Investigation of a New Thermosensitive Block Copolymer Micelle: Hydrolysis, Disruption, and Release

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Thermosensitive polymer micelles are generally obtained with block copolymers in which one block exhibits a lower critical solution temperature in aqueous solution. We investigate a different design that is based on the use of one block bearing a thermally labile side group, whose hydrolysis upon heating shifts the hydrophilic−hydrophobic balance toward the destabilization of block copolymer micelles. Atom transfer radical polymerization was utilized to synthesize a series of diblock copolymers composed of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(2-tetrahydropyranyl methacrylate) (PTHPMA). We show that micelles of PEO-*b*-PTHPMA in aqueous solution can be destabilized as a result of the thermosensitive hydrolytic cleavage of tetrahydropyranyl (THP) groups that transforms PTHPMA into hydrophilic poly(methacrylic acid). The three related processes occurring in aqueous solution, namely, hydrolytic cleavage of THP, destabilization of micelles, and release of loaded Nile Red (NR), were investigated simultaneously using ^{1}H NMR, dynamic light scattering, and fluorescence spectroscopy, respectively. At 80 °C, the results suggest that the three events proceed with a similar kinetics. Although slower than at elevated temperatures, the disruption of PEO-*b*-PTHPMA micelles can take place at the body temperature (∼ 37 °C), and the release kinetics of NR can be adjusted by changing the relative lengths of the two blocks or the pH of the solution.

Introduction

Thermosensitive block copolymer (BCP) core−shell micelles are of interest for controlled delivery applications. Such micelles can be destabilized either in response to a change in temperature or at body temperature (∼37 °C) with appropriate kinetics, which enables the drug pre-encapsulated by the hydrophobic micelle core to be released in a controlled manner. Generally, thermosensitive polymer micelles are obtained with BCPs that have one block exhibiting a lower critical solution temperature (LCST), below which the block is soluble in water, while above which it is insoluble. The most used polymer is poly(*N*-isopropylacrylamide) (PNIPAAm) having an LCST of ∼32 °C.[1−5] To mention only a few examples, with poly(ethylene oxide) (PEO) as the hydrophilic block, PNIPAAm was used as the hydrophobic block at $T >$ LCST, whose micelles can be dissolved at $T <$ LCST and are expected to give a quick release of loaded drugs.[1] Whereas with poly(butyl methacrylate) (PBMA) as the hydrophobic block, PNIPAAm was also utilized as the hydrophilic block at $T <$ LCST.[2] In this case, the micellar destabilization occurs at $T >$ LCST, with the outer shell of PNIPAAm becoming insoluble in water and precipitating; the perturbation was shown to give rise to release of a loaded drug due to the low-T_g micelle core of PBMA (∼20 °C < LCST of PNIPAAm). Moreover, it is known that the LCST of PNIPAAm can be adjusted to a higher or lower temperature by incorporation of hydrophilic or hydrophobic comonomer units, respectively.[4] An interesting development of thermosensitive BCP micelles is the use of a diblock copolymer composed of PEO and a random copolymer of NIPAAm and *N*-(2-hydroxypropyl)methacrylamide lactate) (NHPMAAm-lactate).[5] At physiological conditions (pH 7.4, 37 °C), the hydrolysis of the lactate side groups increases the amount of hydrophilic comonomer units and thus the LCST of the polyacrylamide block; when the LCST rises to above 37 °C, the micelles are destabilized. PNHPMAAm-lactate can also be used without PNIPAAm to design thermosensitive BCP micelles based on the same principle of changing LCST upon hydrolysis of the lactate groups.[6] A recent study also explored the combined use of thermo- and photosensitivity of micelles formed by a BCP of PEO and poly(ethoxytri(ethylene glycol) acrylate-*co*-*o*-nitrobenzyl acrylate) (PEO-*b*-P(TEGEA-*co*-NBA).[7] Upon UV irradiation, the photoinduced cleavage of *o*-nitrobenzyl groups increases the amount of acrylic acid group in the thermosensitive P(TEGEA-*co*-NBA) block and thus its LCST. In another example of study, methacrylamide-based copolymers with pendent *ortho* ester groups were found to form micelles that are both thermo- and pH-sensitive.[8]

The purpose of the present study was to investigate a different design for thermosensitive BCP micelles, which is inspired by our studies of photocontrollable polymer micelles.[9] We have synthesized an amphiphilic diblock copolymer composed of hydrophilic PEO and hydrophobic poly(2-tetrahydropyranyl methacrylate) (PTHPMA). The use of PTHPMA allows PEO-

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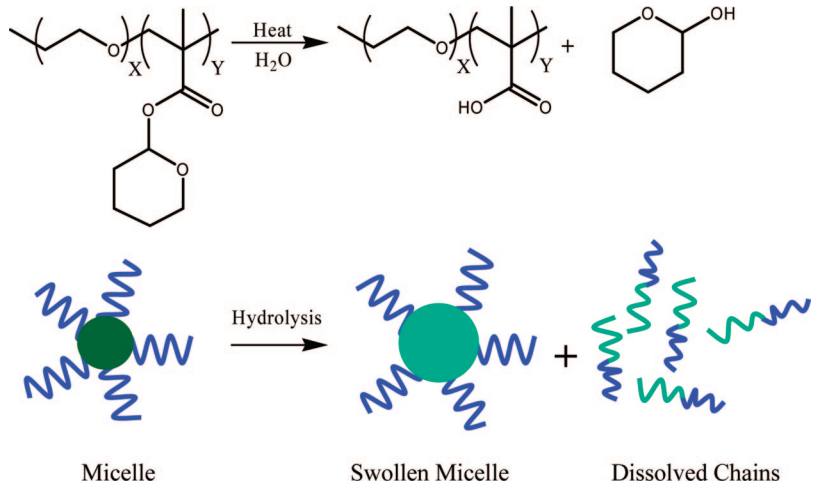
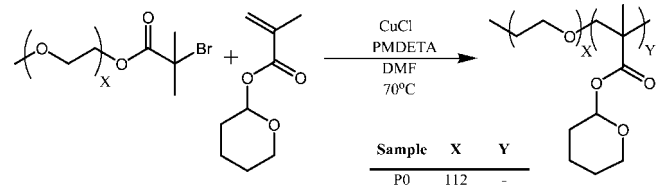


Figure 1. (a) Thermosensitive hydrolysis of the BCP composed of PEO and PTHPMA (PEO-*b*-PTHPMA). (b) Schematic illustration of the disruption of BCP micelles in aqueous solution as a result of the hydrolysis (swelling and dissolution).

b-PTHPMA micelles to be thermosensitive since the tetrahydropyranyl (THP) side groups are known to be thermal-labile. Indeed, THP moiety has been widely used as a protecting group in organic[10] and polymer synthesis[11] and can be removed through thermolytic cleavage in the solid state.[12] Therefore, as depicted in Figure 1, it was expected that the hydrolytic cleavage of THP groups from PEO-*b*-PTHPMA micelles in aqueous solution would be sensitive to temperature. Additionally, for the micelles, the cleavage of THP means destabilization (swelling and dissolution as will be discussed later), because the reaction converts the hydrophobic PTHPMA into water-soluble poly(methacrylic acid) (PMAA) and, consequently, shifts the hydrophilic−hydrophobic balance toward the destabilization of the micelles.[9] Compared to the aforementioned PEO-*b*-PNHPMAAm-lactate,[5,6] the present BCP design is not just about a different polymer, it is of interest for at least two reasons. First, the use of PTHPMA extends the hydrolysis-based approach to poly(meth)acrylates that do not have an LCST, such as polyacrylamides. Second, although the present study mainly dealt with the thermal effect, the micelles of PEO-*b*-PTHPMA are also pH-sensitive. PTHPMA has been used as a photoresist that, in the presence of a photoacid generator, can undergo acid-mediated cleavage reaction.[13] However, contrarily to the hydrolysis of lactate groups that is enhanced by basic pH,[6] the hydrolysis of THP is sensitive to acidic pH. This may make PTHPMA-based micelles more suitable as drug delivery nanocarriers that respond to relatively acidic tumor tissues (pH ∼ 6.8) and the endosomal and lysosomal compartments of cells (pH ∼ 5−6) with respect to the physiological pH of 7.4.[14] Additionally, pH-mediated dissolution of PTHPMA homopolymer nanoparticles prepared by emulsion methods were shown to be good for drug delivery applications because of, among other things, the low toxicity of PTHPMA.[15] More importantly, the first simultaneous investigation of the kinetics of three related events (hydrolysis, micellar disruption, and release) by using ^{1}H NMR, dynamic light scattering (DLS), and fluorescence spectroscopy provides new physical insight into the complicated processes.

Scheme 1. Synthetic Route to BCPs of PEO-*b*-PTHPMA of Various Compositions



Sample	X	Y
P0	112	-
P1	112	24
P2	112	54
P3	112	164
P4	45	22
P5	112	42

Experimental Section

1. Synthesis. *Materials.* Dichloromethane (DCM, 99%) was purified by distillation from calcium hydride. Tetrahydrofuran (THF, 99%) was purified by distillation from sodium with benzophenone. Tetrahydropyranyl methacrylate (THPMA, 99%) was provided by St-Jean-Photochemicals (Quebec, Canada) and passed through a column of basic alumina silica before use. 2-Bromoisobutyryl bromide, Nile Red (NR), copper chloride (Cu(I)Cl, 98%), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), and poly(ethylene glycol) methyl ether with number-average molecular weights of 2000 and 5000 g mol^{-1} were purchased from Aldrich and used without further purification.

Synthesis of Diblock Copolymers of Ethylene Oxide and THPMA (PEO-b-PTHPMA). As shown in Scheme 1, diblock copolymers were synthesized using atom transfer radical polymerization (ATRP). Bromine end-capped PEO macroinitiators with molecular weight of 2000 or 5000 g mol^{-1}, designated as PEO_{45}−Br or PEO_{112}−Br, were prepared following a literature method;[16] details are not repeated here. What follows is an example of ATRP of the THPMA monomer using a macroinitiator PEO_{112}−Br. THPMA (4.5 g, 26.4 mmol), PMDETA (64.9 mg, 0.37 mmol) and Cu(I)Cl (16.1 mg, 0.16 mmol) were added to a solution of PEO_{112}−Br (547 mg, 0.11 mmol) dissolved in dimethylformamide (3.4 g). The reactive mixture placed in a flask was degassed three times using the freeze−pump−thaw procedure. After 30 min of stirring at room temperature, it was immersed in a preheated oil bath at 70 °C for 2 h. Afterward, the

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mixture was passed through a neutral Al_2O_3 column with THF as the eluent to remove the catalyst. The solution was concentrated upon solvent evaporation under reduced pressure and then precipitated twice in cold ether (ice bath). The white diblock copolymer power (2.02 g, yield ~ 45%) was collected by filtration and dried in a vacuum oven. This reaction gave the sample of PEO_{112}-*b*-$PTHPMA_{164}$ (P1 in Scheme 1). ^{1}H NMR ($CDCl_3$), δ (ppm): 5.95 (broad, 1H, −OCHO−, THPMA), 3.90 (m, 2H, −OCHOCH_2−, THPMA), 3.65 (s, 4H, −CH_2CH_2O−, PEO), 1.60 (broad, 6H, (OCH$CH_2CH_2CH_2$−, THPMA), 1.25 (s, 3H, CH_3CCOO−, THPMA). M_n (^{1}H NMR) = 32500 g mol^{-1}, M_n (GPC) = 32800 g mol^{-1}, M_w/M_n = 1.20.

2. Preparation of Aqueous Micellar Solutions with or without Loaded NR. The samples of PEO-*b*-PTHPMA synthesized for this study are insoluble directly in water. Therefore, the conventional "dialysis" method of preparing aqueous micellar solutions was utilized. A sample was first dissolved in THF, which is a good solvent for both PEO and PTHPMA, and water was then added slowly to the THF solution to induce the formation of micelles with a hydrophobic PTHPMA core and a hydrophilic PEO shell; finally, THF was completely removed through dialysis of the micellar solution against water, resulting in an aqueous solution of PEO-*b*-PTHPMA micelles. The loading of the hydrophobic dye NR was easily achieved using the same method, only, in this case, both NR and a PEO-*b*-PTHPMA sample were dissolved in THF prior to the addition of water; while upon micelle formation when water was mixed with THF, an amount of NR could be solubilized by the hydrophobic PTHPMA core. The NR-loaded micelles in aqueous solution were prepared as follows. PEO_{112}-*b*-$PTHPMA_{164}$ (3.0 mg) was dissolved in dry THF (4.6 mL); the solution was then mixed with a THF solution of NR (25 μL, 0.2 mg mL^{-1}). Under vigorous stirring, 1.2 mL of water was added slowly (about 25 μL over every 30 s) to the THF solution of polymer and NR, which was followed by quick addition of another 1 mL of water at the end. The solution was stirred for 1 h at room temperature before being placed in a dialysis bag (Specrum, MW cutoff 3500) for dialysis against water for three days (water was frequently refreshed); unloaded NR, precipitated in water, was removed through microfiltration (0.45 μm cellulose acetate membrane). For all measurements at acidic pH, a 1 M HCl solution was added in the aqueous micellar solution, the pH change being measured with a pH meter (Orion 410A plus).

3. Characterizations. Unless otherwise stated, ^{1}H NMR spectra were obtained with a Bruker Spectrometer (300 MHz, AC 300). To monitor the hydrolysis reaction at elevated temperatures as a function of time, a Varian spectrometer (600 MHz, INOVA system) was used to record spectra at a time interval of 5 min, by inserting an NMR tube filled with the micellar solution into the preheated and thermostat probe. DLS experiments were performed on a Brookhaven goniometer (BI-200) equipped with a highly sensitive avalanche photodiode detector (Brookhaven, BI-APD), a digital correlator (Brookhaven, TurboCorr) that calculates the photon intensity autocorrelation function $g^2(t)$, a helium−neon laser (wavelength λ = 632.8 nm), and a thermostat sample holder. The hydrodynamic diameter (D_H) of micellar aggregates was obtained by a cumulant and CONTIN analysis, and the change in scattered light intensity was measured at 90°. UV−vis absorption and steady-state fluorescence emission spectra were recorded using a UV−vis (Varian Cary 50 Bio) and a fluorescence spectrophotometer (Varian Cary Eclipse), respectively. The excitation wavelength was 540 nm for NR (excitation and emission slit widths set at 5 nm, and the scan rate at 10 nm s^{-1}); a single cell Peltier was used for controlled-temperature measurements. Scanning electron microscopy (SEM) observations were carried out using a Hitachi S-4700 Emission Gun Scanning Electron Microscope. Samples for SEM were prepared by deposing one drop of diluted micellar solution on a silicon wafer, followed by drying at room temperature. Gel permeation chromatography (GPC) measurements were conducted on a Waters system equipped with a refractive index detector (RI 410), a photodiode array detector (PDA 996) and one column (Styragel 5HE, 7.8 mm × 300 mm). THF was used as the eluent (elution rate, 1 mL min^{-1}), and polystyrene standards were used for calibration. Thermal properties of the polymers were investigated using a Perkin-Elmer DSC-7 differential scanning calorimeter with a heating or cooling rate of 10 °C min^{-1}.

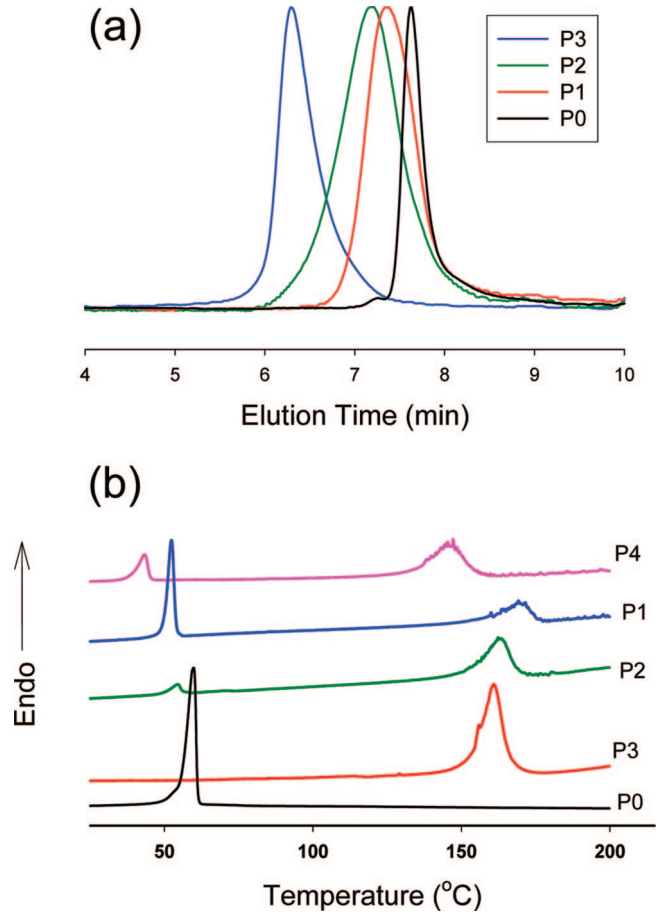

Figure 2. (a) GPC and (b) DSC curves of the PEO-*b*-PTHPMA samples of various compositions as indicated in Scheme 1.

Results and Discussion

1. Syntheses and Characterization. A series of PEO-*b*-PTHPMA diblock copolymers with various relative block lengths were obtained, as indicated in Scheme 1. Using the known molecular weight of PEO, the number of THPMA units was estimated on the basis of the BCP composition as revealed by ^{1}H NMR spectra, comparing the resonance signals of the two blocks (the peaks at 5.95 and 3.65 ppm for PTHPMA and PEO, respectively). Before studying micelles in aqueous solution, BCP samples were characterized by means of a number of techniques. An example of the results is given in Figure 2.

Figure 2a presents the GPC curves of PEO_{112}−Br and three BCP samples obtained with the same macroinitiator. The controlled growth of the PTHPMA block can be noticed from the single peak shifting to shorter retention times, whereas the polydispersity indexes (M_w/M_n) remain low from ~1.06 for the macroinitiator to about 1.20 for PEO-*b*-PTHPMA. Figure 2b shows the DSC heating curves for the same series of samples, together with PEO_{45}-*b*-$PTHPMA_{22}$. The thermolytic cleavage of THP groups for the copolymers in the solid state occurs at temperatures above 140 °C, indicated by the appearance of an endothermic peak. In addition to the observation that the cleavage heat is proportional to the content of THPMA groups, being highest for PEO_{112}-*b*-$PTHPMA_{164}$, it is interesting to note the mutual influence of the two blocks on their properties. Indeed, compared to the PEO macroinitiator, the melting endotherm of crystalline PEO of the BCP appears at a lower temperature and

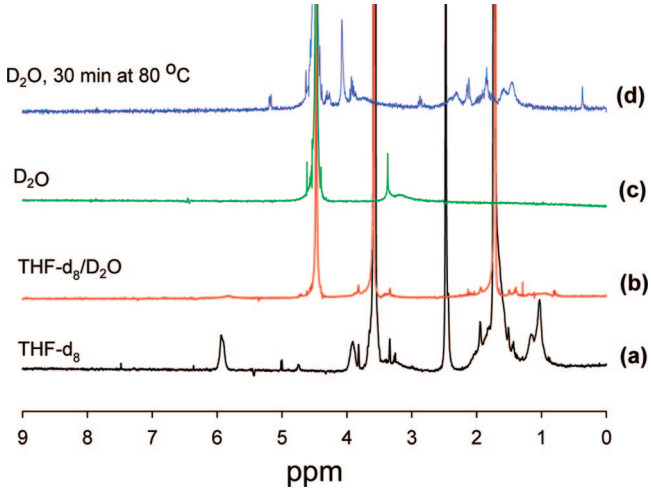

Figure 3. ^{1}H NMR spectra of PEO_{112}-*b*-$PTHPMA_{164}$ (a) dissolved in THF-d_8, (b) upon addition of D_2O in THF solution inducing the formation of micelles, (c) aqueous micellar solution obtained by dialysis against D_2O, and (d) aqueous micellar solution heated to 80 °C for 30 min.

becomes less and less prominent upon increasing the content of PTHPMA (indiscernible for PEO_{112}-*b*-$PTHPMA_{164}$), while, on the other hand, the crystalline PEO block apparently increases the thermolytic cleavage temperature of THP groups, which is mostly clear for PEO_{112}-*b*-$PTHPMA_{24}$. Comparing PEO_{45}-*b*-$PTHPMA_{22}$ with PEO_{112}-*b*-$PTHPMA_{54}$, it is seen that, at a similar relative block lengths ratio (PEO/PTHPMA ~ 2:1), shorter block lengths lower the melting temperature of crystalline PEO as well as the thermolytic cleavage temperature of the side groups of PTHPMA.

2. Thermosensitive Micelles in Aqueous Solution. Unless otherwise stated, the experiments described in this paper used a sample of PEO_{112}-*b*-$PTHPMA_{164}$. Figure 3 compares ^{1}H NMR spectra of the BCP sample in deuterated THF (THF-d_8), upon addition of heavy water (D_2O), after dialysis against heavy water, and after heating of the aqueous micellar solution at 80 °C. As indicated in the figure, in THF-d_8, the characteristic resonance signals of both PEO (3.55 ppm, partly overlapped by a signal of THF-d_8) and PTHPMA (5.93 ppm) are visible (spectrum a). As D_2O is added into the solution, inducing the formation of micelles, the peak of PEO remains, while the signals of PTHPMA have almost completely disappeared, indicating that PTHPMA chains are aggregated into the compact micelle core and are no longer solvated (spectrum b). The situation is the same after THF is removed through dialysis against water, the proton signal of THF-d_8 (3.58 and 1.73 ppm) being gone (spectrum c). Spectrum d was recorded after heating the aqueous micellar solution to 80 °C for 30 min; the disruption of the micelles is revealed by the spectral changes. Indeed, the characteristic peak of tetrahydropyran-2-ol (5.33 ppm) appears, which is formed after the hydrolytic removal of THP groups from the BCP (Figure 1). Other resonance signals of PTHPMA or from the resulting PMAA also appear, indicating either dissolved polymer chains or hydrated BCP micelle cores as a result of the hydrolysis of PTHPMA at 80 °C. This preliminary result confirms that, in aqueous solution, the hydrolysis of PTHPMA can be thermally activated at temperatures much lower than what is required for the thermolytic cleavage in the solid state, and induce the destabilization of the micelles of PEO-*b*-PTHPMA.

The destabilization of the micelles in aqueous solution at 80 °C was further monitored using DLS. Figure 4a shows the distribution of hydrodynamic diameters of micellar solution before and after 60 min heating. It is seen that, prior to the temperature increase, the micelles are uniform in size, and their average hydrodynamic diameter is centered at 70 nm. After 60 min heating, we can notice that the micelle peaks become larger, shift to higher diameter (~ 85 nm), and decrease in intensity, while aggregate peaks above 200 nm can be observed. The accompanying change in the scattered intensity by the micellar solution is shown in Figure 4b. Despite some fluctuations of the data, the scattering intensity decreases continuously after an increase at the beginning of the process. The early increase in scattering intensity (about the first 10−20 min) was observed in all experiments carried out at elevated temperatures. Combining these results we can suggest that, prior to dissolution or disintegration, micelles first undergo a swelling that induces an increase of the scattered intensity. As the hydrolytic cleavage of THP groups takes place, leading to the formation of methacrylic acid (MAA) groups, the increased hydrophilicity of the micelle core can result in more absorption of water molecules. The subsequent decrease in the scattered intensity implies that a majority of swollen micelles start to fall apart and be dissolved. Thus, after 60 min heating, swollen micelles and large aggregates apparently coexist in solution, and the presence of large aggregates prevents detection by DLS of molecularly dissolved PEO-*b*-PMAA BCP. It was also observed that the hydrolysis only resulted in a small decrease in pH of the micellar solution (about 0.2), presumably because of the very low concentration of the PTHPMA block in the solution (about 0.37 mg mL^{-1}) and an incomplete hydrolysis reaction under the used conditions. Now, how does one explain the formation of the large aggregates with the concomitant decrease in scattered light intensity? On one hand, the complexation of dissolved polymer chains is unlikely to occur for the following reason. Although complete hydrolysis of PEO-*b*-PTHPMA gives rise to PEO-*b*-PMAA, at pH 7, the ionized carboxylic acid groups should prevent the polymer chain complexation driven by H-bonding between PEO and PMAA chains.[17] On the other hand, even for polymer micelles in equilibrium, chain exchange can take place through two mechanisms: insertion and expulsion of single chains and merging and splitting of micelles.[17,18] It is conceivable that this dynamic nature would be enhanced in the present system, which undergoes a continuous structural change and perturbation. As the hydrolysis goes on, the micelle core becomes increasingly hydrophilic and absorbs more and more water, leading to the swelling of the micelle. But the disruption process is not simply the dissolution of micelles into individual chains; rather it is a constant structural and morphological evolution over time that may involve micelles swelling, their merging upon encounter, splitting, and polymer chains dissolution. In other words, the large aggregates could be

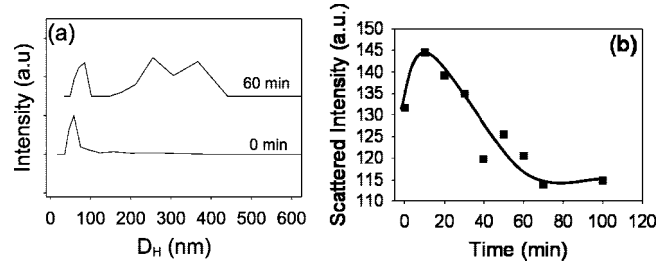

Figure 4. Aqueous micellar solution of PEO_{112}-*b*-$PTHPMA_{164}$ heated to 80 °C: (a) evolution of the distribution of hydrodynamic diameters of micellar aggregates before and after 60 min, and (b) changes in the scattered intensity measured at 90°.

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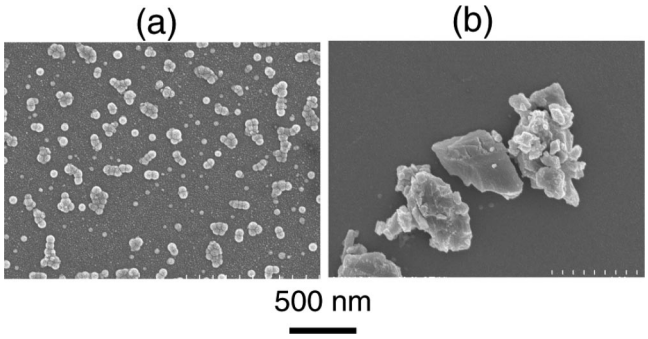

Figure 5. SEM images of (a) micelles of PEO_{112}-*b*-$PTHPMA_{164}$ observed by casting the micellar solution at room temperature, and (b) large aggregates formed after heating the micellar solution to 80 °C for 100 min.

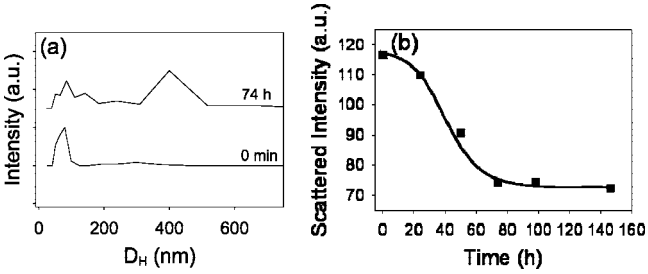

Figure 6. Aqueous micellar solution of PEO_{112}-*b*-$PTHPMA_{164}$ heated to 37 °C (pH 7): (a) evolution of the distribution of hydrodynamic diameters of micellar aggregates before and after 74 h, and (b) changes in the scattered intensity measured at 90°.

formed by merging swollen and hydrated micelles through chain rearrangement in the course of the evolution process. A thorough understanding of the complicated dynamic processes is the subject of future investigations.

The dissolution of the micelles is consistent with the visual observation that the solution turned clearer over time. SEM observations also confirm the analysis. Figure 5 shows images obtained with the micellar solution before and after heating to 80 °C for 100 min, deposited on a silicon wafer and dried. Before heating, dried micelles with an average diameter of about 62 nm are visible, and they are quite uniform in size. After the hydrolysis at 80 °C, large aggregates are observed. It is easy to imagine that large hydrated micelles are easier to collapse and coalesce during the film drying, giving rise to the observed large aggregates in the solid state. Since the hydrolysis of PEO-*b*-PTHPMA gives rise to PEO-*b*-PMAA, it should be mentioned that the self-complexation of the latter due to hydrogen bonding between PEO and PMAA would not happen in the present case because of the ionization of MAA groups under the used conditions (pH 7).[17]

Interestingly, the thermosensitivity of the hydrolysis of PTHPMA makes the micelles unstable at the body temperature, suggesting the relevance of PTHPMA-based BCP micelles for drug delivery applications. Figure 6 shows the results obtained at 37 °C (pH=7). From the changes in the size distributions and scattering intensity, it appears that the destabilization of the micelles at 37 °C follows the same path as that at 80 °C, with the only difference that the process is much slower. Because of the slow kinetics, no initial increase of the scattering intensity was captured.

3. Simultaneous Investigation of Hydrolysis, Destabilization, and Release. For an aqueous solution of PEO-*b*-PTHPMA micelles, as the thermal hydrolysis of THP groups progresses, the initially hydrophobic PTHPMA core becomes increasingly hydrophilic as the micelle core, being made up with a random copolymer of THPMA and MAA, contains more and more carboxylic acid groups. It is easy to imagine that the micelle core, absorbing an increasing amount of water, swells and eventually dissolves into unimolecular chains. If the BCP micelle is loaded with a hydrophobic compound solubilized by the PTHPMA core, it would be interesting, and challenging, to investigate simultaneously the three related events, i.e., hydrolysis of THP groups, micelle disruption, and release of the loaded guest into the aqueous medium. We made an attempt using NR-loaded BCP micelles. The release of NR can readily be monitored through fluorescence measurements since the emission of NR is quenched by water in which it is insoluble.[14a] The experiment was conducted at 80 °C to have a faster hydrolysis, while using (1) ^{1}H NMR to observe the cleavage of THP groups from BCP micelles in aqueous solution, (2) DLS to monitor the micelles disruption, and (3) change in fluorescence emission of NR to follow its release in solution.

The most challenging is to observe the kinetics of the hydrolytic cleavage of THP groups from the micelles. To do this, micelles in heavy water (D_2O) were prepared using the same method as described in the Experimental Section, while the removal of THF was carried out by dialysis against D_2O. With the more sensitive 600 MHz NMR spectrometer, the possibility to obtain a spectrum with a short acquisition time (79 s in the present study) allows the hydrolysis kinetics to be monitored by recording spectra at a time interval of 5 min. Figure 7a shows examples of the ^{1}H NMR spectra of the micellar solution before heating (spectrum a), after 12, 42, and 62 min at 80 °C, respectively. Despite the high noise level and some remained THF traces in the solution, the signal at 5.33 ppm, arising from cleaved tetrahydropyran-2-ol molecules, increases its intensity in time, indicating the progress of the hydrolysis reaction. In parallel, as the hydrolytic cleavage goes on, the resonance signals of the remaining PTHPMA and resulting PMAA in other regions become more and more prominent, indicating an increased hydration of the micelle cores. The two other events occurring in the same time can be investigated more easily. Figure 7b shows the change in the distribution of hydrodynamic diameters of micellar aggregates in the solution upon heating to 80 °C. The micellar disruption via swelling and disintegration or dissolution as discussed above is clearly visible. It is worth noting that, prior to the hydrolysis at elevated temperature, micelles loaded with NR (average hydrodynamic diameter $\sim$ 89 nm) do not display the same size distribution as the micelles without loaded dye molecules (Figure 4a), which is not unusual since the encapsulation is known to affect the micellar aggregates. Figure 7c shows the concomitant change in fluorescence emission of NR as a function of time at 80 °C. Not only does the intensity peaked at 603 nm decrease, but the emission maximum also displays a significant red-shift (by about 32 nm after 100 min). This is characteristic of NR's quenching by water.[14a] Of course, the quenching can occur either with NR molecules released into water due to the dissolution of polymer micelles or with NR molecules retained inside the swelling micelles upon absorption of water.

The simultaneous characterizations make it possible to compare the kinetics of the three events, providing information on the relationship between them. Figure 8 shows the plots of three normalized quantities versus hydrolysis time at 80 °C for the micellar solution. These are (1) the integral of the 5.33 ppm peak that is indicative of the amount of cleaved THP groups, (2) the light scattering intensity related to the overall disruption of micelles, and (3) the decrease in fluorescence intensity revealing

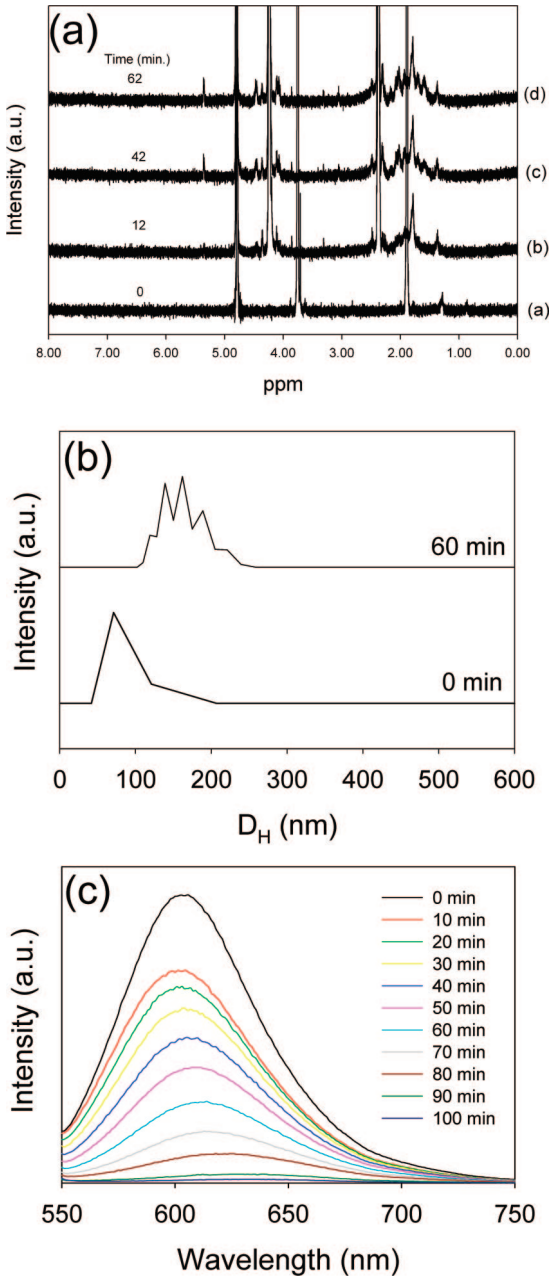

Figure 7. Simultaneous characterizations of the aqueous micellar solution of PEO_{112}-*b*-$PTHPMA_{164}$ heated to 80 °C by combining the use of three techniques: (a) ^1H spectra showing the hydrolytic cleavage of THP groups over time, (b) DLS results showing the evolution of the size distribution of the aggregates upon micellar disruption, and (c) fluorescence emission spectra of NR loaded in the micelles ($\lambda_{ex} = 540$ nm) showing its release into an aqueous medium.

the contact of loaded NR molecules with water. We feel that the decrease in scattering intensity better describes the kinetics of the structural and morphological changes of polymer micelles in solution than the apparent changes in the hydrodynamic diameter. Similar to the case of micelles without NR loading (Figure 4), the early time increase in light scattering is likely to be caused by swelling of the micelles without dissolution. As the swelling develops over time, some micelles can be dissolved while others absorb more water inside, leading to the observed decrease in light scattering. Inspection of Figure 8 shows that, within experimental error, the three events basically follow the same kinetics, indicating that they are closely related to each other. This is not illogical. Once the hydrolytic cleavage of THP groups takes place in the aqueous solution, the micelle core

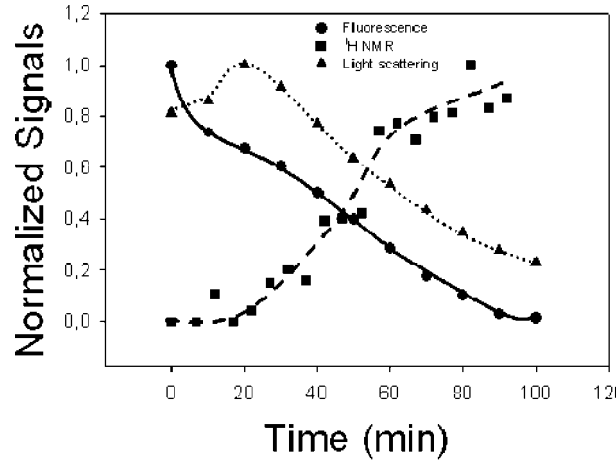

Figure 8. Comparison of the kinetics of the three events occurring in a micellar solution of PEO_{112}-*b*-$PTHPMA_{164}$ at 80 °C: (1) the hydrolytic cleavage of THP groups indicated by the change in the normalized integral of the resonance signal at 5.33 ppm, (2) the resulting micellar disruption related to the decrease in the normalized light scattering intensity by the solution, and (3) the concomitant release of loaded NR from the micelles into the aqueous medium as revealed by the change in the normalized fluorescence intensity at the maximum emission wavelength.

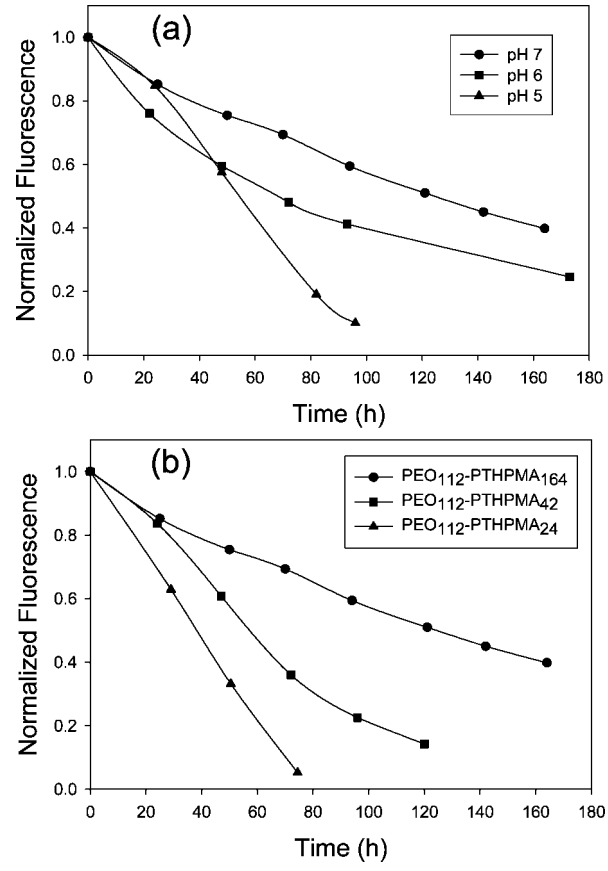

Figure 9. (a) Normalized fluorescence intensity of NR vs time for a micellar solution of PEO_{112}-*b*-$PTHPMA_{164}$ at 37 °C for three pH values (5, 6, and 7). (b) Normalized fluorescence intensity of NR vs time for micellar solutions at pH 7 prepared with three copolymer samples having the same PEO block but different lengths for the PTHPMA block.

absorbs more water molecules. This triggers micelle swelling, while the encounter of NR molecules with water leads to the quenching of their fluorescence emission. That the fluorescence quenching of NR follows the same kinetics as the hydrolysis

reaction and the decrease in scattered light intensity also suggests that the release of the chromophore into an aqueous media is mostly determined by the micellar swelling and dissolution, while the observed large hydrated aggregates should have little effect on the release process.

4. Effects of pH and BCP Composition. As mentioned earlier, the hydrolysis of PTHPMA block can also be enhanced by acidic pH, in contrast to lactate-based polymers whose hydrolysis is favored by basic pH.[6] Using aqueous solutions of micelles formed by PEO_{112}-*b*-$PTHPMA_{164}$ loaded with NR, the effect of pH on the micelles disruption and release was investigated by monitoring the fluorescence emission of NR. Figure 9a compares the plots of normalized fluorescence of NR versus time for the same micellar solution thermostatted at 37 °C under three different pH values. Indeed, as pH is decreased from 7 to 6 and to 5, the rate of fluorescence quenching increases continuously, indicating faster micelle disruption at acidic pH.

Another parameter that can affect the micelles disruption and the release of loaded molecules is the BCP composition. This was investigated by comparing the release rate of NR loaded in micelles of three samples with different relative lengths of the two blocks, PEO_{112}-*b*-$PTHPMA_{164}$, PEO_{112}-*b*-$PTHPMA_{42}$, and PEO_{112}-*b*-$PTHPMA_{24}$ (P3, P5, and P1 in Scheme 1), all measurements being carried out at fixed pH = 7 and $T = 37$ °C. As can be seen from the results in Figure 9b, with the same hydrophilic corona (the same PEO_{112} block), the rate of NR fluorescence quenching becomes faster as the hydrophobic core of PTHPMA becomes smaller as a result of the shorter PTHPMA block. This is understandable. With a smaller micelle core, water molecules are more accessible to PTHPMA (and loaded NR). This not only may favor the hydrolysis reaction, but also, as the hydrolysis takes place, can shift the hydrophilic−hydrophobic balance more quickly toward the micellar destabilization, leading to a faster micelle disruption and thus quenching of NR fluorescence.

Conclusions

We synthesized a new amphiphilic diblock copolymer composed of PEO and PTHPMA, and investigated the thermal sensitivity of its micelle in aqueous solution. In contrast to other thermosensitive polymer micelles usually related to the LCST of one block, the thermal sensitivity of the present system stems from the hydrolytic cleavage of the thermally labile THP groups. The hydrolysis of THP transforms the initially amphiphilic PEO-*b*-PTHPMA into double-hydrophilic PEO-*b*-PMAA, which destabilizes the micelles. The hydrolysis of PEO-*b*-PTHPMA micelles can occur slowly under physiological conditions (37 °C and pH = 7), while the reaction rate becomes faster at elevated temperatures. For the first time, we were able to use ^{1}H NMR, DLS, and fluorescence spectroscopy to simultaneously monitor the removal of THP groups, the disruption of micelles, and the release into aqueous medium of loaded NR molecules. The results suggest that the three seemingly distinct events are closely related to each other, and follow basically the same kinetics. The disruption of micelles as a result of the hydrolysis proceeds mainly through a process involving swelling, disintegration, and dissolution of the micelles in aqueous solution (depicted in Figure 1). Moreover, the rate of micelles disruption and thus the rate of release of NR are also enhanced by acidic pH or by using micelles with a smaller PTHPMA core.

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