Original Article

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Fabrication of Janus particles composed of poly (lactic-co-glycolic) acid and hard fat using a solvent evaporation method

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Summary **The feasibility of fabricating Janus particles based on phase separation between a hard fat and a biocompatible polymer was investigated. The solvent evaporation method used involved preparing an oil-in-water (o/w) emulsion with a mixture of poly (lactic-co-glycolic) acid (PLGA), hard fat, and an organic solvent as the oil phase and a polyvinyl alcohol aqueous solution as the water phase. The Janus particles were formed when the solvent was evaporated to obtain certain concentrations of PLGA and hard fat in the oil phase, at which phase separation was estimated to occur based on the phase diagram analysis. The hard fat hemisphere was proven to be the oil phase using a lipophilic dye Oil Red O. When the solvent evaporation process was performed maintaining a specific volume during the emulsification process; Janus particles were formed within 1.5 h. However, the formed Janus particles were destroyed by stirring for over 6 h. In contrast, a few Janus particles were formed when enough water to dissolve the oil phase solvent was added to the emulsion immediately after the emulsification process. The optimized volume of the solvent evaporation medium dominantly formed Janus particles and maintained the conformation for over 6 h with stirring. These results indicate that the formation and stability of Janus particles depend on the rate of solvent evaporation. Therefore, optimization of the solvent evaporation rate is critical to obtaining stable PLGA and hard fat Janus particles.**

Keywords: Janus particle, PLGA, hard fat, phase separation, solvent evaporation

1. Introduction

Microparticles have been widely used in pharmaceutical formulations including as carriers for oral (*1-5*) and pulmonary drug delivery (*6*) or injectable formulations for long-term drug release (*7*). Developing microparticles as oral drug carriers to improve drug absorption in the gastrointestinal (GI) tract is an attractive challenge in the pharmaceutical field and a current focus. Strategies for improving intestinal drug absorption have been investigated in numerous research studies, especially for drugs with low stability and permeability such as peptides, proteins, and nucleotides, and are an attractive research focus. Several methods such as using absorption enhancers (*8,9*), enzyme inhibitors (*10*), and mucoadhesive polymers (*11*) have been proposed to improve intestinal drug absorption. Microparticles used for improving drug absorption should ideally possess the desired characteristic of protecting the loaded drug against enzymatic attack until it reaches the absorption window to ensure intact drug delivery. In contrast, drug release from conventional microparticles lacks directional specificity and, so, drugs released on the opposite side of the mucosa or toward the luminal side are easily degraded by enzymes in the lumen.

To overcome this problem, Takada (*12,13*) proposed the hemispherical patch system as a new formulation for improving intestinal drug absorption, which releases drug only from the flat and not the spherical surface, thereby limiting the release direction. The flat surface

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also plays a role in attaching the system to the mucosal surface to maintain the drug concentration near the site of absorption. In contrast, the spherical surface prevents drug release toward the lumen, which is the opposite side of the mucosa and acts as a barrier against enzymatic degradation. They demonstrated that the system enables the intestinal delivery of peptide drugs such as granulocyte-colony stimulating factor (*14*) and interferon (*15*). Thus, the hemisphere formulation appears to be a promising intestinal delivery system. However, there are some technical challenges in fabricating micro- or nano-sized hemispherical preparations. The hemispheric delivery system can be produced using several fabrication processes that may require entirely different manufacturing systems from those used for any conventional preparation. In addition, it should be costly effective.

The Janus particle is a heterogeneous particle consisting of two distinct hemispheres of different materials that impart different characters or functions to each hemisphere. Janus particles have been reported to show unique properties that conventional homogeneous particles do not possess, and have been proposed for use in various applications including as amphiphilic surfactants (*16*), elementary particles of the display in an electric field (*17*), and particles for magnetolytic therapy (*18*). Recently, the pulmonary delivery of hydrophilic and hydrophobic anticancer drugs has been reported (*19*). In this study, we hypothesized that Janus particles could be used to enhance drug absorption, and the concept is illustrated in Figure 1. Janus particles consist of biocompatible polymeric and thermosensitive lipid hemispheres and can reach the epithelium while blocking the leakage of the loaded drug and the attack by enzymes. Then, the lipid hemisphere attaches to the surface of the epithelium before melting to produce the micro-hemisphere of the polymer. The drug incorporated in the lipid hemisphere is subsequently released in a selective direction, and the polymeric hemisphere acts as a barrier against the leakage of encapsulated drug to the luminal side and enzymatic attack from the luminal side (*20*).

Here, we report the results of the designed preliminary step for proving the concept. Specifically, the feasibility of fabricating polymer-lipid Janus particles was investigated using poly (lactic-co-glycolic) acid (PLGA) and hard fat as the materials for the polymeric and lipid hemispheres, respectively. The solvent evaporation method was used to fabricate the Janus particles.

2. Materials and Methods

2.1. *Materials*

PLGA (molecular weight [MW] 20,000, lactic:glycolic, 50:50) was purchased from Wako Pure Chemicals Co., Ltd. (Japan). Suppocire® AM pellets were a kind gift from Gattefossé, s.a., (France) and were used as the hard fat. Polyvinyl alcohol (PVA, POVAL® 220C) was obtained from KURARAY Co., Ltd. (Japan). All the other chemicals used were of reagent grade.

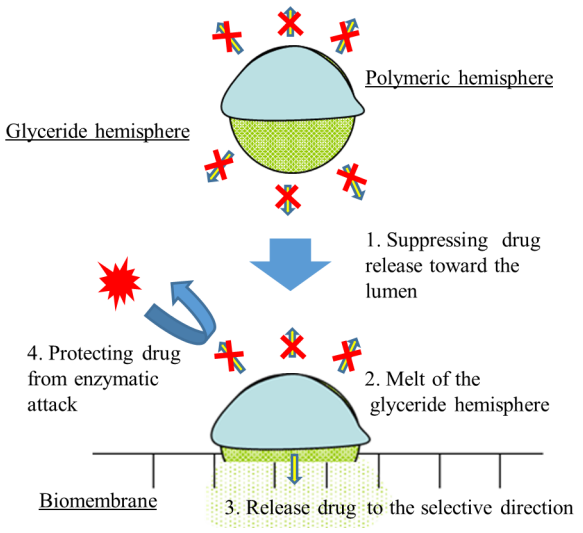

Figure 1. Janus particle-mediated drug absorption through biomembrane (concept). (1) Janus particles consist of polymeric and lipid hemispheres. **(2)** Slight luminal drug release. **(3)** Lipid hemispheres melt, and Janus particles change to micro-hemispheres. **(4)** Drug encapsulated in lipid hemispheres would be released to selective directions. Polymeric micro-hemispheres attach to biomembrane as a barrier against leakage of encapsulated drug to luminal side and enzymatic attack from luminal side.

2.2. *Analysis of phase diagram and identification of each phase*

The mixture of PLGA and Suppocire® AM with various ratios was dissolved in methylene chloride or ethyl acetate. The solvent was slowly evaporated at 20-23°C until phase separation was observed, and the solution was weighed at that time. The concentrations of polymer and lipid were calculated. In addition, we identified each phase in the separated solution. A mixture of 200 mg each of PLGA and Suppocire® AM was dissolved in 0.3 mL methylene chloride and Oil Red O was added to the resultant separating solution, which was then centrifuged. The state of the solution was visually observed.

2.3. *Fabrication of Janus particles*

The Janus particles were fabricated using an oil-in-water (o/w) emulsion solvent evaporation method (Figure 2). PLGA and Suppocire® AM were dissolved in methylene chloride or ethyl acetate to prepare the oil phase. Then, Oil Red O (1 mg) was added to the oil phase as needed, and this was emulsified into a 0.5% PVA aqueous solution using a homogenizer (ULTRA-TURRAX® T18, IKA®, Germany) for 5 min at 20-23°C. The resultant emulsion was added to the water all at once as needed

and stirred at 20-23°C to remove the solvent, which constitutes the solvent evaporation process. For Ex.4, the emulsion was weighed during the solvent evaporation process at predetermined times to evaluate the residual solvent or the PLGA- Suppocire® AM concentration in oil phase. The details of the amount of each component and conditions are summarized in Table 1. The obtained hardened particles were sieved through 149-μm sieves to remove any aggregates.

2.4. *Microscopic observations*

During the solvent evaporation process, the emulsion and fabricated Janus particles were observed using an optical microscope system (Motic PA300, Shimadzu Co., Ltd., Kyoto, Japan).

2.5. *Determination of Janus particle size*

The sizes of the fabricated Janus particles were determined using a laser diffraction particle size analyzer (SALD-2200, Shimadzu Co., Ltd., Kyoto, Japan).

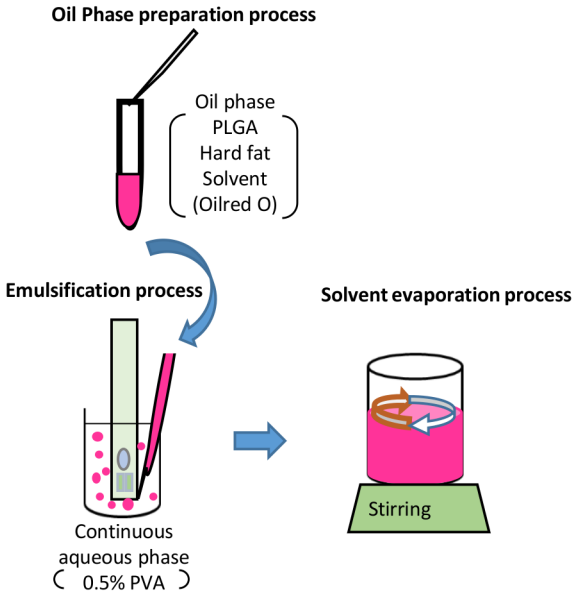

Figure 2. Illustration on fabrication process of Janus particles. Fabrication process is based on oil-in-water (o/w) emulsion solvent evaporation method.

3. Results

3.1. *Phase diagram of PLGA-hard fat solution*

The phase diagram of PLGA-hard fat in methylene chloride or ethyl acetate is shown in Figure 3. PLGA and the hard fat dissolved in common solvents such as methylene chloride and ethyl acetate. However, phase separation occurred above a certain concentration. The critical concentrations at which a phase separation was observed differed between the methylene chloride and ethyl acetate solutions. Phase separation occurred at a higher concentration in methylene chloride than it did in ethyl acetate.

3.2. *Conformation of Janus particles*

The optical micrograph of the obtained particles is shown in Figure 4, and phase separation was observed in the particles fabricated using both methylene chloride and ethyl acetate solutions. The Janus particles consisted of two clearly observed hemispheres. The Janus particles fabricated using the Oil Red O-containing oil phase were only stained on one side of the hemispheres. Figure 5 shows the state of the oil phase following the addition of Oil Red O, which exhibited phase separation. The bottom layer showed high viscosity and was slightly stained by

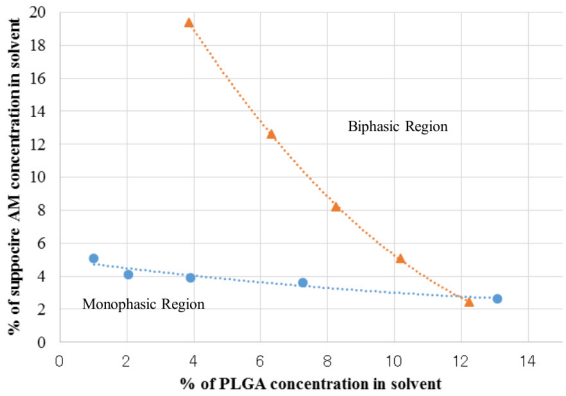

Figure 3. Phase diagram of poly (lactic-co-glycolic) acid (PLGA)-Suppocire® AM-organic solvent. Critical line between monophasic and biphasic region, using (▲) methylene chloride and (●) ethyl acetate as organic solvents.

Table 1. Fabrication condition

Number	A	B	C	D	E
Ex.1	MC 2 g	5 mL	5,000 rpm 3 min	5 mL	3 h
Ex.2	EA 3 mL	5 mL	5,000 rpm 3 min	5 mL	3 h
Ex.3	MC 2 g + Oil Red O	5 mL	5,000 rpm 3 min	5 mL	3 h
Ex.4	MC 2 g + Oil Red O	3 mL	3,000 rpm 5 min	3mL	4 h
Ex.5	MC 2 g + Oil Red O	3 mL	5,000 rpm 3 min	3mL	6 h
Ex.6	MC 2 g + Oil Red O	3 mL	5,000 rpm 3 min	100 mL	6 h
Ex.7	MC 2 g + Oil Red O	3 mL	5,000 rpm 3 min	200 mL	6 h

A: solvent of oil phase, B: volume of water phase at emulsification process, C: strength and duration of emulsification, D: volume of solvent evaporation medium, E: duration of solvent evaporation process, MC: methylene chloride, and EA: ethylacetate.

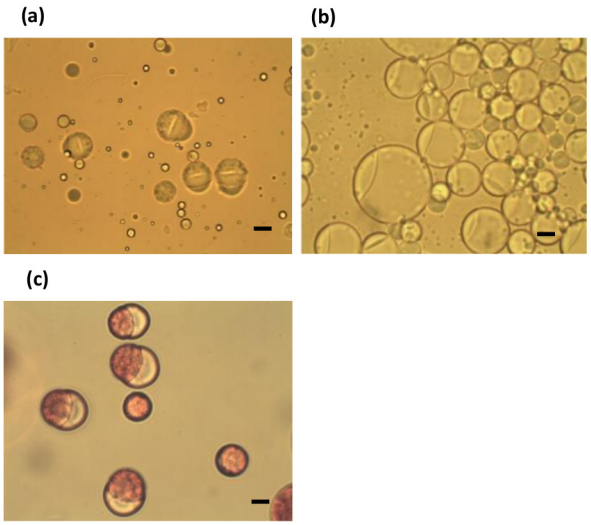

Figure 4. Optical micrograph of Janus particles. Janus particles fabricated using **(a)** methylene chloride; Ex.1 and **(b)** ethyl acetate; Ex.2 as oil phase solvents. **(c)** Janus particles fabricated using methylene chloride adding Oil Red O to oil phase solvent; Ex.3. Scale bar, 20 µm.

Figure 5. Partition in phase separated solution of poly (lactic-co-glycolic) acid (PLGA)-Suppocire® AM-methylene chloride. Poly (lactic-co-glycolic) acid (PLGA) and Suppocire AM (200 mg each) were dissolved in 0.3 g 0.02% Oil Red O-methylene chloride, and resultant solution was centrifuged.

the hydrophobic dye. In contrast, the upper layer had low viscosity and was strongly stained with the dye.

3.3. *Relationship between residual solvent profiles and Janus formation*

The PLGA-Suppocire® AM concentration monitored during the solvent evaporation process and the emulsion state are shown in Figure 6. The dispersed phase was solidified as the residual solvent decreased. Phase separation occurred at 90 min when the estimated concentration in the dispersed oil phase corresponded to the critical concentration in Figure 3. The Janus particles were obtained at over 90 min.

3.4. *Effect of volume of solvent evaporation medium*

After the oil phase containing 2 g methylene chloride was emulsified with 0.5% PVA solution, the resultant

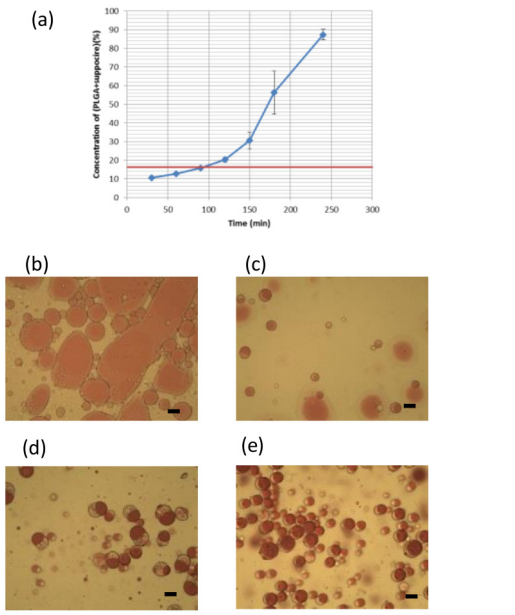

Figure 6. PLGA-Suppocire® AM concentration profile and state of emulsion during solvent evaporation process. (a) Residual solvent profile during solvent evaporation process. Red line indicates critical concentration of phase separation. The state of emulsion was **(b)** 60, **(c)** 90, **(d)** 150, and **(e)** 240 min of solvent evaporation time; Ex. 4. Scale bar, 20 µm.

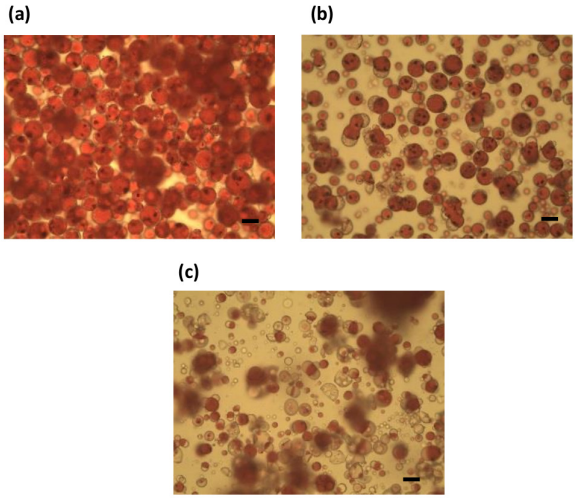

Figure 7. Effect of addition of aqueous phase after emulsification process on formation of Janus particles. Optical micrograph of emulsion after 360 min of solvent evaporation process. Addition of **(a)** 200 mL water (condition A); Ex.7, **(b)** 100 mL water (condition B); Ex.6, and **(c)** no water (condition C); Ex.5. Scale bar, 20 µm.

emulsion was added to varying volumes of water and solvent evaporation was performed. The particles obtained after 360 min of the solvent evaporation process are shown in Figure 7. Monolithic particles and a few Janus particles were observed in condition A (200 mL solvent evaporation medium). We observed that most of the particles in condition B were Janus particles (100 mL solvent evaporation medium). Furthermore, stained and unstained particles were observed in condition C (3 mL solvent evaporation medium).

4. Discussion

Research on Janus particles has drawn increasing attention over the last decade. Janus particles with an anisotropic structure likely possess numerous unknown functions and have not been utilized as much as conventional microparticles with homogeneous properties have been. In the pharmaceutical field, anisotropic formulations such as bilayer tablets have been available on the market. In a bilayer tablet, two active ingredients are loaded into distinct layers to avoid a chemical interaction between them, or the drug release from the tablet is controlled by the different release profiles of the two layers. However, even in the pharmaceutical field, few investigations on anisotropic microparticles have been performed, and so their functional applicability remains unknown. As mentioned in the introduction section, we hypothesized that Janus particles can attach and form hemispheres on the biomembrane to localize intact drug at its site of absorption and thereby enhance drug absorption (Figure 1). We performed these investigations to prove our hypothesis using PLGA and hard fat.

Several fabrication methods for Janus particles have been reported, such as the selective surface modification of isotropic particles (*21*) using a microfluidic device (*22*) and seed polymerization (*23*). Among these methods, we selected phase separation using a solvent evaporation method (*24*) because it is simple and does not require the use of synthetic organic chemistry. Moreover, the solvent evaporation method is a proven fabrication method for the commercial production of prolonged release injection formulations such as Lupron depot®. When phase separation is adopted as the fabrication method of Janus particles, it is necessary to induce phase separation between two components. First, we monitored the phase separation process between PLGA and Suppocire® AM. In the common solvent used, phase separation occurred above A certain concentration, which differed between methylene chloride and ethyl acetate. The critical concentration that induced phase separation in methylene chloride was higher than that in ethyl acetate (Figure 3). In addition to this result, the effect of the PLGA/ Suppocire® AM ratio on the critical concentration differed between the two solvents. In ethyl acetate, phase separation was less independent of PLGA concentration and occurred near 3-5% of Suppocire® AM. This phenomenon is similar to the precipitation of polymer by adding poor solvents. In contrast, in methylene chloride, the phase separation occurred when the total concentration of two components exceed a critical concentration, more independent of the mixing ratio of two components. This phenomenon is similar to the phase separation that occurs between polymers such as polylactic acid and PLGA in solvents (*25*). Dobry and Boyerkawenoki (*26*) reported the spontaneous combination of various polymers in an organic solvent. Polymers generally have higher mixing entropy than low molecular weight substances, so phase separation tends to occur between two polymers. The phase separation of PLGA and Suppocire® AM in methylene chloride may be related to the high mixing entropy.

Next, we investigated the formation of Janus particles from methylene chloride or ethyl acetate using the solvent evaporation method, and particle formation was confirmed in both solvents (Figure 4). This indicates that phase separation occurred even in the oil droplets of the o/w emulsion similar to the results shown in Figure 3. When Oil Red O, a non-polar dye used for staining triglycerides in cells in cytology, was added to the oil phase of methylene chloride, only one side of the hemispheres was stained. To identify the hemispheres stained, we analyzed the properties of each phase in the oil phase. Because the density of Suppocire® AM is lower than that of PLGA, the PLGA and Suppocire® AM layer are considered as the bottom and upper layers, respectively. Based on the results showing that the less stained bottom layer had a high viscosity, the unstained hemisphere in the Janus particle was estimated to be PLGA. The strongly stained upper layer had a low viscosity and, therefore, the stained hemisphere in the Janus particle was estimated to be Suppocire® AM.

Next, the relationship between the PLGA-Suppocire® AM concentration in the emulsion and Janus formation were investigated. Methylene chloride has 1.3% w/w solubility in water at 20°C, so approximately 40 mg methylene chloride can be dissolved in 3 mL of the continuous aqueous phase. Therefore, this amount was considered negligible compared to the 2 g of methylene chloride in the dispersed oil phase. Hence, the residual solvent in the emulsion corresponded to the approximate residual solvent in the oil phase. The formation of Janus particles was observed at a concentration over the critical concentration for phase separation (Red line in Figure 6a). The result also indicates that the phase separation occurred even in the oil droplets of the emulsion similar to the results shown in Figure 3.

Finally, we examined the effect of the medium volume used in the solvent evaporation process on the formation of Janus particles. The medium volume is related to the extraction rate of the solvent from the dispersed oil phase to the continuous aqueous phase. If the volume of the solvent evaporation medium is enough to dissolve the oil phase solvent, the solvent in the dispersed oil phase is rapidly extracted into the continuous aqueous phase. In contrast, if the volume of solvent evaporation medium is not sufficient to dissolve the oil phase solvent, The solubility of the solvent rapidly reaches at the saturation point and the solvent remains in the oil phase. Furthermore, the solvent is extracted slowly as it evaporates from the saturated aqueous phase. Condition A consisted

of 200 mL solvent evaporation medium, which was sufficient to dissolve the solvent of oil phase solvent, produced a few Janus particles (Figure 7a). To form Janus particles, the droplets produced by phase separation in the dispersed oil phase need to aggregate and coalesce before the coalescing droplets migrate to form two layers. When the rate of solvent extraction from the dispersed oil phase is rapid, the viscosity of the dispersed oil phase increases so rapidly that the droplets produced by phase separation do not have time to coalesce and migrate. Contrastingly, in condition C with 3 mL solvent evaporation medium (Figure 7c), a small amount of solvent was extracted into the continuous aqueous phase from the dispersed oil phase, and there is enough time for the separated phases to coalesce and migrate near the point of phase separation. However, the low viscosity lasts long so that the separated phases can completely migrate. The complete of migration reduces the cohesion of both hemispheres, which may be too weak to maintain the Janus conformation and both hemispheres completely separated ultimately. In this study, condition B with 100 mL solvent evaporation medium (Figure 7b) was the optimum condition required to produce stable Janus particles. Approximate 65% (1.3 g) of solvent in the dispersed oil phase is estimated to be extracted into the continuous aqueous phase at the beginning of the solvent evaporation process in condition B in the view of the solubility of methylene chloride in water. However, phase separation occurred in the dispersed oil phase when approximate 1.1 g of solvent was extracted in the continuous aqueous phase, which is close to the saturation point of methylene chloride in water. The dissolution rate near the saturation point reduces in comparison with that in a sink condition. Hence, the dissolution rate of methylene chloride in condition B is slower than that in condition A and is faster than that in condition C. The optimum dissolution rate is considered to provide the optimum balance among the coalescing and migration of the separated phases and the cohesion of both hemispheres.

In conclusion, we demonstrated that Janus particles composed of PLGA and hard fat could be fabricated using the solvent evaporation method from an o/w emulsion formation. Furthermore, this study revealed that the formation of Janus particles was affected by the extraction rate from the dispersed phase. In addition to the extraction rate, there may be other factors that affect the formation of Janus particles including the balance of interfacial tension. The conformation of particles composed of two materials has been reported to be affected by the interfacial tension between the phases of both materials in the dispersed oil phase and between the continuous aqueous phase and each phase in dispersed oil phase (*27*). The investigation of the effect of the balance in the interfacial tension will be the focus of our next investigation. In addition to demonstrating the feasibility of the formation of Janus particles, the localization of Oil Red O in one side of the particle hemispheres is another notable finding of this study. These observations indicate that the distribution of materials in Janus particles may be controllable, which is also another potential focus of future investigations.

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