

& Block Copolymers

Direct Synthesis of Polymer Vesicles on the Hundred-Nanometer and Beyond Scale Using Chemical Oscillations

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Abstract: The direct synthesis of block copolymer vesicles on the scale of tens to hundreds of nanometers using reversible addition–fragmentation chain transfer (RAFT) dispersion polymerization as an effect of chemical oscillations is reported. RAFT polymerization is successfully accomplished between poly(ethylene glycol) containing a RAFT agent (PEG-CTA) and ethyl acrylate monomer in the presence of the Belousov–Zhabotinsky (B-Z) reaction in oscillatory mode. The self-assembly of poly(ethylene glycol)-*b*-poly(ethyl acrylate) unimers gives rise to spherical micelles. The self-assembled micelles reorganize and transform to vesicles. All the chemistry of polymerization, self-assembly and self-organization, of macromolecules takes place in a single pot using only a few simple raw materials in aqueous solution.

The self-assembly of block copolymers in aqueous environments is important for many different applications such as nanomedicine,^[1] nanoreactors,^[2] and mimicking some features of life.^[3] After polymer synthesis, the typical self-assembly of block copolymers into collective structures is achieved by dissolving one block in a favourable solvent, enabling reorganization of the other block to form some desirable morphology.^[4–7] The only variable in these methods is the solvent properties as the block copolymer used has a fixed molecular weight and a fixed diblock composition. They involve separate multistep and complicated synthesis together with rigorous purification of polymer, which are not only time consuming but also require more direct human intervention.

Polymerization-induced self-assembly (PISA) is a recently developed polymerization approach that generates multiple morphologies of polymer nano objects depending on the hydrophilic–hydrophobic ratio and interfacial energy.^[8,9] Typically, a hydrophilic polymer precursor is extended with a hydrophobic

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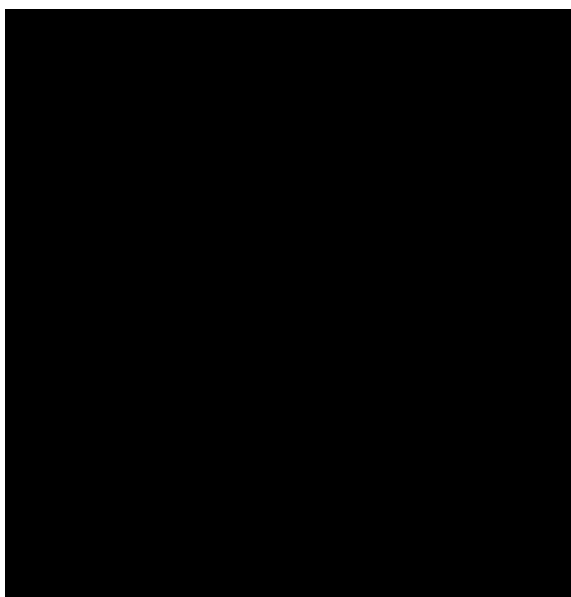
second block in an aqueous environment. Previous authors have studied the reversible addition–fragmentation chain transfer (RAFT) aqueous dispersion polymerization of 2-hydroxypropyl methacrylate on poly(ethylene glycol).^[10] The growth of a hydrophobic block on the short hydrophilic stabilizer leads to the formation of higher-order morphologies depending on the nature and structure of the resulting amphiphilic block copolymer. Using a high temperature (>50°C) in most of PISA, because a thermal initiator is usually used as a radical source, limits the in situ encapsulation of biomolecules and makes it difficult to work with temperature-responsive materials.^[11] The use of a visible light activated catalyst as a source of radicals will be effective to some extent and offers numerous new scenarios that are not easily accessible using conventional polymerization.^[12–14] Recently Albertsen et al. observed the macromolecular self-assembly of an amphiphilic block copolymer under visible light controlled PISA leading to micron sized vesicles.^[3]

Here, we report self-assembly and self-organization of nanometer-sized polymer nanoaggregates at room temperature in the absence of light through RAFT aqueous dispersion polymerization. Instead of using a photo- or thermo-initiator as normal in radical polymerization, we use radicals from the Belousov–Zhabotinsky (B-Z) reaction in oscillatory mode. B-Z is the most studied oscillatory redox chemical reaction.^[15,16] It consists of the oxidation of an organic substrate (e.g., malonic acid) by the bromate ions in acidic conditions in the presence of metal ions (Ru²⁺, Fe³⁺, Ce³⁺, Mn²⁺) as catalyst. Many recent studies are aimed at developing practical applications coming from the oscillatory nature of the B-Z reaction.^[17–20] Yoshida and co-workers developed self-oscillating gels that undergo spontaneous swelling–deswelling cycles by introducing B-Z catalyst in the polymer backbone.^[19] The chemically bound catalyst undergoes redox oscillation. The

hydrophilicity of the polymer chain increases for the oxidized state (Ru^{3+}) of catalyst and decreases for the reduced state (Ru^{2+}). Washington et al. studied the role of different B-Z intermediates for the homopolymerization of different monomers.^[20]

The B-Z reaction oscillates with continuous generation of free radicals. The motivation to choose B-Z reaction as an initiator is not only due to the generation of free radicals during oscillation but also to impart some functionality to the resulting polymer structures, because B-Z can be used, for example, in chemical communication^[21,22] or as a chemical Turing machine.^[23] As the hydrophobic block grows on the end of the hydrophilic block (PEG-CTA), the dynamically evolving copolymer undergoes self-assembly to form polymer nanoaggregates and minimize the free energy. The reaction within the self-assembled system assists the self-organization of polymer chains to form vesicles on the scale of tens to hundreds of nanometers. The synthesis of the block copolymer, self-assembly, and self-organization of polymer vesicles all take place in a single pot under facile conditions. At the end of polymerization, the B-Z reaction is still active and could confer functionality to the vesicles.

PEG-CTA was synthesized via esterification of the terminal hydroxyl group of PEG with 4-cyano-4-(phenyl- carbonothioylthio) pentanoic acid.^[24] RAFT polymerization of ethyl acrylate was carried out using a PEG-CTA. The synthetic scheme is shown in Scheme 1. We coupled the B-Z reaction with poly-



Scheme 1. Synthesis of PEG₄₄-b-PEA₉₂ block copolymer.

merization to form a polymeric nanocompartment. The progress of the B-Z reaction was monitored through the periodic change in redox potential of $\text{Ru}^{3+}/\text{Ru}^{2+}$ ion pairs. The color change during polymerization is due to catalytic participation of tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate in the redox reaction. $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^{2+}$ have different colors. The polymerization does not occur without all reactants for the B-Z reaction. Figure S1-a shows the oscillation of B-Z during polymerization. The oscillation

of pure B-Z is also shown for comparison (Figure S1-b, Supporting Information).

The upper peaks of oscillation represent the catalyst oxidized state (Ru^{3+}), whereas the lower correspond to the reduced (Ru^{2+}). The oscillation was greatly perturbed in polymerization.^[17] The induction time was delayed and the amplitude of the oscillation also decreased. The presence of macromolecules, consumption of radicals, and increased viscosity are the major cause of perturbation on the B-Z oscillation. The interesting part is that the B-Z reaction is still active even after completing its main functions in the process (polymerization, self-assembly, and self-organization).

During its course and oscillations, the reaction proceeds with generation of radicals and intermediate species, as shown in Figure S2 (Supporting Information). Malonyl radical ($\text{MA}^{\bullet}$) is an oxidized product of malonic acid. Similarly, $\text{BrO}_2^{\bullet}$ radical is generated from BrO_3^- , which could change HBrO_2 as a result of the redox reaction with Ru^{2+} ions.^[25] The generation of $\text{MA}^{\bullet}$ and $\text{BrO}_2^{\bullet}$ free radicals in B-Z oscillations inspired us to couple B-Z reaction with radical polymerization in the context of PISA. These radicals behave as initiators in free-radical polymerization.

A specific amount of poly(ethylene glycol) containing reversible addition-fragmentation chain transfer agent (PEG-CTA) and ethyl acrylate monomers were added to the oscillating B-Z reaction under constant stirring at 200 rpm. Stirring the solution accelerates the mixing and removes the CO_2 bubbles produced from the oxidation of malonic acid. Initially the solution mixture was homogeneous, however, after some time it becomes turbid confirming the polymerization between PEG-CTA and the ethyl acrylate monomer. The progress of polymerization was visually observed. Photographs of the solution at different time intervals clearly indicate the polymerization and self-assembly of the resulting block copolymer (Figure 1). The

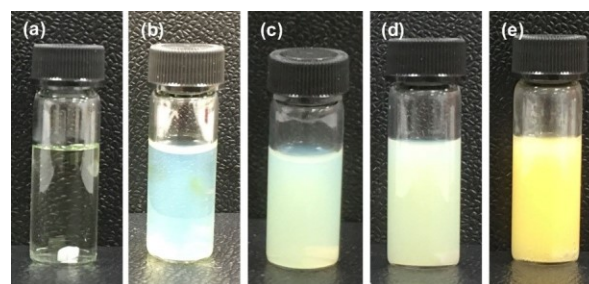


Figure 1. Photographs of polymer solution at different times of polymerization. (a) 0 min, (b) 15 min, (c) 30 min, (d) 45 min, and (e) 60 min.

polymerization was further confirmed by Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy. $\text{C}=\text{O}$, $\text{C}=\text{O}$, and CH stretching of the polymer backbone are clearly observed in FTIR spectra (Figure S3, Supporting Information). The conversion and degree of polymerization of poly(ethyl acrylate) (PEA) were calculated from the integrated vinyl proton signals of ethyl acrylate (δ 5.5–6.5) and peaks of terminal CH_3 (δ 1.15) and CH (δ 3.22) in the backbone of PEA (Figure S4). The degree of polymerization for PEA was 92 with 88% conversion of monomers.

The unimers undergo self-assembly and form polymer nanoaggregates in aqueous solution. Dynamic light scattering measurements of the hydrodynamic diameter (D_h) of PEG-bPEA nanoaggregates at different time intervals were performed and showed that the D_h increases from 240 to 350 nm (Figure 2 and Figure S5). The polydispersity index is less than 0.05 for all measurements, indicating that the nanoaggregates are fairly monodispersed (Figure S5). To gain more insight into the internal structure of these nanoaggregates, we performed time dependent TEM measurements (Figure 3 and Figure S6).

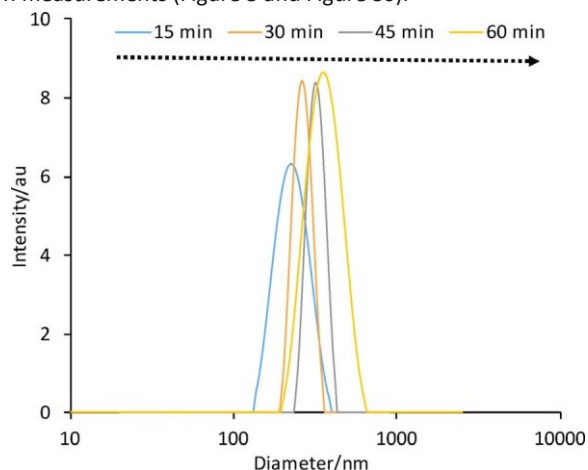


Figure 2. Intensity distribution of polymer nanoaggregates at different times of polymerization as determined by DLS.

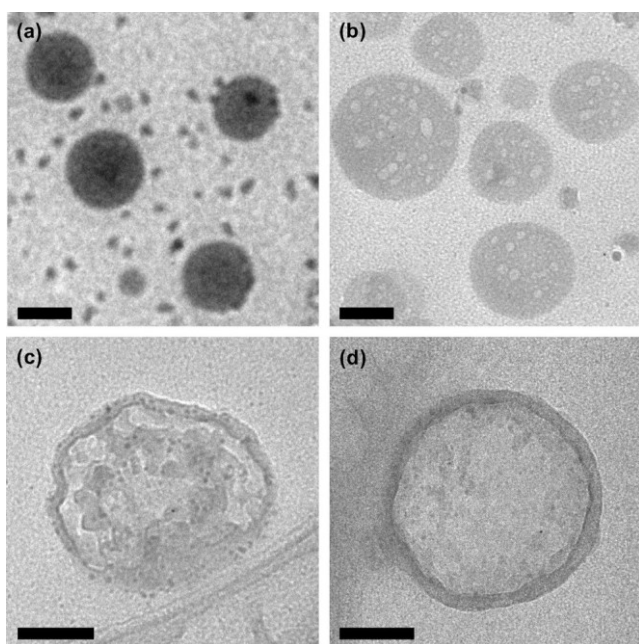


Figure 3. TEM images of polymer nanoaggregates of PEG-b-PEA block copolymer at different time of polymerization (a) 15 min, (b) 30 min, (c) 45 min, and (d) 60 min. The scale bar is 50 nm.

The Ru-based B-Z catalyst also served as a convenient staining agent for TEM studies. Spherical micelles with a diameter of 70 nm were observed after 15 min of polymerization. This indicates that the self-assembly of the PEG-b-PEA block copolymer had already

started and explains the observed turbidity and the DLS measurements (Figure 1 and Figure 2). Micelles with patchy voids were observed after 30 min of polymerization (Figure 3b). These voids in micelles could provide a unique platform for making highly efficient nanofactories and drug carriers based on potential bio-compatible extensions of the phenomena reported here.^[26] The unreacted monomers, PEGCTA, and B-Z components are enclosed into the spherical polymer vesicles during self-assembly. The difference in the chemical environment inside and outside the polymer micelles and the subsequent addition of ethyl acrylate on PEG-CTA promotes the growth of micelles/vesicles and stimulates the formation of voids. The polymerization within micelles supports self-organization. During the self-assembly and self-organization, solvent may be entrapped inside the incomplete vesicles (Figure 3). This favors the further rearrangement of hydrophilic and hydrophobic blocks to form complete vesicles.^[27]

We successfully synthesized a PEG-b-PEA block copolymer using the B-Z chemical reaction in its oscillatory mode as initiator. The radicals from the reaction initiate polymerization between the PEG macro RAFT agent and ethyl acrylate monomers. The attachment of hydrophobic units on the long chains of the hydrophilic block induces the self-assembly. As the reaction proceeds, the rearrangement of hydrophilic and hydrophobic blocks leads to the spontaneous formation of polymer vesicles. All chemical steps (polymerization, self-assembly, selforganization) were achieved in a single system under the control of the B-Z reaction. We believe this method has the potential to expand the concept of solution-induced self-assembly of polymer nano objects with unique morphologies for future materials applications.

Experimental Section

Materials

Polyethylene glycol methyl ether (Aldrich, Mn=2000), 4-cyano-4(phenylcarbonothioylthio)pentanoic acid (Aldrich), ethyl acrylate (EA; Sigma-Aldrich), sodium bromate (NaBrO_3 ; Sigma-Aldrich), malonic acid (MA; Sigma-Aldrich) sulphuric acid (H_2SO_4 ; Ricca Chemical Company), tris(2,2'-bipyridyl) dichlororuthenium(II) hexahydrate (Sigma-Aldrich) are used without further purification. Ultra-pure water (molecular grade) from Hardy Diagnostic was used to perform all the experiments.

Synthesis of block copolymer

PEG₄₄-CTA (3.6 mg) was dissolved in distilled water (2265 mL). Malonic acid (90 mL, 1M), sodium bromate (300 mL, 1M), H_2SO_4 (290 mL, 5M), ethyl acrylate monomers (20 mL), and $\text{Ru}(\text{bpy})_3\text{Cl}_2$ (35 mL, 8.5 mM) were added sequentially and stirred at 200 rpm at room temperature in the dark. The total volume of the solution was 3000 mL. The electrochemical behavior of the solution was monitored just after mixing all the reactants.

Characterization

A Pt electrode was used to monitor the change in redox potential during polymerization. The hydrodynamic diameter was measured using a

Beckman Coulter DelsaNano C particle size analyzer. Morphology of the samples was observed under transmission electron microscopy (TEM; JEOL JEM-2100). The proton NMR spectra were acquired in [D₆]DMSO using a 500 MHz Varian Unity/Inova spectrometer. Fourier transform infrared spectra were collected on PerkinElmer FTIR Spectroscopy using a Diamond attenuated total reflection method.

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Conflict of interest

The authors declare no conflict of interest.

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