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The Chinese University of Hong Kong
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One-Step Formation of W/O/W Multiple Emulsions Stabilized by Single Amphiphilic Block Copolymers

Liangzhi Hong,*,[†] Guanqing Sun,[‡] Jinge Cai,[‡] and To Ngai[†,‡]

[†]Faculty of Materials Science and Engineering, South China University of Technology, Guangzhou 510640, China
[‡]Department of Chemistry, The Chinese University of Hong Kong, Shatin N.T., Hong Kong

Ⓢ *Supporting Information*

ABSTRACT: Multiple emulsions are complex polydispersed systems in which both oil-in-water (O/W) and water-in-oil (W/O) emulsion exists simultaneously. They are often prepared accroding to a two-step process and commonly stabilized using a combination of hydrophilic and hydrophobic surfactants. Recently, some reports have shown that multiple emulsions can also be produced through one-step method with simultaneous occurrence of catastrophic and transitional phase inversions. However, these reported multiple emulsions need surfactant blends and are usually described as transitory or temporary systems. Herein, we report a one-step phase inversion process to produce water-in-oil-in-water (W/O/W) multiple emulsions stabilized solely by a synthetic diblock copolymer. Unlike the use of small molecule surfactant combinations, block copolymer stabilized multiple emulsions are remarkably stable and show the ability to separately encapsulate both polar and nonpolar cargos. The importance of the conformation of the copolymer surfactant at the interfaces with regards to the stability of the multiple emulsions using the one-step method is discussed.
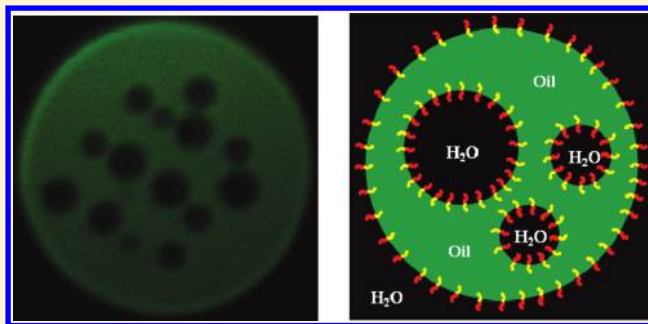
■ INTRODUCTION

Multiple emulsions of both the water-in-oil-in-water (W/O/W) and the oil-in-water-in-oil (O/W/O) have attracted significant interest due to their potential applications in various fields, including pharmaceutics, cosmetics, and food industries.[1] In addition, they are widely used as templates for the preparation of microcapsules to carry both polar and nonpolar cargos for improved synergistic delivery[2−5] or as microreactors for chemical reactions.[6]

In most cases, multiple emulsions are prepared according to a two-step process.[7] For producing a W/O/W emulsion, a primary water-in-oil (W/O) is first prepared and then re-emulsified to form W/O/W by subsequently pouring it in an aqueous solution. Whereas the two-step emulsification process has been extensively studied, this method is complex and introduces possible destabilization pathways like rupturing of the primary emulsion droplets in the second re-emulsification step.[8,9] This destabilization pathway, however, may be avoided when the one-step process via the phase inversions or microfluidic method is employed.[10−12]

Phase inversions including either catastrophic inversion or transitional inversion in nonionic surfactant−water−oil system have been well documented.[13−21] The produced W/O/W or O/W/O multiple emulsions before the catastrophic inversion are often termed as temporary or abnormal emulsions because they are extremely unstable. It has been stated that the intervening film between the colliding abnormal emulsion drops behaves as if surfactant is absent.[21,22] Only recently, some

reports have shown that stable multiple emulsions can be produced with surfactant blends as a result of the simultaneous occurrence of catastrophic and transitional phase inversion.[22−25]

The main problem regarding to the formulation and stability of multiple emulsions is the presence of two thermodynamically unstable interfaces, namely, the W/O interface of the primary emulsion and the O/W interface of the multiple emulsion. Hence, a combination of hydrophilic and lipophilic surfactants is typically required whatever by one-step method or by two-step method in order to formulate a multiple emulsion, one to stabilize the inner droplets and another to stabilize the outer droplets. Hanson et al.[26] were the first time to show that W/O/W multiple emulsions could be prepared in a direct process using single-component, synthetic amphiphilic diblock copoly-peptide surfactants with polydimethylsiloxane as oil phase.

We report herein a single catastrophic inversion process to prepare the W/O/W multiple emulsions stabilized solely by a synthetic diblock copolymer that is, in the absence of a combination of surfactants. In contrast to many previous studies,[23,26] we made the multiple emulsions with volatile and less viscous oil, which is very important in the preparation of microcapsules containing hydrophilic pharmaceuticals using solvent-evaporation method.[1−3] The formulated multiple

emulsions were not temporary systems, but were stable for over 6 months. Moreover, they could simultaneously encapsulate both water-soluble polyelectrolytes and oil-soluble dye molecules, demonstrating a versatile platform for precise encapsulation of incompatible actives.

■ EXPERIMENTAL SECTION

Materials. 2-Bromoisobutyryl bromide (98%, Aldrich), N,N,N',N'',N''-pentamethyl diethylenetriamine (99%, Aldrich, PMDE-TA), and p-toluenesulfonyl chloride (≥99%, Sigma-Aldrich) were used as received. Copper(I) bromide (98%, Aldrich) was purified with glacial acetic acid and washed with absolute ethanol and then vacuum-dried and stored under argon.[27] Triethylamine (Fisher Scientific, AR) was refluxed with p-toluenesulfonyl chloride for 12 h before it was distilled over CaH_2. Styrene (≥99%, 10–15 ppm 4-tert-butylcatechol inhibitor, Sigma-Aldrich) was dried over finely grounded CaH_2 for 1 day and then fractionally distilled. Poly(ethylene glycol) monomethyl ether (Fluka, AR, MPEG−OH, M_n = 2000 g/mol) was dissolved in tetrahydrofuran (THF) and precipitated in diethyl ether to obtain polymer powder before it was vacuum-dried. THF was distilled over sodium with benzophenone.

Synthesis of PEG-b-PS. The block copolymer used to stabilize and control the emulsion inversion in this study is composed of two blocks: the first one is poly(ethylene glycol) (PEG) block and is therefore hydrophilic, whereas the second one consists of polystyrene (PS) block and is lipophilic. We synthesized the poly(ethylene glycol)-b-polystyrene (PEG-b-PS) copolymers by atom-transfer radical polymerization (ATRP) using α-(2-bromoisobutyrylate)-ω-methyl PEG-2000 macroinitiator.[28] In a typical procedure, α-(2-bromoisobutyrylate)-ω-methyl PEG-2000 macroinitiator (2.0459 g, 1.02 mmol), which was synthesized following the literature method,[29] was dissolved in 6.4 mL styrene prior to degassing by three freeze−pump−thaw cycles. The solution was transferred, via cannula, to a second Schlenk flask containing copper(I) bromide (174.0 mg, 1.21 mmol) and PMDETA (0.64 mL, 3.07 mmol) in 0.5 mL styrene, previously degassed by three freeze−pump−thaw cycles and backfilled with nitrogen. Polymerization was carried out at 105 °C for 6 h. The contents of the Schlenk flask were diluted with THF, passed through a basic alumina column, and precipitated three times into n-hexane. The polymer was subsequently dried and analyzed by ^{1}H NMR and size exclusion chromatography (SEC). The n/m ratio in the PEG_n-b-PS_m diblock copolymer was determined by ^{1}H NMR from the ratio of methylene group of the PEG block and aromatic group of the PS block. Table 1 summarizes the characteristics of the block copolymer used in this study.

Table 1. Characteristics of PEG-b-PS Diblock Copolymers

polymer	n/m^a	M_n (g/mol)^a	PDI^b
PEG_{45}-b-PS_{66}	1.47	8.86×10^3	1.30

^{a}Calculated from ^{1}H NMR ^{b}From SEC; the SEC graph is shown in Figure S1, Supporting Information.

Emulsion Preparation and Characterization. The emulsions stabilized by the block copolymers, PEG_{45}-b-PS_{66}, were prepared by homogenizing a specific internal fraction of toluene and DI water using an Ultra Turrax T25 homogenizer (10 mm head) operating at 13 500 rpm for 120 s. The total emulsion volume was kept at 4 mL for all samples. The copolymers were dissolved in toluene before emulsification and trace amount of perylene was added for confocal imaging. For samples adding RhB-PDMAEMA, this polymer was first dissolved in water before emulsification. The confocal microscopy images were taken on a Nikon Eclipse Ti inverted microscope (Nikon, Japan). Lasers with wavelength of 543 and 408 nm were used to excite the Rhodamine B molecules on the PDMAEMA polymer and perylene molecules dissolved in toluene, respectively. An oil immersion objective (60×, NA = 1.49) was used to view the samples. The multiple emulsions were placed on the cover slides, and a series of x/y

layers were scanned. All confocal images were the cross section of the emulsion droplets.

■ RESULTS AND DISCUSSION

The emulsification of water and toluene using the copolymer surfactants was prepared by mechanically shearing a mixture containing water, toluene, and PEG_{45}-b-PS_{66} copolymers at different internal fraction of oil for 2 min with an Ultra Turrax T25 homogenizer (10 mm head) operating at 13 500 rpm. The total emulsion volume was kept at 4 mL for all samples, and the volume ratio of toluene was changed from 15% to 80%. The block copolymers were dissolved in the toluene before mixing, and the concentration was kept at 1.0 wt %. To better probe the exact structure of the resulting emulsions by confocal laser scanning microscopy (CLSM), we made use of a lipophilic dye (perylene, ∼0.1 wt %) in toluene, which allowed an unambiguous distinction between dispersed and continuous phase during imaging.

The confocal images shown in Figure 1 illustrate how a versatile synthetic diblock copolymer can form single and
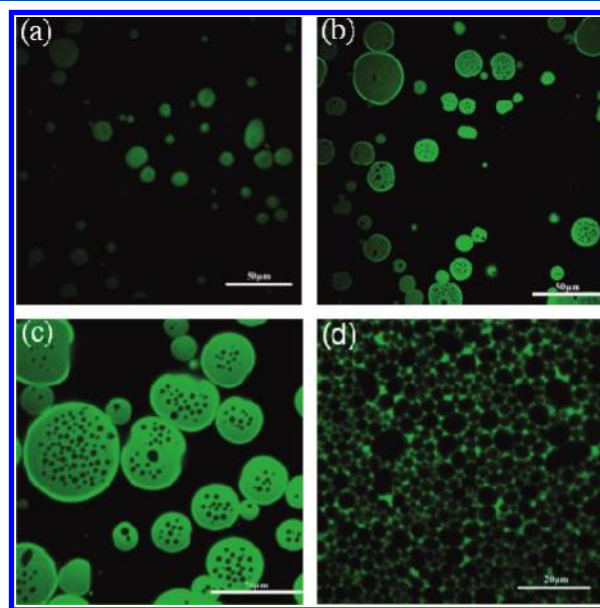

Figure 1. CLSM images of copolymer-stabilized emulsions prepared by shearing a mixture containing toluene, water, and PEG_{45}-b-PS_{66} copolymers at different volume fractions of oil: (a) 15% toluene, (b) 22% toluene, (c) 40% toluene, and (d) 80% toluene.

multiple emulsions depending on the internal volume fraction of oil. At the volume fraction of toluene below 15%, stable O/W emulsions were formed as confirmed by the confocal images Figure 1a. Most of the oil droplets (the green color arises from the dissolved perylene) are spherical and dispersed in water, and therefore, water is the continuous phase. Some multiple emulsions were found when the volume fraction of toluene reaches at 20% as shown in Figure S2 in the Supporting Information. Yet most interestingly, at the intermediate volume fraction of toluene (20−70%), multiple W/O/W emulsions were obtained via the one-step emulsification process. Figure 1b and c clearly shows that multiple globules entrapping a large number of small internal droplets with relatively narrow size distribution were observed when the volume fractions of toluene were 22% and 40%, respectively. The droplet size has remained constant, and the multiple emulsions have maintained

structural integrity since they were prepared (Figure S3, Supporting Information), indicating no sign of coalescence. A further increase of the oil fraction (80%) resulted in a typical catastrophic phase inversion. The emulsions formed such that toluene remained the continuous phase and were thus W/O type (Figure 1d).

While many studies have described the appearance of W/O/W multiple globules during a one-step phase-inversion process, the emulsions produced that have been reported until now are usually temporary and/or unstable systems, especially when small molecule surfactant combinations are used.[15,30] As a result, the development of multiple emulsions for application is largely limited. In general, two mechanisms based on diffusion controlled process have been proposed to account for the instability of the multiple emulsions stabilized by small molecule surfactants.[8] As illustrated in Figure 2a, the oil
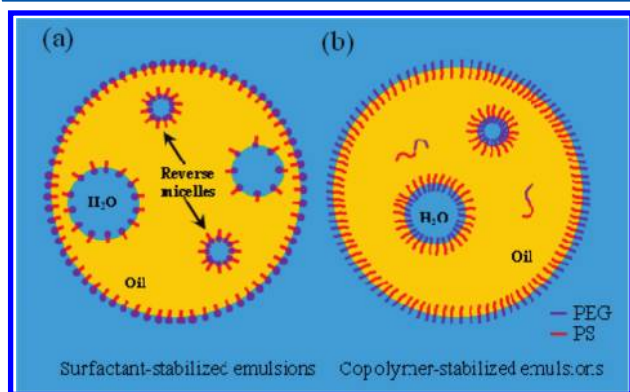

Figure 2. Schematic illustration of the influence of surfactants and block copolymers on emulsion stability control:L (a) W/O/W multiple emulsions stabilized by small molecule surfactants and (b) W/O/W emulsion stabilized by PEG_{45}-b-PS_{66} block copolymers.

phase of a formed W/O/W emulsion is commonly regarded as a sort of liquid membrane separating the internal and external aqueous phase. However, in the presence of an osmotic gradient and relatively thin film interlayer formed by the surfactants at the water–oil interface, the thermodynamic driven diffusive exchange of water and oil between the internal phase and external phase can result in a swelling or shrinkage of the inner droplets and followed by rupture of the oily membrane. This phenomenon leads to the production of a simple emulsion or even disappearance of the multiple globules.[9] The second mechanism regarding to the destabilization of a W/O/W multiple emulsion is due to the presence of reverse micelles in the oil phase. This is because the amount of surfactants commonly used to prepare the multiple emulsions is significantly above the critical micelle concentration (CMC). In this way, the formed micelles may solubilize the surfactants both in the oil phase and interfaces, which in turn leads to the rupture of the oily membrane and loss of the internal water phase.[7] Long-term stability so far is still a major problem of surfactant-stabilized multiple emulsions.

The designed PEG_{45}-b-PS_{66} diblock copolymer surfactants shown here provide a unique feature that stabilizes multiple emulsion droplets. Although hydrophilic PEG block is soluble in both toluene and water, PEG is expected to be preferentially wetted by water phase that allows the migration of the copolymers from the initial toluene phase to the water–oil interface,[31] stabilizing both the outer O/W and inner W/O

droplets, as schematically described in Figure 2b. In addition, PS was chosen as a lipophilic block because it is soluble in toluene but not in water. The high solubility of the lipophilic PS chain in the oil phase could facilitate the extension of the PEG chain to outer water phase in order to shield the PS blocks interaction with water molecules. As a result, the more extended conformation of PEG in the water phase significantly enhances the stability of outer O/W emulsion through the steric repulsion mechanism.[32,33] Similarly, in the inner water droplets, the extension of the PS chain to the toluene phase is also facilitate as the PEG block is shielded from interactions with oil molecules. This shielding effect leads to a more extended conformation of PS in the oil phase and allows the efficient stabilization of the inner water droplets. Our designed copolymers thereby differ greatly from typical surfactant-stabilized emulsions where single component surfactants generally do not stabilize multiple emulsion droplets. Another important feature of using PEG_{45}-b-PS_{66} block copolymers to stabilize the multiple emulsions is that both PEG and PS blocks are soluble in toluene. In contrast to the typical surfactants, no reverse micelles are formed in the middle toluene phase. This striking difference can account for the longer shelf life of our formulated emulsion because there is no the solubilization of the surfactants in the oil phase and interfaces, which eventually prevents the rupture of oil membrane and loss of the internal droplets.

Having discussed the long-term stability of one-step formulated emulsions, we demonstrate their encapsulating ability by loading both water-soluble and oil-soluble fluorescent markers into copolymer-stabilized multiple emulsions. Water-soluble Rhodamine B end-labeled linear poly(2-(N,N-dimethylamino)ethyl methacrylate) (RhB-PDMAEMA) polymer and oil-soluble perylene were dissolved in water and toluene, respectively, before emulsification. The synthesis of RhB-PDMAEMA has been detailed elsewhere.[34] It is worthy to mention that the RhB-PDMAEMA, which will avoid intra-molecular cyclization (spirocyclic form), should maintain its fluorescence and shows pH-independent emission spectrum. This is analogous to rhodamine derivatives bearing an ester functionality at the 2′ position, e.g., rhodamine 6G.[35]

Using confocal microscope, we imaged both markers in the multiple emulsion droplets. Figure 3a shows the copolymer-stabilized multiple emulsions with merged green and red channels at pH 7. The green color arises from the dissolved perylene clearly distinguish the oil phase, while it is hard to resolve the RhB-PDMAEMA in this image. Keep in mind that the fluorescence intensity of RhB-PDMAEMA is weak because there is only one rhodamine B molecule per polymer chain. By turning off the green channel, Figure 3b clearly shows RhB-PDMAEMA is also preferentially located in oil phase at pH 7. However, we obtained high loading in inner water phase at a lower pH (Figure 3c) because low pH increases the protonation of the tertiary amine group of PDMAEMA, thus enhancing the solubility of RhB-PDMAEMA polymer chains in water.[36] Comparing Figure 3c and d, there are almost no RhB-PDMAEMA polymers distributed in oil phases at pH 3 as shown in the darker part in Figure 3d. Figure 3 clearly shows that the lipophilic perylene (green) can be encapsulated into the oil phase, but the loading ability of RhB-PDMAEMA polyelectrolytes depends on the pH of the medium. This result is of great interest, because polyelectrolytes may serve as a model for study the enzymes and proteins encapsulation.
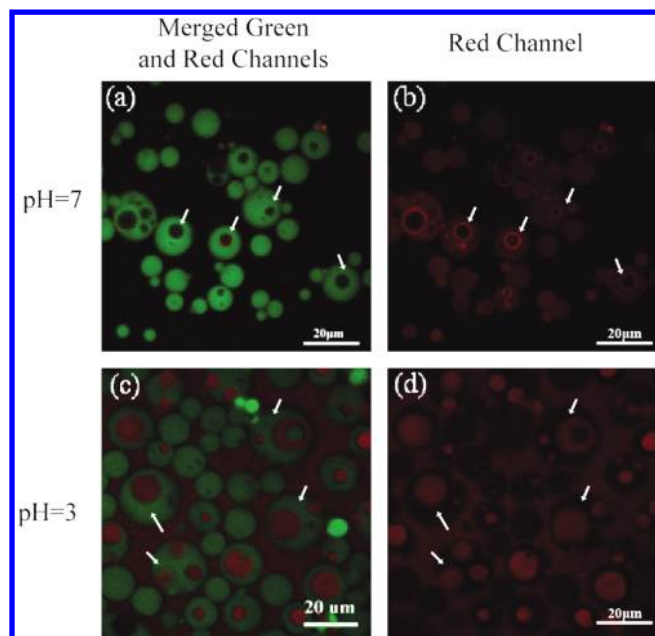
Figure 3. CLSM images show the separately loading of both water-soluble *RhB*-PDMAEMA polyelectrolytes and oil-soluble dye molecules into copolymer-stabilized W/O/W multiple emulsions: (a) with merged green and red channels and (b) with red channel at pH 7; (c) with merged green and red channels and (d) with red channel at pH 3. The multiple emulsions were prepared by using 1 wt % block copolymer as stabilizer at 50% volume fraction of toluene. After exciting by a 408 nm laser, the oil phase showed fluorescence green due to entrapped perylene, while the internal aqueous phase showed fluorescence red because of encapsulation of *RhB*-PDMAEMA polyelectrolytes. The arrows indicate the oil phase.

It is worth noting that the size of the internal water droplets in Figure 3 is larger than that in Figure 1. To demonstrate the encapsulating ability of multiple emulsions, we have added 1% *RhB*-PDMAEMA polyelectrolytes in aqueous phase in Figure 3, which may increase the original water phase viscosity. When the viscosity of the dispersed phase increases, it will take longer time to deform a drop of that liquid phase, as well as increase the size of the dispersed phase.[30] Salager et al. has also demonstrated that when the viscosity of any phase increases for a given water−oil-ratio, the critical dispersed phase fraction at which the catastrophic phase inversion is trigger decreases.[20] All these factors likely cause the multiple emulsions in Figure 3 contain larger but less internal water droplets than Figure 1c.

CONCLUSIONS

In conclusion, this study has demonstrated that the designed PEG-*b*-PS diblock copolymer can be very efficient surfactants to stabilize the W/O/W multiple emulsions during the one-step emulsification process. The efficiency stems from the fact that diblock copolymers with correct wettability were adsorbed at the interfaces with the lipophilic PS blocks extended on the oil side, and the hydrophilic PEG block extended on the water side, allowing the efficient emulsion stabilization through the steric repulsion mechanism. More importantly, no inverse micelles were formed in the oil phase. The prepared multiple emulsions were very stable and exhibited no significant structural change during a storage period of six months. In addition, the resulting emulsions demonstrate the ability of loading polyelectrolytes through simple pH changes. Using these types of multiple emulsions will enable the design of novel capsules for encapsulation of enzymes and proteins in drug delivery.

ASSOCIATED CONTENT

Supporting Information

SEC graph of the block copolymer and CLSM images of block copolymer stabilized multiple emulsions. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*E-mail: mslzhong@scut.edu.cn. Telephone: (86)-2223 6372.
Notes
The authors declare no competing financial interest.

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