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Fabrication of starch-based nanospheres to stabilize pickering emulsion

Ying Tan[a], Kun Xu[a], Chang Liu[a,b], Yangling Li[a,b], Cuige Lu[a,b], Pixin Wang[a,*]

[a] Key Laboratory of Ecomaterial Polymers, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China
[b] Graduate School of Chinese Academy of Sciences, Bejing 100039, PR China

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ABSTRACT

Starch is successively modified by acetic anhydride and phthalic anhydride, and then starch-based nanospheres are fabricated by a simple nanoprecipitation procedure. These pH-responsive amphiphilic starch-based nanospheres of uniform size are used as particulate emulsifiers to stabilize Pickering emulsions. CLSM and SEM characterization find that starch-based nanospheres adsorbed at the interface between oil phase and water phase. Moreover, the catastrophic inversion and transitional inversion of such emulsions can be easily achieved by varied the water/oil volume ratio and pH of aqueous dispersion, respectively.

1. Introduction

There has been growing interest in Pickering emulsions because the particulate emulsifiers can give superior stability than conventional surfactant molecules (Binks, 2002; Pickering, 1907). Meanwhile, these solid-stabilized emulsions avoid the application of hazardous surfactants and open up a new application prospect of emulsion (such as colloidsomes) (Friberg, 2010; Rossier-Miranda, Schroen, & Boom, 2009). Therefore, a wide variety of micro- and nanoscale particles with different sizes and shapes have been used as emulsifiers to stabilize the emulsion (Dinsmore et al., 2009; Fujii, Read, Binks, & Armes, 2005; Kim et al., 2007; Noble, Cayre, Alargova, Velev, & Paunov, 2004).

In consideration of the practical application of emulsion in food, cosmetics and pharmaceutics fields, the particulate emulsifiers used in such emulsions should derivate from natural biopolymers (Augustin & Hemar, 2009; Dickinson, 2010). Although some particles based on viruses, protein or cells have been explored to stabilize Pickering emulsions, there are still no inexpensive particles of natural origin to meet the larger scale application in industrial products (Fujii et al., 2009; Russell et al., 2005; Semo, Kesselman, Danino, & Livney, 2007).Another interesting approach in the design of particulate emulsifiers is the utilization of hydrophobically modified polysaccharide. Micro-size amphiphilic starch granules and cellulose fibers have been used as emulsifier to stabilize emulsion (Tzoumaki, Moschakis, Kiosseoglou, & Biliaderis, 2011; Wege, Kim, Paunov, Zhong, & Velev, 2008; Yusoff & Murray, 2011). However, these larger particles are unstable against the gravity force and tend to sediment, resulting in a poor stability for the obtained emulsion.

Starch, as the second largest component of the biomass, and is an excellent material to meet food and biomedical requirements due to its biodegradability, derivability and low cost. We have shown recently that hydrophobically modified starch could form uniform nanospheres and vesicles through a nanoprecipitation procedure (Tan et al., 2009; Tan, Xu, Li, Sun, & Wang, 2010; Tan et al., 2010b). Herein, a new facile approach is presented to fabricate amphipathic starch-based nanospheres as particulate emulsifier for Pickering emulsion.

2. Experimental

2.1. Materials

Waxy maize starch with molecular weight (Mw) of 180,000 was obtained according to a previously published method (Chang, Lin, & Lii, 2004). Acetic anhydride (AC), phthalic anhydride (PTA), fluorescein isothiocyanate (FITC), dibutyltin dilaurate (DBTDL) and *N*,*N*-dimethylaminopyridine (DMAP), were obtained from Alfa Chemical Co. Ltd. (America). All other chemicals were obtained from Beijing Chemical Works (China) and were used as received.

2.2. Preparation of starch mixed ester (S-AC-PTA)

Three grams of waxy maize starch dispersed in 30 mL of *N*,*N*-dimethylformamide (DMF) was reacted with 4.8 g of acetic

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* Corresponding author. Fax: +86 431 5262629.
E-mail address: pxwang@ciac.jl.cn (P. Wang).

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anhydride in the presence of 10 mL of pyridine and 0.05 g of DMAP for 3 h at 80 °C, and then 1.4 g of PTA was added and the mixture was allowed to react for 3 h at 80 °C under stirring. The S-AC-PTA was precipitated, washed with water three times, and dried at 50 °C.

2.3. *Preparation of fluorescein labeled starch (FS)*

One gram of the waxy maize starch dissolved in 10 mL of dimethyl sulfoxide (DMSO) was reacted with 25 mg of FITC in the presence of 1.5 mL of DBTDL for 5 h at 100 °C. The polymer was precipitated, washed repeatedly with acetone until filtrate free of fluorescin as detected by fluorescence spectroscopy. The final product was dried at 60 °C in the oven.

2.4. *Preparation of fluorescein labeled starch mixed ester (FS-AC-PTA)*

One gram of FS dispersed in 20 mL of DMF was reacted with 1.6 g of acetic anhydride in the presence of 5 mL of pyridine and 0.05 g of DMAP for 3 h at 80 °C, and then 0.5 g of PTA was added and the mixture was allowed to react for 3 h at 80 °C under stirring. The FS-AC-PTA was precipitated, washed with water three times, and dried at 50 °C.

2.5. *Preparation of amphiphilic starch-based nanospheres*

The amphiphilic starch-based nanospheres were prepared by a nanoprecipitation process. Typically, 20 mg of starch mixed ester was dissolved in 20 mL of tetrahydrofuran. Alkalescent distilled water (20 mL, pH = 7.5) was then added dropwise to the polymer solution. The resulting nanoparticle suspensions were stirred at room temperature until tetrahydrofuran was completely vaporized from the aqueous suspension.

2.6. *Preparation of pickering emulsion*

Stock solutions (1 wt% solids) of the starch-based nanospheres with different pH were adjusted as required by adding a few drops of either concentrated HCl or NaOH. These aqueous dispersions were then homogenized with certain amounts of oil phase for 3 min at 20 °C using an IKA Ultra-Turrax T18 homongenizer at 12,000 rpm. Immediately after the homogenization, the emulsion type was determined by a conductivity measurement and also by observing the outcome of a drop of each emulsion added to either pure oil or pure water.

2.7. *^{1}H NMR characterization*

The 400 MHz ^{1}H NMR spectra were measured on a Bruker AV400 spectrometer (Ettlingen, Germany). The samples of S-AC-PTA dissolved in d_6-DMSO were recorded for ^{1}H NMR at 25 °C.

^{1}H NMR (DMSO-d_6): $\delta = 7.7–7.5$ (PTA H), 5.5–3.5 (Starch H), 2.1–1.9 (AC H).

2.8. *Nanospheres characterizations*

2.8.1. *SEM observation*

The appearance of nanospheres was characterized by scanning electron microscopy (SEM) using a model XL30 ESEM microscope (Philips). A droplet of suspension was placed on a silica surface. After the water was evaporated at room temperature, the system was covered by gold before measurement.

2.8.2. *DLS characterization*

The particle size and polydispersity of nanoparticles in aqueous solution were measured by dynamic light scattering using a 90 Plus particle size analyzer (PSA; Brookhaven) at 25 °C. The suspensions were diluted with distilled water to a concentration of about 0.01%. The mean particle size was approximated as the diameter and the combined polydispersity as the polydispersity index (PDI).

2.9. *Emulsions characterizations*

2.9.1. *Optical characterization*

The morphology of emulsion droplets was observed with a digital optical microscopy (MOTIC, China). The emulsion droplet size was obtained by processing the image using the microscopic image analysis software.

2.9.2. *CLSM characterization*

The distribution of fluorescein labeled nanospheres in emulsion droplets interface was observed by confocal laser scanning microscopy (CLSM) using a Leica TCS SP2 CLSM. The samples were excited by using a 554 nm He/Ne laser. A droplet of suspension was placed on a glass surface, and visualized directly.

2.9.3. *SEM characterization*

The arrangement of nanospheres on the surfaces of emulsion droplets was investigated by scanning electron microscopy using a model XL 30 ESEM microscope (Philips). First, 4 mL of emulsion prepared using 2 mL of stock solutions (1 wt% solids) and 2 mL of toluene. A droplet of emulsion was air-dried on a silica surface. After the water was and toluene evaporated at room temperature, the system was covered by gold before measurement.

3. Results and discussion

3.1. *Synthesis of S-AC-PTA*

In this process, the commercially available corn starch was successively esterized by acetic anhydride and phthalic anhydride. The acetic ester groups (AC) afforded sufficient hydrophobility for starch mixed ester so the aggregation and collapse of the nanospheres in water could be avoided. And the phthalic ester (PTA) was introduced as pH responsive amphiphilic groups, by which the hydrophobic phenyl and hydrophilic carboxyl could sustain the obtained nanospheres a certain hydrophobic/hydrophilic balance to stabilize the O/W or W/O emulsions. The structural feature of S-AC-PTA is illustrated in Fig. 1. The degree of substitute (DS) of AC and PTA determined by ^{1}H NMR is 2.34 and 0.35, respectively (Chi et al., 2008).

3.2. *Preparation of amphiphilic starch-based nanospheres*

The nanospheres were prepared by the dropwise addition of alkalescent water into a tetrahydrofuran solution of S-AC-PTA under stirring. After the complete removal of tetrahydrofuran, sphere-shaped particles were obtained. DLS characterization indicated that these nanospheres exhibit a size of ca. 270 nm in aqueous media (Fig. 2). The formation of such nanosphere is essentially based on the interfacial deposition of amphiphilic polymers, following displacement of a semi-polar solvent miscible with water from a lipophilic solution (Liebert, Hornig, Hesse, & Heinze, 2005). It is noted that the pH of added water must be alkaline to facilitate the deprotonation of carboxyl groups, or else flocculation and sedimentation is difficult to be avoided during the nanoprecipitation process. Moreover, an appropriate DS of PTA is crucial for such system. Higher PTA amounts tend to increase the solubility of S-AC-PTA in water and hence no nanospheres form, whereas lower PTA amounts can not maintain the nanospheres sufficiently amphipatic to stabilize an emulsion.

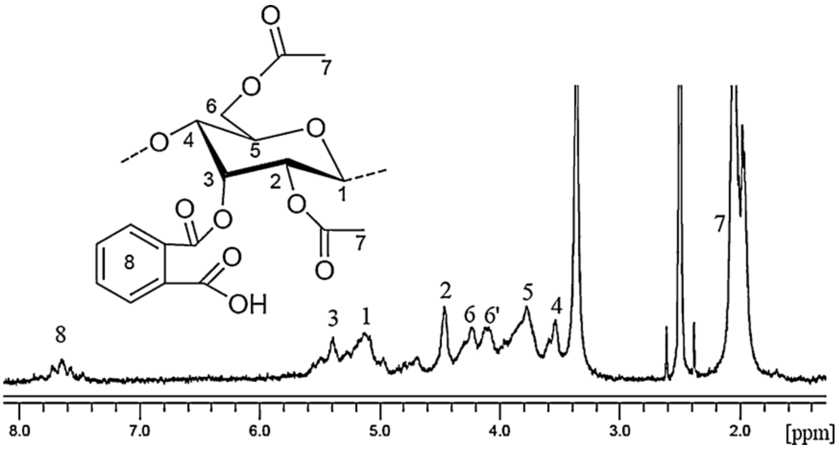

Fig. 1. The ^{1}H NMR spectrum and potential structural character of S-AC-PTA.

3.3. Preparation of emulsion

A 1 wt% aqueous dispersion of nanospheres was used as the particulate emulsifier to stabilize an emulsion with undecanol as the oil phase. Electrical conductivity measurement and droplet tests indicated that O/W emulsions were obtained at pH 7.5 for the water and undecanol volume ratio of 1/1 (Table 1). Typical optical microscopy images of the emulsion droplets are shown in Fig. 3A. These O/W emulsions droplets were spherical, with diameters of 24 μm according to the statistical analysis from image analysis.

3.4. Characterization of emulsion

To explore the distributed situation of nanospheres in emulsion, a fluorescent group was introduced in starch-based nanospheres by covalently bonding starch with fluorescein isothiocyanate (Li et al., 2011). As shown in Fig. 4A, a green halo was present surrounding the emulsion droplet when an emulsion was prepared using fluorescently labelled particulate emulsifier. Moreover, most of the fluorescence dot aggregated to form a dense film around the dispersed drops impeding coalescence. This fact gave direct evidence that the nanospheres were adsorbed at the interface between oil phase and water phase.

SEM characterization was also used to detect the microappearance of emulsion in its dry state. We adopted toluene as the oil phase, which possess the similar boiling point as water. It enables both the oil and aqueous phases of an emulsion to be simultaneously removed from the droplet at ambient. This method is easily conducted and different from the complicated critical point drying technique (Fujii, Armes, Binks, & Murakami, 2006; Read, Fujii, Amalvy, Randall, & Armes, 2004). Fig. 4B shows typical SEM micrographs obtained from a toluene-in-water emulsion with a volume ratio of 1/1. Collapsed emulsion droplets with 10 μm of diameter could be easily distinguished from the background. The surface of the collapsed droplet is coated with nanospheres (Fig. 4C), further supporting the notion that the nanospheres adsorb at the oil–water interface. It is noted that the size of emulsion droplets in dry state is less than its size in aqueous (23 μm). Therefore, this large interfacial area reduction may result to the ejection of nanospheres from the interface during the drying process. And this fact can be confirmed by two factors, some separated fragments could be observed in the background (Fig. 4B), and the amplified image indicate that the droplets are not densely covered by the nanospheres (Fig. 4D).

3.5. Properties of emulsion

Fig. 3A shows image of emulsion, spherical droplets with size of 24 μm and even size distribution can be obtained. The emulsion is O/W type according to the conductivity analysis (Table 1). Generally, phase inversion of particle-stabilized emulsions from oil in water to water in oil can be achieved either by variation of the oil/water ratio of emulsion (catastrophic phase inversion) (Binks & Lumsdon, 2000a; Binks, Fletcher, Holt, Beaussoubre, & Wong, 2010; Sun, Li, & Ngai, 2010). Therefore, the effect of water/oil ratio on the emulsion type was investigated to detect such inversion in our system. Emulsions were prepared separately at different water/oil

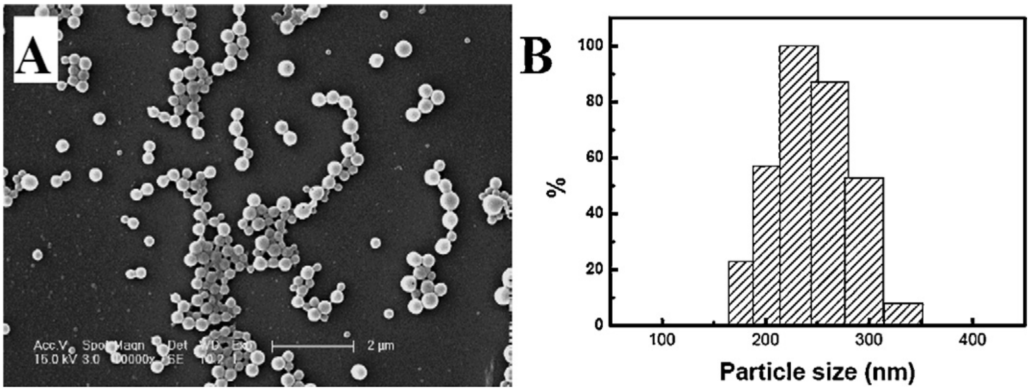

Fig. 2. (A) SEM image of amphiphilic starch-based nanospheres. (B) Particle size distribution of amphiphilic starch-based nanospheres according to DLS characterization.

Table 1

Characteristics of the emulsion prepared by homogenizing 1 wt% nanospheres dispersion with undecanol and toluene at different water/oil volume ratio and pH. The emulsified experiment was carried out at 12,000 rpm for 3 min at 20 °C.

Oil	pH of aqueous dispersion	Volume of water/oil	Conductivity of emulsion (μs cm^{-1})	Type of emulsion[a]	Mean droplet size[b] (um)
Undecanol	7.5	1/1	66	O/W	24.4
	7.5	3/1	99	O/W	27.6
	7.5	1/3	<1	W/O	20.0
	5.5	1/1	<1	W/O	20.3
Toluene	7.5	1/1	113	O/W	23.1

[a] The type of emulsion determinded by conductivity measurement and droplet test.

[b] Mean droplet size determinded by the statistic analysis from optical image.

ratio by direct mixing of the components. According to Table 1 and Fig. 3A–C, the conductivity falls gradually on increasing the volume fraction of oil since the oil drops formed act as obstructing entities in the conduction of the aqueous phase. When the water/oil ratio in volume is 1/3, the conductivity falls precipitously to very low values indicative of inversion to w/o emulsions. Systemic studies indicated that the catastrophic phase inversion from w/o to o/w emulsions occurs at a volume fraction of oil about 0.7–0.75 without altering the particle wettability. Although the droplet size was slightly lower with increasing oil phase volume, phase inversion is not accompanied by dramatic changes in the stability and size distribution of the emulsions. Importantly, all of the above emulsions are stable to coalescence with no sign of the dispersed phase separating in over 1 month. This is quite remarkable contrast to normal surfactant systems.

For particle-stabilized emulsions, phase inversion can also be obtained by variation of the particle hydrophobicity at constant oil/water ratio (transitional phase inversion) (Binks & Lumsdon, 2000b; Binks & Murakami, 2006). In our case, the surface of starch-based nanospheres is hydrophility because of the deprotonation of PTA units in an alkaline aqueous media. And the inherent wettability of starch-based nanospheres could be altered through the protonation and deprotonation of carboxyl in PTA units. Therefore, we conducted the emulsified experiment at a lower pH of 5.5, which below the pKa of S-AC-PTA (6.0). As shown in Table 1 and Fig. 2D, a stable W/O emulsion can be obtained when the pH of the aqueous dispersion was decreased to 5.5, and the droplet size is slightly decreased from 24 to 20 μm. However, further decrease in pH lead to a rapid demulsification because of the absolute protonation of carboxyl, which caused desorption of nanospheres from oil and water interface. Moreover, we found that the emulsions show no sign of ageing after one month if kept pH at about 7. While poor emulsion stability would be presented when the pH is greater than 8, this coalescence may

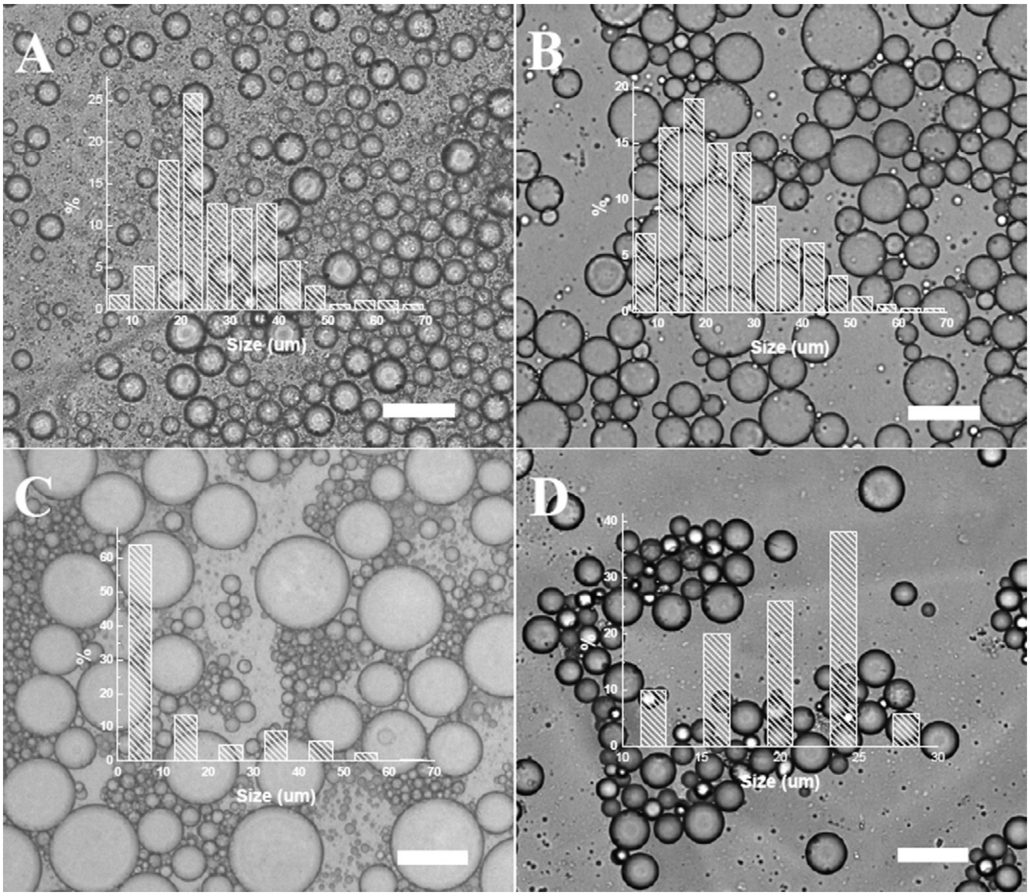

Fig. 3. Optical images of emulsions prepared at pH 7.5 for undecanol as oil phase with water/oil volume of (A) 3/1, (B) 1/1 and (C) 1/3, respectively. (D) The W/O emulsions prepared at pH 5.5 for undecanol as oil phase with water/oil volume of 1/1. All the scale bars are 50 μm. Inset is the distributions of droplet sizes of emulsions according to statistical analysis.

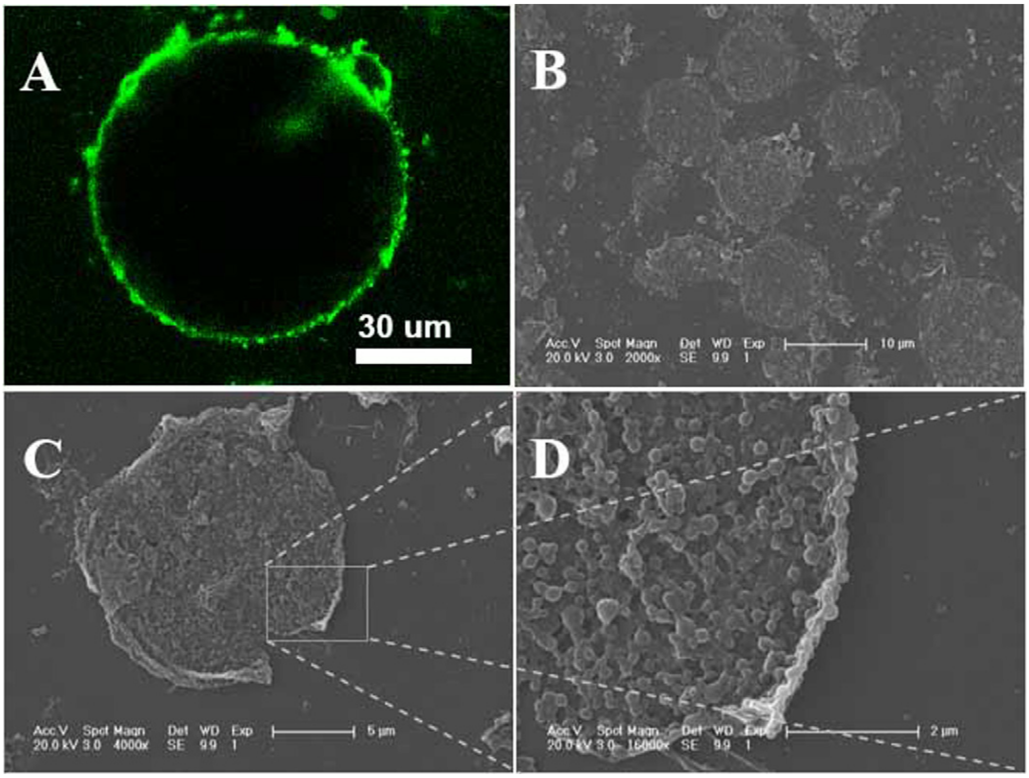

Fig. 4. (A) CLSM image of emulsion droplets prepared at pH 8 using fluorescent starch based nanospheres as emulsifier for undecanol-in-water emulsions. (B) SEM images of toluene-in-water emulsion dried at ambient. (C) A single droplet and (D) a magnified area of the small rectangle shown in part C.

result from the gradual hydrolysis of AC groups under basic conditions.

4. Conclusions

In summary, a simple and robust method was presented to fabricate amphiphilic starch-based nanospheres to stabilize Pickering emulsion. CLSM and SEM characterization indicated that these nanospheres adsorbed at the interface between oil phase and water phase. Moreover, the catastrophic inversion and transitional inversion of such emulsions could be easily achieved by varying the water/oil volume ratio and pH of the aqueous dispersion, respectively. Because of the favorable biocompatibility of polysaccharide-based polymer, this inexpensive and readily accessible particulate emulsifier may be potentially used in biomedical and food fields.

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