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Communication

Biocompatible microcapsules with a water core templated from single emulsions

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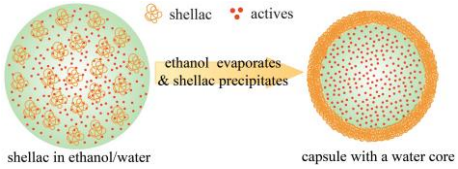

We use single emulsions as templates to fabricate monodisperse biocompatible microcapsules with a water core. These microcapsules are fabricated using FDA-approved polymer and non-toxic solvents and are of great use in drugs, cosmetics and foods.

ABSTRACT

Biocompatible microcapsules with a water core are widely used to encapsulate hydrophilic actives. Here, a facile method to fabricate monodisperse biocompatible microcapsules with a water core in large quantity is reported. Microfluidic technology is utilized to emulsify the inner aqueous phase containing the shell polymer into monodisperse drops in the outer oil phase. As the cosolvent in the inner aqueous phase diffuses into the outer oil phase, the solubility of the shell polymer decreases, which eventually precipitates. Since the shell polymer, shellac, contains both hydrophilic and hydrophobic groups, it tends to wet both the inner aqueous phase and the outer oil phase, thus forming a solid shell at the periphery of the drop. We show that the diffusion rate of hydrophilic molecules encapsulated in the water core decreases as their molecular weight increases and the property of the microcapsules could further be modified by polyelectrolyte multilayer coating. These microcapsules are fabricated using FDA-approved polymer and non-toxic solvents and are of great use in drugs, cosmetics and foods.

Keywords: Microcapsule, Microfluidic, Biocompatible, Single emulsion,

Hydrophilic actives

Microcapsules with a core-shell structure are ideal vehicles for delivery and have been used in various applications [1]. The core of the microcapsules provides an amber space to encapsulate the desired materials and the shell protects the materials in the core from the harsh external environments, such as UV radiation. With the delicate core-shell microstructure, microcapsules can further be functionalized to achieve desired properties, such as stimuli-responsive release, targeted release, sustained release and burst release [2]; these functions are able to meet the specific requirements in different applications. To encapsulate hydrophobic materials, it generally requires an oil core in the microcapsules, while an aqueous core is preferred to encapsulate hydrophilic materials. In our daily life, a lot of active materials [3], such as insulin [4], vitamin C [5,6], peptides [7] and probiotics [8], are hydrophilic; therefore, microcapsules with a water core are important carriers to encapsulate these materials and achieve desired functions.

To fabricate microcapsules with a water core, different techniques have been developed, for example, interfacial polymerization [9] and internal phase separation [10]. However, to be used in drug delivery, foods and cosmetics, microcapsules are preferentially fabricated using biocompatible polymers and nontoxic solvents; solidification of biocompatible polymers in the shell from water-in-oil-in-water (w/o/w) double emulsions is a promising green method to meet these requirements. Traditionally, w/o/w double emulsions are prepared by two separate steps of emulsification. The inner aqueous phase is first dispersed in the middle oil phase dissolved with the shell polymer. The middle oil solution containing the w/o single emulsions is subsequently emulsified in another outer aqueous phase, forming w/o/w double emulsions. As the solvent in the middle oil phase evaporates, the polymer precipitates, forming a solid shell [11]. However, the widespread application of the method is limited by its poor encapsulation efficiency, since a lot of double emulsions are broke in the second emulsification step, and its poor control over the size of the microcapsules and thus their release profile.

With the advent of microfluidic technology, the size and the internal structure of w/o/w double emulsions can be precisely controlled [12,13]. However, the scale-up of w/o/w double emulsions fabricated by microfluidic technology remains a big challenge, which

strongly limits their industrial applications. An alternative way to produce monodisperse microcapsules with a water core, which holds a great promise for scale-up, is to use single emulsions as templates; the shell materials are co-dissolved in the inner phase using a cosolvent. When the inner phase is emulsified in the outer phase and the cosolvent subsequently diffuses into the outer phase, the shell materials under delicate control could gradually precipitate at the periphery of the drops and ultimately form a solid shell, encapsulating hydrophilic materials in the water core. Different from double emulsions, the scale-up of single emulsions with tunable size using microfluidic techniques is already realized [14-18]. However, there are still limited examples of fabricating microcapsules with a water core from single emulsions, especially using biocompatible polymer and non-toxic solvents through the whole process.

In this paper, we develop a green method to produce biocompatible microcapsules with a water core, having uniform and tunable size. We use ethanol as our cosolvent and FDA-approved shellac as our shell material, both of which are non-toxic and biocompatible. We dissolve shellac and hydrophilic actives together in the ethanol/water mixture. We then emulsify the solution into drops in the outer oil phase using a flow-focusing microfluidic device. When ethanol continuously diffuses into the outer phase, the solubility of shellac decreases and eventually precipitates at the periphery of the drops, forming a solid shell. The encapsulated hydrophilic molecules in the water core are subsequently released in a controlled manner. Our work provides a facile route to fabricate monodisperse biocompatible microcapsules, which are promising carriers for the applications, such as drug delivery.

The procedure to fabricate the microcapsules is schematically illustrated in Fig. 1. The inner aqueous phase of shellac in the ethanol/water mixture is emulsified in a flow-focusing microfluidic device, forming monodisperse drops in the outer oil phase, as shown in Fig. 1a. After emulsification, the drops are collected in an open vial. When ethanol diffuses into the oil phase and then evaporates into the air, the solubility of shellac in the drop decreases and eventually precipitates at the periphery of the drop, forming a microcapsule with a water core, as illustrated in Fig. 1b. Because shellac barely dissolves in DI water (deionized water), its solubility in the ethanol/water mixture decreases dramatically when the concentration of water is more than 22.5 vol%, as shown by the blue curve in Fig. 1c. However, the ethanol/water mixture with a water content below 22.5 vol% has a very low surface tension and could not be emulsified. Therefore, we use a small amount of Na_2CO_3 to increase the solubility of shellac in the ethanol/water mixture with a higher water content, thus increasing the surface tension of the inner aqueous solution, as shown by the red curve in Fig. 1c. A typical solution of the inner phase used in the study contains 25 mg/mL shellac and 0.33 mg/mL Na_2CO_3 in the ethanol/water mixture with a volume ratio of ethanol:water = 2:1.

In experiment, monodisperse water-in-oil (w/o) single emulsions are generated in a PDMS microfluidic device, operating in the dripping regime, as shown in Fig. 2a. The uniform drops of shellac in the ethanol/water mixture are dispersed in the oil phase and collected in an open vial, which allows the evaporation of ethanol into the air, as shown in Fig. 2b. As the water concentration increases and the solubility of shellac decreases, shellac eventually precipitates at the periphery of the drops and forms a solid shell around the water core. The shells are relatively robust and the microcapsules maintain their spherical shape even when they are dried, which is directly confirmed by the SEM image shown in Fig. 2c. Since the microcapsules are fabricated using single emulsions as templates, the desired size of the microcapsules could be achieved by adjusting the flow rates, changing the dimensions of the device or varying the concentration of water in the inner aqueous phase. The facile control over the size of the microcapsules is particularly important in food-related applications. Because the tongue could barely resolve the texture of the microcapsules when they are smaller than 20 μm, it could barely sense the granular texture of our microcapsules with an average diameter of 9.87 ± 0.66 μm. To further confirm the core-shell microstructure, the shells of the microcapsules are broke by crushing the microcapsules using a sharp blade or by vigorous shaking and the empty cores are directly visualized, as shown in Figs. 2d and e, respectively. The shell thickness estimated from the SEM image is ~0.8 μm.

Generally, the final morphology of the w/o single emulsions after solvent evaporation and polymer precipitation is determined by the spreading coefficients and they could form core-shell, acorn, heteroaggregated or occluded structures [19]. The core-shell morphology is mainly governed by the spreading coefficient of the middle shellac shell, S_s, which is defined as $S_s = \gamma_{wo} - \gamma_{ws} - \gamma_{so}$, where γ_{wo}, γ_{ws}, and γ_{so} are the interfacial tensions between the water and oil, the water and shellac, and the shellac and oil, respectively. To form the core-shell structure, it requires shellac to preferentially wet both the water and oil phases, that is $\gamma_{wo} > \gamma_{ws} + \gamma_{so}$, as illustrated in Fig. 3a. The dual wettability of shellac benefits from its intrinsic chemical structure, which contains both hydrophilic groups, such as the alkoxy groups highlighted in red circles, and hydrophobic groups, as shown in Fig. 3b. To quantitatively calculate the spreading coefficient S_s, we measure the contact angle of an oil drop on the shellac surface and calculate the interfacial tension between them using Young's equation, $\gamma_{sv} = \gamma_{sl} + \gamma_{lv}\cos\theta$, where γ_{sv}, γ_{sl} and γ_{lv} refer to the interfacial tension between the solid and vapor phases, the solid and liquid phases, and the liquid and vapor phases, respectively, and θ is the contact angle, as shown in Fig. 3c. If we take the interfacial tension of shellac in air γ_{sv} ~36 mN/m [20], the interfacial tension of fluorocarbon oil in air γ_{ov} ~17.1 mN/m [21], and the contact angle θ ~14°, the calculated interfacial tension between the shellac and oil γ_{so} is ~19.41 mN/m. Similarly, the calculated interfacial tension between the water and shellac γ_{ws} is ~6.71 mN/m, as shown in Fig. 3d. Therefore, the spreading coefficient of the middle shellac shell, $S_s = \gamma_{wo} - \gamma_{ws} - \gamma_{so}$ = 45 mN/m – 6.71 mN/m – 19.41 mN/m = 18.88 mN/m [22], is indeed positive, and shellac, which is amphiphilic and similar to surfactants, prefers to precipitate and solidify at the periphery of the drops.

To test the release profile of hydrophilic molecules from the microcapsules, we use FITC-dextran of different molecular weight as our model actives and encapsulate them in the core. The hydrophilic molecules and shellac are dissolved together in the inner aqueous

solution. Due to the strong affinity, the hydrophilic molecules remain dissolved in water during ethanol evaporation and shellac precipitation and are thus automatically encapsulated in the water core of the microcapsules. We disperse the microcapsules in a water reservoir and monitor the release of hydrophilic materials from the microcapsules over time by measuring the UV-vis absorption of FITC-dextran in the water reservoir at 494 nm. Small hydrophilic molecules diffuse into the water reservoir quickly and their diffusion rate decreases when their molecular weight increases, as shown in Fig. 4. The results suggest that the polymer matrix of the shellac shell is relative incompact as it is prepared by solvent evaporation and polymer precipitation, and thus these microcapsules are more useful for the sustained release of large molecules. Since the microcapules are made of FDA-approved material and the solvents are green, the prepared microcapsules are readily edible. We encapsulate caffeine in the core so that the microcapsules could mask the bitter taste of caffeine but quickly release it in the stomach, as shown by the black curve in Fig. 4.

Generally, the diffusion rate of materials from the microcapsules could be tailored by polyelectrolyte multilayer (PEM) coating of the microcapsules, which provide an additional barrier at the surface. When dispersed in water, the microcapsules are negatively charged due to the partial ionization of carboxylic groups at the surface. Therefore, positive polyelectrolytes could electrostatically bind to the surface of shellac microcapsules. However, due to the complex configuration of polymers, there are extra positive charges left at the outer surface, making the microcapsules positively charged. The microcapsules could subsequently be coated with a layer of negative polyelectrolytes and the extra charges at the outer surface make the microcapsules negatively charged again. The process could be repeated, as illustrated in Fig. 5a. The surface of neat microcapsules are smooth, as shown in Fig. 5b, and the changes of the surface morphology after coating with a layer of positive polyelectrolytes (PDDA) and then a layer of negative polyelectrolytes (PAA) are obvious, as shown in Fig. 5c and d, respectively.

A current challenge for the applications of microcapsules in the fields of drugs, foods and cosmetics is fabricating them using biocompatible polymer and non-toxic solvents through the whole process. Here, we present a green method to make biocompatible microcapsules that encapsulate hydrophilic actives in the water core. We use shellac as our shell material, which preferentially wets both the water and oil phases and thus is able to form a solid shell at the periphery of the water drops upon solvent diffusion and polymer precipitation. Since the microcapsules are fabricated using single emulsions as templates, their size and internal structure can be precisely controlled and their production can easily be scaled up. The technique can be extended to encapsulate various hydrophilic molecules in the microcapsules and opens up a wide range of applications. The green recipe could also possibly simplify some complicated structure. For example, if we use oil-in-water-in-oil double emulsions as templates, we would expect to obtain microcapsules with an oil-in-water core, which are previously only achievable when using oil-in-water-in-oil-in-water triple emulsions as templates.

Acknowledgments

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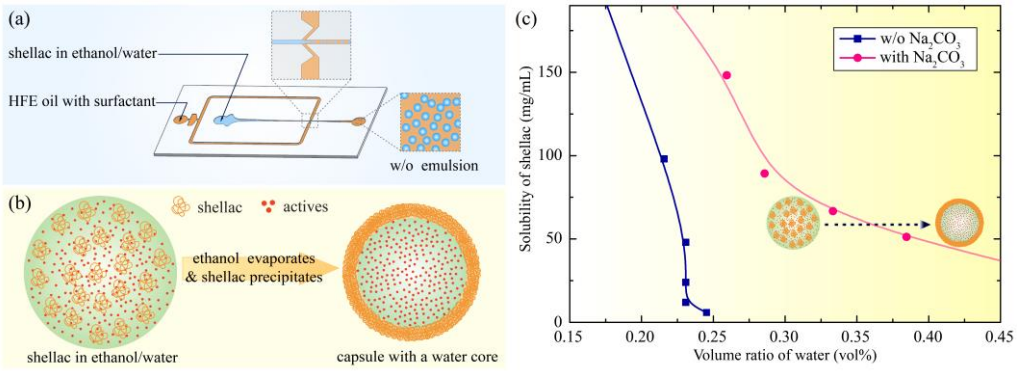

Fig. 1. Schematic illustration of the formation of microcapsules upon ethanol evaporation and shellac precipitation. (a) The inner aqueous solution of shellac dissolved in the ethanol/water mixture is emulsified in a flow-focusing microfluidic device, forming monodisperse w/o single emulsions. (b) As the cosolvent, ethanol, evaporates, the solubility of shellac in the drop decreases and shellac eventually precipitates at the periphery of the drop, forming a solid shell that encapsulates the hydrophilic actives in the water core. (c) Solubility of shellac in the ethanol/water mixtures. The solubility of shellac decreases dramatically when the concentration of water is greater than 23 vol% (blue curve). To increase the solubility of shellac in the ethanol/water mixture with a higher water content, thus increasing the surface tension of the inner aqueous solution, a small amount of Na_2CO_3 is added (red curve).

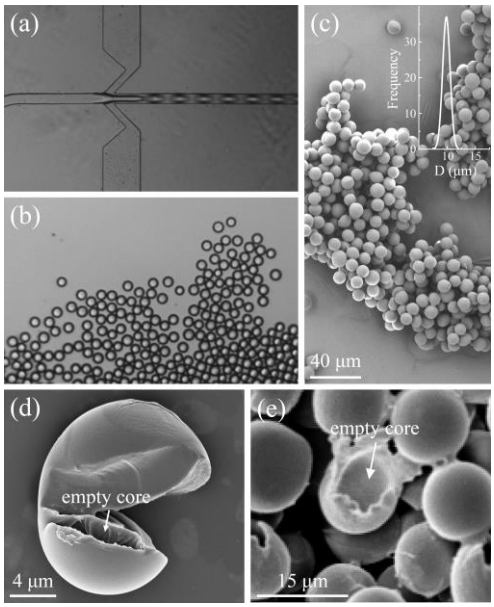

Fig. 2. Preparation and characterization of microcapsules with a water core. (a) Snapshot of a PDMS microfluidic device generating monodisperse w/o single emulsions by flow focusing. (b) Uniform drops of shellac dissolved in the ethanol/water mixture, which are dispersed in the outer oil phase and collected in an open vial. (c) SEM image of solid microcapsules after ethanol evaporation and shellac precipitation. The inset shows the distribution of the microcapsules' diameter. (d) and (e) The empty core of dried microcapsules is revealed by crushing the microcapsules using a sharp blade or by breaking the shell *via* vigorous shaking, respectively.

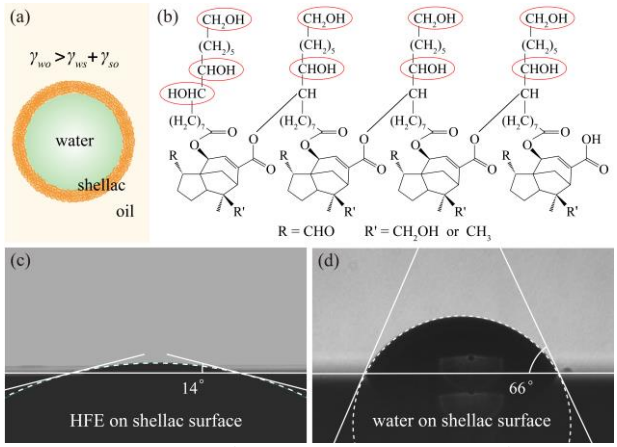

Fig. 3. Wetting property of shellac. (a) The precipitation of shellac at the periphery of the water drop depends on the spreading parameter, which requires shellac to wet both the inner water and outer oil phases ($\gamma_{wo} > \gamma_{ws} + \gamma_{so}$). (b) The chemical structure of shellac, which contains both hydrophobic and hydrophilic (highlighted in red circles) groups. (c) The contact angle of a fluorocarbon oil drop on the shellac surface is ~14°. (d) The contact angle of a water drop on the shellac surface is ~66°.

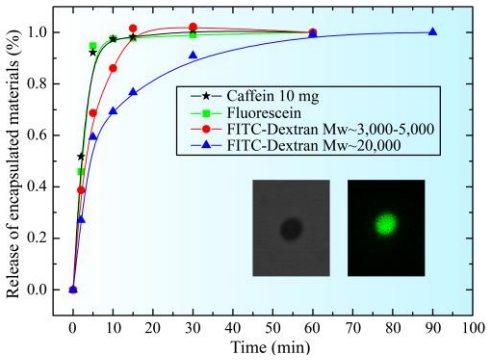

Fig. 4. Release profile of encapsulated hydrophilic materials from the microcapsules. Hydrophilic FITC-dextran molecules of different molecular weight are used as model actives to test the release performance of the microcapsules. As the molecular weight increases, it takes longer time for the molecules to diffuse into the aqueous phase. The inset shows a microcapsule with FITC-dextran encapsulated in the core.

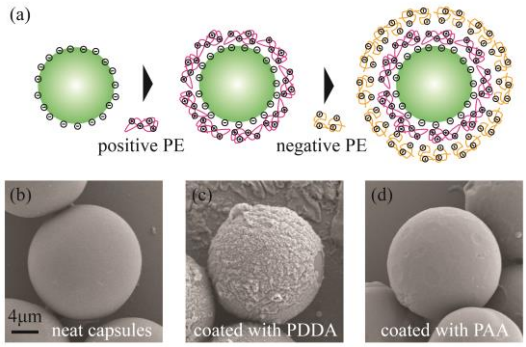

Fig. 5. Polyelectrolyte multilayer (PEM) coating of the microcapsules. (a) Schematic illustration of the PEM coating on the surface of the microcapsules. Due to the partial ionization of the carboxylic groups at the surface, shellac microcapsules are negatively charged. (b) A close-up of shellac microcapsules, showing a smooth surface. (c) SEM image showing the surface morphology of the microcapsules coated with a layer of positive polyelectrolyte, PDDA. (d) The surface morphology of the microcapsules changes again when further coated with a layer of negative polyelectrolyte, PAA.