MRI.^[97-98]

Like hyperthermia treatment, the presence of superparamagnetic NPs is sufficient for MRI applications; a simple core shell structure will suffice. To increase the selectivity as well as the uptake, additional modification on the silica shell surface with target peptides or molecules can be performed.

In many cases, MRI are often combined with other types of imaging, such as fluorescent, for multi-modal imaging.^[93] For example, Chen et al.^[96] have also synthesized a simple core shell structure (silica coated with magnetic NP) loaded with fluorescent dyes and demonstrated multimodal imaging of human stem cells using MRI and fluorescent.

In addition, Chen et. al.^[97] has functionalized fluorescent polymer layer containing rhodamine B (red fluorescent dye) on silica shell coated iron oxide NPs. Like the ones used in drug delivery, the silica matrix can be used to load drug molecules while the surface was functionalized with folic acid (a targeting ligand) for selectivity to cancer cells.

In a different design, instead of modification with another polymer layer, Lee et al. had reported on attaching both fluorescent molecules (Rhodamine B or FITC) and superparamagnetic NPs on the surface of a silica particle, for simultaneous MRI and fluorescent imaging. Like most cases, the silica structure was also used as an anticancer drug carrier. Thus it can be used in both imaging and drug delivery applications.

1.3.2 CATALYTIC APPLICATIONS

The nanostructure designs for application in catalysis often involved a catalytically active surface and a supporting material (either colloidal or bulk surface). The active site is the main location where catalyzed reactions took place, while the supporting material was used to enhance stability as well as to act as a platform for us to ‘manage’ the catalyst (bulk substrate). For nano-sized colloidal catalyst, silica coated magnetic NPs has been a common material used in conjunction with different types of catalytic NP. Silica is often used as a protective shell while magnetic properties are used to ease the purification method and to recycle the catalyst.^[99-101]

In year 2007, Mohammadreza et al.^[101] from Taeghwan Hyeon’s research group have reported a simple magnetically recyclable nanocomposite catalyst using the platform of silica coated iron oxide NPs. The Molybdenum oxide NPs were synthesized and incorporated into the silica shell.

Next, Ge et al.^[102] has synthesized a novel catalyst structure by immobilizing AuNP on the silica coated iron oxide using surface ligands with amine functional groups (figure 1.11). A second silica layer was then coated over the AuNP before slightly etched to form a porous outer silica shell, protecting the satellite AuNP without affecting the catalytic performance. The catalytic capability of the attached AuNP was demonstrated using a model reaction involving the reduction of 4-nitrophenol by NaBH₄. Due to the magnetic NP core, complete separation of the catalyst from the solution was achieved, allowing easy recyclability of the catalyst.

In addition, magnetic material such as nickel and α -Fe₂O₃ NPs has also been used as catalyst. Using silica as protective shell, Park et al.^[103] has synthesized

Figure 1.11: The above scheme depicting the formation of AuNPs adhered onto a modified silica shell encapsulating a magnetic NP core. Next, an additional shell was grown over and was slightly etched to produce a rigid and highly porous shell forming a catalyst with high stability and magnetic recyclability. The TEM image below showing the structure of the catalyst. Reprinted with permission from ref. ^[102], Copyright © 2008 WILEY-VCH Verlag Gm bH & Co. KGaA, Weinheim.

yolk shell structure of Ni@SiO₂ as catalyst for hydrogen transfer reactions of acetophenone. The silica shell was prepared through reverse microemulsion method coating the Nickel NP. The Nickel core was then etched using acid, forming a yolk shell structure. The Silica coating was porous thus allowing reactants to pass through, catalyzed by the Nickel NP core and react within the silica compartments. Silica coated α -Fe₂O₃ NPs had also been prepared as catalysis reported by Feyen et al,^[104] for ammonia decomposition reaction. The structure design are rather similar focusing on using silica as a protective shell while iron oxide NPs act as both catalyst as well as to recycle the catalysis through magnetic attraction.

1.3.3 SENSING AND PURIFICATION APPLICATIONS

NP-based nanosensors have attracted much attention due to their high precision and accuracy in selectively detecting and separating minute concentration of target species, such as toxic metal ions, and biomolecular species. The detection of targeted molecules normally takes place in a binding event on the surface of the structure which then be transmitted to emit signals, through colour changes (light absorption and emission or fluorescent).^[49] Purification can be done in via centrifugation methods or through magnetic separation.^[13, 105] Centrifugation methods are more rapid and commonly used in separation of nano-size colloids in laboratory, but for purification applications that involved large volume of solution, magnetic separation are more suitable.^[13]

Silica coated magnetic NP are very commonly used for designing nanosensors, due to low cost, the ease of modification and stable property of silica shell and magnetic properties of the magnetic core.^[13] For detecting

different species, suitable ligands or capping molecules are synthesized and bound to the surface of the silica. For example, selective detection of toxic metal ions in water or even in blood such as Pd^{2+} (shown in figure 1.12),^[106-107] Cu^{2+} ,^[108] Hg^{2+} ,^[109] and UO_2^{2+} .^[110]

Figure 1.12: Schematic showing the surface functionalization of Ni@SiO₂ with specific ligands (BODIPY) for the detection of Pb²⁺. Once bound, with Pb²⁺, fluorescents will turn ‘on’ as illustrated in the picture. Reprinted with permission from ref. ^[106], copyright © 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

1.3.4 NOVEL SELF ASSEMBLY OF MAGNETIC NPs

In the literature, many magnetically assemble structures has been reported which included 1D chains,^[59] rings,^[60-64, 66] 2D nanorrafts^[66] and labyrinth.^[65] Formation of magnetic chain structures has been reported in almost all publications about magnetic NPs. In a nut shell, chains will form whenever magnetic NPs are put close to a magnetic field given that they are not too diluted. However, without any means to preserve the assembled structure, it will collapse either by aggregations or during drying for characterizations. Hence, rigid shells (such as silica) are quite common to be used as coating.

One beautiful demonstration is from Ge et. al..^[111-112] They have reported that by aligning clusters of small superparamagnetic NPs into chains, tunable magnetic colloidal photonic crystals can be formed. The colours can be tuned by controlling the regular interparticle spacing (of several hundred nanometers) between the particles which can be controlled using a common magnet. They have demonstrated that the diffraction wavelength can be tuned across the whole visible spectrum. To preserve the tuned colour of the structure, the interparticle spacing between the particles has to be locked (figure 1.13). The chains structure is coated with silica shell, to preserve the structure integrity, thus preserving the colour.^[113-114] Instead of using the strength of magnetic field, the interparticle distance was controlled by the silica shell thickness (which can be controlled by incubation time in Stöber method) before aligned into chains. After coating, the colour in the solution becomes uniform and appears different colour when they were aligned to a magnetic field to different directions.^[115]

Figure 1.13: Schematic showing silica encapsulation of magnetic nanochains. The interspacing between the NPs can be tuned by controlling the starting time of chain alignment during silica formation; longer waiting time will produce NPs with larger spacing, as it allowed silica shell to form before chains formation. Graph (b) showing the increase in interparticle spacing with time while graph (c) showed the absorbance wavelength changes with the waiting time before encapsulation and size of the superparamagnetic particles (inlet). Reprinted with permission from ref. ^[113], copyright © 2011 WILEY-VCH Verlag Gm bH & Co. KGaA, Weinheim.

Beside chains, Qiao et al^[65] from the same group, has also reported on assembling magnetic particles to form 2D wall structure (photonic labyrinths) on a substrate by placing a magnet underneath (figure 1.6e). To assemble magnetic NPs into such wall structure, concentrated NPs are required. The morphology of the labyrinth can be further control using a different lithographically patterned non-magnetic substrate.

In addition to preserving assembled structure, silica shell can also be used to provide steric hindrance, via magnetic janus structure, to assist in the formation of different kinds of magnetic assemblies. The general assembled structure using magnetic janus structure (spherical structure) is to form staggered chains.^[116-117] In another case, non-spherical janus structure can be use for assembled. Yan et. al.^[118] has prepared rod-like janus magnetic structure (Figure 1,14a) by coating a layer of Ni metal on silica micro-size rods, these structure can be tuned to form 1D ribbon like structure in the presence of a magnetic field. These ribbons maintained their chain structure even after removal of the magnetic field, and will bend, splay and even twist (Figure 1.14b). Unlike spherical janus structure, chains form from rod shaped janus structures are more flexible in one plane than the other, hence, their bending can be observed more easily. In their work, by reversing direction of the magnetic field, they can observe progression from magnetic chains to rings

Figure 1.14: Magnetic assembled structure of rod-like magnetic janus structure. TEM in (a) showing formation of ribbon-like structure while (b) showing part of the ribbons formed bend, splay and twisted chain structure. (c) showing that the ribbons can stack in trans or cis formation. Reprinted with permission from ref. ^[118], copyright © 2013 Nature Publishing Group

1.4 RESEARCH MOTIVATION AND SCIENTIFIC CONTRIBUTION

The research field of developing multifunctional nanostructure, using the combination of iron oxide and silica, has been largely explored. In fact, there are quite a number reviews dedicated on this field.^[7, 12, 119-120] However, as described in the sections above, most design is simple; almost all are simple core-shell structures (silica shell coating iron oxide NP core). Hence, we strongly believed that, there is a window of opportunity for developing multifunctional nanostructures with more complexity to perform better in many novel applications and to further bring the field forward using the combination of silica and iron oxide NPs.

One of our strategies is by using iron oxide in a different way. Instead of using iron oxide NP, we have synthesized a hydrophobic polymer with iron and oxygen as its backbone and then coated it with silica to form a liquid encapsulation (droplet encapsulation). The main advantage is in the high compatibility between iron oxide polymer and silica, hence allowed easy and stable liquid encapsulation. Due the liquid core, we showed high loading of various different hydrophobic molecules. In addition, we can also incorporate any combination of hydrophobic NPs. (for example, the oily vesicles can be both magnetic and fluorescent by incorporating magnetic NPs and quantum dots). With this work, we have solved many challenges regarding liquid encapsulation. For example, convenient and effective loading and release method, as well as the ability to load many different molecules and nanoparticles.

In another work (chapter 3 and 5), we have include slight complexity into the core shell regime, by first making Fe₃O₄ NPs a magnetic chain before coat with

silica shell, and developed the world's smallest magnetic nano stir bars for rapid mixing inside tiny and confined systems, for example inside a picoliter emulsion droplets. The chain can be rotated by putting them on top of a common magnetic stirrer just like a common stir bar. Moreover, we have further developed the nano stir bars with fluorescent properties, to potentially be used for tracking different droplets with simultaneous stirring.

From the literature works, it is clear that nanostructures, formed using iron oxide and silica, are very useful in many applications. We recognized the potential importance of this combination, and intended to further explore by designing better nanostructures based on this combination.

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CHAPTER 2: OILY LIQUID FILLED SILICA VESICLES WITH GENERAL LOADING AND RELEASE OF HYDROPHOBIC MOLECULES.

Figure 2.1: (a) Drawn image of oily vesicles cross section (b) TEM and (c) SEM Images of oily vesicles incorporated with iron oxide NPs.

2.1 INTRODUCTION

In the field of nanomedicine, the delivery of therapeutic molecules into the body is of high importance.^[1] However, there are many pharmacologically potent molecules that do not have suitable pharmacokinetics and biodistribution properties to reach target cell, especially hydrophobic drug molecules.^[2-3] Conventionally, these drug molecules can be efficiently modified to increase solubility and permeability through altering or masking functional groups.^[2] However, such optimizations may scarify efficacy.^[3] Furthermore, drug molecules with easier optimization will be more favoured, thus, leaving many physiological potent compounds uncultivated.^[4] Hence, such lipophilic and sensitive drugs necessitate the use of hydrophobic drug delivery vehicles (DDV) for a general, low cost and effective system that could smoothly deliver any hydrophobic drugs with low pharmacokinetic properties to specific target destinations.^[3, 5]

The few examples of popular hydrophobic DDV includes liposomes, polymersomes, serum albumins, lipid nanocapsules and mesoporous silica structures. The general architecture of these hydrophobic DDV consist of a stable shell and a hydrophobic reservoir for storage of drug molecules. For an ideal DDV, the shell should serve three main purposes: (1) enhance structural stability and solubility in aqueous environment, (2) control permeability for containment and release of the cargo molecules, and (3) allow easy fabrication to add advance functionalities into an effective DDV.⁵ Polymer shells have been widely studied due to their controllable triggered released properties. However, it can be quite challenging to incorporate of additional properties onto the polymer shells. Recently, mesoporous silica has been developed as an ideal shell

for DDV. While silica based DDV have low toxicity, they are physically more rigid and stable in comparison to polymer shell. In addition to that, the largest advantage of using silica shell is their facile synthesis to coat on many different nanomaterials. The silica surface can also be easily modified for additional properties such as, attachment of targeted cell recognition molecules, fluorescent imaging as well as gated channels for triggered released of cargo molecules. To increased biocompatibility, an additional polymer (PEG) layer can also be grown on the silica surface.

For hydrophobic reservoirs (storage of drugs), high loading capacity with secluded and protected hydrophobic environment is preferred.^[3] Generally, hydrophobic molecules can be stored via two different architectures, (1) through surface binding or (2) through maximising hydrophobic interactions.^[3, 6] The latter option allowed high payload of hydrophobic molecules. As such, among the many different types of reservoirs, hydrophobic liquid reservoir are well known to pack huge payload and store a generality of hydrophobic molecules but they are highly unstable in aqueous solution.^[3] Hence a coating is often required.^[3] Unlike solid material, due to their unstable, formless properties⁷⁻⁸ it is very challenging to encapsulate hydrophobic liquid with a solid stable shell.^[3]

In the research of emulsion droplets encapsulation, microfluidic methods have been widely reported.^[7-8] This method is versatile, allowing controlled formation of different types of droplet encapsulations (e.g. double emulsion and multi droplet encapsulations, as well as aqueous and non-aqueous droplets encapsulations^[7]) with different type of shells. However, relatively to colloidal synthesis, their production is relatively slow and the minimum sizes formed are always in tens of microns^[7]. For colloidal methods, block copolymers^[9] and

inorganic metal oxide^[10-17] are commonly used as shell materials for liquid encapsulations, sometimes, NPs^[18] as well. However, in general, most polymers assemblies have low stability against physical sheer force and sensitive to changes in environment conditions.^[9] For silica encapsulation, although they are relatively more difficult to coat on a liquid droplet, they are more stable in comparison to polymer shells. Additionally, silica shell can be easily modified for specific applications.

In these emulsion droplet encapsulations, the loading are often done by first mixing with the core droplet, while the released are triggered by breaking the shell structure releasing everything including the core liquid as well as the loaded cargo molecules. While there is a need to synthesis better liquid encapsulations, the loading and releasing method needs to be improved as well.

Here, we report the silica coated emulsion droplets, termed here as Oily Vesicles (figure 2,1). We demonstrate that large generality of hydrophobic molecules (dyes) can be externally loaded in high capacity into the Oily Vesicles, post-formation. The release of the cargo molecules are control by diffusion through the shell without breaking or rupturing the structure. The synthesis is facile and low cost. Through altering the amount of oily liquid and ammonia, the size and silica shell thickness of the Oily Vesicles can be tuned at ease. Remarkably, different types of nanoparticles with any combination can be incorporated securely within the shell for additional functions thus, allowing ultra-tuning of properties of the Oily Vesicles.

Oily Vesicles are silica encapsulation of oily liquid emulsion droplets via a modified Stöber method. In a typical synthesis, the oily liquid is first synthesized through a modified thermal decomposition process reported elsewhere⁹ and was

purified and diluted with THF solution. The resulting solution is then added into a mixture of 2-propanol and water solvent to form emulsion droplets before shaken. After that, TEOS and aqueous ammonia is added to initiate silica coating and finally, the solution is incubated overnight for silica shell to form. To incorporate NPs into the structure, hydrophobic NPs are added in desired ratio into THF solvents containing the oily liquid before the addition into alcohol water system. The structure is then purified by centrifugations and magnetic separation before re-dispersed into water for loading of hydrophobic molecules as well as for characterization purposes.

2.2 RESULTS AND DISCUSSION

Actually, the oily liquid is a by-product formed during the synthesis of iron oxide nanocrystals.^[19-20] As such, being a random mixture, accurate identification is difficult. Hence, for ease of explanation, we termed the by-product species as oily liquid.

In the reported nanocrystals synthesis,^[19-20] iron oxide nanocrystals were synthesized from thermal decomposition process of iron oleate (Fe(oleate)_3) in high temperature. Like many synthesis of NPs, an incomplete reaction would produce intermediate species liken to polymer species before the full formation of desired nanocrystals.^[19-21] They are not large enough to be observed as NPs but already formed large polymer species. As such, these species would have the same composition of the nanocrystals.^[19-20] In our case (figure 2.2), the oily liquid would be an intermediate polymer species with a similar molecular

Figure 2.2: (a) Schematics showing the formation of Oily Liquid from thermal decomposition reaction of Iron oleate. Oily liquid is a mixture of polymer of iron oxide clusters with many random branching; the molecule shown is only a representation of the mixture. (b) Photograph image of Oily Liquid purified with Ethanol (c) Schematic drawings depicting the proposed mechanism of Oily Vesicle formation. TEM image of Oily Vesicles with (d) incomplete shell and (e) fully formed silica shell. Both scale bars represents 100 nm.

structure with the iron oxide nanocrystals (polymer species that contain iron and oleic acid with random branching).^[19-22] Hence, it will have a high affinity towards silica formation. The formation of such intermediate species has already been characterised and explained in detail in another publication on the mechanism studies of the iron oxide nanocrystals synthesis.^[19-20, 22-23] Such speculation was also suggested during a private discussion with Assoc. Prof. Yin Yadong from University of California, Riverside.

With the understanding of the identity of the oily liquid, we demonstrated that it can be produced in large scale with one slight modification of the original nanocrystals synthesis; the lowering of reaction temperature from 320°C to 280°C. Such decrease in temperature would result in insufficient energy needed for complete thermal decomposition process, thus forming only the polymer species, oily liquid.^[19-20] Although oily liquid consist of mixture of products which complicates its accurate identification, its functional groups can be speculated (Figure 2.2).

Here, we propose a mechanism for the formation of Oily Vesicles. The synthesized oily liquid (diluted in THF solution) will form emulsion systems when added into the Stöber conditions. The oily liquid on the surface of the water have a high affinity for silica growth. Hence, when in contact with TEOS and aqueous ammonia on the droplet surface, the TEOS molecules would hydrolyzed to form silica eventually covering the droplet with a rigid silica shell, allowing it to be stable in aqueous solution.

Figure 2.3: TEM images of oily vesicles, with estimated shell thickness of (a) 25nm (b) 60nm (c) 110nm synthesized using different amount of aqueous ammonia, and with average size of (d) 180nm (e) 250nm (f) 400nm controlled through the amount of oily liquid added

The shell thickness and average sizes of oily vesicles can be tuned with easy alteration on the experimental conditions. As Stöber method was used in the encapsulation, the thickness of the outer silica shell can be increase by adding larger amount of aqueous ammonia for higher TEOS hydrolysis rate. 5 μ l of aqueous ammonia added would produce 25 nm silica shell thickness while 8 μ l of aqueous ammonia would result in 60 nm shell thickness and 15 μ l of ammonia would give 110 nm shell. In addition to that, as observed from TEM images, oily vesicles have range of sizes due to the uneven droplet sizes. The average sizes of oily vesicles can be controlled by increasing the droplet size with larger volume of oily liquid added (figure 2.3d-f).

In addition to dimension control, we also demonstrated tunable properties through integration of various NP combinations desired (figure 2.4 and 2.5). During the synthesis, hydrophobic NPs are pre-mixed with oily liquid before silica encapsulation. The NPs which resided in the droplets will be “trapped” within the liquid core and some can be observed to have encapsulated in the silica shell. Since the loading of hydrophobic NPs are achieved via pre-mixing with the oily liquid before encapsulation, hence all hydrophobic NPs can be incorporated into oily vesicles. As a proof of concept, we have incorporated different combinations of NPs which include iron oxide NP, gold NP, iron platinum (FePt) NP and Cadmium Selenide (CdSe) quantum dots as well as their combination as shown in Figure 2.4 and 2.5. After the incorporation, these oily vesicles will retain the properties of the incorporated NPs such as magnetic properties and fluorescent (figure 2.4i and i). As a control, oily vesicles can also be syntheses in the absence of NPs.

Figure 2.4: Magnified TEM images of oily vesicles (a) without NP, with (b) Fe₂O₃ NP, (c) Au NP, (d) FePt NP, (e) CdSe@CdS NP (QD NP), with combination of (f) Au NP and Fe₂O₃ NP, (g) Au NP, Fe₂O₃ NP and QD NP, as well as (h) Fe₂O₃ NP + QD NP. Picture images of Solution of oily vesicles with Fe₂O₃ NP + QD NP (i) put under UV light and (j) put under UV light in the presence of a strong magnet. The figure shows the generality of NP incorporations with retained of properties such as magnetic responsive and fluorescent emission of NP after encapsulation.

Figure 2.5: TEM images at low magnification showing oily vesicles loaded with different NPs as well as their combinations.

2.3 LOADING OF HYDROPHOBIC MOLECULES

For the loading of hydrophobic molecules into hydrophobic liquid encapsulations, in common cases, hydrophobic molecules are loaded by first added into the hydrophobic liquid before coating, trapping the molecules within. However, not all hydrophobic molecules are suitable because they may interfere with the coating process. Hence, in our case, loading of the hydrophobic molecules were achieved via post-loading process; molecules were loaded into the structure after Oily Vesicles are formed. To demonstrate, Nile Red, a common hydrophobic fluorescence dye, will be used as a representation of the hydrophobic drug molecules.

In a typical loading process, oily vesicle incorporated with iron oxide NP was first prepared and concentrated by means of centrifugation. Nile red, initially dissolved in THF, was then added into the oily vesicles followed by slow addition of water (water:THF $\rightarrow$ 2:1). The slow addition of water would slowly increase the polarity of the solvent mixture, driving the diffusion of Nile red from the solution across the silica shell into the oily liquid core. The oily vesicles loaded with Nile red were then thoroughly washed, removing the excess Nile red in the solution through magnetic separation (Detailed procedure for drug loading will be described in the supporting information at the end of the chapter). From the naked eye, an intense reddish colour can be observed from the purified oily vehicles indicating high loading of Nile red molecules. When the loaded oily vesicles are positioned close to a magnet, the purplish red colour of the solution appear to be concentrating towards to the magnet leaving a clear solution, indicating the confirmed residency of Nile red within the oily vesicles

Figure 2.6, (a) Picture drawing depicting diffusion of Nile red into Oily Vesicles through external loading. (b) Schematic of loading process of Nile red. Photograph pictures showing Oily Vesicles (c) before and (d) after the loading process; colour changes from pale yellow to purplish color signifying a successful loading process. (e) and (f) are TEM images of the Oily Vesicles corresponding to (c) and (d) respectively. The TEM images show no obvious rupturing or damage on Oily Vesicle before and after loading of Nile red.

(figure 2.6d). From the TEM images, there were no rupturing or breaking observed in oily vesicles both before and after loading (figure 2.6e and f).

Even though an intense reddish colour can be observed for Nile red loaded oily vesicles, their fluorescence is low, suggesting possible quenching effect. Fluorescence will re-emerge upon release from the oily vesicles. There are three possible reasons for the quenching of fluorescent signal. (1) The NP embedded on the silica shell, (2) paramagnetic iron species of the oily liquid (3) due to self quenching as they were packed into a small compartment. We believe that all the above are possible, but the main factor that caused quenching should be due to the oily liquid and self quenching, since most dye molecules were positioned within the oily liquid, far from the NP (>100 nm). This quenching phenomenon is also consistent with the fact that dyes are loaded inside the oily liquid because simple molecule absorbance or shallow embedding in the thin silica shell will not have such quenching effect. In addition to that, the quenching behaviours and slow re-emergence of the fluorescent will allow the kinetic studies on the Nile red release profile using fluorescent spectroscopy.

To demonstrate the generality in loading hydrophobic molecules into oily vesicles, we demonstrated the loading with 14 different hydrophobic fluorescence dye molecules (figure 2.7). Distinct and intense colours were shown showing obvious high loading of the dye hydrophobic molecules. Similar to the loading process of Nile red dyes, when the dye loaded oily vesicles are put close to a magnet, oily vesicles will concentrate towards the magnet, leaving a clear solution, indicating residency of the dyes with the oily vesicles.

Figure 2.7: Photograph images of oily vesicles loaded with different hydrophobic dye molecules showing the dyes' colors in the absence and presence of a strong magnet. When was positioned near to a strong magnet, the oily vesicles were attracted towards the magnet, the color from the dyes followed the movement of oily vesicles proves successful loading into the oily vesicles.

2.4 RELEASE OF THE LOADED HYDROPHOBIC MOLECULES

Using oily vesicles, the loaded hydrophobic molecules can be released in two different methods, termed here as organic release and aqueous release. Their names imply which medium were used for the drug molecules to be extracted. Organic release was used to extract all hydrophobic dyes from the loaded oily vesicles using a polar organic solvent such as THF or DMF while aqueous release was used to transfer hydrophobic molecules to another biological structure with a hydrophobic compartment (nano-acceptors) across the aqueous solution, such as in bovine serum albumin, BSA.

2.4.1 ORGANIC RELEASE: RELEASING IN POLAR ORGANIC SOLVENT.

Typically, organic released involved adding polar organic solvent into purified dye-loaded oily vesicles so the loaded hydrophobic molecules can diffuse out into the organic solvent (figure 2.8). For the case of Nile red, the initially loaded oily vesicles will appear purplish red, but once the THF is added, the colour will immediately change into bright red. When a magnet was placed closely, the purified oily vesicles appear to have regained its original pale yellow colour while the remaining solution still appeared bright red. This indicates that majority of the Nile red molecules have been released into the THF solvent. This release method is convenient for determining the amount of molecules loaded into the oily vesicles. In our calculations (section 2.7), there are about 0.7 million Nile red molecules loaded in the oily vesicles (average diameter ~350 nm).

Figure 2.8: (a) Schematic drawing depicting the release process of Nile red from oily vesicles via organic release process. Photograph pictures showing oily vesicles (b) before and (c) after the release process. Obvious color changes signifying Nile red molecules were released into the THF solvent. (d) and (e) TEM images of the oily vesicles corresponding to (b) and (c) respectively. Similar to Figure 2.6, the TEM images show no obvious rupturing on oily vesicle before and after the release of Nile red molecules. The picture images showed in the insets are the color of the Oily Vesicles before and after released.

It is important to note that, for organic release, only polar organic solvent such as THF and DMF allowed the immediate extraction of the loaded hydrophobic molecules; the extraction using non-polar organic solvent such as hexane and cyclohexane only takes place after vigorous stirring for a day, suggesting that solvents are required to be able to diffuse across the aqueous inorganic silica matrix (shell) to ease the diffusion (release) of dye molecules from the oily liquid into the outer solvent.

Observing from TEM images, oily vesicles were stable after the release process; they are able to withstand the polarity changes in the solvent as well as the mass diffusion of Nile red molecules, thanks to the robust and porous silica barrier. On top of that, the UV absorption signals of the released Nile red showed no obvious changes suggesting that almost no oily liquid leaked into the solution (graph 2.3 in section 2.7). We speculated that, due to the polymeric nature of the oily liquid, they are both too big and too hydrophobic to diffuse across the tiny pores of the silica shell.

2.4.2 AQUEOUS RELEASE: RELEASING INTO NANOACCEPTORS.

The second released method demonstrated is the aqueous release (figure 2.9). Differing from organic release method, which involved diffusion of loaded molecules into an organic solvent (bulk state acceptor), aqueous release involved the release or transferring of the hydrophobic molecules across an aqueous solution into another nanostructure, (nano-acceptors). Here, the loaded oily vesicles were used as a nanocarrier while Bovine Serum Albumin, BSA, was used as the nanoacceptors.

AQUEOUS RELEASE - Release in aqueous solution through nanoacceptors

Figure 2.9: (a) Schematic drawing depicting the release process of Nile red from oily vesicles through aqueous release process. BSA was used as nanoacceptors as a model system to study the slow release across the aqueous solution. Photograph pictures showing oily vesicles (b) before and (c) after the release process. The color changes signifying Nile red molecules were released from the oily vesicles. (d) is the fluorescence spectrum of Nile red molecules released via organic release. The gradual release profile of Nile red was plotted against time in (e).

In a typical release method, Nile red loaded oily vesicles were first purified before mixed with a BSA solution. The fluorescent emission of Nile red in the resulting solution was measured every minute using fluorescence kinetic spectroscopy for a day. From the spectrum, the fluorescent emission peak of Nile red was observed to have increased upon addition of BSA which signifies the release of Nile red from oily vesicles across aqueous solution into BSA. Based on the fluorescent intensities, saturation was reached in about 24 h.

2.5 CONCLUSIONS

In summary, we had demonstrated encapsulation of oily liquid using silica shell via a facile, scalable and low cost method. The size and structure can be easily tuned. To our knowledge, this is the first reported system of hydrophobic liquid encapsulation that allowed (1) external loading of a generality of hydrophobic molecules, (2) different types of releasing methods of cargo molecules without rupturing of the structure, and (3) versatile incorporation of any combinations of hydrophobic NPs.

2.6 DETAILED EXPERIMENTAL SECTION

Materials and Characterizations: All chemical reagents were purchased and used without further purification. Sodium oleate $\geq 99\%$ (GC), FeCl_3 , Oleic acid $\geq 99\%$ (GC), 1-octadecene $\geq 95.0\%$ (GC), and tetraethyl orthosilicate (TEOS) were purchased from Sigma Aldrich; Ammonia 25%-28% w/w was purchased from Chemical Reagent. Copper specimen grids (200 meshes) with formvar/carbon support film were purchased Beijing Xin Xing Bai Rui Technology Co. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 kV. Field emission scanning electron microscope (FE-SEM) images were collected on a JEOL JSM-6700F.

Formation of iron oxide NPs: The NPs were synthesized using a reported method by Park et al.^[19]

Formation of Oily Liquid: In a typical synthesis, solvent mixture containing ethanol (80 mL), water (60 mL) and hexane (140 mL) were first added into the mixture of FeCl_3 (1g) and Na(oleate) (3g) in a RBF before attached with a condenser. The solution was then heated and incubated at 80 °C for 4 h to form $\text{Fe}(\text{Oleate})_3$. After cooled to room temperature, the resulting brown solution were washed 3 times with water (30 mL) using a separating funnel to remove the aqueous solvents and the un-reacted Na (oleate) and finally the remaining hexane were evaporated leaving a dark brown viscous liquid. Next, 1g of the viscous liquid was mixed with 1-octadecene (240 mL) and oleic acid (6 μL) before heated (at a rate of 3 °C/min) and incubated at 280 °C. After cooled to room temperature, the mixture was purified by washing with ethanol (Figure

2.2b). Lastly, the dark liquid are collected and dispersed into THF to form a 5% concentration to be use as oily liquid.

Synthesis of Oily Vesicles: Oily Vesicles are synthesized using a modified Stöber method. Typically, 40 μL of oily liquid solution (5% in THF) were first added into a solvent mixture of 2-propanol (1000 μL) and water (400 μL). The solution turned turbid signifying the formation of emulsion droplets. The solution were vortex for ~ 10 s. Next, TEOS (5 μL) and ammonia (10 μL) are added and the mixture is then incubated overnight (~ 16 h) for complete growth of silica shell. Finally, Oily Vesicles are purified through centrifugation methods and magnetic separation before re-dispersed into water or ethanol. As described in the main text, size of the Oily Vesicles can be tuned with increased of oily liquid while the shell can be controlled by amount of ammonia added.

Loading of dye molecules (Nile red) into the oily vesicles: Purified Oily Vesicles are first added into 100 μL of water / propanol (1:1) solution. 100 μL of Nile red@THF (10mM) is then added and the resulting solution is incubated for 10 min to initiate diffusion of Nile red molecules into the Oily Vesicles. Next, water (600 μL) is added drop wise to increase the polarity of the solvent in a slow manner. This is important to allow time for Nile red molecules in the solution to accumulate into the hydrophobic compartment of Oily Vesicles. Finally, the Oily Vesicles is purified at least 5 times to remove the unloaded dyes in the solution. After purification, the colour of the Oily Vesicles will turned according to the colour of the loaded dyes.

Dye release from Oily Vesicles (Nile red): Organic Release of dye molecules: The purified Nile red loaded Oily Vesicles are mixed with THF (100 μ L). bright red color will appear immediately once THF is added, and the purified Oily Vesicles will revert back into its original pale yellow colour. The Dye@THF can then be used for UV characterizations.

Aqueous Release of dye molecules: Purified dye-loaded Oily Vesicles is added into 1000 μ L of BSA@water (20mg/mL) and the mixture is left alone for about a day for releasing of dyes into BSA.

2.7 CALCULATIONS

2.7.1 CALCULATION OF CONCENTRATION OF LOADED NILE RED MOLECULES IN OILY VESICLES VIA ORGANIC RELEASE

Graph 2.1: UV absorbance of nile red (NR) in different concentration

Graph 2.2: Calibration curve of NR in THF solvent.

Graph 2.3: UV absorbance curve of the released NR molecules from oily vesicles via organic release method.

Table 2.1

Concentration calculation of NR release			
Sample name	intensity	Concentration	Number of mol (3.5ml)
NR1	0.818	0.024mM	8.4×10^{-8}
NR2	0.986	0.029mM	1.015×10^{-7}
NR3	0.851	0.025mM	8.75×10^{-8}
NR4	0.793	0.023mM	8.05×10^{-8}

Average total number of mol of NR = 8.84×10^{-8} mol

Calculation of the amount of NR dyes loaded into one Oily Vesicles

(average diameter 315 nm)

Volume of Oily Liquid (5%) used = 25 μ L

assumed that only very small amount of THF left in the droplet.

hence, volume of pure Oily Liquid used = 1.25 μ L

$$= 1.25 \times 10^{-9} \text{ m}^3$$

Total Volume of one Oily Vesicles with width of 315 nm (in diameter)

$$r = 315 \div 2$$

$$\frac{4}{3}\pi r^3 = 1.668 \times 10^{-20} \text{ m}^3$$

$$\text{The total number of oily vesicles} = \left(\frac{1.25 \times 10^{-9} \text{ m}^3}{1.668 \times 10^{-20} \text{ m}^3} \right)$$

$$= 7.494 \times 10^{10} \text{ Particles}$$

From the UV data,

the total number of mole Nile red loaded = $8.84 \times 10^{-8} \text{ mol}$

The total number of molecules loaded = $(8.84 \times 10^{-8}) \times N_A$

$$= 5.32 \times 10^{16} \text{ Molecules}$$

hence,

$$\text{the number of Nile red molecules per one oily vesicle} = \left(\frac{5.32 \times 10^{16}}{7.494 \times 10^{10}} \right)$$

$$= 710177.2 \text{ molecules per oily vesicle}$$

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CHAPTER 3: STIRRING IN SUSPENSION: NANOMETER-SIZED MAGNETIC STIR BARS

Figure 3.1: (a) Schematics illustrating the synthesis of nano stir bars. (b) TEM and (c) SEM images of nano stir bars. (d) Schematic and (e) photograph showing parallel stirring of droplets (5 μL) on a common magnetic stir plate. (f) Photographs showing the dispersing of dye solution (rhodamine 6G) in a water droplet (30 μL) with stirring nano stir bars (see also Video S2).

3.1 INTRODUCTION

Rapid mixing is essential for achieving effective chemical and biological reactions. Because passive diffusion is a slow process, it is often necessary to agitate or stir a solution. Generating such disturbance must involve external input of energy, for example, transduction of magnetic field energy through the use of commercial magnetic stir bars. But such use is impractical for tiny channels and droplets, which are of great importance for lab-on-chip application^[1-4] and microliter bioassay.^[5-7] The main challenge lies in the fabrication of low-cost stir bars that are sufficiently small but still able to transduce external energy for the mixing. Moreover, one should be able to introduce, operate, and extract the stir bars with ease.

In microfluidic research, a number of methods have been developed to improve mixing. For a lamellar flow confined in a channel, turbulence can be induced by forcing the solution through a complex winding channel,^[2, 8-9] or by pulsed injection of additional solution/bubbles from the side channels;^[10-13] thermal gradient can be used to induce convection;^[7, 14] direct physical agitation can be achieved using ultrasound or piezoelectric transducer;^[15-19] and finally, in-channel stirring was achieved using magnetic turbines that were fabricated by lithographic methods.^[20-22] These mixing schemes were often fabricated together with the channel systems, because it will be too difficult to introduce them after the fabrication.

In static microdroplets, however, transducing external energy for mixing remains a challenge, particularly in arrays of microdroplets. Ultrasonication and violent stirring can break up the droplets, while thermal gradient across tiny droplets is impractical. So far, magnetic stirring is still the most convenient

option. Micro-sized stir bars have been reported, for example, linear chains of polymer beads embedded with magnetic nanoparticles (NPs),^[23-29] star-shaped micro-stirrers made by soft lithography,^[30] and Co-based magnetic bars cut by laser micromachining.^[31] Because of gravitational and magnetic attraction, micro-sized stir bars tend to stir only at the bottom,^[31] leaving most part of the solution unstirred. While facile and scalable synthesis remains a challenge, the main problem of micro-sized stir bars is still their size: they are too large to remain suspended, but too small to churn up the whole solution.

Here, we report a simple and scalable method for fabricating magnetic stir bars (figure 3.1), which are tunable from 75 nm – 1.4 μm in width (figure 3.2) and from 2 μm to ~15 μm in length (Figure 3.3). They are straight single-line chains assembled from 40 nm magnetic NPs and then preserved in silica shells of variable thickness. These rigid magnetic chains showed immediate response to a common magnetic stir plate and can be easily recovered. Being small and in large number, they can remain suspended and stir independently in all parts of the solution. These nano stir bars can be readily dispensed for parallel stirring of droplets down to picoliter in volume (4 picoliter).

3.2 RESULT AND DISCUSSION

In a typical synthesis, oleic acid stabilized Fe_3O_4 NPs^[32] ($d = 40$ nm) were first modified with citric acid to render them soluble in water.^[33] After purification, these NPs were dispersed in water/2-propanol mixture ($v/v = 3:5$). To align the NPs with simultaneous silica encapsulation,^[34-36] tetraethyl orthosilicate (TEOS) and ammonia were added to the solution and the reaction vessel was immediately placed close to a magnet. After incubated overnight, the

Figure 3.2: Nano stir bars of different thickness. TEM image of nano stir bars with (a) no shell, (b) ~10 nm, (c) ~50 nm, (d) ~100 nm, and (e) ~520 nm thick shell around the magnetic core NP chains.

Figure 3.3: Nano stir bars with different lengths. SEM image of nano stir bars with average length of (a) $\sim 1\ \mu\text{m}$, (b) $\sim 5\ \mu\text{m}$, and (c) $15\ \mu\text{m}$. Scale bar of (a) and (b) represented $1\ \mu\text{m}$ while (c) represented $10\ \mu\text{m}$.

products were isolated and purified by means of centrifugation. To control the thickness of the chains, the amount of TEOS is modified (details in the experimental section.) while length of the chains can be tuned by the concentration of Fe_3O_4 NPs added.

The product Nano Stir Bars were too small to be directly observed by naked eyes, but remarkably, droplets containing them “blinked” in sync to the rotating magnetic field (Video S1 attached). The “blinking” appeared more obvious when thicker magnetic chains were used, suggesting that it was caused by light scattering. As the rigid chains spun in unison, their different scattering efficiency in the transverse and longitudinal directions caused the “blinking” of the entire droplet.

As shown in the transmission electron microscopy (TEM) image (Figure 3.1b), the dark Fe_3O_4 NPs formed linear single-line chains, which were coated with a grayish silica shell. The gaps between the NPs were barely noticeable, suggesting that the aggregation had probably occurred at the initial stage before the silica encapsulation. In this method, changing both the solvent and the surface ligand of the as-synthesized Fe_3O_4 NPs were important for promoting aggregation and achieving their alignment. In images where the Nano Stir Bars were stacked together (*e.g.*, Figure 3.1c), it can be observed that they were straight and rigid. There was no obvious morphological change after stirring, which was remarkable considering the sheer forces involved in the process.^[37] It appeared that the silica shells were critical in maintaining their structural integrity. In control experiments without silica encapsulation, the resulting NP chains were much shorter and often in randomly curved conformations (Figure 3.2a).^[38-43]

Figure 3.4: (a) Schematic illustrating the setup for observing nano stir bars during their slow stirring. (b-d) Typical optical microscopy images showing the alignment of nano stir bars in accordance to the rotating magnetic field (black arrows). See also Video S3

To observe the effects of stirring, we added 3 μL of fluorescent dye (rhodamine 6G, 0.25 mM) to static water droplets (30 μL) containing nano stir bars of ~ 200 nm in width (Figure 1f and Video S2). Under UV irradiation ($\lambda = 254$ nm), the initial droplet was dark, thus the homogenization of the bright dye can be clearly observed. When stirring was off, complete dispersion occurred at about 100s after the addition of dye, whereas it took only 12 s when stirring was on. From the video, it can be observed that the bright cloud of dye actually swirled around in the droplet along the direction of stirring, almost in the same way swirling is induced by a typical macroscopic stir bar. However, the swirling was much less pronounced in that there was no whirlpool formation.

There are several possible modes of stirring: the nano stir bars may either rotate individually at their local positions; or “swimming” around like a school of fish; or a combination of these two motions.

While the “real” stirring was too fast to be observed, we designed a setup using an optical microscope to directly observe the stirring in slow motion: a droplet containing Nano Stir Bars was positioned on a fixed platform between two opposing magnets, which were placed on a plate beneath the platform (Figure 3.4). As the plate was manually spun, the movement of the nano stir bars can be directly observed. From the images and video (Figure 3.4b-d and Video S3), the nano stir bars were too small to be clearly distinguished. But we can still discern their alignment from the fiber-like appearance, which was always aligned in the direction of the magnetic field (*i.e.*, rotating at the same speed). Hence, it appeared that the nano stir bars were all individually rotating at their local positions. It is logical to expect a similar rotating motion under faster

stirring conditions. Indeed, this was supported by the “blinking” phenomenon discussed above.

However, the swirling of dyes in the droplet (Figure 3.1f) is indicative of a concerted motion of the entire droplet, as opposed to local agitation of the solution. The rate of swirling appeared much slower than that of the rotating magnetic field. Probably, the rotating Nano Stir Bars can induce their surrounding solution to move along a same direction, causing slow swirling of the entire droplet. When the magnetic field was manually spun at a low speed, the concerted motion of the solution was likely negligible. Though we cannot directly observe the Nano Stir Bars during fast stirring, there are good reasons to believe that they were being carried along with the slow swirling solution, as they were rotating individually.

To view the magnetic alignment in greater detail, we dried the droplets containing Nano Stir Bars when the stirring was either on or off (Figure 3.5). The resulting samples were characterized by scanning electron microscopy (SEM). When stirring was off, the Nano Stir Bars in the dried sample were all aligned in the direction of magnetic field. In contrast, when stirring was on, the Nano Stir Bars stacked with each other in a criss-cross fashion, as if they were trapped during their rotation at different points in the final stage of drying. Most importantly, the Nano Stir Bars did not arrange head-to-tail like a school of fish, consistent with the above analyses.

Figure 3.5: (a) Schematics illustrating the drying of water droplets containing nano stir bars. SEM images of the dried samples when the stirring was (b) off; or (c) on. Similar setup using nano stir bars of different sizes is shown at figure 3.10.

The smallest Nano Stir Bars in our system (average 75 nm in width and 4 μm in length) were still larger than the typical colloidal particles. Thus, when there was no stirring, they usually sedimented after several hours (5-10 h). But remarkably, they remained almost permanently suspended in solution (for at least 24 h) when the stirring was on (Figure 3.6a and b), probably because of their constant agitation. As such, they can stir all parts of the solution, unlike larger stir bars which tend to stir only at the bottom (or on top¹⁸) of the solution. To make a fair comparison, we synthesized large stir bars ($\sim 1.4\ \mu\text{m}$ in width and $\sim 10\ \mu\text{m}$ in length) by coating a thick layer of silica, making them close to but still smaller in size than the typical micro-sized magnetic stir bars in the literature.^{17e, 19} While these larger stir bars were slightly more effective in terms of stirring, they were still far too small to create a whirlpool. At this size, most of these stir bars sediment after ~ 10 min even when the stirring was on (Figure 3.6c and d). In the literature, most of the micrometer-sized stir bars were in the range of 30 – 400 μm in width; they should sediment more quickly than our largest stir bars.^{16, 18-19}

However, the swirling of dyes in the droplet (Figure 3.1f) is indicative of a concerted motion of the entire droplet, as opposed to local agitation of the solution. The rate of swirling appeared much slower than that of the rotating magnetic field. Probably, the rotating nano stir bars can induce their surrounding solution to move along a same direction, causing slow swirling of the entire droplet. When the magnetic field was manually spun at a low speed, the concerted motion of the solution was likely negligible. Though we cannot directly observe the nano stir bars during fast stirring, there are good reasons to

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The Nano Stir Bars in our systems were small and thus, they were made and used in large numbers. On the basis of the average 97 NPs per stir bar, we estimated that there were about 410 million stir bars in the stirring experiments in Figure 1f (30 μ L droplets). Hence, the amount of the dispensed Nano Stir Bars can be easily controlled according to their concentration. This is an advantage over similar systems, where ensuring at least one micro-size stir bar per droplet is a challenge. On the other hand, extracting the Nano Stir Bars was also easy: As illustrated in Figure 3.7, a static droplet (8 μ L) containing nano-stir bars (200 nm in width) was placed on a piece of parafilm on top of a static magnet. After 5 min, the cloudy brownish droplet turned clear and the Nano Stir Bars were all attracted to the bottom of the droplet. The clear solution on top was moved away using a micropipette (Figure 3.7a-c, Video S4), allowing the recovery of both the solution and stir bars.

Figure 3.6: Photographs showing the sedimentation process of the following 30 μL solutions when stirring was on: (a) suspension of nano stir bars of ~ 75 nm in width; and (c) suspension of nano stir bars of ~ 1.4 μm in width. Typical TEM images of the nano stir bars are shown in (b) and (d), respectively.

As a proof of concept, we tried to introduce Nano Stir Bars for stirring inside picoliter droplets, which were generated by channeling water into the flow of olive oil using a simple cross-flow microfluidic chip (Figure 3.8a). The resulting water-in-oil emulsion was characterized by optical microscope. By increasing the flow rates, tiny droplets could be generated ($d = 20\ \mu\text{m}$, Figure 3.8b-c), but their monodispersity was not as good as that obtained at a low flow rate. For easy observation, Nano Stir Bars of $\sim 200\ \text{nm}$ in width were used in low concentration. They were dispensed into the droplets as part of the initial water phase. As shown in Figure 3.8c and Video S5, the Nano Stir Bars in the droplets were initially aligned to the magnetic field but were induced to rotate when the stirring was turned on. Such stirring would not be possible if not given the extremely small size of the stir bars.

3.3 IMPLICATION ON THE USE OF NANO STIR BARS

Nano Stir Bars is a nanometer size stirring device that can be very useful for rapid mixing in tiny and confined environments such as inside picoliter droplets. The advantage is in its ability to have control in their stirring (for e.g. turning off stirring any time by switching off the stir plate). To our knowledge, it is the most convenient device to stir inside water-in-oil emulsion droplets. Since mixing of droplets (in microfluidic chip) normally take place in complex winding channels, hence the development of nano stir bars will potentially simplify the channel designs; mixing reactions inside the droplets can also be accurately controlled.

In addition, nano stir bars is also suitable for parallel mixing in arrays of micro-reactors, particularly in screening experiments (figure 2.1e). Despite their small size, they are effective in mixing in small amount of liquid (from a few picoliters up to tens of micro-liters in volume) and are easy to handle. Their stirring is also relatively gentle, and will not caused breaking or accidental mixing between neighboring droplets. We strongly believe that the development of nano stir bars will potentially enable a simpler design of microreactor arrays with smaller reactors size.

3.4 CONCLUSION

In summary, we have developed a new method for fabricating the world's smallest magnetic stir bars. Their small size turned out to be of great importance: The nano stir bars can be used for stirring inside extremely small droplets; they can remain suspended and stir all parts of the solution; they can be easily confined inside a droplet instead of being pulled out by an external magnetic field; they can stir effectively without breaking up the droplet; and finally, they can be easily fabricated in great numbers, allowing economic usage, easy dispensing, and effective stirring.

Figure 3.8: (a) Schematics illustrating the formation of picoliter droplets containing nano stir bars using a cross-flow microfluidic system. (b) Optical image of the suspended water droplets in oil. (c) High resolution optical images of a picoliter droplet containing nano stir bars (black arrows). See them stirring in Video S5.

Figure 3.9: Optical image showing nano stir bars inside of another picoliter droplet ($\sim 100\ \mu\text{m}$ in diameter) when the magnetic stir plate was (a) off; or (b) on. Arrows showing the same nano stir bars in (a) and (b) accordingly, changed direction when the magnetic field is on signifying rotating motion.

3.4 DETAILED EXPERIMENTAL SECTION

Materials and Characterizations: All chemical reagents were purchased and used without further purification. Oleic acid-stabilized iron oxide NP (Fe_3O_4 NPs, $d = 40 \text{ nm}$) was purchased from Ocean NanoTech LLC; Dimethylformamide (DMF), 1,2-dichlorobenzene (DCB), citric acid 99% and tetraethyl orthosilicate (TEOS) were purchased from Sigma Aldrich; Ammonia 25%-28% w/w was purchased from Chemical Reagent. Copper specimen grids (200 meshes) with formvar/carbon support film were purchased Beijing Xin Xing Bai Rui Technology Co. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 kV. Field emission scanning electron microscope (FE-SEM) images were collected on a JEOL JSM-6700F. Optical microscopy images and videos were recorded using a CCD camera (DP70, Olympus), mounted on a Nikon Eclipse Ti-U.

Preparation of TEM Sample: TEM grids (as purchased) were first treated with oxygen plasma using Harrick plasma cleaner/sterilizer for 2 min to improve the hydrophilicity of the surface. 5 μL of diluted sample solution was then dropped on the treated surface of the TEM grid and was dried in air for 1 h.

Preparation of SEM Sample: Silicon wafer was cut into smaller pieces with dimension of approximately 1 cm x 1 cm. They were cleaned by sonicating in acetone, deionized water and ethanol for about 15 min each and then kept in ethanol solution for storage. For sample preparation, the cleaned silicon wafers were first taken out and dried under a flow of N_2 gas. 5 μL of sample solution were dropped on the treated silicon wafer and dried in air for 3 h.

Ligand exchange process of Fe₃O₄ NPs: The ligand exchange process was carried out according to a reported method.^[1] Citric acid (100mg) was first weighed into a vial followed by the addition of DMF (1 mL), DCB (1 mL) and Fe₃O₄ NPs (20 µL). The resulting solution was incubated at 100 °C for 24 h. Then, the product was isolated by centrifugation at 5200 g for 5 min. Finally, the hydrophilic Fe₃O₄ NPs was dispersed in 1 mL of deionized water.

Synthesis of Nano Stir Bars with ~75 nm, ~140 nm and ~200 nm in width: The synthesis of Nano Stir Bars was achieved using modified Stöber method in the presence of a magnet. In a typical synthesis of ~140 nm width nano stir bars, The citric acid-stabilized Fe₃O₄ NPs in water (200 µL) was first added into the solvent mixture of water (400 µL) and 2-propanol (1000 µL) followed by the addition of TEOS (10 µL) and ammonia (30 µL). The resulting solution was then vortex for 5 s to ensure thorough mixing and was immediately positioned next to a neodymium magnet to assemble the Fe₃O₄ NPs into chains. After incubating overnight, the brownish Nano Stir Bars were adhered to the wall closer to the magnet due to magnetic attraction. The transparent solution was removed, and the nano stir bars in the vial were then redispersed in water and purified by centrifugation at 2000 g for 3 min. For the synthesis of nano stir bars with ~75 nm and ~200 nm in width, 20 µL and 45 µL of TEOS were added, respectively.

Synthesis of Nano Stir Bars with ~1.4 µm in width: The synthesis method is similar to that of smaller width Nano Stir Bars described above. Citric acid-

stabilized Fe_3O_4 NPs (200 μl) were added into the solvent mixture of water (110 μl) and 2-propanol (1000 μl) followed by the addition of TEOS (10 μL) and ammonia (90 μL). The resulting solution was immediately positioned next to a magnet, incubating overnight. The product was isolated by centrifugation at 800 g for 2 min. Finally, the process was repeated five times to produce Nano Stir Bars with 1.4 μm in width.

Synthesis of Nano Stir Bars controllable different lengths (average) from ~1 μm , ~5 μm , ~12 μm and ~20 μm : The length of Nano Stir Bars were controlled and synthesized using the same Stöber method as explained in above, but with modification in the amount of Fe_3O_4 NP solution. For Nano Stir Bars of 1 μm in length, 10 μL of Fe_3O_4 NP solution were added. For synthesizing nano stir bars of ~5 μm , ~12 μm and ~20 μm , 30 μL , 100 μL and 300 μL of NP solution were added respectively.

Stirring in picoliter droplets: Picoliter droplets (~100 μm in diameter) were produced in a simple cross-flow microfluidic chip with flow-focusing design (figure 3.9). The chip was made of polydimethylsiloxane (PDMS) via a standard soft lithography process. The dimension of the channels in the cross-shaped junction were 50 μm x 50 μm . Picoliter droplets were generated when water was channeled into the flow of olive oil using syringe pumps. Nano Stir Bars can be dispensed into the droplet for stirring by addition into the water phase prior to the flow. These droplets were then placed near to a magnetic stir plate, in which the stirring motion of the Nano Stir Bars within the droplet were observed and recorded using a CCD camera mounted to the optical microscope.

Figure 3.10: Photograph of picoliter droplets containing nano stir bars being generated from a cross-flow microfluidic chip.

Figure 3.11: Large-area SEM images showing dried samples of nano stir bars when the stirring was (a, c) on; or (b, d) off. Only magnetic stir plate was used; no magnet was used. (a) and (b) are nano stir bars of 200 nm in width while (c) and (d) are 75 nm in width.

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CHAPTER 4: THE CONTRACTION OF SILICA SHELL

Figure 4.1: (a) Schematic showing the formation of contracted structure as shell thickness increases. (b) and (d) showing SEM and TEM images of chains with thin silica shell while (c) and (e) showing chains with thick silica shell respectively. When a thick silica shell was coated on a magnetic chain, the structure formed a “twisted” looking structure (c), the chain becomes contracted as well, in (e).

4.1 INTRODUCTION

Silica has been very commonly used in synthesizing useful multifunctional nanostructures.^[1] In most cases, it is only used as coating onto complex structures partly because it could not directly cause or induce any formation of complex nanostructures. Silica by itself, do not show any distinctive shapes (other than spherical shape); they will just follow the shape of core material. Even with core structures that contained edges such as cubes or star shapes, the edged parts will be smoothen by the silica coating, forming a rounded shape.^[2-10] it is important to note that, smooth silica coating will only form when the core structures have low interfacial energy or has high compatibility with silica. For example, in the work of Kotov et al.^[11-12], silica can formed bristled shaped silica coating when there is a high interfacial energy between the CdTe NW (core) and silica. Generally, the role of silica encapsulation is mostly to stabilize as well as to preserve structure integrity. Even in the formation of chiral mesoporous silica, it does not induce any structural changes but only to preserves the self assembly structure of the chiral molecules.^[3, 13-15]

Here, remarkably, we have discovered an uncommon property of silica encapsulation during coating of Fe₃O₄ NP chain. Instead of the smooth silica surface, a “twisted”-like morphology was observed (Figure 4.1c). Upon close inspection, their surface morphology resembles a twisted nylon rope which is chiral, but with less order. Interestingly, this structure will only form when thick silica shell was coated (Figure 4.1). In addition to that, although the thick shell chains appeared roughly straight overall, the inner core chains appear highly contracted (Figure 4.1 and 4.2); chains with thin shell remained very straight in relative with no contraction. While direct analysis of such phenomena remained

challenging, we resort to detail reactions to probe the reasons of such formation. We were able to provide evidence that such morphology is dependable on the thickness of silica coating. And by elimination process based on rational control experiments, we showed consistent results that the “twisted”-like morphology and the contracted inner NP chains are caused by a new and unusual property of silica; a compression force originated from thick silica shell.

The chain structure discussed in this chapter is actually the same structure as the nano stir bars discussed in the previous chapter. However, to avoid confusion, this structure will be known as magnetic chain in this chapter. In addition to that, throughout this chapter, ‘contraction of chains’ will be used to described the curvy state of the inner NP chain as observed in TEM images throughout the chapters, while “twisted”-like morphology will be used to described the surface morphology of the thick chains. We named the morphology as “twisted”-like morphology because it appeared twisted (figure4.1c), we were not able to provide strong evidence to prove such twisted morphology.

Figure 4.2: Schematic (a) showing the method used to control the strength of magnetic field via controlling the distance of the reaction vials from the magnet. While the SEM images in (b) to (e) showing the increased in contraction of the chains in relation to the strength of magnetic field respectively.

4.2 RESULTS AND DISCUSSIONS

The chains are synthesized through a modified Stöber method, similar to the Nano Stir bars, described in the previous chapter. Typically, hydrophilic Fe_3O_4 NPs were first prepared by surface modification using citric acid before adding into a solvent mixture of 2-propanol and water (10:3). Next, TEOS and aqueous ammonia were then added and the solution was vortex to mix. Immediately after the addition, the solution was put close to the magnet to align the NPs into chains before silica shell growth. Shell thickness can be controlled by tuning the solvent ratio, amount of TEOS and ammonia as well as the incubation time controlled from $\sim 4\ \mu\text{m}$ to $17\ \mu\text{m}$ in length while the width of the silica coating can be tuned from 75 nm to 500 nm. Generally, the straightness of the chains can be controlled by tuning the strength of the magnetic field, in which straighter chains can be synthesized by stronger field, demonstrated in figure 4.2. To ensure the accuracy of our study, the same magnetic field strength (distance = 0 cm) will be used for all the experiment unless specifically mentioned.

Upon close inspections of the SEM and TEM images, the morphology of the thick chains are can be observed as visible sections of silica ‘folded in’ to form grooves in between, ranging from 1 side to another, throughout the chain. These sections do not have ordered separated segments and they appeared to be connected and ‘coiling’ around the chain just like a twisted nylon rope. In addition to that, from the TEM images, the inner NP chains appear to be contracted along the chain.

There were two uncommon phenomena, (1) the formation of contracted morphology and (2) the contraction of the chains, in which both can be clearly observed when the thickness of the silica coating reached $\sim 120\ \text{nm}$ or more;

chains with thin silica shell (~15 nm) are much straighter. It is important to note that all samples were purified and characterized using the same condition, and since the thin shell chain structures remained straight, the contracted surface should not be a result from the purification process such as centrifugation and drying.

In the nature of silica: firstly, silica coating will always form a rounded surface even when coating on rough surfaces. Secondly, coating from the same material, thin and thick, should behave the same, because almost every condition is the same, and third given the same magnetic field, the straightness of the chain should be the same. Hence, it is highly uncommon that the formation of such surface morphology as well as the contracted chain only form in thick silica coating.

4.3 COMPRESSION RATIO: DEFINING THE DEGREE OF CHAIN CONTRACTION.

To study the mechanism of formation, it is important to characterise the phenomena. While it is difficult to quantify the “twisted-like” morphology, we resort to estimate the degree of contraction in the chains by measuring the compression ratio. The compression ratio is a simple calculation to compare the degree of contraction of the chains, in which the larger the compression ratio, the higher degree of contraction in the chain (figure 4.3), and hence, logically, the “twisted”-like morphology should become more pronounce. The variables can be obtained from TEM images, α is the length of the contracted chains and β is the product of number of NPs in part of the chain times with the diameter of each NP. Putting both α and β into the equation in figure 4.3b will give a

Figure 4.3: TEM and schematic in (a) and (b) showing the calculation of compression ratio to describe the contraction of the magnetic chains. The sample size used in the calculation was at least 600 NPs for each data point. Graph (c) depicting an increased in compression ratio when shell thickness is increased by changing the condition of the Stöber method which include the amount of water, TEOS and ammonia added as well as different reaction time.

Figure 4.4: TEM images of chains with different shell thickness synthesized using different modifications of Stöber method. For the first row, the thickness of the silica shell was tuned using the water:2-propanol, (a) 1:1, (b) 1:2 and (c) 1:5. The silica shell in the second row was tuned by amount of TEOS, (d) 1 μL , (e) 3 μL and (f) 20 μL . In the last row, the shell thickness was tuned by amount of ammonia, in which (g) 10 μL , (h) 30 μL and (i) 60 μL . Detailed synthesized is explained in the experimental section at the end of this chapter. Even from these different conditions, all thin shells appeared to be straight while the thick shells appeared twisted with contracted chain.

compression ratio, which estimate the degree of contraction that has occurred in the same part of the chain, (as compared to its own ideal straight chains).

To pinpoint the driving force for the “twisted”-like morphology as well as the contraction of the chain, different condition in the Stöber method that can be used for tuning shell thickness were investigated, which include varying the solvent ratio, amount of TEOS and ammonia (as shown in figure 4.4). In addition, shell thickness can also be tuned via controlling the reaction time; aliquots of the sample were taken out for TEM studies at different time. Using these controls, samples with different shell thickness were synthesized, their compression ratio were calculated and plotted in graph (figure 4.3c). From the graph, a clear trend can be observed; as the shell thickness was increased, the compression ratio increases.

These results suggest that (1) there is a driving force are gradually increase with increase of the shell thickness and (2) is independent on the reaction condition.

4.4 POSSIBLE MECHANISM FOR THE CONTRACTION

For the next section, several possible mechanisms that could have led to such occurrence will be discussed. From the above control experiments (figure 4.3), we pinpoint that the driving force should not have caused by any changes in the Stöber method condition, and most probably, were originated during silica formation. Hence, there are a few possible mechanisms, (1) the relative speed of silica shell growth to the distance to the magnet, hence, strength of the magnetic field, (2) aggregation of small silica NPs to the chain before shell formation, and (3) the nature of the silica shell, where the silica matrix may have contracted and

cause a compression force towards the inner NP chain. First, the strength of the applied magnetic field affects the straightness of the chain which can be tuned by adjusting the distance from the magnet as demonstrated in figure 4.2; NPs closer to the magnet will form straighter chains. In the synthesis, NPs will first form chains before gradually attracted towards the magnet and resulted in straighter chains. In order to have large difference in compression ratio (straightness), the difference in rate of silica shell formation must be quite substantial, with relative to the speed of NP chain moving towards the magnet.

To study this possibility, aliquot from both thick and thin shell reaction (by tuning water solvent ratio) was taken for characterizations. From the graph showed in Figure 4.5a, silica shell from thick shell condition formed within 5 minutes while silica from the thin shell reaction formed within 7 minutes. In both reactions, only after 15 min, some NP chains can be seen concentrated near the magnet. Hence, the difference in rate of silica formation is too short to have caused the difference in compression ratio.

Second, another possible reason would be due to the aggregation of silica aggregates adhering to the chain surface to cause formation of ‘sections’. Such aggregation onto the surface should be random, hence will only formed rough surfaces. However, from the TEM images (Figure 4.1e and f), the silica coatings on every chain were highly uniform (even in areas where NP chain bent). Therefore, this possibility is unlikely.

Lastly, both observations could be originated from the nature of silica. The hypothesis is that: silica could have contracted due to its nature. The contraction of silica would cause a reduction in size and hence, will apply a force compressing the NPs in the core. The NPs can then have 2 possible ways to

Figure 4.5: The rate of silica shell formation of different shell thickness. Graph (a) depicting an increased in compression ratio when shell thickness is increased (by tuning the water amount) at different incubation time as showed in the TEM images as well. These thin and thick shells are formed almost at the same time; it is unlikely that speed of silica formation would be the reason for the twisted morphology as well as the contracted chains.

respond, either “gives in” and the whole structure will reduced in size, or the NP chains could have resisted the compression. We believe NPs should be solid and incompressible but the chains were only connected by a weak force from the magnetic field. Hence, the result of resisting compression should caused contraction of the chain, hence, forming a twisted-like morphology.

There can be 2 possible ways for silica to contract, either (a) the lost of polar molecules that were embedded in the silica matrix during drying process for further TEM or SEM characterizations,^[5] and (b) further cross-linking in the silica matrix after silica formation.^[5b]

Firstly, for (a) to be true, the contraction will only occurred after drying; in other words, the “twisted”-like morphology can be prevented if the inner NPs were to be removed before drying. However, shown in figure 4.6b, contraction and the morphology can still be clearly observed, even when NPs were completely etched before drying. This suggests that contracted morphology and the contracted chain occurred before the etching and drying process. In another control experiment (figure 4.6c), the silica shells were slightly etched to introduce more pores in the silica matrix before drying process to prevent contraction. However, both “twisted”-like morphology and contracted NP chains can still be clearly seen, hence it is logical to speculate that contraction has already occurred before drying. From these two control experiments, contraction by drying process is unlikely as well.

Figure 4.6: The TEM images showing the before (a) and after etching of inner NPs chain in (b) as well the silica shell in (c). In (a) and inlet of (b), the background appeared to be ‘checked’ because it was purposely brighten to allow clear observation of the NP chains encapsulated within the thick silica shell. “Twisting”-like morphology as well as crooked chains can still be seen after etching hence, it is likely that drying effect did not caused the phenomena.

Lastly, in another possibility, contraction of silica may have occurred in solution, during the silica shell formation. From the previous work in our group, there are different kinds of silica; the inhomogeneity in the silica shell, the soft and hard silica, denoting lesser and more cross-linked silica respectively.^[5b, 6] Generally, in common Stöber method, the initial silica shells formed were soft with very little cross-linking. Cross-linking will gradually take place as the reaction aged to form a hard shell.^[5b]

We proposed that it is possible for silica shell to have heavily cross-linked and induced a contraction in the silica matrix which in turn compressing the NP chains, which would be consistent with the gradual increase in compression ratio as silica shell is formed (figure 4.3c and d). To prove this hypothesis (shown in figure 4.7), chains with thick shell were acquired at different reaction time (10 min and 30 min) because the samples showed large increase in compression ratio, shown in figure 4.3c. Both chain samples were etched in hot water for 10 min (similar method used to differentiate soft and hard silica). The 10 min chain sample (~120 nm in diameter) was almost completely etched, while the 30 min chain sample (~206 nm) was hardly etched. This indicates that the degree of cross-linking has increased in ~20 min, which is consistent with the increase in compression ratio.

Figure 4.7: Etching in silica in hot water can be used to assess if the silica shell is cross-linked. TEM images showing silica shell before etching in (a) and (d) and after etching in (b) and (d). For (a) and (b) are from silica shells that were formed in 10 min while (c) and (d) are formed in 30 min. As compared. The silica formed in the earlier stage are completely etched, less cross-linked as compared to the one formed within 30 min.

In another control experiment (figure 4.8), to demonstrate the presence of a compression force from the contraction of silica shell, a second thick silica shell was coated on a thin silica shell with a hollow inner chain (the NPs were etched away). The diameter of the inner hollow NPs was measured before and after the thick shell coating. After the thick coating, we observed a trend, the thicker the 2nd silica shell, the smaller the size of hollow NPs. When the second thick silica shell (152 nm) was coated on the thin shell hollow chain, there is a total of ~4.3 nm decrease in diameter for each hollow NP area. This is indicative of a compression force from the silica shell, compressing the inner hollow NP chains, causing reduced in size of the hollow NP chains.

It is important to note that, the above NP lengths were taken through TEM images. Under TEM, the diameter of the hollow NP can be affected by the thick silica; edge of the hollow NP may be unclear. In addition, It is also possible that, the reduction of diameter of the hollow NP areas may be caused by silica diffusing inward, growing on the wall of the hollow NP areas. Hence, to improve accuracy of this method, length of 10 straight hollow NPs was measured for each data point (sample size is at least 220 NPs) instead of measuring individual NPs. Then, the measurement was divided by 10 to get the average diameter for each hollow NP.

To summaries the above discussions, the thicker the silica shell, the more dangling bonds, and the more cross-linking site in silica. There will be more volume it can contracts, hence, the higher the compression force, which will result in more obvious “twisted”-like morphology and contracted of the chains.

Figure 4.8: The compression force of second thick shell silica on the first thin silica shell. Hollow NP core were used to allow the reduction of diameter to assess any compression force from the second thick shell. TEM images of (a) showing the thin silica shell with hollow NP chain, and (b) showing the chain after encapsulated with a second thick shell (diameter of 317.71 nm). Table (c) depicting a trend; the diameter of hollow NP chain will decrease when a thicker shell was coated, signifying an increase in compression force.

From our understanding in this system, thick silica shell is the key for such “twisted”-like morphology. The role of NP chains is to resist such compression. Hence, we try to encapsulate different NWs with thick silica shell (figure 4.9) to recreate the phenomena in other NWs. However, from the SEM images, although some of the ultrathin Au NWs appeared to be slightly contracted, none showed obvious contracted morphology. Since these NWs are not straight in nature, it is difficult to judge if there are any contraction took place. We speculate that, even for a flexible 1D structure, to form contracted morphology, the inner 1D structure should be able to rotate sideways, and hence, NWs are unable to form similar “twisted”-like morphology.

4.5 CONCLUSION

In summary, through various control experiments and compression ratio measurements, we have demonstrated a hidden property of silica. When thick silica shell were coated on a magnetic NP chain, “twisted”-like morphology will form, the chain will also becomes contracted. Such morphology cannot be seen in thin silica coating. From our control experiments, we believe such phenomena were caused by contraction of silica due to further cross-linking occurred during silica growth

However, such twisting morphology could not be observed when other types of NWs were coated with thick silica shell. Most probably, the contraction required not only a thick silica shell and a flexible chain, but a flexible chain that can rotate sideways without much restriction.

Figure 4.9: TEM (left column) and SEM (right column) images showing thick silica encapsulation using different types of NW. (a) and (b) $\text{MnO}_2\text{NW@SiO}_2$, (c) and (d) AgNW@SiO_2 , (e) and (f) hydrophobic ultrathin AuNW@SiO_2 while (g) and (h) are hydrophilic ultrathin AuNW@SiO_2

4.6 DETAILED EXPERIMENTAL PROCEDURES

Materials and Characterizations: All chemical reagents were purchased and used without further purification. Oleic acid-stabilized iron oxide NP (Fe_3O_4 NPs, $d = 40 \text{ nm}$) was purchased from Ocean NanoTech LLC; Dimethylformamide (DMF), 1,2-dichlorobenzene (DCB), citric acid 99% and tetraethyl orthosilicate (TEOS) were purchased from Sigma Aldrich; Ammonia 25%-28% w/w was purchased from Chemical Reagent. Copper specimen grids (200 meshes) with formvar/carbon support film were purchased Beijing Xin Xing Bai Rui Technology Co. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 kV. Field emission scanning electron microscope (FE-SEM) images were collected on a JEOL JSM-6700F.

Preparation of TEM Sample: TEM grids (as purchased) were first treated with oxygen plasma using Harrick plasma cleaner/sterilizer for 2 min to improve the hydrophilicity of the surface. 5 μL of diluted sample solution was then dropped on the treated surface of the TEM grid and was dried in air for 1 h. During imaging, the brightness of the electron beam was increased to for clearer image of the inner NP chains. Hence, the background may appear checked.

Preparation of SEM Sample: Silicon wafer was cut into smaller pieces with dimension of approximately 1 cm x 1 cm. They were cleaned by sonicating in acetone, deionized water and ethanol for about 15 min each and then kept in ethanol solution for storage. For sample preparation, the cleaned silicon wafers

were first taken out and dried under a flow of N₂ gas. 5 µl of sample solution were dropped on the treated silicon wafer and dried in air for 3 h.

Synthesis of magnetic chains with different shell thickness: The synthesis of magnetic chain was achieved using modified Stöber method in the presence of a magnet. In a typical synthesis, citric acid-stabilized Fe₃O₄ NPs in water was first added into the solvent mixture of water and 2-propanol followed by the addition of TEOS and aqueous ammonia. The resulting solution was then vortex for 5 s to ensure thorough mixing and was immediately positioned next to a neodymium magnet to assemble the Fe₃O₄ NPs into chains. After incubating overnight, a brownish precipitate can be found adhered to the wall close to the magnet due to magnetic attraction. The transparent solution was removed, and the precipitate in the vial were then re-dispersed in water and purified by centrifugation. In the discussion, shell thickness of the silica coating were controlled by tuning either the water : 2-propanol ratio, the amount of TEOS, and the amount of ammonia as well as the incubation time.

Etching of silica shell: The silica shell were etched using a previously reported method.^[16] After the chains were purified and collected from the above reaction, it was re-dispersed into 1 mL of water. The samples were then incubated in 90° C for 10 min. Next, the vial was place under flowing water for 1 min for cooling and was centrifuged twice with ethanol before TEM characterizations.

Synthesis of Au NP@Sodium Citrate: The Au NPs are prepared according to a previously reported method.^[17-18] Typically, 100 μ L of HAuCl₄ (10mg/ml) were added into 100 mL of water. The solution was stirred vigorously using magnetic stir bars, and was heated to 100° C. After incubating for 15 min, 1 mL of sodium citrate (1% v/v in water) was added into the reaction. The resulting solution was incubated for another 15 min before was cooled to room temperature.

Synthesis of α -MnO₂ NW@PVP: α -MnO₂ NWs were synthesized via a reported method.^[19-21] Typically, Mn(CH₃COO)₂·4H₂O (0.49 g), (NH₄)₂S₂O₈ (0.46 g), and (NH₄)₂SO₄ (0.13 g) were added into mixture of 10 mL of deionized water, with 30 mL of 1-octanol and was transferred into a 45 mL Teflon-lined autoclave before heated at 140 °C for 12 h in a digital oven. Next, the product was then obtained by centrifugation with deionized water and ethanol. Lastly, the α -MnO₂ NWs were then incubated into 10 mL of PVP solution (1% in DMF) at 70 °C for 2 h.

Synthesis of Ag NW@PVP: Ethylene glycol (50 mL) was first heated at 151 °C for 1 h in a 250 mL rbf, with stirring at ~260 rpm. CuCl₂ (4mM, 400 μ L) was introduced into the solution and continue heated for 15 min. Next, PVP (0.147 M, MW = 55000, 15 mL) was added into the mixture followed by a slow addition of AgNO₃ (94 mM, 15 mL) using a syringe pump at a rate of 500 μ L min⁻¹. The solution will turn lighter in colour with swirly precipitates after several hours of reaction. Lastly, the RBF is moved to an ice water bath to

quench the reaction. The AgNW were purified with ethanol using low speed centrifugation and were re-dispersed in water.

Synthesis of ultrathin AuNW@4-MBA: The hydrophilic ultrathin NWs were grown on a silica surface as reported previously.^[22] In a typical synthesis, APTES solution was first prepared by adding 2 μL APTES into solvent mixture containing Ethanol (250 μL) and water (750 μL) before sonicated for 15 min. A silicon substrate was treated with plasma cleaner for 10 min and was then soaked in the APTES solution for another 15 min. After rinse several times in Ethanol and water, the substrate was soaked in a solution containing Au NPs prepared as described for 5 min before wash. A monolayer of Au NP will be stick on the substrate. Next, the substrate is put inside a growth solution containing, ethanol (700 μL), HAuCl_4 (50 μL , 17 mg/ml in water), 4-MBA (150 μL , 10mM in ethanol) and finally Ascorbic acid (600 μL , 10mM in water). The solution will turn colourless and the substrate will turn pale gold. The NWs can be obtained by re-dispersed them in water via sonication.

Synthesis of ultrathin AuNW@oleylamine: This modified synthesis was adapted from Prof Xing's group.^[23] Briefly, 3 mg of HAuCl_4 was mixed into 2.5 mL of Hexane containing 100 μL oleylamine and 150 μL triisopropylsilane. The solution were shaken for a few seconds and left alone for ~5 h. The NWs were centrifuged with ethanol and were re-dispersed into THF before use for encapsulation.

Silica encapsulation of MnO₂ NW, Ag NW, and ultrathin AuNW@4-

MBA: Similar to the Stöber method used for encapsulation of magnetic chain described above. The hydrophilic NWs were added into 2-propanol (1000 µL), water (400 µL), TEOS (10 µL) and ammonia (90 µL). The resulting solution was then incubated for ~16 h and the samples were purified by centrifugations.

Silica encapsulation of ultrathin AuNW@oleylamine: oleylmine capped NW are hydrophobic. For encapsulation using Stöber method, the ultrathin NW were added into THF solvent (400 uL) and were mixed into the 2-propanol (1000 uL), water (300 uL) and TEOS (20 uL), and ammonia (20 uL). Lastly, the resulting solution was then incubated for ~16 h and the samples were purified by centrifugations.

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CHAPTER 5: GENERAL DYE INCORPORATION IN SILICA SHELLS FOR MULTIPLEXED FLUORESCENT NANO STIR BARS.

Figure 5.1: (a) Drawn image, (b) TEM, and (c) SEM image of the fluorescent nano stir bars. (d) and (e) are drawn image, (f) and (g) are confocal microscopy images (scale bar: 10 μm), while (h) is the fluorescent spectra showing that our method are better in synthesizing brighter fluorescent nano stir bars in comparison to the surface grafting method.

5.1 INTRODUCTION

Silica NPs with incorporated dyes are used as fluorescent labels for sensing and imaging in biomedical applications.^[1-4] Despite the large number of literature reports, only a few specific dyes were commonly used. They typically contain a reactive functional group for bio-conjugation, for example, –SCN group for reacting with –NH₂ surface groups. These reactive dyes are usually available in mg scale and very expensive.^[3, 5]

Coating dye-impregnated silica on functional NPs can lead to synergistic effects and novel photophysical properties.^[6-7] However, such examples are rare and limited, because the colloidal stability of NPs is generally incompatible with the process of dye incorporation. Rational design is thus essential in avoiding the complications among the three components (dye, silica, and NPs). To the best of our knowledge, to date there has been no systematic study on the general incorporation of dyes in silica shells.

Previously (In chapter 3), we reported the synthesis of nano stir bars and their stirring inside tiny emulsion droplets.^[8] They were formed by first aligning citrate-stabilized Fe₃O₄ NPs into single-line chains using an external magnet, followed with silica coating to preserve their chain structure. As such, they are robust enough to stir and small enough to enter picoliter droplets and resist sedimentation.^[8] If we view emulsion droplets as convenient micro-reactors, color coding of the nano stir bars would enable multiplexed tracking of the emulsion droplets with simultaneous stirring. But the limited capability in dye impregnation into silica shells becomes a bottleneck.

5.2 RESULT AND DISCUSSION

In this work, we improved the literature methods to greatly enhance dye incorporation into the silica shells of nano stir bars. Traditionally, dye molecules are chemically grafted onto the surface of silica NPs^[3, 9-10] (as shown in figure 1e). As such, the surface provides limited capacity and overloading of dyes can lead to excimer formation. We were surprised that no one has tried to load dyes homogeneously in the silica shells (Figure 1d), but later realized that avoiding NP aggregation was essential for our goal. By simply applying PVP in the reaction and modifying the preparative steps, we were able to load significantly more dyes than the traditional methods can achieve (Figure 1f-h), that is, the ceiling of excimer formation became much higher. We showed that this method can be generally applied to 9 typical dyes, including inexpensive dyes without a reactive functional group. Proof-of-concept demonstration was achieved by tracking the fluorescent nano stir bars inside picoliter droplets with simultaneous stirring.

At the beginning, we followed a method in the literature^[3] to graft fluorescent dyes on the silica surface of our nano stir bars. Basically, the silica surface of the nano stir bars was first functionalized with -NH_2 groups by reacting with APTES. After purification to remove the excess chemicals, FITC (Dye 1) was added so that its -SCN group can react with the surface amine groups. The nano stir bars were then purified again to remove the excess dyes and chemicals. In the second method, Dye 1 was first conjugated to APTES and the reaction mixture was directly used to treat nano stir bars, where the hydro. The -SCN group will readily react with -NH_2 group to form amide bond, linking the dye molecules on the silica surface. After incubation, the product was

purified and coated with additional silica shell to improve both stability and solubility. However, there were almost no visible fluorescent observed by naked eye under UV light (Figure 1d); their fluorescent can only be seen using a confocal fluorescent microscope (Figure 1e).

When we released the loaded dyes by etching away the silica shell, bright fluorescent can be observed in the supernatant under UV light. This observation indicates a quenching effect, most likely, due to excimer formation of the loaded dyes. Since the method limits dyes incorporation only to one molecular layer densely on the silica surface, it is not optimized in terms of dye loading, creating a case of dilemma; low loading of dyes would lead to low fluorescent signal, while high loading would lead to excimer formation which will also resulting in loss of fluorescence signal.

It is important to note that, inexpensive dyes that do not contained reactive groups can also be conjugated with -NH_2 group using a less reactive functional group (-COOH) via prior activation with EDC. However in comparison, their conjugation is much lower in efficiency, hence, are less preferred for fluorescent labelling, for example 1-pyrene butyric acid (dye **4**), 7-hydroxycoumarinyl-4-acetic acid (dye **5**), calcein blue (dye **6**) 5(6)-carboxy fluorescein (dye **7**), calcein (dye **8**) and rhodamine B (dye **9**).

Here, we have further developed the surface grafting method to avoid aggregation and to greatly enhance dye loading, so that even inexpensive dyes without reactive conjugation groups can be loaded in high amount onto the nano stir bars (Figure 1). In our method, dye **7** was used as a representation of the inexpensive dyes. It was first conjugated with APTES in the presence of EDC. Basically, EDC will activate the -COOH group in **7** which will form amide bond

with the -NH_2 groups of APTES, forming 7-APTES conjugation. 7-APTES were then incubated overnight with ethanol and ammonia for pre-hydrolysis of its silane group. Nano stir bars and polyvinylpyrrolidone, PVP were then added before introducing TEOS to initiate silica shell formation. Co-condensation of 7-APTES with TEOS will ensure incorporation of the dyes covalently into the silica matrix. The solution was then aged overnight, ensuring the silica coating to be highly cross-linked. Lastly, additional silica was coated to further increase its stability in aqueous solvent. The same method was used for the incorporation of dye **4** to **9**. For dyes with reactive conjugation groups such as dye **1**, Rhodamine B isothiocyanate (dye **2**) and 5-Carboxy-X-Rhodamine, succinimidyl ester (dye **3**), the addition of EDC is not necessary.

Our fluorescent nano stir bars showed bright fluorescent under UV light, observed by naked eyes, in comparison to the literature method. Such high loading is due to the better incorporation method, by embedding dyes into the entire silica shell instead of only on the surface. From the fluorescent microscopy image (figure 1f), the bright green bars are the fluorescent nano stir bars and dark backgrounds indicates clean purification of by-products and unloaded dye molecules. These fluorescent nano stir bars appeared to be aligned to a single direction due to the presence of a magnet.

From the transmission electron microscopy (TEM) image (Figure 1b), the dark dots in chains are the Fe_3O_4 NPs while the surrounding grey layers are the silica shells. The lighter silica layer in between is embedded with PVP and dye molecules; the lighter contrast is due to the lower atomic weight of the C in PVP and dyes, in comparison to Si.

Figure 5.2: (a) Pictures showing fluorescent nano stir bars loaded in various types of dye molecules with their molecular structures. Dye 1 to 3 are common dyes with reactive functional groups while Dye 4 to 9 are relatively inexpensive dyes with less reactive functional groups (-COOH). (b) fluorescent spectra of the fluorescent nano stir bars in (a).

Similar to the previous nano stir bars,^[8] obvious blinking can also be observed when fluorescent nano stir bars were put close to a rotating magnetic field. As they were rotating in unity, the difference in light scattering efficiency in the transverse and longitudinal directions will result in the blinking effect. This blinking effect is actually very useful as a fast indication for aggregation. Since blinking depended on the clear contrast of light scattering at different direction during rotation, blinking will 'stop' when the fluorescent nano stir bars are aggregated and unable to rotate.

Fluorescent of fluorescent nano stir bars do not enhance blinking because fluorescent are emitted in all directions and do not contribute towards the difference between the light scattering efficiency. In fact, the low light surroundings under UV light will cause the blinking effect to be less obvious.

To prove the generality of this dye incorporation method, we demonstrated loading of 9 different dyes both with and without reactive conjugation group into our nano stir bars, all showing bright fluorescent under UV light (figure 5.2). Consistent with the literature reports, dyes with reactive conjugation groups have higher efficiency in loading. Hence, for dyes without special conjugation group, 20 times more dyes-APTES are added for bright fluorescent.

From our estimation using UV spectroscopy, our brightest fluorescent nano stir bars (shown in figure 1d) contained about 420k dye **1** molecules per nano stir bars, about 6.7 times more than that of the surface grafting method (62k molecules). The higher loading is because dyes are distributed in the entire silica shell rather than limited only onto the surface (figure 1d and e). The amount of loaded dyes can be further increased 3.6 times more, about 1.51 mil dyes/nano

stir bar, but such increase in loading will result in a reduced in fluorescent intensity and a red shift due to excimer formation (figure 5.3).

Figure 5.3: Fluorescent spectrum showing an increase in fluorescent quenching when more dye **1** molecules were incorporated into fluorescent nano stir bars. The maximum emission wavelengths were red shifted indicating excimer formations.

5.3 MECHANISM OF FORMATION AND THEIR MASSIVE AGGREGATIONS

Our synthesis of bright fluorescent silica shell is only possible due to the addition of PVP to prevent aggregations (Figure 5.3). Dye-covered silica nanostructures are often slightly insoluble in aqueous environment and will tend to form agglomerations. During dye incorporation, small silica NPs from the by-product will tend to accumulate onto the agglomerations. Further silica growth on these accumulations will lock the agglomeration, forming large aggregations as observed in the SEM image in figure 4b.

To prevent such massive aggregation, PVP were added to stabilize the dye covered silica structure, and preventing accumulation of the silica nucleates. Interestingly, among the many surfactant molecules, for example, PEI, PEG, PAA and others, only the addition of PVP can prevent the aggregations. Most likely because PVP is a weak ligand;^[11] hence, they will only tend to reversibly bind to the silica surface. Polymers with slight positive charge such as PEI may have interacted strongly with all silica surface and promoting their aggregations. Other polymers or surfactants such as PEG and PAA that carries slight negative charge will be repelled by the –OH group on silica, hence will only be a ‘bystander’ in the reaction.

Figure 5.3: (a) Schematic showing the effect of PVP addition to prevent massive aggregations. SEM images and picture image showing the synthesized fluorescent nano stir bars (c) with and (b) without the addition of PVP.

5.4 PROOF OF CONCEPT: LABELING AND STIRRING INSIDE PICOLITER DROPLETS.

As a proof of concept, we have inserted fluorescent nano stir bars (loaded with dye 2) into picoliter droplets (figure 5.4). The droplets were generated by channelling water containing fluorescent nano stir bars into a flow of olive oil via a simple cross-flow microfluidic channel. Under the fluorescent microscope (figure 5.4a) the fluorescent nano stir bars are stable and was distributed evenly inside the droplets. When a rotating magnetic field were applied, the fluorescent nano stir bars can be spun. For easy imaging (figure 5.4b to d), these fluorescent nano stir bars were rotated slowly by moving the magnets by hand. Without our optimized method to load large amount of dye molecules, the imaging will never be so clear.

5.5 CONCLUSION

In summary, we report a systematic study on a general method to incorporate fluorescent dyes into silica shells including inexpensive dyes with no reactive functional groups. In comparison to the surface grafting method, we can load more dye molecules homogenously within silica shell. This cannot be achieved without solving the aggregation problem during dye incorporation. We demonstrated the loading of many different dye molecules into the nano stir bars. With their varieties of colours, these fluorescent nano stir bars can be used to label and stir simultaneously inside emulsion droplets.

Figure 5.4: (a) Fluorescent microscopy images showing fluorescent nano stir bars rotating inside an emulsion droplet. (b) to (d) are zoomed in images of the same droplet showing them rotating at different directions.

5.6 DETAILED EXPERIMENTAL PROCEDURES

Materials and Characterizations: All chemical reagents were purchased and used without further purification. Oleic acid-stabilized iron oxide NPs (Fe_3O_4 NPs, $d = 40$ nm) was purchased from Ocean NanoTech LLC; Dimethylformamide (DMF), 1,2-dichlorobenzene (DCB), citric acid 99%, tetraethyl orthosilicate (TEOS), and all dye molecules (such as 5(6)-carboxyfluorescein, Rhodamine B, Calcein and Calcein Blue) were purchased from Sigma Aldrich; Ammonia 25%-28% w/w was purchased from Chemical Reagent. Copper specimen grids (200 meshes) with formvar/carbon support film were purchased Beijing Xin Xing Bai Rui Technology Co. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 kV.

Ligand exchange process: The ligand exchange process was carried out according to a reported method.^[1] Citric acid (100 mg) was first weighed into a vial followed by the addition of DMF (1 mL), DCB (1 mL) and Fe_3O_4 NPs (20 μL). The resulting solution was incubated at 100 °C for 24 h. Then, the product was isolated by centrifugation at 5200 g for 5 min. Finally, the hydrophilic Fe_3O_4 NPs was dispersed in 1 mL of deionized water.

Synthesis of Nano Stir Bars with ~200 nm in width: The synthesis of Nano Stir Bars was achieved using modified Stöber method in the presence of a magnet.[3, 7] In a typical synthesis, The citric acid-stabilized Fe_3O_4 NPs in water (210 μL) was first added into the solvent mixture of water (400 μL) and 2-

propanol (1000 μL) followed by the addition of TEOS (10 μL) and ammonia (90 μL). The resulting solution was then vortex for 5 s to ensure thorough mixing and was immediately positioned next to a neodymium magnet to assemble the Fe_3O_4 NPs into chains. After incubating overnight, the brownish Nano Stir Bars were adhered to the wall closer to the magnet due to magnetic attraction. The transparent solution was removed, and the Nano Stir Bars in the vial were then re-dispersed in water and purified by centrifugation at 2000 g for 2 min.

Synthesis of Fluorescent Nano Stir Bars: Here, Dye 4 was used as the model dye molecules. Typically, the dye molecules are conjugated with APTES first by mixing dye **4** (10 mM in ethanol) into a mixture of EDC and APTES with molar ratio of 5:6:6 in an overnight incubation. Next, 300 μL of the resulting solution, now containing **4**-APTES, were diluted into 1.3 mL ethanol and 100 μL ammonia for at least 12 h. After that, 200 μL of purified nano stir bars in water and 100 μL PVP (MW = 40,000, 0.5M in water by unit) in water were added and incubated for 2 h. After stirring for another 30 min, 10 μL of TEOS are added and the solution was incubated for at least 2 h before purified with ethanol many times with low speed centrifugation at 1600 g for 5 min. To improve stability, an additional silica shell were coated using a standard Stöber method which involved, 1 mL ethanol (with the fluorescent nano stir bars), 300 μL water, 10 μL TEOS and 90 μL ammonia. After aged overnight, the product was purified with water and is ready for further characterizations. The same condition can be applied for incorporating dye **5** to **9**. For dye **1**, **2** and **3**, the same method was applied without adding EDC.

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CHAPTER 6: CONCLUSION AND OUTLOOK

6.1 CONCLUSION

To summaries, my research has been focusing on silica coated iron oxide nanostructures. In my system, silica were used mainly to improve stability, to preserve structure integrity and to allow additional functionalizations, incorporating new properties, while iron oxide were used for its magnetic properties in movement controlled of multifunctional nanomaterials as well as for easy purification. Improvising the many similar systems reported in the literature, novel multifunctional nanostructures based on silica coated iron oxide nanomaterials were designed and synthesized, for example, briefly, as described in chapter 2, Oily Vesicles, a hydrophobic liquid encapsulation has been prepared as drug delivery vehicle for hydrophobic molecules. While in chapter 3, 4 and 5, Nano Stir Bars, magnetic chains of iron oxide NPs were used for stirring in extremely small and highly confined areas such as in picoliter size droplets or in microfluidic systems.

In chapter 2, iron oxide materials were used in a very novel way, as hydrophobic liquid for loading of hydrophobic molecules. The liquid is a mixture of polymers consisting iron oxide as backbone and oleic acid as branches, termed here as Oily Liquid. Thanks to the unique structure, it can be easily encapsulate with silica to form silica shell vesicles (Oily Vesicles). Due to its hydrophobic and liquid nature, high amount of drug molecules can be loaded, protected by the silica shell. In this chapter, we demonstrate their application in

storing, delivering and release of hydrophobic molecules, using 14 different types of dye molecules.

The advantages of Oily Vesicles, in contrast to other types of liquid encapsulations, are (1) the loading of dye molecules are achieved after the synthesis, hence, allowing a general method for cargo loading (this is important as some molecules may affect liquid encapsulations), (2) leakage will not occur even in hydrophobic polar solvent such as THF due to the polymeric nature of the oily liquid species, while the hydrophobic molecules stored inside can diffuse across the shell freely, (3) rupturing is rare, thanks to the rigid and stable silica shells, diffusion of dye molecules across the oily vesicles as well as drying for characterization will not cause rupturing. In other words, the structure can be re-used many times. Furthermore, NPs (such as Au, Fe_3O_4 , FePt and CdSe) with any desired combinations can be easily incorporated into the oily vesicles by just mixing the NPs with the oily liquid before silica encapsulation.

In chapter 3, to extend the use of magnetic property of Fe_3O_4 NPs, we have assembled them into single line chains and then coated with silica shell to form Nano Stir Bars; Presently, in our knowledge, the world's smallest magnetic stirring bar. The dimension can be easily controlled from 75 nm to 1.4 μm in width and from 1 μm to 20 μm in length. The synthesis method is facile; billions of them can be synthesized in a 1 mL solution.

These Nano Stir Bars can be used for stirring inside extremely small droplets (~4 picoliters); they can remain suspended in solution without settling down, stirring all parts of the solution; they will be confined stably inside a droplet instead of being pulled out by an external magnetic field; they can stir effectively without breaking up the droplet; and finally, they can be easily

fabricated in great numbers, allowing economic usage, easy dispensing, and effective stirring.

Next, in chapter 4, upon close inspection on some of the thick shell Nano Stir bars, a novel twisted like structure can be observed. These twisted structures will always formed when thick coating is applied, independent of the different conditions using Stöber methods. By imaging the chains at different time, we showed that twisting is a gradual process during silica shell growth, in which the chain is straight initially and were twisted as the shell grows thicker. Via various control experiments, we believe that these twisted structures were formed due to a compression force exerted by the thick silica shell uniformly towards the inner chain core, caused by the cross-linking of the silica matrix. It is logical to assume that cross-linking will result in a decrease in space due to bond formation, causing uniform contraction of the silica matrix. The contraction will then creating a compression force towards the inner chain core, but were resisted by the incompressible but flexible Fe_3O_4 NP chains, hence, forming a twisted morphology. Surprisingly, such twisted morphology will only form when NP chains were used as cores; even ultrathin AuNW do not show the same twisted morphologies. This is most likely because these NPs in a chain can be independently rotate sideways in comparison with the NWs.

Lastly, as described in chapter 5, taking advantage of the silica shell, a fluorescent silica layer were grown onto the Nano Stir Bars using cheap fluorescent dyes. Dye molecules with carboxylate group(s) were chosen for conjugation with APTES in the presence of EDC. The dye incorporation was done using Stöber method with co-condensation of dye-APTES and TEOS. While this has been adapted many times to synthesized ultrabright fluorescent

silica NPs, it will cause massive aggregations when growing a fluorescent shell onto larger silica nanostructures.

The aggregation is due to two main reasons, (1) the low solubility of dye-covered silica nanostructure during incorporation, which will cause reversible agglomeration, (2) the accumulation of silica deposited onto these reversible agglomerations, hence locking the agglomerates with an additional silica shell. We have modified the synthesis and have successfully prevent massive aggregation by introducing PVP to coat on the dye-covered Nano Stir Bars to prevent initial agglomeration, hence, the massive aggregations. Harsh stirring which commonly prevent agglomeration, will break the Nano stir Bars during the synthesis, hence, only gentle stirring will be used.

Using this method, bright fluorescent Nano Stir Bars can be synthesized, as observed under the fluorescent microscope as well as under UV light. Images from fluorescent microscopy suggested that there almost no aggregations formed between the Nano Stir Bars, which is consistent with their blinking effect observed as they were spun on magnetic stir plate.

6.2 OUTLOOK

Based on the several inventions described in this thesis, several future directions can be explored.

6.2.1 OILY VESICLES FOR POTENTIAL TEXTILE APPLICATIONS

For Oily Vesicles, Its ability to store large amount of hydrophobic molecules and to release them to the biological entities in aqueous solution as well as to be able to incorporate and embed any NPs within the silica shell would allow it to be great for drug delivery. However, to be suitable for in vivo applications, the diameter should be reduced to below 50 nm. Besides that, a system to remove the oily vesicles from the body is crucial if we wanted to use it in the body due to the hydrophobic liquid within the oily vesicles.

Although it may not be suitable for drug delivery, it can be used externally such as on textiles (such curtains, clothes, socks shoes and other types of garment), and on hard surfaces (such as walls or patches). The Oily Vesicles can be easily incorporated into the surfaces of clothing or meshes through either via dry casting method or through charge-charge interaction via amine group functionalizations (figure 6.1). More common hydrophobic molecules such as mosquito repellent, fragrances, anti-fungi medicines, can be loaded into a readymade patches or surface containing Oily Vesicles and release to the air. Theoretically, air is hydrophobic, hence hydrophobic molecules should be able to slowly diffuse close to the surface to prevent diseases or odours (similar to the case with nanoacceptors). If required, organic polar solvent such as acetone or ethanol can be used to spray on these oily vesicles to help release.

Moreover, the synthesis of Oily Vesicles is low cost because only cheap materials are required such as iron chloride, sodium citrate, TEOS and others. It is non toxic since it contained mainly iron oxide and silica. However, the main problem is that it will be difficult to study the release of materials because it involved measuring small amount of release in air.

Figure 6.1: Picture showing oily vesicles can be embedded in the mesh of textiles. The spherical structures are the oily vesicles while the black strings represent the textile mesh,

6.2.2 NANO STIR BARS TO STIR IN MICROREACTORS

As described in the previous chapters, our low cost Nano Stir bars can be very efficient for mixing in extremely small and confined areas. Beside microfluidic systems, it can be inserted and stir inside colloidal microreactors. For example, small yolk shell systems or liquid core shell encapsulations containing inner compartments can use such precise stirring capability to churn up the reaction inside every microreactors in the solution (in the billions), which we believe cannot be achieved other than using the small size Nano Stir Bars.

Figure 6.2: Nano Stir Bars can be inserted and stir inside a microreactor

The magnetic properties of Nano Stir Bars would also allow these microreactors to be easily purified. Moreover, due to the silica shell, we can easily incorporate different metal NPs onto the shell, to form a catalyst

that can stir. Here, we showed that several different kinds of nanomaterials can be incorporated onto the Nano Stir Bars such as AuNP, AgNP, Ag Nanoplates, AuNR, AuNWs as well as AgNWs. While the direct assembly of longer 1D nanomaterials onto the Nano Stir Bars are more difficult and messy to control in comparison to the NPs (Figure 6.3), we can grow a forest of NWs on the surface of the Nano Stir Bars (Figure 6.4) for high surface area catalyst.^[1] However, after the growth of this ultrathin forest of AuNWs, they become easily agglomerate, hence impeding the stirring of Nano Stir Bars.

Figure 6.3: Different types of NPs can be adhered onto the surface of the nano stir bars. However, adhering NWs appeared messy.

Figure 6.4: Using a reported method,^[1] forest of ultrathin AuNW can be grown on the AuNPs, previous assembled on the surface of nano stir bars. Following the arrow key, from (a), (b), (c) to (d), TEM and SEM images showing the increasing length of AuNW forest grown on the nano stir bars.

6.2.3 NANO STIR BARS: SPINNING TO FORM SPIRAL STRUCTURES.

In addition to that, the spinning ability of the Nano Stir Bars can be used in creating novel spiral nanostructures. The idea is to attach soft flexible NWs onto the two ends of the Nano Stir Bars and spin rapidly, pulling these NWs into spiral shapes. Silica shell can then be coated, locking the structure.

The challenge is to selectively grow or attach NWs on to the two ends which can be very difficult. As shown in Figure 6.5 as well as in the previous paragraphs, attaching NWs can be very messy, due to the sticky amine functionalized silica surface. However, we have successfully synthesized 1D structure at the end of the Nano Stir Bars by using magnetic NPs. By attaching these NPs onto the Nano Stir bars, aligning them in an external magnetic field followed by thin silica shell formation, long chains of NPs can be grown at the two ends. However, while they can spin under the rotating magnetic field, they are could not form any spiral structure, even without any silica shell. The structure many have too rigid with silica shell and too easy to break without silica shell during spinning.

Figure 6.5: (a) Picture showing spiral structure when nano stir bars are spun with 2 flexible NWs attached on the 2 ends. (b) TEM image of using magnetic NPs to form the 2 side chains however, were not able to form spiral structure yet.

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The product can be reached online at

<http://nanoseedz.com/nanostir%20bar.html>