

Enzyme-assisted Photoinitiated Polymerization-induced Self-assembly in Continuous Flow Reactors with Oxygen Tolerance

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Abstract Polymerization-induced self-assembly (PISA) is an emerging method for the preparation of block copolymer nano-objects at high concentrations. However, most PISA formulations have oxygen inhibition problems and inert atmospheres (e.g. argon, nitrogen) are usually required. Moreover, the large-scale preparation of block copolymer nano-objects at room temperature is challenging. Herein, we report an enzyme-assisted photoinitiated polymerization-induced self-assembly (photo-PISA) in continuous flow reactors with oxygen tolerance. The addition of glucose oxidase (GOx) and glucose into the reaction mixture can consume oxygen efficiently and constantly, allow the flow photo-PISA to be performed under open-air conditions. Polymerization kinetics indicated that only a small amount of GOx (0.5 $\mu\text{mol/L}$) was needed to achieve the oxygen tolerance. Block copolymer nano-objects with different morphologies can be prepared by varying reaction conditions including the degree of polymerization (DP) of core-forming block, monomer concentration, reaction temperature, and solvent composition. We expect this study will provide a facile platform for the large-scale production of block copolymer nano-objects with different morphologies at room temperature.

Keywords Polymerization-induced self-assembly; Reversible addition-fragmentation chain transfer (RAFT) polymerization; Photoinitiation; Oxygen tolerance

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INTRODUCTION

Block copolymer nano-objects with various morphologies are of widespread interest for applications in the field of drug delivery, catalysis, optoelectronic device, biomineralization, functional coating, etc.^[1–8] Cosolvent self-assembly is one of the most commonly used methods for the preparation of block copolymer nano-objects, which involves the synthesis of well-defined amphiphilic block copolymers and the subsequent self-assembly in a selective solvent.^[9] The morphology of block copolymer nano-objects can be controlled by changing packing parameter of the block copolymer. However, a diluted solution (solids content <1%) is required in the cosolvent self-assembly method, making the large-scale production of block copolymer nano-objects challenging. Moreover, organic solvents are usually needed, which is not beneficial to the preparation of biofunctional block copolymer nano-objects.

Over the past decade, the development of reversible addition-fragmentation chain transfer (RAFT)-mediated polymerization-induced self-assembly (PISA) by Pan, Armes, Charleux and others has enabled the preparation of block copoly-

mer nano-objects at much higher polymer concentrations (10 wt%–50 wt%).^[10–27] In RAFT-mediated PISA, a soluble homopolymer prepared by RAFT polymerization is chain-extended with a second block. The second block becomes insoluble in a selective solvent when the molecular weight increases to a critical value, leading to the occurrence of *in situ* self-assembly and therefore the formation of block copolymer nano-objects. The morphology of block copolymer nano-objects can be easily controlled by varying reaction parameters (e.g. molecular weight of the core-forming block, monomer concentration, temperature etc.). Most RAFT-mediated PISA formulations are performed *via* thermal initiation at relatively high temperatures (e.g., 70 °C). The high temperature feature of thermally initiated RAFT-mediated PISA limits the preparation of various functional block copolymer nano-objects e.g. biofunctional block copolymer nano-objects. In 2015, our group and others have developed a room-temperature PISA method based on type I photoinitiation, termed photoinitiated polymerization-induced self-assembly (photo-PISA).^[28–45] The photo-PISA method has greatly expanded the scope of RAFT-mediated PISA for the preparation of functional block copolymer nano-objects.^[46–48] Several novel PISA systems such as sequence-controlled PISA^[49], temperature-programmed PISA,^[50–52] and Z-RAFT-mediated PISA^[53] have also been developed based on photo-PISA.

Despite the fact that many achievements have been made

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in photo-PISA, external light irradiation is inevitable to perform the polymerization. Therefore, light penetration is a critical issue that should be considered in photo-PISA, which may limit the large-scale production of block copolymer nano-objects. To solve the light penetration problem, conducting photo-PISA in continuous flow reactors is an attractive strategy to achieve uniform light irradiation of the reaction mixture.^[54–56] Currently, several flow photo-PISA formulations have been developed by Boyer, Junker, and others.^[57,58] However, prior deoxygenation with nitrogen and sealed environment are required in these flow photo-PISA systems. The oxygen-sensitive characteristic of RAFT-mediated PISA greatly reduces the applicability of flow photo-PISA for the preparation of block copolymer nano-objects. In this study, we report an enzyme-assisted photo-PISA method using continuous flow reactors in the presence of glucose oxidase (GOx) and glucose. Oxygen and glucose can be converted into hydrogen peroxide and gluconolactone *via* the catalysis of GOx, respectively.^[59] Therefore, the flow photo-PISA reported in this study exhibited excellent oxygen-tolerant property. The effect of GOx on the polymerization kinetics was investigated in detail, demonstrating the importance of GOx on the oxygen-tolerant property. By changing the length of core-forming block, monomer concentration, reaction temperature, and solvent composition, block copolymer nano-objects with various morphologies can be readily prepared and two morphological phase diagrams were constructed.

EXPERIMENTAL

Materials

2-Hydroxypropyl methacrylate (HPMA, Aladdin), monomethoxy poly(ethylene glycol) (mPEG, 2000 and 5000 g/mol, Sigma-Aldrich), GOx (Aladdin), glucose (Aladdin), anhydrous dichloromethane (DCM, Tianjin Zhiyuan), hydroquinone (Aladdin), *N,N*-dimethylformamide (DMF, Aladdin), dicyclohexylcarbodiimide (DCC, Aladdin), 4-dimethylaminopyridine (DMAP, Aladdin), 1,3,5-trioxacyclohexane (Aladdin), and 4,4'-azobis(4-cyanovaleic acid) (ACVA, Aladdin) were used as received. 4-Cyano-4-(ethylthiocarbonothioylthio) pentanoic acid (CEPA) was synthesized according to a literature procedure.^[33] Sodium phenyl-2,4,6-trimethylbenzoylphosphinate (SPTP) was synthesized according to a literature procedure.^[33] Silicone tubes were purchased from Maijialong (Shanghai). LED lamps (405 nm) were purchased from Riyuefeng Electronics (Shenzhen, Guangdong). LED strips (405 nm) were purchased from Jincheng LED Lighting (Shenzhen, Guangdong).

Characterization

The obtained samples were diluted with water. A drop of the dispersion was placed on a copper grid for 3 min and then blotted with filter paper to remove excess solution. A drop of uranyl acetate solution (0.5 wt%) was soaked on the same copper grid for another 3 min, and then blotted with filter paper to remove excess strain. Transmission electron microscopy (TEM) observations were carried out on an HT7700 instrument operated at 100 kV.

¹H-NMR spectra were recorded in CDCl₃, D₂O or DMSO-*d*₆ using a Bruker Avance III 400 MHz NMR spectrometer at 25 °C.

The molecular weight and polydispersity of the block co-

polymers were measured by gel permeation chromatography (GPC) using a Waters 1515 GPC instrument with DMF as the mobile phase and Waters styragel HR1 and HR4 columns. The eluent was DMF containing 10 mmol/L LiBr and was filtered prior to use. The flow rate of DMF was 1.0 mL/min. Linear poly(methyl methacrylate) polymers with narrow molecular weight distributions were used as standards to calibrate the apparatus.

UV-Visible absorption spectra were recorded with a 1.0 cm quartz cuvette using a UV2450 spectrometer.

Intensity-average hydrodynamic diameters of the copolymer dispersions were determined by dynamic light scattering (DLS) using a Brookhaven nanoparticle size-zeta potential and molecular weight analyzer. Dilute aqueous dispersions were analyzed using disposable cuvettes and all data were averaged over three consecutive runs.

Synthesis of mPEG₁₁₃-CEPA

A solution of CEPA (2.37 g, 9 mmol) in anhydrous DCM (60 mL) was added in a dry flask under nitrogen atmosphere containing mPEG₁₁₃ (30.0 g, 6 mmol). Then a solution of DCC (1.86 g, 9 mmol) and DMAP (0.11 g, 0.9 mmol) in anhydrous DCM (20 mL) was added dropwise to the reaction mixture at 0 °C. The esterification reaction proceeded with stirring at room temperature for 48 h. The polymer was collected by precipitation of the reaction mixture in cold hexane. The product was then further purified by silica column chromatography (DCM:CH₃OH=15:1, V:V), and finally dried at 45 °C under vacuum to obtain a yellow powder.

Enzyme-assisted Photo-PISA in a Flow Reactor

In a typical experiment for the synthesis of mPEG₁₁₃-PHPMA₂₀₀ (10 wt%) at room temperature, HPMA (0.4 g, 2.77 mmol), mPEG₁₁₃-CEPA (0.073 g, 0.014 mmol), glucose (0.072 g, 0.1 mol/L), and GOx solution (64 µL, 5 mg/mL, to ensure the final concentration of GOx is 0.5 µmol/L) were weighed into a 10 mL round bottom flask. Then a certain amount of water (3.249 g in this case) was added to the flask to dissolve all reagents to form a homogeneous solution. The reaction mixture was incubated for 15 min to remove dissolved oxygen. Then a SPTP solution (287 µL, 5 mg/mL) was added to the reaction mixture. The reaction mixture was then pumped into the flow reactor *via* a peristaltic pump while the LED strips (λ=405 nm) were on. By regulating the speed of the peristaltic pump, the reaction mixture was exposed to purple light (λ=405 nm) at room temperature for 30 min. The polymerization was quenched by exposure to air and the addition of a small amount of hydroquinone.

Kinetic Study of Enzyme-assisted Photo-PISA in a Flow Reactor

In a typical experiment for the synthesis of mPEG₁₁₃-PHPMA₃₀₀ (10 wt%, [GOx]=0.5 µmol/L), HPMA (2.0 g, 13.9 mmol), mPEG₁₁₃-CEPA (0.243 g, 0.046 mmol), SPTP solution (956 µL, 5 mg/mL), glucose (0.36 g, 0.1 mol/L), and water (16.40 g in this case) were added into a 25 mL round bottom flask to form a homogeneous solution. The reaction mixture was then separated into ten centrifugate tubes. For each centrifugate tube, a certain amount of GOx solution glucose (2.5 mg/mL, to ensure the final concentration of GOx is 0.5 µmol/L) was added and incubated for 15 min to remove dissolved oxygen. The reaction

mixture was pumped into the flow reactor via a peristaltic pump at room temperature while the LED strips ($\lambda=405$ nm) were on. The irradiation time in the flow reactor was controlled by changing the flow rate. The reaction was quenched by exposure to air and the addition of a small amount of hydroquinone. The samples were then analyzed by $^1\text{H-NMR}$ spectroscopy and GPC.

Enzyme-assisted Photo-PISA in Batch

In a typical experiment for the synthesis of $\text{mPEG}_{113}\text{-PHPMA}_{200}$ (20 wt%), HPMA (1.2 g, 8.3 mmol), $\text{mPEG}_{113}\text{-CEPA}$ (0.218 g, 0.042 mmol), glucose (0.108 g, 0.1 mol/L) and GOx solution (96 μL , 5 mg/mL, to ensure the final concentration of GOx is 0.5 $\mu\text{mol/L}$) were weighed into a 10 mL round bottom flask. Then a certain amount of water (3.844 g in this case) was added to the flask. The reaction mixture was incubated for 15 min to remove dissolved oxygen. SPTP solution (860 μL , 5 mg/mL) was then added to the reaction mixture. The reaction mixture was exposed to a LED lamp ($\lambda=405$ nm, 0.9 mW/cm^2) at room temperature for 30 min. The polymerization was quenched by exposure to air and the addition of a small amount of hydroquinone.

RESULTS AND DISCUSSION

Synthesis and Characterization of Macro-RAFT Agent

The macro-RAFT agent was prepared by coupling mPEG_{113} with CEPA in dry DCM for 48 h using a DCC/DMAP chemistry, denoted as $\text{mPEG}_{113}\text{-CEPA}$. Fig. 1 shows $^1\text{H-NMR}$ spectra of CEPA, mPEG_{113} , and $\text{mPEG}_{113}\text{-CEPA}$ in CDCl_3 . A new $^1\text{H-NMR}$ signal (signal f in Fig. 1C) at 4.25 ppm was observed in the $^1\text{H-NMR}$ spectrum of $\text{mPEG}_{113}\text{-CEPA}$, indicating the successful esterification between mPEG_{113} and CEPA. The esterification efficiency can be determined by comparing integral areas of signal a (or f) and signal i in Fig. 1(C), and a value of $\sim 98\%$ was obtained. UV-Vis spectroscopy was also utilized to measure the esterification efficiency (Fig. 2). Fig. 2(a) shows a Lambert-Beer linear calibration plot of CEPA at a wavelength of 310 nm. By comparing the calibration plot of CEPA with the absorbance of $\text{mPEG}_{113}\text{-CEPA}$ (Fig. 2c), an esterification efficiency of $\sim 98\%$ was also obtained.

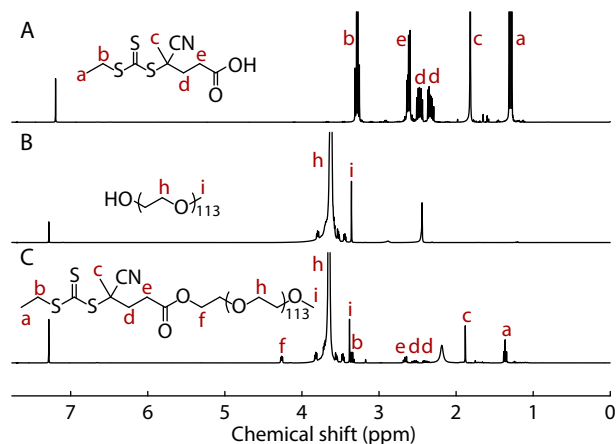


Fig. 1 $^1\text{H-NMR}$ spectra of (A) CEPA, (B) mPEG_{113} , and (C) $\text{mPEG}_{113}\text{-CEPA}$ in CDCl_3 .

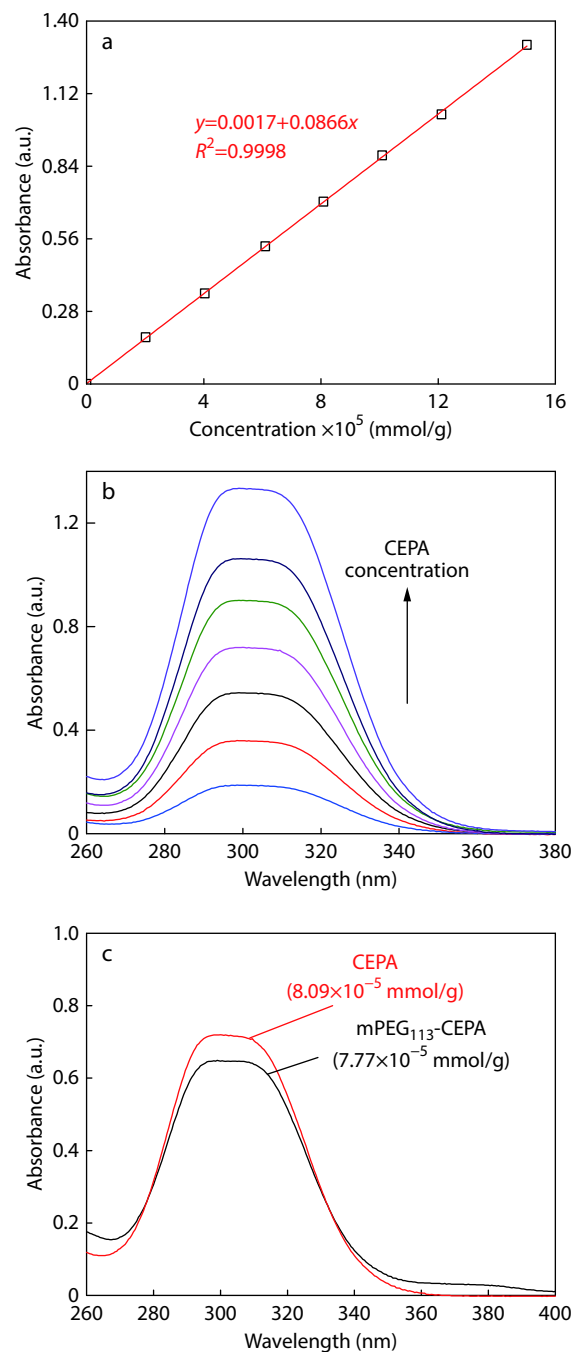
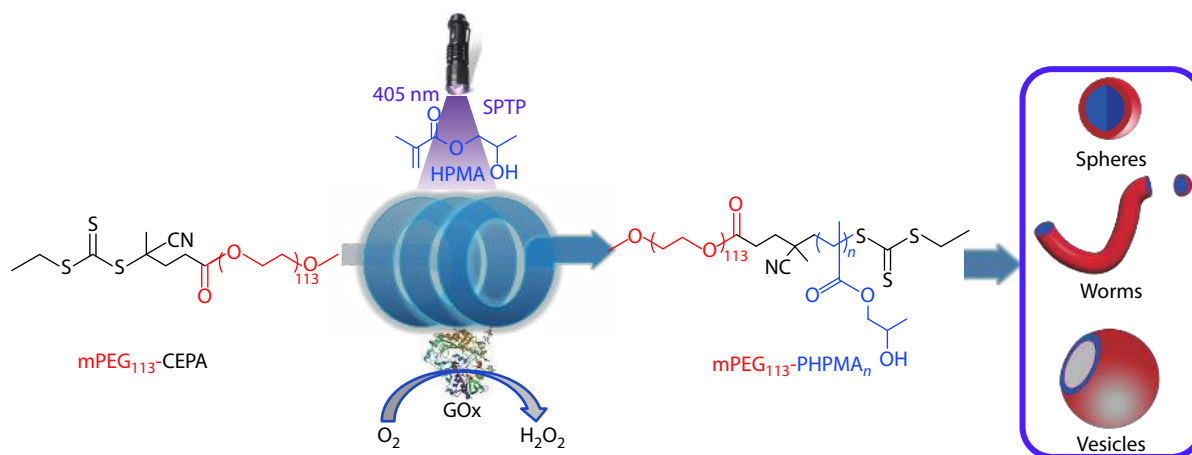


Fig. 2 (a) Lambert-Beer linear calibration plot obtained for CEPA at a wavelength of 310 nm. (b) UV-Vis absorption spectra recorded for CEPA at different concentrations as Fig. 2(a). (c) UV-Vis absorption spectra of $\text{mPEG}_{113}\text{-CEPA}$ and the CEPA precursor.

Enzyme-assisted Photoinitiated Polymerization-induced Self-assembly in Continuous Flow Reactors

The obtained $\text{mPEG}_{113}\text{-CEPA}$ was then used to mediate enzyme-assisted flow photo-PISA of HPMA in water using SPTP as a water-soluble photoinitiator (Scheme 1). To ensure good RAFT control during the polymerization, the $[\text{mPEG}_{113}\text{-CEPA}]/[\text{SPTP}]$ ratio was maintained at 3/1 in this study. Because of the amphiphilic feature, $\text{mPEG}_{113}\text{-PHPMA}_n$ diblock copolymer nano-objects would be formed after the chain extension of PHPMA in



Scheme 1 Schematic illustration for the enzyme-assisted photoinitiated polymerization-induced self-assembly of HPMA mediated by mPEG₁₁₃-CEPA in continuous flow reactors.

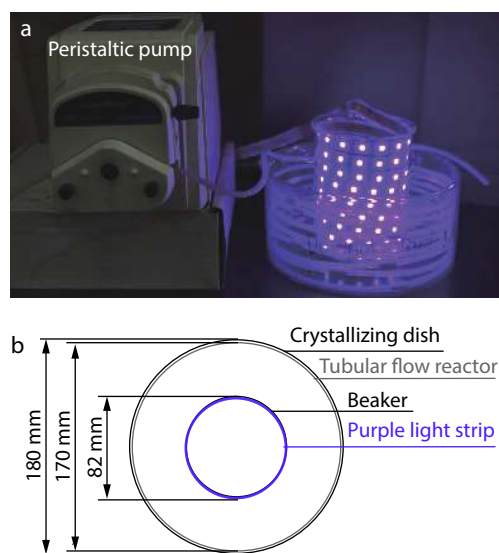


Fig. 3 (a) A digital image of the flow reactor used in enzyme-assisted flow photo-PISA. (b) Schematic illustration of the flow reactor including all dimensions.

water. Purple light ($\lambda=405$ nm) was used to perform the aqueous flow photo-PISA, since radicals can be generated rapidly *via* the decomposition of SPTP under purple light irradiation.^[60] Fig. 3 shows a custom-made flow reactor, which consists of silicone tubing wrapped inside a crystallizing dish. A LED strip (purple light, $\lambda=405$ nm) wrapped inside a transparent beaker was placed in the middle of the crystallizing dish and faced toward the silicone tubing. Reaction solutions were pumped into the reactor *via* a peristaltic pump under open-air conditions and the irradiation time was controlled by changing the flow rate. In our experiments, we found that the change of flow rate had little effect on the morphology of block copolymer nano-objects. The light intensity in the flow reactor was maintained at 0.9 mW/cm².

In this study, GOx was utilized to consume oxygen in the presence of glucose. Mild reaction conditions of aqueous flow photo-PISA such as aqueous medium, room temperature (30 °C in this study), and visible light are critical to main-

taining the bioactivity of GOx and ensuring good oxygen tolerance throughout the polymerization. In the GOx deoxygenation process (Scheme 1), it is clear that a small amount of H₂O₂ would be generated. In our previous study,^[59] we have demonstrated that H₂O₂ generated from the GOx deoxygenation process had no effect on the RAFT controllability. To evaluate the effect of GOx on the flow photo-PISA, polymerization kinetics of aqueous flow photo-PISA of HPMA (10 wt%, target DP of 300) mediated by mPEG₁₁₃-CEPA were studied by using different GOx concentrations. It should be noteworthy that the glucose concentration was maintained at 0.1 mol/L to ensure constant removal of oxygen during the polymerization. Samples collected at different irradiation time points were dissolved in DMSO-*d*₆ immediately and characterized by ¹H-NMR spectroscopy. Monomer conversions can be determined by monitoring the decrease in vinyl signals relative to an internal standard. Fig. 4(a) shows ¹H-NMR spectra of reaction mixtures collected at different time points during the kinetic study of aqueous photo-PISA with 0.5 μmol/L GOx. A small amount of *N,N*-dimethylformamide (DMF) was added as the internal standard. Vinyl signals of HPMA at 5.65 and 6.05 ppm decreased significantly as the polymerization proceeded, indicating the rapid consumption of HPMA during enzyme-assisted flow photo-PISA. Fig. 4(b) shows plots of monomer conversion versus irradiation time for enzyme-assisted flow photo-PISA with different GOx concentrations. In the absence of GOx, only 38.2% monomer conversion was achieved within 30 min of purple light irradiation. In our previous research on batch photo-PISA with nitrogen deoxygenation, we have demonstrated that full monomer conversions could be achieved within 15 min of purple light irradiation.^[28] The low polymerization rate and incomplete monomer conversion in the flow photo-PISA can be ascribed to the oxygen inhibition toward radicals. When 0.1 μmol/L GOx was added, a monomer conversion of 59.4% was achieved within 30 min of purple light irradiation and a significantly higher polymerization rate was observed compared to that of 0 μmol/L GOx. Upon increasing the GOx concentration to 0.5 μmol/L, near quantitative monomer conversion was achieved within 30 min of purple light irradiation, exhibiting a fast polymerization behavior. Further increasing the GOx concentration to

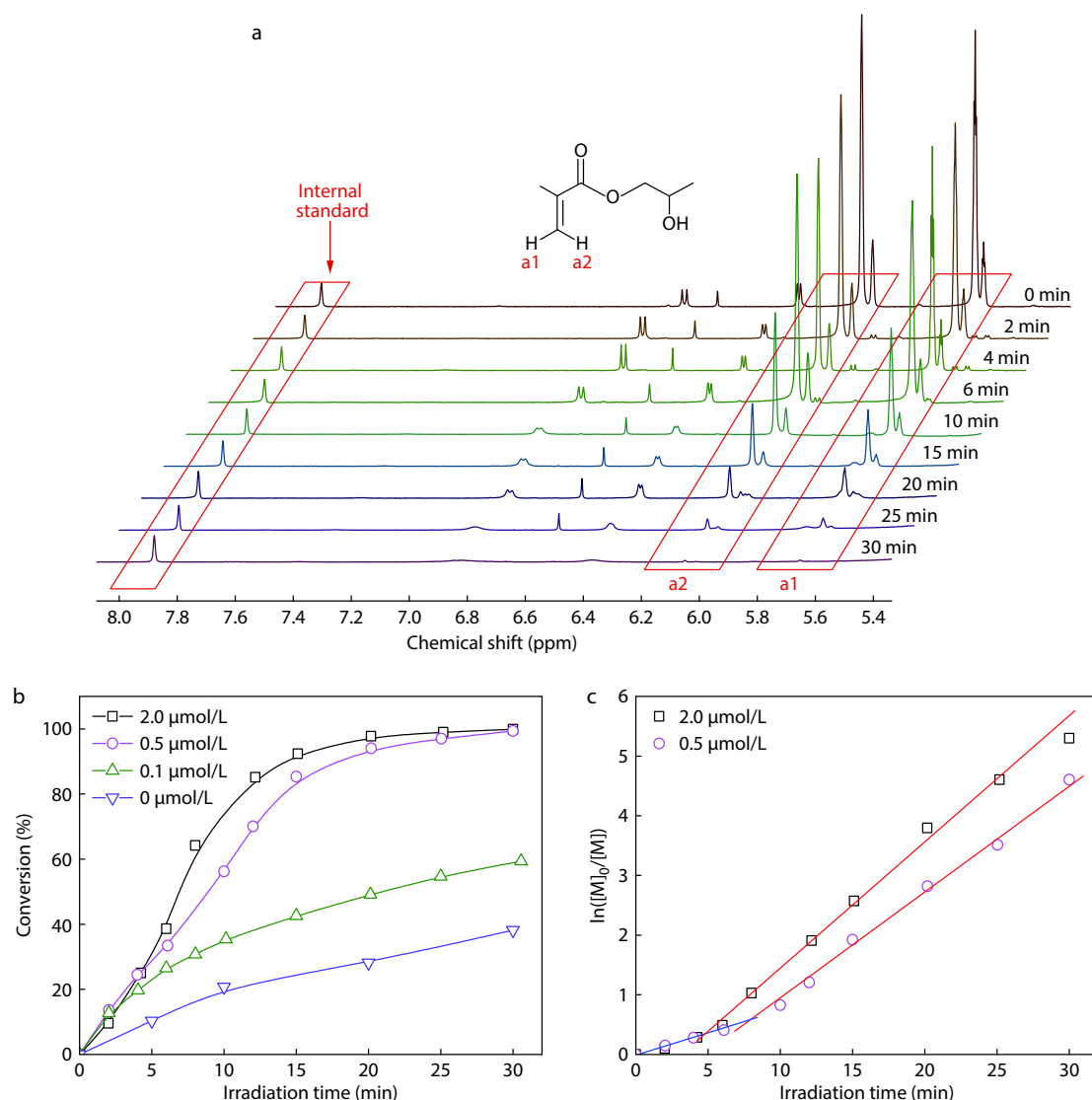


Fig. 4 (a) ¹H-NMR spectra of samples collected at different irradiation time points during the kinetic study of aqueous photo-PISA with 0.5 μmol/L GOx. A small amount of DMF was added as the internal standard. (b) Plots of monomer conversion versus irradiation time for enzyme-assisted flow photo-PISA with different GOx concentrations. (c) Semilogarithmic plots of enzyme-assisted flow photo-PISA with 0.5 and 2.0 μmol/L GOx.

2.0 μmol/L has little influence on the polymerization kinetics. These results suggest that flow photo-PISA with excellent oxygen tolerance can be achieved by adding a small amount of GOx (0.5 μmol/L or higher in this case) in the reaction. Fig. 4(c) shows semilogarithmic plots of enzyme-assisted flow photo-PISA with 0.5 and 2.0 μmol/L GOx. Two distinct regimes were observed in both cases, which correspond to homogenous polymerization stage and heterogenous polymerization stage. The rate enhancement observed during the heterogenous polymerization stage can be attributed to the high local monomer concentration in the micelles. These results indicate that self-assembly can still occur even conducting photo-PISA in a flow reactor with the addition of GOx under open-air conditions.

Samples collected during the kinetic study of enzyme-assisted flow photo-PISA (with 0.5 μmol/L GOx) were freeze-dried directly and characterized by DMF GPC. As shown in Fig. 5(a),

each GPC trace shifted gradually to a higher molecular weight as the irradiation time increased, indicating the successful chain extension under enzyme-assisted flow photo-PISA conditions. It was found that mPEG₁₁₃-CEPA consumed gradually and two GPC peaks were observed during the early stage of the polymerization. A monomodal and symmetric GPC curve was observed for the final sample, indicating the achievement of a relatively high blocking efficiency. It should be noteworthy that the amount of GOx used in this study was too small to be detected by GPC equipment. A linear increase in number-average molecular weight (M_n) with monomer conversion and narrow molecular weight distributions ($M_w/M_n < 1.30$) confirmed that good RAFT control was maintained under the enzyme-assisted flow photo-PISA conditions (Fig. 5b).

One significant advantage of RAFT-mediated PISA is its ability to produce a variety of block copolymer nano-objects at high concentrations.^[61] A series of mPEG₁₁₃-PHPMA_n dib-

lock copolymer nano-objects were then prepared by systematically changing the target DP of PHPMA block and the HPMA concentration. Mean DPs were determined by measuring monomer conversions via $^1\text{H-NMR}$ spectroscopy and the morphology was checked by transmission electron microscopy (TEM). A morphological phase diagram was then constructed, as shown in Fig. 6. Similar to other PISA phase diagrams,^[61] the phase diagram reported here is strongly concentration-dependent. Spheres can only be prepared at a HPMA concentration of 10 wt%. In contrast, pure vesicles can only be obtained at a HPMA concentration of 15 wt% or higher with a high DP of PHPMA (500 in this case). This can be explained by the fact that higher monomer concentrations in RAFT-mediated PISA favor the plasticization of micelles with monomer as well as the sphere-sphere fusion, promoting the formation of higher-order morphologies. Moreover, a large

region of mixed morphology was also observed in this phase diagram. Comparing this phase diagram with our previous phase diagram obtained in batch photo-PISA,^[28] it can be concluded that conducting RAFT-mediated PISA in bath or in flow has a significant influence on the morphology of block copolymer nano-objects. To make a direct comparison between these two techniques, enzyme-assisted photo-PISA of HPMA mediated by $\text{mPEG}_{113}\text{-CEPA}$ was also conducted in batch (a 10 mL round bottom flask). Fig. 7 shows DMF GPC traces of $\text{mPEG}_{113}\text{-PHPMA}_n$ ($n=200, 300, 400, 500$) diblock copolymers (20 wt% HPMA) prepared by enzyme-assisted flow photo-PISA (top) and enzyme-assisted batch photo-PISA (bottom). It can be seen that the GPC data of $\text{mPEG}_{113}\text{-PHPMA}_n$ diblock copolymers prepared in the flow reactor were very close to those prepared in the batch reactor. These results suggest that conducting enzyme-assisted photo-PISA in flow

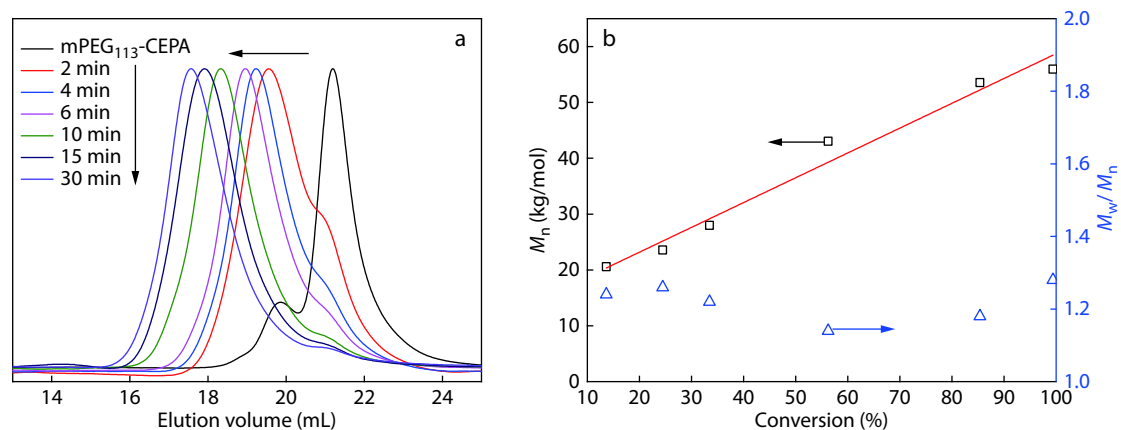


Fig. 5 (a) DMF GPC traces of $\text{mPEG}_{113}\text{-CEPA}$ and $\text{mPEG}_{113}\text{-PHPMA}_n$ prepared by enzyme-assisted flow photo-PISA with $0.5 \mu\text{mol/L}$ GOx (target DP of 300). (b) M_n and M_w/M_n versus monomer conversion for enzyme-assisted flow photo-PISA with $0.5 \mu\text{mol/L}$ GOx.

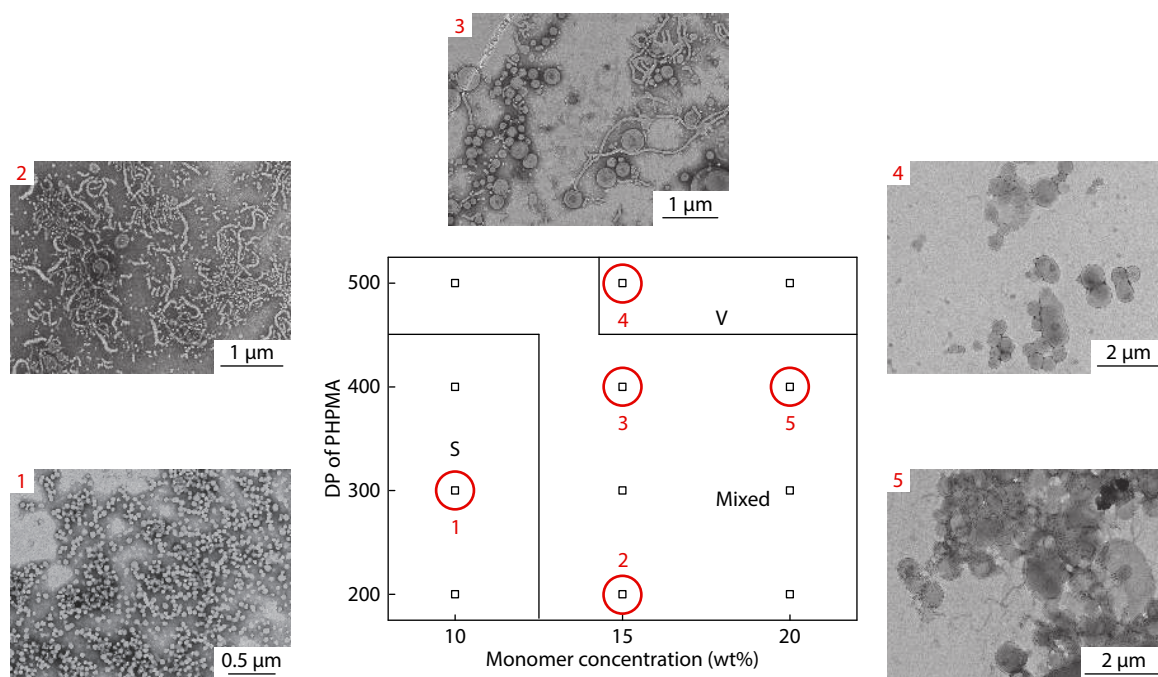


Fig. 6 Representative TEM images and morphological phase diagram constructed for enzyme-assisted flow photo-PISA of HPMA mediated by $\text{mPEG}_{113}\text{-CEPA}$ at room temperature. Phase regions consist of spheres (S), mixed morphologies (Mixed), and vesicles (V).

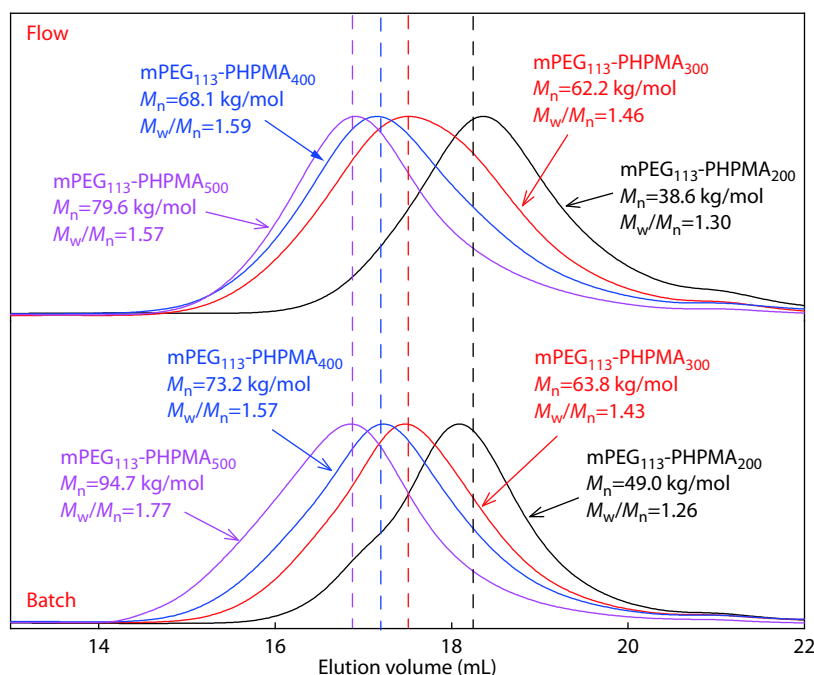


Fig. 7 DMF GPC traces of $m\text{PEG}_{113}\text{-PPHMA}_n$ ($n=200, 300, 400, 500$) obtained by enzyme-assisted photo-PISA of HPMA (20 wt%) in flow (top) and in batch (bottom).

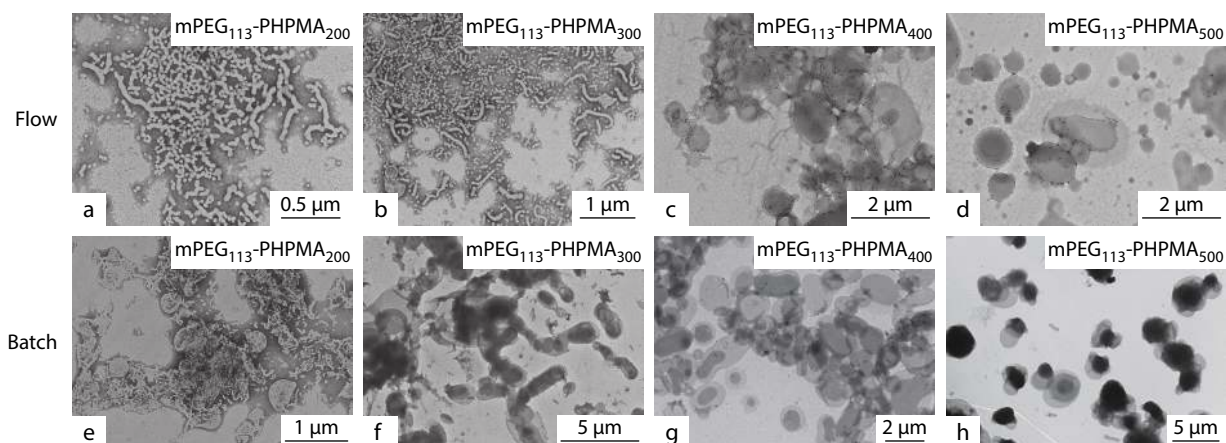


Fig. 8 TEM images of $m\text{PEG}_{113}\text{-PPHMA}_n$ ($n=200, 300, 400, 500$) diblock copolymers obtained by enzyme-assisted photo-PISA of HPMA (20 wt%) (a–d) in flow and (e–h) in batch.

or in batch had little effect on the composition of the diblock copolymers. These samples were also characterized by TEM, as shown in Fig. 8. However, it was found that performing enzyme-assisted photo-PISA in batch was more likely to obtain higher-order morphologies. For example, a mixed morphology consisting of spheres and short worms was obtained at a DP of 300 when the enzyme-assisted photo-PISA was performed in flow (Fig. 8b). In contrast, pure vesicles were obtained at a DP of 300 when the enzyme-assisted photo-PISA was performed in batch (Fig. 8f). Moreover, for the enzyme-assisted flow photo-PISA, DP of PPHMA up to 500 was required to obtain pure vesicles (Fig. 8d). The morphological difference between flow photo-PISA and batch photo-PISA can be attributed to two reasons: (i) Continuous magnetic stirring in batch photo-PISA can enhance the sphere-sphere

fusion and therefore the formation of higher-order morphologies; (ii) Fluidic dynamic is different in both cases, for a lamellar flow exists in the flow photo-PISA and a turbulent flow exists in the batch photo-PISA.

Temperature is another important factor that can further control the morphology of block copolymer nano-objects prepared by RAFT-mediated PISA. In our previous research, we have demonstrated that higher-order morphologies are likely to form at a higher reaction temperature in RAFT-mediated PISA.^[50,62] Herein, the effect of reaction temperature on enzyme-assisted flow photo-PISA of HPMA (20 wt%) was also investigated by varying the reaction temperature from 30 °C to 50 °C. Near quantitative monomer conversions were achieved in all cases when the reaction temperature was 50 °C or lower. Low monomer conversions were observed when the

reaction temperature was higher than 50 °C (e.g. 55 °C). This can be ascribed to the loss of bioactivity of GOx at such high temperatures, and thereby leading to the occurrence of oxygen inhibition during aqueous flow photo-PISA. Fig. 9(a)

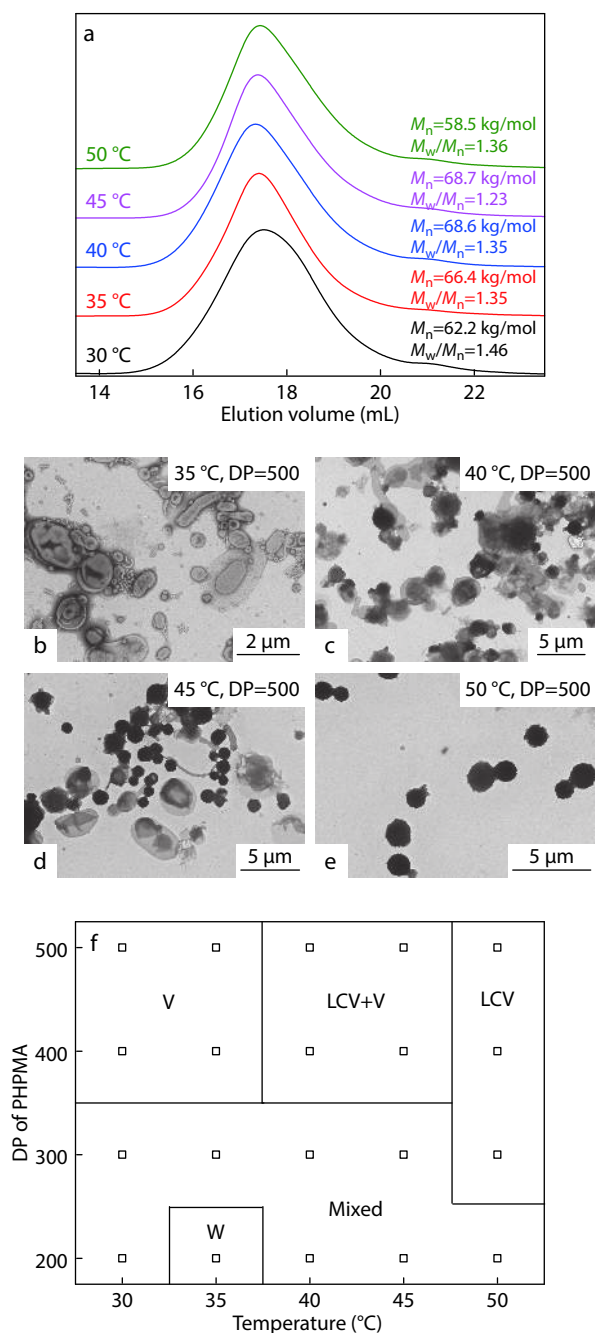


Fig. 9 (a) DMF GPC traces of mPEG₁₁₃-PPHMA₃₀₀ diblock copolymers prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at different temperatures. (b–e) TEM images of mPEG₁₁₃-PPHMA₅₀₀ diblock copolymer nano-objects prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at different temperatures. (f) Morphological phase diagram constructed for enzyme-assisted flow photo-PISA of HPMA (20 wt%) by varying the DP of PHPMA and reaction temperature. Phase regions consist of worms (W), mixed morphologies (Mixed), vesicles (V), and large compound vesicles (LCV).

shows that GPC traces of mPEG₁₁₃-PPHMA₃₀₀ diblock copolymers prepared at different temperatures are very close, suggesting the change of reaction temperature had little effect on the composition of the formed diblock copolymers. Figs. 8(d) and 9(b)–9(e) show TEM images of mPEG₁₁₃-PPHMA₅₀₀ diblock copolymer nano-objects prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at different temperatures. Pure vesicles were obtained when the reaction temperature was 35 °C or lower (Figs. 8d and 9b). When the reaction temperature was increased to 40 or 45 °C (Figs. 9c and 9d), mixed morphologies containing vesicles and large compound vesicles (LCVs) were obtained. Further increasing the reaction temperature to 50 °C led to the formation of LCVs (Fig. 9e). A morphological phase diagram was also constructed by varying the reaction temperature and the DP of PPHMA (Fig. 9f). It is clear that increasing the reaction temperature facilitates the formation of higher-order morphologies. This phenomenon can be explained by the fact that increasing the reaction temperature can decrease the solubility of mPEG₁₁₃ and HPMA in the reaction medium.^[50] Therefore, the packing parameter of mPEG₁₁₃-PPHMA will increase as the reaction temperature increases, favoring the formation of higher-order morphologies.

To further tune the morphology of block copolymer nano-objects prepared by enzyme-assisted flow photo-PISA, water mixed with a certain amount of non-functionalized mPEG (2000 g/mol) was then used as the solvent. The Zhang group^[63] reported that the addition of solvophilic homopolymer in RAFT-mediated PISA can promote the formation of block copolymer nano-objects with higher-order morphologies. Similar results were observed in our enzyme-assisted flow photo-PISA of HPMA (20 wt%, target composition of mPEG₁₁₃-PPHMA₅₀₀). Quantitative monomer conversions were observed in all cases, suggesting that the addition of mPEG in water did not lead to the loss of GOx activity. Fig. 8(d) shows that pure vesicles were obtained in the absence of mPEG. When the mPEG content was 5%, similar vesicular morphology was obtained (Fig. 10a). When the

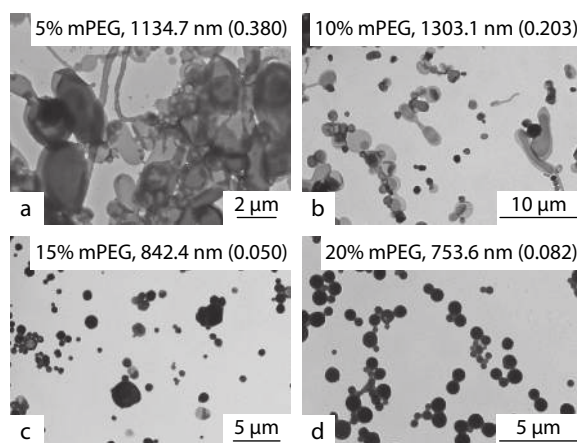


Fig. 10 TEM images of mPEG₁₁₃-PPHMA₅₀₀ diblock copolymer nano-objects prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at room temperature using water mixed with different amounts of mPEG (2000 g/mol) as solvents: (a) 5% mPEG, (b) 10% mPEG, (c) 15% mPEG, (d) 20% mPEG.

mPEG content was increased to 10% or 15%, a mixed morphology containing vesicles and LCVs was formed (Figs. 10b and 10c). Further increasing the mPEG content to 20% led to the formation of LCVs (Fig. 10d). This phenomenon can be explained by the fact that mPEG acts as a plasticizer for mPEG₁₁₃-PHPMA_n block copolymer nano-objects in aqueous photo-PISA and therefore enhances the mobility of core-forming block.

CONCLUSIONS

In summary, an enzyme-assisted flow photo-PISA of HPMA mediated by mPEG₁₁₃-CEPA was developed by adding GOx and glucose in the reaction mixture. Polymerization kinetics showed that GOx played an important role in achieving oxygen tolerance. Near quantitative monomer conversions were achieved within 30 min of purple light irradiation with 0.5 or 2.0 μmol/L GOx. Good RAFT control was maintained as confirmed by the linear evolution of M_n with monomer conversion and relatively narrow molecular weight distributions ($M_w/M_n < 1.30$). By systematically changing the DP of PHPMA and the HPMA concentration, a series of mPEG₁₁₃-PHPMA_n block copolymer nano-objects were prepared and a morphological phase diagram was constructed. The morphology of block copolymer nano-objects prepared by the enzyme-assisted flow photo-PISA was also controlled by changing reaction temperature. It was found that increasing the temperature facilitated the formation of higher-order morphologies. Moreover, the addition of non-functionalized mPEG in the reaction medium also promoted the formation of higher-order morphologies. This study will provide a scalable and robust method for the preparation of block copolymer nano-objects at room temperature.

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