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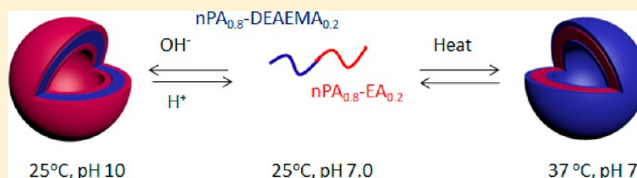
Switchable Vesicles Formed by Diblock Random Copolymers with Tunable pH- and Thermo-Responsiveness

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Supporting Information

ABSTRACT: The thermo-responsiveness of polymers in aqueous media can be tuned by the choice of comonomers used in the synthesis of block copolymers made of random sequences of the same comonomers but of different molar ratios. The same synthetic approach may be applied to other stimuli and we have made diblock random copolymers with both pH- and thermo-responsiveness and studied the formation of vesicles whose membrane core and coronas may be inverted in aqueous media. Sequential reversible addition–fragmentation chain transfer (RAFT) polymerization was used to prepare well-defined block copolymers in the form of $A_nB_m-b-A_pC_q$, where A, B, and C are *N*-*n*-propylacrylamide (*n*PA), 2-(diethylamino)ethyl methacrylate (DEAEMA), and *N*-ethylacrylamide (EA), respectively. This polymer shows interesting “schizophrenic” behavior in aqueous solutions. Both blocks are thermo-responsive, and one block is pH-responsive in which the tertiary amine group of DEAEMA may be protonated at a lower pH. A molecularly dissolved polymer is obtained at neutral pH and ambient temperature. At pH 7 and 37 °C, the polymer self-assembles into vesicles with the poly(*n*PA_{0.8}-co-EA_{0.2}) block as the membrane core (mean hydrodynamic diameter of the vesicles $D_h = 148$ nm). In an alkaline medium (pH 10) at 25 °C, the membrane core and the coronas of the vesicles are inverted with poly(*n*PA_{0.8}-co-DEAEMA_{0.2}) block forming the core ($D_h = 60$ nm). In addition, two-step phase transitions are observed in both alkaline and neutral solutions corresponding to the cloud points of the individual blocks. Here, the random nature of the blocks allows fine-tuning the thermo-responsiveness based solely on lower critical solution temperatures and its combination with pH-sensitivity provides vesicles with switchable membrane core and corona in aqueous solution.



INTRODUCTION

The first examples of so-called “schizophrenic” stimuli-responsive block copolymers were introduced in the late 1990s by Armes and co-workers.¹ These systems form micelles capable of inverting their shell and core in aqueous solutions without adding any organic solvent, in response to an external stimulus that alters the relative hydrophilicity of the blocks. Each block is sensitive to a certain stimulus and can respond individually as it becomes either more hydrophilic or more hydrophobic.² They are also sometimes called “confused” block copolymers for their dual behavior.³ Such stimuli include temperature,^{4–7} pH,^{8–12} and combinations of ionic-strength pH^{1,13,14} or temperature pH.^{15–26} All the systems introduced so far benefit from a change in their solution properties under fixed pH or temperature value, since tuning such responsiveness may not be easy given the limited choice of the monomers, and they are composed of blocks consisting of a single monomer,^{16,19–25} even though multiblock copolymers may exhibit multiple responses to external stimuli.^{27–34} On the other hand, temperature-dependent inversion of core and shell blocks in the micellar aggregates of “schizophrenic” block copolymers has been reported in aqueous solutions based on lower and upper critical solution temperatures (LCST and UCST) of the blocks,^{5,7} but sometimes observing a clear UCST is difficult as the transition may be broad. To the best of our knowledge, there has been no report on such invertible systems

with dual thermo-responsiveness exploiting the LCST-like behavior of both blocks. We attempt to address these issues here by preparing an invertible system made in quite a controlled manner, where both tunable blocks in a diblock copolymer show separate cloud points (CPs) in water.

We have prepared random copolymers that allow the tuning of the CP by adjusting the monomer composition in the blocks.^{35–38} Block random copolymers showing tunable solution properties for each block have recently been synthesized and studied.^{39,40} Inspired by the natural and synthetic polymers responsive to multiple stimuli, our approach is to make diblock copolymers from random copolymer blocks of pH- and thermo-sensitive comonomers. Here, the pH-sensitive block is also thermo-sensitive, while the second block responds only to temperature changes. Controlling the block length and the composition of the blocks allows the design of systems with tunable responsiveness to a desired pH, a property that may be useful for the rapid release of an encapsulated guest molecule in response to external stimuli. The diblock copolymer consists of two blocks showing separate CPs, and a variety of techniques may be used to study their “schizophrenic” self-assembling process into vesicles. The

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stimuli-responsiveness of individual blocks and the pH-dependent thermo-sensitivity of diblock random copolymers should lead to the inversion of core and corona blocks in the vesicle membranes under suitable conditions and their aggregation at high temperatures.

EXPERIMENTAL SECTION

Materials. 2,2'-Azobisisobutyronitrile (AIBN, Eastman Kodak) was recrystallized from methanol. Sodium deuteroxide, deuterium chloride, acryloyl chloride, ethylamine (70% aqueous solution), and *n*-propylamine were purchased from Aldrich and were used without further purification. 2-(Diethylamino)ethyl methacrylate (DEAEMA) was purchased from Aldrich and vacuum-distilled prior to use. *N*-*n*-Propylacrylamide (*n*PA) and *N*-ethylacrylamide (EA) were synthesized according to a procedure reported by Shea et al.⁴¹ 3-(Benzylsulfanylthiocarbonylsulfanyl)propionic acid (BPA) was prepared according to a procedure described by Stenzel et al.⁴² and used as a chain transfer agent (CTA). Anhydrous and oxygen-free tetrahydrofuran (THF) was obtained by passage through columns packed with activated alumina and supported copper catalyst (Glass Contour, Irvine, CA). Water was purified using a Millipore Milli-Q system.

Polymer Synthesis. A previously reported method was used to synthesize the diblock random copolymer.³⁹ In this work, THF was used as the solvent and BPA as the RAFT agent. The monomers used for the first copolymerization are *n*PA and DEAEMA, while *n*PA and EA are used to prepare the second block. Since DEAEMA is prone to sublimation in the freeze–thaw process, nitrogen bubbling was selected as the degassing method. The reaction mixture of [monomer]/[BPA]/[AIBN] in the molar ratio of 200:1:0.1 and with a total monomer concentration of 0.3 g/mL was purged with N₂ for 30 min prior to immersing it in a preheated oil bath (70 °C). After 90 min reaction time, the polymerization was terminated and the random copolymer was precipitated in petroleum ether, filtered, and dried in vacuum at room temperature to yield poly(*n*PA_{0.8}-*co*-DEAEMA_{0.2})-CTA. The resulting random copolymer was then used as the BPA-ended macro-CTA in the second step to prepare a diblock copolymer, poly(*n*PA_{0.8}-*co*-DEAEMA_{0.2})-*block*-poly(*n*PA_{0.8}-*co*-EA_{0.2}), using the reactant molar ratio [monomer]/[macro-CTA]/[AIBN] of 200:1:0.1. The procedure for the diblock copolymerization was the same as that for the random copolymerization, except that the comonomers were different and BPA was replaced by macro-CTA (Scheme 1). Poly(*n*PA_{0.8}-*co*-EA_{0.2}) and poly(*n*PA_{0.7}-*co*-DEAEMA_{0.3}) were also made separately with the same method to study the LCST of the second block individually and the effect of the DEAEMA content on the thermal behavior, respectively.

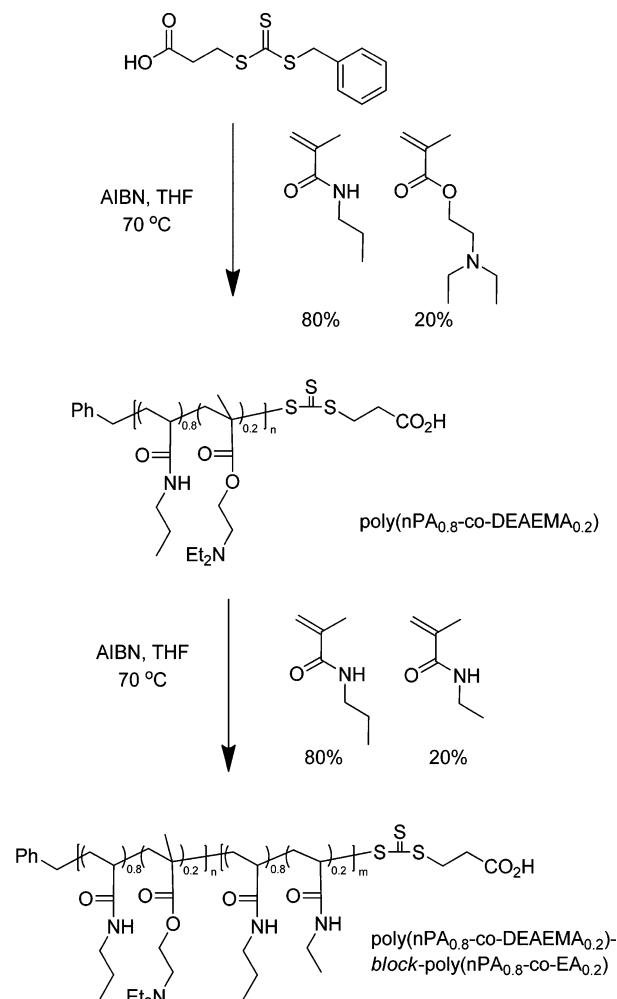
Polymer Characterization. Molar masses and polydispersity indices (PDI) of the polymers were determined by SEC on a Waters 1525 system equipped with three Waters Styragel columns and a refractive index detector (Waters 2410) at 35 °C. *N,N*-Dimethylformamide (DMF) containing 0.01 M LiBr was used as the mobile phase at a flow rate of 1 mL/min. The system was calibrated by poly(methyl methacrylate) standards.

The NMR spectra of the monomers and polymers in deuterated chloroform (CDCl₃) were recorded on a Bruker AV-400 NMR spectrometer operating at 400 MHz for protons, and the temperature-dependent NMR spectra of the diblock random copolymer were recorded on a Bruker AV-500 spectrometer working at 500 MHz in deuterated water (D₂O). The pH of the solutions was adjusted by adding NaOD or DCl solution in D₂O. The theoretical molar masses were calculated from the conversions given by ¹H NMR according to

$$\bar{M}_{n,th} = M_{CTA} + \frac{[\text{monomer}]}{[\text{CTA}]} \times M_{\text{monomer}} \times \text{conversion} \quad (1)$$

where M_{CTA} is the molecular weight of the chain transfer agent and [monomer] and [CTA] are the initial monomer and CTA concentrations, respectively. M_{monomer} is the average molecular weight of the comonomers and calculated from

Scheme 1. Synthesis of Dual Responsive Diblock Random Copolymer Poly(*n*PA_{0.8}-*co*-DEAEMA_{0.2})-*block*-poly(*n*PA_{0.8}-*co*-EA_{0.2}) via RAFT Copolymerization of *n*PA and DEAEMA Followed by a Chain Extension with *n*PA and EA



$$M_{\text{monomer}} = xM_1 + (1 - x)M_2 \quad (2)$$

where x is the molar fraction of monomer 1 (obtained by ¹H NMR) and M_1 and M_2 are the molecular weights of monomers 1 and 2 in the random copolymer, respectively. For a block copolymer, [CTA] in eq 1 is replaced by the concentration of macro-CTA.

Atomic force microscopy (AFM) images were acquired in air at room temperature using tapping mode on a (Digital Instruments Dimension 3100 microscope, Santa Barbara, CA) Intermittent contact imaging (i.e., “tapping mode”) was performed at a scan rate of 1 Hz using aluminum-coated etched silicon cantilevers (ACTA tips from App Nano Inc.) with a resonance frequency around 300 kHz, a spring constant of ~42 N/m, and a tip radius of <10 nm. All images were acquired with a medium tip oscillation damping (20–30%). The samples were analyzed in the dried state via drop deposition of the 0.05 mg/mL aqueous solution of the polymer onto a mica surface at desired temperature.

Transmission electron microscopy (TEM) images were recorded on lyophilized aqueous samples (0.05 mg/mL) deposited on copper grids (300 mesh, Carbon Type-B, Ted Pella, Inc.) at desired temperature. The images were acquired on FEI Tecnai 12 TEM at 120 kV, equipped with AMT XR80C CCD camera system.

Temperature-dependent zeta potential measurements were conducted in pure Milli-Q water on a Zetasizer instrument (Nano ZS) from Malvern. The zeta potential, Z , is determined through the electrophoretic mobility UE with Henry’s equation:

$$UE = \frac{2\varepsilon Zf(\kappa a)}{3\eta} \quad (3)$$

where ε is the dielectric constant, η is the viscosity, and $f(\kappa a)$ is the Henry's function. The value of 1.5 is used for $f(\kappa a)$ in aqueous solutions of moderate electrolyte concentration, which is referred to as the Smoluchowski approximation. The final values were the average of three measurements. The cloud points (CPs) of the polymers were determined from the optical transmittance measured on a Cary 300 Bio UV–vis spectrophotometer equipped with a temperature-controlled sample holder. The samples at a concentration range of 0.5–0.05 mg/mL were prepared by dissolving the copolymers in deionized water cooled in an ice–water bath, after which the solutions were homogenized by ultrasonication. The pH of the solutions (5 mL) was adjusted by adding microliter quantities of 0.1 N HCl or 0.1 N NaOH. The stability of the pH was checked after 24 h and fine-tuned by further addition of acid or base, if necessary. The absorbance was measured at different wavelengths for the aqueous solution of polymers by continuous heating at a rate of 0.1–0.3 °C/min over various temperature ranges. For individual blocks, the cloud point is given as the temperature at which 50% transmittance was lost upon heating. For block copolymers, the CP is determined from the middle point between the onset and the offset of the transmittance curve as a function of temperature.

Light scattering studies on the pH- and temperature-dependent aggregation behavior were conducted on a CGS-3 compact goniometer (ALV GmbH) equipped with an ALV-5000 multi tau digital real time correlator at selected temperatures using a Science/Electronics temperature controller. The laser wavelength was 632 nm. In dynamic light scattering (DLS) experiments, the scattering angle was fixed at 90°. All solutions were prepared at a concentration of 0.05 mg/mL, and dust was removed by filtering through 0.22 μ m Millipore filters. The DLS results were analyzed by the cumulant method. The decay rate distributions were transformed to a diffusion coefficient, and the apparent intensity-weighted hydrodynamic diameters of the polymers were obtained from the Stokes–Einstein equation. In static light scattering (SLS) experiments, apparent weight-average molar masses ($M_{w,app}$) of the diblock copolymer and its aggregates were measured in dilute solutions (0.05 mg/mL) from the angular dependence of the excess absolute time-averaged scattering intensity, known as the Rayleigh ratio $R(\theta)$. The angular range was between 30° and 150° with increments of 10°. The values of $M_{w,app}$ were obtained from the extrapolation of partial Zimm plots.^{43,44} The refractive index increments (dn/dc) were determined by a Brookhaven (BIC-DNDC) differential refractometer for three temperature ranges in relation to the two cloud points of the block copolymer, CP₁ and CP₂. The values were 0.143 ($T < CP_1$), 0.171 ($CP_1 < T < CP_2$), and 0.174 ($T > CP_2$).

RESULTS AND DISCUSSION

Preparation of the Polymers. We demonstrated in an earlier study³⁹ that the cloud point of the random copolymers made from *n*PA and EA can be tuned over a wide temperature range of 20–85 °C by adjusting the ratio of comonomers. In addition, the diblock copolymer consisting of such blocks showed two separate phase transition temperatures. As the reactivity of monomers in the RAFT polymerization depends on the chain transfer agent (CTA), a suitable CTA must be chosen to efficiently polymerize both acrylamide- and methacrylate-based monomers. For instance, 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DMP) has been used in our group as a CTA for the polymerization of acrylamides, but DEAEMA was not polymerized with DMP as the RAFT agent. Therefore, 3-(benzylsulfanylthiocarbonylsulfanyl)propionic acid (BPA) was our choice for the (block) copolymerization of *N*-*n*-propylacrylamide (*n*PA) with 2-(diethylamino)ethyl methacrylate (DEAEMA) or *N*-ethylacrylamide (EA). The composition of the blocks obtained was

similar to the feed ratio (Table 1), and frequent sampling from the reaction mixture showed the statistical nature of the

Table 1. Conversions and Compositions of Mono- And Diblock Random Copolymers

polymer	monomer ratio in the blocks ^a	conversion (%) ^a	M_n ($\times 10^3$ g/mol)		
			theor ^b	SEC ^c	PDI ^c
P(<i>n</i> PA _{0.8-co} -DEAEMA _{0.2})	81.1:18.9	31	7.9	6.5	1.18
P(<i>n</i> PA _{0.8-co} -DEAEMA _{0.2})- <i>b</i> -P(<i>n</i> PA _{0.8-co} -EA _{0.2})	78.2:21.8	34	13.9	14.6	1.35

^aDetermined by ¹H NMR. For the block copolymer, the given composition corresponds to that of the second block. ^bCalculated from eq 1. ^cDetermined by SEC with poly(methyl methacrylate) as calibration standards for the molecular weight.

random copolymer of an acrylamide (*n*PA) and methacrylate (DEAEMA, Supporting Information Table S1). An earlier study on the kinetics of copolymerization of *n*PA and EA indicated a deviation from linearity at high conversions ($\geq 70\%$), suggesting the presence of dead chains.³⁹ Therefore, the conversions of the current polymerizations were kept low ($\sim 30\%$) to ensure the livingness of the chains and to achieve similar molar masses for the two blocks, controlled by the [monomer]/[CTA]/[AIBN] ratios. We found that the block containing the methacrylate-based monomer DEAEMA allowed easy addition of the second block, while the block containing only acrylamide monomers is not easily extended to form a diblock copolymer.

The purification of poly(*n*PA-*co*-DEAEMA) copolymer by precipitation was necessary prior to its use as a macro-CTA in the subsequent block copolymerization. The properties of the macro-CTA and the resulting block copolymer are listed in Table 1. The compositions of the polymers and the molar masses were determined by ¹H NMR (Figure S1) and size exclusion chromatography (Figure S2), respectively. An increase in the polydispersity is observed for the diblock copolymer which is frequently reported for the RAFT polymerization of macro-CTA.^{39,45,46} As the block symmetry is one of the critical parameters influencing the solubility and the micellization process,¹ block ratio 1:1.25 for poly(*n*PA-*co*-DEAEMA)/poly(*n*PA-*co*-EA) was selected. The block ratio was calculated from the compositions of the blocks and the molar masses given by SEC. The monomer ratios have been selected to yield two well-separated thermal transitions at neutral pH, one of which is slightly below the body temperature. Based on our earlier study on the effect of *n*PA/EA molar ratio on the cloud point of the copolymer, the latter transition is obtained at a ratio of 80:20.³⁹ The solution properties of individual blocks are discussed below. Although it is known that the end groups of thermo-responsive polymers influence the micellization and lower critical solution temperatures,⁴⁷ the end group was not removed to avoid the possible cleavage of the ester bond of DEAEMA. Furthermore, the selected RAFT agent (BPA) is water-soluble, and hence, its effect on the solution properties of the copolymers is expected to be minimal.

Solution Properties of the Copolymers. The thermal transitions of individual blocks of the diblock random copolymer were measured by UV–vis spectroscopy. Figure 1

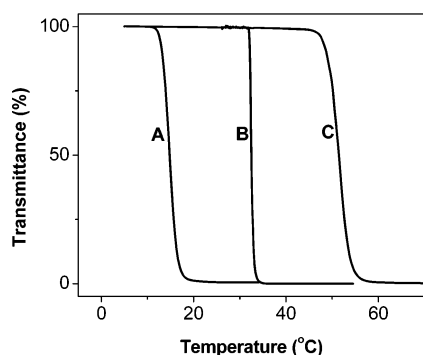


Figure 1. Temperature-dependent transmittance of 0.5 mg/mL aqueous solutions of (A) poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) at pH 10.0, (B) poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) and (C) poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) at pH 7.0, observed at a wavelength of 300 nm.

shows the transmittance curves of aqueous solutions of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) with the properties mentioned in Table 1 and poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) with molar mass of 16 500 g/mol, and PDI of 1.21.

While the CP of poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) at 33 °C does not change with pH, a strong pH-dependence is observed for poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$), as shown in Figures 2 and 3. The

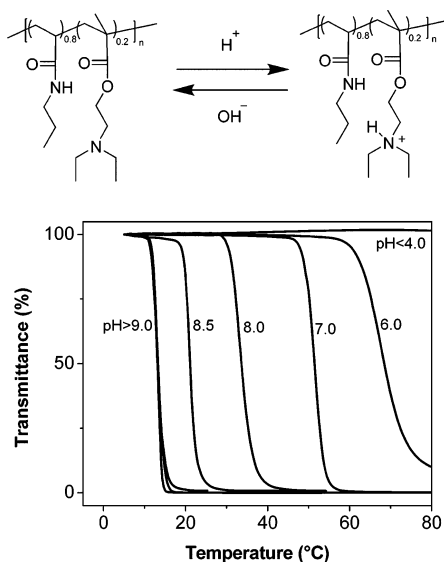


Figure 2. Thermosensitivity of 0.5 mg/mL aqueous solution of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) measured by UV-vis spectroscopy at 300 nm and heating rate 0.3 °C/min at different pH values. The protonation and deprotonation of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) random copolymer depending on the pH is also shown.

tertiary amine residues of DEAEMA become protonated and positively charged in acidic medium, making the copolymer more hydrophilic and, thus, leading to the complete disappearance of CP at pH 4. While the ester bonds of polyacrylates are subject to hydrolysis in aqueous solutions,⁴⁸ those of polymethacrylates show better stability even in acidic and basic solutions.⁴⁹ NMR data showed that poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) was stable against hydrolysis at both pH 7 and 10 used in this study. The pK_a of PDEAEMA homopolymer is 7.3,¹⁶ giving an idea of the degree of protonation of DEAEMA moieties in the pH range studied. The solubility of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) decreases with increasing pH upon depro-

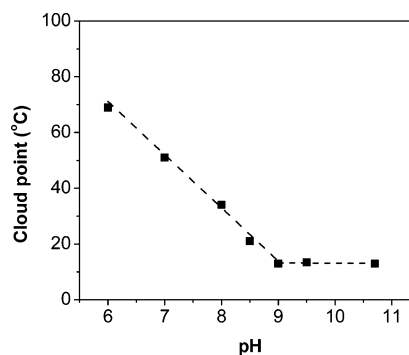


Figure 3. Cloud points of the 0.5 mg/mL aqueous solutions of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) as a function of pH (extracted from Figure 2). The dashed lines are added as visual guides.

nation, and, as a result, the CP decreases gradually from 68 (pH = 6) to 13 °C (pH > 9, Figure 3). The PDEAEMA homopolymer is water-insoluble in its deprotonated form.¹¹ Therefore, the CP of fully deprotonated poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) is lower than that of PnPA (20 °C, M_n = 12 800 g/mol).³⁹

As shown in Figure 1, at both pH 7 and 10, the CP of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) is well distinguishable from that of poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) and, hence, two well-separable transitions could be observed also with their block copolymer at these pH values. The composition dependence of the CP for poly($n\text{PA}_x\text{-co-DEAEMA}_{1-x}$) was also observed. For instance, the CP of a copolymer with higher DEAEMA content, poly($n\text{PA}_{0.7}\text{-co-DEAEMA}_{0.3}$) (M_n = 7400 g/mol, PDI = 1.20) at pH 10 lies at 10 °C, which is lower than that of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) (13 °C, M_n = 6500 g/mol, PDI = 1.18). Thus, the cloud point of a poly($n\text{PA-co-DEAEMA}$) random copolymer at any desired pH can be adjusted by tuning its composition.

Solution Properties of Diblock Random Copolymer.

The dual thermo-responsive behavior of the diblock random copolymer poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) at pH 7 and 10 was studied by UV-vis, ¹H NMR spectroscopy, TEM, AFM imaging, zeta potential measurements, and laser light scattering (LLS).

UV-Vis Spectroscopy. At pH 7 (Figure 4A), the more hydrophobic poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) block starts to collapse with increasing temperature at its cloud point of 33 °C, accompanied by a reduction in transmittance. TEM images show that at this point, vesicles with poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) membrane core are formed (Figure 5A). The second aggregation step starts at around 52 °C, corresponding to the cloud point of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) at pH 7 (Table 2).

The effect of particle size on the apparent turbidity has been discussed previously.³⁹ The strong wavelength dependence of scattering ($\sim \lambda^{-4}$) means that a radiation of shorter wavelength is scattered more strongly than that of a longer wavelength.⁵⁰ Therefore, fewer particles are visible when observed at higher wavelength, leading to a lower apparent turbidity.³⁰ Due to the large size of particles formed in the course of the first transition, resulting in a strong shift in transmittance, the second step is not as clear at a low detection wavelength (Figure S4).

At pH 10 (Figure 4B), the first cloud point starting at ~14 °C is assigned to the collapse of the poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) block, where the deprotonated DEAEMA units make this block more hydrophobic. Hence, the poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) block will form the core of the vesicle membranes

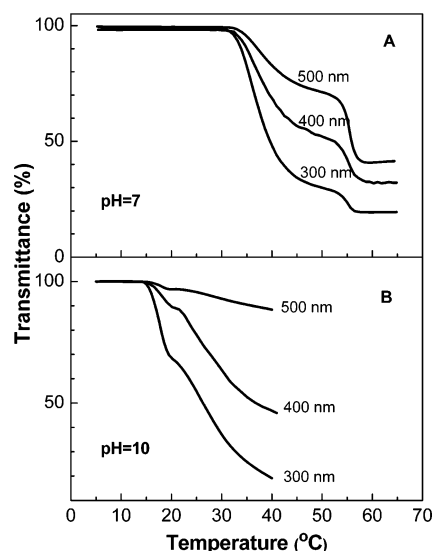


Figure 4. Dual stimuli-responsive behavior and the two-step transition observed for aqueous solutions of diblock random copolymer poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) (0.05 mg/mL) at (A) pH 7 and (B) pH 10 measured by UV–vis transmittance at three different wavelengths at a heating rate of 0.1 °C/min.

upon its collapse (Figure 5B). The second step in the transmittance curves starting at 21 °C is rather broad and the smaller total shift in the transmittance at 500 nm compared to pH 7 reflects the smaller size of the particles at pH 10. This is confirmed by the dynamic light scattering results discussed later. Larger aggregates at pH 7 could be due to the slightly longer poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) block, which collapses first at pH 7.

We have previously observed the interdependence of thermal transitions of a double thermosensitive diblock random copolymer due to a difference in the relative hydrophilicities of the blocks. Thus, the CP of the less hydrophilic block of the diblock copolymer shifts to a higher temperature, while that of the more hydrophilic block moves to a lower temperature, and such a behavior would also be expected for poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$).

Table 2. Cloud Points (CP °C) of Individual Blocks and the Block Copolymer at Different pH Values Measured by UV–Vis Transmittance at 300 nm^a

pH	P($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)	P($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$)	P($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)- <i>b</i> -P($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$)	
			CP ₁	CP ₂
7.0	52	33	39	53
10.0	13	33	18	32

^aThe CPs measured at higher wavelengths are generally 1–3 °C higher.

DEAEMA_{0.2})-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$). Therefore, at pH 10, the CP of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) block is slightly higher and that of poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) block is lower than the corresponding transition temperatures of the individual copolymers. This effect is also seen at pH 7 with the first transition corresponding to the CP of the poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) block, which is slightly higher than the corresponding transition of the individual copolymer. At pH 7, the more hydrophilic and partially protonated poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) block is much less affected by its neighboring block and the onset of the transition is close to the CP of the individual copolymer.

¹H NMR Spectroscopy. ¹H NMR was used to study the dual responsive behavior of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) solutions in D₂O. Figure 6 shows the temperature-dependent ¹H NMR spectra at pH 7 and 10. In Figure 6A (pH 10), no difference is observed in the spectra at 10 and 15 °C, but peaks *b* and *c*, both characteristic for poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$), start to show reduced intensity upon heating to 20 and 25 °C, indicating the collapse of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$), which is now more hydrophobic. Peaks *d* and *f* belong to the poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) block, whereas peak *a* comes from both blocks. Further heating to 30 °C induces no change to peak *f* and a small change to peaks *a* and *d*, but they show greater attenuation at higher temperatures (35 and 40 °C), where the more hydrophilic block poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) is collapsed. In Figure 6B (pH 7), the characteristic peaks of poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) only start to attenuate at 35 °C,

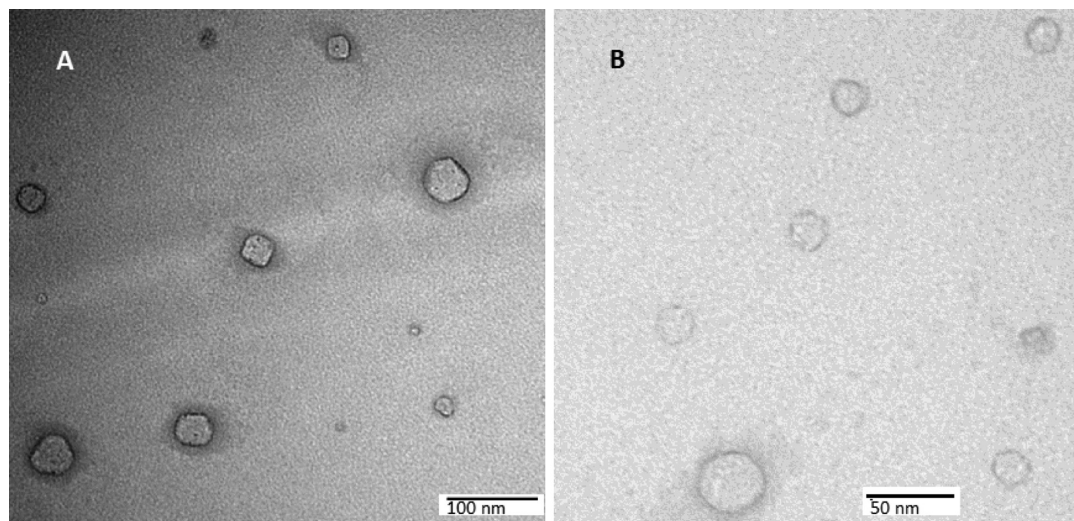


Figure 5. Representative TEM images of 0.05 mg/mL aqueous solutions of diblock random copolymer poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) deposited on copper grids at (A) pH 7, 37 °C and (B) pH 10, 25 °C, showing vesicles at both conditions. Note that the scale bars are 100 and 50 nm, respectively.

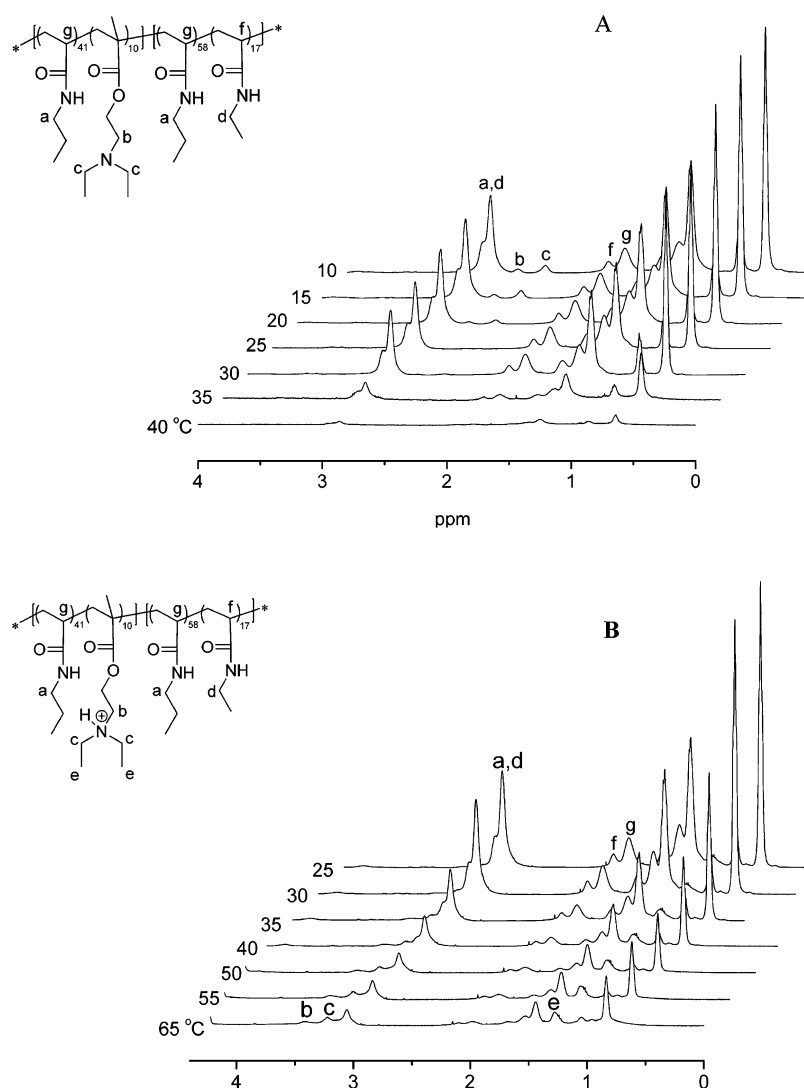


Figure 6. ^1H NMR spectra of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-block-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) as a function of temperature at (A) pH 10 and (B) pH 7.

Table 3. Micellar Properties of a Diblock Random Copolymer Poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-block-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) in 0.05 mg/mL Aqueous Solutions Studied by LLS

	temperature ($^{\circ}\text{C}$)	D_h (nm) ^a	PDI ^a	$M_{w,\text{app}}$ (g/mol) ^b	N_{agg} ^c	zeta potential (mV)
pH 10	37	116	0.029	1.2×10^8	6.7×10^3	-23 ± 2
	25	60	0.184	7.1×10^6	4.0×10^2	-20 ± 1
pH 7	25	7		1.8×10^4	~ 1	4 ± 1
	37	148	0.164	1.8×10^8	1.0×10^4	22 ± 1
	55	328	0.266	7.1×10^9	3.9×10^5	25 ± 2

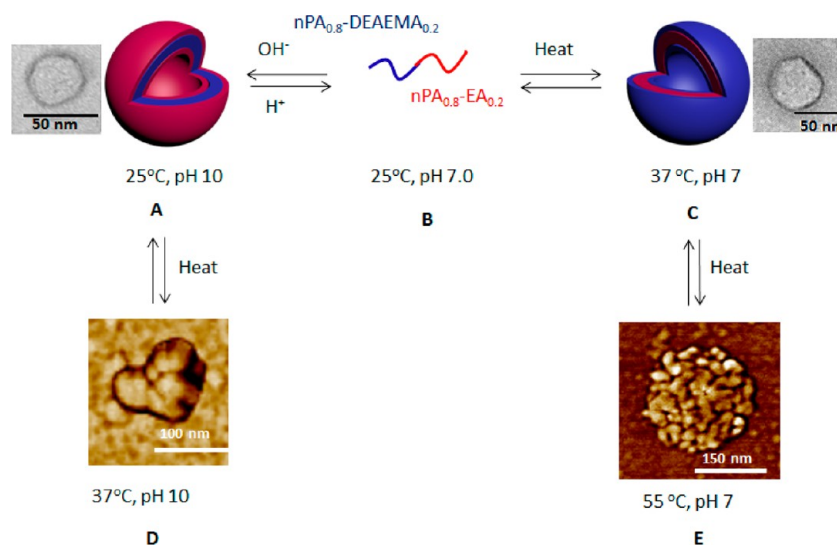
^aMean intensity-weighted hydrodynamic diameter and polydispersity index (PDI) determined by DLS. ^bApparent weight-average molar mass determined by SLS. ^cAggregation number calculated from $M_{w,\text{app}}$.

indicating that poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) is now the more hydrophobic block and collapses first. Interestingly, the signals of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$) do not disappear even at 65 $^{\circ}\text{C}$, probably due to the charged nature of this block. Since the pK_a of this block is 7.3,⁷ about 67% of amine groups are protonated at pH 7. These charged moieties are still swollen, and peaks *b*, *c*, and *e* even seem to become more visible at higher temperatures, which could suggest high mobility of the protonated domains. While the ^1H NMR and UV-vis spectroscopy provide evidence for the “schizophrenic” self-assembling behavior, the aggregate morphologies cannot be deduced. Hence, LLS, TEM and AFM imaging, and zeta

potential measurements were undertaken for a better understanding of the aggregation process.

Dynamic and Static Light Scattering. According to the light scattering results shown in Table 3, the solution of random block copolymer at pH 7 and 25 $^{\circ}\text{C}$ mostly contains molecularly dissolved polymer chains with zeta potential lower than 5 mV. Even though we used PMMA as SEC standards, which is different from the polymers studied, the apparent weight-average molar mass obtained by SLS (1.8×10^4 g/mol) agrees quite well with the one given by SEC in DMF (1.9×10^4 g/mol). The samples were heated at 1 $^{\circ}\text{C}$ intervals within 20 min equilibration time, corresponding to an average heating

Scheme 2. Self-Assembling Process of Poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) into Vesicles, and Then Aggregates upon Heating^a



^aThe images of representative particles are taken from larger TEM and AFM images (Figures 5 and 7, respectively).

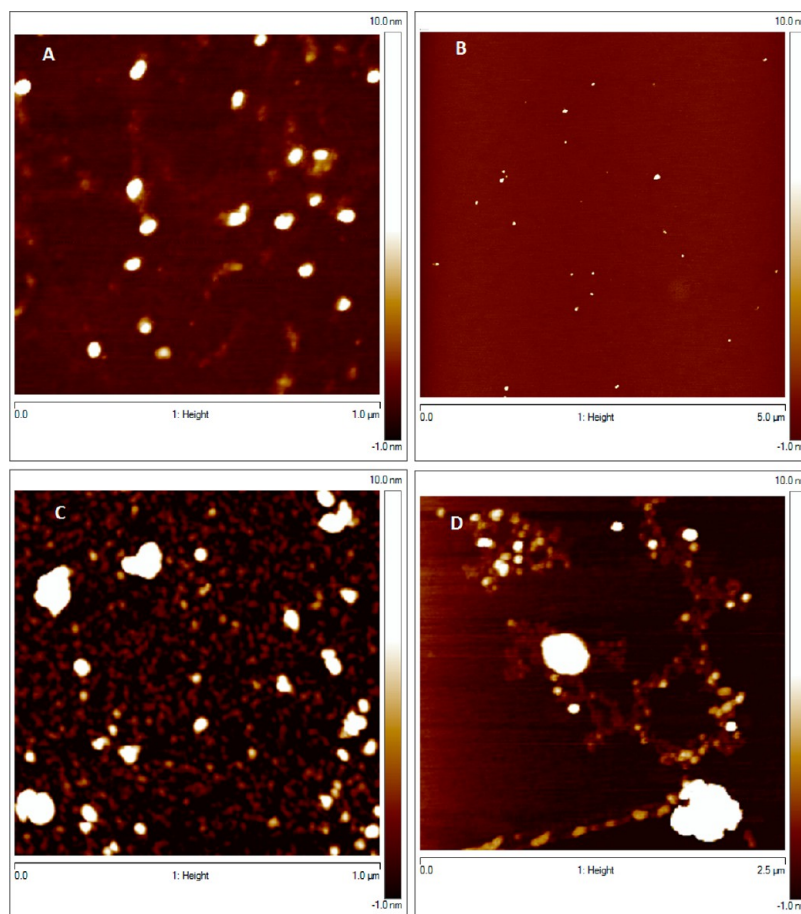


Figure 7. AFM images of 0.05 mg/mL aqueous solutions of poly($n\text{PA}_{0.8}\text{-co-DEAEMA}_{0.2}$)-*block*-poly($n\text{PA}_{0.8}\text{-co-EA}_{0.2}$) diblock random copolymer deposited on mica at (A) pH 10 and 25 °C, (B) pH 7 and 37 °C, (C) pH 10 and 37 °C, and (D) pH 7 and 55 °C. Note that the image width (scale) is 1.0 μm for (A) and (B), 5.0 μm for (C), and 2.5 μm for (D).

rate of 0.05 °C/min. At such a slow heating process, the polymer chains have more time for interchain interactions and aggregation before the temperature-induced coil-to-globule transition; therefore, larger aggregates are expected.⁵¹ It must

be emphasized that the selected temperatures in Table 3, both at pH 7 and 10 (and at 37 and 25 °C, respectively), correspond to conditions where self-assembled nanostructures are formed, namely, the temperatures where the transmittance in Figure 4

starts to decrease and self-assembling occurs. At temperatures above CP_2 , the corona-forming blocks of the vesicles will collapse, leading to aggregation.

Based on the cloud points of the individual blocks and the block copolymer, the core of the vesicles membrane is composed of the poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) block at pH 7 and the poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) block at pH 10. The size distributions obtained during the experiment are depicted in Figure S3.

The proposed self-assembling process is depicted in Scheme 2. At pH 10, the DEAEMA moieties of poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) block and also the carboxylic end group of the CTA are deprotonated. Thus, the poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) block becomes more hydrophobic at 25 °C above its cloud point, while the poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) block is still solvated along with the attached anionic CTA end group. This leads to the formation of vesicles with mean hydrodynamic diameter of 60 nm and $N_{agg} \sim 400$. The zeta potential of the vesicles is -20 mV due to the anionic chain ends, which are located on the coronas. At pH 7, the poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) block is dehydrated at 37 °C, thus forming the core of the vesicle membrane, while the poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) block is solvated due to the partial protonation of amine groups. The partially protonated block contributes to the positively charged coronas of inverted vesicles with zeta potential of 22 mV. The hydrodynamic diameter of the vesicles is 148 nm and the aggregation number ($N_{agg} \sim 1.0 \times 10^4$) is substantially higher than at pH 10. The large hydrodynamic diameter and high aggregation number are typical to the vesicular structures. AFM studies at both pH 10 and 7 (Figure 7A and C, respectively) showed spherical particles with mean number-average diameters of 60 and 67 nm for poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$)-core (pH 10) and poly($nPA_{0.8}$ -*co*- $EA_{0.2}$)-core (pH 7), respectively. The diameters obtained by AFM have been determined in a dried state and are therefore lower than those by DLS in solution. The charged particles at pH 7 are more affected by the removal of water.

The block ratio is known to influence the morphologies of self-assemblies, even when the blocks are stimuli-responsive and capable of changing their relative hydrophilicities in the block copolymer. For example, the pH- and temperature-responsive poly(*N,N*-diethylaminoethyl methacrylate)-*block*-poly(*N*-isopropylacrylamide) (PDEAEMA-*b*-PNIPAM) forms spherical micelles in aqueous solution in spite of the core block if the mass fractions of blocks are similar, that is, $\sim 50:50$ (wt %). However, if one block is much longer, different self-assembled morphologies can be observed and, for example, the mass ratio of $\sim 70:30$ (wt %) of PDEAEMA-*b*-PNIPAM leads to the formation of either spherical micelles or vesicles, depending on which block has higher relative hydrophilicity with the selective stimuli.¹⁶ In another example, a double temperature-responsive poly[2-(dimethylamino)ethyl methacrylate-*block*-di(ethyleneglycol)methyl ether methacrylate] (poly(DMAEMA-*b*-DEGMA)) at $\sim 50:50$ (wt %) formed multi- or unilamellar vesicles depending on temperature.⁵² In our case, the mass ratio of the poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) and poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) blocks is 45:55 (wt %), and with this composition the formation of either micelles or vesicles could be expected. The higher degree of aggregation at pH 7 could stem from the slightly longer poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) block collapsing at pH 7 and 37 °C.

If the solution of vesicles at pH 10 is heated from 25 °C (A in Scheme 2) to 37 °C (D), above the cloud point of the corona-forming poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) block, the coronas

collapse and the vesicles aggregate into larger clusters ($D_h = 116$ nm, $N_{agg} \sim 6.7 \times 10^3$). This process corresponds to the second step in the shift in transmittance shown in Figure 4B and is accompanied by a decrease in zeta potential to -23 mV. This step is illustrated by AFM imaging in Figure 7, and the collapsed particles coexist with the single ones in a polydisperse system with diameters ranging from 25 to 103 nm. On the other hand, the vesicles with both temperature- and pH-sensitive poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) coronas at pH 7 (C in Scheme 2) show pH-dependent aggregation upon heating. When the sample (pH 7) is heated to 55 °C, above the cloud point of poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) block, $D_h = 328$ nm and $N_{agg} \sim 3.9 \times 10^5$ (E) are obtained with the zeta potential of 25 mV. The molar mass of the aggregates measured by SLS is almost 40 times higher at 55 °C (E) than at 37 °C (C), suggesting that the vesicles at pH 7 show the behavior similar to pH 10, aggregating into larger clusters upon heating. According to the AFM image in Figure 7, large aggregates are present in a polydisperse system with diameters between 40 and 400 nm. According to the phase diagram proposed in Scheme 2, the pH-sensitive and thermo-sensitive poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) block can contribute either to the core of the vesicle membranes or to stabilizing the coronas, depending on the external stimulus. This feature in addition to the tunability of the temperatures for switching between vesicles and inverted vesicles contributes to the versatility of these smart materials.

CONCLUSIONS

The synthetic approach developed in our group for making blocks of random copolymers allows the making of block copolymer responding to a desired condition almost at will within a reasonable range for the external stimuli. This work provides an illustrative example for the design and synthesis of such polymers. Combining pH- and temperature-responsive poly($nPA_{0.8}$ -*co*- $DEAEMA_{0.2}$) and temperature-responsive poly($nPA_{0.8}$ -*co*- $EA_{0.2}$) random copolymers to a diblock copolymer resulted in a system that is responsive to multiple stimuli. The composition-dependent response to the external stimuli allows the design of a wide range of responsive polymers. pH-switchable vesicles exhibiting “schizophrenic” behavior were formed in aqueous solutions, and their sizes depended on both pH and temperature. The block copolymer was molecularly dissolved in water at 25 °C (pH 7), forming vesicles at 37 °C (pH 7), while the core and corona of the vesicle membranes could be switched at 25 °C (pH 10). The behavior of the polymers is clearly of theoretical interest. There may be potential applications where responsiveness to both temperature and pH is needed. For instance, the thermoresponsive “schizophrenic” diblock copolymer PNIPAAm-*b*-PSBMA has recently been studied for its anticoagulant behavior in the human blood in the range of 4–40 °C.⁴ The advantage of the current design is the possibility to tailor such responsiveness to a desired temperature and pH by adjusting the chemical composition of the monomers during the polymerization. The two-step self-assembling process is also an interesting issue that could be the subject of further detailed studies.

ASSOCIATED CONTENT

Supporting Information

¹H NMR spectra and SEC traces of mono- and diblock random copolymer, particle size distribution by DLS, and short

wavelength UV–vis spectra. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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