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ARTICLE TYPE

Facile fabrication of nanocomposite microcapsules by combining layer-by-layer self-assembly and Pickering emulsion templating

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Nanocomposite polysaccharide microcapsules composed of biocompatible polyelectrolyte complex via electrostatic layer-by-layer (LbL) self-assembly on Pickering emulsions template method are prepared. polyethyleneimine (PEI)/Laponite based Pickering emulsions are obtained regardless of the polarity and viscosity of the oils at PEI/Laponite mass ratio of 0.50 and Laponite concentration of 0.25 wt%, and these emulsions show good long-term stability for more than two months. Four bilayers sodium alginate/chitosan microcapsules with the dimension of about 43.9 μm and wall thickness of 55 nm were prepared by alternate adsorption of negatively charged sodium alginate and positively charged chitosan on Pickering emulsions. Hollow microcapsules are obtained after core removal at a mild condition of washing with excess 2-propanol. Ibuprofen (IBU) as a model drug is loaded into hollow microcapsules, and the release rate of IBU from the microcapsules at pH 7.4 is obviously faster than the release rate at pH 2.0. The more polyelectrolyte layers of IBU-loaded microcapsules, the more difficult for IBU release. Consequently, nanocomposite microcapsules composed of natural polysaccharides fabricated by Pickering emulsion templated LbL assembly method offer great potential applications in the food and medical industries.

Introduction

Over the past decades, nano- or microcapsules with unique and tailored properties, biocompatible and biodegradable, or environment response have been extensively studied as an important class of materials because of their potential application in self-healing materials,[1] catalyst,[2] environment protection,[3] drug delivery or controlled release systems,[4] and so on. A number of approaches have been proposed to prepare these capsules: interfacial condensation polymerization,[5] in-situ radical polymerization,[6] internal phase separation,[7] coacervation,[8] and layer-by-layer (LbL) assembly.[4,9-11]

Among various ways, the LbL assembly is one of the prominent methods. Hollow microcapsules can be produced by the LbL assembly on sacrificial templates with charged polyelectrolytes and/or charged inorganic nanoparticles, followed by subsequent decomposition of cores. The properties of microcapsules, such as the thickness, particle size, composition, surface features, wall permeability, can be tailed on a nanoscale range.[12-14] These sacrificial templates can be solid particles[3,4,10,11,15] or soft bodies such as emulsion droplets.[9,16,17] Especially, the development of emulsion-based microcapsules has gathered increased interest in the pharmaceutical fields. LbL-based microcapsules are emerging as a novel potential therapeutic tool because of mild template removal condition, high loading capability of guest materials, good biocompatibility, high stability to environmental stresses and stimulus responsive behaviour.[12-14]

In the previous literatures, oil-in-water (O/W) emulsion-based microcapsules using the LbL technique have been prepared using small molecule surfactants,[9,18] proteins,[16,19] and phospholipids[17] as emulsifiers.

Recently, colloidal particles acted as particulate emulsifiers to stabilize emulsion droplets have aroused great interest, named Pickering emulsion.[20] Once colloidal particles adsorb at oil-water interfaces, the particles need high energy to desorb from the interfaces, in contrast to surfactant molecules.[21,22] Thus, compared to conventional emulsions, Pickering emulsions have unique advantage in stabilization, which is helpful for using as templates to prepare functional materials or structures.[23-27]

However, to the best of our knowledge, only Stöver and co-worker[28] used Pickering emulsions as templates to make poly(diallyldimethylammonium chloride) (PDADMAC)/ poly(sodium styrenesulfonate) (PSS) and PSS/PDADMAC/LUDOX HS/PDADMAC microcapsules via LbL assembly.

Usually, the polyelectrolyte materials for the LbL assembly of microcapsules mainly focus on non-degradable synthetic polyelectrolytes, such as PDADMAC,[18,28] PSS,[18,28,29] poly(allylamine hydrochloride) (PAH),[29,30] polyethyleneimine (PEI),[31] and poly(acrylic acid) (PAA).[28] But for practical and biomedical applications, the use of nature polyelectrolytes seems an attractive alternative because of their biocompatibility and biodegradability. Sodium alginate (ALG) is a natural biopolymer extracted from brown algae. It is composed of linear chains of the α-L-guluronic acid (G) and the β-D-mannuronic acid (M).

Sodium alginate is a weak acid, a polyanion with a pK_a between 3-4.[15] Chitosan (CS) is a basic linear polysaccharide containing β [1→4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose units. CS is a weak base, a positively charged polyelectrolyte in acidic medium with a pK_a 6.5.[23] They are economical and nontoxic biomaterials, and have received considerable research attention over the recent years.

Herein, the LbL deposition of nature polysaccharide of ALG and CS on Pickering emulsion droplets, which were stabilized by Poly (ethylene imine) (PEI) surface-modified Laponite particles, was performed for the first time. A mild method was used to obtain hollow microcapsules after core removal by washing with excess 2-propanol. A schematic drawing of the production of hollow microcapsules is presented in Fig. 1. Ibuprofen (IBU) was used as the model drug to study the release behaviour of the hollow microcapsules. The advantage of this method or microcapsule are: (i) the high stability of Pickering emulsions can suppress deformation and flocculation of emulsion droplets during LbL assembly process, (ii) the encapsulation of molecules both in the polyelectrolyte shell (IBU) and oil core simultaneously, and (iii) the scalability of this method to obtain other microcapsules.

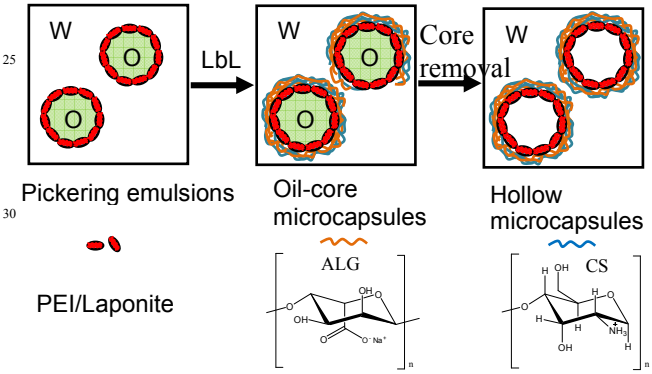

Fig. 1. Formation of multilayer microcapsules by the Pickering emulsion templated LbL assembly.

Experimental

Materials

Laponite RD was kindly provided from another laboratory. Poly (ethylene imine) (branched, M_W = 10,000 by GPC; M_W = 25,000 by light scattering), chitosan (CS, degree of deacetylation ⩾ 90 %, viscosity-average molecular weight M_v = 60,000), sodium alginate (ALG, M_W = 120,000), ibuprofen (IBU), fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich, and used without further purification. Xylene, chloroform, sunflower oil, dodecane, liquid paraffin, 2-propanol, glacial acetic acid, tetrahydrofuran (THF), dimethyl sulfoxide (Guangzhou Chemical Reagent Factory, China) were of analytical grade. Water used in all experiments was purified by deionization and filtration with a Millipore purification apparatus to the resistivity higher than 18.0 MΩ•cm.

Preparation of Pickering Emulsions

PEI/Laponite complex were prepared according to Ref.[32] First, stock aqueous solutions of PEI and Laponite were prepared. Then, these two solutions were mixed to fabricate aqueous solution of 0.25 wt% Laponite, 0-1.0 wt% PEI. The obtained PEI-coated Laponite aqueous suspension was added into a 14 mL glass vial, followed by addition of 2 mL oil (xylene, chloroform, sunflower oil, dodecane, liquid paraffin). The mixture was homogenized at 12,000 rpm for 2 min by an IKA Ultra Turrax T25 homogenizer equipped with a 10 mm dispersing tool in an ice bath.

Table 1 Parameters of organic reagents used as oils.

oil	Xylene	Chloroform	SFO[a]	Dodecane	LP[b]
ε	2.4	4.4	~3	2	1.9
Viscosity (cP, 20 °C)	0.65	0.57	~50	1.34	~40
Diameter (μm)	43.9±19.9	22.1±16.7	24.3±10.8	40.5±25.4	19.7±8.9

[a]Sunflower oil [b]Liquid paraffin

The primary Pickering emulsion was purified as follows. The resulting emulsion was diluted with deionized water. The excess water was removed from the bottom using a syringe after a few minutes of quiescent storage. This dilution/quiescence cycle was repeated three times. The volume of clean Pickering emulsion was kept in 6 mL.

Microcapsule Fabrication via LbL Assembly

Stock 1 mg mL^{-1} ALG and CS solution were prepared by dispersing ALG and CS in aqueous solution, NaCl concentration in these two solutions were 0.5 M. The pH of CS solution was adjusted to 4 with glacial acetic acid. The resulting CS solution must be stirred overnight to ensure complete dispersion, followed by filtered in order to remove the impurities before use.

The clean Pickering emulsions stabilized by PEI/Laponite (0.25 wt% Laponite, PEI/Laponite (mass ratio) = 0.5) were added into a vial. Then, 5 mL ALG solution was added, and the mixture was oscillated at 300 rpm for 20 min to ensure ALG adsorption. This is followed by 3 washing cycles that involved creaming of the secondary emulsions after a few minutes of quiescent storage, removing the excess liquid from the bottom by syringe, and replenishing with a 0.5 M NaCl solution to keep constant volume. The adsorbing of CS on the resulting cleaned, secondary Pickering emulsion template was prepared in exactly the same manner for the ALG coating, including three washing steps. This coating sequence was repeated until desired double layers of ALG/CS had been deposited.

Tetrahydrofuran Challenge and Hollow Microcapsules Preparation

The obtained $(ALG/CS)_4$ (6 mL) at optimized condition were transferred to a 14 mL vial, followed by addition of excess THF (5 mL) and violent shaking. The same THF challenge was used to assess the corresponding Pickering emulsions stabilized by PEI/Laponite (0.25 wt% Laponite, PEI/Laponite = 0.5).

Hollow microcapsules were prepared by dissolving the oil core with excess 2-propanol for 20 min and centrifuging at 5000 rpm for 5min.This dissolving process was repeated two times to ensure the complete removal of the oil. Then, the microcapsules were washed two times with water and centrifuged to remove 2-propanol, and finally redispersed in water.

Loading and release experiments

0.01 g hollow $(ALG/CS)_3$ microcapsules were dispersed in 20 mL phosphate buffer solution (PBS, pH = 7.4) containing 0.5 mg mL^{-1} IBU. After being incubated for two hours at 37 °C, the IBU-loaded microcapsules were centrifuged to remove the excess IBU, followed by washing twice with water.

The resulting IBU-loaded microcapsules was dispersed into 20 mL PBS (pH = 7.4 or 2.0), followed by transferred into a dialysis bag (M_w cut-off of 3500). And then, the dialysis bag was immersed into 180 mL of the PBS at 37 °C under magnetic stirring. After desired time intervals, 2.0 mL sample solution was taken out to analyse the IBU concentration. Following, this 2.0 mL solution was poured back into the PBS. This process proceeded until the concentration of IBU in the PBS remained unchanged. The quantification of IBU can be analysed with UV-vis spectrophotometer. The relationship between fluorescence intensity and concentration of IBU was linear in our calibration curve, which was established from standard solutions of IBU at PH = 7.4 or 2.0.

Further adsorption of ALG and CS layers on the IBU-loaded microcapsules was performed as the same procedures used above, except that the IBU-loaded microcapsules acted as self-assembly templates. All the supernatants produced during the washing process were collected. Loss of IBU during this process was calculated by counting the total IBU amount in the supernatants.

Characterization

Pickering emulsion droplets were observed with an optical microscope (Carl Zeiss, German) and the average diameter was measured by Laser Scattering Particle Size Distribution Analyzer (LA950, Horiba). Zeta potential of Pickering emulsion was determined with a Malvern Zetasizer Nano ZS90 and the ζ-potential value was the average of three measurements. The confocal micrographs were taken with a Leica TCS-SP2 confocal laser scanning microscope (CLSM) with a 10 × objective. CS was visualized by FITC-labelled polymer at excitation wavelength of 488 nm. FTIR spectra were recorded using a Bruker Vector-33 FTIR spectrometer under ambient conditions. The spectrum was taken from 400 to 2000 cm^{-1}. Scanning electron microscopy (SEM) was carried out with a Zeiss EVO 18 electron microscope equipped with a field emission electron gun. The samples were sputter-coated with gold prior to measurement. An Auto Probe CP Research atomic force microscopy (AFM, XE-100, Park systems) with a silicon nitride (Si_3N_4) cantilever of force constant of 0.58 N m^{-1} was used for observing the morphology and topology of microcapsules in tapping mode at room temperature. The sample was prepared by drying a drop of the microcapsule suspension on a freshly cleaved mica substrate. The changes in frequency (Δf/3) and dissipation (ΔD) of an Au sensor crystal during the LbL assembly were monitored by quartz crystal microbalance equipped with dissipation (QCM-D) (Q-Sense E1). Prior to the ALG and CS deposition, PEI/Laponite particles were firstly coated onto the Au surface to ensure a positive charged coating. Then, five bilayers films of alginate/chitosan were built by alternating ALG and CS depositions onto quartz crystal. During the coating, fresh polyelectrolyte solutions were injected into the measurement chamber for 20 min at a flow rate of 150 μL min^{-1}. A rinsing step of 10 min with a 0.5 M NaCl solution was included after the adsorption of each polyelectrolyte. For all measurements, the temperature was set up at 25 °C. The quantification of IBU concentrations were evaluated by a Hitachi U-3010 UV-vis spectrophotometer at 223 nm.

Results and discussion

Particle Surface Modification and Pickering Emulsion Preparation

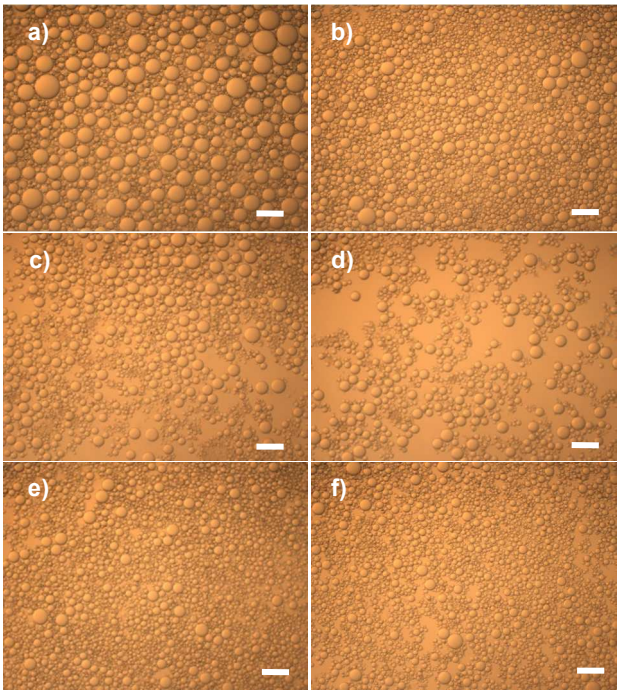

Fig. 2 Optical micrographs of xylene-in-water Pickering emulsions prepared at PEI/Laponite mass ratio of (a) 0.2, (b) 0.4, (c) 0.5, (d) 0.6, (e) 0.7, (f) 0.8. The Laponite concentration is 0.25 wt%, the oil to water volume ratio is kept at 1:2. All scale bars represent 100 μm.

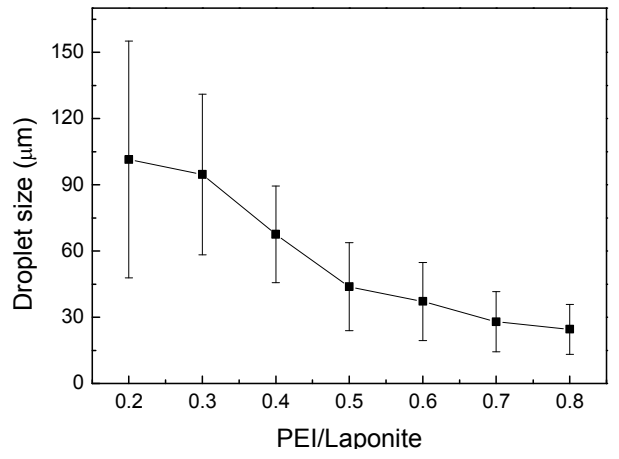

Fig. 3 Mean droplet diameters of xylene-in-water Pickering emulsions as a function of PEI/Laponite mass ratio.

It is well known that Laponite alone is too hydrophilic to act as a particulate emulsifier.[33,34] In order to produce effective emulsifiers of Laponite particles, many literatures were reported to hydrophobic Laponite by adding various small molecule additives or polymeric modifiers, such as salt,[33,35] amines,[34] and other polymer.[36] In this study, the properties of Laponite particles

were tailored by positively charged PEI,[32] which could bind irreversibly with negatively charged Laponite particle surfaces to produce an effective Pickering emulsifier. Moreover, PEI could give enough positive charge to absorb the first layer of ALG. The resulting polyelectrolyte-modified particles should form stable Pickering emulsions that could serve as robust templates for LbL assembly of ALG and CS, as shown in Fig. 1.

The morphologies of Pickering emulsions were investigated at different PEI/Laponite mass ratios and constant Laponite concentration of 0.25 wt%, as shown in Fig. 2. Drop test[23] confirmed that we had prepared xylene-in-water type emulsion. It is noted that emulsion droplets were discrete, spherical at various mass ratios. Besides, the mean droplet diameter of the emulsions gradually decreased with increasing the mass ratio until a plateau was reached (Fig. 3).

ζ-Potential value of colloid particle represents the hydrophilicity, which is crucial for the particle to form stable Pickering emulsion. According to Armes et al.,[32] with increasing PEI content, ζ-potential value of Laponite particles gradually changed from highly negative charge to positive charge, remaining at a plateau of about 15 mV. At first stage, Laponite particles modified by few PEI were relatively hydrophilic, so emulsions prepared at this condition exhibited relatively poor stability toward coalescence, resulting in big droplets. With increasing PEI/Laponite mass ratio, suitable hydrophilicity of PEI/Laponites ensured stable Pickering emulsions and small droplets. Further increasing PEI content played no obvious role in reducing the size of Pickering emulsions.

Minimum droplet diameter of around 59 μm was achieved at mass ratio of 0.5 in Ref. 32. However, in this study, the droplet diameter of xylene-in-water emulsion at mass ratio of 0.5 was about 43.9 μm which is not minimum value. Presumably, this difference was attributed to different oil and oil to water ratio. Significantly, the xylene-in-water emulsions at mass ratio of 0.5 displayed good stability against coalescence for several months.

Then, four other oils (chloroform, sunflower oil, dodecane, liquid paraffin) were chosen to prepare Pickering emulsions for comparison with xylene. The parameters of the oils are listed in Table 1. The polarity can be characterized by the dielectric constant ε. Xylene and chloroform are polar oils. Sunflower oil, dodecane and liquid paraffin are weakly polar oils. Liquid paraffin has a high viscosity of approximately 40 cP, but the viscosities of the other oils are very low. In all cases, these emulsions showed good long-term stability for more than two months regardless of the polarity and viscosity of the oils at PEI/Laponite mass ratio of 0.50 and Laponite concentration of 0.25 wt%. The morphologies of the corresponding emulsions are shown in Fig. S1 (ESI), and the droplet diameters of the emulsions are listed in Table 1. In this system, xylene was selected as the model oil of Pickering emulsions because of its low toxicity and high volatility. Thus, it was convenient to make hollow microcapsules. For practical requirement, the oil could be easily replaced by other oils.

The use of Pickering emulsions as templates for LbL assemblies of polyelectrolytes requires proper charged emulsion droplet surfaces, and sufficient stability to survive the removal of excess polyelectrolyte with water. We performed all LbL assembly studies at PEI/Laponite mass ratio of 0.50, Laponite concentration of 0.25 wt%, and the oil to water volume ratio of 1:2. This condition ensured that there was no PEI or Laponite remaining in the aqueous continuous phase after creaming of the emulsion droplets, which could reduce the aggregates of particulate emulsifiers after adding the first polyelectrolyte layer. In addition, the adsorption of PEI onto Laponite closed to monolayer coverage[32] at this condition, which availed the electrostatic deposition of ALG and formed well ordered and compact microcapsules.

Polyelectrolyte Complex Shells by LbL Assembly

During the LbL assembly process, the main problem of using the LBL technique to prepare multilayer emulsions is the tendency for droplet aggregation.[19,38] To avoid droplet aggregation during preparation, different volume of polyelectrolyte solutions were added to the clean emulsions to form a series of emulsions with different polyelectrolyte concentrations.

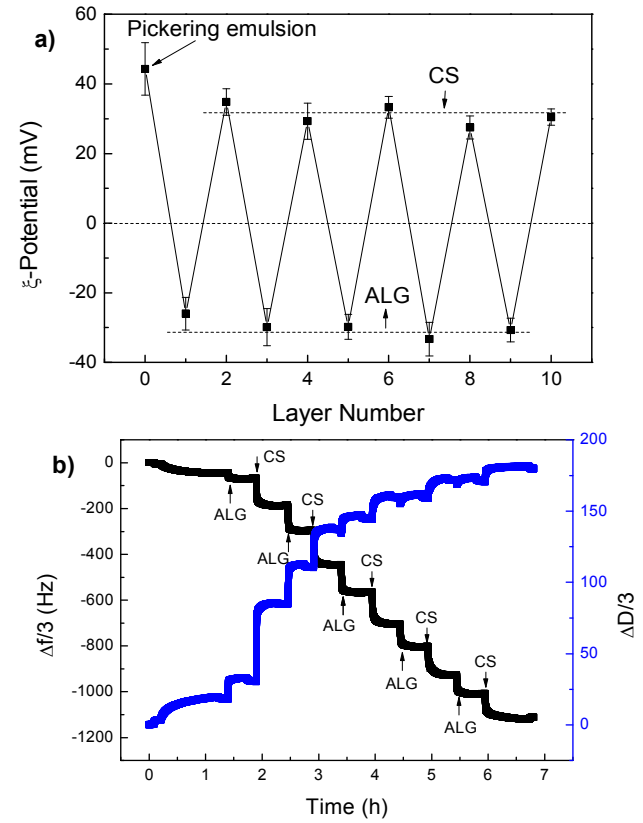

Fig. 4 (a) ζ-potential values as a function of layer number for ALG and CS LbL assembly on Pickering emulsion template. The Laponite concentration is 0.25 wt%, PEI/Laponite mass ratio is 0.5 and the oil to water volume ratio is kept at 1:2; (b) QCM monitoring of the growth of ALG/CS multilayers on the PEI/Laponite-coated QCM electrode. The poyelectrolyte concentrations are 1 mg/mL with 0.5 M NaCl.

In the experiments, when the ALG concentration was 0-0.04 wt% or above 0.06 wt%, obvious droplet aggregation was observed at the top of the aqueous solution due to bridging flocculation or depletion flocculation.[9,37] Therefore, 0.045 wt% of ALG and CS solutions (by adding 5 mL fresh ALG and CS solutions of 1 mg mL^{-1} to the 6 mL emulsions) were chosen to prepare microcapsules in the following experiments.

The surface charge is important for the LbL process,

especially, the initial Pickering emulsion template has to be sufficiently charged. If not, the adsorbed layers may be partially removed upon adsorption of the next layer.[9] The ζ-potential value of clean primary emulsion stabilized by PEI/Laponite particles is +44.3 mV, this charge value is sufficiently applied to absorb the next polyelectrolyte layer. Fig. 4a shows a typical charge inversion plot found for the investigated LbL systems. The surface charge changed from +44.3 mV to about -30 mV, indicating successful adsorbing with oppositely charged polyelectrolyte of ALG. The following deposition of the cationic CS leaded to the achievement of positive ζ-potential values (+34.8 mV). Overall, an obvious alternate ζ-potential value with sequential deposition of polysaccharides was observed. This suggested successful alternate adsorption of ALG and CS on the Pickering emulsions.

The deposition of ALG and CS on planar surfaces by means of QCM-D technique was applied to simulate the assembly behaviour on emulsion templates (Fig. 4b). The decrease of $-\Delta f/n$ and increase of D after each polyelectrolyte adsorption step demonstrated that mass was being deposited on the PEI/Laponite hybrid surface. The first layer of ALG only showed a decrease in frequency of 40 Hz, while for the other polyelectrolyte layer, a decrease in frequency of about 100 Hz was observed. Presumably, the inhomogeneous and incomplete coating layer of PEI/Laponite on Au surface, whereby there was not enough positive charge, resulted in difficult adsorption of the first ALG layer. Actually, PEI was usually chosen to firstly complete coating on the Au surface, ensuring uniform and enough charge to support LbL assembly.[38] Further adsorption of polyelectrolytes generated stable frequency decrease due to uniform charged coating after ALG and CS deposition. With the increment of adsorption number of polyelectrolyte, only small change of D was attributed to a transformation from a dissipative viscoelastic film to a compact and rigid structure, given that the change of each frequency remained unchanged.[39,40]

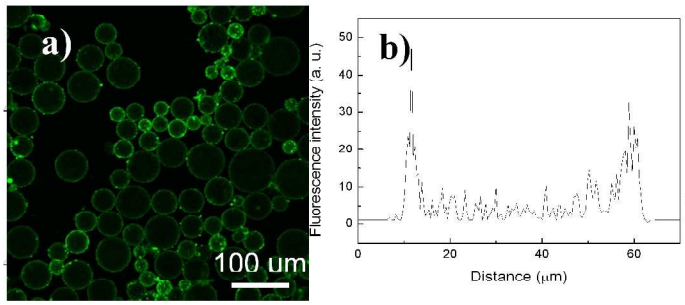

Fig. 5 (a) Confocal microscopy of $(ALG/CS)_4$ microcapsules with oil cores. (b) Corresponding fluorescence intensity profile along the line indicated in (a).

ALG was labelled by FITC, the CLSM image is shown in Fig. 5a. The microcapsules exhibited a highly localized green fluorescence in their shells. Besides, fluorescence intensity profiles along its diameter demonstrated green fluorescence existed in the microcapsules shell, with just a small trace of green fluorescence in the core of the microcapsule (Fig. 5b). This result with the experiment data of ζ-potential, QCM-D experiments allowed us to confirm that the ALG and CS were successfully assembled on the surface of Pickering emulsion droplets.

Furthermore, LbL assembly on Pickering emulsions was assessed by a THF challenge. Intact microcapsules are observed by optical microscopy after inserting a small sample of the microcapsules suspension into excess THF (Fig. S2b). In contrast, only a small number of droplets were observed when the original Pickering emulsions were subjected to THF challenge (Fig. S2a). Most of emulsion droplets broke up immediately after addition of THF. It also proved that the microcapsules coated by ALG and CS were successfully prepared and displayed that the microcapsules had a high stability against to outside environment, which is important in the further potential applications. [9,17]

Characterization of Hollow Microcapsules

The FTIR (Fig. S3) spectrum indicates the component of the hollow microcapsules. The characteristic peaks of above materials, such as 1417 cm^{-1} ($ALG–COO^+$), 1380 cm^{-1} ($–CH_2$ bending, CS), 1355 cm^{-1} (C-N stretching, PEI), 1006 cm^{-1} (Si–O–Si stretching frequencies, Laponite), were presented in the FTIR spectra of the microcapsules (Fig. S3c), indicating that we had successfully fabricated composite microcapsules.

Optical images of four bilayers sodium alginate/chitosan ($(ALG/CS)_4$) microcapsules before and after the complete removal of xylene by washing with excess 2-propanol are presented in Fig. S4. The morphologies and droplet diameters before core removal (Fig. S4a) were identical to the cleaning emulsion in Fig. 2c and the microcapsules in Fig. 5a. After core removal, hollow microcapsules (Fig. S4b) were obtained with similar size. Because the shells of microcapsules consisted by nature polyelectrolyte are very thin, the microcapsules after core removal are difficulty to be found in the condition of optical microscope.

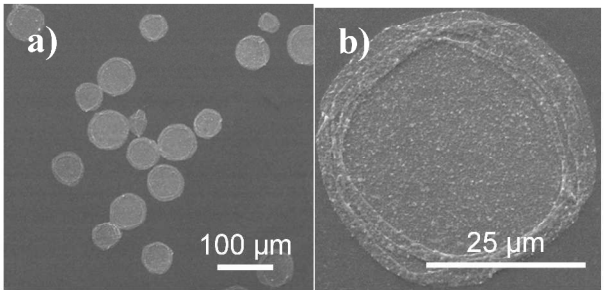

Fig. 6 (a) SEM images of $(ALG/CS)_4$ microcapsules with water cores after drying. (b) The high magnification image.

The surface morphology of the hollow microcapsules was also investigated by means of SEM. Fig. 6 shows SEM images of hollow $(ALG/CS)_4$ microcapsules. All microcapsules preserved intact and well dispersed sphere structure, which were similar to the morphologies in Fig. S4b. This intact structure may be attributed to low osmotic pressure induced by oil dissolved in 2-propanol. As expected, the ζ-potential value of hollow microcapsules showed a positive charge, given that the outer layers were coated by cationic CS, which accounted for the well dispersed microcapsules in the SEM images. There were many creases and folds in the microcapsules because of the collapse during the drying process caused by the evaporation of the aqueous. It was noteworthy that the textures of the microcapsules had a "grainy" appearance (more noticeable in Fig. 4b), probably

reflecting the presence of Laponite particles inside the microcapsules after the core dissolution process, or local aggregation of the polyelectrolyte components.[42,43]

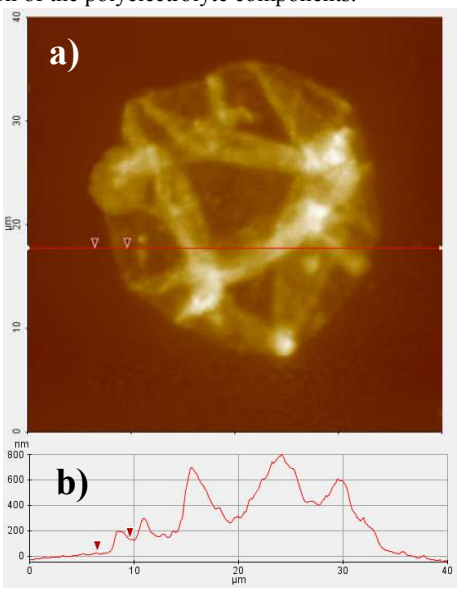

Fig. 7 (a) Confocal microscopy of $(ALG/CS)_4$ microcapsules with oil cores. (b) Corresponding fluorescence intensity profile along the line indicated in (a).

The structural study of the hollow microcapsules was further complemented with AFM observations (Fig. 7). A single hollow $(ALG/CS)_4$ microcapsule also exhibited many folds and creases, which was in accord with the SEM images. AFM measurements showed that the thickness of $(ALG/CS)_4$ microcapsules reached about 110 nm. Given that the measured height is twice the thickness of a single microcapsule wall. The height was estimated to be about 55 nm (Fig. 7b). This height was much larger than previous $(ALG/CS)_n$ microcapsules[43] made by LbL assembly, which was attributed to existence of Laponite, or local polyelectrolyte aggregation, as described above.

Controlled release from $(ALG/CS)_n$ microcapsule

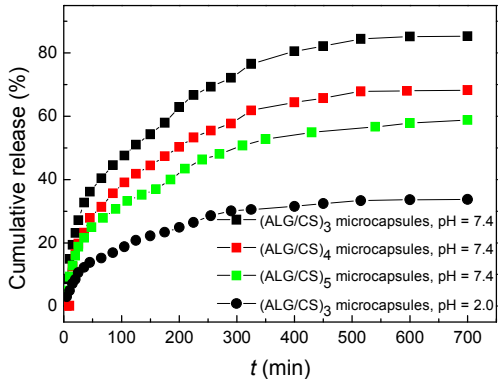

Fig. 8 IBU release from $(ALG/CS)_n$ microcapsules at pH 7.4 (squares) or 2.0 (circles).

Nanocomposite microcapsules composed of natural polyelectrolytes of ALG and CS were widely studied in controlled release filed.[4,43] IBU is widely used as a clinical drug, which is a good candidate for the development of oral controlled release formulations. The research on IBU delivery and release has attracted many interests. In this part, IBU as a model drug was loaded into $(ALG/CS)_n$ microcapsules. The loading time was fixed at two hours, that was long enough for IBU loading of $(ALG/CS)_n$ microcapsules. The loading capacity of IBU in the microcapsules was determined by the difference drug concentrations in PBS before and after loading, and the capacity value was 150 mg g^{-1}, which is comparable to other CS microcapsule.[44]

The IBU release from the $(ALG/CS)_3$ microcapsules as a function of time was investigated at pH 7.4 and 2.0 under 37 ^oC (Fig. 8). It's observed that IBU was gradually released at pH 2.0, where only about 33.7 % of the loaded IBU was released after 11 hours. Compared to the release at 2.0, the release of IBU at pH 7.4 increased sharply with prolonging release time (within 100 min), and then the release rate decreased until a equilibrium after 500 min with a cumulative release amount of 85.3 %. This release is the typical release pattern of IBU from ALG/CS microcapsules.[4] This significant difference of IBU release at different pH derived from a different solubility of IBU at pH 7.4 and 2.0.[4,44] IBU molecule exhibits a better solubility at pH 7.4 than at pH 2.0 because that it is an acidic molecule. The initial rapid release of IBU mostly could be attributed to the release of IBU on the surface and near the exterior surface.

Then, $(ALG/CS)_3$, $(ALG/CS)_4$ and $(ALG/CS)_5$ microcapsules were prepared to investigate the effect of different adsorption double layers on IBU release. IBU-loaded $(ALG/CS)_4$ microcapsules were obtained by additional polyelectrolyte layers coated on IBU-loaded $(ALG/CS)_3$ microcapsules, and IBU-loaded $(ALG/CS)_5$ microcapsules were obtained by additional polyelectrolyte double layers coated on IBU-loaded $(ALG/CS)_4$ microcapsules. During self-assembly based on previous microcapsule and washing process, the loss dosage of IBU was 25-35 wt%. The IBU release from these three microcapsules at pH 7.4 is also shown in Fig. 8. As expected, with increasing polyelectrolyte double layers, the release rate was obviously reduced and the remaining IBU in the microcapsule increased correspondingly, indicating that additional deposited layers on former microcapsules can result in more obstacles in the release channel of IBU.[44] Thus, the permeability of hollow microcapsules can be controlled by tuning adsorption layers coated on Pickering emulsion template.

Conclusion

In summary, ALG/CS multilayer microcapsules were firstly fabricated through the LbL self-assembly on Pickering emulsion template. No matter what were the polarity and viscosity of oils, PEI/Laponite based Pickering emulsions exhibited good long-term stability for more than 2 months at PEI/Laponite mass ratio of 0.50 and Laponite concentration of 0.25 wt%. The Pickering emulsion not only provided high stable LbL assembly template that could efficiently suppress deformation and flocculation of emulsion droplets during LbL assembly process, but also ensured the control of the size distribution of the resulting microcapsules.

Hollow microcapsules were obtained after core removal after washing with excess 2-propanol. The mild washing process could preserve the integrality and mechanism strength of the hollow microcapsules. The experimental data confirmed that the successful formation of ALG and CS multilayer on the Pickering

emulsion template, and the formation of intact and well dispersed hollow $(ALG/CS)_n$ microcapsules. The size of microcapsules can be tuned from several to tens of micrometers by varying the size of Pickering emulsion template, which was easily changed by either PEI/Laponite mass ratio or Laponite concentration.

IBU-loaded microcapsules were prepared by incubating these microcapsules into IBU solution of PBS, and the release rate of IBU from $(ALG/CS)_n$ microcapsules was obviously faster at pH 7.4 than the release rate at pH 2.0. The permeability of the microcapsules can be controlled by tuning adsorption layers coated on the Pickering emulsion template according to the particular final purpose. The more polyelectrolyte layers of IBU-loaded microcapsules, the more difficult for IBU release.

Herein, the proposed approach to prepare biocompatible polyelectrolyte microcapsules via Pickering emulsion template can be considered as a general method that can be easily extended to other emulsion systems. Besides, nanocomposite microcapsules composed of nature polysaccharide prepared by Pickering emulsion templated LbL assembly method offer great potential applications in the food and pharmaceutical industries.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Optical micrographs, THF challenge experiments, FTIR spectra. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

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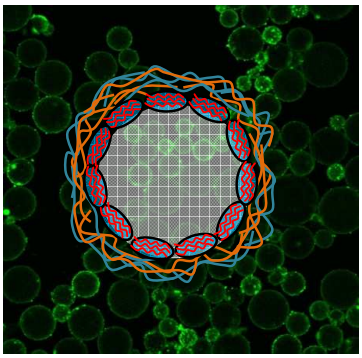
A table of contents entry



Nanocomposite multilayer microcapsules are prepared by layer-by-layer self-assembly based on Pickering emulsions.

Electronic Supplementary Information

Facile fabrication of nanocomposite microcapsules by combining layer-by-layer self-assembly and Pickering emulsion templating

Hao Liu, Xiaoyu Gu, Meng Hu, Yang Hu, and Chaoyang Wang*

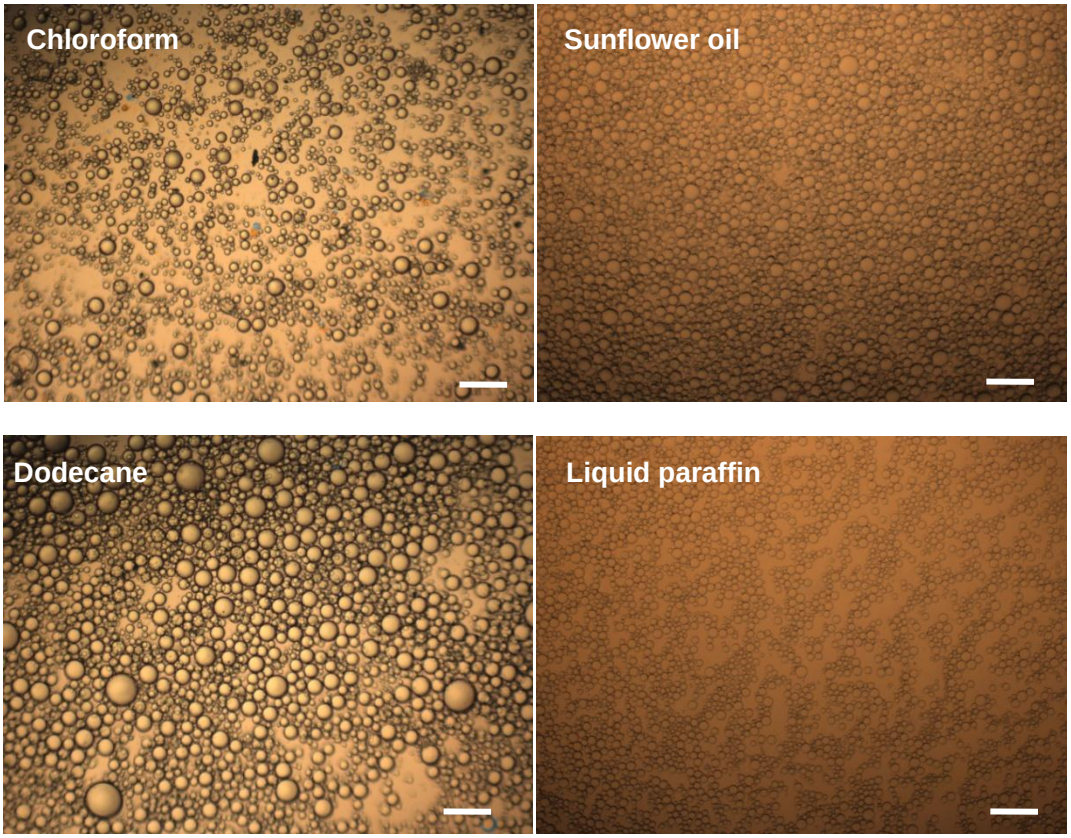


Fig. S1 Optical micrographs of Pickering emulsions prepared using different oils. The concentration of Laponite is 0.25 wt%, PEI/Laponite mass ratio is 0.5. All scale bars represent 100 μm.

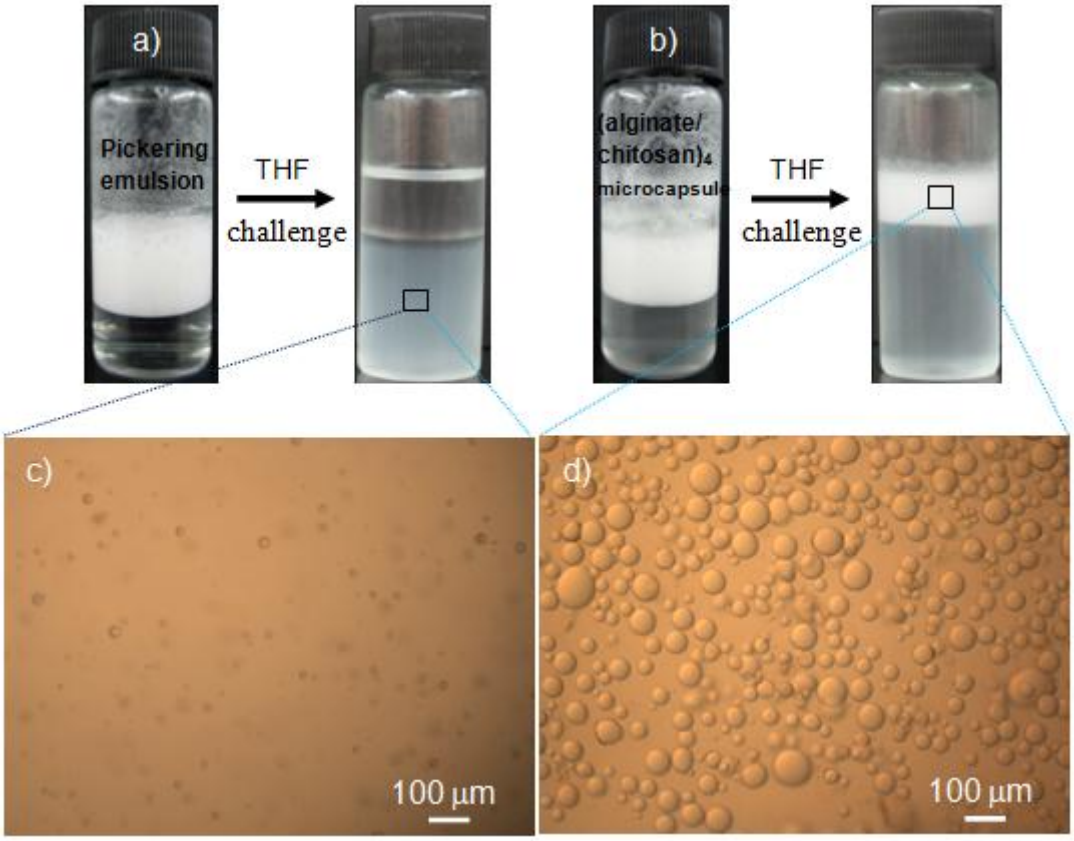


Fig. S2. Digital photographs of the clean Pickering emulsion (a) and $(ALG/CS)_4$ microcapsules (b) following a THF challenge. Optical microscopy images of the clean Pickering emulsion (c) and $(ALG/CS)_4$ microcapsules (d) after a THF challenge. Laponite concentration is 0.25 wt. %. PEI/Laponite mass ratio is 0.5.

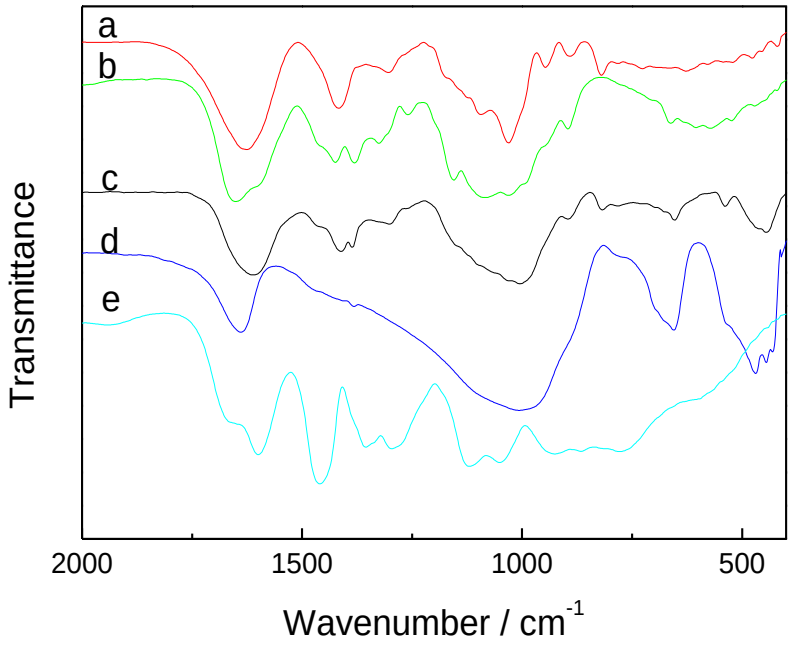


Fig. S3. FTIR spectra of (a) ALG, (b) CS, (c) microcapsule, (d) Laponite, and (e) PEI.

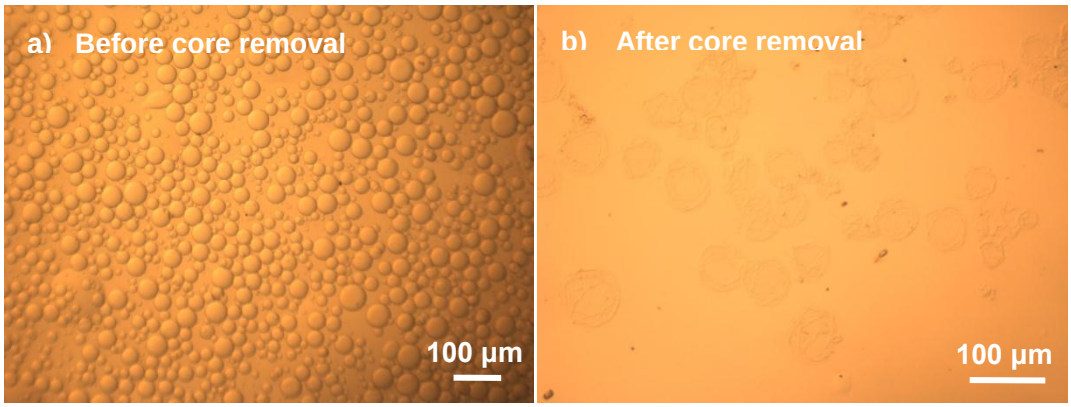


Fig. S4. Optical images of the $(ALG/CS)_4$ microcapsules before and after core removal.