

Bi-Continuous Emulsions Stabilized by pH-Responsive Self-Assembled Aggregates of Amphiphilic Random Copolymer with One-Step Emulsification

Hao Du ¹, Yun Zhang ², Yuyun Yang ¹, Tongtong Cao ¹, Ming Li ¹ and Yuhua Cao ^{1,*}

¹ School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, China; 18735491524@163.com (H.D.); 6230606047@stu.jiangnan.edu.cn (Y.Y.); 7230610001@stu.jiangnan.edu.cn (T.C.); leeming@jiangnan.edu.cn (M.L.)

² Suzhou Green Leaf Daily Commodity Co., Ltd., Suzhou 215000, China; alex.zhang@szgreenleaf.com

* Correspondence: yuhuacao64@gmail.com

Abstract

We reported a simplified one-step emulsification strategy to prepare bi-continuous emulsions with a gel-like property using the pH-responsive self-assembled aggregates of an amphiphilic random copolymer poly (styrene-*co*-methacrylic acid) (P(St-*co*-MAA)) as the interfacial stabilizers. Using caprylic/capric triglyceride (GTCC) as the oil phase, 1.0% P(St-*co*-MAA) aqueous solution with a pH between 7.0 and 8.0 as the water phase, and an oil/water phase ratio of 6:4, bi-continuous emulsions could be formed directly through one-step emulsification. Systematic characterization with a fluorescence microscope, scanning electron microscope, and confocal laser scanning microscope confirmed the formation of the bi-continuous emulsions. The three-phase contact angle measurements confirmed that the surface wettability of the self-assembled aggregates changed with pH, and the three-phase contact angles of the bi-continuous emulsions formed at a pH between 7.0 and 8.0 were close to 90°. Furthermore, rheological analysis of the bi-continuous emulsion showed the storage modulus (G') dominating over the loss modulus (G''), which verified that the bi-continuous emulsion was attributed to the existence of a three-dimensional elastic gel network. The pH-dependent wettability of the self-assembled aggregates as the stabilizers enabled pH to control the emulsion type from O/W to bi-continuous to W/O. The work provides a simple, rapid, and robust approach to preparing bi-continuous emulsions without intricate particle modifications and cumbersome procedures.

Keywords: amphiphilic random copolymer; bi-continuous emulsion; pH-responsive; self-assembled aggregates

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1. Introduction

Bi-continuous emulsions are unique emulsion systems composed of two immiscible fluids, formed by solid particles or surfactants jammed at the interface between the two phases [1]. The structural configuration attracts great attention owing to its high surface-to-volume ratio [2]. Moreover, the unique interfacial architecture of bi-continuous emulsions, characterized by balanced positive and negative curvatures, not only exhibits efficient and sustained transfer and supply of substances between the oil and water phases [3], but also provides an ideal application platform for tasks involving two-phase interface

transport, such as battery electrodes [4], catalytic engineering [5], filtration membranes [6,7], chemical reactors, [8,9] and so on.

Bi-continuous emulsion channels were initially fabricated through thermal quenching, solvent transfer, and vapor-induced phase separation [10–12]. While thermal quenching needs to control the temperature precisely, the other two methods require the removal of the cosolvents. Currently, a direct mixing strategy is employed to fabricate bi-continuous emulsions. Cai et al. [2] developed a two-step mixing protocol for fabricating a bi-continuous emulsion using SiO₂ nanoparticles with neutral wettability modified by cetyltrimethylammonium bromide to stabilize high-viscosity glycerol and polydimethylsiloxane. Huang et al. [13] reported a one-step homogenization method to prepare a bi-continuous emulsion with a submicron-scale structure using SiO₂ nanoparticles combined with hydrophilic carboxylic acid-functionalized polystyrene and hydrophobic amine-functionalized polydimethylsiloxane to stabilize the water–toluene interface.

In addition, it is necessary to utilize modified particulate stabilizers with neutral wettability to fabricate bi-continuous emulsions. This can be achieved by bonding hydrophobic or hydrophilic groups [14], adsorbing anionic and cationic surfactants [15–18], multivalent ions [19], or polyelectrolytes [20] to the surfaces of SiO₂ [21], Janus particles [22], nanocrystals [23], nickel carbonate nanospheres [24], Sm₂S₃ nanospheres [25], reduced graphene oxide, [26] and so on. The amphiphilicity of the particulate emulsifiers is represented by their three-phase contact angle θ at a liquid–liquid interface. When $\theta < 90^\circ$, the particles exhibit stronger hydrophilicity, favoring O/W emulsion formation, while when $\theta > 90^\circ$, W/O emulsion becomes predominant. As θ approaches 90° , the particles have equal wettability in both phases and generate zero-curvature and bi-continuous liquid domains to form bi-continuous emulsions [27–29]. The necessity of carefully adjusting particle wettability indeed introduces significant complexity in bi-continuous emulsion fabrication.

Bi-continuous emulsions have demonstrated great potential in the field of materials science in recent years due to their unique fully interconnected dual-network structure. Their primary application lies in serving as templates for the fabrication of porous materials [30]. Lee et al. [31] utilized ultraviolet irradiation to cure the oil phase of a bi-continuous emulsion, forming a universal polymer bi-continuous template. Subsequently, they further produced bi-continuous macroporous ceramics, a copper-coated macroporous polymer, nickel networks, and a spinodal nickel shell via chemical conversion. Pizzetti et al. [32] innovatively introduced alginate into the aqueous phase of a bi-continuous emulsion, cross-linked it with CaCl₂ to form a hydrogel, and applied it in a drug delivery system. This approach successfully resulted in the fabrication of a biphasic porous drug delivery system capable of synergistic release of both hydrophilic and hydrophobic drugs. Wang et al. [33] successfully fabricated a bi-continuous emulsion with uniform submicron-scale structures using a two-step solvent removal method. Through UV curing, a porous polymer film was formed. Optical tests revealed that the film exhibited excellent broadband reflection performance across the visible and near-infrared spectra, showing potential applications in passive cooling coatings, solar cells, and LED devices.

Our latest research revealed that amphiphilic random copolymer poly (styrene-co-methacrylic acid) (P(St-co-MAA)) can self-assemble to form aggregates due to pH adjustment. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS) confirmed that the copolymer self-assembled to form aggregates at pH < 9.0. By using P(St-co-MAA) as the stabilizer at the water–oil interface and adjusting the pH of the water phase to modulate the wettability of the self-assembled P(St-co-MAA) aggregates, pH-responsive Pickering emulsions with reversible phase transformation between W/O and O/W were successfully achieved. At pH 8.0,

larger oil domains appeared in the O/W emulsion, while at pH 7.0, larger aqueous zones were observed in the W/O emulsion [34].

Based on this, it is reasonably speculated that a bi-continuous emulsion may be formed within the pH window. In the present work, the pH of the copolymer aqueous solution between 7.0 and 8.0 was adjusted finely to modify the wettability of the self-assembled aggregates, and stable bi-continuous emulsions were achieved by a one-step emulsification strategy. The surface wettability of self-assembled aggregates used to stabilize bi-continuous emulsions at the water–GTCC interface was demonstrated by three-phase contact angle measurements. The bi-continuous emulsions were also characterized by fluorescence microscopy, SEM, and CLSM from microscopic perspective. Furthermore, the gel-like structure of the bi-continuous emulsion was characterized by rheological analysis. Although a one-step mixing method for bi-continuous emulsion preparation has been documented, the use of self-assembled aggregates from the amphiphilic random copolymer as the stabilizers to prepare bi-continuous emulsions has not been reported.

2. Materials and Methods

2.1. Materials

Caprylic/capric acid triglyceride (GTCC, 98%) was obtained from Shandong Yusuo Chemical Technology Co., Ltd. (Linyi, China). Nile red (BR) and fluorescein isothiocyanate (FITC, 90%) were obtained from Shanghai Titan Technology Co., Ltd. (Shanghai, China) and Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium hydroxide (NaOH, AR) and hydrochloric acid (HCl, AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Solid paraffin was obtained from Nantong Haizhixing Laboratory Equipment Co., Ltd. (Nantong, China) Poly(styrene-co-methacrylic acid) (P(St-co-MAA)) (monomer ratio of St to MAA of 6:4, $M_n = 9655$, $M_w = 13,942$) was laboratory-made, and the characterization data can be found in the literature [34].

2.2. Preparation of the Polymer Aqueous Solution

A total of 1.0 g P(St-co-MAA) with a monomer ratio of 6:4 was dissolved in 42 mL of 0.1 M NaOH solution and stirred overnight, then diluted with deionized water to 100 mL to obtain a 1.0% (*w/v*) aqueous solution.

2.3. Preparation of the Emulsions

With Nile-red-stained GTCC as the oil phase and an FITC-stained 1.0% (*w/v*) P(St-co-MAA) aqueous solution with pH 7.3 as the water phase, and the oil and water phases maintained at 25 °C, emulsions with different oil-to-water mass ratios were prepared by emulsifying for 2 min at 10,000 rpm using a high-speed disperser (XHF-DY, Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China) at room temperature.

The aqueous polymer was adjusted to different pH values using 0.1 M HCl. Using GTCC as the oil phase and 1.0% aqueous polymer solutions with different pH values as the aqueous phase, emulsions at different pH values were prepared under an oil-to-water mass ratio of 6:4 following the same method as described above.

2.4. Characterization of the Emulsions

Fluorescence microscope characterization: Polymer aqueous solutions with pH 8.5, pH 7.9, pH 7.6, pH 7.3, pH 7.1, and pH 6.5 were stained by FITC, and the emulsions were prepared according to the method described in Section 2.3. Then, 100 μ L of the emulsions at different pHs were dripped onto glass slides. The emulsions were observed using a fluorescence microscope (Nikon Corporation, Shinagawa, Japan) under an excitation

wavelength of 485 nm, and fluorescence microscopic images of the emulsions were captured using 4× and 10× objective lenses.

Appearance observation: Polymer aqueous solutions with pH 8.5, pH 7.9, pH 7.6, pH 7.3, pH 7.1, and pH 6.5 were stained by FITC, and GTCC was stained by Nile red. Then, the emulsions were prepared according to the method described in Section 2.3. The prepared emulsions were photographed after 5 min static equilibration at room temperature to observe phase separation, color, and other macroscopic characteristics. The flowability was assessed by inverting the vials containing the emulsions.

Three-phase contact angle measurement: 50 mL of the aqueous polymer solutions with pH 8.5, pH 7.9, pH 7.6, pH 7.3, pH 7.1, and pH 6.5 were freeze-dried to obtain solid powders, which were then compressed to form solid discs with a diameter of 1.5 cm using an automatic powder tablet press machine (C0803-PCD-100S, Tianjin Pinchuang Technology Development Co., Ltd., Tianjin, China). The solid discs were placed at the bottom of a glass container and submerged with GTCC. The pH of water drops dripped onto the discs was the same as the pH of the corresponding freeze-dried solid powder. The optical contact angle was measured with a contact angle measuring device (OCA15EC, German Data Physical Instruments Co., Ltd., Filderstadt, Germany).

Laser scanning confocal imaging: 100 µL of an emulsion at pH 7.3 was placed in a confocal dish with a glass slide diameter of 20 mm. The emulsion was observed under a confocal laser scanning microscope (CLSM, TCS SP8, Leica Microsystems, Wetzlar, Germany) equipped with a 10× objective lens, with parameters of 512 × 512 resolution, 600 Hz scanning speed, and excitation wavelengths of 285 nm and 525 nm.

SEM characterization: Aqueous polymer solutions with pH 8.5, pH 7.3, and pH 6.5 and paraffin were thermostatically treated in a constant-temperature water bath at 65 °C, serving as the aqueous phase and oil phase, respectively. A series of emulsions was prepared by emulsification at 10,000 rpm for 2 min. Then, the emulsions were freeze-dried to obtain solid sample. An appropriate amount of sample was placed on conductive adhesive, sputter-coated with gold, and characterized using a scanning electron microscope (SEM, S-4800, Hitachi, Ltd., Tokyo, Japan) at an accelerating voltage of 3.0 kV.

Rheological analysis: Emulsions at pH 8.5, pH 7.3, and pH 6.5 were prepared according to the method described in Section 2.3. After being stored at room temperature for 24 h, an appropriate amount of sample was placed on the testing platform of a rotational rheometer (DHR-3 TA Instruments, New Castle, DE, USA). Using a 40 mm diameter cone-plate fixture with a constant temperature of 25 °C, the apparent viscosity of the samples was measured under shear rates from 0.01–100 s^{−1}. The steps for testing the emulsion modulus are as follows: First, the linear viscoelastic region (LVR) of emulsion was determined under the strain sweep mode. The strain range was set at 0.01% to 20%, with a constant frequency of 1 Hz and a test temperature of 25 °C, and a 40 mm cone plate was selected for the strain sweep measurement of the LVR. Subsequently, an oscillatory frequency sweep test was conducted on the emulsion within the LVR. A constant strain of 0.7% was applied, with a frequency sweep range from 0.1 to 100 rad/s, and the test temperature was maintained at 25 °C. During the test, the dynamic rheological data of the storage modulus (*G'*) and loss modulus (*G''*) as a function of frequency were collected and recorded in real time.

Stability test: A bi-continuous emulsion at pH 7.3 was stored at room temperature in the dark for 1 day and 7 days. The fluidity of the sample was observed by inverting the emulsion to determine whether the gel characteristics maintained. The stability was further verified using a fluorescence microscope.

3. Results and Discussion

3.1. The Effect of Oil–Water Mass Ratio on the Type of the Emulsion

To determine the optimal oil-to-water mass ratio to prepare a bi-continuous emulsion, a series of emulsions with different oil-to-water mass ratios were prepared using Nile-red-stained GTCC as the oil phase (red) and FITC-stained aqueous polymer solution with pH 7.3 as the aqueous phase (green) and observed with a fluorescence microscope. Figure 1 demonstrates that the emulsions with oil-to-water mass ratios ranging from 1:9 to 5:5 were O/W emulsions. At oil-to-water mass ratios of 6:4 and 7:3, the emulsions exhibited a bi-continuous structure forming bi-continuous emulsions. However, at oil-to-water ratios of 8:2 and 9:1, the emulsions were identified as W/O emulsions. Although an emulsion also exhibited bi-continuous structure at an oil-to-water mass ratio of 7:3, the bi-continuous emulsion formed at a ratio of 6:4 exhibited a more complete oil–water interpenetrating structure and more uniformly distributed oil–water channels. Therefore, the optimal oil-to-water ratio was chosen as 6:4 for in-depth characterization of bi-continuous emulsion properties.

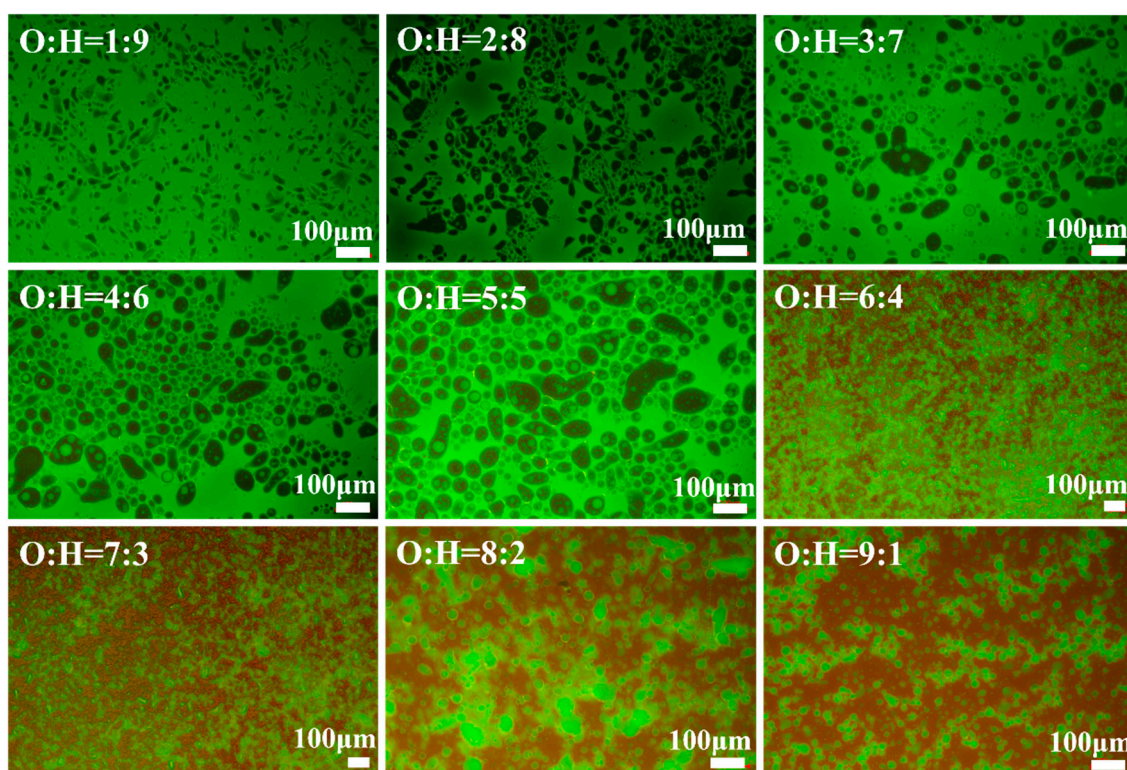


Figure 1. Fluorescence photographs of the emulsions under excitation wavelength of 485 nm. The emulsions with different oil-to-water mass ratios were formulated using FITC-stained 1.0% (*w/v*) P(St-co-MAA) aqueous solutions with pH 7.3 as the aqueous phase and Nile-red-stained GTCC as the oil phase, via emulsification at 10,000 rpm for 2 min.

3.2. Fluorescence Microscopy Characterization

The emulsion type can be altered by adjusting the pH of the copolymer aqueous solution. The microstructures of different emulsion types were characterized by fluorescence microscopy. The green regions correspond to the aqueous phase stained by FITC excited at 485 nm, while the dark regions represent the oil phase in Figure 2. At pH 8.5, the oil droplets were dispersed in the aqueous phase, forming an O/W emulsion. As the pH decreased to 7.9, the oil droplets became larger and began to coalesce, locally forming a continuous network structure. At pHs 7.6, 7.3, and 7.1, the dispersed oil droplets coalesced completely, forming a continuous oil phase in the system, while the aqueous phase

was either interconnected or fragmented by the continuous oil domains. The mutual interpenetration of the oil and aqueous phases resulted in bi-continuous emulsions. When the pH further decreased to 6.5, the oil phase dominated as the continuous phase, and the water droplets were dispersed in the oil phase, forming a W/O emulsion. Hence, pH was demonstrated to be the pivotal parameter in controlling emulsion type. This phenomenon originated from the pH-dependent ionization degree of MAA segments on the surface of the self-assembled aggregates. At pH 8.5, the carboxy groups of MAA segments at the aggregate surfaces exhibited a high ionization degree, resulting in pronounced hydrophilicity of the aggregates. At pH 6.5, the ionization degree of MAA decreased and the hydrophobicity of the self-assembled aggregates increased, which led to the formation of a W/O emulsion. At a pH between 7.0 and 8.0, close to pK_a (7.76) of P(St-co-MAA) with a monomer ratio of 6:4 [34], the self-assembled aggregates exhibited nearly equal proportions of $-COO^-$ and $-COOH$ groups on their surfaces. This equal wettability between oil and water phases led to the formation of bi-continuous emulsions. The insets in Figure 2 show that these emulsions at pH 7.9, pH 7.6, pH 7.3, and pH 7.1 remained at the bottom when the vials were upside-down. The lack of the flowability demonstrated that the bi-continuous emulsions had gel-like characteristics [13].

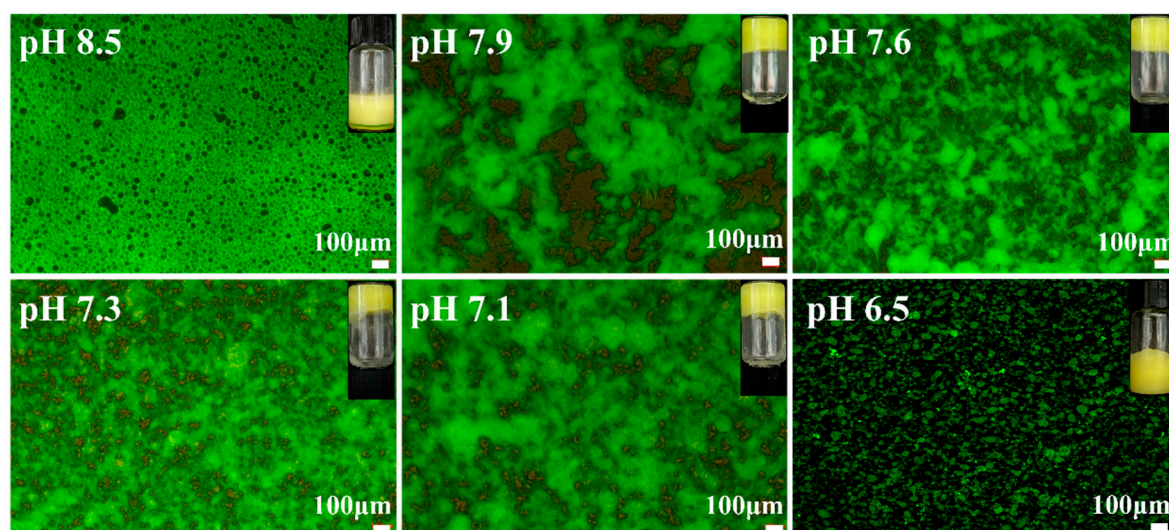


Figure 2. Fluorescence photographs of the emulsions at different pHs under excitation wavelength of 485 nm using 4× objective. The insets in the micrographs represent the visual appearance of the emulsions at pH 8.5 and pH 6.5, and the inverted vial images of the emulsions at pH 7.9, pH 7.6, pH 7.3, and pH 7.1. The emulsions were formulated using FITC-stained 1.0% (*w/v*) P(St-co-MAA) aqueous solutions with different pHs as the aqueous phase and unstained GTCC as the oil phase, with an oil-to-water ratio (*w/w*) of 6:4, by emulsification at 10,000 rpm for 2 min.

Additionally, the emulsions prepared using Nile-red-stained GTCC as the oil phase (red) and FITC-labeled aqueous polymer solutions (green) at different pHs exhibited obvious macroscopic differences. In Figure 3a, the emulsion at pH 8.5 shows phase separation, with the pink emulsion phase at the upper layer and the green water phase at the bottom. Owing to the density difference, the oil droplets in an O/W emulsion would float up and the water phase sink down. None of the phase separation occurred in the emulsions at pH 7.9, pH 7.6, pH 7.3, pH 7.1, or pH 6.5. However, the emulsion at pH 6.5 was pink, and the emulsions at pH 7.9, pH 7.6, pH 7.3, and pH 7.1 appeared orange. The oil phase stained with Nile red was red, and the water phase stained by FITC was green. The pink emulsion at pH 6.5 indicated that the oil phase served as the continuous phase and the water phase as the dispersed droplets encapsulated in the oil phase. This verified the formation of a W/O emulsion at pH 6.5. In contrast, the emulsions at pH 7.9, pH 7.6, pH

7.3, and pH 7.1 were orange in color, a combination of red and green, which suggested that the continuous phase was not a single phase, but rather that the aqueous and oil phases were interpenetrating each other to form a unique bi-continuous structure. Then, fluorescence microscopy was used to characterize the microstructure of these emulsions (Figure 3b), and the results were the same as in Figure 2. Similarly, the emulsions at pH 7.9, pH 7.6, pH 7.3, and pH 7.1 were located at the bottom of the vials after inversion (Figure 3b). This also proved that these emulsions had gel-like properties.

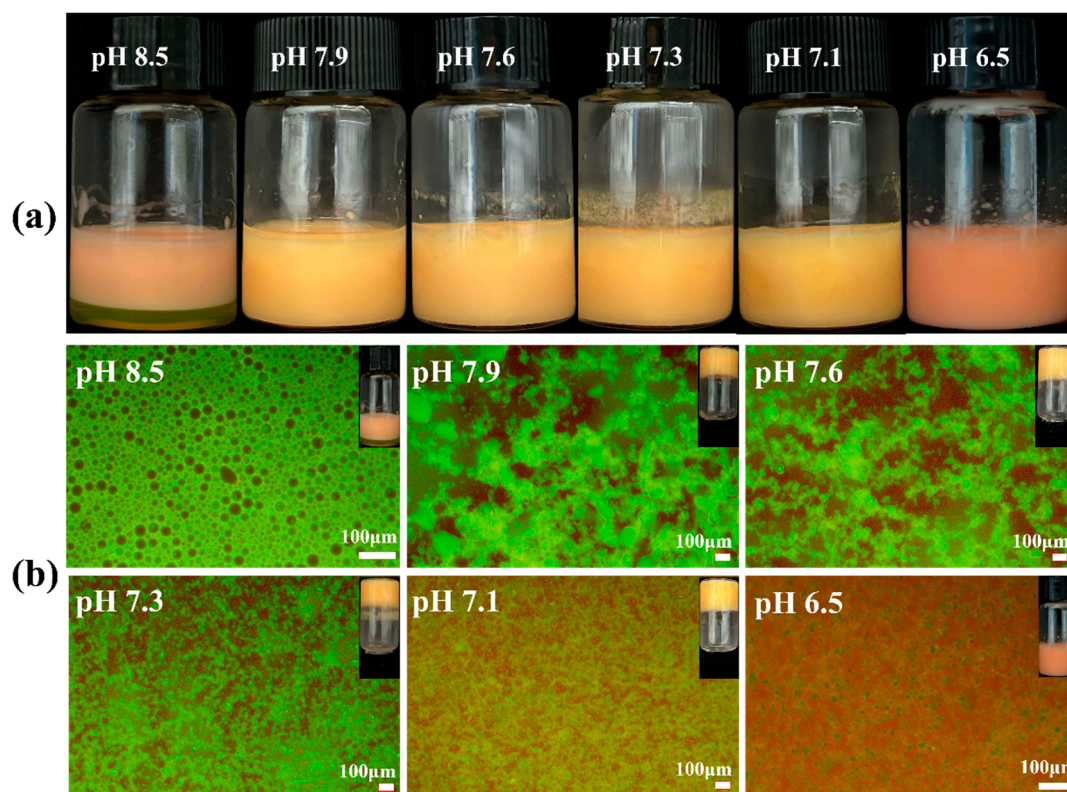


Figure 3. (a) Appearance and (b) fluorescence microscopy images of the emulsions prepared using FITC-stained 1.0% (*w/v*) P(St-co-MAA) aqueous solutions with different pHs as the water phase and Nile-red-stained GTCC as the oil phase. The fluorescence images of the emulsions at pH 8.5 and pH 6.5 were obtained using a 10× objective, and the other conditions were the same as in Figure 2.

3.3. Measurement of the Three-Phase Contact Angle

The neutral wettability of the self-assembled aggregates is the key factor in the fabrication of a bi-continuous emulsion. Thus, the surface wettability of the aggregates with different pHs was explored by measuring the water–GTCC–aggregate contact angles (θ) [35], with the results shown in Figure 4. At pH 8.5, the three-phase contact angle was 74.9°, less than 90°, indicating the aggregates exhibited pronounced hydrophilicity favoring O/W emulsion formation. When pH was adjusted to 6.5, the aggregates were more easily wetted by GTCC at $\theta > 90^\circ$, resulting in the formation of a W/O emulsion. With θ approaching 90° at pH from 7.0 to 8.0, the aggregates exhibited equal wettability between the water and GTCC phases, resulting in the formation of a bi-continuous structure. Because the ratio of the hydrophobic -COOH to the hydrophilic -COO⁻ groups on the surfaces of the aggregates varied with pH changes, the three-phase contact angle of the aggregates at the water–GTCC interface also changed, which led to the transition of the emulsion types.

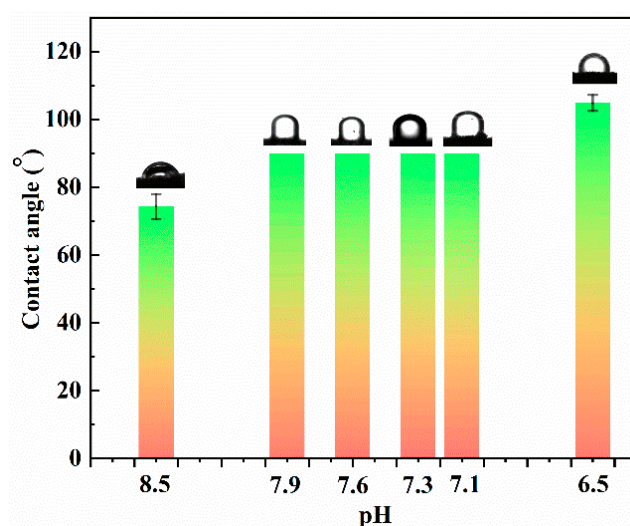


Figure 4. The three-phase contact angles (θ) of the self-assembled aggregates from P(St-co-MAA) under different pH conditions at the water–GTCC interface.

3.4. Scanning Electron Microscopy Characterization

With a decrease in temperature, paraffin wax will undergo a transition from liquid to solid. Thus, it was able to lock the microstructure of an emulsion rapidly when it served as the oil phase. It was believed that the gray areas in the SEM images (Figure 5) were the oil areas and the black areas were the water areas after freeze-drying treatment. Owing to the rapid evaporation of water by lyophilization, the oil droplets in the O/W emulsion became deformed. Thus, cube-shaped paraffin wax as the dispersed phases in the water phase at pH 8.5 is shown in Figure 5a, and O/W emulsion was testified. At pH 6.5, a W/O emulsion with paraffin wax as the continuous phase was formed, and the black points left by water droplets can be seen in Figure 5c. As is clearly seen in Figure 5b, the large black areas (indicating the aqueous phase) existed around the interconnected paraffin phase, and the two phases mutually interpenetrated, in contrast to the oil or water phase as the dispersed droplets in the other continuous phase shown in Figure 5a or Figure 5c. These findings were consistent with the results presented in Figures 2 and 3. All of them demonstrated that the surface wettability of the self-assembled aggregates acting as the particulate stabilizers could be controlled by adjusting the pH of the aqueous phase. Thereby, controllable transition of the emulsion type would be achieved.

3.5. Laser Confocal Microscopy Imaging

Furthermore, taking the emulsion at pH 7.3 as an example, the distribution of the oil and aqueous phases on different axial layers of the bi-continuous emulsion was scanned and stacked to establish a complete three-dimensional scanning image using CLSM. Excitation of the water-soluble dye FITC at 285 nm resulted in green fluorescence emission. So, the green areas represent the aqueous phase labelled with FITC, and the black areas indicate the oil phase in Figure 6a. Although some isolated water domains were present, the bi-continuous structure formed by three-dimensional interpenetration of the aqueous and oil domains predominated in the emulsion. Furthermore, this architecture was consistent with the bi-continuous morphology reported by Zhang et al. [35]. Additionally, a three-dimensional scanning image of the emulsion prepared using Nile-red-labelled GTCC as the oil phase and the aqueous polymer solution at pH 7.3 as the aqueous phase was obtained. Excitation of the oil-soluble dye Nile red at 525 nm resulted in red fluorescence emission. As shown in Figure 6b, the oil phases on different axial planes were interconnected, while the aqueous phases were either interconnected or fragmented by the

continuous oil domains. Figure 6 displays the spatial connectivity between the aqueous and oil phases among the different axial planes in a bi-continuous emulsion. The interconnected aqueous networks spanned all axes and formed a continuous water domain, while mutually interpenetrating oil–water areas generated a bi-continuous architecture.

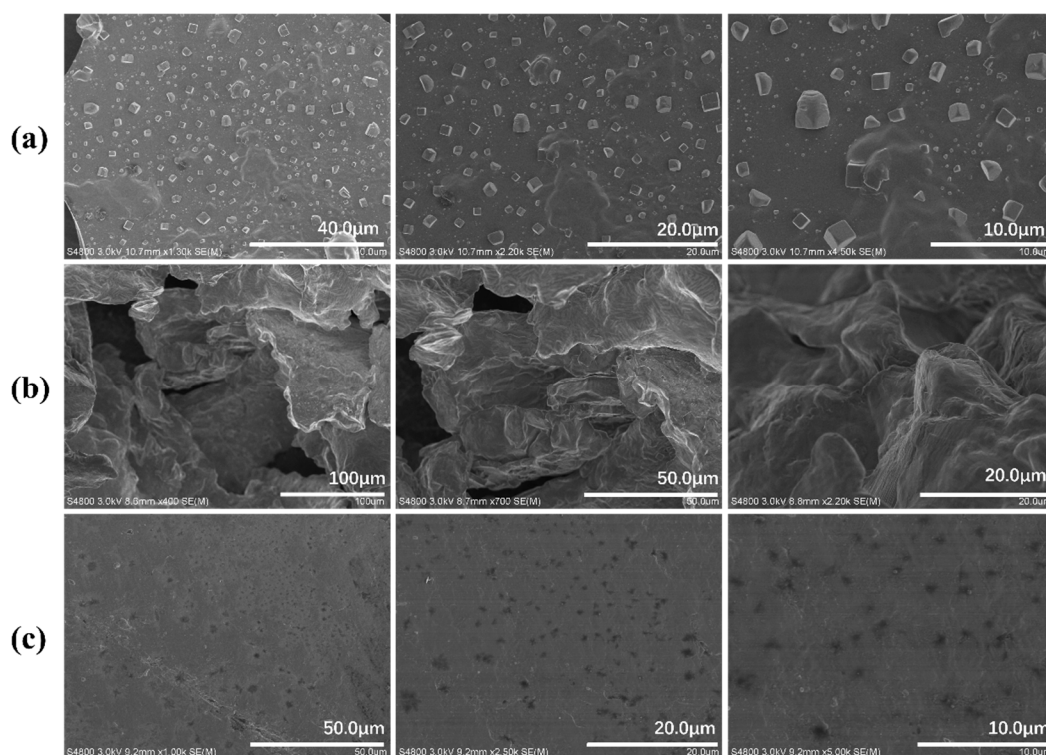


Figure 5. SEM images of the emulsions after freeze-drying prepared with 1.0% (*w/v*) P(St-co-MAA) aqueous solutions with (a) pH 8.5, (b) pH 7.3, and (c) pH 6.5 as the aqueous phase and melted paraffin wax as the oil phase and a mass ratio of oil to water of 6:4, emulsified at 10,000 rpm for 2 min.

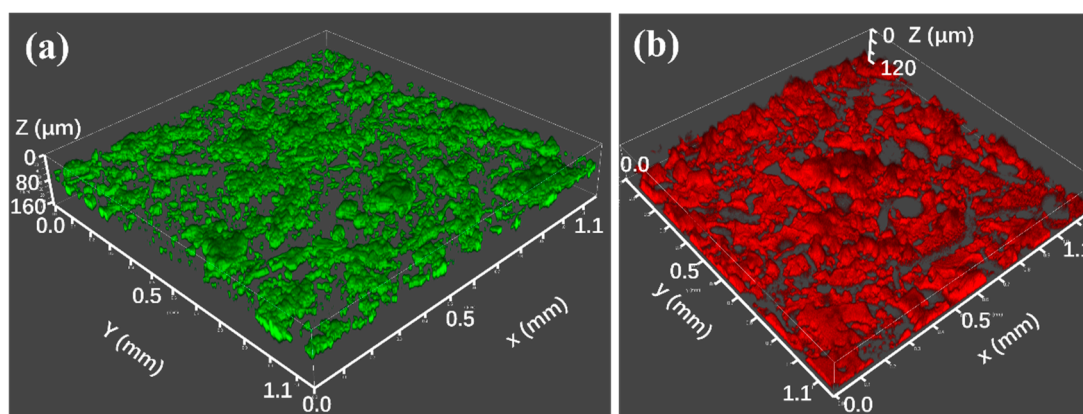


Figure 6. Laser confocal microscopy 3D images of the emulsion at pH 7.3. The scanning images were captured using excitation wavelengths of (a) 285 nm and (b) 525 nm, and the emulsion preparation conditions were the same as in Figure 2.

3.6. Rheological Analysis of Emulsion

As shown in Figure 7a, the apparent viscosity of three kinds of emulsions, W/O, bi-continuous, and O/W, gradually decreased with increasing shear rate, demonstrating distinct shear-thinning behavior [36]. At lower shear rates, the viscosity of the bi-continuous emulsion was the largest, followed by the W/O emulsion, and the viscosity of O/W emulsion was the smallest. As can be seen from Figure 7b, both the bi-continuous and W/O

emulsions exhibited pronounced gel-like behavior due to the storage modulus (G') exceeding the loss modulus (G'') [37]. Furthermore, in Figure 7c, the phase angles for both the W/O and bi-continuous emulsions were below 45° , indicating their elastic-dominated gel state [38]. Therefore, the gel-like characteristics of both the bi-continuous and W/O emulsions were determined not by the viscosity of the oil or aqueous phases, but by the self-assembled aggregates adsorbed at the interface. These aggregates formed a solid-like interfacial film with high elasticity at the oil–water interface [30]. Figure 7b,c clearly show that the bi-continuous emulsion exhibited a higher G' and G'' but a lower phase angle compared to the W/O emulsion. These rheological results collectively demonstrated that the gel property and elastic network rigidity of the bi-continuous emulsion were superior to those of the W/O emulsion.

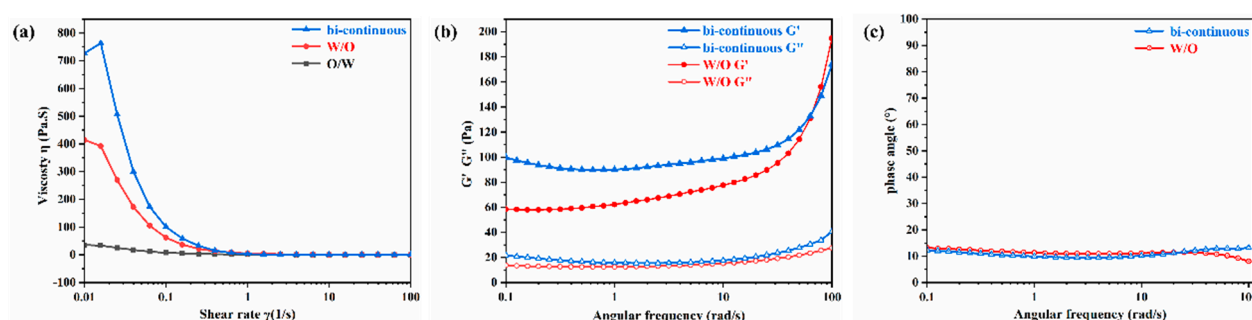


Figure 7. (a) Viscosity tests of W/O (pH 8.5), bi-continuous (pH 7.3), and O/W (pH 6.5) emulsions; (b) modulus tests and (c) phase angle results of bi-continuous (pH 7.3) and W/O (pH 6.5) emulsions.

3.7. Stability Determination of the Bi-Continuous Emulsion

Taking the bi-continuous emulsion formed at pH 7.3 as an example, the photographs of the upside-down emulsion were captured after 1 day and 7 days of storage in the dark, as shown in Figure 8a. It can be observed that the emulsion maintained its original state without significant changes after 1-day and 7-day storage. Both samples remained at the bottoms of the vials upon inversion, which demonstrated that the gel characteristics of the bi-continuous emulsion remained unchanged over time. The microscopic structures of the emulsion were captured using a fluorescence microscope on different days. As evidenced in Figure 8b, the microstructure was maintained after 7-day storage. These findings confirmed that both the gel-like property and bi-continuous architecture of the bi-continuous emulsion possessed stability.

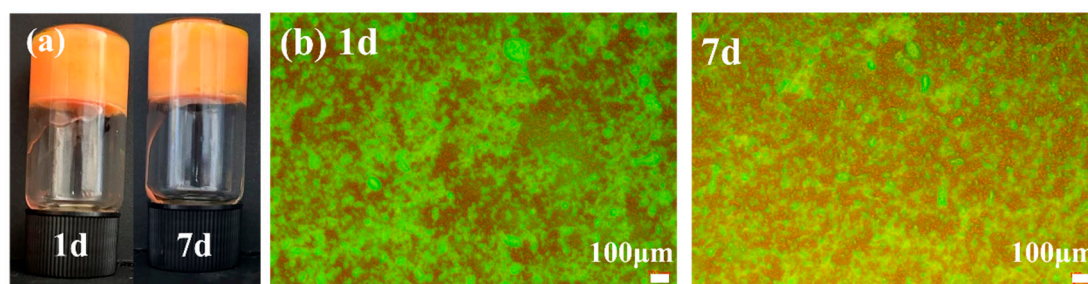


Figure 8. (a) Inverted appearance photographs of the bi-continuous emulsion at pH 7.3 stored in the dark at room temperature for 1 day and 7 days and (b) corresponding fluorescence microscopy images.

4. Conclusions

In this study, a one-step emulsification strategy to fabricate a bi-continuous emulsion with a gel-like property using pH-responsive self-assembled aggregates from P(St-co-

MAA) was developed. By adjusting pH of the aqueous phase, the surface wettability of the self-assembled aggregates was finely tuned to achieve a contact angle close to 90° at the water–GTCC interface, as verified by three-phase contact angle measurements, and a stable bi-continuous emulsion with interpenetrating bi-continuous networks was confirmed by fluorescence microscopy, SEM, and CLSM. Rheological analysis described a strong gel-like behavior for the bi-continuous emulsion, where the storage modulus (G') consistently surpassed the loss modulus (G'') and the phase angle was below 45°. The bi-continuous emulsion could persist for seven days. This approach circumvented the requirements for complicated surface modifications of particulate stabilizers as well as the laborious and tedious process to fabricate bi-continuous emulsions. This work not only expands the application of an interfacial stabilizer with self-assembled aggregates from an amphiphilic copolymer, but also provides a versatile platform for designing functional soft materials with bi-continuous morphologies.

Author Contributions: H.D.: methodology, investigation, validation, formal analysis, and writing—original draft; Y.Z.: supervision and methodology; Y.Y.: conceptualization, data curation, and software; T.C.: software, data analysis, and writing—review and editing; M.L.: visualization and writing—review and editing; Y.C.: conceptualization, supervision, methodology, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: We confirm that all authors have no conflicts of interest to declare regarding this work. Author Yun Zhang was employed by Suzhou Green Leaf Daily Commodity Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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