ACS APPLIED MATERIALS & INTERFACES

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Gelatin Particle-Stabilized High Internal Phase Emulsions as Nutraceutical Containers

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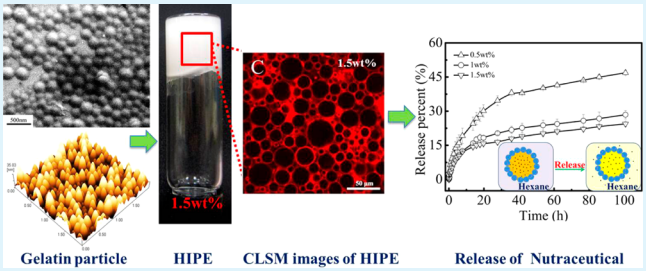
ABSTRACT: In this paper, we report for the first time the use of a well-dispersed gelatin particle as a representative of natural and biocompatible materials to be an effective particle stabilizer for high internal phase emulsion (HIPE) formulation. Fairly monodispersed gelatin particles (~200 nm) were synthesized through a two-step desolvation method and characterized by dynamic light scattering, ζ-potential measurements, scanning electron microscopy, and atomic force microscopy. Those protein latexes were then used as sole emulsifiers to fabricate stable oil-in-water Pickering HIPEs at different concentrations, pH conditions, and homogenization times. Most of the gelatin particles were irreversibly adsorbed at the oil−water interface to hinder droplet coalescence, such that Pickering HIPEs can be formed by a small amount of gelatin particles (as low as 0.5 wt % in the water phase) at pH far away from the isoelectric point of the gelatin particles. In addition, increasing homogenization time led to narrow size distribution of droplets, and high particle concentration resulted in more solidlike Pickering HIPEs. In vitro controlled-release experiments revealed that the release of the encapsulated β-carotene can be tuned by manipulating the concentration of gelatin particles in the formulation, suggesting that the stable and narrow-size-distributed gelatin-stabilized HIPEs had potential in functional food and pharmaceutical applications.



KEYWORDS: *gelatin particle, Pickering emulsion, high internal phase emulsion, controlled release, nutraceutical containers*

INTRODUCTION

In the past few years, there has been growing attention on emulsion structures serving as nutraceutical carriers in the food industry because they not only impart food with new and improved palatability properties but also greatly improve the bioavailability when active compounds are dispersed into small droplets (typically in the range of 0.5−100 μm).[1,2] In particular, a particular kind of emulsion, termed high internal phase emulsions (HIPEs), recently revealed outstanding talent in a vast number of applications including as templates for gels,[3] porous materials,[4−6] foams,[7] and scaffolds[8] and in capillary electrochromatography.[9] HIPEs are commonly defined as highly concentrated emulsions, in which the volume fraction of the internal phase (Φ) exceeds 0.74.[10] The advantages of applying HIPEs in functional food engineering rely on the fact that they possess outstanding large Φ and tunable viscoelasticity properties. Furthermore, the water-dispersible HIPEs provide better microbiological stability by virtue of their very low water activity.[2] Recently, Ribeiro and Cruz[11] reported a carotenoid-loaded oil-in-water (O/W) HIPE stabilized by Tween 20 and xanthan gum, in which the dispersed phase is 94% but the emulsion still exhibited a viscoelastic flow behavior.

Conventionally, HIPEs are stabilized by large amounts (5−50 vol %) of low-molecular-weight surfactants or macromolecules, such as polymers and proteins.[12] Over the past decade, there has been increasing interest in the replacement of surfactants with solid colloidal particles, which has led to an exponential growth in research on particle-stabilized emulsions (often called Pickering emulsions).[13] Colloidal particles are known to adsorb irreversibly at the oil−water interfaces. This characteristic associated with the ability of particles to develop strong interactions at interfaces to form rigid layers protecting the droplets is the origin of the outstanding stability of Pickering emulsions toward coalescence, Ostwald ripening, and creaming. Because of these peculiar properties, particle-stabilized HIPEs have been an active research area during the past decade and bring numerous interesting applications.

However, most of the particles used in these fundamental studies are synthetic or inorganic particles (e.g., silica, hydroxyapatite, and TiO_2 particles) that have greatly limited applicability in cosmetic, pharmaceutical, and food applications. Therefore, the development of environmentally friendly biocompatible particles as effective stabilizers that could replace the hazardous surfactants and be used on a commercial scale is

Received: May 28, 2014
Accepted: August 8, 2014
Published: August 8, 2014

desirable. Latex particles such as proteins,[14] starch granules,[15] and cellulose particles[16] have recently been proposed to stabilize emulsions and, consequently, used in food systems. However, the requirements to serve as an effective particle stabilizer, such as intermediate wettability and insolubility in both liquid phases, are so challenging that not many such biocompatible materials fulfill them. Moreover, these employed particles are often highly polydispersed and poorly characterized. That is why few fully natural materials, especially proteins, are available as effective stabilizers to prepare HIPEs without any surface or chemical treatment.

In that context, herein we report the study of gelatin particles as a new class of natural particle stabilizers of HIPEs and explore the formulated emulsions to serve as nutraceutical carriers, toward food and pharmaceutical applications. It is well-known that gelatin is a denatured, biodegradable, and nonimmunogenic protein obtained by controlled hydrolysis of the triple-helix structure of collagen into single-strain molecules. As an amphiphilic biopolymer, gelatin can easily assemble into different kinds of aggregates under the defined processing conditions of temperature and pH and thus has been extensively used in the food industry and considered one of the most versatile hydrocolloids. In the present paper, we first demonstrated the preparation of surfactant-free and edible O/W HIPEs using relatively monodispersed colloidal gelatin particles as the sole stabilizers. Because they are soft, it is likely that they can deform and occupy large areas on the interface, leading to the formation of viscoelastic absorbed layers, which, in turn, enhance the emulsion stability. In addition, we examined the role of parameters such as the particle concentration, pH, and mixing time on the emulsion formulation and stability against coalescence. Furthermore, the viscoelastic properties of the resulting gelatin-particle-stabilized HIPEs were characterized, and the encapsulation and release of the nutraceutical material, β-carotene, was investigated.

EXPERIMENTAL SECTION

Materials. Gelatin type B (~250 g bloom) was obtained from Aladdin. Acetone (Sigma-Aldrich), sodium hydroxide (NaOH; 99%, LAB-SCAN), glutaraldehyde (International Laboratory, 25% aqueous solution), hexane (Sigma-Aldrich), Tween 80 (Duksan), and β-carotene (96%, Aladdin) were used without further purification. Sunflower oil (Standard Foods) was used as received. Water was purified by a Milli-Q system and used for all of the experiments.

Preparation of Gelatin Particles. The gelatin particles were prepared by a two-step desolvation method as previously reported by Coester et al.[17] In a typical preparation of gelatin particles, 1.25 g of gelatin type B was dissolved in 25 mL of distilled water under constant heating and stirring. Then 25 mL of a desolvating agent, acetone, was added to the gelatin solution to precipitate the high-molecular-weight (HMW) gelatin. After that, the supernatant was discarded and the remaining HMW gelatin was redissolved in 25 mL of distilled water with the pH of the solution adjusted to 12.0. In order to obtain the gelatin particles, 75 mL of acetone was added dropwise to the gelatin solution under constant heating. After 10 min of stirring, 250 μL of a glutaraldehyde solution was added to cross-link the particles, and the resulting mixture was stirred at 50 °C for 3 h. Finally, the dispersion was centrifuged at 10000g (Sigma, 3−18 K) for 20 min, and the particles were purified by 3-fold centrifugation and redispersion in an aqueous acetone mixture (30 vol %, acetone). After the last centrifugation, the particles were dispersed in distilled water and the residual acetone was removed by slow vaporization.

Particle Size Measurement. Measurement of the particle sizes of the gelatin dispersions was performed by a Malvern Nano-ZS ZEN3600 instrument using a Zetasizer at a detection angle of 90° at 25 °C. The concentration of gelatin particles was 0.5 wt %. The mean particle size and polydispersity index were obtained in the cumulant mode using the built-in Malvern software.

ζ-Potential Measurements. The ζ potential of the gelatin particles was measured with the same Malvern Nano-ZS ZEN3600 instrument. The concentration of gelatin particle solutions was diluted to 0.5 wt % with 10 mM NaCl, followed by adjustment of the pH to values between 2.0 and 12.0 using 0.25 M HCl or 0.25 M NaOH.

Atomic Force Microscopy (AFM). The size and morphology of the gelatin particles were also imaged by an atomic force microscope (SPM-9600, Shimadzu, Japan). The particles were dispersed in distilled water, and 1 drop of the dilute dispersion was placed on a freshly cleaved mica substrate and dried for 1 day at room temperature in a desiccator with silica gel. The samples were analyzed in air with a Dimension 3100 Nanoscope IV equipped with UrtlalveerB probes using tapping mode.

Scanning Electron Microscopy (SEM). SEM images were carried out using a Christ ALPHA 1−2 LD operating at 15 kV. A total of 1 drop of the dilute gelatin dispersion was placed on a silica wafer and dried in air for 12 h, and then all of the samples were sputtered with gold for 3 min in an argon atmosphere before observation.

Preparation and Characterization of HIPEs Stabilized by Gelatin Particles. The HIPEs were prepared by mixing a 20 vol % gelatin particle solution and 80 vol % hexane or sunflower oil by mechanical shearing with an Ultra Turrax T25 homogenizer (10 mm head) operating at 13500 rpm for 30 s. The concentration of gelatin particles was varied from 0.3 to 2.0 wt %. We also examined the effect of the pH on HIPE formation at a fixed gelatin particle concentration (0.5 wt %) by varying the solution pH from 2.0 to 12.0. The confocal laser scanning microscopy (CLSM) pictures were obtained on a Nikon Eclipse Ti-inverted microscope (Nikon, Japan) using a 543 nm laser to excite the samples (the gelatin particle possesses autofluorescent properties resulting from glutaraldehyde cross-linking[18]), and an objective of 60× was used. The emulsions were directly placed on the cover slides, and a series of x/y layers were scanned. Three different sets of experiments were performed. Also, the droplet size of the emulsions was determined using image tool software (Nikon EZ-C1FreeViewer).

Rheology Measurement. The rheology measurement of the resulting emulsions was carried out at room temperature with a rheometer (Anton Paar Physica MCR 301) by a plate−plate mode PP 25. The strain sweep was performed at a fixed frequency of 1 Hz. The temperature was maintained with a water bath.

In Vitro Release. To determine whether the gelatin-particle-stabilized HIPEs have the potential to be nutraceutical containers, we carried out an in vitro release study based on the membrane-free model.[19] Herein, crystalline β-carotene served as a model for lipophilic nutraceutical material. Initially, 10 mg of β-carotene was dissolved in 12 mL of sunflower oil at 50 °C for 5 min and then stirred at ambient temperature for about 1 h to ensure that all β-carotene was dissolved. The HIPEs were prepared by homogenizing 3 mL of different concentrations of gelatin particle aqueous solutions (0.5, 1.0, and 1.5 wt %, respectively) with the oil phase at 13500 rpm for 30 s. Then, 1.0 g samples of the corresponding HIPEs were placed on the bottom of glass bottles, followed by the careful addition of 4 mL of a 3 wt % Tween 80 aqueous solution and 10 mL of hexane. The bottles were kept at 25 °C in a shaking chamber at 70 rpm, and 100 μL of the supernatants was periodically removed for measurement of the absorbance at 450 nm with a UV−visible spectrometer (Alpha-1, Shanghai Lab-spectrum Instruments Co., Ltd.). A calibration curve was created from varying amounts of a known β-carotene solution to quantify the β-carotene-released content. Because the HIPEs were less dense than a Tween 80 aqueous solution but heavier than hexane, they remained floating at the middle interface of the two referred solutions.

RESULTS AND DISCUSSION

Preparation and Characterization of Gelatin Particles. The preparation of protein particles is based on classical

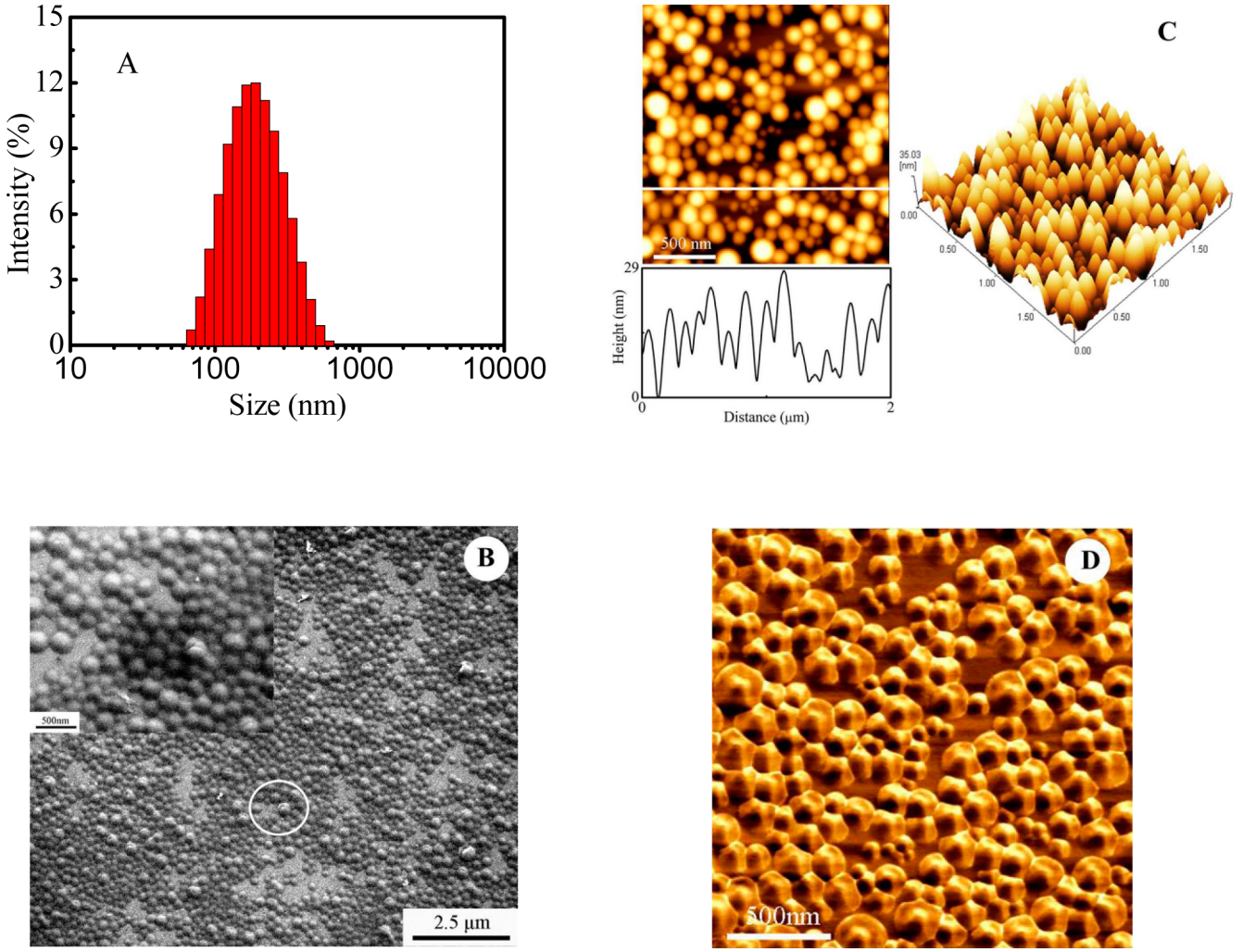


Figure 1. (A) Size distribution of the synthesized gelatin particles as measured by DLS. (B) SEM image of the synthesized gelatin particles, where the inset is the corresponding high-magnification SEM image. (C) 2 μm × 2 μm AFM topography images of the synthesized gelatin particles. (D) Phase graph of the synthesized gelatin particles.

desolvation, followed by chemical cross-linking or heat denaturation, a method that has been widely used for the synthesis of various protein particles, including human serum albumin and gelatin.[20,17] In an aqueous solution, protein keeps its three-dimensional shape by organization of the water molecules around the solute owing to noncovalent interaction. When a solvent of different polarity and hydrogen-bond-forming capacity, like acetone or ethanol, is added to the solution, it will replace some or all of the water molecules, resulting in shrinkage of the hydrated protein chains. At a certain point, hydration is too low and the protein chains precipitate as fine particles.[21,22] After removal of the organic solvent by evaporation, an aqueous dispersion containing protein particles is obtained. Although this preparation method is versatile enough to fabricate the protein particles, the resulting particle size and distribution would greatly be influenced by the desolvation agent, pH, temperature, and molecular weight of the proteins.[20,22−24] To obtain narrowly distributed protein particles, we carried out a two-step desolvation on the coarse gelatin solution and adjusted the pH value of the dispersion away from the IEP of the gelatin. At the same time, the temperature was kept at 50 °C to ensure that the protein chains were sufficiently uncoiled. Figure 1A shows the size distribution of the synthesized gelatin particles as measured by dynamic light scattering (DLS), which indicates that the average size of a prepared gelatin particle is around 200 nm in diameter and fairly monodispersed. The SEM image (Figure 1B) shows that gelatin particles are fairly monodispersed with a spherical morphology and tend to form large aggregates on the stub upon air drying. Figure 1C shows the in situ height images of the gelatin particles and the corresponding cross-sectional analysis of the line shown in the images. The domain diameter of the particles is around 200 nm, which agrees well with the SEM results, whereas the vertical height of the particles is ∼20 nm, indicating the soft nature of the gelatin particles. Furthermore, both parts B and C of Figure 1 show that the particles are isolated without aggregation, revealing that the cross-linker (glutaraldehyde) mostly reacts with amino groups within the same particle and wraps inside rather than linking two particles together, so that this preparation method leads to narrowly distributed protein particles. Additionally, it is established that the phase images can be used to visualize the local surface property of heterogeneous regions because the phase shift of the cantilever oscillation is very sensitive to the viscoelasticity like rigidity of the surfaces during tapping.[25] Figure 1D reflects the variation of the surface property of the

gelatin particles by the contrast of cores (dark) and edges (light). It is likely that the density of the gelatin particles is not homogeneous,[26] and the edge parts seem looser than the cores, which will lead to superiority for the particle stabilizer because the less cross-linked chains around the edges may help infusion of the particles at the interface by interpenetration.

The ζ potential of the gelatin particles, determined by electrophoretic mobility measurement, was plotted against the pH of the aqueous dispersion in Figure 2. On lowering of the pH to 2.0, the gelatin particles become positively charged ($\zeta \sim +15$ mV), with the IEP close to pH 4.5. With increasing pH, the particles become negatively charged ($\zeta \sim -15$ mV). The pH value of the aqueous phase not only triggered the change in the charge of the gelatin particles but also impacted their sizes, as can be seen in Figure 2B. Because gelatin particles are soft and poorly cross-linked particles swollen by water, their swelling behavior closely relates to the degree of solvation of the constitutive protein units as well as to the attractive or repulsive interactions between their charges. A minimum of the size is found around pH 4.5, relating to the domain of charge neutralization, i.e., IEP. In contrast, the gelatin particles swell largely at higher and lower pH, where their net charges are higher. Note that the swelling degree is more pronounced at basic conditions, indicating that the distribution and degree of ionization of the lateral $-COO^-$ groups of the protein amino acids are much more pronounced under alkaline conditions than those of $-NH_2$ in acidic solutions, which is, in turn, attributed to the consumption of $-NH_2$ in the preparation of gelatin particles.

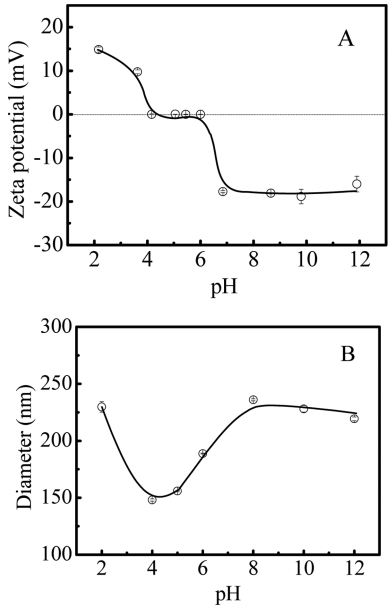


Figure 2. (A) ζ potenial of the synthesized gelatin particles as a function of the pH at 10 mM NaCl. (B) Diameter of the synthesized gelatin particles as a function of the pH at 10 Mm NaCl.

Effect of the Particle Concentration on the Emulsion Stability. After having described the preparation and characterization of the gelatin latexes, we now consider the ability of those particles to stabilize real liquid–liquid interfaces and the characteristics of the resulting HIPEs. Traditionally, protein particles are widely valued as functional ingredients for the the formation and stabilization of emulsions or foams in the food, cosmetic, and pharmaceutical industries. While the surface activity of protein particles is an important issue, the lowering of the interfacial free energy by themselves does not explain the stability of protein-particle-based emulsions or foams. It has been argued that the essential stabilizing function of protein particles is the formation of coalescence-arresting viscoelastic skins on the surfaces of droplets and bubbles.[27] To examine the emulsification power of our formulated gelatin particles, hexane-in-water HIPEs stabilized by gelatin particles were prepared as a function of the particle concentration and are shown in Figure 3. In all of the emulsions, the pH of the dispersion was neutral, and thus the particles were slightly negatively charged (Figure 2A). Figure 3 shows that, at a gelatin particle concentration above 0.5 wt %, stable HIPEs are obtained by using hexane as the internal phase. The formulated emulsions are gel-like emulsions, and they can hold their own weights even though the vials are inverted. Moreover, they show very little phase separation even after 3 months, highlighting the ability of gelatin particles to stabilize the emulsions. However, upon a further increase of the particle concentration to 2.0 wt %, no stable HIPE can be formed. Instead, macroscopic phase separation occurred (Figure 3). It is likely that gelatin particles form large aggregates and agglomerates at high concentration via hydrogen bonds among the particles, which sediment very quickly and subsequently obscure their surface activity.[28] The CLSM pictures of the HIPEs stabilized by different concentrations of particles are shown in Figure 4. Because the gelatin particles display autofluorescent properties owing to the Schiff's base formation, they show a red color when excited by a laser with a wavelength of 543 nm.[18] It can be seen that, at 0.5 and 1.0 wt % particle concentration, most of gelatin particles are irreversibly adsorbed at the oil–water interface to hinder droplet coalescence, and not many gelatin particles remain in the aqueous phase. At higher particle concentration (1.5 wt %), we envisage that, in addition to adsorption at the interface, the particles in the aqueous phase are probably contiguous with those adsorbed, so the gelatin particles from different oil droplets are bound together in a three-dimensional network, which, in turn, traps the oil droplets in the gel matrix (Figure 4). Therefore, increasing the particle concentration leads to a stronger gel-like emulsion. Indeed, this finding is very similar to the HIPEs stabilized by soft particles, like microgel particles, as reported before in our group.[29]

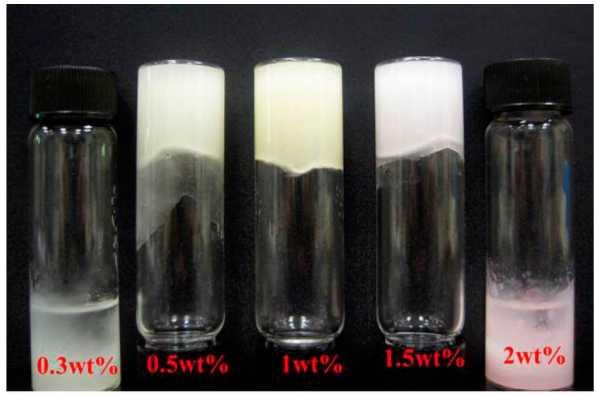


Figure 3. Photographs of HIPEs stabilized by different concentrations of synthesized gelatin particles. The photographs were taken 3 months after preparation.

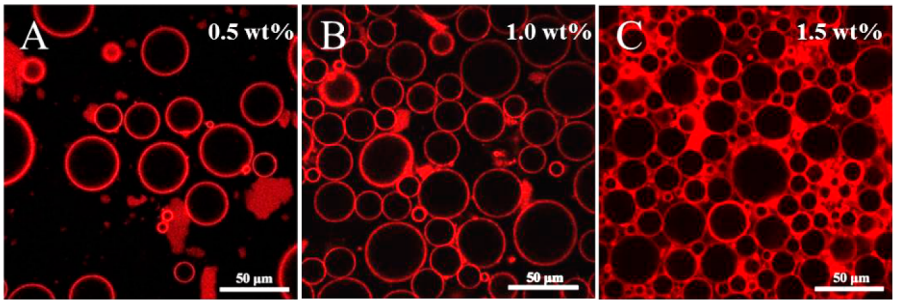


Figure 4. CLSM images of HIPEs stabilized by (A) 0.5 wt %, (B) 1.0 wt %, and (C) 1.5 wt % synthesized gelatin particles.

The CLSM images show the tendency that increasing particle concentration results in a decrease in the droplet size. Figure 5 shows the average droplet size distribution of the gelatin-stabilized HIPEs at various particle concentrations measured by CLSM with the incorporated image software. As observed, the droplet decreases with increasing particle concentration. An obvious phenomenon is the presence of oil droplets, whose sizes range less than 10 μm when the particle concentration exceeds 1 wt %. Moreover, it is interesting to see that the droplet size distribution is quite narrow; in particular, a high particle concentration is used to stabilize the emulsions.

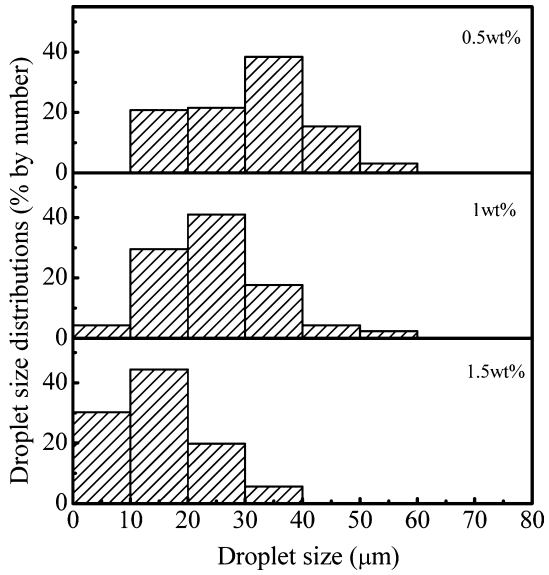


Figure 5. Droplet size distributions of HIPEs stabilized by different concentrations of synthesized gelatin particles.

Effect of the Homogenization Time on the Emulsion Droplet Size Distribution. We have also investigated the effect of the homogenization time on the HIPEs stabilized by gelatin particles. We used 20 vol % (1 mL) of a 1.5 wt % gelatin particle aqueous solution in 80 vol % (4 mL) of hexane oil. The emulsions were formed by emulsifying the biphasic layers using an Ultra Turrax T-25 rotor stator homogenizer fitted with a 10 mm stainless steel shaft rotating at 13500 rpm at different times. As the homogenization proceeds, all of the hexane is gradually incorporated into the emulsion, and this ultimately leads to HIPE formation. Figure 6A shows CLSM images of the emulsions formed by different homogenization times. It can be seen that increasing homogenization time leads to decreasing droplet size when the shearing rate is fixed. This finding is consistent with the reported results[30,31] because increasing the homogenization time causes the particles to be more dispersed, and in the meantime, the large droplets are broken up. The better dispersed particles then have more opportunity to migrate onto the interface to stabilize the emulsions. On the other hand, we also conjecture that, like microgels, gelatin particles are soft and deformable. The longer shear emulsification process may favor particle spreading at the interface. As a consequence, the drop coalescence process is stopped at an early stage, resulting in a small droplet.[32] The statistical analysis, as shown in Figure 6B, further confirms that a longer shear emulsification process not only decreases the droplet size but also leads to a narrow size distribution of the resulting Pickering HIPEs. This is significantly important in practice because the loading capacity of active compounds in each droplet can be tuned by increasing the homogenization time.

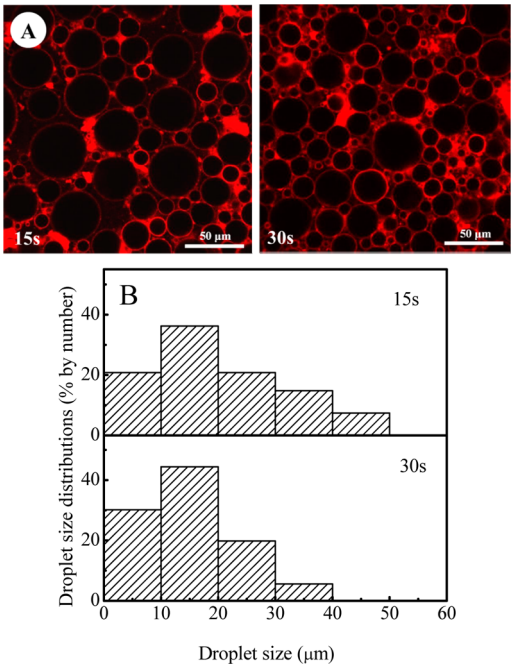


Figure 6. CLSM images (A) and pore size distributions (B) of gelatin-stabilized HIPEs prepared with different homogenization times using hexane as the oil phase.

Effect of the pH Conditions on the Emulsion Stability. As shown in Figure 2, the pH has a strong influence on the surface charge and stability of gelatin particles. They can acquire either positive surface charges below the IEP region (pH ~4.5) or negative charges above it. We therefore studied the effect of the pH on the emulsion stability against coalescence at a constant gelatin particle of 0.5 wt %. The

dx.doi.org/10.1021/am503341j | ACS Appl. Mater. Interfaces 2014, 6, 13977−13984

pH of the aqueous gelatin dispersion was then adjusted to different values. Figure 7 shows that, at pH 7.0, 8.0, and 10.0,

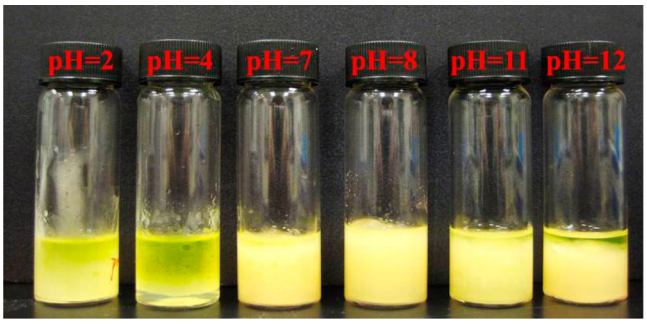


Figure 7. Photographs of HIPEs stabilized by the synthesized gelatin particles at different pH conditions.

where the particles are negatively charged, they can act as effective emulsifiers to stabilize Pickering HIPEs. However, some oil is not incorporated in the internal phase when the solution pH is adjusted to 12.0. This difference reflects the fact that the adsorption efficiency of particles strongly depends on the surface charge or the ionic strength of the aqueous solution. At very high pH values, the surface charges of gelatin particles are screened by the increased ionic strength owing to NaOH addition, and thus the particles are less swelled (see Figure 2). For emulsion stabilized by soft particles, some recent studies have highlighted the importance of particle swelling on the packing density and, consequently, on the emulsion stability.[33,34] It has been shown that when soft particles are charged, the interface exhibits a soft gel-like behavior, leading to more stable emulsions, while the interface is rather brittle when covered by neutral soft particles and the formulated emulsions are not stable. Indeed, when the pH is close to the IEP region, substantial macrophase separation occurred immediately after homogenization at pH 4.0. However, it is surprisingly to see that, at pH 2.0, far away from the IEP, the stable HIPE is still not formed. This may have resulted from the damage of Schiff's base cross-linking under acidic conditions within the particles, indicating that acidic conditions are not beneficial to the system stability. The results presented above suggest that the system of gelatin-particle-stabilized HIPEs is "pH-dependent", and the different balance between the hydrophilic and hydrophobic properties of the particles influences their swelling ability and, consequently, the emulsion stability.

Rheological Measurement. The rheology of the emulsions plays an important role in controlling the emulsion stability, and thus it is considered to be a valuable method to provide insight into the complex structures. In addition, for practical application such as in food technology, the rheological data can be used to develop the relationship of the food between process and product optimization. It has been shown that the rheological properties are of relatively limited value to understand the breakdown pathway of the food materials during mastication; i.e., the rheology can be an indicator of food textural perception.[35−37] Figure 8 shows the strain dependence of the storage modulus (G') and loss modulus (G'') of gelatin-stabilized emulsions as a function of the particle concentration measured at a fixed frequency (1 Hz). At low applied strains, G' is always larger than the corresponding loss modulus G'' regardless of the particle concentration, indicating that all produced emulsions contain elastic or solidlike

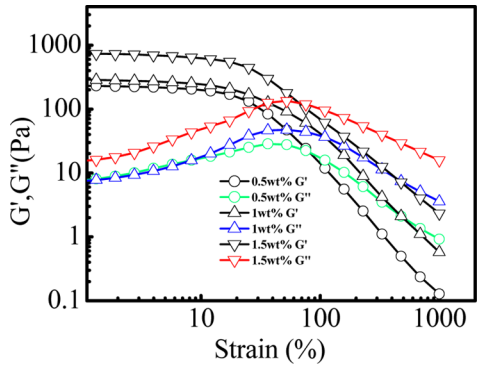


Figure 8. Strain dependence of the storage (G') and loss (G'') moduli of the HIPEs stabilized by different concentrations of gelatin particles measured at a frequency of 1 Hz.

behavior. Conversely, G'' gradually becomes larger than G' when the strain increases, revealing the viscoelastic solution behavior of the emulsions. This may be due to the structural rearrangements or flow of the emulsion droplets at high applied strains. In addition, it can be seen from Figure 8 that, at low strains, G' increases as the gelatin particle concentration increases, indicating that the emulsions become more solidlike at high particle concentration. Note that the measured G' values are higher than those of conventional surfactant-stabilized HIPEs because of the close packing of oil droplets and the viscoelastic protein layers formed at the oil−water interfaces.[38] Moreover, the obtained values are comparable to those of many other concentrated food emulsions[39,40] without the incorporation of polysaccharides to improve stability, benefiting from the striking features of HIPEs' high viscosity.

Release of Nutraceutical Material from HIPEs. A vehicle-like O/W emulsion-based delivery system is considered to be an efficient way to increase the dispersibility, stability, and bioavailability of nutraceutical materials.[41] It has been previously demonstrated that the large voids among particles on the interfaces give the encapsulated compounds a chance to diffuse out of the internal phase.[42] However, most of the reported findings[43,44] used fluorescent dyes as the drug model, which may not reflect the real controlled-release behavior in practice. Therefore, to gain more insight into the release process of an encapsulated bioactive lipid from the Pickering HIPEs, β-carotene, which is of interest as a nutraceutical material, serves as a controlled-release model for small-molecule lipophilic drugs in this work. Measurement of the release profile of β-carotene involves a process of extracting it from inside the emulsion droplets across the continuous phase (aqueous phase) into the receptor phase. Because the solubility of β-carotene in various solvents is solubility $_{\text{hexane}}$ > solubility $_{\text{sunflower oil}}$ > solubility $_{\text{water}}$, the concentration of β-carotene in hexane during the release measurement is far from its saturated value because of the high solubilizing power. So, a chemical potential difference from sunflower oil to hexane for diffusion of β-carotene from the inside to the outside phase exists.

Figure 9 shows the release curves of different concentrations of gelatin-particle-stabilized HIPEs encapsulating the same content of β-carotene. It can be seen that, in all samples, β-carotene steadily diffuses out of the emulsions to a greater extent. At the initial stage, all of the samples present a fast release, and then the diffusion is going on a sustained release at a very low rate. At low particle concentration (0.5 wt %), the initial fast release is finished at 36 h and about 35%

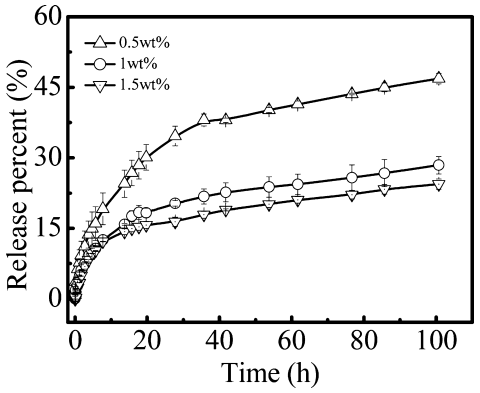


Figure 9. Release curves for β-carotene diffusing from the HIPEs stabilized by different concentrations of the synthesized gelatin particles.

encapsulated β-carotene is released. However, the initial fast release times of 1.0 and 1.5 wt % Pickering HIPEs shift to an earlier time (14 h). In addition, only about 15% of the encapsulated content is released. This finding can be due to the fact that a higher concentration of gelatin particles leads to a thicker packing density, and thus the thick layers can retard the diffusion. Furthermore, like the CLSM images presented above (Figure 4), the continuous phases of 1.0 and 1.5 wt % HIPEs are still rich in gelatin particles, although the majority of gelatin particles are adsorbed on the interfaces to stabilize the emulsions. Hence, the concentrated continuous phase can also play a crucial role in hindering the release. The CLSM images and droplet size distribution in Figures 4 and 5 also indicate that the difference between 1.0 and 1.5 wt % HIPEs is very small; that is why the release behaviors of the two samples are so similar. Collectively, the present study explores the possibility that the nutraceutical release can be tuned by manipulating the concentration of gelatin particles in the formulation.

Compared to other food-grade protein-stabilized emulsion loading with β-carotene,[45−48] the internal phase volume of gelatin-particle-stabilized HIPEs is higher (80%); they thus possess higher loading capacity with the same emulsion volumes. On the other hand, previous findings suggest that soft particles can be much more closely packed on the surface than solid colloidal particles; thus, transport through the shell is slower for the former one.[42] Fielding and Armes[44] reported the release behavior of a dye from solid-particle (modified silica)-stabilized microcapsules; full release was reached within 24 h, which is significantly earlier than that in our results, for which the balance release time of β-carotene is not observed even beyond 100 h. Presumably, the controlled-release properties of gelatin-particle-stabilized HIPEs can be used in long-term drug delivery by encapsulating different lipophilic drugs. In conclusion, the good biocompatibility and biodegradable properties of gelatin particles impart the emulsions higher superiority than other synthetic materials.

CONCLUSIONS

Gelatin particles are prepared by a two-step desolvation and employed as an active emulsifier to prepare stable O/W Pickering HIPEs with an internal phase of 80%. We demonstrate that factors like the particle concentration, homogenization time, and pH conditions have a great effect on the formation and droplet size distribution of the resulting emulsions. The rheological properties of the Pickering HIPEs can be tuned within a certain range by varying the particle concentration. Moreover, the Pickering HIPEs are inherently permeable and can retain lipophilic compounds for the long term. As opposed to many other synthetic materials, the gelatin-stabilized HIPEs are based on fully versatile food-grade protein and biocompatible natural renewable resources. Encapsulating the nutraceutical material into food-grade products offers new opportunities for the use of gelatin-particle-stabilized HIPEs in functional food and pharmaceutical applications.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support of the National Natural Science Foundation (NNSF) of China (Grants 21276166 and 21176159) and National High-tech Research and Development Program (863 program) of China (Grant 2013AA06A306) is gratefully acknowledged. T.N. acknowledges support by the Hong Kong Special Administration Region General Research Fund (Grants CUHK402712 and 2130304).

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