Solute induced phase separation and droplet encapsulation

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Solute Induced Phase Separation and Droplet Encapsulation

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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

2019

Special Statement

The work presented in this thesis has been completed under the supervision of professor Dr. CHEN, Hongyu.

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Abstract

The scope of this research is to expand the design of nano-synthesis from solid particles to liquid droplets. In the research area of nano-synthesis, a variety of strategies have been developed based on the understanding of fundamental principles of solid nucleation and growth. We aimed on following this example to lay the foundation of droplet nano-synthesis (**Chapter 1**). Solid nano-synthesis starts from building solid materials bottom-up from a liquid phase. Top-down method such as grinding down solid chunks would hardly yield complicated nano-structures in a controllable manner. However, traditional method of producing small droplets is through breaking continuous liquid phase down, which is considered as top-down. Therefore, it would be crucial to develop a bottom-up method of building liquid materials so that the way towards droplet nano-synthesis can be paved.

Solute induced phase separation is the key. The most common one among this kind of phenomena is salting out, which represents the phase separation of two previously miscible solvents induced by the addition of salt. By controlling the release of salt in a water-ethanol mixture, uniformed droplets with a narrow size distribution have been synthesized. Silica nano-encapsulation and thickeners were introduced to capture these droplets for characterizations (**Chapter 2**). The generality of salts has also been verified based on the Hofmeister series. Inspired by the kosmotrope-chaotrope theory, the choice of solute has been further generalized to a series of kosmotropes other than salts.

A variety of substances can be loaded into droplets synthesized through the phase separation process. Therefore, nano-encapsulation of those substances has been achieved through capturing the loaded droplets (**Chapter 3**). There are three routes of

loading, pre-, peri-, and post loading. In pre-loading, the loaded substance itself induces the phase separation. Peri-loading indicates the loading during the formation and growth of droplets. Post-loading happens after the formation of droplets. Following these routes, Au(III), Ag(I), Cu(II), Pt(IV), Pd(II) complex salts, cisplatin and doxorubicin have been loaded into silica nano-capsules. The advantages and shortcoming of the three routes have also been analyzed.

Other materials have been applied to the nano-encapsulation of droplets (**Chapter 4**). Nano-capsules made from polydopamine, resorcinol formaldehyde resin, copper-trimesic acid metal organic framework, iron oxide, other metal oxide and insoluble salts have been synthesized. Also, a series of polydopamine-silica hybrid capsules were applied in the nano-encapsulation of droplets generated from phase separation. By manipulating the droplet growth, a double layer structure with a gap between both layers has been synthesized through a one-pot reaction.

The fluidity of droplet could also lead to complicated asymmetric nano-structures. Based on a work in a literature, water post-loading was applied to synthesize nano-bottles with multiple bellies and dumbbell-like hollow structures (**Chapter 5**). The mechanism of their formation has been further analyzed and elaborated.

Finally, based on the data above and the result of a series of new experiments, two droplet nucleation hypotheses in the solute induced phase separation system have been raised (**Chapter 6**). The liquid particle hypothesis is a proximation modified from solid particle nucleation and growth. The condensation and extraction hypothesis is inspired from the polygonal-shaped capsules observed under certain reaction conditions. After a thorough analysis and reasoning, the latter hypothesis has been improved to explain two trends observed in experiments.

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Chapter 1 Introduction

The synthesis of nanoparticles in human history can be dated back to as early as 4th century, when artisans of Rome managed to endow a luxury burgundy color to stained glass vessels.¹ This technique has been lost and was rediscovered in 17th century, and with the invention of electron microscope people can finally glance into the world of nanometers. Yet for a long time, the discovery of new nanostructures has been mainly at the mercy of luck, or pure enumerations.

Scientists were not satisfied. If they want to make more complicated nanostructures, specific design strategies based on a deeper understanding of their mechanisms would be necessary, instead of groping blind and expecting wonders to pop out by themselves. Hence, vast efforts have been put into this area. As a result, the tuning of shape, symmetry, permeability and many other features has been achieved, and a large variety of complicated nanostructures have been engineered, all thanks to the further understanding of general principles in nanosynthesis explored by the pioneers. To tribute to their achievements, our group have attempted to systemically sum up and expound these principles^{2,3}.

Now, standing on the shoulders of giants, we would like to look beyond the old horizon. One step further from the designed nanosynthesis of solid objects, we would appreciate if the nanosynthesis of liquid droplets could be made possible. Not stopping at simply making droplets smaller, but to produce and manipulate nanoscale droplets like how scientists have done to nanoparticles. The synthesis of nanoscale droplets is not only of theoretical significance but may also revolutionize the methodologies of nanosynthesis through manipulation of the shifting nature of liquid. The fluidity of liquid may be utilized to break symmetry which leads to unconventional shapes; the

merging of droplets may serve as a driving mechanism for self-assembly, and the philicity of droplets may also be tuned to enrich certain substances at our disposal.

In order to synthesis liquid droplet at nanoscale, we plan to firstly learn from how scientists have done to solid particles. We need to comprehend and master the methodologies and the principles of nucleation and growth lie beneath. Several classic examples of complicated nanostructures would be discussed in this chapter, to unravel their design strategies and the mechanisms behind their sophisticated techniques.

1.1 Nanosynthesis methodology of solid particles

In general, there are two ways to shape a nanostructure. One is by manipulating the nucleation and growth process of the designated solid material, the other is through the assist of nanoscale templates. By combining such methods together should we be able to engineer even more complicated nanostructures.

1.1.1 Manipulation of nucleation and growth

The concept of this method is to control the factors governing the spontaneous aggregation of the designated solid material monomers, such as concentration, temperature, reaction speed, etc., so that they could arrange themselves into the shape we want.

1.1.1.1 Principle of particle nucleation and growth

A basic example of this method is the synthesis of high-quality gold nanospheres. Even though gold nanoparticles is considered to be one of the earliest synthesized nanostructures by man, to yield larger (diameter > 50 nm) regular shaped gold nanosphere with a very narrow size distribution has always been challenging, due to the whimsy of gold reduction reaction and the nightmarish aggregation of novel metal particles. Thus, synthesis of idea gold nanospheres requires accurate control based on the understanding of what lies beneath the nucleation and growth process. It

was made possible through a one-pot 12-step citrate reduction optimized by Feng ⁴. At the first step, a classic citrate reduction is carried out to generate the seeds. Before the addition of excessive citrate, the solution should be kept refluxing under vigorous stirring for 30 min to make sure the temperature is high and steady. After adequate incubation, boiled water, NaOH solution, citrate solution and HAuCl₄ solution were dropwise added in sequence followed by a 20 min incubation for each cycle, for a total of 11 cycles.

Figure 1.1 (a) Plot of monomer concentration against time. (b) A burst homogeneous nucleation leads to uniform sized particles. (c) An ongoing homogeneous nucleation time leads to a wider size distribution. Reprinted from ref 3, which has been modified from ref 10, 11 with permission. Copyright © 2015, 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, and 1950, American Chemical Society, respectively.

The theory regarding such factors can be explained in Figure 1.1. Plot a is drawn from monomer concentration in a solution system against time. The energy barrier of

homogeneous nucleation is higher than that of heterogeneous nucleation, therefore the monomer concentration needed for the former would be higher than the later. Due to the high energy barrier of nucleation, the concentration of Au^0 monomers will rise and start to oversaturate. However, the early stage of the nuclei which are supposed to be gold nanoclusters, are not stable in this situation, they may catch the coming Au^0 monomers as well as losing them through dissolving. This is due to the high energy barrier of homogeneous nucleation. At a certain point, as the monomer concentration goes up with the chance of collision, the balance will finally break so that the size of the clusters reaches a critical point. They will become the nuclei. After this stage, if the monomers in the solution stop building up, they will be consumed almost instantly, because when nuclei are present, monomers will switch to heterogeneous nucleation which is easier, instead of going through the harder homogeneous way. It is indicated by the lower energy barrier of heterogeneous nucleation in the plot. This will result in uniform sized seeds. Shorter homogeneous nucleation time leads to better uniformity, as is shown in Figure 1.1 b.

However, If the oversaturation of monomers in the solution cannot go down, for example, they are continuously generated or added, the heterogeneous nucleation may not be able to exhaust the monomers. Hence, they may keep colliding into new nuclei while the old ones are growing. This will lead to a wide size distribution, just as what Figure 1.1 c represents, which is what we want to avoid. Hence, we need to prevent monomer building up after homogeneous nucleation starts, AKA to make sure all the precursors turned into monomers as fast as possible before the early stage of homogeneous nucleation. That is why excessive citrate was added in the first step and the temperature was kept high. The total amount of the Au^0 monomers are limited by the HAuCl_4 precursors already in the solution. Both high concentration of the reductant

and high temperature could secure a burst reduction and quickly turn all gold into value 0. This could prevent more Au^0 coming out after the initial nucleation stage since there is little precursors left, and result in uniform seeds. Also, excessive citrate could stabilize the gold seeds as ligands.

During the following 11 cycles of growth, we want to promote heterogeneous nucleation only, to let new Au^0 monomers only deposit on existing seed without forming any new nuclei. However, there is already excessive citrate in the solution, which may cause a local oversaturation spike when more HAuCl_4 is added. That is why it must be added in 11 cycles to keep the amount introduced in each cycle low enough. Also, a dropwise addition under high speed stirring is necessary so that it can average the local concentration as fast as possible, preventing the possibility of local oversaturation. NaOH is added to neutralize the HCl generated from the gold reduction, which turns into NaCl . The addition of water is to reduce the salt concentration which, if too high, could induce aggregation of gold nanoparticles.

1.1.1.2 Facet control of nanocrystals

Aside from nanosphere, nanocrystals with sharp edge and polygonal shape are also well-established. In general, there is a trend to regulate the shape of a nanostructure towards a sphere, which is preferred by its low surface energy. As shown in Figure 1.2 , an internal atom is surrounded by its peers, so that they can hold each other in place by interactions such as chemical bonds or intermolecular forces ³. However, for an atom on the surface, part of itself is exposed and unable to bond to its peers. Therefore, being a surface atom is not welcomed. Statistically, it will lead to a minimization of the surface atoms, AKA the shape with minimum surface to volume ratio will be preferred, which is the sphere.

However, for an atom in a crystal, not all interactions from its peer are equal. Some are more stable; therefore, the lowest energy state may not be a sphere, but the shape that exposes the less preferred interactions, which will lead to a shape expose certain facets. Therefore, by controlling the priority of those interactions, AKA through facet control, polygonal shaped nanostructures can be synthesized (Figure 1.2).

Figure 1.2 (a) Schematic of the surface energy caused by the difference of internal and surface atoms. (b) The effect of facet control. Reprinted from ref 3, Copyright © 2015, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

1.1.2 Templating nanosynthesis

As its name suggested. Templating nanosynthesis utilizes nanoscale objects, such as macromolecules, micelles, vesicles, and even other nanoparticles as the template to guide the deposition of specific solid materials.

1.1.2.1 Traditional templating nanosynthesis

Traditionally, templates can be categorized into hard and soft ones. Hard templates refer to solid particles as the template ⁵, as well as soft templates are generally

micelles, vesicles and other flexible structures connected by intermolecular forces such as liposome and polymersome⁶⁻⁸.

On the other hand, templating synthesis also follows the rule of solid nucleation and growth. The deposition of the designated material onto the template can be considered as a heterogeneous nucleation process, in which another material heterogeneously nucleated onto the template. This especially applies to hard templating, since the production of the template itself may also rely on the manipulation of nucleation and growth process.

1.1.2.2 Molecular skeleton templates

With more powerful and controllable templates, it is possible to fabricate nanostructures of specific dimensions with high uniformity. DNA and polysaccharides may serve this purpose since their structures are regulated by abundant strong intermolecular forces such as hydrogen bonds. For example, in the work of Lin group's⁹, novel metal rods and wires of designated length and thickness have been produced. Their template is a kind of molecular skeletons called "molecular brushes" which are built on cellulose backbones with specific lengths (AKA degree of polymerization). Block polymer branches are functioned onto hydroxy groups of the cellulose chain. Those branches are composed of a section that can enrich the precursor by coordination and hydrophilicity, while other sections that reject the precursor. Hence, after the reduction, metal would only deposits onto their desired sections. Since the length of the backbone as well as branches can be tuned by controlling the polymerization, the size and uniformity of the nanostructures are as a result, highly controllable (Figure 1.3). They also managed to fabricate core-shell and hollow nanoshells through this method¹⁰.

Such templates can hardly be categorized into hard-soft ones since even though they are not solid particles, the rigidity of the molecular skeletons is significantly

superior than traditional soft templates. Though extraordinarily powerful, the controllability of such templates comes solely from the controllability of the molecular skeletons which could also be its greatest limitation. It based on pinpoint control of polymerization and sophisticated organic modifications which consists most of the workload in a project. Instead of fabricating a complicated nanostructure, it is to fabricate complicated polymers then turn it into nanostructures. Thus, in a sense, it is like transferring a problem instead of solving it. The capability of nanosynthesis this way may fall into the subject of polymer science. Moreover, such methods are extremely pricy on both time and money, which exacerbates the already high cost that have long troubled the application of nanomaterials. Therefore, molecular skeletons as template could only serve in limited situations.

Figure 1.3 Synthetic strategy of plain nanorods (A) and core-shell nanorods (B). Reprinted from ref 9 with permission. Copyright © 2016, American Association for the Advancement of Science.

1.1.3 Synthesis of complicated nanostructures

With the above methods at hand, it is possible to design more complicated nanostructures other than spheres and polygons.

1.1.3.1 Hollow nanostructures and etching

Traditionally, hollow nanostructures are made through the removing of the inner part of a solid particles. The inner part can be the template, or simply the core of a

homogenized nanoparticle. Yin group designed a method to produce gold nanoparticles of certain diameters by controlling the nucleation process ¹¹. This method is capable of making AuNPs of which the diameter ranges from 10 to 200 nm through a one-step seeded growth. The growth of large gold particles (>50 nm diameter) was considered to be quite difficult that may require either a tedious step-by-step reaction ¹²⁻¹⁴ or strict control of the reduction rate by a very weak reductant and high purity of the precursors ^{15,16}. The author's design was to suppress the homogeneous nucleation of gold by promoting its energy barrier extensively, leaving only heterogeneous nucleation possible. Therefore, by controlling the amount of seed added, they would be able to strictly control the particle size. The less seeds added, the larger for each nanoparticle, since the total amount of Au⁰ monomers are constant, the size of the designated gold nanoparticles is proportion to the amount of seed added. They successfully achieved their goal by introducing an iodide ligand that stabilizes the Au⁰ monomers even at a strong reductive circumstance with excessive ascorbic acid. This approved the efficacy of their design. However, the boundary of their nanoparticles is rugged and polygonal. This is due to the fast reaction time when seeds are added thanks to the extremely high monomer oversaturation.

Also, hollow nanostructures can be synthesized. An intuitive way of making hollow structure is to dig the center of a solid object. Following this design, Zheng group managed to etch the center of silica particles, leaving only the outer layer as a hollow capsule ¹⁷. The mechanism of why the inner parts are easy to be etched out was later unveiled by Song and co-workers ¹⁸. They hypothesized that the growth of a silica nanosphere is composed of two different stages. On stage one, as is shown in Figure 1.4, tetraethyl orthosilicate (TEOS) hydrolyzes and polymerizes into poly(silicic acid) chains with different length. When certain chains become too long, they will

oversaturate the solvent and homogeneous precipitate out from, entangling into cores. After that, short poly(silicic acid) oligomers will start to deposit on these cores since heterogeneous nucleation is much easier. During the entire process, the pairing of cations (in this case, NH_4^+) plays an important role for it inhibits the precipitation. In a classic Stöber system, the higher ratio of water against isopropyl alcohol (IPA) leads to a higher polarity of the solvent, and a higher polarity will result in a better solubility to the cation paired poly(silicic acid), which postpones the oversaturation. On the other hand, ion pairing promotes the dissolving of the silica during the etching process similar to how it inhibits the precipitation. Therefore, a higher water ratio leads to more etchable silica cores.

Figure 1.4 Schematic of (a) condensation and etching process of silica particles. (b) How ion pairing affects the condensation and etching by changing the solubility. (c-f) Behaviours of poly(silicic acid) chains in different solvents. (g) Silica particles after drying. Reprinted from ref 18 with permission. Copyright © 2015, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

To verify this theory, they designed an experiment (Figure 1.5), to make double layered silica particles by a two-step growth. For one of them, the inner layer was made in a low polar (low water ratio) solvent and the outer later was made in a high polar one.

The other was the opposite. After etching, the former one becomes a yolk-shell structure where a non-etchable core was left; the later one leaves only a hollow shell. It approves that higher cation pairing does promotes the etching of silica. Also, they tried to abduct the NH_4^+ cations by treating the later one's inner core with acid before the second step growth. After etching, a product similar to the first one was yield, indicating that cation pairing is indeed the key to this solubility difference.

Figure 1.5 (a) Nanosynthesis design schematic. (b-d) TEM pictures before etching. (e-g) TEM pictures after etching. Scale bar: 50nm. Reprinted from ref 18 with permission. Copyright © 2015, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

What has pushed the etching technique to a new level is the work of Xia group¹⁹. Their design is to synthesize an ultra-thin, sub nanometer platinum nanocages by deposition and etching. They chose palladium nanocrystals as the core for their well-established uniformity and morphology. Then they coated a layer of several atoms of Pt (in this case, 4). Due to the lower energy barrier, the Pt atom prefers to be embedded

into the Pd lattice, turning the outermost layers of each nanocrystal into a composite of Pt and Pd (Figure 1.5). After etching out the Pd, an ultra-thin Pt nanocage will be left. The significance is how they managed to achieve an extremely high specific surface area through a pin-point control of Pd deposition and etching. As a result, the structure they designed and synthesized showed an ideal catalyst activity and stability. It is also admirable for promoting the design and synthesize of hollow nanostructures from amorphous to crystal, from polymer and silica to novel metal.

Figure 1.6 Schematics of (A) Pt deposition and embedding into the surface of the surface of Pd nanocrystals. (B) the surface layer composition and etching process. (C-F) TEM pictures of the Pt coated Pd nanocrystals after (C) 0 (D) 10 (E) 30 (F) 180 min of etching. Reprinted from ref 19 with permission. Copyright © 2015, American Association for the Advancement of Science.

The work of Zhao group represented a typical methodology of multi-layer growth²⁰ (Figure 1.7). They firstly synthesized mesoporous silica particles from 1,2-Bis(triethoxysilyl)ethane (BTSE) and TEOS, with a surfactant of cetyltrimethylammonium bromide (CTAB) for tuning of the pore size. After the formation of the

core, they added more BTSE and TEOS for a second round growth, and then, a third one. After that, they hydrothermally treated the nanoparticles at 120 °C to etch the gap and crosslinking the remain silica. Finally, the product was treated by acidic alcohol to abduct the surfactant molecules so that pores can be freed. Their design shows a general schematic of building the layers one by one, then treated it to yield a designated structure. Similar strategies have been utilized by many other works ²¹⁻²³ and the generality of this methodology has been firmly approved.

Figure 1.7 Schematics of multi-layer growth of mesoporous silica particles, etching and extracting of surfactant templates. Reprinted from ref 20 with permission. Copyright © 2015, American Chemical Society.

1.1.3.2 Symmetry breaking and Janus nanostructures

By far all the nanostructure synthesized are highly radial symmetrical. In order to synthesize Janus nanostructures which, have distinct features on both size, the breaking of symmetry would be necessary.

1.1.3.2.1 Masking

The most intuitive way of Symmetry breaking is through a masking process, which is to cover one side of each particle, functionalize the exposed side, then remove

the covering. A further modification of the other side is optional. This method is ideal for micro-particles and larger sub-micron particles. For example, Velev group²⁴ firstly applied a thin layer of glucose film onto a glass plate. Then they align a layer of particles onto it. The glucose film could hold the particles in place as well as masking their bottom. The process was followed by a modification on the top side. Kopelman group²⁵ introduced a similar plate masking process to produce magnetic Janus particles. Granick²⁶ group pushed this method to a new level, by introducing a wax-water emulsion as the masking system. When heated, paraffin wax turns into an oil and acquires the potential of forming an emulsion with water. Then, particles are added to the system to turn the system into a Pickering emulsion²⁷ in which the particles lie on the interface between water and wax oil. Followed by a cooling process, the wax will solidify and therefore freeze the particles. After that, their exposed side can be modified. The total surface area of wax droplets are several magnitudes larger than any glass plates that can be practically used in a lab, making the synthesis of Janus particles scalable.

If a diameter smaller than 100 nm is required, ligands can be used to induce the Janus growth. Chen and co-workers^{28,29} managed to achieve Janus growth on gold seeds by applying different ligands to different sides of the seed. The philicity of the ligand can either attract the monomer of coating material or repel it, hence the degree of Janus can be controlled by the coverage of ligands which can be tuned through ligand ratio, and the polarity of the solvent (Figure 1.8), by balancing the competition between different ligands on the gold surface.

Figure 1.8 Schematic of (a) the coordination of different ligands on gold nanoparticle seeds. (b, e) Core-shell. (c, f) Less degree of Janus. (d, g) Higher degree of Janus. Reprinted from ref 29 with permission. Copyright © 2008, American Chemical Society.

1.1.3.2.2 Depletion sphere

However, sometimes the masking method may not be ideal. If the gold nanospheres are too small for the masking method, and introduction of multiple ligands may interfere the following reactions since they are hard to eliminate once bonded to the surface. For example, Feng and co-workers³⁰ successfully synthesized an Ag@Au complex nanostructures, where a single silver nanowire with polygonal tip was grown from a gold nanoparticle (Figure 1.9 a). Their design was based on the concept of depletion sphere and facet control, the former was further elaborated in a follow up

research³¹. Based on their hypothesis, they managed to design and synthesize gold nanospheres with specific numbers of silver islands (Figure 1.9 b).

Figure 1.9 Schematic of (a) tipped silver nanowire on gold nanospheres and (b) island number controlling by depletion sphere theory. Reprinted from ref 30, 31 with permission. Copyright © 2017, The Royal Society of Chemistry, and 2015, American Chemical Society, respectively.

In their design, the introduction of multiple ligands would interfere the following growth of silver nanowire and tips. Therefore, the subtle controlling technique based on the depletion sphere theory was introduced. With the gold nanospheres existing in the reaction solution, silver could deposit on the gold surface.

Due to the relatively small lattice mismatch between silver and gold, the growth of silver on gold surface would be easier than to homogeneously nucleating into free silver particles in the solution. The energy barrier of silver growth on gold would be in between those of the silver homogeneous nucleation and of the silver growth on silver, which make the synthesis of Ag@Au Janus particles possible. However, the crucial problem is how to secure that only one silver island could grow on the gold nanosphere

(and one step further, to control the number of silver islands, which has been achieved in another work ³¹).

Figure I.10 (a) Schematic of depletion sphere and the concentration gradient. (b) Plot showing the relationship between monomer concentration, time and radius. Reprinted from ref 31 with permission. Copyright © 2017, The Royal Society of Chemistry.

The concept of depletion sphere was introduced. Before the concentration of the Ag^0 monomers reaches a critical point, silver clusters undergo a similar balanced catching and dissolving process. When the critical point is reached, one of the clusters on the gold surface finally becomes a stable island, and from then on, almost all the free

monomers within a certain distance will deposit on it in a short period of time due to its energy barrier privilege. Since it almost depletes all monomers, other potential nucleation centers within this space can no longer compete for monomers with it. Therefore, there is not likely to be another island to form. The concept can be further expounded by Figure 1.10.

The Schematic 1.10a represents the depletion sphere by a circle line. The black dots indicate monomers while the color gradient shows the concentration gradients of the monomers. The 3D plot 1.10b is drawn from the relationship between monomer concentration, time and the radius. The color of the lines corresponds to the same color in the color gradient. Each intersection of the 3D plot is similar to the plot in 1.1a. This theory hypothesizes the concentration of all locations would be even, until the first silver island nucleates out. Right after the formation of the first silver nucleus, the concentration around it will start to go down. The closer to the silver nucleus the faster. The concentration on location 1 with the pink color would decrease faster than the green location 4, for instance. The term “depletion sphere” indicates where the Ag^0 concentration is “depleted” and below the concentration required for heterogeneous nucleation. Outside this sphere, the Ag^0 concentration is in between with the requisition of homogeneous and heterogeneous nucleation, thus if there is a gold seed outside the depletion sphere, it could still grow a silver island. In the case of a $\text{Ag}@\text{Au}$ Janus particle, if the depletion sphere covers the whole surface of the gold seed, there would not be two spots for silver to emerge. Therefore, by controlling the monomer concentration alone, the second step of making $\text{Ag}@\text{Au}$ janus particles can be achieved.

Besides, in order to turn this Ag island into a plate tipped wire, facet control was applied by a two-step ligand addition. To shift the growth mode of the Ag nucleus from spherical to wire, excessive 2-mercapto-benzoimidazole-5-carboxylic acid (MBIA) as

ligand was introduced, and a slower reduction rate is also favored. As is shown in Figure 1.11, MBIA is able to restrict the growth of Ag. If at a relatively low level, they will spread onto Ag nucleus and apply an even restriction over the entire surface, which results in a near spherical shape. Yet when the ligand concentration is high enough, and the generation of Ag^0 monomers is slow enough, the “old” surface will be packed with the ligand, which makes the deposition on those surfaces increasingly unpreferable. On the other hand, for the “fresh” surface that lacks ligands, the deposition of Ag^0 becomes easier. This generates a “hot spot” on the nucleus and leads to a wire shaped growing pattern. In order to make the plate tip, citrate is added later on. As a stronger reductant, it will even out the deposition privilege on the “fresh” surface as well as drastically increase the speed of monomer generation; as a ligand, it will further switch the growth pattern into isotropic by promoting the $\text{Ag}(111)$ facet.

Figure 1.11 (a) Schematic of the growth of silver nanowire and the tip on gold sphere. (b) The packing of MBIA ligand and how it shift the growth pattern into wire. (c) TEM picture of silver nanowire on gold sphere. (d) TEM picture of plate tipped silver nanowire on gold sphere. Reprinted from ref 31 with permission. Copyright © 2015, American Chemical Society.

Examples that combines both masking and depletion sphere have been published. Wang and co-workers³² managed to cover certain parts on a nanostructure, then depositing another material on the exposed sites. More interestingly, they were

able to control the masking shape by the ligand and surface tension. Firstly, they coated gold nanostructures by a layer of polystyrene-polyacrylic acid block co-polymer (PS-PAA), then heat it to induce the transformation of the PS-PAA coated (Figure 1.12). The resulted pattern varies due to the different interactions provided by ligands. After that, other novel metal such as Ag and Pd can be deposit onto the exposed.

Figure 1.12 (a) Schematics of two-step contraction of the masking and consecutive deposition of Pd and Ag on gold nanorods. (b-g) STEM, EDX and TEM picture of the barbell nanostructure. Masking, transformation and deposition on (h) bipyramid and (i) nanoplates. Scale bar: 100 nm for (a-g), 50nm for (h, i). Reprinted from ref 32 under a Creative Commons Attribution 4.0 International License.

The PS-PAA masking can be transformed even further, exposing more site for a second step modification, leading to more complicated structures. If applied to gold nanorods, they were able to synthesize a barbell like nanostructure where the shaft is the gold rod and the sleeves would be multiple other novel metals. This method can also be applied to bipyramid and nanoplates. Especially, in the case of bipyramid, an asymmetric structure can be produced, even though the exposed sites are indeed symmetrical. This can be explained by depletion sphere theory mentioned above ³¹. This work further approved that multiple design strategies can be combined together to achieve more complicated and irregular shape in nanosynthesis.

1.1.4 Assembly of nanostructures

Besides the modification of specific nano-objects, more unique structures can be synthesized through assembly of multiple objects. The concept of self-assembly of nanostructures was introduced by Granick group ³³⁻³⁵ while they managed to build chiral helices and kagome networks with Janus particles as building blocks. The key to nano-object self-assembly is the driving force, and in these cases, it was van der Waals force. They utilize the distinct philicities on the different sites of Janus particles to guide them into organized pattern. Later, controlled assembly of non-Janus particles has been achieved. Wang and co-workers ³⁶ designed a 1-D chain assembly system driven by the interaction between phospholipid ligands at a relatively high yield.

In order to further improve the design methodology, stronger and more selective driving force was introduced. Chong and co-workers ³⁷ designed a series of nanostirring bar by aligning and encapsulation of magnetic particles (Figure 1.13). They firstly exchanged their ligands to enhance the stability of individual particles, making them dispersed better and suppress irregular aggregation. Then a strong magnetic field was introduced to force align the particles into 1-D chain array. After that, a layer of silica

coating was applied to freeze the array as well as serving as a protection layer. The thickness and length can be tuned by the silica and strength of the magnetic field applied. Those nanostirring bars can be as thin as 75 nm at the length of 4 μm , which was smaller enough to suspend in a colloid system, resulting an ideal stirring capability both in bulk volume and small droplets. This work provided us another self-assembly driving force other than intermolecular forces, which expanded the arsenal of nanosynthesis.

Figure 1.13 (a) Schematics of synthesis of nanostirring bars. (b) TEM picture (c) SEM picture. (d-f) Stirring of the nanostirring bars. Reprinted from ref 37 with permission. Copyright © 2013, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

All the examples above could inspire us on how to initiate our research methodology if we want to study droplets at nanoscale and utilize them in nanosynthesis.

1.2 The meaning of droplets in nanosynthesis

To be clarified, the term of “nano droplet” which appears in many literatures actually refers to droplets of the size of nanoliters (10^{-9} L), of which the diameter is within the range of millimeters (10^{-3} m). As a contrast, the term of “nanoparticle” and “micro-particle” are based on their diameters. Hence, in this thesis, the scale of droplets also refers to their diameter rather than volume, so that they are comparable to micro and nanoparticles.

1.2.1 Droplets in synthesis of micron-sized objects

Droplets have been adopted as a general tool in synthesis of micro-scale objects. A general application is direct loading. A droplet is a natively self-contained solution environment, after encapsulation, it could serve as a perfect carrier of the solutes. The most common way to encapsulate a water droplet is through gelation, which could be stable in a wide temperature range. Hydrogel particles have been used in drug delivery for over half a century. In 1960, Wichterle and Lim³⁸ developed a hydrogel material from functionalized glycol monomethacrylate and tested its bio-compatibility. Recently, the most popular hydrogel material is calcium alginate. Alginic acid is an anionic polysaccharide extracted from seaweeds. The solution of around 1% w/w sodium alginate would gelate instantly when it meets cations such as Ca^{2+} and Mg^{2+} . Its greatest advantage is simplicity (Figure 1.14). Just dissolve the targeted drug in an alginate solution, turn it into microdroplet either through spray^{39,40}, micro-injection⁴¹ or break it into an emulsion^{42,43}, then mix it with a calcium chloride solution, done. There are two crucial limits for this method. Firstly, the target chemical must not be able to induce the gelation by itself. For example, metal ions with multiple charges cannot be loaded

since they could play a similar role like Ca^{2+} and Mg^{2+} . Secondly, even though the permeability of alginate hydrogel can be controlled to some extent ^{44,45}, the pore size would still be too big even tuned to the minimum. Therefore, only macromolecules and polymers are suitable for this method. That is why the typical loadings would be DNA, enzyme and even living cells ⁴⁶⁻⁴⁸.

Figure I.14 Schematics of an example of the device to produce alginate hydrogel particles. Reprinted from ref 41 with permission. Copyright © 2017, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Another way is droplet encapsulation, which means coat one or multiple layers of protective membranes or shells onto the droplet. It can also be considered as growing solid material on a droplet template, depending on the perspective. This concept was introduced in 1964 by Chang ⁴⁹. Nowadays, such coating methods are usually based on material deposition on the interface between two liquids. Microfluidic device is an ideal tool to generate uniform aqueous droplets in a hydrophobic continuous phase. De Vos group managed to synthesize hollow capsules from a copper-trimesic acid metal organic frameworks (MOFs) material ⁵⁰. They separated the two ingredients of the MOF, The ligand and the copper salt, into two phases. The oil dispersant was dissolved

by the trimesic acid ligand, and the aqueous droplet generated from the microfluidic device was enriched by copper acetate. On the interface where the two phases meet, the ligand will be able to combine with the copper ion, leading to the formation of a layer of MOF that covers the entire water droplet. Inspired by this concept, Abell group introduced a biocompatible supramolecular material into a similar system (Figure 1.15), and successfully encapsulated living bacteria through a more complicated one-step microfluidic device⁵¹.

Figure 1.15 Schematics of (a) Microcapsules produced from microfluidic devices. 1a: AuNP functionalized with ligands; 2a: functionalized co-polymer; 3 and 4: neutral and functional ligands, respectively. (b) Picture and schematic of the mixing channel. (c) microscopic image of the microcapsules and their size distribution. Reprinted from ref 51 with permission. Copyright © 2015,

1.2.2 Other applications

There are other potential uses of droplets. For example, liquid droplets are fluidic by nature, so their shape can be changed, which could become a potential tool that could bring a new degree of freedom in nanosynthesis. For example, Wang group utilized the shape changing of the droplet template to synthesize a bottle-like structure⁵². When the semi-shell over a droplet grew thicker, it may squeeze the water out of the

uncovered parts, breaking the symmetry and finally forming a bottleneck (Figure 1.16). Such tuning can only be made possible by the fluidity of a liquid droplet.

Figure 1.16 (a-c) Schematic of the formation of bottleneck. CPTMS: (3-chloropropyl) trimethoxysilane; TBOS: tetrabutyl orthosilicate. (d, e) SEM picture. (f) TEM picture. Reprinted from ref 52 with permission. Copyright © 2016, WILEY-VCH Verlag GmbH & Co. KGaA,

We may notice that most of the synthetic concepts mentioned above that based on micron-sized droplets are actually not size dependent. If we replace them with nanometer-sized droplets, there is a good chance that they will work as well. The limiting effect is that generating ideal droplets smaller than micron-size is quite challenging. The limit of mechanical devices such as spray and microfluidics is sub pico-liter scale, so they are not capable for droplets smaller than tens of micrometers. Also, droplets dispersed in traditional emulsion systems are also too big for nanosynthesis, needless to say their wide size distribution and poor stability. So, the key to this problem would be finding a way to generate uniform stable droplets at nanoscale.

What makes it so challenging is the difference between the growing patterns of solid particles and liquid droplets.

1.3 Comparison between the nucleation and growth of solid and liquid

1.3.1 Traditional way: top-down

Traditionally, in order to make small droplets in an emulsion system, large mechanical energy input is necessary to break the droplets down^{43,53}. We describe it as a top-down method of generating droplets, since it starts with a large constant liquid phase and ends in a large number of small droplets scattered in the dispersant liquid. However, if we go through this route, the knowledge and experience in solid particle nanosynthesis would help us little. Since we always make nanostructures bottom-up from ingredients, then grow it larger, rather than grinding macroscopic chunks into powders. Therefore, in order to correspond the synthesis methodology of nanoparticles and droplets, we need a bottom-up method to generate droplets.

1.3.2 making droplet bottom-up: salting out effect

Figure 1.17 A demonstration of salting out process. Potassium carbonate was added into a mixture of water and methanol, and it results in two separated phases.

The concept of salting out finally drew our attention. A typical scene of salting out can be described as follows (Figure 1.17). When certain salt is added to a mixture of water and another miscible solvent, for example, methanol, it will induce the mixture

to separate into two phases, with one enriches water and salt, the other one is mostly methanol {Shakhashiri:uh}. We assume the second phase is emerged from the initial one, and it has to start from the nucleation and growth of small droplets, which is similar to how solid nanoparticles do in traditional nanosynthesis. That is why we want to base our study on solute induced phase separation. More details about salting out effect will be covered in later chapters of this thesis.

1.3.3 Comparison between droplets and particles

1.3.3.1 Merging / collection growth

The most crucial difference between the synthesis of particles and droplets is that liquid can merge. When we discuss about growth of a solid particle, we are referring to the deposition of monomers on the nucleus and its size increases. However, for a liquid, deposition is not the only choice. Droplets can become bigger not only by deposition, but also by merging. In atmospheric physics, when talking about raindrop growth, they are described as condensation and collection, respectively ⁵⁴. The merging/collection growth refers to two or more droplets collide into each other through brown motion and become a larger sized droplet. This additional pathway provides the growth of droplets one extra degree of freedom over that of particles, which drastically increases the difficulty of control. On the other hand, if we could suppress the merging/collection, our old tools in the solid nanosynthesis arsenal may still be valid, in case the nucleation and deposition process work similar between the two systems. Hence, we are going to first propose some potential solutions to the merging/collection, then compare and analyze the other two processes.

The most intuitive way to alleviate droplets' merging/collection is to reduce their chance of collision. For example, by physically separate each droplet away. In AFM studies, nanometer sized droplet can be obtained by wiping macroscale water

spots on a graphite plate⁵⁵. Yet the size uniformity cannot be controlled, not to mention that the droplets generated this way is practically not applicable to nanosynthesis. Another choice is to enhance the microfluidic device so that it could generate nanoscale droplets one by one, eliminating the risk of merging. However, in a foreseeable future it is not likely to happen due to the limitation of mechanical precision. Therefore, the only practical way is to reduce the total volume of the liquid droplet per volume of the dispersant. Presumably the size of each droplet nucleus is constant, the number of nuclei per volume in the dispersant will decrease, leading to a decreased colliding chance.

Increasing the viscosity of the dispersant may also help lowering the colliding chance. We assume a droplet as a liquid “particle”, which means the shape changing and turbulent within the droplet are ignored. Therefore, a moving droplet will apply a shearing force to the dispersant and suffers from its resistance. The higher the resistance, the slower the droplet moves, which decreases the chance of collision. Also, resistance increases with the viscosity. That is why more viscous dispersant should have a better chance of suppressing merging/collection.

Besides just choosing a high viscosity solvent as the dispersant, we could also increase the viscosity by adding thickeners. This could widen our choice of dispersants. However, some thickeners can also serve as surfactants, and may initiate self-assembly or forming of micelles, which may interfere the nucleation and deposition of the droplet. Hence, we want to avoid amphiphiles as thickeners, since they have a higher chance of self-assembly.

Another way of decreasing merging/collection is to “freeze” the droplets before they meet each other. Most droplet based nanosynthesis requires the encapsulation of the droplet. Since it is a necessary step, why not let it happen before the merging vastly happens? It could preserve the droplets without deviant from its practical purpose.

Furthermore, the method of “freezing” droplets with encapsulation may also serve as an important tool to study the droplets at different stages. Since direct characterization of droplets are extremely challenging, the capsule shells may provide us indirect evidences to bridge the gap.

In summary, we could attempt to minimize the influence of merging/collection by reducing the volume of water added, thickening the dispersant, and quickly capturing the droplets by encapsulation.

1.3.3.2 Nucleation and deposition

Unfortunately, the study of the nucleation process of liquid droplets in another liquid phase is poorly established, due to the lack of its potential utilities in the past. Therefore, we initiated two hypotheses, one is similar to the nucleation of solid particles, the other is inspired by the raindrop nucleation. The first hypothesis is preferred in nanosynthesis design, since we can directly adopt the solid nucleation theory simply by replacing the monomers with free water molecules. Taking the salting out of water-ethanol mixture as an example, free water molecule “monomers” is under a balance between aggregating into clusters and being redissolved into alcohol. When the concentration of “monomers” builds up the trend of aggregation will gradually overwhelm that of redissolving, after reaching the critical point, the droplet nucleus can be stabilized, and other water “monomers” will instantly heterogeneously condense on it. It would be of great convenience if our future experiment facts could correspond to this hypothesis. However, it can only be considered as an approximate theory, because the solute is not involved in its narrative.

As the title “solute induced phase separation” suggests, the phase separation itself is caused by the addition of solute. The salting out of water and ethanol can be considered as water being abducted from ethanol by its strong interaction with salt.

Therefore, it may not be reasonable to ignore the role of the solute. For example, water clusters may not be able to accumulate into large size before being separated by entropy, without the help of the solute. Moreover, in the case of salts as the solute, ionization makes it even more complicated, since the solute contains both ions as well as the non-ionized salt since in a solution with a high content of ethanol, salts may not be able to fully ionize.

Moreover, even if we could take all of the above into account, we still lack an efficient characterization method to verify it. The insufficiency of analytic method of reactions in solution has long haunted nanosynthesis. Therefore, by now our only choice is to reluctantly accept the simplified theory mentioned above.

Another alternative hypothesis was inspired by raindrop nucleation. In the case of solid particle nucleation, the energy barrier of homogeneous nucleation is higher than that of heterogeneous nucleation, so does in the raindrop nucleation. The difference is, the gap between these two nucleation types for a raindrop is vastly larger than that for a solid particle. Actually, water vapor may supersaturate to over ten times of its saturated concentration without any homogeneous nucleation taking place^{56,57}. Hence, in practice, the formation of raindrop requires a hetero-core. It can be ice, dust, or silver iodide powder in the case of rainmaking. In a salting out process, salt crystals may play a similar role as a hetero-core in the miscible solution. Since heterogeneous nucleation for the water is now possible, homogeneous one will be un-preferable. Salt crystals may be able to attract water molecules in the mixture and get dissolved into salt solution droplets. Those salt droplets can further extract water from the mixture, representing the deposition growth. In some cases, this hypothesis is in line with experiment results. It will be further discussed in later chapters.

From a practical point of view, we decided to base our design and study mainly on the first hypothesis, with the second one in mind as well.

1.3.3.3 Ripening

The influence of ripening is also taken into consideration. For a droplet dispersed in another immiscible continuous phase, the molecules on the surface of the droplet are less stable than the molecules inside. An inside molecule is surrounded by its peers, so it can develop more interactions with others. As to a molecule on the surface, part of it is exposed to the dispersant molecules which could only provide much weaker interactions (Figure 1.18). Therefore, the inside molecules are energetically preferred. If the total volume of the droplet is constant, it will tend to minimize the number of surface molecules, leading to a spherical shape, since sphere has the minimum surface area per volume. Moreover, if the volume can be changed, smaller droplets will be thermodynamically less preferable than larger ones, since smaller size leads to a higher specific surface area.

Figure 1.18 In order to minimize the energy of a system, less surface atoms is thermodynamically preferred. Reprinted from ref 3. Copyright © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

In a water-ethanol salting out system, each droplet is under a balance of deposition and redissolving, since the water molecules in the droplet can go back into the ethanol phase, while the droplet can also catch water molecules from the dispersant

mixture. Since smaller droplets are less favorable than larger ones, they will have higher chance of losing water than obtaining it. Hence there will be a thermodynamic trend that the larger droplets become larger and the smaller ones smaller.

However, the effect of ripening in nanoscale droplets may not be discernable, since it may be concealed by the merging/collection process. Both of them has a similar trend to make the size distribution wider. In studies of ripening of millimeter sized fluidic objects, air bubbles in liquid for example ⁵⁸, the ripening can be observed *in situ* by a camera to differentiate ripening with merging. It can hardly be applied to nanoscale droplets even with *in situ* TEM technology. On the other hand, solid particles tend to become smoother and rounder after ripening, but liquid droplets are always quite spherical. We have to admit that by far we are not able to distinguish the influence of ripening from that of the merging/collection. Therefore, we will ignore ripening in our design and research until crucial evidence emerges.

1.4 Potential applications of droplet in nanosynthesis

1.4.1 Nanocapsules and hollow nanostructures

One of the potential applications is the synthesis of loaded nanocapsules. For example, drug loaded mesoporous silica nanocapsules has been put into *in vivo* tests and yielded some encouraging results ⁵⁹. Luo and co-workers' design is a five step nanosynthesis. Firstly, they made a sacrificial silica particle template through Stöber method. Then a layer of CTAB incorporated mesoporous silica was coated onto the core, followed by etching of core and removal of CTAB. After that the surface of these hollow mesoporous silica capsules were modified by functional groups. Finally, doxorubicin was loaded into the empty nanocapsules by a long-time diffusion process. The first three steps are modified from previous works ⁶⁰ which used octadecyltrimethoxysilane (C₁₈TMS) instead of CTAB to generate the pores (Figure1.19).

However, there are two crucial limitations of this design strategy. Firstly, for the removal of the sacrificial template, the capsule shell must be permeable to the etching agent, which put a strict limit over the choice of the shell materials. It would be perfectly fine if a porous shell is exactly what we need, but what if the case that a non-porous shell is necessary? For example, doxorubicin is highly toxic for normal cells as well as cancer cells. We want to minimize its leaking before the capsule reaches its destination. Therefore, a non-porous biodegradable capsule shell that can be break down when attached to the tumor may be a better choice. However, the template etching method is not capable of synthesizing such nanocapsules.

Figure I.19 Schematic of the synthesis of hollow mesoporous silica. Route A is etching through Na_2CO_3 , and route B is through ammonia. Route A was chosen by the synthesis of doxorubicin loaded nanocapsules. Reprinted from ref 60 with permission. Copyright © 2009, American Chemical Society.

On the other hand, the loading of drugs relies solely on diffusion, which is extremely inefficient. The diffusion loading is exactly a reversed process of the

releasing, which means if we want to reduce the releasing rate, the loading rate will go down respectively. Needless to say, the loading process along with the synthesis and etching may take too much time and human resource.

These two limitations can be overcome by encapsulation of nanoscale droplets dissolved with the loadings, just like what have been done to micron sized droplets^{50,51}. A wider choice of capsule shell materials and faster, more efficient synthetic routes can be achieved.

1.4.2 Merging driven self-assembly

Another potential application is self-assembly of nanostructures driven by droplet merging. In a unpublished work, Han and co-workers managed to induce assembly of nanobowls through the merging of the droplets they incorporate (Figure 1.20). Each of the nanobowl is built over a droplet. When the droplets meet each other and merge into one, the bowls will be attached together at the same time. The assembly pattern can be tuned by the opening of the bowls.

Figure 1.20 Schematic of (a) dimer assembly. (b) Tetramer assembly. (c) TEM picture of dimers. (d) SEM picture of tetramers

1.4.3 Solving the difficulties of droplet nucleation and growth research

On the other hand, as is mentioned before, the encapsulation of droplet may also serve as a tool to study the nucleation and the early stage of droplet growth. Chemists are familiar with reactions in solution system, because it is what basic chemistry experiments start with. However, in the realm of nanosynthesis, there is still not much tool to figure out what exactly is happening in a reacting vial. Spectroscopies may be able to collect statistic data of the decreasing of precursors and the formation of nanoparticles, similar does dynamic light scattering (DLS). However, as to the shape and topology of the nanostructures in a solution, even the most advanced technology could only provide limited help.

For example, AFM requires direct contact with the object measured, while for a nanostructure in solution, it is covered by a thick layer of solvent, which blocks it from the tip of the microscope. Traditional TEM requires a high vacuum condition to eliminate the interference as well as protect the filament and sensors. It would be disastrous if a drop of solution is put into the chamber of a traditional TEM machine. *in situ* TEM technology seems to be able to shed some light on this research area. Liquid cell is a pocket filled with reaction solutions made of two layers of ultra-thin and ultra-strong materials, such as silicon nitride or graphene. Recent technology allows an open loop to be connected to the liquid cell^{61,62}. Therefore, new reaction ingredient can be pumped in, as well as the liquid flow could somewhat mimic the stirring in a flask (Figure 1.21). This technology opens a new window of a thoroughly understanding to the nucleation and growth of solid nanoparticles.

However, even *in situ* TEM may not be suitable to study droplet nucleation. The first limitation factor is its imaging contrast. Almost all of works of *in situ* TEM study

of nucleation and growth are based on novel metal crystals, because only heavy elements could provide an acceptable contrast. In a traditional TEM, besides the sample, the electron beam only needs to penetrate the near vacuum chamber and a layer of polymer membrane, while in the case of a liquid cell, it needs to go through two layers of graphene or silicon nitrate and a thick layer of solvent. It will reduce the contrast drastically. In fact, even silica could not generate a clear enough image in a liquid cell. By far, there are several attempts of monitoring silica growth on gold nanoparticles, but no breakthrough has been made. While in a salting out system, most of the ingredients are even lighter than silicon and oxygen.

Figure 1.21 Schematic of (a) a liquid cell. (b) The open loop connected to the liquid cell. Reprinted from ref 62 with permission. Copyright © 2015, American Chemical Society.

The other limitation is the electron induced reactions. It has been reported that electron beam could generate bubbles in the liquid cell ⁶³. It would be a disastrous interference for the droplet nucleation and growth. Especially, if we want to overcome the contrast issue mentioned above, we may need more powerful electron beam, which could exacerbate the bubbling.

Due to the insufficiency of direct monitoring method, we turned our head to indirect ones. By encapsulating different stages of a droplet, the shell left could represent the condition of the droplet it captured. Actually, our group has long been preserving nanostructures by encapsulating them before TEM tests ^{4,30,31}. It is reasonable to place our expectation on droplet encapsulation.

1.5 Summary

In this chapter, typical previous nanosynthesis cases based on solid-particle nucleation and growth, their design strategies, and the mechanisms lie beneath have been summarized and analyzed. A comparison between droplet and particle nucleation has been made, in order to pave the way towards nanosynthesis through droplets.

Hypotheses regarding the process of droplet nucleation and growth at nanoscale have been proposed. Finally, its potential applications have been discussed. We expect the introduction of droplet nucleation and growth could support the design of many novel and complicated nanostructures, as well as provide us a feasible tool for indirect study of the nucleation process itself.

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Chapter 2 Solute induced phase separation and droplet encapsulation

In this chapter, we want to explore whether the solute induced phase separation could build droplets bottom-up, and its compatibility with nanoencapsulation techniques. We aim on screening for a standard system as the basis of future studies. At this stage, modified Stöber process was chosen for the encapsulation, due to its well-establishment in the literature ¹⁻⁴ and the stability of the silica material. Since this project was inspired by salting out effect, salt would be on the first priority in our list of potential solute, followed by other potential substances that could serve as phase separation initiators.

2.1 Introduction

2.1.1 Salting out effect

As mentioned above, salting out effect in general refers to the phenomena of the phase separation of two previously miscible liquids induced by the addition of salt ⁵. However, this doesn't mean that any salt plays this role well. For example, addition of ammonium acetate in a mixture of water and ethanol will finally lead to a saturation and precipitation of the salt itself from the mixture as a whole. Hence, a general principle is needed to guide our selection among salts. Fortunately, there is such a rule named Hofmeister series, even though its scope of applicability may be a little different from what we seek.

2.1.1.1 Hofmeister series

Hofmeister series was based on the study of salting out effect of proteins, instead of solvents. It was summarized by Franz Hofmeister at 1888 describing the ability of

precipitating proteins among a variety of ions⁶. A protein molecule is huge, therefore on each region of the molecule, there can be relatively strong local charges, some are positive and others negative. In pure water, positively charged regions may attract the negative regions of another protein molecule, which will lead to aggregation, and further, a poor solubility. When a certain amount of ions are introduced, they will help to balance the regional charges of proteins and separate them from each other. It is called the “salting in” of proteins. However, when the concentration of ions increases, the overwhelming ions will dehydrate the protein molecules, causing them to aggregate together and precipitate out again. This process is called the “salting out” of proteins⁷⁻⁹. The salting out of proteins may seem different from that of solvents, but the mechanisms lie within are the same – the abduction of water from the salted-out substance due to the strong interaction between ions and water molecules. In fact, salting out is a general phenomenon for substances that can interact with water. For example, it has been applied in purification of DNA¹⁰⁻¹².

Hofmeister series was first based on how efficient an ion could salt out proteins. Later it was revealed that their stabilizing effect on the secondary and tertiary structures of proteins also follows the same trend. A typical representation of this series is as follows:

Anions are considered to have a significantly stronger impact than cations¹³. Also, from a practical point of view, the salts we need must have a relatively high solubility in water. Hence, only ammonium, potassium and sodium are commonly chosen in our research. As a result, we would mainly focus on the comparison of anions instead of cations.

2.1.1.2 Hofmeister series in non-protein salting out process

If we want to apply Hofmeister series in a solvent salting out system, we need to first verify its validity. Even though theoretically water-miscible solvents should follow a similar trend like proteins. Fortunately, it has already been proved by Qiu group ¹⁴. In their research, they tested the salting out efficiency of a water-ethanol mixture. As is shown in Table 2.1, $K_4P_2O_7$, K_3PO_4 , K_2HPO_4 and K_2CO_3 are tested. The α is Setschenow constant, which is indicated by the Setschenow equation:

$$\text{Log } S_O/S_A = \alpha \cdot C_{\text{salt}} \quad (2.1)$$

S_O and S_A indicates the solubility of the specific salt in organic phase and aqueous phase respectively, C_{salt} means the molar concentration of that salt, and α is the Setschenow constant. Hence, the absolute value of α represents the effectiveness of salting out for that salt.

Table 2.1 The salting out effectiveness of different salts. α indicates the Setschenow constant, β are the intercepts of their fitted curves, R^2 are the statistic determinations of their data. Printed from ref 14 with permission. Copyright © 2017, The Korean Society of Industrial and Engineering Chemistry. Published by Elsevier B.V.

System	Salt	α	β	R^2
40 wt% ethanol	$K_4P_2O_7 \cdot 3H_2O$	-1.7228	3.8772	0.9981
	$K_3PO_4 \cdot 3H_2O$	-1.212	4.2693	0.9982
	$K_2HPO_4 \cdot 3H_2O$	-0.6466	3.5581	0.9939
	K_2CO_3	-0.6328	3.7907	0.9967

According to the data provided, the effectiveness of salting out of ethanol among those anions follows an order of $P_2O_7^{4-} > PO_4^{3-} > HPO_4^{2-} > CO_3^{2-}$, which in general corresponds to the Hofmeister series, especially for the phosphates. On the other hand, both as 2 valued anions, HPO_4^{2-} and CO_3^{2-} showed a similar salting out strength, which does not conflict with the trend mentioned either. By choosing the right anion, they were able to salt out more than 99.9% of all ethanol in the mixture, as well

as keeping the salt concentration in the ethanol phase below 0.01% wt. This result could serve as a solid evidence that Hofmeister series can also be adopted to the solvent salting out system.

2.1.2 Kosmotropes and chaotropes

2.1.2.1 Aqueous two-phase systems

Another concept has drawn our attention due to its close resemblance of our system. The aqueous two-phase system (A2PS) are liquid systems composed of two separated aqueous phases. It was first discovered by a Dutch biologist Martinus Beijerinck in 1896¹⁵. The most common application of these systems is also protein extraction and purification, thanks to the mild condition of aqueous solutions. A classical A2PS is made of water, poly(ethylene glycol) (PEG) and dextran which is a polysaccharide. Aqueous solutions of PEG and dextran at concentrations of as low as around 5~6% w/w would not merge into one phase when mixed, resulting in two visually immiscible phases (Figure 2.1).

Figure 2.1 A2PS made of aqueous solutions of PEG and dextran. System A: 5.0% PEG 4,000 and 6.7% Dextran 40,000; system B: 6.2% PEG 4,000 and 8.3% Dextran 40,000, w/w, both in pH 7.0 phosphate buffer. (a) right after mixing. (b) 2 min later. (c) 30 min later. Reprinted from ref 15 with permission. Copyright © 2000, Humana Press Inc.

2.1.2.2 Kosmotropes and chaotropes, non-ionic ones

The two phases in an A2PS are usually described as one solution of a kosmotrope and another solution of a chaotrope. The term “kosmotrope” means “order-

maker” while “chaotrope” means “disorder-maker”. This classification method was raised firstly based on the behaviors of different salts ¹⁶ in protein purification but was later applied to a large variety of other substances, even though the exact definition has not been nailed down ¹⁷⁻¹⁹. In this thesis we would not join this debate and simply adopt the most well-accepted classical explanation ¹⁹. The kosmotrope refers to chemicals that can enhance the structure of water clusters or those that can greatly promote the polarized alignment of water molecules. The chaotrope means substance that weaken the water structure or providing weak supports to water molecule alignment ^{18,19}. Also, kosmotropes are supposed to stabilize proteins while chaotropes increasing the chance of denaturation.

In the case of the PEG-dextran system described above, the former is a chaotrope while the latter a kosmotrope. PEG molecules are actually long hydrophobic chains with only two highly hydrophilic ends ²⁰. Within a certain range of length, the longer the molecule, the more hydrophobic for the chain, but the more hydrophilic for the ends. This makes PEG soluble in water, yet the long hydrophobic chain could severely disturb the structure of water, leading to a more disordered solution. On the other hand, carbohydrates such as dextran have tremendous amount of hydrogen bonding along the entire molecule, which greatly enhance the structure of water, making it more ordered. Therefore, a disordered water solution of PEG and a highly ordered water solution of dextran will repel each other.

Similar to PEG, other water soluble/miscible chemicals with hydrophobic structures can be seen as chaotropes, such as Triethylamine (three hydrophobic ends, but can enhance the hydrogen bonding of the nitrogen), ethanol and most water miscible organic solvents. On the other hand, chemicals like carbohydrates could serve as good kosmotropes.

2.1.2.3 Ionic kosmotropes, chaotropes and Hofmeister series

Ions can also be classified into kosmotropes and chaotropes. Ionic kosmotropes can be considered as anti-chaotropes due to their ability to enhance the polarized alignment of water molecules ¹⁹. In practice, some salts such as phosphates are able to form A2PSs with chaotropic polymers like non-ionic kosmotropes are ¹⁵. The efficiency of forming A2PSs of a salt was defined as the degree of kosmotropicity, where a better phase separator is attributed to be more kosmotropic and a poor phase separator to be more chaotropic. Hence, the series of kosmotropicity is parallel to Hofmeister series in general. For example, for anions, there is –

Kosmotropic

Chaotropic

Generally, smaller anions with more charges tend to be more kosmotropic while larger ions with less charges more chaotropic. Even though ionic kosmotropes and chaotropes are different from non-ionic ones in nature, they play similar role in phase separation, which is exactly what we seek.

To this extend, a salting out system can also be seen as a generalized A2PS, since both phases contains water, even in the “organic phase”. In an ethanol-water salting out system, the ethanol phase can be considered as a chaotrope-rich aqueous phase, and the salted aqueous phase is naturally kosmotrope-rich. Inspired by this concept, it is possible for us to promote salting out into a more generalized solute induced phase separation system.

2.1.2.4 Generalized solute induced phase separation

As mentioned above, since ionic kosmotropic salts such as K_3PO_4 can induce phase separation in a water-ethanol “A2PS” system, could non-ionic kosmotropes do the same? Bearing this question in mind, we would test a series of typical kosmotropes

aside from classical salts to see if they could also induce phase separation and further serve as a potential tool for nanosynthesis.

2.1.3 Objectives and design principles of the experiments

Theoretical discussion has approved the possibility of solute induced phase separation. The goal of the experiments in this chapter is to testify that whether this phenomenon could generate nanoscale liquid droplets with kosmotropic solutions from a chaotropic liquid phase. Since droplet at this size cannot be observed by conventional equipment, a modified Stöber process would be introduced to capture them. This would result in the formation of silica nanocapsules templated from the droplets. By observing and evaluating those nanocapsules should we collect indirect information from the droplets.

2.2 Material and methods

2.2.1 Materials

Tetraethyl orthosilicate (TEOS), Citric acid (H_3Ct), hydroxypropyl cellulose (HPC, Average Mw $\sim 80,000$, Average Mn $\sim 10,000$), polyvinylpyrrolidone (PVP, Average Mw 40,000), glucose (D-(+)-glucose, GC), sucrose (BioXtra, $\geq 99.5\%$, GC), oxalic acid (ReagentPlus[®], $\geq 99\%$), poly(acrylic acid) (PAA, Mw 1800), polyphosphoric acid (PPA, reagent grade, 115% H_3PO_4 basis), phosphoric acid solution (85%, 99.99% metal trace), acetone (for HPLC, $\geq 99.8\%$), tetrahydrofuran (THF, anhydrous, $\geq 99.9\%$, inhibitor-free), dimethyl sulfoxide (DMSO, GC $\geq 99.5\%$) were purchased from Merck (AKA Sigma-Aldrich). Sodium citrate (dihydrate, 99%) was purchased from Alfa Aesar. Ammonium solution (28%) and dimethylformamide (DMF, $>99.5\%$ GC) were purchased from TCI. Concentrated sulfuric acid (H_2SO_4 , 98%) was purchased from QR $\ddot{e}$ C, ethanol (absolute, 'Baker analyzed') was purchased

from J.T.Baker, sodium hydroxide (NaOH, pellets ACS) was purchased from VMR chemicals, acetonitrile (ACN, HPLC) was purchased from Macron, Deionized water (resistance $> 18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was used in all reactions.

2.2.2 Methods

Characterization. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 ~ 120 kV. Dynamic light scattering (DLS) data were collected from a Malvern ZETTASIZER NANO DLS machine.

Preparation of TEM Samples. TEM grids were treated with oxygen plasma in a Harrick plasma cleaner/sterilizer for 45 s to improve the surface hydrophilicity. The hydrophilic face of the TEM grid was then placed in contact with the sample solution. Samples were then dried under vacuum for at least 30 min.

Preparation of 4% w/w standard hydroxypropyl cellulose solution. 1.0 g of HPC was added to 30.42 mL of ethanol. The solution was stirred at 60 °C for 2 hours. After cooled down, it was centrifuged at 16,000G for 30 min. The supernatant was passed through a 0.45 μm syringe filter, and collected as a 4% w/w standard HPC solution.

Preparation of PVP stock solution. 10 mL of ethanol was added to 3000 mg of PVP. The mixture was stirred for 1 hour at 65 °C. The solution was cooled down before use.

Standard synthesis of silica nanocapsules from citric acid and sodium hydroxide. 250 μL of 4% HPC solution was diluted by 742 μL of ethanol, then 8 μL of 500 mM citric acid ethanol solution was added. The solution was pre-mixed by vortex. After that, under vortex, 50 μL of 260 mM sodium hydroxide aqueous solution was quickly added into the mixture, followed by 5 seconds of vortex. The mixture was incubated for 1 minute, then 6 μL of TEOS was added under vortex. It was then put into a 30 °C oven for 2 hours. In order to isolate the capsules, the mixture was centrifuged at 8,000G for 15 minutes. The precipitant was washed by 500 μL water for 2 times, and 500 μL

ethanol once. 5 minutes of centrifugation was adequate for washing. The final precipitant was dispersed in 1000 μ L of ethanol.

Synthesis of silica nanocapsules from sodium citrate solution. 250 μ L of 4% HPC solution was diluted by 750 μ L of ethanol. The solution was pre-mixed by vortex. After that, under vortex, 50 μ L of 20 mM sodium hydroxide aqueous solution was quickly added into the mixture, followed by 5 seconds of vortex. The mixture was incubated for 1 minute, then 6 μ L of TEOS was added under vortex. It was then put into a 30 °C oven for 2 hours. The washing as re-dispersion was the same as the standard operation.

Cloudiness test. A 2mL Eppendorf tube was filled with 900 μ L of Ethanol. It was then added by 100 μ L of 0.1 M sodium citrate aqueous solution. Another tube was filled with 880 μ L of ethanol and 20 μ L of 0.5 M citric acid ethanol solution, followed by an addition of 100 μ L 0.32 M sodium hydroxide aqueous solution. Video of both operation were collected.

Synthesis of silica nanocapsules from sulfuric acid and sodium hydroxide. 1M sulfuric acid ethanol solution was made by adding 55 μ L of 98% H_2SO_4 into 945 μ L of ethanol. 1 mL of 6 mM and 0.23 mM H_2SO_4 ethanol solution with 1% w/w HPC was made from the 1M H_2SO_4 , 4% HPC solution and ethanol, respectively. The solutions were pre-mixed by vortex. After that, under vortex, 50 μ L of 260 mM and 29.2 mM sodium hydroxide aqueous solution were quickly added into of the mixtures, respectively, followed by 5 seconds of vortex. The mixtures were incubated for 1 minute, then 6 μ L of TEOS was added into each one under vortex. It was then put into a 30 °C oven for 2 hours. The washing as re-dispersion was the same as the standard operation.

Synthesis of silica nanocapsules from phosphoric acid and sodium hydroxide. 1M phosphoric acid ethanol solution was made by adding 66 μL of 85% H_3PO_4 into 934 μL of ethanol. 1 mL of 1.2 mM and 0.12 mM H_3PO_4 ethanol solution with 1% w/w HPC was made from the 1M H_3PO_4 , 4% HPC solution and ethanol, respectively. The reaction process, washing and re-dispersion were similar the standard operation. The concentration of sodium hydroxide solutions were adjusted to keep an excessive 1 mM of sodium hydroxide.

Synthesis of silica nanocapsules from oxalic acid and sodium hydroxide. 1 mL of 1.2 mM and 0.36 mM oxalic ethanol solution with 1% w/w HPC was made from the 1M oxalic stock solution, 4% HPC solution and ethanol, respectively. The reaction process, washing and re-dispersion were similar the standard operation. The concentration of sodium hydroxide solutions were adjusted to keep an excessive 1 mM of sodium hydroxide.

Synthesis of silica nanocapsules from citric acid and sodium hydroxide. 1 mL of 80, 120 and 160 mM citric acid ethanol solution with 1% w/w HPC was made from the 0.5M citric acid stock solution, 4% HPC solution and ethanol, respectively. For 80 mM and 120 mM reactions, 50 μL of sodium hydroxide solutions made from 192.8 mg in 1 mL water and 289.2 mg in 1mL water were added, respectively. For the 160 mM one, 100 μL of sodium hydroxide solutions made from 192.8 mg in 1 mL water was added. The reaction process, washing and re-dispersion were the same as the standard operation.

Thickener free synthesis of silica nanocapsules from citric acid and sodium hydroxide. The whole reaction process, washing and re-dispersion were similar the standard operation. Expect no HPC solution was added.

Synthesis of PVP thicken silica nanocapsules from citric acid and sodium hydroxide. The whole reaction process, washing and re-dispersion were similar the standard operation. Instead of diluting from 4% HPC solution, the PVP stock solution was used. 150 mg/mL was produced by diluting the stock solution by 1 time, 75 mg/mL was by 3 times. The standard incubation time before the addition of TEOS was 10 min. The product needs to be washed by water for 1 more time in order to remove excessive PVP.

Synthesis of silica nanocapsules from citric acid and ammonium solution. The whole reaction process, washing and re-dispersion were similar the standard operation. Except instead of NaOH solution, a ~10 M ammonium solution made from 38% concentrated solution was added.

Synthesis of shrunken silica nanocapsules from citric acid and sodium hydroxide. The whole reaction process, washing and re-dispersion were similar the standard operation. Except the 2 hour incubation was done at 21 °C.

Synthesis of silica nanocapsules from PPA and bases. The whole reaction process, washing and re-dispersion were similar the standard operation. Except instead of NaOH solution, a ~10 M ammonium solution made from 38% concentrated solution was added.

Synthesis of silica nanocapsules from PAA and bases. 72 mg PAA was dissolved in 1 mL of ethanol to produce a stock solution of 1M as monomer. 250 μ L of 4% HPC solution was diluted by 720 μ L of ethanol, then 30 μ L of PAA stock solution was added. The whole reaction process, washing and re-dispersion were similar the standard operation. 50 μ L of 0.62 M NaOH solution and ~10 M ammonium solution were added, respectively.

Synthesis of silica nanocapsules from glucose. 250 μ L of 4% HPC solution and 2.1 μ L of 1M NaOH ethanol solution was diluted by 748 μ L of ethanol. Under vortex, 74 μ L

of 2M glucose aqueous solution was added. After a 2 min incubation, 6 μ L TEOS was added. The mixture was incubated for 1 minute, then 6 μ L of TEOS was added under vortex. It was then put into a 30 °C oven for 2 hours. In order to isolate the capsules, the mixture was centrifuged. The precipitant was washed by 500 μ L water for 2 times, and 500 μ L ethanol once. The final precipitant was dispersed in 1000 μ L of ethanol.

Synthesis of silica nanocapsules from sucrose. The procedure is similar to that from the glucose. Glucose solution was replaced by 73 μ L of 1M sucrose aqueous solution.

Synthesis of silica nanocapsules from sulfate in water-ACN system. The procedure is similar to that of the water-ethanol system. The HPC ethanol solution was replaced by an ACN solution with 100 mg/mL PEG. NaOH solution was replaced by ~10M ammonia solution.

Synthesis of silica nanocapsules from sulfate in water-acetone system. The procedure is similar to that of the water-ethanol system. The HPC ethanol solution was replaced by an acetone solution with 1% w/w HPC. NaOH solution was replaced by ~10M ammonia solution.

Synthesis of silica nanocapsules from citrate in water-DMF system. The procedure is similar to that of the water-acetone system. The HPC acetone solution was replaced by a DMF solution with 150 mg/mL PVP.

Synthesis of silica nanocapsules from citrate in water-THF system. The procedure is similar to that of the water-acetone system. The HPC acetone solution was replaced by a THF solution with 1% w/w HPC.

2.3 Results and discussions

2.3.1 Silica droplets encapsulation through salting out systems

The first step to design droplet based nanosynthesis is to make uniform water droplets bottom-up, just like how we started to design nanoparticle from making

uniform gold nanospheres. One step further, in order to preserve the droplet structures, we decided to encapsulate them in silica shells for characterization. A combination of the two steps would result in the synthesis of small and uniform silica nanocapsules.

2.3.1.1 Synthesis of uniform hollow silica nanocapsules

To smooth our route towards droplet based nanosynthesis, there are two crucial problems to solve so that we can adopt our experiences in making nanoparticles. One of them is to build droplets bottom-up from liquid “monomers” which can be achieved by a salting out process. The other one is to suppress merging. In a standard water-ethanol salting out system, hydroxypropyl cellulose (HPC) was introduced as a thickener. Also, sodium hydroxide instead of ammonium in traditional Stöber processes are applied due to its higher efficiency on catalysing silica polymerization ^{4,21}, so that we would be able to capture droplets faster before severe merging happens.

Therefore, the design of droplet based nanosynthesis started from our experience in particle based one with adjustment to fill the gaps between liquid and solid. As is shown in Figure 2.2, under a vigorous vortex mixing, a slightly excessive NaOH aqueous solution was quickly added to an HPC thickened ethanol solution of citric acid. The mixture was incubated for 1 minute then TEOS was added. The mixture was let stand at 30 °C for 2 hours for silica to complete its coating on the water droplet surface. After centrifugation and washing, silica hollow capsules preserving the shape of droplets can be examined under TEM.

There are three crucial details that must be taken care of in this seemingly simple process. First is to generate the salt during the reaction instead of directly adding it in. The second one is to choose the right salt and the volume of water introduced. Last but not the least, the choice of thickener could also affect the shape and uniformity of capsules to a large extent. They would be further discussed to unveil the mechanisms lie within. Besides, the reaction temperature, though may not truly influencing the shape and uniformity of droplets, may play a special role in the silica polymerization process. It will be discussed in later chapters.

2.3.1.2 Salt VS acid + base

The breakthrough in synthesizing uniform droplets at nanoscale is by abandoning of the mindset in a salting out system – to directly add salt into a solvent mixture. Without any doubt, salt is necessary in a salting out of a water-ethanol system. However, kinetically doing so would ruin the nucleation process, since the salt crystals are huge compare to nanoscale objects, they may also switch the nucleation process to heterogeneous. An intuitive modification is to add a salt solution instead to solid salt. However, in practice it would still result in a very wide size distribution (Figure 2.3 b c). We hypothesize that it was induced by a local oversaturation and early nucleation during the addition.

Figure 2.3 TEM images of capsules induced by (a) Acid+base. (b) Salt. (c) Dynamic light scattering data of both situations. Scale bar: 200 nm. (d) Schematic plot of water "monomer" concentration against time and local ethanol ratio. The shades indicate the nucleation period in both cases.

Assuming that a drop of sodium citrate aqueous solution was added to ethanol. When it was in contact with water, there would be a local concentration and solvent ratio during the mixing process until everywhere in the solution is averaged out. Keep in mind that the solubility of citrates in ethanol is vastly lower than that in water, hence the higher the ethanol ratio, the lower the solubility. On the other hand, if the local salt concentration is constant, the higher the ratio, the higher the oversaturation. Then the mixing process started to happen, and the local solvent ratio (ethanol:water) increases as well as the oversaturation of citrate, therefore water “monomers” started to build up. If the oversaturation was not high enough so that the water “monomer” concentration cannot break the energy barrier of homogeneous nucleation before the mixing is done, AKA no droplet would start to form during the mixing process, it would go through an ideal pathway of burst homogeneous nucleation which results in uniform droplet size. However, if the local oversaturation of citrate could reach a critical level, which breaks the energy barrier of homogeneous nucleation during the mixing process, droplet seeds would start to come out. However, at the same point, as the local solvent ratio increases, the saturated concentration of citrate decreases. It means the energy barrier of homogeneous nucleation would drop at the same time (Figure 2.3 d), since it is directly related to the saturated concentration. This means that even though the local concentration of citrate remains constant, its oversaturation increases. In other words, the oversaturation would not go down after nucleation started. This will lead to a continuous homogeneous nucleation, which results in a wide size distribution. That would possibly be the reason why direct adding of sodium citrate aqueous solution wouldn't give us small and uniform capsules.

To compensate this problem, there are two most intuitive measures. One is to further increase the mixing efficiency, to complete the mixing process faster so that the

early nucleation would not be early at all. However, it is not practical since vortex is one of the highest efficient laboratory mixing method, and our reaction vial is already small enough. The other one is by reducing the concentration of citrate added to keep local oversaturation below critical level. However, decreased total citrate concentration means a low global oversaturation, which leads to a drastic decrease of capsule yield. The nanocapsule dispersion would be too diluted that after washing, even after being highly concentrated, it was still not possible to produce a practically observable TEM sample. Needless to say, the extremely low yield would render most application impossible.

Therefore, we decided to separate the sodium citrate salt into citric acid and sodium hydroxide and let them react to generate the salt *in situ*. Since both citric acid and sodium hydroxide are soluble in both water and ethanol, they would not cause any precipitation or phase separation by themselves.

The purpose of this adjustment is to postpone the nucleation timeline compare to the mixing (Figure 2.3 d). we suppose that if the formation of salt could take some time, it would be possible to complete the mixing before the nucleation starts, or at least before they accumulate too much to pass the critical point. The question is, since acid-base reactions are considered to be one of the fastest chemical processes. In practice, would it be possible to buy us enough time? We would like to make an estimation about the time scale of this process based on existing data and equations.

Donten and co-workers systemically studied the reaction rate of acid-base reactions in aqueous solution ²². They tested the dissociation of acid in Deuterium replaced water by ultrafast spectroscopy. For acetic acid, there is

$$\text{pKa} = \log k_- - \log k_+ \quad (2.3)$$

Where Ac^- indicates acetate anion and k_+ and k_- refer to the reaction rate of the forward and the backward reaction respectively. According to their research, the k_- is limited by the proton diffusion and therefore would be relatively constant at a given concentration. In most cases it would be around $8 \cdot 10^{10} \text{ M}^{-1}\text{s}^{-1}$. Therefore the forward reaction rate is directly related to the pKa. This principle also applies to other weak and moderately strong acid. Where the weaker the acid, the larger the pKa, and the slower it dissociates. In the case of 1M acetic acid dissociation in heavy water, the reaction rate would be $2.8 \cdot 10^5 \text{ s}^{-1}$.

On the other hand, the pKa of an acid would decrease significantly in organic solvents rather than in water. According to Sarmini and co-woker's research and calculation²³, for a variety of monoprotic acid, their pKa in ethanol increased from 1.2 to 13 times. Unfortunately, multiprotic acids have not been tested. Hence, the exact increase of pKa on citric acid is unknown.

Maybe we could compare citric acid with acids of a similar pKa. According to the data provided by our chemical supplier²⁴, for the three protons, $\text{pKa}_1=3.13$, $\text{pKa}_2=4.76$, $\text{pKa}_3=6.4$. We need to understand that in order to initiate a typical salting out process, dissociation of the second proton would be necessary. This is because citrate is a relatively large ion, if only one proton is dissociated, as a single charged anion it is not likely to be kosmotropic enough to induce phase separation. The pKa_2 of citric acid is quite similar to the pKa of acetic acid, where the latter increased 1.2 times from water to ethanol. Hence, we assume the pKa_2 of citric acid in ethanol would be around 10.47. If we apply this data into equation 2.3 with $k_- = 8 \cdot 10^{10} \text{ M}^{-1}\text{s}^{-1}$, the reaction rate $k_+ = 2.71 \text{ M}^{-1}\text{s}^{-1}$. In our standard reaction system, the concentration of citric acid in ethanol is 4 mM. Therefore, the dissociation would happen in the scale of 10 seconds.

By far we evaluated the reaction rate of the acid dissociation in ethanol based on several proximations. Hence, we want to put some redundancy into our estimation by decreasing it by 2 magnitudes. It would still be in the scale of 100 milliseconds, which would be enough for the mixing to take place. On the other hand, even though the other steps such as the dissociation of sodium hydroxide in water may be faster for multiple magnitudes, they will at least be bottlenecked by the limiting step of citric acid dissociation. Therefore, we could still assume that the time scale of the salt formation process could be comparable to that of the vortex mixing.

Figure 2.4 Cloudiness test. (a) 0.1M sodium citrate aqueous solution 100 μL added to 900 μL of EtOH. (b) 0.3M sodium NaOH aqueous solution 100 μL added to 900 μL of EtOH.

Our assumption was further proved by the experiment result, where the capsules in the case of *in situ* salt formation is significantly smaller and more uniform than in

the case of salt solution addition. On the other hand, it can be further proved by the cloudiness test (Figure 2.4). The total concentration of citric acid was decreased to prolong the limiting step of the reaction, since the actual reaction time is inversely proportional to the molar concentration. We can see that in the case of addition of salt solution (Figure 2.4 a), the mixture almost instantly turned cloudy, indicating a large amount of droplet forming in ethanol. On the other hand, when sodium hydroxide aqueous solution was added into an ethanol solution of citric acid, it turned cloudy gradually in a few seconds. This could approve that the formation of salt can be postponed.

2.3.1.3 Influence of different anions

Besides citrate, a series of typical kosmotropic anions were chosen to verify their ability to synthesize nanometer scaled droplets. Based on our studies in citrate system, the salts were introduced as the form of corresponding acids and sodium hydroxide. The procedures are exactly the same as that of the citrate system, except replacing citric acid with sulfuric acid, phosphoric acid and oxalic acid.

In the case of sulfuric acid and phosphoric acid, their standard concentrated solutions were used. 85% phosphoric acid solution is considered as of a molarity of 15.2M while 98% sulfuric acid solution is 18.4M. The water introduced from these solutions is lower than the water content of an analytic reagent ethanol, hence is neglectable.

We started with an equivalent proton concentration of 12 mM, so that the sodium hydroxide amount can be standardized. However, the addition of NaOH can cause severe precipitation in the case of phosphoric acid and oxalic acid, leading to the formation of silica clusters. Hence, concentrations of these acids were reduced until hollow silica nanostructures can be produced. Strangely, as is shown in Figure 2.5 a c

e, the silica nanocapsules generated in the case of sulfuric acid, phosphoric acid and oxalic acid are all polygonal, instead of spherical in the citrate system. Some of the nanostructures shown in those TEM images strongly resemble salt crystals. Theoretically, it would not be possible that they are salt nanocrystals since all the samples has been washed with water for at least two times. Silica is very permeable for small molecules and ions and sodium salt of all the three anions are highly soluble. It is possible that they are actually silica coated nanocrystals, or at least nanocrystals are somewhat involved in the nanoencapsulation process. During the washing process, water dissolved the salt by penetrating the porous silica capsule, leaving only hollow shells resemble the shape of their former templates.

This result provides a strong evidence to our second hypothesis, which suggests that solid salt crystals exist at the earliest stage of the nucleation process and serve as seeds for water “monomers” to heterogeneously deposit on. However, the inconsistency among anions also haunts the generality of our design strategy. Therefore, we have to verify whether our standard citrate system is just a lucky exception, or all the salts share a similar trend. Those inconsistent results may merely be different sections captured from the same curve.

We assume it is due to the solubility difference between these sodium salts. As mentioned before, citrate anion is relatively larger compare to traditional inorganic anions and oxalate. It has three arms with the length of two carbon-carbon single bonds, so that the three negative charged centers could be able to keep a certain distance between each other. Therefore, according to the trend shown in Hofmeister series, it would be relatively less kosmotropic even with its three charges, which leads to a slightly higher solubility for its sodium salt in ethanol. Therefore, the equivalent concentration for citrate may be too high for other anions.

Figure 2.5 TEM image of salting out system with the acid of (a) 6 mM H_2SO_4 , (b) 0.23 mM H_2SO_4 , (c) 1.2 mM H_3PO_4 , (d) 0.12 mM H_3PO_4 , (e) 1.2 mM oxalic acid, (f) 0.36 mM oxalic acid, (g) 80 mM citric acid, (h) 120 mM citric acid, (i) 160 mM citric acid with double the water added. (j) Schematic of the capsule shape changing with concentration. Scale bar: 200 nm.

We also compared the solubility of those sodium salts in water ²⁵, which could shed some light on how difficult to break their crystal packing (Table 2.2) during the dissolving process. As we can see, the solubility of sodium citrate is indeed significantly higher. Therefore, we would expect that at a lower concentration, other salts may show a similar behavior as citrate's.

Table 2.2 The solubility of the four sodium salts at 25°C and their molarity.

	Sodium citrate	Sodium phosphate	Sodium sulfate	Sodium oxalate
Solubility (g/L)	682	145	139	37
Molarity	2.64	0.88	0.98	0.28

As is shown in Figure 2.5 b d f, all three anions, if the concentrations are low enough, can yield in spherical capsules similar to citrate does. At a concentration of 0.23 mM, though not completely spherical, the edge of the sulfate capsules starts to turn smoother, and the ratio of their length and width decreases. Similar trends can also be observed in the case of oxalate and phosphate. Especially, for phosphate, at a concentration of 0.12 mM, the capsules are already as spherical as in our standard citrate system. It is difficult to further reduce the concentration of anions, since the yield of capsules could be too low, and homogeneous nucleation of silica is increasing.

On the other hand, when the concentration of citrate increases, a trend of becoming more polygonal is revealed (Figure 2.5 g h i). Even though we were unable to obtain a sample with typical polygon shape due to the increasingly severe cracking on silica shells and the high viscosity of concentrated NaOH solutions. In the case of 160 mM citrate, double the volume of NaOH aqueous solution was introduced to compensate the high concentration. An obvious change in their shape can be observed. Sharp edges start to come out, which resembles the capsule shape of sulfate and oxalate at low concentration. It would be reasonable to assume that in the case of citrate, the

shape of capsules also undergoes a change from spherical to polygonal when its concentration increases (Figure 2.5 j).

Besides, the cracking on the silica shell may be caused by the high concentration of sodium. It has been suggested that alkali metal cations would dope into silica and change its interfacial energy with other substance. Song and co-workers have manipulated the degree of Janus on Au@SiO₂ Janus particles through this method²⁶. We assume a high sodium doped silica in our system could also change the interfacial energy, preventing silica islands on the droplet surface to grow together into a constant capsule.

From the datum at hand, it is reasonable to assert that our citrate standard system is not special. In general, kosmotropic anions showed such a trend that the shape of their capsule's changes from spherical to polygonal as their concentration increases. The most interesting parts is the intermediates between spheres and polygons, how could a droplet maintain a shape between a polygon and a sphere? Are there truly droplets or the silica smoothed their crystal template during its growth so that we can see hollow spheres under TEM? Moreover, could it serve as an evidence to our second hypothesis, or weaken it? These questions would be discussed in chapter 6.

2.3.1.4 Role of the thickener

One thing has long been haunting us in our research is the role of the thickener. Most thickeners are basically surfactants which are known to serve as templates by themselves, especially in silica nanosynthesis²⁷⁻²⁹. We intentionally chose HPC because it is not a typical amphiphile surfactant. However, further research is needed to verify the actual role of thickener in our system.

Figure 2.6 (a) TEM image of thickener free encapsulation of droplets. (b) TEM images and schematic of examples that encapsulation happens during the merging process. (c) TEM images and schematic of examples of proto-encapsulation before merging, followed by the silica shell growth. Scale bar: 100 nm.

As is shown in Figure 2.6, removing of HPC would result in a significantly decrease in uniformity. However, hollow structure can be observed all the way even in a thickener free reaction. This could serve as an evidence that the hollow structures themselves were not a direct result of surfactants. The droplets can be generated through the salting out process even without any thickener. However, it is still necessary to have HPC in our system if a better uniformity is desired.

Especially, we should notice that without the thickener, some partially merging capsules can be observed. This could serve as a solid evidence of the presence of liquid droplets, since merging/collection is one of the crucial distinctions between solid and liquid substances. More exactly, there are two types of partially merging. One is captured during the merging process of two droplets (Figure 2.6 b). The other is different since there are very thin choke points between spheres. We assume that during the early stage of silica proto-encapsulation, the silica shell on the droplet surface may be very thin and highly permeable. Therefore, liquids in droplets can exchange through

these permeable shells, so that they could stick the proto-capsules together. When the shells grew thicker, they become the second type of merging. In conclusion, both merging types could approve the existence of nanoscale droplets.

Figure 2.7 TEM image of (a) 300 mg/mL PVP as thickener. (b) 150 mg/mL PVP as thickener. (c) 75 mg/mL PVP as thickener. (d) Addition of TEOS without incubation. 300mg/mL PVP as thickener. Scale Bar: 200 nm.

We also tried to replace the thickener with PVP (Figure 2.7), thanks to its good solubility in both ethanol and water, its stickiness, and non-amphiphile-like structure. Unfortunately, PVP only shows a moderately suppression of merging even at a very high concentration. Interestingly, in some experiments the capsules made from PVP thicken solutions showed some strange shapes, like pulled balloons. This phenomenon

is more common in larger capsules. We finally figured out that it was caused by a quick addition of TEOS after vortex. If we let the mixture incubate for 10 minutes, the chance of forming those strange shaped capsules would be greatly decreased. We suppose that it could be explained by the extraction of PVP. Since PVP prefer a higher polar solvent, the water droplet will extract PVP from ethanol. However, the concentration of PVP in ethanol is already very high. Therefore, water droplets may accumulate a near saturate concentration of PVP, which make them extremely sticky. Those droplets saturated with PVP may behave like a micron-sized jelly ball and get pulled by the shear stress caused by vortex. Especially, shear stress tends to affect larger objects more than smaller ones. Given enough time, a twisted jelly ball may return to its spherical shape since it is thermodynamically preferred. This special feature of PVP may be utilized in some specific applications.

2.3.1.5 Other factors

If we replace sodium hydroxide with ammonium, like a standard Stöber process would do (Figure 2.8 a), we would observe that the size distribution is significantly wider. We attribute it to the slower reaction rate catalysed by ammonium. Also, even though less TEOS was added, there are significant amount of homogeneously nucleated solid silica particles in the case of ammonium. Thanks to the larger size of capsules, they can be purified under low speed centrifugation by leaving solid silica particles in the supernatant. This phenomenon could be explained by the ion doping theory ³. Sodium hydroxide, as a strong base, will release more hydroxide as well as sodium cations, and a strong ion doping will inhibit the condensation of poly(silicic acid) which composes the silica. Therefore, sodium hydroxide could inhibit unnecessary precipitation of silica. This could also explain why the silica shells in the case of ammonium are generally thicker, since more silica are condensed out.

Figure 2.8 TEM image of (a) ~ 5 M ammonium solution as catalyst instead of NaOH. (b) Standard system at 21 °C, capsules are shrunken. Scale bar 200 nm.

Reaction temperature could affect the shape of capsule. If the reaction was taken place at a temperature lower than 25 °C, there is a chance that the capsules will be shrunken (Figure 2.8 b). That is why the reaction temperature in our standard system is set to 30 °C. However, it may not be a direct result of salting out process. Its mechanism would be discussed in chapters 3.

2.3.2 General solute induced phase separation

We have achieved encapsulation of droplet made from salting out processes. Now we want to push our system a step further, to induce phase separation from a more generalized list of chemicals.

2.3.2.1 Polyelectrolytes

Polyelectrolytes are basically ions with a larger molecular weight and more charges. Even though the Hofmeister series doesn't include polyelectrolytes in it, it is reasonable to assume that its trend also works for polyelectrolytes. They are larger, but also bearing more charges, therefore there would be a chance for them to induce phase separation like typical anions do.

Figure 2.9 TEM image of (a) PPA and NaOH. (b) PPA and ammonium. (c) PAA and NaOH. (d) PAA and ammonium.

As is shown in Figure 2.9, poly(acrylic acid) (PAA) and polyphosphoric acid (PPA) are introduced as separation initiators. When coupled with ammonium, both PAA and PPA can produce nanoscale droplets for silica capsules. Strangely, in the case of sodium hydroxide, PAA was not able to induce phase separation. This is counterintuitive since ammonium salts are considered to be even more soluble than sodium ones. However, in this case, PAA sodium salt showed a great solubility in ethanol rich solvent. This indicates a complexation in the case of polyelectrolytes and the limitation of Hofmeister series.

2.3.2.2 Carbohydrates

Figure 2.10 TEM image of Silica capsules made from (a) Glucose induced phase separation. (b) Sucrose induced phase separation.

Carbohydrates are typical non-ionic kosmotropes due to their strong interactions with water. Therefore, they are expected to induce phase separation similar to ionic kosmotropes – like citrate and sulfate – would do. Monosaccharide glucose and disaccharide sucrose were introduced in this test. As expected, (Figure 2.10), both of them could induce phase separation and generate nanosized droplet at certain concentration.

2.3.2.3 Other neutral polymers

The name “other neutral polymers” actually refers to traditional polymers that we are familiar with. Unfortunately, it is not easy to choose a proper traditional polymer for this purpose. Hofmeister series doesn’t include these substances. Hence, we need to conclude the screening conditions.

We could see the solute induced phase separation as a competition between water, ethanol, and the solute. If the interaction between water and the solute is stronger than that between either of them and ethanol, the solute and water will stay together and detach from the grasp of ethanol, causing the phase to separate. If the interaction

between water and ethanol is stronger, neither of them likes the solute, the solute will be kicked out as a solid precipitation. If the interaction between the solute and ethanol is stronger, there can be two possible outcomes. If the solute could repel water, for example, a highly hydrophobic one, it may be able to stay with ethanol and kick the water out. However, this is not quite possible in practice since the polarity of ethanol is relatively high and its interaction with water is already strong. It is more likely that ethanol prefer water rather than the highly hydrophobic solute. The other result is just everything mixed together without any phase separation.

Therefore, our screen conditions are as follow. Firstly, the polymer must to be water soluble, so that it interacts with water to exclude it from ethanol. Secondly, its solubility in ethanol must be lower than that in water. Last but not least, it must not be a polyelectrolyte, since we have already done it before and want a traditional neutral polymer this time.

Finally, we narrowed down our list to two “traditional” polymers – PEG and PVA. Both of them follow the conditions mentioned above. Unfortunately, neither of them could generate droplets from phase separation. In the case of PEG, no phase separation can be observed. Even addition of a high concentration PEG water solution into ethanol would result in a one phase solution. What makes thing worse is that PEG solutions are highly viscous. May be the highly hydrophobic chain of PEG could get along with ethanol while the two ends with strong hydrogen bond are welcomed by water.

PVA, on the other hand, would precipitate out without abducting of water even at a low concentration. Its intensive hydroxyl branch may be the cause. Water and ethanol prefer each other so the solvent as a whole cannot dissolve PVA.

These unsuccessful attempts indicate that traditional polymers may not be quite suitable for this purpose.

2.3.3 Other solvent systems

Aside from water-ethanol system, other combinations of water and common water miscible solvents have been tested. Water mixture with acetone, acetonitrile (ACN), tetrahydrofuran (THF), dimethylformamide (DMF) and dimethyl sulfoxide (DMSO), respectively have been tested. Citrate and sulfate were chosen as separation initiators, and ammonia solution was the catalyst. Different thickeners were chosen according to their solubilities in those organic solvents. Except DMSO, all other solvent combinations could generate aqueous droplets and synthesized nanocapsules. These experiments are merely proofs of concept to verify the generality of solvent combinations. We believe given enough time, it would be possible to optimize the condition for each pair, even water-DMSO, to synthesize uniform droplets and nanocapsules. The solute induced phase separation is not limited to water-ethanol system, it has a much wider applicability.

Figure 2.11 TEM image of Silica capsules made from different solvent combinations. (a) Sulfate in ACN. (b) Sulfate in acetone. (c) Citrate in DMF. (d) Citrate in THF. Scale bar: 200 nm.

2.4 Conclusion

In order to build liquid droplets bottom-up, we started from an extrapolation of the salting out effect. In order to optimize the liquid nucleation and growth process, we separated salt into acid and base, and introduce them as different solutions, to slow down the supply of salt and therefore reduced excessive local oversaturation. Also, thickeners were introduced to suppress the merging of droplets, to shift the liquid nucleation and growth to a similar pattern as solid particles. By doing so, silica nanocapsules with uniform shape and a narrow size distribution have been synthesized from the droplets generated from a controllable salting out system. The potential of other salts has also been verified, and the polygonal shape related to the concentration of salt may serve as an evidence of the extraction hypothesis. Finally, we promoted the phase separator from salt to more generalized solutes such as polymers and carbohydrates. The concept of our design is proved to be valid. Next step, phase separation would serve as a tool for chemical loading and synthesis of nanocapsules from other materials.

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Chapter 3 Loading of capsules

We have discussed solute induced phase separation and its potential on synthesis of hollow nanocapsules. In this chapter, we want to switch our topic on its application. We would like to evaluate the generality of loading capabilities in this system, expecting it could compensate some of the disadvantages of traditional methods.

3.1 Instructions

When talking about what to do about a capsule, the first answer comes up with would be to put something in it. Hence, loading is the most intuitive potential applications of capsules, and capsules in nanoscale could secure an enormous total surface area which would be extraordinarily beneficial for some purposes such as catalyst, drug releasing and nanoreactors.

3.1.1 Application of loaded hollow capsules

3.1.1.1 Drug delivery

Drug delivery has long been a popular application of nanosynthesis. Since most drugs of chemotherapy could damage normal tissue as well as cancer cells, scientist expect that drug loaded nanocarriers could be able to hold the toxic chemicals away from healthy organs and release it only close to their target – the tumor ^{1,2}. Generally speaking, there are three different types of nanocarriers: polymeric carriers, liposomes and nanoshells (Figure 3.1). Polymeric carriers are more likely drug molecules entangled into polymer doughs rather than typical capsules ³⁻⁶. Their drug release relies solely on the bio-degradation of polymer material. A crucial problem is the philicity of the entrapped chemical must fit the polymer's otherwise it may be repelled. Another challenge is their wide size distribution and the poor synthetic controllability. Even though in rare cases the size can be fine-tuned ⁵. Liposome is another promising system

for drug loading, due to its intrinsic bio-compatibility⁷⁻¹⁰. However, it also suffers from several shortages. Firstly, liposomes can be targeted by the mononuclear phagocyte system by the body, so they cannot easily reach the destination at a desirable concentration. On the other hand, if a large amount of liposome carriers is introduced, it may also cause an uncontrolled burst release of the loadings, even though it may be compensated by chemical modification of the drug loaded. An advantage of nanoshell is that it provides a functionable surface outside the chemical. Therefore, modifications don't need to be focused on the loaded drug molecules, but can be on the shell, which is considered to be less destructive to the medication efficiency¹¹⁻¹⁶.

Figure 3.1 Three different modes of drug loaded nanocapsules. Reprinted from ref 5 with permission. Copyright © 2007, Nature Publishing Group.

3.1.1.2 Nanoreactors

In the case of drug delivery, the chemical loaded is expected to come out, either by penetrating the shell, or by the decomposition of the capsule itself. On the other hand, for nanoreactors, the loading is supposed to stay inside the capsule during reaction happens. Nanoreactors can be used as a mold to constrain the product synthesized within, therefore control its size and morphology¹⁷. Another popular application of nanoreactor is the entrapment of catalysts¹⁸⁻²². It would be ideal to keep the catalyst inside nanocapsule, but let the reaction substrates come inside, complete the reaction, and leave. The catalysts encapsulated can be nanosized novel metal structures as well

as homogeneous ones. Even though simply coating on novel metal nanoparticles could serve a similar purpose, it is approved that with gaps between the NP and the capsule shell, the catalyst efficiency would be higher¹⁸. For the homogeneous catalyst loaded capsule, the nanoreactor practically turn it into a heterogeneous catalyst with huge surface area, which is both efficient and economically recyclable.

3.1.2 Traditional synthetic strategies of nanocapsules

3.1.2.1 Template and etching process

Figure 3.2 Schematics of typical template and etching. (a) Template $\rightarrow$ LBL $\rightarrow$ etching. (b) Template $\rightarrow$ interfacial coating $\rightarrow$ etching. (c) Stober silica $\rightarrow$ etching. Reprinted from ref 23, 22, 26 with permission. Copyright © 2006, Elsevier Ltd; 2013, American Chemical Society; 2009, The Royal Society of Chemistry, respectively.

As mentioned before, the most “orthodox” method for to synthesize nanocapsules is to first make a hollow capsule, then load it by diffusion. To make the hollow shell, traditionally, a sacrificial template was first introduced, followed by a coating of other materials either by a layer-by-layer (LBL) method ^{23,24} or deposition guided by interfacial energy ^{22,25}. Later, the template must be removed to release an empty capsule for the loading process. On the other hand, Stöber silica particles can serve as an ideal self-template precursor for hollow shell ^{1,26} since its core and outer layer are composed of different types of silica and the shell is much more resistant to etching ²⁷. All the processes need multiple steps of operations, and the diffusion loading is especially inefficient. Also, the shell material must be permeable otherwise the template inside cannot be removed (Figure 3.2).

3.1.2.2 Nanoemulsion and microemulsion

The terminology of nanoemulsion and microemulsion are quite misleading. Both are dispersions with droplets at sub-micron scale. Ironically, the droplets in microemulsion are actually smaller than that in the nanoemulsion ^{28,29}, this may because they were named by scientists from different subdisciplines. The difference between these two emulsions were shown in table 3.1.

Table 3.1 Comparison between microemulsions and nanoemulsions. Modified from ref 28 with permission. Copyright © 2017, Elsevier Inc.

System	Microemulsions	Nanoemulsions
Appearance	Transparent	Opalescent
Droplet size	<150 nm	100 - 1000 nm
Formation	Spontaneous	Agitated
Stability	Thermodynamically stable	Kinetically stable

As mentioned before, if we can generate nanoscale droplets and put designated chemicals inside, then the encapsulation of such loaded droplets could result in our desired products in one step. Microemulsions and nanoemulsions both rely on specific

solvent and surfactant ratios. Especially for microemulsions. A microemulsion system is actually formed at a subtle balance point between the solvents and multiple types of surfactants. Nanoemulsions require mechanical power input but are usually able to keep stable for a reasonably long period of time ³⁰. Those emulsions do provide nanoscale droplets, some of them can serve as templates for nanocapsules. However, it would be very difficult to put a variety of loading chemicals inside these already sensitive systems. The interval for the ratio between the ingredients is very narrow, introducing a new compound could easily break the balance and the emulsions will be no more. Even though Shchukin group managed to incorporate $\text{Mg}(\text{NO}_3)_2$ into microemulsion droplets and made them into nanocapsules ³¹, such methods showed very poor generalities.

We believe the solute induced phase separation could serve as a universal chemical loading tool for nanoencapsulations.

3.2 Material and methods

3.2.1 Material

Tetraethyl orthosilicate (TEOS), Citric acid (H_3Ct), hydroxypropyl cellulose (HPC, Average Mw $\sim 80,000$, Average Mn $\sim 10,000$), polyvinylpyrrolidone (PVP, Average Mw 40,000), hydrogen chloroaurate(III) (HAuCl_4 , $\sim 52\%$ Au basis), chloroplatinic(IV) acid (H_2PtCl_6 , 38~40% Pt basis), Potassium hexachloroplatinate(IV) (K_2PtCl_6 , reagent grade, 98%), hydratesodium tetra-chloropalladate(II) (Na_2PdCl_4 , 98%), silver(I) nitrate (AgNO_3 , ACS reagent, $\geq 99.0\%$), copper(II) nitrate ($\text{Cu}(\text{NO}_3)_2$, puriss. p.a., 99-104%), doxorubicin hydrochloride (98~102%), dopamine hydrochloride, ascorbic acid (AA), sodium borohydride (NaBH_4 , powder, $\geq 98.0\%$) and cisplatin (pharmaceutical secondary standard), were purchased from Merck (AKA Sigma-Aldrich). Sodium citrate (dihydrate, 99%) was purchased from Alfa Aesar.

Ammonium solution (28%) was purchased from TCI. ethanol (absolute, 'Baker analyzed') was purchased from J.T.Baker, sodium hydroxide (NaOH, pellets ACS) was purchased from VMR chemicals, Deionized water (resistance $> 18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was used in all reactions.

3.2.2 Methods

Characterization. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 ~ 120 kV. UV-Vis spectrum data was collected on a 100 Conc (Cary) UV-Visible spectrophotometer. EDX mapping data was collected on a JEOL-2100F field emission electron microscope with an EDS (energy dispersive x-ray spectrometer) module.

Preparation of TEM samples. Procedures were the same as mentioned in the previous chapter.

Preparation of 4% w/w standard hydroxypropyl cellulose solution. Procedures were the same as mentioned in previous chapter.

Preparation of PVP stock solution. Procedures were the same as mentioned in the previous chapter.

Silica nanocapsules pre-loaded with gold(III) salt. 100 μL of 100 mM HAuCl_4 ethanol solution was added into a mixture with 250 μL PVP stock solution, 20 μL of 500 mM citric acid ethanol solution, and 630 μL of ethanol under vortex. Then, 100 μL of ~5 M ammonia solution was quickly added to the mixture under vortex, followed by a 10s of vortex. The mixture was incubated for 5 min and 6 μL of TEOS was then added. It was let stand at room temperature for 6 hours. To isolate the capsule, 1000 μL of water was added to the reaction mixture to reduce the viscosity, followed by a centrifugation at 16,000 G for 15 min. It was then washed by water for 3 times and 2

times by ethanol. 5 min at 8,000 G was suffice. Finally, it was topped with 1000 μ L of ethanol.

Silica nanocapsules pre-loaded with gold(III) salt with HPC as thickener.

Procedure was similar to the above. PVP stock solution was replaced by 4% w/w HPC ethanol solution. During the isolation process, no water needs to be added since its viscosity was not as high as the PVP system's. The centrifugation speed of the first run was 8,000G. Only 2 times of washing by water is required.

Silica nanocapsules pre-loaded with platinum(IV) salt. Procedure was similar to that of the gold(III) system. HAuCl_4 was replaced by H_2PtCl_6 .

AA reduction of gold(III) loaded silica nanocapsule. 100 μ L of the dispersion of gold(III) loaded nanocapsules was mixed with 800 μ L of ethanol. Then 100 μ L of 0.1M AA aqueous solution was added under vortex. The mixture was sealed and placed in a 65°C oven for 2 hours. It was then washed with water and ethanol before re-dispersed in 1000 μ L of ethanol.

Citric acid reduction of gold(III) loaded silica nanocapsule. 100 μ L of the dispersion of gold(III) loaded nanocapsules was mixed with 800 μ L of ethanol. Then 100 μ L of 0.5 M citric acid aqueous solution was added under vortex. The mixture was sealed and placed in a 95°C oven for 2 hours. It was then washed with water and ethanol before re-dispersed in 1000 μ L of ethanol.

Sodium borohydride reduction of gold(III) loaded silica nanocapsule. 100 μ L of the dispersion of gold(III) loaded nanocapsules was mixed with 800 μ L of ethanol. 100 μ L of freshly made 1M NaBH_4 aqueous solution was diluted by 900 μ L of ethanol. Under vigorous stirring, 100 μ L of the above NaBH_4 solution was added to the diluted gold(III) nanocapsule dispersion. It was stirred for 30 min, washed by 90% ethanol for 2 times and re-dispersed in 1000 μ L of ethanol.

Silica nanocapsule peri-loaded with cisplatin. 250 μL of 4% HPC solution was diluted by 742 μL of ethanol, then 4 μL of 500 mM citric acid ethanol solution was added. The solution was pre-mixed by vortex. 1 mL of 350 μM cisplatin aqueous solution was mixed with 0.5 mL of 210 mM NaOH aqueous solution. After that, under vortex, 100 μL of the solution with NaOH and cisplatin was quickly added into the mixture, followed by 5 seconds of vortex. The mixture was incubated for 1 minute, then 6 μL of TEOS was added under vortex. It was then put into a 30 $^{\circ}\text{C}$ oven for 2 hours. In order to isolate the capsules, the mixture was centrifuged at 8,000G for 15 minutes. For UV-vis characterization, it was washed by 300 μL water for 5 times, the eluates were combined. Otherwise, the precipitant was washed by 500 μL water for 2 times, and 500 μL ethanol once. 5 minutes of centrifugation was adequate for washing. The final precipitant was dispersed in 1000 μL of ethanol.

Silica nanocapsule peri-loaded with copper(II) salt. 250 μL of 4% HPC solution was diluted by 742 μL of ethanol, then 8 μL of 500 mM citric acid ethanol solution was added. The solution was pre-mixed by vortex. 1 mL of 400 mM $\text{Cu}(\text{NO}_3)_2$ aqueous solution was mixed with 1 mL of 28% ammonia solution. After that, under vortex, 100 μL of the solution with tetraaminecopper(II) salt was quickly added into the mixture, followed by 5 seconds of vortex. The mixture was incubated for 5 minute, then 2 μL of TEOS was added under vortex. It was then put into a 30 $^{\circ}\text{C}$ oven for 2 hours. In order to isolate the capsules, the mixture was centrifuged at 8,000G for 15 minutes. The precipitant was washed by 500 μL water for 2 times, and 500 μL ethanol once. 5 minutes of centrifugation was adequate for washing. The final precipitant was dispersed in 1000 μL of ethanol.

Silica nanocapsule peri-loaded with silver(I) salt. The procedure was similar to that of the copper(II) peri-loading experiment. $\text{Cu}(\text{NO}_3)_2$ was replaced by AgNO_3 . The vial for preparation of Ag containing solutions and the vial for the reaction were all covered by aluminum film. Instead of water, the sample was washed by a mixture of water:ethanol = 1:4 for 3 times followed by a washing with pure ethanol. The product was also wrapped with aluminum film.

Silica nanocapsule peri-loaded with palladium(II) salt. 250 μL of 4% HPC solution was diluted by 742 μL of ethanol, then 8 μL of 500 mM citric acid ethanol solution was added. The solution was pre-mixed by vortex. 1 mL of 300 mM Na_2PdCl_4 aqueous solution was mixed with 0.5 mL of 210 mM NaOH aqueous solution. After that, under vortex, 100 μL of the solution with NaOH and Na_2PdCl_4 was quickly added into the mixture, followed by 5 seconds of vortex. The mixture was incubated for 5 minute, then 6 μL of TEOS was added under vortex. It was then put into a 30 °C oven for 2 hours. In order to isolate the capsules, the mixture was centrifuged at 8,000G for 15 minutes. The precipitant was washed by a mixture of water:ethanol = 1:4 for 3 times followed by a washing with pure ethanol. The final precipitant was dispersed in 1000 μL of ethanol.

Silica nanocapsule peri-loaded with doxorubicin. 10 mg of HPC was added into 1255 μL of 400 μM doxorubicin ethanol solution, followed by a stirring of 2 hours. The solution was passed a through a 0.45 μm syringe filter. 996 μL of the above solution was mixed with 4 μL of 500 mM citric acid ethanol solution. . After that, under vortex, 100 μL of the 70 mM NaOH solution was quickly added into the mixture, followed by 5 seconds of vortex. The mixture was incubated for 1 minute, then 6 μL of TEOS was added under vortex. It was then put into a 30 °C oven for 2 hours. In order to isolate the capsules, the mixture was centrifuged at 8,000G for 15 minutes. For the UV-Vis

characterization, 500 μL of mixture with water:ethanol = 1:1 was added to wash out the doxorubicin until no visible orange color in the precipitant can be observed after centrifugation. It may requires 12 times of washing. Otherwise, the precipitant was washed by water for 2 times followed by a washing with pure ethanol. The final precipitant was dispersed in 1000 μL of ethanol.

Silica nanocapsule post-loaded with copper(II) salt. 250 μL of 4% HPC solution was diluted by 742 μL of ethanol, then 8 μL of 500 mM citric acid ethanol solution was added. The solution was pre-mixed by vortex. Then 50 μL of 260 mM NaOH aqueous solution was added under vortex. Followed by a 1 min incubation, 6 μL of TEOS was added. The mixture was let stand at room temperature for 30 min. 1 mL of 400 mM $\text{Cu}(\text{NO}_3)_2$ aqueous solution was mixed with 1 mL of 28% ammonia solution. After the 30 min stand was finished, 50 μL of the solution with tetraaminecopper(II) salt was quickly added, followed by 5 seconds of vortex. The mixture was then put into a 30 $^\circ\text{C}$ oven for 1.5 hours. In order to isolate the capsules, the mixture was centrifuged at 8,000G for 15 minutes. The precipitant was washed by 500 μL water for 2 times, and 500 μL ethanol once. The final precipitant was dispersed in 1000 μL of ethanol.

Silica nanocapsule post-loaded with palladium(II) salt. The procedure was similar to that of the copper(II) post-loading experiment. $\text{Cu}(\text{NO}_3)_2$ was replaced by Na_2PtCl_4 . The precipitant was washed by a mixture of water:ethanol = 1:4 for 3 times followed by a washing with pure ethanol.

Silica nanocapsule post-loaded with silver(I) salt. The procedure was similar to that of the copper(II) post-loading experiment. Instead of citric acid alone, a mixture of 250 mM citric acid and 375 mM oxalic acid ethanol solution was chosen. $\text{Cu}(\text{NO}_3)_2$ was replaced by AgNO_3 . . The vial for preparation of Ag containing solutions and the vial for the reaction were all covered by aluminum film. Instead of water, the sample was

washed by a mixture of water:ethanol = 1:4 for 3 times followed by a washing with pure ethanol. The product was also wrapped with aluminum film.

Silica nanocapsule post-loaded with platinum(IV) salt. The procedure was similar to that of the copper(II) post-loading experiment. Instead of citric acid alone, a mixture of 250 mM citric acid and 375 mM oxalic acid ethanol solution was chosen. The tetraamine copper(II) solution was replaced by a 100 mM K_2PtCl_6 aqueous solution. The precipitant was washed by a mixture of water:ethanol = 1:4 for 3 times followed by a washing with pure ethanol.

Silica nanocapsule post-loaded with doxorubicin. The procedure was similar to that of the copper(II) post-loading experiment. $\text{Cu}(\text{NO}_3)_2$ was replaced by 8 mM of doxorubicin. For the UV-Vis characterization, 500 μL of mixture with water:ethanol = 1:1 was added to wash out the doxorubicin until no visible orange color in the precipitant can be observed after centrifugation. It may requires 6~7 times of washing. Otherwise, the precipitant was washed by water for 1 times followed by 2 times of washing with pure ethanol. The final precipitant was dispersed in 1000 μL of ethanol.

Synthesis of shrunken silica nanocapsules. The procedure is similar to that of the standard silica nanocapsules experiment with citrate induced phase separation. The only difference is after the addition of TEOS, the reaction mixture was let stand at a temperature of 21 °C.

Water post loading of silica nanocapsules. 250 μL of 4% HPC solution was diluted by 742 μL of ethanol, then 8 μL of 500 mM citric acid ethanol solution was added. The solution was pre-mixed by vortex. Then 50 μL of 260 mM NaOH aqueous solution was added under vortex. Followed by a 1 min incubation, 6 μL of TEOS was added. The mixture was let stand at 21 °C for 4, 8, 16, 32, 60 min respectively. Then 50 μL of water was quickly added, followed by 5 seconds of vortex. The mixture was then kept at 21

°C for 2 hours. Then it was washed by 500 μ L water for 2 times, and 500 μ L ethanol twice. The final precipitant was dispersed in 1000 μ L of ethanol.

Synthesis of double-layer PDA-silica hybrid capsules. 4 mg of dopamine hydrochloride was dissolved in 1 mL of ethanol. 500 μ L of the above solution was mixed with 250 μ L of 4% HPC solution, 242 μ L of ethanol, then 8 μ L of 500 mM citric acid ethanol solution. The solution was pre-mixed by vortex. Then 50 μ L of 260 mM NaOH aqueous solution was added under vortex. Followed by a 1 min incubation, 6 μ L of TEOS was added. The mixture was let stand at room temperature for 30 min. Then 50 μ L of 5M ammonia solution was quickly added, followed by 5 seconds of vortex. The mixture was then kept at 30 °C for 6 hours. Then it was washed by 500 μ L water for 2 times, and 500 μ L ethanol twice. The final precipitant was dispersed in 1000 μ L of ethanol.

Measurement of the aqueous phase volume change at different temperature.

Mixtures of 50 μ L 130 mM NaOH aqueous solution and 1 mL of 4 mM citric acid ethanol solution was incubated at room temperature (21 °C) and 40 °C for 3 hours, respectively. Then, they were centrifuged at 16,000 G for 1 min. For each sample, 500 μ L of the supernatant, AKA the ethanol phase, was collected and diluted by 1500 μ L of water. The rest of the supernatant was removed. The precipitant droplet, AKA the aqueous phase, was vacuum dried, re-dissolved by 500 μ L of water. 300 μ L of the above solution was collected and mixed with 900 μ L of water and 300 μ L of ethanol. A mixture of 50 μ L 130 mM NaOH aqueous solution and 1 mL of 4 mM citric acid aqueous solution was chosen as the standard. The sample from the supernatant, precipitant, and the standard diluted by 3, 7, 15 times was tested by UV-Vis spectrum. The calculation details would be covered later in this chapter.

3.3 Results and discussions

3.3.1 Three windows of time for droplet loading

During a solute induced phase separation process, there can be three windows of time for introducing the chemicals. The first chance would be at the initiation of phase separation when droplets starts to appear. For example, in a citrate salting out system, the water droplets formed are literally solutions of the citrate salt. Therefore, after encapsulation, citrate – the phase separation initiator – would be loaded into nanocapsules as a result. We define this type of loading as pre-loading, since according to our condensation and extraction hypothesis, the loading substance exists even before the formation of droplets (Figure 3.3 a).

The second chance of loading is during the growth of droplets. In a typical water-ethanol system salted out by a solute A for instance, when a droplet starts to form, it provides a water rich condition, which would be preferred by hydrophilic substances. Assumedly no merging/collection would take place, if we see the solubility of A in the ethanol phase is zero, then the amount of A in a specific droplet would be constant all along. Since A and ethanol is incompatible, and water molecules could form interactions between both of them, the growth of a droplet is like a tug of war between the salt A inside the droplet and the surrounding ethanol. They would compete for water molecules. Hence, the growth of the droplet is like an extraction of water by A from the ethanol phase.

On the other hand, just like how A can extract water, water can also extract other hydrophilic substances in the system. If there is another solute B in the system when the above-mentioned growth process happens, and B also prefers water to ethanol, it would be extracted into the growing water droplet similar to that in a traditional extraction process. When the droplet is growing, it will have more water and therefore

more B will be enriched inside. As a result, after an encapsulation, B could be loaded into nanocapsules. This process is called peri-loading of solute B because its enrichment is happened during the growth of droplets (Figure 3.3 b).

Figure 3.3 Schematics of (a) Pre-loading process. The loading substance itself serves as the phase separation inducer. (b) Peri-loading process. The initiator generates the droplet. During its growth, loading substance is extracted in. (c) Post-loading process. After the formation of droplet, loading substance is added, and gets extracted into the droplet.

There is one thing need to be mentioned. The solubility difference between water and ethanol of A would be significantly higher than that of B. Because if contrariwise, B would be the initiator of droplet instead of A. Thus, the process would

become a B induced phase separation that extracts A. In the case that A and B have a similar solubility difference, the model would be too complicated, and the initiation may be caused by both of them. We would ignore such a non-typical situation during this stage of our research.

The third window of time to load substances into droplets is after the growth of droplets. We define it as post-loading (Figure 3.2 c). After the droplets reached a relatively stable stage, they can still extract hydrophilic substances. If a substance C is introduced at this moment, it could be incorporated into nanocapsules as well. It seems to be an ideal loading chance since the droplet growth and loading process could be separated to avoid complexions. The problem is how to introduce C. Since the enrichment of C in droplets depends on its solubility difference in water and ethanol, the solubility of C in ethanol would be relatively low. Therefore, if C is introduced as its ethanol solution, the total amount of C would be low unless a large amount of solution is added, which will introduce excessive ethanol into the balancing system between salt A, water and ethanol, and results in an abduction of water from the droplets. Less water in droplets results in less C loaded, which will exacerbate the already poor loading efficiency. Therefore, the practical way to introduce C is through its aqueous solution. To avoid a further disturbance on the uniformity of droplets, we also decide to do the encapsulation during the post-loading process, so the forming capsule could help on stabilizing the droplets.

In practice, pre-, peri and post-loading each fits different situations. In the work, we would like to conclude from typical cases and make a comparison between them, so that we could possibly chose the best strategy for a specific loading substance.

3.3.2 Typical case analysis

We would like to firstly go through several typical cases of different loading strategies. Silica was chosen as the capsule material in this chapter, due to its chemical inertia. Other shell materials would be discussed in chapter 4. A series of cases will be discussed to demonstrate the generality of the system.

3.3.2.1 Pre-loading of gold(III) salt

It can be expected that even though pre-loading may be the most efficient and convenient among all strategies, it also has the most limitations. As mentioned in previous chapters, not all solute is capable of separating the phases. Even if it could, generating of uniform nanoscale droplets for encapsulation may still not be possible. Therefore, the generality of pre-loading is supposed to be relatively low. Although if a loading substance could meet the needs, the phase separation and encapsulation would be smooth and efficient.

Pre-loading of gold (III) salt would serve as a good demonstration. Ammonia solution was added into an ethanol solution of chloroauric(III) acid, citric acid and a thickener, either HPC or PVP, under vortex. The mixture was let stand for 5 minutes, followed by an addition of TEOS. Then, after 2 hours, uniform silica capsules with gold(III) complex ion was made. This procedure is quite simple, but the result is surprisingly successful, as is shown in Figure 3.4 a-d.

As we can see, after centrifugation, the compressed precipitant shows a dark yellowish color. After re-dispersed in water, an opalescent yellow dispersion was produced, indicating the presence of gold(III) (Figure 3.4 f). TEM image shows an ideal narrow size distribution in the case of PVP, while as to the case of HPC, the monodispersity is slightly worse but still acceptable. It was further approved by EDX mapping that gold is indeed entrapped inside silica capsules (Figure 3.4 e). The salt

trapped within is sensitive to electron beam, therefore only 1 round of scan is possible before severely breaking the sample, which result in a relatively weak signal. However, the profile of gold is still discernable.

Figure 3.4 TEM images of (a-b) Gold(III) loaded silica capsule with PVP thickener. (c) HRTEM image. (d) TEM image of Gold(III) loaded silica capsule with HPC thickener. (e) EDX mapping of gold for Gold(III) loaded silica capsule with PVP thickener. (f) Gold(III) loaded silica capsule after centrifugation and re-dispersed. Scale bar for a, b, d: 200 nm.

The pre-loading of gold(III) was made possible thanks to the low solubility of fulminating gold^{32,33} – a series of gold(III) ammonium complexes – which could serve as an ideal phase separation initiator. Interestingly, without citric acid, no phase separation can be observed, and it will end in a transparent yellow solution. This indicates that citrate has played a part in the phase separation process. On the other

hand, it is possible to get almost as good spherical capsules by simply replacing citric acid with oxalic acid. It has been demonstrated that citric acid and oxalic acid showed distinct behavior in phase separation. The former tends to yield spherical capsules while the latter leads to polygonal ones at similar concentration. The spherical shape of the gold(III) loaded capsules produced by oxalate could serve as an evidence that the phase separation was not induced by a simple oxalate salt. According to the literature, the exact component of a specific fulminating gold depends on the solvent and other ingredients in the system. Hence, we assume that the real initiator would be a gold-ammonia-citrate or gold-ammonia-oxalate complex.

Figure 3.5 (a-d) Gold(III) loaded capsules reduced by NaBH_4 . (a-b) TEM images. (c) After centrifugation and redispersed. (d) UV-Vis spectrum. (e) Gold(III) loaded capsules reduced by citric acid. (f) Gold(III) loaded capsules reduced by ascorbic acid. Scale bar: 200 nm.

In order to further approve the existence of gold(III) ion, as well as verifying the nanoreactor potential of this system, we also performed reduction reactions to the nanocapsules and tried to turn the ions into gold nanoparticles (Figure 3.5). It was proved that under a high content of ethanol, sodium borohydride could reduce the

gold(III) ions into nanoparticles within the capsules. It was indicated by the ruby color of the reduced dispersion, and further approved by its UV-Vis spectrum (Figure 3.5 a-d). Other typical reductants such as ascorbic acid (AA) and citric acid have also been tested. However, they tend to yield larger gold nanoparticles outside silica capsules (Figure 3.5 e f) which result in dark purple dispersions. We assume that the concentration of gold(III) ions inside the capsules are too high so in an acidic condition, they would easily get washed out in an acidic condition. Since AA and citric acid are not as powerful as sodium borohydride, the actual reduction would happen after gold ions have left their capsules, leading to free gold nanoparticles. Sodium borohydride provides a basic condition as well as more powerful reducing ability, therefore could turn gold(III) into nanoparticles before the washing-out happens.

3.3.2.2 Peri-loading of cisplatin

Cisplatin is a charge neutral complex. It is a well-known medication for chemotherapy which could be applied into the treatment of a variety of cancers. However, like other similar drugs, it could induce a series of side effects³⁴. That is why the encapsulation of such medications has always been a goal of nanosynthesis. Cisplatin is not suitable for pre-loading, due to its negligible solubility in ethanol. Actually, even in water, cisplatin can only reach a maximum concentration of several mM³⁵ since it's not ionic. Therefore, our only choice would be the other two strategies.

In order to avoid ligand exchange of the two chloride, ammonia solution is ruled out as the catalyst. Therefore, an aqueous solution of sodium hydroxide and cisplatin was added into an ethanol solution of citric acid with HPC as the thickener. Followed by a 1 minute incubation, TEOS was added into this system. After 2 hours of reaction, the cisplatin encapsulated nanocapsules were collected. The existence of the loaded drug was testified by UV-Vis spectrum (Figure 3.6 a) since unlike the previous

experiment, no visible cisplatin remnant can be seen on the TEM image due to its low total concentration (Figure 3.6 b). As we can see, almost two third of the cisplatin added was entrapped into the capsule. Given that only 10% volume of water was added, and the actual volume of the aqueous phase would be even less due to the partition of water between the two phases. We would expect the partition coefficient of cis-platin between water and ethanol to be quite high.

Figure 3.6 (a) Uv-vis spectrum of the capsule eluate and standard cisplatin solutions. (b) TEM image of cisplatin loaded capsules after a round of washing. Scale bar: 200 nm.

As we can see, the size distribution of the cisplatin loaded capsules is less narrow than capsules without cisplatin introduced. This may be because the introduction of cisplatin interfered the droplet nucleation process. The solubility of cisplatin in ethanol is almost zero, therefore it may precipitate when its aqueous solution mixed with the alcohol, which may compete with the sodium citrate induced phase separation. This is a limitation of the peri-loading strategy. The presence of the loading substance during the droplet nucleation and growth may shift the condition and finally compromise the uniformity. The concept of post-loading was introduced if size uniformity would be an issue.

3.3.2.3 Post-loading of copper(II) salt

Copper(II) would be a good example of post-loading of ions, even though it is also suitable for peri-loading. The details and comparison would be covered later. At a basic condition, which is needed for a Stöber process, copper would form an insoluble hydroxide which would ruin the loading and encapsulation process. Fortunately, the tetraammonium complex of copper(II) is a classical soluble complex ion stable in basic conditions. Also, its blue color could serve as an indicator for a convenient monitoring of the reaction. Hence, it would be an ideal loading substance in our system, is it right?

However, as mentioned in the previous chapter, replacing sodium hydroxide with ammonia in the citrate system would lead to a much wider size distribution of the capsules, as well as homogeneous solid silica particles. To compensate these issues, we would like to keep the system free of tetraamminecopper(II) ion until the droplets are formed and sealed. Like in a standard system, sodium hydroxide aqueous solution was added into the ethanol solution of citric acid thickened by HPC. After a 1 minute incubation, TEOS was added. Then the mixture was let stand for a while until a concentrated tetraamminecopper(II) aqueous solution was added during vortex. After that, the mixture was kept standing for the rest of the time, and copper(II) loaded nanocapsules could be then washed and collected.

As is shown in Figure 3.8, after centrifugation, the blue precipitant could serve as an evidence that there is indeed ionic copper instead of oxides or copper(0) particles. The loading can be washed out by 0.1M hydrochloric acid. When topped with excessive concentrated ammonia solution, the eluate will turn blue, indicating the formation of copper-ammonium complex, which further indicates the existence of copper(II) inside the capsule. TEM image shows a great uniformity and narrow size distribution, which is significantly different than nanocapsules made from ammonium catalyst systems.

Under Hi-Res TEM, the diffraction pattern of the lattice can be observed. Since it has been approved not to be copper(0), there can be two possible explanations to this crystal structure. It could be the dried crystal of tetraamminecopper(II) salt, either nitrate or citrate. It could also be copper(0) reduced during the TEM imaging, since cases of electron beam induced novel metal reduction have been reported ^{36,37}. EDX mapping can also be an evidence, though for the same reason as in the case of gold(III), the signal is not very strong.

Figure 3.7 Copper(II) loaded capsules. (a-b) TEM images. (c) HRTEM image. (d) After centrifugation and re-dispersed. (e) EDX mapping of copper. Scale bar of a and b: 200 nm.

Copper(II) is especially suitable to post-loading, thanks to the ideal solubility of tetraamminecopper(II) ion. It is high enough to evenly disperse in ethanol so that they would not cause significant homogeneous nucleation. On the other hand, its high solubility difference between water and ethanol allows adequate amount of copper(II) complex to be extracted into water droplets.

3.3.3 Other loading results

Besides typical examples discussed before, a series of substances have been loaded into silica nanocapsules through pre-, peri- and post-loading processes.

3.3.3.1 Pre-loading of platinum(IV) salt

Fortunately, platinum(IV) can also be loaded through a similar pre-loading reaction as that of gold(III). As is shown in Figure 3.8, TEM images of loaded nanocapsules showed an ideal uniformity and narrow size distribution.

Figure 3.8 TEM images of platinum(IV) loaded silica nanocapsules. Scale bar: 200 nm.

3.3.3.2 Peri-loading of novel metal salts

As mentioned before. Peri-loading has a better generality and can be applied to a variety of novel metal ions. We have managed to apply this method on copper(II), silver(I) and palladium(II) salts. As is shown in Figure 3.9, the size distributions and morphologies of these capsules are not ideal. For example, comparing to post-loaded

ones, the peri-loaded capsules with copper(II) are more irregular by shape. Also, the loading amount in each capsule is not consistent. Also, in the case of silver(I), large amount of homogeneous silica is produced. This represents the shortage of peri-loading discussed before.

Figure 3.9 TEM images of peri-loaded capsules. (a) Copper(II). (b) Silver(I). (c) Palladium(II). Scale bar: 200 nm.

3.3.3.3 Post-loading of novel metal salts

Besides copper(II), silver(I), palladium(II) have been loaded into silica nanocapsules through this method (Figure 3.10). Their uniformity and size distribution are significantly better than those made from peri-loading. However, leaking of the loading substances can be occasionally observed. This may due to the good solubility of the diamminesilver(I) salt and sodium tetrachloropalladate(II) in both water and ethanol. These may unveil a potential limitation to the post-loading method.

On the other hand, if the solubility of loading substance is too low, it would also put great challenge in the post-loading process. We also tested potassium

hexachloroplatinate(IV) and the loading was considered unsuccessful (Figure 3.10 c). Its solubility in ethanol is too low thus will precipitate when added. Since little chloroplatinate(IV) ions would go into the ethanol phase, they cannot have the chance of being extracted by water droplets. Instead, they may heterogeneously nucleate on the surface of silica capsules in solid form.

Figure 3.10 TEM images of peri-loaded capsules. (a) Palladium(II). (b) Silver(I). (c) Platinum(IV). Scale bar: 100 nm.

3.3.3.4 Loading of doxorubicin hydrochloride

Doxorubicin is another representative chemotherapeutic medication for cancer treatment³⁸. We also managed to encapsulate it as its chloride salt by both peri- and post-loading method. The iconic bright orange color can be observed in the product precipitants, which indicates the presence of doxorubicin in the capsules (Figure 3.11). UV-vis spectrum of the eluate could further verify its existence. Unfortunately, due to the low concentration of doxorubicin and the low molecular weight of its component elements, the loading substance cannot be observed under TEM.

In our experiment, we also noticed that doxorubicin in the post-loaded capsules are significantly easier to wash out. We assume that during peri-loading, doxorubicin hydrochloride may have undergone an ion exchange into its citrate salt, which is supposed to be much less soluble in ethanol.

Figure 3.11 (a) TEM images of doxorubicin post-loaded capsules after 1 round of wash. (b) After centrifugation. (c) UV-Vis spectrum of eluates. Scale bar: 200 nm.

3.3.4 Novel applications of loading

Besides synthesizing nanocapsules incorporated with specific substances, loading strategies can also be used to manipulate the shape of nanostructures.

3.3.4.1 Post-loading of water and shrunken capsules

In the previous chapter, we have reported the shrunken capsule in the standard citrate salting out system. It has long been haunting our research that sometimes the exact same operation procedure results in different products. The shell of those capsules seems unable to support a spherical shape and collapsed. It was attributed to insufficiency of the silica shell thickness. Therefore, we tried to increase the amount of

sodium hydroxide, TEOS and prolonged the reaction time. However, none of them helped.

Later, we noticed that the temperature in our lab is around 25 °C at dry seasons in Singapore, yet during wet season, the temperature might drop to below 20 °C. The occurrence of shrunken capsules corresponds to the weather change. Therefore, after setting our reaction temperature at 30 °C, we have successfully eliminated this problem.

Figure 3.12 (a) Volume difference of aqueous phases when IM sodium citrate aqueous solution was mixed with equal volume of ethanol. (b) Shrunken capsule. (c) No shrunken capsule when water added after 60 min. (d-g) Water added at different time point. (d) 4 min. (e) 8 min. (f) 16 min. (g) 32 min. Scale bar: 200 nm.

During this period, we started to notice that the actual volume of the aqueous phase changes with temperature. Generally speaking, in a salting out system, if the ratio of salt, water and ethanol is constant, the volume of the aqueous phase increases with the temperature. It could be demonstrated by a prove-of-concept experiment (Figure

3.12 a). The water level in the 30 °C tube is observably lower than that in the 65 °C tube after the emulsions were force broken by centrifugation. Later, we have calculated the volume difference of aqueous phase at 21 °C and 40 °C in the standard system. The volume of aqueous at 40 °C is about 17.5% larger than that at 21 °C. The original data and calculation would be covered in later parts of this chapter.

Hence, we hypothesis that the shrinking at low temperature may be caused by the insufficiency of water. The formation of Stöber silica consumes water, since TEOS need to first undergo a hydrolysis to produce the precursor. Therefore, TEOS actually takes part in the competition of water between salt and ethanol. Silica shell cannot grow thicker is due to too much water in the droplet has been consumed. That is why adding more TEOS, sodium hydroxide or increase the reaction time would help little, because the limiting factor is water. If we increase the water added with sodium hydroxide, we could prevent the shrinking of capsules.

Therefore, it is reasonable to assume that if more water could be added, even at a low temperature, we could still prevent shrunken capsules from appearing. We define this process as post-loading of water.

As is shown in Figure 3.11 d-g, by post-loading of pure water, we managed to synthesize spherical capsules at 20~22 °C. Furthermore, we tested the influence of post-loading time when we add the water. It can be seen that if water is added too early, it would cause a bursting of capsules, which further approved that the water added indeed went into existing droplets. It could also serve as an evidence of the validity of our post-loading strategy.

3.3.4.2 Double-layer hybrid capsules

We have demonstrated that the post-loading of water could refuel the existing droplets. Theoretically, the new surface of the refueled droplets could also serve as a

template for other interfacial reactio. Based on this design, several attempts were made to grow multiple layers of silica with gaps between each other, yet none of them succeeded.

Later, we switched the material of the second layer to polydopamine (PDA) and finally managed to synthesize double-layer capsules with hybrid shells (Figure 3.13 b). The gap between the two layers can be clearly observed. The proposed mechanism is shown in Figure 3.13 a. Post-loading of water will increase the diameter of a droplet, forming a new water-ethanol interface. However, since the new boundary is still very close to the old silica shell, any silica nucleation would prefer the heterogeneous way to deposit on the old shell rather than forming a new one on the interface. However, PDA is a unique material that can deposit on almost every kind of interface ³⁹, therefore its deposition on the new water-ethanol interface was made possible.

Figure 3.13 (a) Schematic of double layer growth. (b) TEM image of double hybrid layer capsules. Scale bar: 200 nm.

We noticed that the uniformity of the capsules is not ideal. It may be due to the introduction of chloride from the dopamine salt. Sodium chloride is very insoluble in ethanol, it may compete or co-precipitate with the citrate, which interferes the subtle

phase separation process. Dopamine salt with a soluble counter ion would be tested in the future.

The application of droplet loading is not limited to loading itself. New technique of nanosynthesis could be made possible as our understanding of the droplet loading process deepens. We would expect more unique nanostructures to come inspired by droplet loading in the future.

3.4 Calculation

Calculation of the proportion of aqueous phase volume at high temperature (40°C) : aqueous phase volume at low temperature (21 °C).

For the experiment, there are–

$$m = m_{Sh} + m_{Ph} = m_{Sl} + m_{Pl} = 4 \text{ mmol} \quad (3.1)$$

$$v = v_{Sh} + v_{Ph} = v_{Sl} + v_{Pl} = 1.05 \text{ mL} \quad (3.2)$$

Where m and v refer to the total mass of sodium citrate and total volume of the system, respectively. m_{Sh} and m_{Ph} are the mass of sodium citrate in the supernatant layer and precipitant droplet at high temperature (40 °C), m_{Sl} and m_{Pl} are the corresponding values at low temperature (21 °C). v_{Sh} , v_{Ph} , v_{Sl} and v_{Pl} are the actual volume of the supernatant layer and the precipitant droplet at high and low temperature, respectively.

$$r_1 = \frac{A_{Ph}}{A_0} = \frac{c_{Ph}}{c_0} = \frac{m_{Ph}}{\frac{8}{3}} \cdot \frac{1}{c_0} \quad (3.3)$$

$$\begin{aligned} r_2 &= \frac{A_{Sh}}{A_0} = \frac{c_{Sh}}{c_0} = \frac{c_{Sh}}{4} \cdot \frac{1}{c_0} = \frac{m_{Sh}}{4V_{Sh}} \cdot \frac{1}{c_0} \\ &= \frac{m - m_{Ph}}{4V_{Sh}} \cdot \frac{1}{c_0} = \frac{v \cdot c_0 - \frac{8}{3}r_1 \cdot c_0}{4v_{Sh} \cdot c_0} = \frac{v - \frac{8}{3}r_1}{4v_{Sh}} \end{aligned} \quad (3.4)$$

$$r_3 = \frac{A_{Pl}}{A_0} = \frac{c_{Pl}}{c_0} \quad (3.5)$$

$$r_4 = \frac{A_{Sl}}{A_0} = \frac{c_{Sl}}{c_0} \quad (3.6)$$

If we see the concentration and absorption of the standard solution as 1, then r_1 indicates the absorbance proportion the high temperature precipitant sample against the absorbance of the standard at the peak around 200 nm, which would be identical to their concentration proportion. r_2 , r_3 , r_4 refer to similar proportion from the high temperature supernatant sample, low temperature precipitant and supernatant samples, respectively, against that of the standard.

Graph 3.1 UV-Vis spectrum and linear fit of the peak value against relative concentration.

r_1	r_2	r_3	r_4
0.179356	0.143478	0.14509	0.175151

$$v_{Sh} = \frac{v - \frac{8}{3}r_1}{4r_2} \quad (3.7)$$

$$v_{Sl} = \frac{v - \frac{8}{3}r_3}{4r_4} \quad (3.8)$$

$$\frac{v_{Ph}}{v_{Pl}} = \frac{v-v_{Sh}}{v-v_{Sl}} = \frac{v-\frac{v-\frac{8}{3}r_1}{4r_2}}{v-\frac{v-\frac{8}{3}r_3}{4r_4}} = \frac{[(4r_2-1)v+\frac{8}{3}r_1]r_4}{[(4r_4-1)v+\frac{8}{3}r_3]r_2} \quad (3.9)$$

The answer of equation 3.9 is the volume of precipitant layer at high temperature against the volume of precipitant layer at low temperature, AKA the proportion of the volume of aqueous phase at 40 °C against the volume of aqueous phase at 21 °C. It is 1.175 from the UV-Vis data, indicating a 17.5% volume increase.

3.5 Conclusion

We have tested three different loading strategies based on three windows of time in the process of the phase separation, droplet nucleation and growth, and encapsulation. Pre-loading aims at the phase separation step, while peri-loading targets the stage of droplet growth. Post-loading is done when droplets reach a relatively stable stage. They all have their advantages and limitations (Table 3.2). The combination of different loading strategies could apply more controllability to the system, it would be evaluated in the future.

Table 3.2 Comparison between different loading strategies. s_e refers to the solubility in ethanol. $p_{w/e}$ indicates the partition of the loading chemical between water and ethanol.

Feature		Pre-loading	Peri-loading	Post-loading
Loading generality	s_e	Very low	no specific requirement	Reasonably soluble
	$p_{w/e}$	Very high	>1, higher is better	>1, higher is better
Capsule uniformity		Good	Varies	Good
Leakage		Very little	Low	Relatively higher

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Chapter 4 Generality of nanocapsule materials

By far, aside from the hybrid double layer capsules in the previous chapter, all of the encapsulations were based on silica. In this chapter, we would like to discuss about the generality of shell materials. A variety of interfacial reactions has been applied to the system of solute induced phase separation to achieve nanoencapsulation.

4.1 Introduction

The encapsulation of droplets is based on reactions of polymerization or condensation on the interface. Following this guidance, a series of potential materials have been evaluated.

4.1.1 Polydopamine

Inspired by a nature adhesion protein Mefp-5 (*Mytilus edulis* foot protein 5), polydopamine (PDA) was introduced as a versatile novel material by Messersmith and co-workers ¹. It is biocompatible, biodegradable, simple to produce, and has the potential to be a conductive polymer. Yet its most unique feature is the ability to wet almost everything to form a hydrophilic layer (Figure 4.1). PDA can easily coat on surfaces such as Polytetrafluoroethylene (PTFE) and perfluoroalkoxy (PFA), which are generally considered as non-sticky ². The production of PDA is quite simple, just adjust a dopamine solution into mildly basic condition by a variety of bases, leave it exposed to oxygen, and it will polymerize by itself. From ammonium, to tris buffer, to sodium hydroxide and carbonate, almost all typical bases can be used to initiate its polymerization ³⁻¹⁰. Sometimes even without base an oxygen, an oxidation agent is

enough for dopamine to polymerize¹¹. What even makes PDA almost mysterious is its complicated structure, which is still not fully unraveled by scientists¹²(Figure 4.1 c).

Figure 4.1 (a) PDA coated PTFE surface and its wettability. (b) PDA coating on various materials. (c) Some structures exist in PDA material. Reprinted from ref 1, 2, 12 with permission. Copyright © 2011, John Wiley and Sons; 2007, American Association for the Advancement of Science; 2013, American Chemical Society, respectively.

PDA was soon adopted in the area of nanosynthesis. Not only can this material itself be conveniently made into nanospheres^{4,13,14}, but also can it be applied to function nanostructures thanks to its unique feature. Since PDA can grow on almost any surface, it is able to gap materials with different affinities. For example, Duan group utilized PDA as a hydrophilic coating to assist metal organic frameworks (MOFs) growth on nanoparticles⁵. Also due to its extraordinary wettability, it is by far not achieved to produce PDA Janus particles via the traditional route of coating PDA asymmetrically on other nanostructures¹⁵⁻¹⁷. However, Janus PDA@Au particles has been synthesized through a reversed method⁹, while a gold island could be asymmetrically grown on a PDA nanosphere. It is reasonable to test the potential of PDA in our phase separation system.

4.1.2 Formaldehyde resins

The famous phenol-formaldehyde resin is one of the members of this family. Aside from phenol, a series of chemicals containing phenyl and amine groups can also undergo similar polymerizations with formaldehyde, such as resorcinol¹⁸, urea¹⁹ and melamine²⁰. They are also known to form nanoparticles²¹ as well as being applied in nanoencapsulations²²⁻²⁵. More complicated structures can be synthesized through a template-coating-etching method mentioned in the previous chapter. For example, Yu group has produced a series of double layer formaldehyde resin hollow structures²⁶, some of them can have invaginated shells (Figure 4.2).

Figure 4.1 Schematic of the synthetic route of mesostructured hollow carbon spheres, invaginated, endo-invaginated and intact one, respectively. Reprinted from ref 26 with permission. Copyright © 2015, American Chemical Society.

4.1.3 Metal organic frameworks

Spoken of the stars in the world of new materials, metal organic framework (MOF) would definitely be among the most popular. MOFs are organic ligand connected metal ions or clusters which could construct into multi-dimensional networks^{27,28}. They are believed to bear the potential to innovate the area of catalyst, gas storage, molecular sieve, semiconductors, etc. MOF nanocrystals can also be produced^{29,30}. Interestingly, amorphous MOF can also be synthesized even though most of their kinds are crystals^{31,32}. Also, MOF is a promising material for interfacial reaction, since sub-

millimeter sized capsules have been fabricated through microfluidic technology³³ (Figure 4.2). Its validity at nanoscale can be expected.

Figure 4.2 Schematics of (a) the microfluidic device. (b) The reaction happened on the interface of aqueous droplet and the organic dispersant. Reprinted from ref 33 with permission. Copyright © 2011, Springer Nature.

4.1.4 Other eligible materials

Magnetic materials such as Fe_3O_4 are one of the materials we interested in. Unfortunately, our system is not suitable for high temperature reactions, hence it would be difficult to produce single crystal Fe_3O_4 directly which has a stronger magnetism per weight³⁴. Therefore, a room temperature reaction would be more promising for our project³⁵. The attempts on other oxides would also be covers in this chapter.

4.2 Material and methods

4.2.1 Material

Tetraethyl orthosilicate (TEOS), Citric acid (H_3Ct), hydroxypropyl cellulose (HPC, Average Mw ~80,000, Average Mn ~10,000), polyvinylpyrrolidone (PVP, Average Mw 40,000), phosphoric acid solution (85%, 99.99% metal trace), copper(II) nitrate ($\text{Cu}(\text{NO}_3)_2$, puriss. p.a., 99-104%), copper(II) acetate ($\text{Cu}(\text{OAc})_2$, 98%), zinc(II) nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2$, reagent grade, 98%), oleylamine (tech grade, 70%), calcium chloride (CaCl_2 , anhydrous, beads, -10 mesh, $\geq 99.99\%$ trace metals basis), barium hydroxide monohydrate ($\text{Ba}(\text{OH})_2$, 98%), trimesic acid (BTC, 95%), resorcinol

(BioXtra, $\geq 99\%$), formaldehyde solution (HCHO, ACS reagent, 37 wt. % in H₂O, contains 10-15% Methanol as stabilizer), sodium acetate anhydrous (NaOAc, 99%), iron(II) chloride anhydrous (FeCl₂, 98%), iron(III) chloride anhydrous (FeCl₃, $\geq 99\%$), iron(II) sulfate hydrous (FeSO₄, 99%), Hexamethylenetetramine (HMTA, ACS reagent, $\geq 99.0\%$), cyclohexane (anhydrous, 99.5%) were purchased from Merck (AKA Sigma-Aldrich). Ammonium solution (28%) was purchased from TCI. Concentrated sulfuric acid (H₂SO₄, 98%) was purchased from QRëC, ethanol (absolute, 'Baker analyzed') was purchased from J.T.Baker, sodium hydroxide (NaOH, pellets ACS) was purchased from VMR chemicals, Deionized water (resistance $> 18.2 \text{ M}\Omega \cdot \text{cm}^{-1}$) was used in all reactions.

4.2.2 Methods

Characterization. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 ~ 120 kV. UV-Vis spectrum data was collected on a 100 Conc (Cary) UV-Visible spectrophotometer. Elemental analysis data was collected on a Euro EA 3000 elemental analyzer (EuroVector).

Preparation of TEM samples. Procedures were the same as mentioned in previous chapters.

Preparation of 4% w/w standard hydroxypropyl cellulose solution. Procedures were the same as mentioned in previous chapters.

Preparation of PVP stock solution. Procedures were the same as mentioned in previous chapters.

Preparation of PDA samples for elemental analysis. 140 mg of dopamine hydrochloride was dissolved in 14 mL of water, followed by addition of 0.5, 5 mL of 28% ammonia solution, respectively. The reaction vial was shake for 10 s, and let stand

at room temperature for 48 h. Then PDA samples were collected by centrifugation, washed by water for 5 times, and vacuum dried for 12 hours.

Preparation of dopamine hydrochloride stock solution. 40 mg of dopamine hydrochloride was dissolved in 10 mL of ethanol. The solution was kept in 4 °C after use and should be replaced when its color starts to turn brown.

Synthesis of pure PDA capsules. 500 μ L of the dopamine hydrochloride stock solution was mixed with 250 μ L of 4% HPC solution, 242 μ L of ethanol, then 8 μ L of 500 mM citric acid ethanol solution. The solution was pre-mixed by vortex. Then 50 μ L of 5M ammonia solution was added under vortex. The mixture was let stand at room temperature, or sealed and kept in a 95 °C oven for 12 hours, respectively. Then it was washed by 500 μ L water for 2 times, and 500 μ L ethanol twice. The final precipitant was dispersed in 1000 μ L of ethanol.

Synthesis of silica capsules with 12 h incubation time. 250 μ L of 4% HPC solution, 742 μ L of ethanol, then 8 μ L of 500 mM citric acid ethanol solution was mixed. Then 50 μ L of 260 mM NaOH solution was added under vortex. The mixture was incubated for 12 hours, followed by an addition of 6 μ L TEOS. it was let stand at room temperature for 2 hours. Then it was washed by 500 μ L water for 2 times, and 500 μ L ethanol twice. The final precipitant was dispersed in 1000 μ L of ethanol.

Synthesis of gapless double-layer PDA-silica hybrid capsules. 500 μ L of the dopamine hydrochloride stock solution was mixed with 250 μ L of 4% HPC solution, 242 μ L of ethanol, then 8 μ L of 500 mM citric acid ethanol solution. The solution was pre-mixed by vortex. Then 50 μ L of 260 mM NaOH aqueous solution was added under vortex. Followed by a 1 min incubation, 6 μ L of TEOS was added. The mixture was let stand at room temperature for 30 min. Then 10 μ L of 28% ammonia solution was quickly added, followed by 5 seconds of vortex. The mixture was then kept at 30 °C for

6 hours. Then it was washed by 500 μL water for 2 times, and 500 μL ethanol twice. The final precipitant was dispersed in 1000 μL of ethanol.

Synthesis of PDA@silica hybrid capsules. The procedure was similar to that of the gapless double-layer hybrid capsules. Instead of 10 μL of 28% ammonia solution, 100 μL of 5M ammonia solution was added.

Synthesis of PDA-silica hybrid capsules. 250 μL of 4% HPC solution, 742 μL of ethanol, and 8 μL of 500 mM citric acid ethanol solution was mixed. Then 50 μL of 260 mM NaOH solution was added under vortex. The mixture was incubated for 1 min, followed by an addition of 6 μL TEOS. it was let stand at room temperature for 30 min. Then 10 μL of 200 mg/mL dopamine hydrochloride aqueous solution and 40 μL ~7M ammonia solution was either added separately or mixed before addition. The mixture was then kept at 30 $^{\circ}\text{C}$ for 6 hours. After the reaction it was washed by 500 μL water for 2 times, and 500 μL ethanol twice. The final precipitant was dispersed in 1000 μL of ethanol.

Preparation of the copper(II)-oleate. 3.8 g of sodium oleate was dissolved in a mixture of 8 mL of ethanol, 6mL of water and 14 mL of hexane. Then 0.68g CuCl_2 dihydrate was added. The mixture was refluxed at 70 $^{\circ}\text{C}$ for 4 hours. The mixture was extracted by 10 mL of hexane for 3 times, then washed with brine for 3 time. The product was evaporated and vacuum dried for 48 hours.

Synthesis of MOF capsules. 250 μL of 4% HPC solution, 430 μL of ethanol, and 320 μL of 25 mM trimesic acid ethanol solution was mixed. Then 100 μL of 246 mM NaOH solution was added under vortex. The mixture was incubated for 5 min, then 16 μL of 32 mM copper(II)-oleate ethanol solution was added. The reaction mixture was sealed and set in a 95 $^{\circ}\text{C}$ oil bath for 60 min. After cooled to room temperature, it was

centrifuged and washed with a mixture of water:ethanol = 1:4 for 5 times, followed by topping with 100 μ L of ethanol.

Synthesis of magnetic iron oxide capsules. 250 μ L of 60 mM NaOAc ethanol solution was mixed with 250 μ L of 4% HPC solution. Then 200 μ L of 37.5 mM sulfuric acid aqueous solution was added. A freshly made aqueous solution with 200 mM of FeCl_3 and 100 mM of either FeCl_2 or FeSO_4 was added to the mixture under mild stirring. After that, 100 μ L of 4M NaOH was added, either under 200 rpm, 500 rpm stirring, or under vortex. The product was collected by centrifugation and washed by water and ethanol for 2 times respectively, followed by topping up with 1000 μ L of ethanol.

Synthesis of zinc oxide nanocapsules. A mixture of 100 μ L 10 mM $\text{Zn}(\text{NO}_3)_2$ ethanol solution, 12 μ L of 500 mM sulfuric acid ethanol solution and 888 μ L of PVP stock solution was added by 100 μ L of 240 mM HMTA aqueous solution. The reaction vial was sealed and put in an oven with 65 $^\circ\text{C}$ for 24 h. The product was collected by centrifugation and washed by water and ethanol for 3 times respectively, followed by topping up with 1000 μ L of ethanol.

Synthesis of copper(II) material nanocapsules. The procedure was similar to that of zinc oxide nanocapsules. $\text{Zn}(\text{NO}_3)_2$ was replaced by $\text{Cu}(\text{NO}_3)_2$. The vial was sealed tight and put into an oven of 95 $^\circ\text{C}$ for 16 hours.

Synthesis of calcium phosphate nanocapsules. 20 μ L of 500 mM H_3PO_4 ethanol solution was added to 980 μ L of PVP stock solution under vortex. Then 100 μ L of $\sim 5\text{M}$ ammonia solution was added also under vortex. The mixture was incubated for 1 min, followed by the addition of 50 μ L 1M CaCl_2 ethanol solution under vortex. The mixture was let stand for 10 min. To isolate the capsule, 1000 μ L of water was added to the reaction mixture to reduce the viscosity, followed by a centrifugation. It was then

washed by water for 3 times and 2 times by ethanol. Finally, it was topped with 1000 μL of ethanol.

Preparation of saturated basic barium acetate solution. 34.2 mg $\text{Ba}(\text{OH})_2$ was added to 2 mL of ethanol, followed by the addition of 11 μL acetic acid. The mixture was stirred overnight at 60 $^{\circ}\text{C}$. The final solution was filtered, followed by addition of 20 μL of ethanol to prevent precipitation from evaporation.

Synthesis of polygonal barium sulfate nanocapsules. The procedure is similar to that of the calcium phosphate nanocapsules. 30 L of H_2SO_4 ethanol solution was introduced instead of H_3PO_4 . Instead of CaCl_2 solution, 500 μL of saturated barium acetate solution was added. The precipitant was washed with 1M nitric acid before washing with water and ethanol to demonstrate the material of the capsule.

4.3 Result and discussion

4.3.1 PDA as capsule material

In the previous chapter, we have already previewed hybrid double-layer capsules with PDA as one of their ingredients. Now we want to focus on both pure PDA and hybrid material as the capsule's shell.

4.3.1.1 PDA alone as capsule shell

Even though the polymerization of PDA generally requires a basic condition, some tuning may still be needed for our standard citrate system. At a pH higher than 11, PDA precursors incline to stay in the solution instead of precipitating out ⁶. On the other hand, simply reducing the amount of sodium hydroxide to decrease the basicity would also bring problems. It was expected that ion doping may play a part in the condensation of PDA according to our data (Table 4.1). The nitrogen to carbon ratio in PDA is higher than the monomer, which does not correspond to the proposed PDA substructure variations (Figure 4.1 c), since there is no loss of carbon in its various

polymerization patterns. Hence, the doping of ammonium would be a reasonable explanation. Moreover, when ammonium amount in a reaction was increased by 9 times, the N/C ratio further increased, suggesting that more ammonium ion may have been doped in.

Table 4.I Elemental analysis of dopamine hydrochloride and different PDA samples.

Sample Name		(N) %	(C) %	(H) %	N/C
Dopamine HCl	Experimental	7.683	51.619	7.033	0.149
	Theoretical	7.382	50.664	6.378	0.146
PDA with 0.5/140 (v/v) 28% ammonia		9.095	56.096	5.096	0.162
PDA with 5/140 (v/v) 28% ammonia		10.502	51.802	4.938	0.203

Therefore, even if the amount of sodium hydroxide can be controlled to reach a proper pH, the lack of ions would lead to an insufficient condensation, which is approved by our experiments. This also explains why in the last chapter, we chose to add ammonia solution instead of plain water to synthesize the double layered capsule, because a certain amount of cation is crucial in PDA condensation. To compensate this issue, we decided to replace NaOH with ammonia solution.

As is shown in Figure 4.3, hollow PDA capsules can be observed. However, the size distribution is not ideal. Also, the diameters are close to 1 μm , which is larger than what we need. We assume the slow polymerization and condensation rate would be the culprit. As mentioned before, droplets size monodispersity suffers from the merging/collection process. We chose Stöber silica because its fast reaction speed so that the influence of merging/collection could be minimized. As a comparison, silica capsules were made by incubating for 12 hours before addition of TEOS (Figure 4.3 c), which is close to the time scale of PDA condensation. As we can see, its size distribution is no narrower than PDA capsules', and capsules larger than 1 μm is commonly seen. We

also increased the temperature to 95 °C to enhance the reaction speed. However, no significant improvement can be observed. This indicates a limitation of pure PDA capsules, since their reaction speed is not ideal and lacks tuneability, its uniformity has to be compromised.

Figure 4.3 TEM images of (a) PDA capsule. (b) PDA capsule from 95 °C reaction temperature. (c) Silica capsule with 12 h incubation before addition of TEOS. Scale bar: 1000 nm.

4.3.1.2 PDA silica hybrid capsules

Naturally, it could expect that the gap between the layers can be tuned. If small enough, it could practically become one. According to the mechanism we proposed in the previous chapter, the second layer growth is due to an overloading of water to the droplet. When the droplet grew larger than it has been before, the PDA deposition on the interface between this new larger water droplet and ethanol became the second layer. Therefore, if we could reduce the amount water introduced, the capsule can be “gapless”. This assumption was further approved by the experiment result (Figure 4.4 b). Furthermore, increasing the amount of water added would also remove the gap (Figure 4.4 d). It may seem paradoxical, however, in the case of less added water, the inner surface of the outer layer was rugged, while in the case of more added water, it was the outer surface that is rugged. The latter was caused by the breaking of the

emulsion, AKA the merging of two phases (Figure 4.4 f). Since too much water was added to the water-ethanol system, the mixture acquired the ability to dissolve citrate salts as a whole, which nullified the phase separation process.

Figure 4.4 Scheme of PDA layer growth on a hybrid system. (a) Relationship between growth pattern and the amount of water added. (b) Gapless hybrid shell. (c) Double layer. (d) PDA@silica shell. (e) Schematic of PDA growth on droplet interface. (f) Schematic of PDA growth when phase merged. Scale bar: 50 nm.

Unfortunately, the gap cannot be made wider. To increase the distance between two layers, a huge amount of water would be added, since the increase of volume is the third power of the distance. It will certainly cause the merging of two phases.

Figure 4.5 (a-b) TEM images of PDA-silica hybrid capsule by adding dopamine from aqueous solution. (c) Schematic of PDA growth pattern. Scale bar: 100 nm.

PDA silica hybrid capsules can also be made by loading water droplet by aqueous solution of dopamine and ammonia (Figure 4.5). Comparing to the double layer, in which the dopamine was dissolved in ethanol, this method never yields double layer capsules. Either added separately or pre-mixed, it would always result in a single layer capsule. We assume that in the case that dopamine dissolved in ethanol, the PDA precursors also mostly stay in the ethanol phase, so it could come from the outside of the droplet and finally depositing on the droplet interface (Figure 4.4 e). In the case of PDA in water, it starts to polymerize in aqueous phase, and therefore deposit within the aqueous phase too, AKA inside the larger droplet (Figure 4.5 c), which would never form double layer structures. Fortunately, this method could produce nanocapsules with a much better monodispersity, thanks to the silica pre-encapsulated droplets as templates.

4.3.2 Formaldehyde resin as capsule shell

Figure 4.6 (a-d) TEM images of RFR capsules made from phase separation system. (e) invaginated capsule reported in literature. Reprinted from ref 26 with permission. Copyright © 2015, American Chemical Society. Scale bar: 200 nm.

Resorcinol formaldehyde resin (RFR) was chosen as the shell material. According to the literature, ammonia is crucial in the polymerization of RFR, it cannot be replaced by sodium hydroxide²¹. Therefore, a reaction similar to that of the ammonia citrate phase separation system was carried out, replacing TEOS with resorcinol and formaldehyde solutions. As expected, large hollow spheres with a wide size distribution has been yield (Figure 4.6). This is due to a similar reason of the pure PDA capsule. The polymerization of RFR is slow so that merging/collection of droplets will ruin the uniformity of capsules. Interestingly, when reaction temperature increases, invaginated capsules can be observed. Similar structure of RFR hollow spheres has been reported²⁶(Figure 4.6 e). However, in that case it was induced by etching and was explained by the thickness of the shell, even though in both cases the difference in shell thickness in not obvious. We also evaluated the possibility of water consuming in droplets, similar to our shrunk silica capsules, but eventually ruled it out. Firstly, the polymerization of RFR releases water instead of consuming it. Secondly, the invaginated ones are also

larger ones, which is the opposite to our silica system, in which the smaller ones tend to become shrunken. The exact reason for this structure is still unknown and requires further research.

4.3.3 MOF as shell material

The difficulty in this case is how to separate the metal ion and the ligands in two different phases, so that they could condense when they meet on the interface. The polarity difference between ethanol and water is small, and the ethanol phase also contains water which makes it even more polar. However, if we choose less polar alcohols, it would be too similar to a traditional water-in-oil emulsion system³³. Finally, we circumvent this problem by synthesizing a copper(II)-oleate complex. It is highly hydrophobic and incompatible with water. Hence, we would be able to keep the copper source in the ethanol phase.

Figure 4.7 TEM images of MOF capsules from phase separation system. Scale bar: 200 nm.

Also, we decided to choose trimesic acid (benzene-1,3,5-tricarboxylic acid, BTC) as the separation initiator instead of directly adopting the citrate system. BTC is a micromolecule anion with three charges, thus is supposed to be a kosmotrope capable of inducing phase separation by its own. Its capability has been tested in a Stöber system

and proved valid. Then, we replaced TEOS with the copper(II)-oleate complex, and heat the mixture to 95 °C for 30 minutes. After centrifugation, precipitant with a bright blue color was collected. Under TEM, its hollow nanostructure can be observed.

Unfortunately, we have not synthesized adequate samples for powder XRD analysis. Further characterization would be carried out in the future.

4.3.4 Magnetic iron oxides as shell material

In order to accommodate the oxides of iron, our standard phase separation system needs to be overhauled. Since both iron(II) and iron (III) would form oxides instantly when meet with OH group, they must be kept away until the last moment of the reaction. Therefore, a phase separation in acidic condition is necessary. We chose to mix an ethanol solution of sodium acetate and aqueous sulfuric acid to produce nanosized droplets in acidic condition. After that, an aqueous mixture of Fe(II) and Fe(III) salt was introduced. Under mild stirring, concentrated sodium hydroxide aqueous solution was then added. As is shown in Figure 4.8, hollow spherical nanostructures can be seen in the TEM images. However, though hollow, there are also some torn and deformed capsules. By increasing the stirring speed, more irregular structures emerge. This could suggest that the deformity may be caused by the shear stress in the solution.

The formation of iron oxides is almost instant; thus, the condensation happens when the mixing is happening. Therefore, the oxide shell will pick up the deformity of the droplets and finally yield in irregular shaped capsules. The magnetism is also testified by an attraction experiment. As is shown in Figure 4.8 g, though slow, capsules can be attracted by strong magnetic fields.

An interesting fact is, Iron cations have to be introduced in aqueous phase. If in their ethanol solution, no hollow structure can be produced. We assume that even

though extraction could happen, solutes tend to stay with its initial solvent. Therefore, if added as an ethanol solution, iron cations may stay in ethanol phase and react with sodium hydroxide outside in the ethanol phase, producing only solid chunks. On the other hand, the anions of the iron salts are not crucial. Both chloride and sulfate work the similar way.

Figure 4.8 TEM images (a) Iron oxides capsules made from 200 rpm stirring. (b) iron oxides capsules made from 500 rpm stirring. (c-e) Spherical capsules and deformed capsules. (f) Highly irregular capsule made from vortex mixing. (g) Capsules attracted by magnetic field. Scale bar: 200 nm.

4.3.5 Other metal oxides as shell material

We want to push the limit of the phase separation system a step further. Previous experiment has demonstrated the weakness of the instant formation of oxides. Therefore, if we could manipulate the reaction speed like we have done in separating

the salt into an acid and a base, then the tunability of oxide capsules could be greatly enhanced. In a work of Sun and co-workers³⁶, Hexamethylenetetramine (HMTA) was creatively introduced as a precursor of base to generate a variety of metal oxides. When heated, it will gradually degrade into ammonia, which adjust the pH of the system at a controllable speed. We expect that HMTA could adjust the formation speed of metal oxide or hydroxide to yield capsules with ideal uniformities.

Figure 4.9 TEM images (a-b) Copper(II) material capsules made from HMTA degradation induced phase separation. (d-e) Zinc(II) oxide material capsules made from HMTA degradation induced phase separation. Scale bar: 200 nm.

As is shown in Figure 4.9, Both zinc(II) and copper(II) can produce structures that resemble hollow sphere to a certain extent. They are more like nanocages instead of capsules with continuous shells. In the case of copper(II), the precipitant was blue after centrifugation, suggesting a hydroxide or complex was formed. The structure may due to the unideal wettability of the shell materials between ethanol and the aqueous salt solution. Usually shell is started from several nucleation islands on the interface and connect together. If the wettability is not ideal the islands may not be able to merge into an entire piece, and form a cage-like structure instead. The wettability can be

further tuned by changing the anions and surfactants, which would be carried out in the future.

4.3.6 Insoluble salts as shell material

Figure 4.10 TEM images (a-b) Capsules made from calcium phosphates. (d-e) Polygonal capsules made from barium sulfate. Scale bar: 100 nm.

Calcium phosphate is a salt which commonly exists in bones and teeth of vertebrates. Therefore, it could serve as a potential biocompatible capsule material. The reaction is quite simple. Droplets were generated by mixing a sodium hydroxide aqueous solution and a phosphoric acid ethanol solution. Then a calcium chloride ethanol solution was added. The calcium(II) would reach the water droplet containing phosphate from outside, forming trisodium phosphate capsules on the interfaces (Figure 4.10). As the TEM image suggests, hollow spheres can be observed. The membrane-like structures may be the reminiscent of broken huge capsules. A similar reaction can also be used to produce barium sulfate capsules. In this case, a barium acetate ethanol solution was introduced after droplets have been generated. The products could withstand the erosion of nitric acid, suggesting the formation of barium sulfate. Like

silica capsules in high concentration of sulfate, the BaSO₄ capsules also show a polygonal shape. The reaction condition of these salts can still be fine-tuned in the future.

4.4 Conclusion

A variety of shell materials have been applied to the phase separation system, from polymers to metal oxides to salts. In most cases, reactions resulted in structures that at least resemble hollow capsules. It suggests that the trend of interfacial coating on nanoscale droplets is very strong, hence approved the generality of this system. However, reaction conditions for specific materials have to be carefully tuned, since unlike TEOS, other precursors may interfere the droplet nucleation and growth process, which may result in irregular shaped capsules. On the other hand, unlike silica which wets the aqueous droplet surface very well, surface energies of other material may not be ideal so that a complete coverage may not be easily achieved. Also, condensation speed of the shell material may influence the shape and size distribution of capsules.

We assume the concept of hybrid capsule could serve as a break through. Like in the PDA-silica system, hybrid capsules could reach a narrow size distribution from a one-pot reaction. Silica could serve as a skeleton of the capsule since it is an ideal material for droplet size control. Other material can then be deposit onto the silica framework to functionalize the entire capsule. Since silica is permeable and its pore size is larger than that of MOFs and zeolites, it would put little interference on the permeability of the functional material. We would expect hybrid capsule to be applied to more systems in the future.

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Chapter 5 Droplet symmetry breaking and bottle-like structures

When talking about asymmetric hollow structures, we emphasize the asymmetry of the structure itself. For example, an etched hollow silica particle with different surface functionalities is not classified, since its shape is almost perfectly symmetrical. Complicated asymmetric hollow structures are scarce to find in the blooming field of nanosynthesis. Yet it is a necessary step if we want to design nanostructures at our will. Droplets may play a crucial role in completing this task.

5.1 Introduction

5.1.1 Basic asymmetric hollow structures

There are two methods to synthesize such nanostructures. Through etching of Janus nanoparticles, or manipulation of nanoscale droplets.

5.1.1.1 Etching of solid Janus nanoparticles

The traditional way of making symmetric hollow structures is to remove the core of solid nanoparticles. Therefore, it would be intuitive to promote this concept to asymmetric solid structures, AKA Janus particles. Not all kinds of Janus particle fit this role. For example, a Janus particle with distinct surface modification on either side¹⁻³ is not likely to generate an asymmetric structure after etching. Particles of which the asymmetry comes from Janus encapsulation is ready for such etching, so that hollow shells can be released.

Figure 5.1 (a) Schematic of the core removal of a Janus encapsulated particle to release asymmetric hollow shell. (b) TEM images of Janus particles before and after the etching of core. Scale bar: 50 nm. Reprinted from ref 4 with permission. Copyright © 2009, American Chemical Society.

The work of Tan and co-workers could serve as a demonstration of this concept⁴ (Figure 5.1). The surface of gold nanoparticles were firstly separated into two sides, one hydrophilic and the other hydrophobic, through the competition of ligands. Then a layer of polymer was coated solely on the hydrophobic side and the hydrophilic side was left untouched, due to the electrostatic repulsion. Finally, the gold core was removed, leaving only the shell with an opening on where it used to be the hydrophilic side of the gold. If it is possible to selectively etch the core material without damaging the shell material, such Janus particles⁵⁻⁹ could be eligible for this method.

5.1.1.2 Manipulation of droplets

A progressive work has been done by Xia group to fabricate asymmetric hollow nanostructures via the manipulation of droplets at the first time¹⁰. They started with adding toluene into a water dispersion of polystyrene (PS) particles. Due to the immiscibility of both solvents, toluene would be absorbed by PS particles and make them swollen. The whole system was frozen by liquid nitrogen, which will cause the toluene density to increase significantly during solidification. This would leave a cavity at the center of each particle since the total volume of toluene shrank started from its surface. After the evaporation of toluene below 0 °C, hollow PS particles each with an opening were synthesized (Figure 5.2). The size of opening can be tuned by replacing the toluene solvent or altering the evaporation speed. They also managed to directly

synthesize bowl-like poly(methyl methacrylate) (PMMA) hollow structures from emulsion droplets. This concept opened our mind about the possibility of droplet control in nanosynthesis.

Figure 5.2 (a) Schematic of formation of hollow PS particles with openings. (b-d) PS hollow particles with different sized openings. (e) PMMA bowl-like structures made from emulsion droplets. Scale bar: 500 nm. Reprinted from ref 10 with permission. Copyright © 2005, Springer Nature.

5.1.1.3 Limitation of solid etching method

Etching of Janus particles may seem simple and intuitive, but by now it is only suitable for one-step reactions. If further fabrication is needed, which may be necessary for engineering more complicated structures, this method could run into obstacles. For example, a second step growth on a Janus encapsulated particle could result in a detach of the core ¹¹. Instead of growing on the exposed gold surface of a Janus particle, the original gold core was pulled out by the secondary grown gold particle due to the stress between interfaces (Figure 5.3), leaving further growth of the capsule shell impossible. Also, etching reagent of the core may also influence the shell ⁴, causing collapse or shrinking which may ruin its asymmetry.

This is where the droplet method shines. The fluidity of liquid could minimize the stress accumulated between interfaces, which opens a window for further growth.

Moreover, the shape shifting nature of liquid can be used as a tool for manipulation of the structure, should we be able to control it.

Figure 5.3 (a) Schematic of gold on a Janus particle being pulled out by a second growth of gold. (b) TEM image of Janus particles before second round growth of gold. (c) TEM image after the second round growth of gold. Reprinted from ref 11 with permission. Copyright © 2009, American Chemical Society.

5.1.2 Bottle-like asymmetric structures

Bottle-like structure is a typical complicated asymmetric hollow structure, since the formation of bottleneck is beyond the reach of common control methods in nanosynthesis. By far, almost all such structures are achieved through manipulation of droplets.

5.1.2.1 Droplet squeezing

Wang group reported a silica nanobottle structure from the co-polymerization of two different siloxanes ¹². Aqueous droplets in an emulsion with 1-pentanol was chosen as the templated. According to their explanation, when the silica semi-shell covering part of the droplet grown thicker, it will squeeze the droplet towards the opening, leaving an asymmetric shape (Figure 5.4). Therefore, further growth would lead to the formation of a bottleneck. Similar squeezing mechanism has also been

suggested in the work of Chen and co-workers¹³ to synthesize a carbonaceous flask-like structure from ribose precursor. In their method, the entire process was completed through hydrothermal reaction in an autoclave. During the reaction, the shell material would deposition from the inner surface of the shell, thus the growth of the shell would squeeze the liquid out, forming a cavity which could be the foundation of the bottleneck.

Figure 5.4 (a-c) Schematic of the formation of bottle-neck. CPTMS: (3-chloropropyl) trimethoxysilane; TBOS: tetrabutyl orthosilicate. (d, e) SEM picture. (f) TEM picture. Reprinted from ref 12 with permission. Copyright © 2016, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

5.1.2.2 Solvent extraction

In the work of Han and co-workers¹⁵, the increase of droplet volume caused by extraction could also induce the growth of bottle-neck on fullerene nanocapsules. A few drops of C₆₀ m-xylene solution was added into dimethylformamide (DMF) under vortex, followed by an addition of isopropyl alcohol (IPA). Since m-xylene and DMF are merely partially miscible, C₆₀ enriched xylene droplets would form during this process. Also due to the low solubility of C₆₀ in DMF, it would deposit on the interface between xylene droplet and DMF, forming a capsule shell. The addition of IPA further dropped the miscibility of xylene in DMF, since it is miscible with DMF but incompatible with

xylene. At the same time, the already partially covered xylene C₆₀ droplets would extract excessive xylene and protrude from the opening, making the formation of bottleneck possible. Its length can be controlled by reaction time.

The method could provide more degrees of freedom of asymmetry tuning, since it is not limited by the amount of liquid inside the droplet but could response to the changes of the entire dispersion system. In other words, in the squeezing method, droplets evolve by their own; while in the extraction system, manipulation through the entire reaction process is made possible. Hence, it would be a promising strategy to synthesize nanostructures with higher complexities.

Figure 5.5 (a) Schematic of the growth of C₆₀ nanobottle. (b-e) Controlling of the length of bottlenecks.

5.2 Material and methods

5.2.1 Material

Tetrabutyl orthosilicate (TBOS), polyvinylpyrrolidone (PVP, Average Mw 40,000), 1-pentanol, ethyltriethoxysilane (ETES, 99%), were purchased from Merck (AKA Sigma-Aldrich). Sodium citrate (dihydrate, 99%) and (3-chloropropyl) trimethoxysilan (CPTMS, >97% (GC)) were purchased from Alfa Aesar. Ammonium

solution (28%) was purchased from TCI. trimethoxy(pentyl)silane (PTMS) and 4-(Chloromethyl)-phenyltrimethoxysilane (CPhTMS) were purchased from ACBR Gute Chemie. Ammonium solution (28%) was purchased from TCI. Ethanol (absolute, 'Baker analyzed') was purchased from J.T.Baker. Deionized water (resistance > 18.2 M Ω ·cm⁻¹) was used in all reactions.

5.2.2 Methods

Characterization. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 ~ 120 kV.

Preparation of TEM samples. Procedures were the same as mentioned in the previous chapter.

Preparation of PVP 1-pentanol solution. 1.0 g of PVP was added to 10 mL of 1-pentanol. The mixture was stirred at 65 °C for an hour and cooled to room temperature before use.

Synthesize of silica nanobottles from ETES and TBOS. 150 μ L of ethanol and 84 μ L of 0.2M sodium citrate in concentrated ammonia solution was added to the above PVP 1-pentanol solution under vortex. Then 15 μ L TBOS and 1.5 μ L ETES was added, followed by a vortex of 10 s. The mixture was let stand for 2.5 h at room temperature. It was then centrifuged and washed twice with water and ethanol, respectively.

Synthesize of silica nanobottles from PTMS and TBOS. The procedure was similar to that from the ETES and TBOS. ETES was replaced by PTMS.

Synthesize of silica nanobottles from CPTMS and TBOS. The procedure was similar to that from the ETES and TBOS. ETES was replaced by CPTMS.

Synthesize of silica nanobottles from CPhTMS and TBOS. The procedure was similar to that from the ETES and TBOS. ETES was replaced by CPhTMS.

Quenching of silica nanobottles from CPhTMS and TBOS. The procedure was similar to that from the CPhTMS and TBOS. Excessive anhydrous cobalt(II) chloride was added to the reaction mixture until there was no pink color left, after 30, 60, 90, 120 min, respectively. To collect the product, a centrifugation at 500 G for 3 min was conducted to remove most of the CoCl_2 . The supernatant was then centrifuged and washed by water for 3 times, followed by twice of washing by ethanol.

Water post-loading of silica nanobottles from CPhTMS and TBOS. The procedure was similar to that from the CPhTMS and TBOS. After 60, 90 and 120 min, 84 μL of water was added to the reaction mixture under mild stirring. The mixture was then let stand for the rest of the time until a total 2.5h was reached.

5.3 Result and discussion

Comparing to the fullerene nanobottle in Han's work ¹⁵, we started to suspect that the reaction condition of silica nanobottles from Wang group ¹² may actually be a salting out system. Firstly, in that work, around 5.5% salt solution was added to 1-pentanol to form an emulsion. However, the solubility of water in 1-pentanol would be higher than that value according to our experience, which indicates that without salt, there should only be a homogeneous water-pentanol mixture instead of an emulsion. Actually, the solubility of water in 1-octanol, which is much less hydrophilic than 1-pentanol, is around 5% at room temperature ¹⁴. Therefore, there is no way that 5.5% water could saturate 1-pentanol without the help of salt. It would be reasonable to assume that the formation of emulsion in their reported situation would be a result of a salting out process.

If our assumption is right, then we could expect other synthetic tools in the solute induce phase separation system could also affect the growth of nanobottles, to yield some unique asymmetric hollow structures. For example, water post-loading in

our standard system has led to a double layer structure. It could also bring more possibilities to the nanobottle system.

Yet firstly, we started from screening of the siloxanes.

5.3.1 Influence of siloxanes

Based on the extrapolation of the mechanism provided by the literature, aside from a 4-branched siloxane like TEOS or Tetrabutyl orthosilicate (TBOS), a 3-branched siloxane is necessary, so that it could serve as a surfactant. The 3-branch indicates that the silicon atom is connected to only 3 alkoxy group, with the last chemical bond connect to a carbon from a hydrophobic group (Figure 5.6).

Following this rule, ethyltriethoxysilane (ETES), trimethoxy(pentyl)silane (PTMS), 4-(Chloromethyl)-phenyltrimethoxysilane (CPhTMS) and the one in the literature – (3-chloropropyl) trimethoxysilan (CPTMS) were tested along with TBOS as the siloxane combinations. As is shown in Figure 5.6, all of them showed the ability to break the symmetry of silica capsules. ETES leads to a jellyfish-like structure with a huge opening, indicating a relatively strong trend towards asymmetry; yet incapable to forming bottlenecks. Both PTMS and CPTMS showed the ability to form typical bottles, while in the case of PTMS, a significant thicker bottom can be observed, this may serve as an evidence of the silica growth sequence, which would be discussed later together with other evidences. CPhTMS provided the most interesting result, as bottles with extraordinarily long neck. It drew our attention because according to the squeezing hypothesis, there must be huge amount of water being squeezed out to form such long necks, which may exceed the total water volume in a droplet, which was indicated by size of the bottle belly. It is reasonable to assume that the formation of bottle neck in all the cases follows a similar rule. Therefore, the result in the CPhTMS experiment

could weaken the validity of the squeezing hypothesis. Hence, we want to focus our research on CPhTMS-TBOS combination.

By the way, experiments with only CPhTMS and TBOS, respectively, were also tested, to verify that the nanobottle is not a result of each of the siloxanes alone (Figure 5.6).

Figure 5.6 TEM images of nanobottles made from different siloxane pairs.

5.3.2 Influence of reaction time

To capture intermediates towards the long-necked bottle, the reactions were quenched at different time. Since the hydrolysis of siloxanes, which is the crucial step in silica polymerization, is governed by water. The quenching was achieved by adding large amount of desiccant. As is shown in Figure 5.7, the silica structure evolved from hemispheres, to bowls, to spheres with a whole, then to a bottle. It could be assumed that the growth of silica from the siloxane combination started from a specific point on the surface of a spherical droplet. Then it grew along the droplet surface as well as perpendicular to it, which made the bottom thicker. When most of the droplet surface was covered, the bottleneck started to form, and grew longer. This phenomenon corresponds to what we observed in extraction systems.

Figure 5.7 TEM images of (a) Quenched after 30 min. (b) Quenched after 60 min. (c) Quenched after 90 min. (d) Quenched after 120 min. (e) Schematic of the nanobottle growth process.

5.3.3 Post-loading of water

Inspired by the double layer growth induced by post loading of water, we also want to apply a similar methodology to the nanobottle system. Also, since the time dependence of bottle neck growth has been revealed, we would expect that the time point of post-loading would greatly affect the final products. As is shown in Figure 5.8,

addition of water would induce the formation of a second belly on the long bottle neck. The earlier the addition, the closer the two bellies, which is corresponded to the result of the quenching experiment. The formation of the second belly instead of a double-layer or thicker layer would suggest that the extraction mainly happens on the opening of the bottleneck. This assumption is reasonable since even though silica shells are permeable, its hinderance would definitely higher than that of the opening, hence water condensation on the latter would have a lower energy barrier than that on the former. Therefore, extraction on the bottleneck opening would be kinetically preferred.

Figure 5.8 TEM image and schematic of (a) Water post-loading after 60 min. (b) Water post-loading after 90 min. (c) Water post-loading after 120 min. (d) Schematic of the formation of bottleneck on the first belly. (e) Schematic of the sealing of the second belly.

Interestingly, even though the belly size between different bottles varies, the size difference between the two bellies on a specific bottle is small. We assume the relationship between the size of opening and belly may play an important role. As we can see, the diameter of the bottleneck, AKA the opening size is proportional to the diameter of the belly. If extraction mainly happen on the opening, larger opening would

secure a higher extraction rate, and finally more water extracted when competing with other bottles. Therefore, a larger initial droplet leads to a larger first belly, which results in a larger opening on the bottleneck and finally results in a larger second belly.

Another phenomenon that drew our attention is the dumbbell-like hollow structures when water was added at a very late stage. Both of the bellies have but one opening. According to the growth pattern of other double-belly bottles in this series, there should be another opening on the second belly to form another bottleneck, regardless of its length. Moreover, it is difficult to distinguish which one is the first belly and which one is the second. We assume the one with a thicker bottom to be the first one, since a similar trend can be observed in other double-belly bottles as well as in the quenching experiment. Furthermore, the thickness of the first belly in the dumbbell-like structure is significantly higher than in the other experiments. We assume that in the late stage of silica growth, siloxane precursors have undergone adequate hydrolysis, the concentration of monomers have reached a high level. Therefore, the deposition rate of silica is increasing through time. This could be proved by the bottle neck growing rate. From 60 min to 90 min, the distance between the two bellies only increased a little bit, while from 90 min to 120 min, the distance increased drastically, indicating a bursting condensation of silica. This high concentration monomer allows the silica deposition to overcome the energy barrier and eliminate the kinetic advantage on the bottleneck opening. The deposition of silica all over the structure would become predominant. Therefore, the first belly, as the oldest silica surface, would accumulate the most material condensed.

The absence of the second opening could be attributed to the low relative deformation rate of the water template at the final stage. During the growth of the bottle neck on the first belly, the silica deposition rate is low. It is no match for the drastic

shape change of its template – the bulging water on the narrow opening. In other words, the silica deposition rate against the shape changing of its template is too low (Figure 5.8 d). Therefore, it would be impossible to seal the opening as the template is not stable. However, in the case of the second belly, at the final stage the silica deposition rate is much higher, so that it could overcome the shape changing of template and finally seal the entire sphere (Figure 5.8 e).

5.4 Conclusion

We have further studied the symmetry breaking from the fluidity of droplets. In contrast to the traditional squeezing out hypothesis, a new extraction hypothesis has been raised. It could explain some phenomena which is inexplicable by previous theories. Furthermore, the concept of post-loading has been applied to this system, leading to a series of unique nanostructures. It would serve as a promising tool for complicated nanosynthesis in the future.

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Chapter 6 Hypothesis of droplet nucleation and growth in solute induce phase separation system

Further experimentations have been conducted to verify our guess on the actual process of droplet nucleation and growth.

6.1 Introduction

As mentioned in previous chapters, two hypotheses have been proposed in order to theorize the nucleation and growth process in a solute induced phase separation system. One is the “liquid particle (LP)” hypothesis based on adding merging/collection in the well-known solid particle nucleation and growth theory ¹. The other can be described as “condensation and extraction (CE)” where water heterogeneously nucleate on solid solute nuclei or clusters and dissolve them into droplets. Besides merging, the aqueous droplets grow through an extraction of water from the water-ethanol mixture.

Our previous observation of polygonal capsules may provide a support of the CE hypothesis. We would like to further study this phenomenon.

6.2 Material and methods

6.2.1 Material

Tetraethyl orthosilicate (TEOS), Tetramethyl orthosilicate (TMOS), Tetrabutyl orthosilicate (TBOS), polyvinylpyrrolidone (PVP, Average Mw 40,000), 1-pentanol, ethyltriethoxysilane (ETES, 99%), were purchased from Merck (AKA Sigma-Aldrich). Ammonium solution (28%) was purchased from TCI. Concentrated sulfuric acid (H₂SO₄, 98%) was purchased from QRëC, ethanol (absolute, ‘Baker analyzed’) was

purchased from J.T.Baker, sodium hydroxide (NaOH, pellets ACS) was purchased from VMR chemicals, Deionized water (resistance $> 18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) was used in all reactions.

6.2.2 Methods

Characterization. Transmission electron microscopy (TEM) images were collected on a JEM-1400 (JEOL) operated at 100 ~ 120 kV.

Preparation of TEM samples. Procedures were the same as mentioned in the previous chapter.

Preparation of PVP stock solution. Procedures were the same as mentioned in the previous chapter.

Synthesis of silica nanocapsules from sulfate induced phase separation system from various siloxanes with 1-min incubation. 1M sulfuric acid ethanol solution was made by adding 55 μL of 98% H_2SO_4 into 945 μL of ethanol. 6 μL of the above solution was mixed with 994 μL of PVP stock solution. Under vortex, 50 μL of $\sim 10\text{M}$ ammonia solution was added, followed by a 1-minute incubation, 1.5 μL of TMOS, TEOS and TBOS was added, respectively. It was then put into a 30 $^\circ\text{C}$ oven for 2 hours. The product was washed by water for three times, and by ethanol for two time. Then it was topped with 1 mL of ethanol.

Synthesis of silica nanocapsules from sulfate induced phase separation system from TEOS with 1-hour incubation. The procedure was similar to the previous experiment, except the incubation time was increased to 1 hour.

Synthesis of silica nanocapsules from sulfate induced phase separation system from various siloxanes with no incubation. The procedure was similar to the previous experiment, except the siloxanes was added first in each experiment, followed by a 1-minute incubation. Then 50 μL of $\sim 10\text{M}$ ammonia solution was added.

6.3 Result and discussion

Figure 6.I (a) Plot of the water "monomer" concentration against time. (b) Schematic of the liquid particle hypothesis. (c) Schematic of the condensation and extraction hypothesis. Modified from ref 1. Copyright © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Firstly, we would like to theoretically validate the CE hypothesis based on the traditional nucleation and growth theory¹⁻³. A prerequisite is that water prefer to nucleate on the salt surface than do it homogeneously. It has been approved that the nucleation of water on a surface of another material from gas phase is tremendously easier than homogeneous nucleation⁴⁻⁶. On the other hand, droplet nucleation on solid hydrophilic nanoparticles has been proposed in literature⁷. It would be reasonable to assume that the energy barrier of water nucleation on salt nuclei is lower than that of the homogeneous one. On the other hand, we would assume that the heterogeneous

nucleation of water on water itself is easier than on other material. Hence, the energy barrier of water nucleation on salt would be in between that of its homogeneous and heterogeneous nucleation. Therefore, the schematic comparing the concept of both LP and CE hypotheses can be described by Figure 6.1.

Based on the concept of CE hypothesis, the intermediates of the evolving from salt nuclei to liquid droplet is to be expected. We decided to capture such intermediates by silica encapsulation.

6.3.1 Capsule shape and its time dependence

We chose sulfate as the salt in this experiment due the uniform polygonal shape of its capsules. In order to preserve the shape as much as possible, a high concentration of PVP thickener was introduced instead of 1% HPC in most systems. The TEM images of the capsule after encapsulation was expected to represent the evolving of salt and droplets.

We separate the standard operation into 4 crucial time points – ① the introduction of sulfuric acid, ② addition of ammonia solution, ③ the addition of siloxane (TEOS), ④ the finish of the reaction. In the standard procedure sequence, we introduced sulfuric acid into the ethanol phase at the start, then added the ammonia in aqueous phase, added the TEOS after a 1-minute incubation, and let it stand for 2h. Hence it would be ①-1 min-②-1 min-③120 min-④, as is shown in Figure 6.2 a. It would be our snapshot **1**. In this process, the droplet evolving would happen during the 1 min incubation between ② and ③, and the earlier stage during the 120 min between ③ and ④ when siloxane is hydrolyzing. Therefore, in order to capture intermediates, we could alter the evolving time from changing the incubation time and choosing siloxanes with different hydrolysis rate. The hydrolysis of TMOS is faster than that of TEOS ⁸, while for TBOS it is the slowest of the three ⁹.

We tried to introduce TEOS before the addition of ammonia and water at our snapshot 2 (Figure 6.2 b). Even though TEOS could hydrolysis at acidic condition ¹⁰, the lack of water in the sulfuric acid ethanol solution would render it impossible. Therefore, the 1-minute incubation between ② and ③ was removed. As a result, the TEM image of silica capsules shows a regular square shape, also with smaller size. This could indicate an earlier stage of salt nuclei in the evolving process. On the other hand, if the incubation time was increased to 1 hour (Figure 6.2 c), corners of capsules became much rounder, and rod-like capsules has been eliminated. This is our snapshot 3. It demonstrates the capsule shape at a very late stage.

Figure 6.2 TEM images with the time bar below indicating the addition order. (a) Snapshot 1. (b) Snapshot 2. (c) Snapshot 3. Scale bar: 200 nm.

We also tried to replace TEOS with TMOS and remove the incubation time to capture the snapshot 4 (Figure 6.3 a). Since hydrolysis of TMOS is the fastest among all traditional 4-branched siloxanes, it is expected to capture a stage even earlier than 2. As is shown by the TEM image, the size of the capsules is significantly smaller, but no obvious sharp corners can be observed. Also, capsules seem to grow into clusters, with flocculated silica structures. Similar impurities can also be seen in the case that addition of TMOS followed by 1 min of incubation (Figure 6.3 b), which is also our snapshot 5.

Therefore, the presence of impurities may be a cause of TMOS. Since its hydrolysis is fast, it is reasonable to induce more homogeneous nucleation in the ethanol phase. **5** is supposed to show a stage slightly earlier than that of **1** since TMOS hydrolysis faster than TEOS.

Figure 6.3 TEM images with the time bar below indicating the addition order. (a) Snapshot **4**. (b) Snapshot **5**. (c) Snapshot **6**. (d) Snapshot **7**. (e) Snapshots arranged in time sequence. Scale bar: 200 nm. (f) Schematic of the capsule shape evolving process. The two ends are extrapolations.

When TEOS was replaced by TBOS, the trend shifts to the opposite side. In snapshot **6** (Figure 6.3 c), the case of TBOS without incubation, rod-like structures dominated the products. If a 1-minute incubation was added in (Figure 6.3 d), the snapshot **7** represent capsule structures very similar to that in **3**. This result corresponds to our expectations, since the slow hydrolysis of TBOS could allow the droplet to evolve longer. However, the exact sequence of **6** and **7** in the evolving process are not crystal clear. For **7**, we can secure that it is later than **1**, but its order comparing to **3** is unclear. We would like to assume that **7** is earlier than **3**, since the slow hydrolysis rate of TBOS is not likely to compensate an entire hour of incubation. However, for **6**, we can only assert that it is later than **4** and **2**, and earlier than **7** and **3**. There isn't enough clue to determine its order comparing to **5** and **1**. We would like to assume that it is earlier than both of them. In **5** and **1**, rod-like capsules co-exist with polygonal ones, while in **7** and **3**, the very late stage of the process, rod-like capsules disappeared. It is reasonable to see the existence of rod-like capsules as a symbol of a relative earlier stage. While in **6**, rod-like capsules are the predominant. Therefore, we could assume that **6** is earlier than **5** and **1**.

By far, we can arrange all snapshots into an order, and summarize the shape evolving during the process (Figure 6.3 e). The sequence would be **4, 2, 6, 5, 1, 7, 3**. Their shape shows a trend from small square to rod, then to polygon, then become smoother, which is demonstrated in Figure 6.3 f. We may extrapolate the shape of both ends – the first stage could be even smaller cubes, while the last stage would be near elliptical or even spherical.

6.3.2 Polygonal or spherical

By far, two trends in the sulfate system have been summarized. On one hand, when salt concentration is constant, as the incubation time increases, the capsule size

increases, its shape develops towards a sphere. On the other hand, as mentioned in the second chapter, when the concentration of salt decreases, the shape of capsules also becomes more spherical. We believe the CE hypothesis is capable to explain both of them. As to the time dependence, capsules become round cornered due to two reasons (Figure 6.4 a). When a thin layer of water is condensed on the nuclei, it would expand evenly along the geometry of the nuclei. Hence, it would form a shape smoother than the nuclei itself, but not completely spherical. When the water layer grows thicker, it would develop towards a sphere to meet the need of a low surface energy. On the other hand, when water starts to dissolve the salt nuclei, the corrosion would happen on the sharp corners and edges of the nuclei first, since at those points the surface energy is higher. Hence, the nuclei itself would become smoother along with the droplet.

Figure 6.4 Schematics based on the CE hypothesis. (a) Two causes of the round corner. (b) How salt concentration affect the capsule shape.

The concentration dependence of shape can be explained by Figure 6.4 b. At a higher concentration, the oversaturation of salt is also higher, which leads to vast salt

nuclei. They would compete for water, leading to an insufficient water condensation and extraction for each nucleus. Therefore, the water layer on a nucleus is thinner as well as the corrosion weaker, leading to a more polygonal capsule after encapsulation. If the salt concentration is low, each nucleus would be distributed with adequate water to dissolve the nuclei completely. Therefore, after encapsulation, a thermodynamically preferred spherical shape would be the result.

6.3.3 Research outlook

In order to further testify the droplet nucleation mechanism, *in situ* TEM could be of great help in the future. If the condensation indeed happens on the salt nucleus, then the introduction of a salt nanocrystal in a liquid cell with a water-ethanol mixture should be able to induce water nucleation on its surface. By monitoring this nanocrystal fixed under *in situ* TEM, the mechanism may finally be nailed down in the future.

6.4 Conclusion

We have identified another trend in a typical solute induced phase separation – the time dependence of the capsule shape. Along with the salt concentration dependence described earlier as evidences, the condensation and extraction hypothesis has been further developed. With the revised hypothesis, the above-mentioned phenomena have been successfully explained.

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Chapter 7 Summary and outlook

7.1 Summary

7.1.1 From solid to liquid

Based on the understanding of solid nucleation and growth, a series of synthetic methodologies have been developed in the research area of nanoscience and technology. Combination of those methodologies would allow the design and synthesis of sophisticated nanostructures from colloid systems. Our research aims on pushing the boundary of nanosynthesis a step further, to promote similar methodologies into liquid-liquid systems.

In order to do so, the first step is to seek a tool of building droplets bottom-up. Precisely fabricated nanoparticles can't be produced from breaking down bulky solid chunks, neither would be droplets at nanoscale. A typical process of the formation of a nanoparticle can be described as the emergence of a solid phase from a liquid phase. Bearing this in mind, salting-out effect drew our attention, since it is a universal phenomenon that one liquid phase emerges from another liquid phase. Its similarity with the traditional nanosynthesis system would be beneficial for the porting of classic methodologies.

We have overcome the influence of merging, which is a crucial deviation from the solid particle growth process. Thickeners were introduced to reduce its chance, while fast silica encapsulation could capture the droplets before merging happens. Instead of addition of salt directly, we chose acid-base reaction to generate salt *in situ*, which minimized the influence of local oversaturation to optimize the uniformity of droplets. By doing so, the foundation of further research has been laid.

7.1.2 Generality of the system

There are three degrees of generality in the nanosynthesis through solute induced phase separation systems. The generality of separation initiator, generality of the loading substance, and generality of the shell material.

The classic separation initiator is salts. Based on the Hofmeister series ¹, a variety of salts have been tested. Inspired by the kosmotrope-chaotrope theory ², we have pushed salting out systems beyond salt. Other kosmotropes could show similar capability of separating liquid phases similar to kosmotropic salts. This approved the generality of the phase separation initiator.

During the encapsulation of a droplet, what inside the droplet is together captured. Therefore, if we could load the droplet with specific substances we want, encapsulation of such droplets would produce nanocapsules loaded with the aforementioned substances. Based on this concept, three different loading strategies were designed, targeting three crucial stages in the nanoencapsulation process. Through this method, a variety of metal ions, complexes, and medications have been successfully loaded into nanocapsules. In theory, anything that has a higher solubility in water than in the organic phase would bear the potential to be loaded into nanocapsules through a phase separation system. This composes the generality of loading substances.

Aside from silica, other materials have been tested for the capsule material. From polymers, including biocompatible ones to metal oxide, from MOF material to insoluble salts, the generality of capsule shell material has been verified. Moreover, silica templated organic-inorganic hybrid capsules have been synthesized, which showed an ideal size and uniformity controllability. Hybrid capsules could be a promising choice in the future since it combines the controllability of silica capsules and the functions of other materials.

7.2 Outlook

7.2.1 New scope

The theory of our research was built upon the concept of aqueous two-phase system (A2PS)^{2,3}. Yet by now our methodologies only runs on systems with an aqueous phase and an organic phase. We would like to push it one step further, to apply similar strategies to systems with two aqueous phases. The mild condition of aqueous solution and the weaker surface tension between aqueous phases could serve as an ideal tool for delicate bio-molecules. In our proof of concept experiments, hollow silica capsules have been synthesized through a phase separation of two aqueous phases, even though the yield and uniformity are still not ideal. Follow up research could be conducted in the future.

7.2.2 Applications

Medication loaded capsules have gone through *in vivo* test for several years. In order to synthesize uniform nanocapsules, traditional method requires to remove the core of solid multi-layer particles, followed by an inefficient diffusion-based loading⁴. Through the nanoencapsulation from a solute induced phase separation system, we would be able to load and seal the nanocapsule in a one-pot reaction, which greatly reduce its cost of time and human resource. On the other hand, it could combine with functional capsule materials, which would bring the practicality of nanocapsule therapy to a new level.

Loaded nanocapsules can also serve as nanoreactors. We have conducted the reduction of gold(III) in the capsule to produce gold nanoparticles. If homogeneous catalyst could be loaded into nanocapsules with the right pore size, it could serve as a highly efficient heterogeneous catalyst with extremely high surface area.

In the long run, other droplet templated nanosynthesis can be adopted into the solute induced phase separation system, since its specialty is to build nanoscale droplets from bottom up. The generality of this system is its greatest advantage.

7.2.3 Mechanism study

We have dabbled in the mechanism study of liquid nucleation in liquid phase. Two hypotheses have been announced and both of them can provide certain guidance to nanosynthesis. However, characteristic tools for this purpose is scarce, as mentioned in previous chapters. In order to nail down the theory, *in situ* characteristic methods may be crucial, just like what scientists have done to their solid nucleation theory^{5,6}.

As mentioned before, *in situ* TEM for liquid droplets suffers from several problems. To compensate the contrast issue, ions with heavier elements could be introduced instead of citrate or sulfate. For example, selenates and tellurates, if chosen as separation initiators, may be able to improve the image quality. Reducing the exposure time could minimize the electron beam induced degradation of the sample, if image quality for each frame is acceptable. If the *in situ* TEM study could be made possible, it would provide solid evidences to our understanding of liquid droplet nucleation and growth.

7.3 Conclusion

This graduate research concentrated on the scope of a new platform to expand the territory of nanosynthesis. Instead of dive deep into each subsystem, we mainly focused on exploring the generality of solute induced phase separation systems, and its mechanism. We hope our study could serve as a foundation for more novel nanosynthetic methodologies in the future.

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