***In situ* nano-objects via RAFT aqueous dispersion polymerization of 2-methoxyethyl acrylate using poly(ethylene oxide) macromolecular chain transfer agent as steric stabilizer**

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ABSTRACT

Poly(ethylene oxide)-poly(2-methoxyethyl acrylate) diblock copolymers (PEO-*b*-PMEA) are synthesized by RAFT aqueous dispersion polymerization of MEA using poly(ethylene oxide) macromolecular chain transfer agent as a reactive steric stabilizer. Both segments are well-known to be bio- and blood-compatible polymers. This formulation enables the production of various particle morphologies such as spheres, worms, and vesicles from the same block copolymer in water. The synthesis starts when both the reactive steric stabilizer and MEA monomer are dissolved in water; however, the growing polymer is not water-soluble and begins to form nano-objects. In the case of the synthesis of PEO₁₁₃-*b*-MEA₃₀₀ diblock copolymers, the nano-objects change from spheres into larger aggregates of worms when the solids concentration in the polymerization increases from 5 to 15 wt% at full monomer conversion. The morphology finally turns into vesicles as the solids concentration increases to 20 wt%. The final block copolymer morphology at full monomer conversion is dictated by not only degree of polymerization of MEA but also the solids concentration in the polymerization mixture.

1. Introduction

Reversible addition fragmentation chain-transfer (RAFT) polymerization is considered to be one of the most established and efficient methods to prepare block copolymers with a controlled molecular weight and molecular weight distribution [1]. In general, block copolymers in selective solvents self-assemble to form spherical micelles, rod/worm-like micelles, vesicles and other higher-order structures [2-21]. However, the self-assemblies can only be achieved in dilute solution (typically, copolymer concentration ≤ 1.0 wt% [2]) by complicated and time consuming processes. Therefore, RAFT aqueous dispersion polymerization presents an efficient strategy

through the polymerization induced self-assembly (PISA) process in water [6-13]. This process enables direct preparation of well-defined block copolymer self-assemblies at high solids concentration. Furthermore, post-polymerization processing is not required and PISA has been achieved in various solutions such as aqueous solutions, non-polar organic solvents, or alcoholic media [14-18]. Another method, RAFT emulsion polymerization, also leads to various morphologies [19]. Thus, PISA is suitable for large scale applications including drug delivery systems (DDS) [20]. Some comprehensive reviews on PISA including the RAFT dispersion and emulsion polymerizations have been written [21].

In the past few years, Sugihara and Armes et al. introduced PISA via RAFT aqueous dispersion polymerization that was dependent on both the degree of polymerization (DP) of the core block and the solids concentration [6-8]. This was achieved through the chain extension of the hydrophilic homopolymer, poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC) with the soluble monomer, (2-hydroxypropyl methacrylate) (HPMA) in water. The self-assembly was induced when PHPMA became insoluble in water during the course of the polymerization. The authors concluded that the diblock copolymer formulation can predict the production of nano-objects with higher-order structures such as worms and vesicles that were previously difficult to prepare due to the narrow phase region. We have generalized this study on PISA by using a useful poly(ethylene oxide) macromolecular-chain transfer agent (PEO macro-CTA) as a steric stabilizer and 2-methoxyethyl acrylate (MEA) as a monomer for PEO-*b*-PMEA. For RAFT aqueous dispersion polymerization of MEA, there is an interesting example using poly(poly(ethylene glycol)methyl ether methacrylate) (PPEGMA) as a macromolecular-chain transfer agent (macro-CTA) [22]. The polymerization enabled the production of nanoparticles 40-60 nm in diameter. However, to our knowledge, there are few reports on the preparation of

various morphologies of PEO-*b*-PMEA not only via RAFT dispersion polymerization and but also by conventional RAFT solution polymerization.

PEO exhibits blood compatible and biocompatible properties because it is nontoxic and nonimmunogenic, thus it is used in biomedical and pharmaceutical applications such as DDS. PEO improves the enhanced permeability and retention (EPR) effect in tumor tissue through PEGylation [23,24]. PEGylated nanoparticles are stable, and further aggregation in the blood stream can be prevented. PMEA also exhibits blood compatibility and low protein adsorption, and has been widely used in commercial biomedical applications [25,26]. The most important feature is that the MEA monomer is highly water soluble, but the PMEA is not [22,27]. This characteristic is suitable for RAFT dispersion polymerization to induce self-assemblies for biomedical applications.

In this study, we demonstrate chain extension using RAFT aqueous dispersion polymerization of MEA in the presence of PEO macromolecular RAFT agent as a reactive steric stabilizer. As the PMEA chains grow during the course of the polymerization, the hydrophobicity is increased. Thus, the resulting PEO-*b*-PMEA diblock copolymers form *in situ* self-assemblies in water. These polymer nano-objects are controlled not only by the length of the PEO block, but also by the total solids concentration of the polymerization of MEA (Fig. 1). The resulting block copolymer aggregates in water are kinetically frozen at the ambient temperature. However, using RAFT dispersion polymerization conducted at high temperature, the aggregates can change their morphology. This is considered to be due to the inter-micelle fusion, which means inelastic collisions between solvated/plasticized nanoparticles leads to an increase in the packing parameter [21(c)]. Thus, unlike other methods, this formulation allows the production of higher-order structures such as worms and vesicles, directly and efficiently in high concentration from the same block copolymer. These predetermined morphologies of the

diblock copolymer in water will offer a new prospect for biomedical applications such as nanocarriers in DDS.

Fig. 1. Synthesis of the PEO macro-CTA (A) and RAFT aqueous dispersion polymerization of MEA using the PEO macro-CTA at 70 °C (B). Various morphologies can be directly prepared, depending on either the total solids concentration or the *DP* of the PMEA block.

2. Experimental

2.1. Materials

2-Methoxyethyl acrylate (MEA) was purchased from Wako (> 98%). The MEA was purified by passage through a prepacked inhibitor-removal column (Sigma-Aldrich) before use. Toluene (Wako; > 99.5%, super dehydrated) was distilled over calcium hydride just before use.

Poly(ethylene oxide) monomethyl ether (PEO-OH, Aldrich; $M_n = 2000 \text{ g mol}^{-1}$ and 5000 g mol^{-1}), 4-dimethylaminopyridine (DMAP, Nacalai Tesque; > 98%) and *N,N'*-dicyclohexylcarbodiimide (DCC, Wako) were used without further purification. 4,4'-Azobis(4-cyanovaleric acid) (V-501, Wako; > 98%) was purified by recrystallization from methanol. RAFT agent, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid, was synthesized according to a literature protocol [28].

2.2. Synthesis of PEO macro-CTA and RAFT aqueous polymerization

PEO macro-chain transfer agents with molecular weights of 2000 g mol^{-1} and 5000 g mol^{-1} were synthesized following the literature protocol as shown in Figure 1(a) [29,30]. The structure of the PEO was determined by ^1H NMR spectroscopy. The typical procedure for RAFT aqueous dispersion polymerization using the PEO macro-CTA is as follows. For commercial PEO having a methyl ether terminus with molecular weight of 2000 g mol^{-1} (Sigma-Aldrich), the actual *DP* of the macro-CTA was 45 as judged by the ratio of oxyethylene moieties and methoxy terminus from ^1H NMR spectroscopy. This PEO_{45} macro-CTA (0.15 g, equivalent to 0.065 mmol PEO repeat units) was mixed with V-501 (6.0 mg, 0.021 mmol; macro-CTA/initiator molar ratio = 3.0), varying amounts of MEA (0.634-2.115 g, 4.872-16.252 mmol; target *DP* 75-250), and deionized water (1.829-20.385 g; 10-30 wt%) for the desired total solids concentration in a 25-50 mL round-bottomed flask, which was equipped with a magnetic stir bar. These solutions were stirred in an ice-bath until all reagents had dissolved. After sparging with nitrogen for approximately 30 min, each polymerization was allowed to proceed at 70°C and then quenched after 23 h via cooling at room temperature and exposure to air. For the longer PEO with a methyl ether terminal with molecular weight of 5000 g mol^{-1}

(Sigma-Aldrich), we determined the DP ($= 113$) by the same protocol and conducted the polymerization of MEA using the PEO₁₁₃ macro-CTA.

2.3. Polymer characterization

Molecular weight distributions (MWDs) of the copolymers were assessed by size exclusion chromatography (SEC) in *N,N*-dimethylformamide (DMF) with 10 mM LiBr at 40 °C using two polystyrene gel columns [TSK gel G-MH_{HR}-M; flow rate 1.0 mL/min] connected to a Tosoh CCPM-II pump and RI-8012 and UV-8000 for refractive and UV detectors, respectively. Number-average molecular weight (M_n) and polydispersity (M_w/M_n) were calculated from GPC curves on the basis of polystyrene (PSt) and PEO calibrations for block copolymer and PEO macro-CTA, respectively. ¹H NMR spectra to determine the structures such as the number-average end functionality of the RAFT (dithioester) terminal (F_n) and the compositions of block copolymers were recorded on a JEOL JNM-EX500 spectrometer (500 MHz). DLS studies were performed using a Zetasizer Nano-ZS instrument (Malvern Instruments) at 25 °C at a scattering angle of 173°. The mean hydrodynamic diameter (D_h) and polydispersity index (PDI, μ_2/Γ^2) of the micelles were calculated by cumulants analysis of the experimental correlation function using Zeta Software version 7.02. Atomic force microscopy (AFM) images were recorded using the dynamic mode at ambient conditions with an SPM-9700 (Shimadzu) scanning probe microscope. A silicon AFM probe tip (Olympus, OMCL-AC160TS-C3) with a radius of 7 nm, a spring constant of 26 N/m, and a resonance frequency of 300 kHz were used. A sample for AFM imaging was prepared by placing a 20 µL drop of the block copolymer solution (0.05 wt% polymer solution) on freshly cleaved muscovite mica (ca. 1 cm × 1 cm, V-4 grade, Alliance Biosystems) and allowing it to dry in air for a day. For scanning electron microscopy (SEM) studies, the aqueous dispersions (0.1 wt% of polymer solution) were

mounted directly on aluminum stubs, dried under air for one day and sputtered with gold under vacuum. They were then visualized in high vacuum using a JEOL JSM-6390 scanning electron microscope. Transmission electron microscopy (TEM) studies were conducted using a JEOL JEM2100 instrument operating at 200 kV equipped with a Gatan ORIUS SC200D CCD camera. Copper grids with carbon-coated support film (ELS-C10, Okenshoji) were glow discharged for 15 sec to create a hydrophilic surface. About 5 μ L of aqueous diblock copolymer dispersions (0.5 wt%) was put on the grid for 30 sec and then blotted with filter paper. The grid was then immersed for 60 s in Gd based stainer (EM stainer, Nisshin EM) for negative staining. The stained grid was then blotted with filter paper and dried.

3. Results and discussion

First, the RAFT agent, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid, was prepared for the RAFT aqueous dispersion polymerizations, and then PEO macro-CTAs were prepared via the esterification process of the carboxylic acid group from the RAFT agent with PEO monomethyl ether in toluene at room temperature. The structure and *DPs* of the resulting PEO macro-CTAs were determined by ^1H NMR spectroscopy (Fig. 2B). The molecular weights of the PEO macro-CTAs were 2300 ($M_w/M_n = 1.05$) and 5500 ($M_w/M_n = 1.08$) calculated from GPC curves on the basis of a PEO calibration, whose actual *DPs* were determined as 45 and 113, respectively, via ^1H NMR spectroscopy. The end-functionality of PEO₄₅ macro-CTA ($F_n = 0.94$) was larger than that of PEO₁₁₃ macro-CTA ($F_n = 0.90$), calculated from the ratio of the peak intensities of ω -end methylene (**g**) and α -end methyl protons (**a**) in Fig. 2B.

Fig. 2. ^1H NMR spectra at 25 °C of (A) 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid, (B) PEO₄₅ macro-CTA with molecular weight of 2300 ($M_w/M_n = 1.05$). PEO₄₅-*b*-MEA₇₅ (entry 1 in Table 1) in aqueous polymerization mixture diluted in D₂O (C) or diluted in CD₃OD (D).

Next, using the resulting PEO macro-CTA, RAFT aqueous dispersion polymerization of MEA was conducted by V-501 initiator in water at 70 °C under various solids concentrations. The solids concentration is defined as $100 \times [\text{PEO macro-CTA (g)} + \text{MEA (g)}] / [\text{all reaction mixture (g)}]$. In the case of the RAFT aqueous dispersion polymerization of MEA, the MEA is expected to be the core monomer for PEO-stabilized self-assembly. As the MEA chain grows from water-soluble PEO macro-CTA, at a certain point, it reaches a critical *DP* and becomes sufficiently hydrophobic so as to induce micellar nucleation. The targeted *DP* of MEA, i.e., $[\text{MEA}]_0 / [\text{PEO macro-CTA}]_0$, was set from 75 to 400. During the course of the dispersion polymerization, the solution changed from transparent to opaque, indicating the progress of the polymerization and *in situ* self-assembly induced by micellar nucleation of PMEA. Finally, the polymerization reached the full monomer conversion of MEA and the final morphology. The self-assembled morphologies induced by the polymerization were confirmed by means of AFM (dynamic mode), TEM, and SEM. The representative polymerization results including morphologies are summarized in Table 1.

Table 1

Representative RAFT aqueous dispersion polymerization of MEA at 70 °C using PEO macro-CTA.^a

entry	sample	solids (wt%) ^b	targeted <i>DP</i> of MEA	actual <i>DP</i> of MEA ^c	M_n ^d	M_w/M_n ^d	D_h (PDI) ^e	morphology ^f
1	PEO ₄₅ - <i>b</i> -MEA ₇₅	25.0	75	76	10300	1.21	104 (0.29)	sphere ^g
2	PEO ₄₅ - <i>b</i> -MEA ₁₀₀	10.0	100	102	14600	1.22	146 (0.22)	sphere
3	PEO ₄₅ - <i>b</i> -MEA ₁₅₀	20.0	150	151	19700	1.24	190 (0.24)	sphere
4	PEO ₄₅ - <i>b</i> -MEA ₂₅₀	10.0	250	254	34300	1.20	223 (0.30)	sphere
5	PEO ₁₁₃ - <i>b</i> -MEA ₃₀₀	5.0	300	301	37500	1.31	129 (0.07)	sphere
6	PEO ₁₁₃ - <i>b</i> -MEA ₃₀₀	10.0	300	304	38900	1.57	161 (0.07)	sphere ^h
7	PEO ₁₁₃ - <i>b</i> -MEA ₃₀₀	15.0	300	306	36600	1.50	209 (0.31)	sphere + worm ⁱ
8	PEO ₁₁₃ - <i>b</i> -MEA ₃₀₀	17.5	300	305	36400	1.55	323 (0.39)	Worm ^j
9	PEO ₁₁₃ - <i>b</i> -MEA ₃₀₀	20.0	300	306	36500	1.51	276 (0.37)	Vesicle ^k
10	PEO ₁₁₃ - <i>b</i> -MEA ₃₀₀	25.0	300	299	35900	1.58	280 (0.07)	Vesicle
11	PEO ₁₁₃ - <i>b</i> -MEA ₄₀₀	15.0	400	405	46400	1.58	197 (0.07)	Sphere ^l
12	PEO ₁₁₃ - <i>b</i> -MEA ₄₀₀	20.0	400	400	44000	1.55	299 (0.11)	Vesicle
13	PEO ₁₁₃ - <i>b</i> -MEA ₄₀₀	25.0	400	408	47000	1.59	329 (0.04)	vesicle
14	PEO ₁₁₃ - <i>b</i> -MEA ₄₀₀	30.0	400	403	46300	1.58	343 (0.09)	vesicle ^m

^a [PEO macro-CTA]₀/[V-501]₀ = 3.0, polymerization time = 24h; ^b 100 × [PEO macro-CTA (g) + MEA (g)]/[all reaction mixture (g)]; ^c Determined by ¹H NMR spectroscopy in *d*₄-methanol, assuming that the block efficiency is 100%; conversion ≥ 99 %; ^d Determined by GPC analysis (PSt calibration), calculated M_n = 2300 (PEO₄₅) or 5500 (PEO₁₁₃) + 130.14 (MEA) × targeted *DP* of MEA; ^e Determined by DLS measurement at 25 °C; ^f by AFM

(dynamic mode), TEM, and SEM measurement; ^g Fig. 4B; ^h Fig. 5A; ⁱ Fig. 5B; ^j Fig. 6B; ^k Fig. 6C; ^l Fig. 7A and 7C; ^m Fig. 7B and 7D.

All the polymerizations using both PEO₄₅ and PEO₁₁₃ macro-CTAs had relatively good control over the polymer chain growth. Representative kinetic results using PEO₄₅ macro-CTA are shown in Fig. 3. The kinetic studies using PEO₄₅ macro-CTA afforded a linear semilogarithmic plot after an induction period (ca. 30 min), indicating first-order kinetics with respect to monomer. This polymerization rate did not change after the induction period (≤ 95 %conversion), indicating similar partition coefficient of monomer in solution and in monomer-swollen particles [14]. The M_n of the block copolymer, PEO-*b*-PMEA, was directly proportion to the monomer conversion and the molecular weight distributions were relatively narrow throughout the polymerization, but increased slightly toward the end of the polymerization as seen in Fig. 3B. At this stage, all the polymers obtained after 40 min polymerization under the condition (10 wt% solids concentration) were spherical morphologies.

Fig. 3. Representative polymerization results to obtain PEO-*b*-PMEA of entry 4; (A) time vs. conversion and first-order kinetic plot, (B) evolution of M_n and M_w/M_n with conversion, and (C) GPC curves for aqueous dispersion polymerization of MEA at 70 °C: $[\text{PEO}_{45} \text{ macro-CTA}]_0/[\text{V-501}]_0/[\text{MEA}]_0 = 3.0/1.0/750.0$ molar ratio, total solids concentration = 10.0 wt%.

In the case of PEO₁₁₃ macro-CTA, the polymerizations of MEA also had sufficient control over the polymer chain growth. All MWDs of block copolymers were shifted toward higher molecular weight relative to the corresponding PEO₁₁₃ macro-CTA (entries 5-14 in Table 1). However, M_n s of the block copolymer were slightly higher than the calculated values and these MWDs were somewhat broader relative to those of PEO₄₅ macro-CTA. These may be due to the difference of end-functionality between PEO₄₅ and PEO₁₁₃ macro-CTAs, and/or due to the morphology change *in situ*. The polymerization proceeds in monomer-swollen particles. As polymerization proceeds, uncontrolled reactions partly occur due to the high dense cores. Thus, final values of M_w/M_n may be relatively high.

In addition, we confirmed the resulting block copolymers formed *in situ* PMEA-core aggregates in water as expected, by the comparison of ¹H NMR spectra of the resulting block polymer in D₂O and *d*₄-methanol as shown in Fig. 2C and 2D, respectively. Specifically, compared to the signals of PEO macro-CTA in Fig. 2B, we confirmed all the signals associated with the PEO macro-CTA were observed in the spectra recorded in D₂O. In contrast, none of the PMEA signals were visible in D₂O. In practice, nano-sized particles were detected by dynamic light scattering (DLS) as shown in Table 1. However, all the proton signals expected for both

the PEO and PMEA blocks were visible in the ^1H NMR spectrum recorded in d_4 -methanol, since this is a good solvent for both blocks. These ^1H NMR observations suggest that the PEO chains act as the reactive and solvated steric stabilizer, while the PMEA chains form the non-solvated micelle core.

To determine all the morphologies, DLS studies were conducted to check the particle size and the polydispersity index (PDI). When PEO₄₅ macro-CTA was used as a steric stabilizer in the RAFT aqueous dispersion polymerization of MEA, the target DP of MEA was set from 75 to 250 (entries 1-4). The representative hydrodynamic diameters (D_h) for various PEO₄₅-*b*-PMEA_m ($m = 75$ -250) diblock copolymer assemblies are shown in Fig. 4A. As the DP of PMEA increased from 75 to 250, the D_h varied from 104 to 223, regardless of the solids concentration (10.0-30.0 wt%). From the relationship between D_h and the mean DP of PMEA, it is clear that the mean D_h increased in nearly linear fashion with the increasing DP of the core. The values of PDI are relatively narrow. It is well-documented that larger micelles are invariably obtained when the chain length of the core-forming block is increased, and the aggregation behavior is essentially the same as that in the post-aggregation using the block copolymer in a selective solvent [31]. However, all diameters determined by DLS were somewhat larger than PPEGMA-PMEA particles [22]. Thus, there may be some aggregation (fusion) of the original small spheres into bigger objects entrapping the block copolymers [32].

From the AFM (dynamic mode) studies on freshly cleaved muscovite mica under the dry condition, all the self-assemblies using PEO₄₅ were spheres, regardless of the solids concentration in polymerization. For the example of PEO₄₅-*b*-PMEA₇₅ diblock copolymer, spherical micelles of 104 nm in D_h determined by DLS were obtained. The AFM images are shown in Fig. 4B. The size determined by AFM (ca. 50-100 nm) was smaller than the D_h by DLS. In addition the AFM topography (height) measurements also indicated flattening (ca. 35

nm) on mica, as shown in Fig. 4C. This may be due to the AFM observation under dry condition.

As the target DP of PMEA further increased up to 300, it was difficult to control the dispersion polymerization even at 10.0 wt% solids concentration. This is likely because PEO₄₅ was too short of a steric stabilizer to prevent precipitation. However, in these diblock copolymers (entries 1-4), no differences were seen on the morphology with the increasing solids concentration, i.e., only spherical micelles were obtained using PEO₄₅ macro-CTA.

Fig. 4. (A) Variation of D_h and PDI with DP of PMEA for PEO₄₅- b -MEA_m ($m = 75$ -250) diblock copolymer assemblies (entries 1-4). (B) Representative AFM (height) image of spheres for PEO₄₅- b -MEA₇₅ diblock copolymer assemblies (entry 1, synthesized at 25.0 wt% solids concentration). (C) Cross-sectional profile was obtained from the line A-B on the image.

Intriguingly, when PEO₁₁₃ macro-CTAs were used in the RAFT aqueous dispersion polymerization with MEA, various morphology transitions were observed. In the case of PEO₁₁₃-*b*-MEA₃₀₀ diblock copolymers (see entries 5-10), it became clear that the transition of self-assembly occurred as the solids concentration changed. Fig. 5 shows AFM (height) images of the largest spheres (Table 1, entry 6) and threshold morphology (entry 7) between spheres and worms, at solids concentrations of 10.0 and 15.0 wt%, respectively. Although these are identical block copolymers, the different solids concentration in polymerization gave different morphologies.

Fig. 5. Representative AFM (height) images with the cross-sectional profiles of (A) spheres and (B) worms and spheres for entries 6 and 7, respectively.

Fig. 6A summarizes the variation of D_h and PDI with solids concentration (5.0-25.0 wt%) for all the polymerizations for PEO₁₁₃-*b*-MEA₃₀₀ diblock copolymer assemblies including entries 5-7. From the DLS studies, the spheres of 130-160 nm in diameter changed into larger aggregates with a diameter of approximately 210 nm when the solids content increased from 5.0 to 15.0 wt%. The PDI gradually became larger. At 17.5 wt%, worms were clearly seen with an average (spherical) diameter of 323 nm, >1 μ m length and broader PDI in Fig. 6B. The dispersion was sufficiently viscous solution. The morphology finally changed into vesicles with an average diameter of 276 nm as the solids concentration increased to 20.0 wt% in Fig. 6C. The formation of vesicles was decided from the dimple by AFM topography (height) measurement. A further increase to 25.0 wt% allowed the full transition into vesicles with diameters of 280 nm. This means that worms were obtained in the narrow phase range from 16.0 to 18.0 wt%, and that the final morphology with increased solids concentration was a vesicle. This variation as solids concentration increased was commensurate with the packing parameter change [6, 33]. Moreover, it can be concluded that PEO with a longer chain ($DP = 113$) can adequately stabilize aggregates in water of various morphologies.

Fig. 6. (A) Variation of D_h and PDI with solids concentration (5.0-25.0 wt%) for the polymerization of PEO₁₁₃-*b*-MEA₃₀₀ diblock copolymer assemblies (including entries 5-10). Upper diagram shows morphological regions; S: spheres, W: worms and V: vesicles. The representative TEM image (B) (bar = 100 nm) and AFM (height) image with the cross-sectional profile (C) (2.00×2.00 μm) for entries 8 and 9, respectively. The inset in the AFM image (C) is the TEM image (bar = 200 nm) for entry 9.

The length of PEO plays an important role in stabilizing the particle and not only imparts dramatic changes in particle diameter, but also determines various *in situ* self-assemblies in water. Here, PEO-*b*-PMEA block copolymers in entries 5-10 are identical (the same block length). Moreover, mere dilution of them with water did not lead to any change in the block

copolymer morphology, confirmed by TEM, AFM and DLS measurements. This means that the resulting block copolymer aggregates in water are kinetically frozen.

When the target DP of PMEA increased to 400 using the same PEO₁₁₃ macro-CTA (entries 11-14), spheres of 209 nm diameter and vesicles of 350 nm diameter were obtained at the solids concentration of 15.0 and 30.0 wt% as shown by the SEM and AFM analysis in Fig. 7. The DLS results and the relationship between D_h , PDI and solids concentration are shown in Fig. 8. The difference was determined from the presence of a dimple in the AFM height image of dried samples, which was also observed in the SEM image of vesicles. In the case of the PEO₁₁₃ macro-CTA, no worms were observed. In practice, a two-step change in D_h was observed in Fig. 8 (15.0 wt% vs. 20.0-30.0 wt%). Thus, the unobserved worm phase may occupy a much narrower region in the series of PEO₁₁₃-*b*-MEA₄₀₀ than that of PEO₁₁₃-*b*-MEA₃₀₀. On the basis of these results, this morphology transition of PEO₁₁₃-*b*-MEA_m occurred under the influence of the DP of the core block, PMEA and the total solids concentration on the final morphology as well as that of PMPC-*b*-PHPMA formulation previously reported [6]. In other words, the production of spheres, worms and vesicles can be tuned and enables the facileness and effective preparation of various self-assemblies by directly changing the concentrated aqueous solution. Since both PEO and PMEA have similar characteristics as biocompatible polymers, they hold great promise for biomedical applications.

Fig. 7. SEM (A and B) and AFM (C and D) images of $\text{PEO}_{113}\text{-}b\text{-MEA}_{400}$ diblock copolymer morphologies polymerized at different solids concentrations of (A and C) 15.0 wt% and (B and D) 30.0 wt%.

Fig. 8. (A) DLS particle size distributions (volume vs. mean hydrodynamic diameter, D_h) and (B) the relationships between D_h or PDI and solids concentration for the resulting PEO₁₁₃-*b*-PMEA₄₀₀ under RAFT aqueous dispersion polymerization of MEA using PEO₁₁₃ macro-CTA.

4. Conclusions

In conclusion, a convenient and feasible method to prepare various morphologies has been developed via RAFT aqueous polymerization using PEO₁₁₃ macro-CTA as a steric stabilizer in the appropriate composition and/or the total solids concentration. As an application for DDS, worms and vesicles are more preferable and efficient for circulation time and cell entry than spherical micelles [20,34]. In this report, we revealed that facile and direct preparation of various morphologies can be obtained by tuning a series of PEO₁₁₃-*b*-PMEA₃₀₀ (sphere, worm, and vesicle) and PEO₁₁₃-*b*-PMEA₄₀₀ diblock copolymers (sphere and vesicle). We anticipate that these PEO-*b*-PMEA formulations will be useful for biomedical applications.

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References

- [1] (a) Chiefari J, Chong YK, Ercole F, Krstina J, Jeffery J, Le TPT, Mayadunne RTA, Meijs GF, Moad CL, Moad G, Rizzardo E, Thang SH. *Macromolecules* 1998; 31: 5559-62; (b) Moad G, Rizzardo E, Thang SH. *Aust J Chem* 2005; 58: 379-410.
- [2] Mai Y, Eisenberg A. *Chem Soc Rev* 2012; 41: 5969-85.
- [3] Hillmyer MA, Bates FS, Almdal K, Mortensen K, Ryan AJ, Fairclough JPA. *Science* 1996; 271: 976-8.
- [4] Wang X, Guerin G, Wang H, Wang Y, Manners I, Winnik MA. *Science* 2007; 317: 644-7.
- [5] Pochan DJ, Chen Z, Cui H, Hales K, Qi K, Wooley KL. *Science* 2004; 306: 94-7.

- [6] Sugihara S, Blanazs A, Armes SP, Ryan AJ, Lewis AL. *J Am Chem Soc* 2011; 133: 15707-13.
- [7] Sugihara S, Armes SP, Blanazs A, Lewis AL. *Soft Matter* 2011; 7: 10787-93.
- [8] Sugihara S. *Kobunshi Ronbunshu* 2012; 69: 567-79.
- [9] Li Y, Armes SP. *Angew Chem Int Ed* 2010; 24: 4042- 4046.
- [10] Blanazs A, Madsen J, Battaglia G, Ryan AJ, Armes SP. *J Am Chem Soc* 2011; 133: 16581-87.
- [11] Warren NJ, Mykhaylyk OO, Mahmood D, Ryan AJ, Armes SP. *J Am Chem Soc* 2014; 136: 1023-33.
- [12] Rieger J, Grazon G, Charleux B, Alaimo D, Jérôme C. *J Polym Sci Part A Polym Chem* 2009; 47: 2373-2390.
- [13] Zhou W, Qu Q, Xu Y, An Z. *ACS Macro Lett* 2015; 4: 495-499.
- [14] Zhou W, Qu Q, Xu Y, An Z. *ACS Macro Lett* 2014; 3: 1220-1224.
- [15] Fielding LA, Derry MJ, Ladmiral V, Rosselgong J, Rodrigues AM, Ratcliffe LPD, Sugihara S, Armes SP. *Chem Sci* 2013; 4: 2081-7.
- [16] Yang P, Ratcliffe LPD, Armes SP. *Macromolecule* 2013; 46: 8545-56.
- [17] (a) Wan W-M, Sun X-L, Pan C-Y, *Macromolecules* 2009; 42: 4950-2; (b) Wan W-M, Sun X-L, Pan C-Y. *Macromol Rapid Commun* 2010; 31: 399-404.
- [18] Pei Y, Lowe AB, *Polym Chem* 2014; 5: 2342-51.
- [19] (a) Boissé S, Rieger J, Belal K, Di-Cicco A, Beaunier P, Li M-H, Charleux B. *Chem. Commun.* 2010; 46: 1950-2; (b) Zhang X, Boissé S, Zhang W, Beaunier P, D'Agosto F, Rieger J, Charleux B, *Macromolecules* 2011; 44: 4149-58.
- [20] Karagoz B, Esser L, Duong HT, Basuki JS, Boyer C, Davis TP. *Polym Chem* 2014; 5: 350-5.

- [21] (a) Charleux B, Delaittre G, Rieger J, D'Agosto F. *Macromolecules* 2012; 45: 6753-65;
(b) Sun J-T, Hong C-Y, Pan C-Y. *Soft Matter* 2012; 8: 7753-67; (c) Warren NJ, Armes SP. *J Am Chem Soc* 2014; 136: 10174-85, (d) Rieger J. *Macromol Rapid Commun* 2015; 36: 1458-1471.
- [22] Liu G, Qiu Q, Shen W, An Z. *Macromolecules* 2011; 44: 5237-45.
- [23] van Vlerken LE, Vyas TK, Amiji MM. *Pharm Res* 2007; 24: 1405-14.
- [24] Nomoto T, Matsumoto Y, Miyata K, Oba M, Fukushima S, Nishiyama N, Yamasoba T, Kataoka K. *J Controlled Release* 2011; 151: 104–9.
- [25] Tanaka M, Motomura T, Kawada M, Anzai T, Kasori Y, Shiroya T, Shimura K, Onishi M, Mochizuki A. *Biomaterials* 2000; 21: 1471–81.
- [26] Mueller X, Jegger D, Augstburger M, Horisberger J, Von Segesser LK, *Int J Artif Organs* 2002; 25: 223-9.
- [27] Steinhauer W, Hoogenboom R, Keul H, Moeller M. *Macromolecules* 2010; 43: 7041-7.
- [28] Lai JT, Filla D, Shea R. *Macromolecules* 2002; 35: 6754-6.
- [29] Rieger J, Stoffelbach F, Bui C, Alaimo D, Jérôme C, Charleux B. *Macromolecules* 2008; 41: 4065-8.
- [30] Boursier T, Chaduc I, Rieger J, D'Agosto F, Lansalot M, Charleux B, *Polym Chem* 2011; 2: 355-62.
- [31] Bütün V, Armes SP, Billingham NC. *Polymer* 2001; 42: 5993–6008.
- [32] Zhang W, D'Agosto F, Boyron O, Rieger J, Charleux B. *Macromolecules* 2011; 44: 7584-93.
- [33] Antonietti M, Förster S. *Adv Mater* 2003; 15: 1323-33.
- [34] (a) Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, Discher DE, *Nat*

Nanotechnol 2007; 2: 249-55; (b) Christian DA, Cai S, Garbuzenko OB, Harada T, Zajac AL, Minko T, Discher DE. Mol Pharmaceutics 2009; 6: 1343-52.

GRAPHICAL ABSTRACT

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TITLE

In situ nano-objects via RAFT aqueous dispersion polymerization of 2-methoxyethyl acrylate using poly(ethylene oxide) macromolecular chain transfer agent as steric stabilizer

GRAPHICAL ABSTRACT FIGURE