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We demonstrate a simple synthesis of renewable high internal phase emulsions (HIPEs) featuring great stability and processability, by centrifuging ultrasonication-produced oil-filled polysaccharide microspheres without the aid of any surfactant or synthetic particles. The properties of HIPEs and corresponding porous structure (closed-/open-cell) are controllable by simply adjusting the ultrasonic intensity and ionization of polysaccharides.

High internal phase emulsions (HIPEs) are a unique system defined by an internal phase volume fraction of more than 0.74.[1] HIPEs have shown great potential in various applications.[2–6] Conventional HIPEs are commonly stabilized against coalescence using a large quantity of surfactants,[7] however, this method can be costly and requires careful choice of the surfactant type. As an alternative, particle-stabilized emulsions, also termed Pickering HIPEs, have been extensively developed over the past few years. The irreversible adsorption of particles at oil-water interfaces can effectively protect Pickering HIPEs from coalescence, creaming, and Ostwald ripening.[8] However, the preparation of stable Pickering HIPEs needs the synthesis of Pickering emulsions as well as the use of special amphiphilic particles using amphiphilic block copolymers[9] or other specific molecules.[10,11] Another issue with traditional HIPEs is the use of synthetic surfactants and nondegradable particles, such as titania, silica, and magnetic nanoparticles, which pose potential toxicity risks and environmental drawbacks.

Recently, researchers have increasingly focused on the utilization of renewable and biocompatible particles as stabilizers for HIPEs, such as starch granules,[12,13] proteins,[14,15] cellulose particles,[16,17] gelatin,[18] and chitin nanocrystals.[19] Unfortunately, these natural compounds are plagued with wettability and solubility problems, thus, they are far from robust at stabilizing HIPEs without the help of surface or chemical modification. Protein/polysaccharide complexes, such as gliadin/chitosan,[20,21] have also been applied to develop Pickering HIPEs. However, the formation of these polyelectrolyte complexes requires strict control of the aqueous phase conditions (ionic strength and pH), which restricts their performance as effective stabilizers.

Herein, we propose a facile synthesis of renewable HIPEs solely stabilized by natural polysaccharides, which does not require specialized equipment, the use of any surfactants, colloidal particles, monomers or synthetic polymers. The procedure involves the preparation of polysaccharide microspheres by ultrasonication and subsequent centrifugation, as illustrated in Fig. 1A. Ultrasonic processing can produce oil-filled microspheres featuring a cross-linked polysaccharide shell through both emulsifying effects and acoustic cavitation, which leads to the formation of reactive superoxides that can then cross-link functional groups (e.g., amino and carboxyl) in the polysaccharides located at the microsphere interfaces.[22,23] A high centrifugal field is then applied to concentrate these microspheres and simultaneously exclude excess continuous phase to obtain a high internal phase volume fraction. In this approach, the generation of the cross-linked interfacial shell is crucial to inhibit phase separation at the high centrifugal forces, thus enabling the formation of stable HIPEs.

For proof-of-concept, chitosan and pectin were chosen as dispersed materials in the aqueous continuous phase due to the fact that they are rich in functional groups, including amino, carboxyl, and hydroxyl moieties. We therefore hypothesized that they could be cross-linked at the interface of the ultrasonically produced microspheres sufficiently to protect the oil droplets against coalescence during centrifugation. The facile HIPE synthesis begins with the preparation of individual

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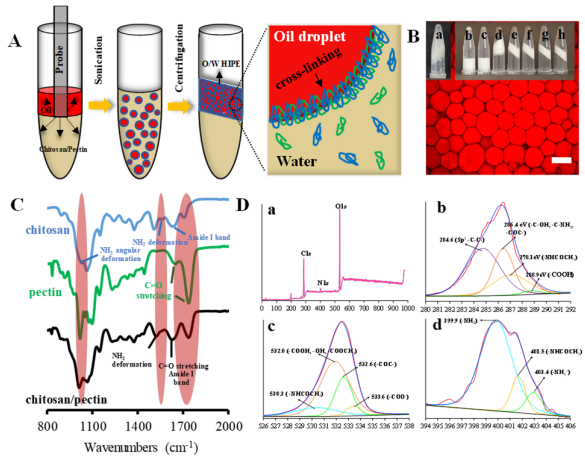

Fig. 1 Formation and characterization of polysaccharide-based HIPEs. (A) Facile synthesis of HIPEs by sequential ultrasonication and centrifugation, and schematic illustration of cross-linking at the oil-water interface. (B) CLSM image from the cream layer shows a typical HIPE structure (10000 *g* for 5 min). Inset shows the visual appearance of polysaccharide/corn oil mixture prepared by (a) homogenization after centrifugation at 10000 *g* for 5 min, and (b-h) ultrasonication (300 W cm^{-2}), (b) before and after centrifugation at (c) 100, (d) 500, (e) 2000, (f) 5000, (g) 10000, and (h) 15000 *g* for 5 min. The scale bar is 1 µm. (C) FTIR spectra of pure chitosan, pure pectin, and sonicated mixture of chitosan and pectin. (D) XPS survey spectra (a) of the sonicated mixture of chitosan and pectin, and high resolution (b) O 1s, (b) C 1s and (d) N 1s spectra. HIPEs were synthesized with polysaccharide solution (0.5 wt%) as the continuous phase and corn oil as the dispersed phase and.

chitosan and pectin aqueous solutions (0.5–1.5 wt%), whose pH was adjusted to 2 so that the molecules were positively charged and would not form polyelectrolyte complexes via electrostatic attraction after mixing. We then ultrasonicated 8 mL of this polysaccharide solution with 4 mL corn oil with the ultrasonic probe positioned at the aqueous-organic interface. The resultant oil-filled polysaccharide microsphere suspension was then subjected to centrifugation, producing an oil-in-water HIPE layer. For comparison, we also prepared a mixture by mechanically mixing the oil and polysaccharide solutions using a homogenizer (see supporting information).

Fig. 1B shows that the homogenized mixture underwent severe phase separation after centrifugation. This was not surprising because high centrifugal force compresses the microspheres into contact with each other, causing extensive coalescence and ultimately destabilizing them (Fig. S1). However, when the mixture was emulsified by ultrasonication, a solid cream layer created on the top of the aqueous phase after centrifugation, which can hold its own weight even when the tube is inverted. We can also modulate the volume fraction of oil in the cream by simply changing the centrifugation speed. At 2000, 5000, 10000, and 15000 *g*, the internal volume fractions were around 79.4%, 82.1%, 86.0%, and 87.8%, respectively. Confocal laser scanning microscopy (CLSM) of the

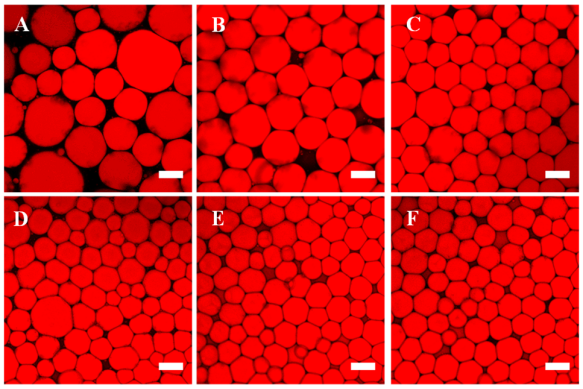

Fig. 2 CLSM observation of HIPEs prepared at different ultrasonic intensities and polysaccharide concentrations: (A) 150 W cm^{-2}, (B) 225 W cm^{-2}, (C) 300 W cm^{-2}, and (D) 375 W cm^{-2} at 0.5 wt% polysaccharide concentration, (E) 1.0 wt% and (F) 1.5 wt% polysaccharide concentration at 375 W cm^{-2}. All scale bars are 1 µm. HIPEs were synthesized with polysaccharide solution (0.5%) as the continuous phase and corn oil as the dispersed phase.

cream layer demonstrated a structure representative of HIPEs, in which the microspheres were closely packed and deformed into polyhedral shapes. Note that further increasing centrifugal force (15000 *g*) would lead to merging of some droplets but without phase separation (Fig. S2). We also demonstrated that HIPEs cannot be formed by either chitosan or pectin alone (Fig. S3), suggesting the key role of cross-linking between chitosan and pectin in HIPEs stabilization.

Fourier transform infrared (FTIR) spectra in Fig. 1C show that after ultrasonication of chitosan/pectin mixture, the peak at 1558 cm^{-1} derived from NH_2 deformation in chitosan shifted to 1523 cm^{-1} and the NH_2 angular deformation at 1031 cm^{-1} disappeared. Meanwhile, the band of C=O stretching of free carboxyl groups in pectin shifted to a lower wavenumber (1627 cm^{-1}) and merged into a broad peak with the amide I band of chitosan. The X-ray photoelectron spectroscopy (XPS) spectra (Fig. 1D) shows the appearance of -NHCO- bonding energy at 278.1, 530.3 and 401.5 eV in C 1s, O 1s and, N 1s, respectively. Thermogravimetric analysis demonstrated an additional peak at high temperature (around 516 °C) after sonicating chitosan and pectin mixture (Fig. S4). All these findings proved the existence of cross-linking via amide linkage between chitosan -NH_2 groups and pectin -COOH groups through amide linkage. Such interfacial cross-linking appears to have enhanced the stability of the microspheres against the high centrifugal forces, enabling the formation of the HIPE. Having determined the feasibility of this strategy, we then further evaluated the influence of ultrasonic intensity (150-375 cm^{-2}) and polysaccharide concentration (0.5-1.5 wt%) on the properties of HIPEs. We found that whatever the ultrasonic intensity applied, HIPEs can be produced successfully without any coalescence (Fig. 2). The increase in the ultrasonic intensity led to smaller microspheres, with the high energy input breaking up larger microspheres to form the homogeneous suspension. Small microspheres also

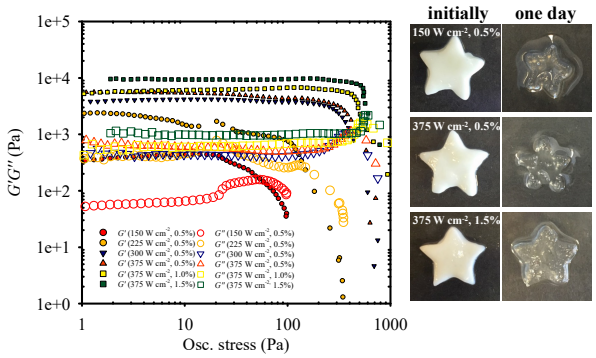

Fig. 3 Stress sweep of the storage (*G'*) and loss (G'') moduli of the HIPEs synthesized at different ultrasonic intensities (150-375 W cm^{-2}) and polysaccharide concentrations (0.5-1.5 wt%). HIPEs were synthesized with polysaccharide solution as the continuous phase and corn oil as the dispersed phase. The photographs at the right show the processability of the HIPEs. Photograph of the star-shaped HIPEs were taken after one-day air drying at room temperature.

favored more closely packed HIPEs. Besides, the polysaccharide concentration did not greatly affect the size of the microspheres or HIPE packing under identical ultrasonic intensity (Fig. 2E-F).

Accompanied by the morphological change, the rheological properties of the HIPEs were altered by the ultrasonic intensity (Fig. 3 and Fig. S5). The frequency sweep measurement presents that the storage modulus *G'* was higher than the loss modulus *G''*, suggesting the formation of stable gelled HIPEs (Fig. S5). Note that as the ultrasonic intensity and polysaccharide concentration increased, the HIPEs exhibited stronger viscoelasticity with higher *G'*, which can be explained by both the formation of the stronger cross-linked network with higher energy input and the higher viscoelasticity of the polysaccharide interfacial layers. The solid-to-liquid transitions in the strain sweep measurement (Fig. 3) further proved that the HIPEs prepared with 1.5% polysaccharides at 375 W cm^{-2} had the yield point of the highest stress value (~620 Pa) to be broken forming fluidic emulsion droplets. Due to their solid viscoelastic gel texture, these HIPEs can be modeled into desired shapes without spreading. The structure of the HIPEs prepared using 375 W cm^{-2} ultrasonic intensity and 1.5 wt% polysaccharide concentration remained self-supporting with very slight oiling off after one-day air exposure.

Furthermore, we can obtain pure polysaccharide foams by using cyclohexane as the oil phase (i.e., the dispersed phase), which can be evaporated during freeze-drying (Fig. 4). Importantly, the porosity and microstructure of these materials could be controlled by adjusting the ultrasonic intensity and the polysaccharide concentration. For the HIPEs prepared at 150 and 225 W cm^{-2} with 0.5 wt% chitosan/pectin, the walls of the microspheres completely collapsed after drying. In contrast, the HIPEs prepared with 300 and 375 W cm^{-2} maintained their integrity, wherein the porosity of the material was controlled by the original microsphere size. More interestingly, increasing the polysaccharide concentration induced the changes from open- to closed-cell structures (375 W cm^{-2} and 1.5 wt% polysaccharides) of the resulting porous materials. We note that more pores were inaccessible at higher polysaccharide concentration. We can conclude that the cross-linked network of polysaccharides became stronger under these conditions and therefore did not rupture to form pore throats during or after freezing.[24]

On the other hand, we calculated the adsorbed amount of polysaccharide on the microspheres (see supporting information) and found that only 41% of polysaccharides can participate the cross-linking reaction during ultrasonication (Table S1). Because of the different ionizations of chitosan (pKa ~6.2) and pectin (pKa ~3.5), we tend to switch the pH (from 2 to 5) of microsphere suspension before centrifugation. Consequently, the excess pectin would become negatively charged with increasing pH, creating an extra layer on the existing positively charged microspheres, and simultaneously interact with the free positively charged chitosan forming polyelectrolyte complexes. This was confirmed by the observation that with increasing pH, the positive charges of microspheres decreased while the particle size increased with the appearance of two intensity peaks (Table S1 and Fig. S6). The morphological observation directly demonstrated the formation of an extra thick layer and complexes coated on the surface of the microspheres at pH 5 (Fig. 5A-B), compared to those at pH 2 (Fig. S7). Accompanying, the participated polysaccharides was increased to 68% at pH 5 (Table S1). After centrifugation, the microspheres were still closely packed forming HIPEs where lots of complexes (i.e., the black dots in

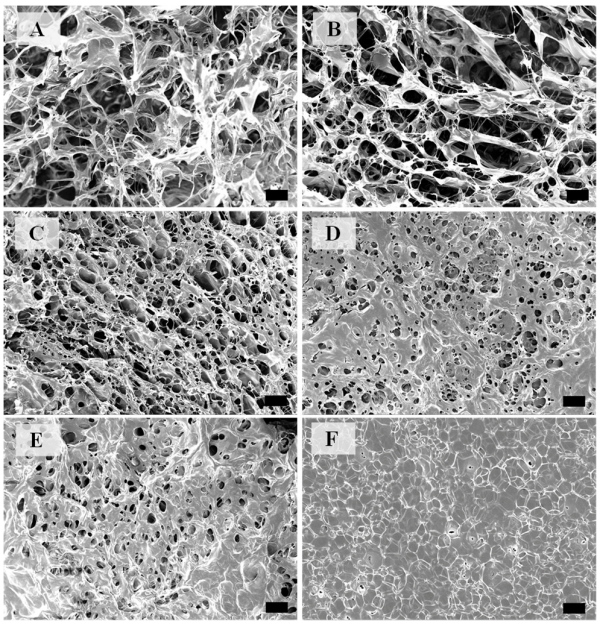

Fig. 4 SEM images of freeze-dried HIPEs prepared at different ultrasonic intensities and concentrations of polysaccharides: (A) 150 W cm^{-2}, (B) 225 W cm^{-2}, (C) 300 W cm^{-2}, and (D) 375 W cm^{-2} at 0.5 wt% polysaccharide concentration, (E) 1.0 wt% and (F) 1.5 wt% polysaccharide concentration at 375 W cm^{-2}. All scale bars are 10 μm. HIPEs were synthesized with polysaccharide solution as the continuous phase and cyclohexane as the dispersed phase.

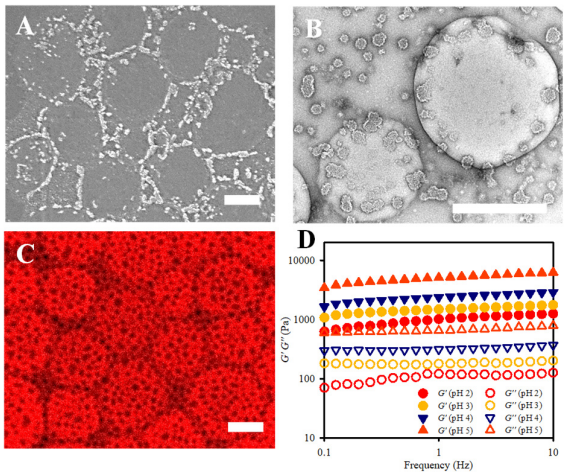

Fig. 5 Influence of extra electrostatic deposition of non-cross-linked polysaccharides. (A) SEM and (B) TEM images of microspheres at pH 5. The microspheres were synthesized by sonicating (375 W cm^{-2}) the polysaccharide solutions (pH 2) and corn oil, followed by adjusting the pH to 5. (C) Corresponding CLSM images of HIPEs. (D) Frequency sweeps of the *G'* and G'' moduli of the HIPEs prepared at different pHs. All scale bars are 1 um.

the CLSM image) attached to the surface of microspheres (Fig. 5C). Particularly, the additional electrostatic deposition further strengthened the HIPEs, as evidenced by the stronger viscoelasticity (Fig. 5D). The storage modulus *G'* for HIPE prepared at pH 5 was increased by approximately 4-fold as compared to that prepared at pH 2 stabilized by the cross-linked shell alone. Finally, we incorporated β-carotene into the HIPEs and evaluate its release in a phosphate buffer of pH 6.2 without and with glutathione (GSH) of 10 mM (simulating the intracellular concentration of GSH). It was found that the release of β-carotene from the HIPEs prepared at pH 2 was accelerated in the presence of GSH, while the HIPEs at pH 5 still maintained a sustained and slow release of β-carotene (Fig. S8), suggesting that the latter hold promise as a long-term delivery system for lipophilic bioactives in cellular environment.

In conclusion, we have developed a novel class of HIPEs made up of polysaccharide microspheres by combining ultrasonication and centrifugation techniques. This approach did not use any surfactants or synthetic particles, and therefore we can fabricate fully natural HIPEs and porous materials for bio-related applications. Furthermore, the morphology, rheology and processability were controllable by simply adjusting the ultrasonic intensity and polysaccharide ionization. The porous polysaccharide foam was also switchable between open- and closed-cell, which demonstrate a new approach to control the microstructure of hierarchical scaffolds by modulating the ultrasonic input and material concentration. Although this study focused on chitosan/pectin for HIPE fabrication, our strategy could be extended to other polysaccharides and polymer materials as long as they can be cross-linked during ultrasonic cavitation process.

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