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Preparation of polydopamine nanocapsules in a miscible tetrahydrofuran–buffer mixture†

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A miscible tetrahydrofuran–tris buffer mixture has been used to fabricate polydopamine hollow capsules with a size of 200 nm and with a shell thickness of 40 nm. An unusual non-emulsion soft template mechanism has been disclosed to explain the formation of capsules. The results indicate that the capsule structure is highly dependent on the volume fraction of tetrahydrofuran as well as the solvent, and the shell thickness of capsules can be controlled by adjusting the reaction time and dopamine concentration.

Hollow polymeric capsules, especially hollow polymeric nanocapsules, have attracted considerable interest in recent years because of their wide application as drug delivery vectors, biomimetic reactors, sensors, and diagnostics.[1–4] Allowing the size, composition, and morphology of the capsules to be finely tuned, template-assisted approaches have been widely used to prepare polymeric capsules.[5–7] Typically, hard[8–10] or soft[11] templates are used as sacrificial cores, and the polymer can be deposited on the cores *via* some methods such as layer-by-layer (LbL) assembly[12,13] or *in situ* polymerization.[14] Hard templates hold the advantages of being stable and monodisperse, but harsh chemical reagents (*e.g.*, acids, organic solvents) are usually required to dissolve these templates, which might bring about some limitations on the applications, especially biomedical applications. In contrast, soft templates, generally based on emulsion systems, can be easily removed under mild conditions like aqueous alcohol solution. Due to this advantage, in the past few decades, various emulsion systems have been developed to prepare polymeric capsules, such as hexadecane-in-water,[15,16] toluene-in-water,[17] and isooctane-in-water.[18] There are two basic requirements for preparing these emulsion systems. One is that the emulsion needs at least two immiscible solvents, and the other is that surfactants are needed to stabilize the emulsion. It is a big problem to remove the surfactants from the finally prepared capsules. So, it is useful and important to develop simpler and surfactant-free soft templates to fabricate hollow nanocapsules.

Polydopamine (PDA), inspired by the mussel adhesive protein, has displayed excellent adhesive properties on different substrates in alkaline solution, and exhibited good biocompatibility.[19–22] It has also been used to prepare hollow capsules *via* hard and soft template approaches. Caruso *et al.*[20] applied SiO_2 particles as hard templates to fabricate hollow PDA capsules with a range of sizes and mesoporous structures. $CaCO_3$ spheres were also used to prepare PDA capsules in order to construct a multienzyme system.[21] Recently, an emulsion method (soft template approach) has been investigated to prepare PDA capsules. Caruso and Hao *et al.*[22] successfully obtained monodisperse PDA capsules using the dimethyldiethoxysilane (DMDES)-in-Tris buffer system, and the size of capsules could be controlled from 400 nm to 2.4 μm, which was the earliest report involving soft template routes to fabricate PDA capsules. Wang and coworkers[23] employed the pristine oil-in-water emulsion technology to prepare PDA capsules with sizes of 1.3–7.5 μm. Rahimipour and co-workers[24] combined the soft emulsion (either canola oil or *n*-dodecane in Tris buffer) technique with a sonochemical method and obtained PDA capsules with a size of around 227 nm. In general, the progress in the preparation of PDA capsules is still limited, especially in the preparation of smaller capsules below 300 nm.

In the present work, we report a new soft template method to prepare PDA nanocapsules of around 200 nm. It is well known that tetrahydrofuran (THF) is macroscopically miscible with water, and it is impossible for them to form an emulsion. However, to our great surprise, PDA hollow nanocapsules were formed when we mixed dopamine with a THF–Tris buffer

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mixture at an appropriate volume fraction of THF (φ). A mechanistic study indicates that THF–Tris buffer mixtures can form microscopically inhomogeneous domains which template the PDA capsules. So, our present work has extended the soft templates from emulsion to non-emulsion systems. In addition, the thickness of the capsule membrane can be controlled by adjusting the dopamine concentration, and is highly dependent on the reaction time. Furthermore, in principle, any kind of agent that is soluble in THF can be easily encapsulated in the capsules after PDA self-polymerization on the THF–water droplet interface, offering potential applications in biotechnology, drug delivery systems and devices.[22] All these characteristics in combination with the advantages of facile preparation, small capsule size, no surfactants and an easy template-removal process by evaporation make this non-emulsion method very promising in the preparation of polymeric capsules.

The synthesis process is very simple involving addition of dopamine into the THF–Tris buffer mixture with a typical concentration of 0.5 mg ml^{-1}. The reaction process of dopamine at room temperature (25 °C) was monitored using a UV-vis spectrometer. As shown in Fig. 1a (φ = 0.7), two new absorption peaks at around 300 nm and in the range of 400–700 nm appear with the reaction, respectively. The former peak is assigned to the oxidation reaction of dopamine into dopachrome; the latter one is attributed to the continuous self-polymerization of dopamine into PDA.[25] We thus measured the absorption at λ = 400 nm with the reaction time to evaluate the self-polymerization dynamics.[23] As shown in Fig. 1b, the absorption increases sharply after 100 h and then levels off after 168 h, meanwhile the reaction system color changes from colorless to dark. All these results clearly support the successful self-polymerization of PDA under our experimental conditions. A proposed PDA self-polymerization mechanism including covalent polymerization and non-covalent self-assembly processes is summarized in the ESI (Fig. S1, ESI†).

The particles obtained from the reaction system were then characterized by TEM (Fig. 1c), which shows that the particles have a clear contrast difference between the inner pool and the outer black wall, indicating the formation of PDA hollow capsules. The capsules are around 200 nm in diameter and are flexible to be deformed during the TEM sampling process. SEM (Fig. 1d) was also used to characterize the capsules and the hollow structure was directly seen according to the cavities of the particles (red arrows). PDA capsules could also be obtained when more THF was added into the reaction systems (φ = 0.8) according to the TEM (Fig. 1e) and AFM (Fig. 1f) measurements. The AFM image clearly shows the capsule structure with a hole in three-dimensional mode (inset of Fig. 1f). A higher temperature of 45 °C was used in order to increase the solubility of dopamine at φ = 0.8.

The data in Fig. 1 prove that PDA nanocapsules have been prepared through self-polymerization of dopamine in the miscible THF–Tris buffer mixtures. As mentioned above, PDA capsules are generally prepared through an immiscible emulsion template, so how can the capsules be formed in a THF–Tris buffer non-emulsion system? To address this, the intermediates with different reaction times were collected for TEM observation. Fig. 2a shows the TEM image of the samples after reacting for 12 h. Many discrete small PDA nanoparticles together with some big hollow spheres are observed, and the hollow spheres are coated with PDA nanoparticles according to the magnified image (inset). Wu *et al.*[26] had studied solution dynamics of a THF–water mixture by using laser light scatter-

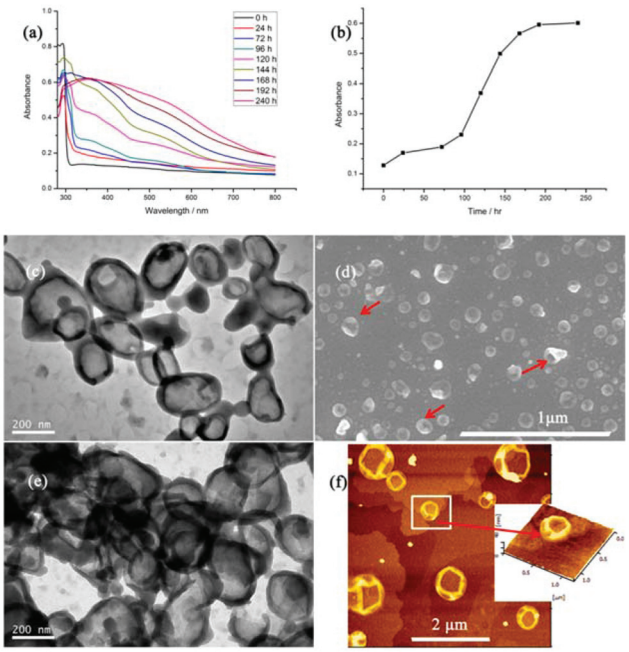

Fig. 1 (a) UV-vis spectra of dopamine polymerization in a THF–tris buffer (φ = 0.7) mixture. (b) Time evolution of the absorbance of dopamine self-polymerization in a mixture at 400 nm. The TEM (c) and SEM (d) images of PDA capsules at φ = 0.7. The TEM (e) and AFM (f) images of PDA capsules reacted at φ = 0.8 and 45 °C.

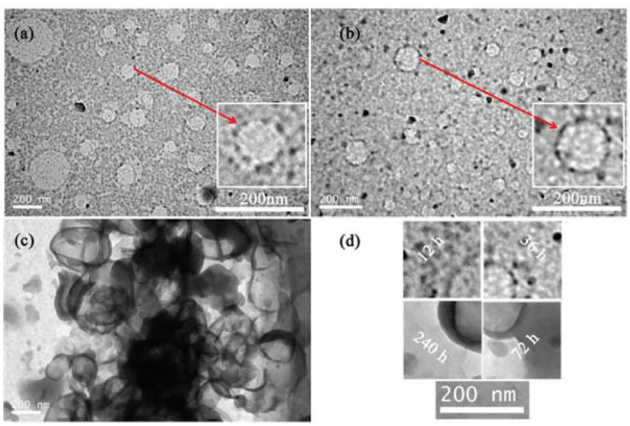

Fig. 2 The TEM images of PDA capsules at φ = 0.7 and 25 °C with different reaction times of 12 h (a), 36 h (b), and 72 h (c). (d) The TEM images of PDA capsule shells when reacted for 12 h, 36 h, 72 h, and 240 h, respectively. The sample solutions reacted at 12 h (a), 36 h (b), and 72 h (c) were directly used for TEM observation without any purification.

ing technology and found that the mixture was not microscopically homogeneous and could form complexes with a dynamic correlation length of 200–600 nm. Our results presented here are in good agreement with Wu's work. It was the microphase-separated THF–water complexes that template the adsorption and self-polymerization of dopamine on the surface, which lead to the final formation of PDA hollow spheres. As reported by Wu, the THF–water complexes are not stable, however in our system PDA nanoparticles spontaneously aggregate onto the surface of the complexes and probably stabilize them through a "Pickering emulsion" mechanism (inset of Fig. 2a).[27,28]

After reacting for 36 h, hollow spheres with a much clearer rim were observed (Fig. 2b), and PDA nanoparticles began to merge together on the surface of the hollow spheres to form a thicker shell according to the amplified image (inset of Fig. 2b). After reacting for 72 h, many hollow spheres with clear shells were obtained (Fig. 2c). Fig. 2d shows the changes of the shell of the PDA capsules with reaction time. The shell thickness progressively increased from about 5 nm, 7 nm, 13 nm to 31 nm after reacting for 12 h, 36 h, 72 h, and 240 h, respectively. A similar result was obtained when we set the reaction time at 192 h and φ = 0.7 but changed the dopamine concentration. The capsule shell thickness increased from 7 nm, 29 nm to 40 nm when the dopamine concentration increased from 0.1 mg ml^{-1}, 0.5 mg ml^{-1} to 1.0 mg ml^{-1} (Fig. S2, ESI†), respectively. So, the capsule shell thickness can be controlled by adjusting either the reaction time or the dopamine concentration. The shell thickness of polymeric capsules plays a crucial role in the permeability and the mechanical strength of the shell wall, which is an important factor for the applications.[29,30]

The abovementioned results strongly support a template-triggered growth mechanism as shown in Fig. 3 for the formation of PDA nanocapsules. The mixture of THF–buffer is macroscopically homogeneous; however, microphase-separated THF–buffer nanodrops of around 200–600 nm will be formed at the microscopic level. These nanodrops serve as the templates to accommodate the self-polymerization of dopamine on the surface. With the continuous accumulation and growth of PDA layers on the surface of the templates, PDA capsules with thick shells are obtained, and the shell thickness increases with the reaction time. The same shell thickness and reaction time dependence has been found in the silica particle-templated PDA capsule system.[20]

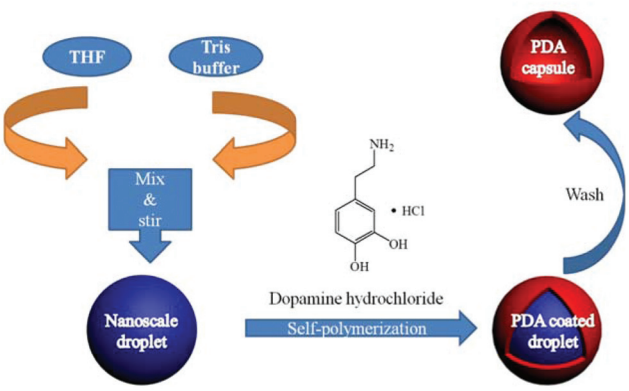

Fig. 3 Preparation procedure of the PDA hollow capsules using THF–Tris buffer mixtures.

Additionally, the stability of PDA capsules in a THF–Tris buffer mixture (φ = 0.7) was examined. The mixture was kept for 5 months at 4 °C. It was found that plenty of PDA nanocapsules still existed in the mixture according to SEM measurements (Fig. S3, ESI†), indicating the stability of the PDA nanocapsules. In fact, the PDA molecules are crosslinked to each other in the nanocapsules, so they are quite stable. Nevertheless, it should be noted that the capsules are more crimpled or deformed than the freshly prepared ones, and some of them tend to aggregate together after 5 months' storage.

The effect of the volume fraction of THF (φ) on the PDA nanocapsules was further studied. For this purpose, the dopamine polymerization in a THF–Tris buffer mixture with different φ values was carried out. The time for the accomplishment of dopamine polymerization in the mixture (φ = 0, φ = 0.2, φ = 0.5) was evaluated using the absorption intensity at 400 nm with the reaction time (Fig. S4, ESI†), and they were around 4 h, 22 h, and 67 h respectively. It indicates that the self-polymerization rate of dopamine decreases with the increase of the volume fraction of THF (φ). This may be attributed to the low concentration of dissolved oxygen in the system with the increase of the THF content, which impedes the oxidation of dopamine and its self-polymerization.

The self-assembled morphology is also dependent on the volume fraction of THF (φ). Fig. 4a and b show the TEM & SEM images of the PDA particles obtained in 100% Tris buffer (φ = 0), which indicates the formation of PDA solid spheres with the size of about 200–300 nm as usual. When φ = 0.1, similar to the pure Tris buffer, only PDA solid particles with the sizes of around 200 nm were formed (Fig. 4c and d). When φ = 0.2, PDA particles with the size of mainly around 200–300 nm were also formed (Fig. 4e and f). However, hollow lumens (red arrows, Fig. 4e) of around 100–200 nm were observed in some of these particles, and the thickness of the shell was about 100 nm. When φ = 0.3, the hollow spheres of around 200 nm and with the shell thickness of 40 nm could be widely observed (Fig. 4g and 4h). So, only when the THF content is above a critical fraction (φ = 0.2) will the microphase-separated THF–water nanodrops act as soft templates to template the PDA hollow capsules. Wu and coworkers[26] also found the formation of the THF–water complex of around 200 nm when the THF molar fraction was larger than 0.13. So our results are in good agreement with their results, which is further evidence to support the mechanism as shown in Fig. 3. The shell thickness of PDA capsules becomes thinner from φ = 0.2 to φ = 0.3, which is probably due to the increase of the concentration of the nanodroplet templates with the increase of THF volume fraction. As a result, less PDA polymers are coated onto the templates, leading to thinner capsule shells. It should be noted that there is no clear morphology difference between

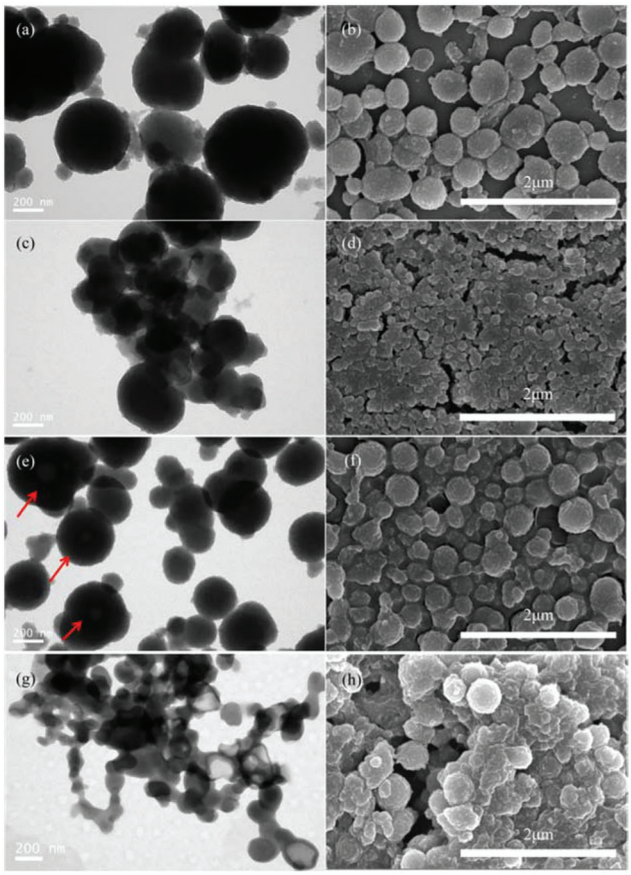

Fig. 4 The TEM and SEM images of PDA particles obtained *via* 10 days oxidation and self-polymerization of dopamine in THF–Tris buffer mixtures at φ = 0 (a,b), φ = 0.1 (c,d), φ = 0.2 (e,f) and φ = 0.3 (g,h). The samples were purified by centrifugation before measurements.

the PDA particles at different THF contents when measured by SEM, and only spherical particles are observed.

Besides, the effects of the buffer and the solvent on the formation of PDA nanocapsules are also studied. In the experiments, DA was added into a THF–phosphate buffer mixture or dimethyl sulfoxide (DMSO)–Tris buffer mixture with a DA concentration of 0.5 mg ml^{-1} and φ = 0.7. The pH value of each buffer was set at 8.0, and the reactions were carried out at 25 °C for 7 days (168 h). It is found that PDA nanocapsules of around 200 nm can also be obtained in a THF–phosphate buffer mixture (Fig. S5, ESI†). For the DMSO–Tris buffer mixture reaction system, only very small PDA nanoparticles with a size of around 2 nm instead of PDA nanocapsules were found (Fig. S6, ESI†). In addition, as reported by Lee and coworkers,[31] only PDA solid spheres with tunable diameters from 70 nm to 300 nm were obtained in an alcohol–Tris buffer mixture. These results partially support that the preparation of PDA capsules is not related to the buffer solution, but is highly related to the solvent. DMSO or alcohol is highly soluble in water, so both of them are unable to form microphase-separated microdomains as the soft templates in DMSO–water and alcohol–water mixtures to mediate the formation of nanocapsules.

Conclusions

In summary, we demonstrate here a simple and non-emulsion method using a miscible THF and buffer mixture to fabricate polydopamine hollow capsules of around 200 nm in size. A mechanistic study indicates that the THF–buffer mixture can form some microphase-separated complexes to template the formation of capsules. The capsule shell thickness can be controlled by adjusting the reaction time and dopamine concentration, and increases with the increasing reaction time and dopamine concentration. The formation of polydopamine capsules is also highly dependent on the volume fraction of THF as well as the water miscible solvent, and the capsules can only be formed when the THF fraction is larger than 0.2, and cannot be formed in DMSO–buffer and alcohol–buffer mixtures. This work has shown the great possibility to produce hollow nanostructures using macroscopically miscible but not microscopically homogeneous mixed solvents as soft templates.

Experimental section

Materials

TRIS buffer consisting of disodium ethylenediaminetetraacetate dihydrate and tris(hydroxymethyl)aminomethane was purchased from Aladdin Industrial Inc. Tetrahydrofuran (THF), Dimethyl Sulfoxide (DMSO), and phosphate buffer consisting of sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. 3-Hydroxytyramine hydrochloride (dopamine) was purchased from Acros and used without purification. The water used in all experiments was prepared using a Milli-Q purification system and had a resistivity greater than 18.2 MΩ cm.

Oxidation and self-polymerization of dopamine in a THF–Tris mixture

Typically, THF and Tris buffer or Phosphate buffer with a desired volume ratio are separately added into a flask. Then, the top of the flask was screwed and the mixture was stirred when dopamine hydrochloride was added to the mixed solution. Unless otherwise specified, the pH value of every buffer is 8.0, the concentration of each buffer is 10 mM, and the reaction was carried out at 25 °C with 0.5 mg ml^{-1} (based on the mixed solvent) for 10 days.

TEM

Transmission electron microscopy (TEM) imaging was implemented using a Tecnai G2 spirit Biotwin (FEI, USA) with a Gatan 832 microscope operated at an acceleration voltage of 120 kV. Unless otherwise specified, the sample was prepared by dropping the reaction solution onto copper grids coated with a thin carbon film and drying for several minutes, and then the grids were washed with deionized water (2–3 drops) with the help of filter paper. The sample was dried at room

temperature for 48 h before TEM measurements. No staining treatment was performed for the measurement.

SEM

Scanning electron microscopy (SEM) images were obtained using a JSM-7401F (JEOL Ltd, Japan) field emission scanning electron microscope operated at an acceleration voltage of 5 kV. Unless otherwise specified, the sample was prepared by dropping the reaction solution onto silica wafers and drying for several minutes, and then the grids were washed with deionized water (2–3 drops) with the help of filter paper. The sample was dried at room temperature for 48 h before SEM measurements. Then the samples were sputter coated with gold to minimize charging.

AFM

Atomic force microscopy (AFM) images were obtained using a Nanonavi E-Sweep (SII, Japan). The surface morphologies of samples were acquired in tapping mode. The samples were prepared by dropping the reaction solution onto a mica sheet and drying for several minutes, and then the mica sheet was washed with deionized water (2–3 drops) with the help of filter paper. The sample was dried at room temperature for 48 h before measurements.

UV-vis

The UV-vis absorption spectra were obtained with a UV/V-16/18 (Mapada, China). Samples of the reaction system were added to a 1 cm quartz cuvette for the measurements.

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