Double-Hydrophilic Block Copolymer for Encapsulation and Two-Way pH Change-Induced Release of Metalloporphyrins

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Received 18 October 2005; accepted 15 December 2005
DOI: 10.1002/pola.21287
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A double hydrophilic block copolymer composed of poly(acrylic acid) (PAA) and poly(4-vinyl pyridine) (P4VP) was obtained through hydrolysis of diblock copolymer of poly(*tert*-butyl acrylate) (P*t*BA) and P4VP synthesized using atom transfer radical polymerization. Water-soluble micelles with PAA core and P4VP corona were observed at low (acidic) pH, while micelles with P4VP core and PAA corona were formed at high (basic) pH. Two metalloporphyrins, zinc tetraphenylporphyrin (ZnTPP) and cobalt tetraphenylporphyrin (CoTPP), were used as model compounds to investigate the encapsulation of hydrophobic molecules by both types of micelles. UV–vis spectroscopic measurements indicate that micelles with P4VP core are able to entrap more ZnTPP and CoTPP as a result of the axial coordination between the transition metals and the pyridine groups. The study found that metalloporphyrins encapsulated by the micelles with PAA core could be released on pH increase, while those entrapped by the micelles with P4VP core could be released on pH decrease. This behavior originates from the two-way pH change-induced disruption of PAA-*b*-P4VP micelles.
 J Polym Sci Part A: Polym Chem 44: 1734–1744, 2006
Keywords: amphiphihilic block copolymer; encapsulation and release; metalloporphyrin; pH-sensitive micelles

INTRODUCTION

Block copolymer micellar aggregates that respond to stimuli such as changes in pH[1] and temperature,[2] exposure to an oxidation reaction,[3] ultrasound[4] and light irradiation[5] have been investigated in view of their potential use as nanocarriers for biologically active substances, such as drugs. Among the many types of stimuli-responsive block copolymer (BCP) systems, double-hydrophilic BCPs, first reported by Armes and coworkers,[6] have the unique feature of being able to form two types of micelles in aqueous solution. When a double hydrophilic BCP is composed of a polybase and a polyacid, two micelles can be formed at ambient temperature just by changing the pH.[6c,7] At high pH (basic medium), the water-soluble polyacid block (an anionic polyelectrolyte) forms the corona of the micelle, while the less hydrophilic polybase block forms the core; at low pH (acidic medium), the micellization occurs with the polybase block (an cationic polyelectrolyte) forming the corona and the polyacid block forming the core. More recently, pH-sensitive flip-flop vesicles of a diblock polypeptide were also reported based on the same principle.[8]

The double-hydrophilic BCPs prepared by Armes group[6] and others[7] have generally a tertiary amine methacrylate-based polybase block,

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Journal of Polymer Science: Part A: Polymer Chemistry, Vol. 44, 1734–1744 (2006)

typically poly [2-(diethylamine)ethyle methacrylate] (PDEA). In the present study, we have synthesized a simple double-hydrophilic BCP composed of poly(acrylic acid) (PAA) as the polyacid block and poly(4-vinyl pyridine) (P4VP) as the polybase block. We mention that PAA and P4VP have been used to prepare a pH-sensitive PAA-*b*-polystyrene (PS)-*b*-P4VP triblock copolymer by Liu and Eisenberg,[1d] which forms vesicles with either PAA (at high pH) or P4VP (at low pH) on the outside of the vesicles. In this paper, we demonstrate that the double-hydrophilic PAA-*b*-P4VP not only forms two types of micelles in aqueous solution at ambient temperature upon changes in pH, but also displays some attractive features that are of interest for polymer nanocarriers. Indeed, in contrast with tertiary amines, the pyridyl groups of P4VP can promote self-assembly through H-bonding and coordination interactions. Knowing that porphyrins are among the most studied photosensitizers for photodynamic therapy of cancers,[9] we chose two metalloporphyrins as a model system and investigated their encapsulation by and release from the two types of PAA-*b*-P4VP micelles. We found that metalloporphyrins entrapped in low pH aqueous solution by micelles with PAA core and P4VP corona can be released through increase in pH, while the guests loaded in high pH aqueous solution by micelles with P4VP core and PAA corona can be released on pH decrease. Moreover, micelles with P4VP core were found to entrap a significantly larger amount of metalloporphyrins than micelles with PAA core, which appears to come from the axial coordination between the transition metals and the pyridine groups, even though possible contribution from π–π stacking interactions cannot be ruled out. To the best of our knowledge, the investigation of encapsulation of hydrophobic compounds by such double-hydrophilic BCP micelles and the demonstration of their two-way pH change-induced release are new.

EXPERIMENTAL

Synthesis

The double-hydrophilic block copolymer (BCP) of PAA-*b*-P4VP was prepared by two steps. First, diblock copolymer of poly(*tert*-butyl acrylate) (P*t*BA)-*b*-P4VP was synthesized using atom transfer radical polymerization (ATRP). Then, *t*BA groups were hydrolyzed resulting in PAA-*b*-P4VP. More details about the synthesis are given here.

Materials

All reagents used in the syntheses and the two metalloporphyrins used in this study, namely, zinc-tetraphenylporphyrin (ZnTPP) and cobalt-tetraphenylporphyrin (CoTPP) were purchased from Aldrich. zinc-tetraphenylporphyrin (ZnTPP) and cobalt-tetraphenylporphyrin (CoTPP), were purchased from Aldrich. 4-Vinylpyridine (4VP) was dried over CaH_2 and distilled before use. *Tert*-butyl acrylate (*t*BA) was distilled with 2,6-di(*tert*-butyl)-4-methyl-phenol as inhibitor before use. CuCl (99.98%) was stirred in glacial acetic acid, washed with methanol and dried in vacuum. *N, N,N′,N′, N″*-Pentamethyldiethylenetriamine (PMDETA) was used without further purification. 1-Phenylethyl chloride (PECl) and tris[2-(dimethtylamino)ethyl]-amine (Me_6-TREN) were prepared according to the methods reported in the literature.[10,11]

*Synthesis of Chlorine-Terminated P*t*BA Macroinitiator*

Matyjaszewski and coworkers showed that the use of chlorine as the halogen is one of the factors that favor the polymerization of 4VP through ATRP by reducing the interfering binding of 4VP or P4VP with the metal catalysts.[12] For this reason, chlorine-terminated P*t*BA macroinitiator, P*t*BA-Cl, was first synthesized by polymerizing *t*BA, using PECl as the initiator and CuCl/PMDETA as the catalyst. In a typical run, *t*BA (11.2 g), PECl (123.0 mg), CuCl (86.5 mg), and PMDETA (151.6 mg) were dissolved in acetone (10 mL). The mixture, placed in a dried round-bottom flask with a magnetic stirring bar, was degassed by three freeze-pump-thaw cycles. The flask was sealed under vacuum, and then immersed in an oil bath at 60 °C. After 10 h of polymerization, the reaction mixture was diluted in THF and passed through a neutral alumina column to remove the metal-containing residues. The macroinitiator was further purified by precipitation in a water/methanol mixture (1/3 v/v), and dried under vacuum. Yield: 85%. M_n = 13,800 and M_w/M_n = 1.27 (GPC). Another macroinitiator of lower molecular weight, M_n = 6700 and M_w/M_n = 1.29, was also prepared by varying the relative amounts of initiator and monomer in the reaction (similar yield).

*Synthesis of P*t*BA-b-P4VP*

To obtain the diblock copolymer of P*t*BA-*b*-P4VP, 4VP was polymerized using P*t*BA-Cl macroini-

Table 1. Characteristics of P*t*BA Macroinitiators and P*t*BA-*b*-P4VP Block Copolymers

	Composition (NMR)	M_n (GPC)	M_w/M_n (GPC)	M_n (NMR)
P*t*BA-*b*-P4VP	$PtBA_{108}$-*b*-$P4VP_{68}$	17,000	1.34	21,000
	$PtBA_{108}$-*b*-$P4VP_8$	14,000	1.41	14,600
	$PtBA_{52}$-*b*-$P4VP_{97}$	5,300	1.40	17,000
P*t*BA-Cl		13,800	1.27	
		6,700	1.29	

tiator and CuCl/Me_6-TREN as the catalyst. The Me_6-TREN ligand was also found to help polymerization of 4VP through ATRP.[12] In one reaction, P*t*BA-Cl with M_n = 13,800 (1.2 g), 4VP (1.1 g), CuCl (22 mg) and Me_6-TREN (52 mg) were added to a round-bottom flask and dissolved in DMF (3 mL). After three freeze-pump-thaw cycles, the flask was sealed and placed in an oil bath (60 °C) for polymerization. The reaction was stopped after 24 h (^{1}H NMR data indicating a conversion of 4VP monomer of about 70%). An excess volume of THF was added in the mixture for dilution, and the solution was then passed through a neutral alumina column to remove the catalyst. The block copolymer was further purified by precipitation in a mixture of water and methanol (1/3 v/v). This reaction gave rise to a diblock copolymer with M_n = 17,000 and M_w/M_n = 1.34 (GPC). Yield: 65%. By reducing the amount of 4VP in the reaction, a sample with a much shorter P4VP block was also obtained. Table 1 summarizes the characteristics of three P*t*BA-*b*-P4VP diblock copolymer samples prepared using two different macroinitiators. The molecular weights and polydispersity were obtained from gel permeation chromatography (GPC) measurement using polystyrene standards and THF as eluent. It can be noticed that the GPC results for these diblock copolymers are different from those of NMR (using M_n of P*t*BA determined by GPC). This is likely caused by the P4VP block whose pyridine groups may have strong interaction with the GPC columns, with THF as eluent.[13] The compositions of the three samples of P*t*BA-*b*-P4VP were thus estimated using the NMR data.

Preparation of PAA-b-4VP

The hydrolysis of *t*BA groups of P*t*BA-*b*-P4VP gives rise to the double-hydrophilic diblock copolymer PAA-*b*-P4VP. Typically, the hydrolysis reaction was carried out by adding concentrated HCl (12 M, 1 mL) in a dioxane solution of P*t*BA-*b*-P4VP (0.5 g of polymer in 20 mL of dioxane) under stirring and heated to 85 °C. After 10 h of reaction, the mixture was added dropwise in methanol to precipitate the polymer. After hydrolysis, infrared spectra of the block copolymers (not shown) displayed the characteristic absorption bands of intermolecular carboxylic acid dimers (the band appearing at about 1700 cm^{-1} and the broadband at around 3000 cm^{-1}). Also, pyridine groups of the P4VP block were protonated as revealed by the appearance of a new absorption band around 1635 cm^{-1}.[14] Once dried, PAA-*b*-P4VP was found to be soluble in water in either acidic or basic pH (actually forming micelles as will be shown later), but insoluble in most organic solvents except in DMSO, in which a slow dissolution was observed. It was assumed that the hydrolysis reaction did not change the composition of the diblock copolymer, that is the number of AA units is the same as that of *t*BA. Used in this study was the sample PAA_{108}-*b*-$P4VP_{68}$.

Characterizations

Diblock copolymers before and after hydrolysis were characterized using a number of techniques. ^{1}H NMR spectra were recorded on a Brucker Spectrometer (300 MHz, AC 300). CCl_3D and D_2O were used as solvents for the NMR measurements; 1 M DCl and 1 M NaOD were used to adjust pH in D_2O. Molecular weights and polydispersity of the samples were measured using a Waters GPC system equipped with a refractive index and a photodiode array detector; using polystyrene standards and THF as eluent (elution rate, 0.5 mL/min). Infrared and UV–vis spectra were obtained using a Bomen MB-100 FTIR spectrometer (CaF_2 crystal used for casting the films) and a Hewlett–Packard 8452A diode array spectrophotometer, respectively. Polymer micelles formed in aqueous solutions were examined using a Hitachi H-7500 transmission electron microscope (TEM) operating at 80 kV. The

samples for TEM observations were prepared by casting one drop of the micellar solution on a carbon-coated copper grid, followed by drying at room temperature.

Preparation of PAA-*b*-P4VP Micelles and Loading of Metalloporphyrins

Two types of micelles were obtained through simple dissolution of the block copolymer in aqueous solutions with predetermined pH values at room temperature. Unless otherwise stated, micelles with PAA core and P4VP corona were prepared at pH = 2, and micelles with P4VP core and PAA corona were obtained at pH = 10. To load the hydrophobic metalloporphyrins, either ZnTPP or CoTPP was first dissolved in THF; the solution was then added dropwise to the micellar aqueous solution under rigorous stirring. The mixture was further stirred for 24 h at room temperature to allow for complete removal of THF and concomitant partial evaporation of water (about 20%). Afterward, the micellar solution was filtered through a 0.45-μm cellulose filter to remove the precipitate of nonencapsulated metalloporphyrin. The dissolution of ZnTPP and CoTPP by the polymer micelles was characterized by UV–vis spectroscopy. Encapsulation was also performed using commercially available buffer solutions at acidic and basic pH; similar results were obtained.

UV–vis spectroscopic measurements were also used to monitor the release of loaded ZnTPP or CoTPP in response to changes in pH. For these experiments, pH of the micellar solution was either increased by adding 1 M NaOH or decreased by adding 1 M HCl solution. The pH change was measured using a pH meter (Orion 410Aplus). Typically, after each addition of either HCl or NaOH, the solution was kept under stirring for 30 min for equilibrium, before an aliquot of the solution was placed in a standard cuvette for the UV–vis spectroscopic measurement.

RESULTS AND DISCUSSION

Formation of Micelles

The sample PAA_{108}-*b*-$P4VP_{68}$ was used in this study. The formation of the two types of micelles in aqueous solution at ambient temperature was first investigated. At low pH, as protonated P4VP is a cationic polyelectrolyte and more water-soluble than PAA, micelles are expected to form with PAA core and P4VP corona. At high pH, as PAA is an anionic polyelectrolyte and more water-soluble than P4VP, micelles with P4VP core and PAA corona could thus be formed. This was indeed confirmed by ^{1}H NMR spectra (Fig. 1) and TEM observations (Fig. 2). Compared in Figure 1 are ^{1}H NMR spectra of P*t*BA-*b*-P4VP in chloroform (before hydrolysis) and the resulting PAA-*b*-P4VP (after hydrolysis) in aqueous solution at pH = 2 and pH = 10. The spectrum of P*t*BA-*b*-P4VP dissolved in chloroform displays the signature peaks of the 4 H of the pyridine ring in the 6–9 ppm region as well as the 9 H of the *tert*-butyl group at about 1.4 ppm. The composition of the diblock copolymer was estimated by comparing the areas of these peaks. As for PAA-*b*-P4VP in water, at pH = 10 the peaks of pyridine are absent, indicating the aggregation of the P4VP block, while the peaks of PAA in the 1.5–2.2 ppm region are visible, indicating the dissolution of the PAA block forming the corona of the core-shell micelle as schematized in the Figure. At pH = 2, the spectral changes are consistent with the formation of micelles under strong acidic condition. Indeed, the peaks of pyridine reappear indicating the dissolution of the P4VP block (the significant peak shifts should be caused by the protonation of pyridine), while the aggregation of the PAA block is indicated by the replacement of their peaks around 2 ppm by those of P4VP in the similar region.

As shown in Figure 2, two types of micelles formed at pH = 10 and pH = 2 were observed by TEM (no staining was used). Note that at pH = 10, we were unable to obtain TEM images with higher concentration of micelles. This is why a much smaller magnification was used to include more micelles in the image to show the variation in the size of micelles. The average size, estimated from the TEM images using a number of micelles, is about 12 nm for the micelles with PAA core and about 20 nm for micelles with P4VP core. In an effort to understand the size difference between the two types of micelles, pyrene was equilibrated with the micellar solutions and its fluorescence emission was measured (spectra not shown). Being a hydrophobic probe, pyrene preferentially partitions into the hydrophobic core of micelle; and it is well known that the intensity ratio of the first to the third emission, I_1/I_3, can be used to assess the polarity of the medium in which pyrene is located,

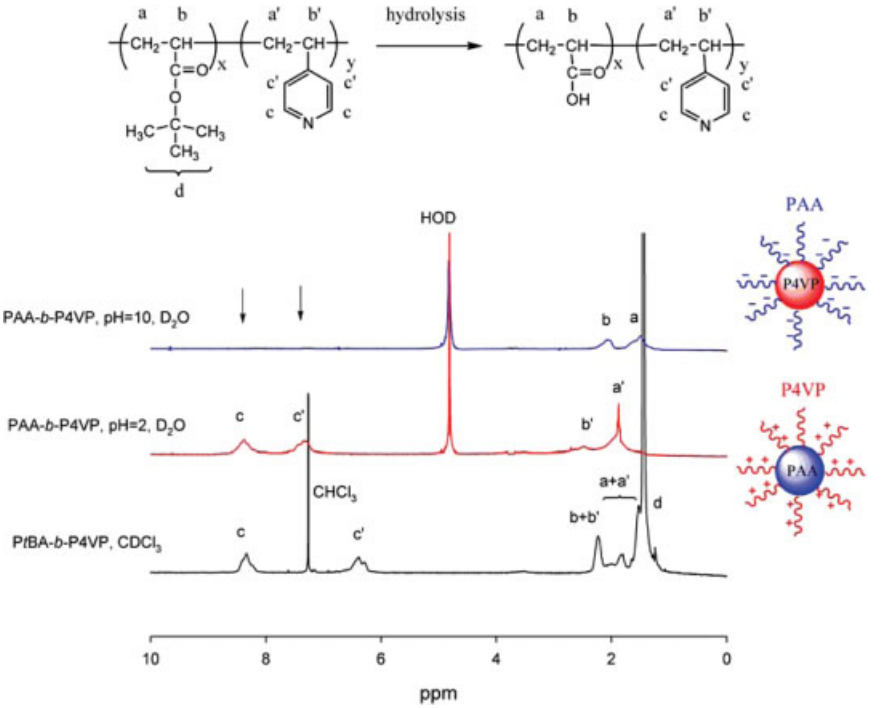

Figure 1. ^{1}H NMR spectra of P*t*BA-*b*-P4VP in chloroform (before hydrolysis) and PAA-*b*-P4VP in water (after hydrolysis) at pH = 2 and pH = 10. The two types of micelles are schematically illustrated. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

a higher value of I_1/I_3 indicating a more polar environment.[15] We found I_1/I_3 = 1.43, 1.60, and 1.86 for pyrene in the micellar solution at pH = 2, the micellar solution at pH = 10 and in water, respectively. This result indicates that the P4VP core at pH = 10 is significantly more polar than the PAA core at pH = 2. In aqueous solution, this difference in core polarity means different degrees of interaction with water molecules. In other words, the P4VP core may absorb more water than the PAA core, which accounts for the larger average size of the micelles formed at pH = 10.

Encapsulation of Metalloporphyrins

We expected that the two types of micelles could encapsulate hydrophobic substances. However, if the guest compound contains a transition metal that can coordinate with pyridine groups of the P4VP core of the micelles formed at high pH, the loading efficiency may improve, since specific interactions or compatibility between the guest and micelle core are known as key factors for encapsulation.[16] To test this, we investigated the encapsulation of ZnTPP and CoTPP, which can form the axial coordination with pyridine,[17] by the micelles formed at both pH = 2 and pH = 10.

The same conditions were used for the encapsulation of the metalloporphyrins by the micelles formed at pH = 2 and pH = 10. In both cases, a concentrated THF solution of ZnTPP or CoTPP (1 mg/mL) was added to a dilute polymer micellar solution (polymer concentration: 0.01–0.05 mg/mL) at a fixed pH, followed by evaporation of THF and part of water (see Experimental). Unless otherwise stated, the weight ratio of ZnTPP (or CoTPP)

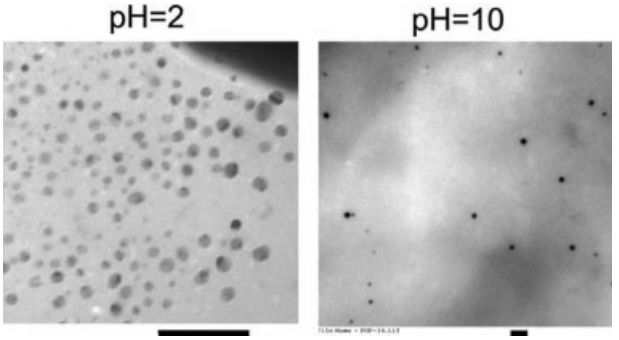

Figure 2. TEM images of the core-shell micelles formed at pH = 2 (PAA core) and pH = 10 (P4VP core). The scale bars are 100 nm.

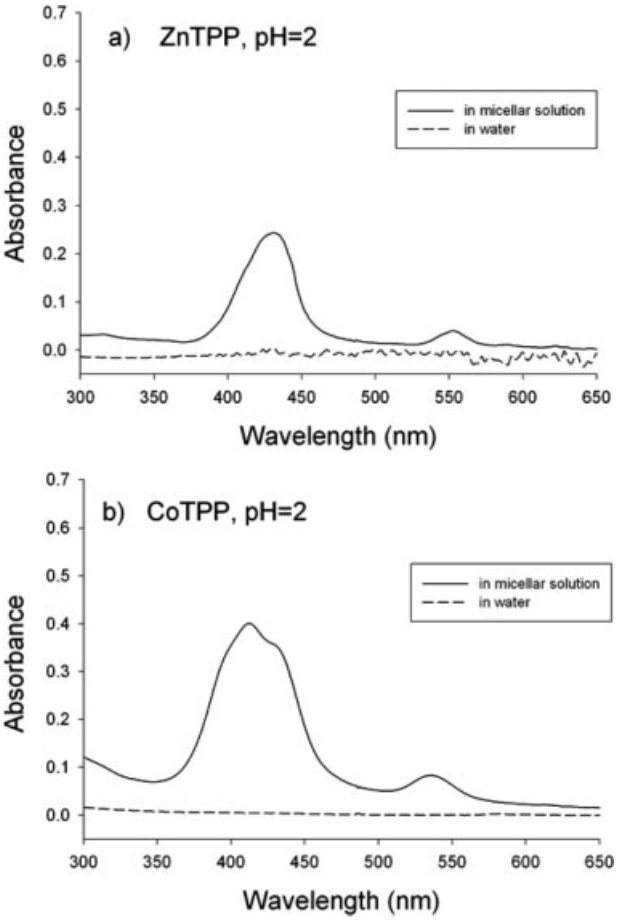

Figure 3. UV–vis spectra of ZnTPP (a) and CoTPP (b) in the micellar solutions at pH = 2 (micelles with PAA core) and in water, showing the solubilization of the metalloporphyrins by the micelles.

to polymer was 4:1. The solubilization of the hydrophobic metalloporphyrins by the polymer micelles was observable from UV–vis spectroscopic measurements; all spectra were taken after the removal of THF. As a control test, we also tried to dissolve the metalloporphyrins in water at pH = 2 and pH = 10 without polymer micelles, under exactly the same conditions as with the micellar solutions. The results obtained with the initial polymer concentration of 0.01 mg/mL were shown as an example, in Figure 3 for the encapsulation at pH = 2 (micelles with PAA core), and in Figure 4 for the encapsulation at pH = 10 (micelles with P4VP core). At pH = 2, the solubilization of ZnTPP and CoTPP by the micelles with PAA core is clearly revealed by the characteristic absorption spectra of the two metalloporphyrins; by contrast, their complete precipitation in water with no polymers is indicated by the undetectable absorption. At pH = 10, the situation is more complicated. Even though much less than in polymer micellar solutions, absorption of both ZnTPP and CoTPP in water were observed, indicating that they had some solubility when added from the THF solution into water at this pH value. In this case, the absorbance displayed by ZnTPP or CoTPP in the micellar solutions cannot directly be correlated with their amount entrapped by the micelles.

To compare the relative amounts of metalloporphyrins loaded in the micelles formed at pH = 2 and pH = 10, it is necessary to know which portion of the absorbance in Figure 4 comes from metalloporphyrin molecules solubilized by the micelle core of P4VP. For this purpose, both the micellar solutions and water solutions were dried, and then redissolved with the same volume of water. UV–vis spectra of these solutions (thick lines in Fig. 4) became very different. No

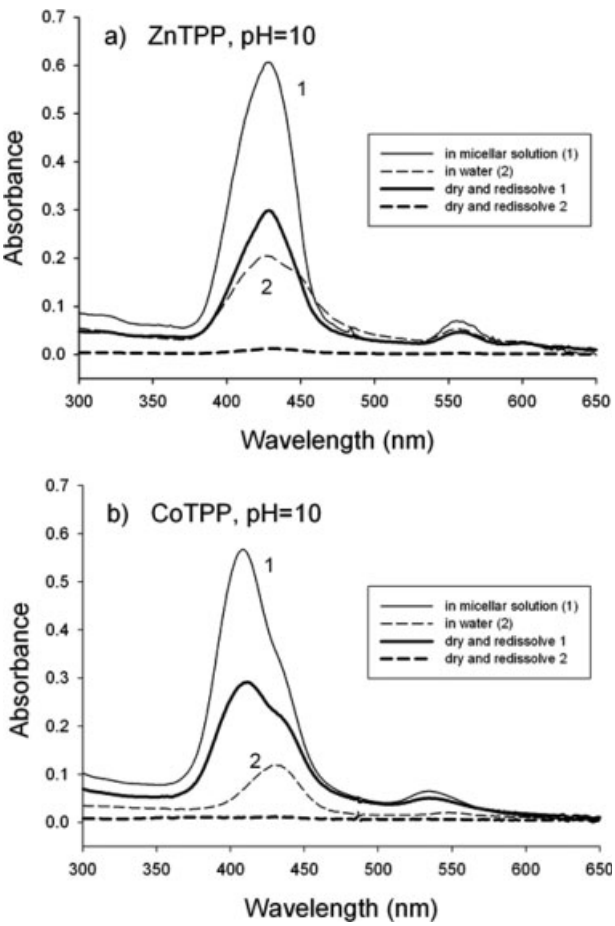

Figure 4. UV–vis spectra of ZnTPP (a) and CoTPP (b) in the micellar solutions at pH = 10 (micelles with P4VP core) and in water, and of the same solutions after drying and redissolution (thick lines). See text for details.

more absorption of ZnTPP and CoTPP could be observed in water with no polymer micelles, indicating that initially solubilized molecules were precipitated during the drying process, and that the precipitate could not be redissolved by water. In the case of micellar solutions, the absorptions of both metalloporphyrins were diminished after drying and redissolution. It is reasonable to assume that ZnTPP or CoTPP molecules that were not entrapped by the micelles but simply dissolved by water at pH = 10, precipitated and could not be redissolved. Considering the fact that water-soluble polymer micelles with loaded hydrophobic agents can be stored by drying and redissolved for use,[18] the remaining absorbance in Figure 4 would correspond to the portion of metalloporphyrin molecules solubilized by the micelles formed at pH = 10, and can be used for comparison with the micelles at pH = 2. To obtain an absorbance of ZnTPP or CoTPP (at the maximum absorption) suitable for comparison of their amounts solubilized by the two types of micelles, the UV–vis spectra were recorded after diluting the initial micellar solutions to various degrees. Taking the dilution degree into account, the micelles at pH = 10 with P4VP core entrapped more ZnTPP (~25% increase) and CoTPP (~80% increase) than the micelles at pH = 2 with PAA core and P4VP corona. As mentioned earlier, higher encapsulation of metalloporphyrins by the micelles with P4VP core was anticipated considering the possibility of axial coordination between transition metals and the pyridyl group. Indeed, as compared with ZnTPP entrapped by the micelles with PAA core (pH = 2), ZnTPP in the micelles with P4VP core (pH = 10) displayed a redshift of about 5 nm for the Q-bands in the 550–600 nm region [compare Figs. 3(a) and 4(a)]. This difference is indicative of the axial coordination between ZnTPP and the P4VP core.[17] A redshift can also be noticed by comparing CoTPP in the PAA core and CoTPP in the P4VP core [Figs. 3(b) and 4(b)].

The general technique used to determine the amount of guest loaded in block copolymer micelles consists in purifying the micelles, dissolving them in a solvent that is good for the two blocks, and then analyzing the amount of released guest. Unfortunately, we could not use this method because we found no good solvents to dissolve the micelles formed in acidic and basic aqueous solutions. In an effort to estimate the loaded amounts of ZnTPP and CoTPP, we performed the following experiment to obtain a rough estimation. ZnTPP and CoTPP were dissolved in chloroform at various concentrations and their absorption spectra were recorded. By plotting the maximum absorbance against the known concentration, a calibration curve (straight line) was obtained for each metalloporphyrin. Afterwards, UV–vis spectra of ZnTPP- and CoTPP-loaded micellar solutions at pH = 2 and pH = 10 with a known polymer concentration were taken (like those in Figs. 3 and 4, and in the case of pH = 10, spectra obtained after the drying and redissolution treatment were used). Assuming that ZnTPP and CoTPP in the two types of polymer micelles had the same extinction coefficients as in chloroform, the use of the two calibration curves allowed the amounts of ZnTPP and CoTPP in the micellar solutions to be calculated, yielding the following estimated loading capacities (weight percentage of metalloporphyrin with respect to the amount of polymer) for the two types of micelles: At pH = 2, 19% of ZnTPP and 20% of CoTPP; while at pH = 10, 30% of ZnTPP and 55% of CoTPP. Even taking into account the experimental uncertainty, these data, obtained from separate sets of experiments using a higher initial polymer concentration (0.02 mg/mL) than that in the experiments described with Figures 3 and 4 (0.01 mg/mL), further confirm the different relative amounts of ZnTPP or CoTPP loaded in the two types of micelles. Micelles with P4VP core are able to encapsulate more metalloporphyrins.

The different loading capacities were corroborated qualitatively by different morphologies of the polymer micelles after encapsulation. Figure 5 shows some typical TEM images of the loaded micelles (initial polymer concentration: 0.02 mg/mL). At pH = 10, the P4VP-core micelles remained firm after the encapsulation, but their average sizes became significantly larger (~32 nm with ZnTPP and ~65 nm with CoTPP) than the micelles without encapsulation (~20 nm, Fig. 2). By contrast, at pH = 2, the PAA-core micelles after encapsulation appeared as larger (>100 nm for many of them) and loose aggregates. The large aggregates in the micellar solution at pH = 2 also resulted in the instability with the polymer and metalloporphyrins precipitated over a period of days, while the micellar solution at pH = 10 remained stable after months.

A number of other loading experiments under different conditions were also carried out and

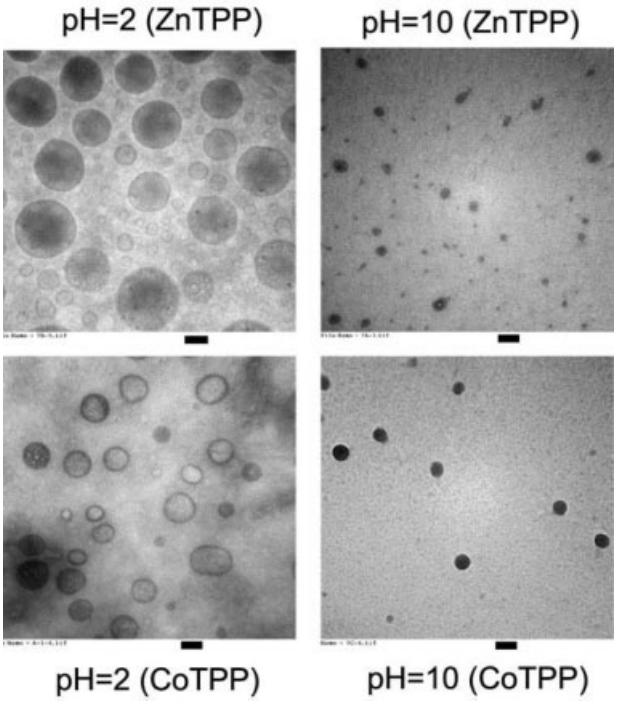

Figure 5. TEM images of ZnTPP and CoTPP-loaded micelles formed at pH = 10, and of ZnTPP and CoTPP-loaded micellar aggregates formed at pH = 2. The scale bars are 100 nm.

some results are worth being mentioned. First, the amount of the THF solution of ZnTPP or CoTPP added to the aqueous solution of PAA-*b*-P4VP seems to affect the encapsulation. In one experiment, a higher initial polymer concentration of 0.05 mg/mL was used; while using the same THF solution of ZnTPP (1 mg/mL) and keeping the same weight ratio of ZnTPP to polymer of 4:1, the volume of THF added was 20% of water (1:5 v/v), as compared with 4% when using the polymer concentration of 0.01 mg/mL. The estimated loading efficiency of ZnTPP was found to be below 5% even at pH = 10. Secondly, the loading efficiency was found to become higher as the weight ratio of CoTPP to polymer increased from 2:1 (~4% loading) to 4:1 (~20%) to 8:1 (~33%). In this experiment, the same micellar solution (polymer concentration: 0.01 mg/mL) and the same amount of THF (4% of water) were used, while the concentration of CoTPP in THF was increased from 0.5 to 1 to 2 mg/mL. It could be expected that many parameters might influence the loading efficiency as revealed by the above two experiments. In the first case, the composition of the mixed solvent can affect the micelles and the interaction between the polymer and metalloporphyrin, since it can change the relative solubility of the two blocks as well as that of the metalloporphyrin. In the second case, the amount of the metalloporphyrin added to the mixture could change its portioning between precipitation in water and solubilization by the micelles.

pH Change-Induced Release of Metalloporphyrins

The two-way pH-sensitivity of the double-hydrophilic PAA-*b*-P4VP, that is reaction to both pH increase and pH decrease, is at the origin of the interconversion between two types of micelles. We anticipated that the metalloporphyrins encapsulated at the acidic pH = 2 and basic pH = 10 could be released on pH increase and on pH decrease, respectively, through a destruction-reorganization process of the micelles.

The pH change-induced destruction and reorganization of the two types of micelles of PAA-*b*-P4VP was first confirmed by the simple turbidity measurement (at 600 nm, using the UV–vis spectrophotometer).[19] Figure 6 shows the change

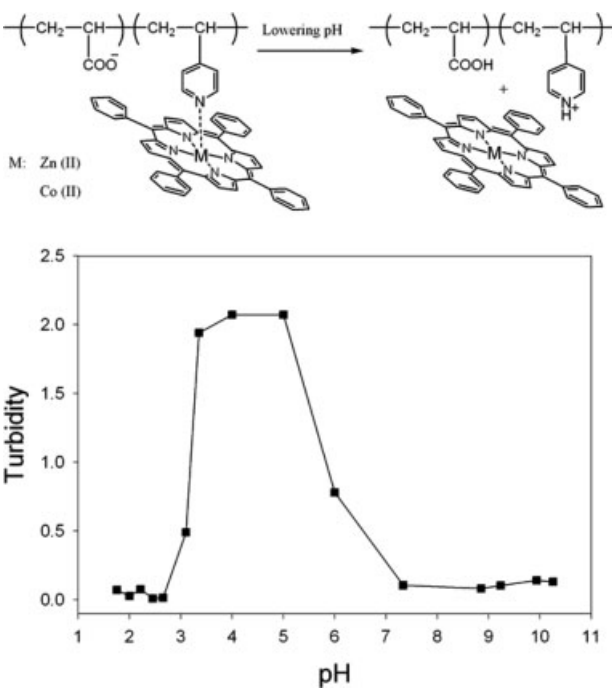

Figure 6. Turbidity of PAA-*b*-P4VP solution (2 mg/mL) as a function of pH, showing the formation of larger aggregates due to the complexation of the two blocks during micelle reversion. The breaking of the axial coordination between the transition metals and the pyridine groups as a result of the protonation, which allows the release of the metalloporphyrins, is also shown.

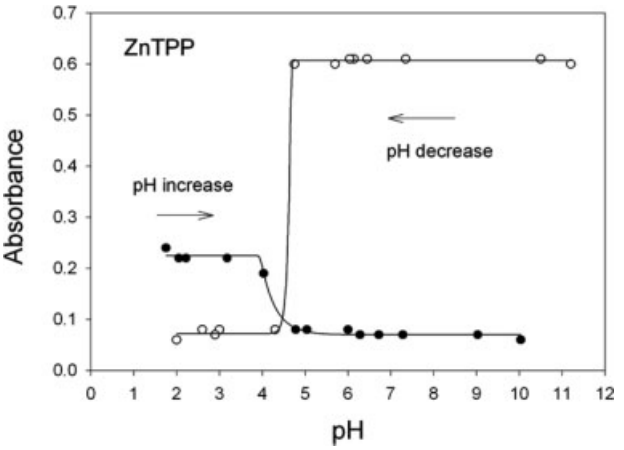

Figure 7. Changes in absorbance of ZnTPP (426 nm) on pH decrease for ZnTPP loaded in micelles formed at high pH, and on pH increase for ZnTPP loaded in micelles formed at low pH, showing the two-way pH change-induced release of ZnTPP.

in turbidity of a micellar solution prepared at acidic pH = 1.8 (polymer concentration, 2 mg/mL) as a function of pH (after each addition of NaOH the solution was stirred for 30 min before the measurement). The turbidity of the solution increases sharply over the pH range of about 3.5–5.5. TEM observation found the transformation of the micelles with PAA core into larger aggregates in this pH range (images not shown), where the two polymer blocks are partially ionized (PAA with COO^- and P4VP with protonated pyridine groups) giving rise to their complexation through H-bonding and ionic interaction.[20] At higher pH (>6.5), the turbidity drops again because of the formation of the micelles with P4VP core. Similar turbidity changes were observed on pH decrease of the micellar solutions prepared at high pH. These results indicate that PAA-*b*-P4VP, which is a zwitterionic diblock copolymer, has an isoelectric point (PI) in the pH range of about 3.5–5.5. At pH close to PI, polymer micelles are destructed, while at pH far enough from PI, they are reformed. Therefore, for both types of PAA-*b*-P4VP micelles with encapsulated ZnTPP or CoTPP, changing the pH would make them go through the destruction-reorganization process, during which the entrapped metalloporphyrin could be released. It should be emphasized that this would occur even with ZnTPP or CoTPP axially coordinated with the pyridine groups of the P4VP core, since the noncovalent chemical bonding is broken on pH decrease due to the protonation of the pyridine groups (depicted in Fig. 6).

As shown in Figures 3 and 4, ZnTPP and CoTPP entrapped by the polymer micelles are absorbed in the visible region. Upon pH change, their release from the micelles into the aqueous solution, where they aggregate due to the insolubility, reduces the absorption. By measuring the absorbance of the Soret band of ZnTPP and CoTPP (the maximum absorptions at 426 and 412 nm, respectively), their release from the micelles with PAA core on pH increase, and from the micelles with P4VP core on pH decrease was investigated. The results shown in Figures 7 and 8 for ZnTPP and CoTPP, respectively, indeed indicate the two-way pH change-induced release of the metalloporphyrins. In these experiments, the initial micellar solution was first diluted to display a maximum absorbance of ZnTPP or CoTPP, suitable for the measurements. After each addition of 1 M HCl to decrease pH, or 1 M NaOH to increase pH, the solution was stirred for 30 min before taking the spectrum. The results in Figure 7 show that for ZnTPP entrapped by both the micelles with P4VP core (at high pH, greater loading leading to higher absorbance) and the micelles with PAA core (at low pH, less encapsulation resulting in smaller absorbance), the release takes place about the same pH range, between pH = 4 and pH = 5. This narrower range as compared to that observed for the turbidity change (Fig. 6) could be explained by the much smaller concentration of the micelles used in this experiment. Moreover, the pH range for the release of ZnTPP may not correspond exactly to the pH range over which the micelles are con-

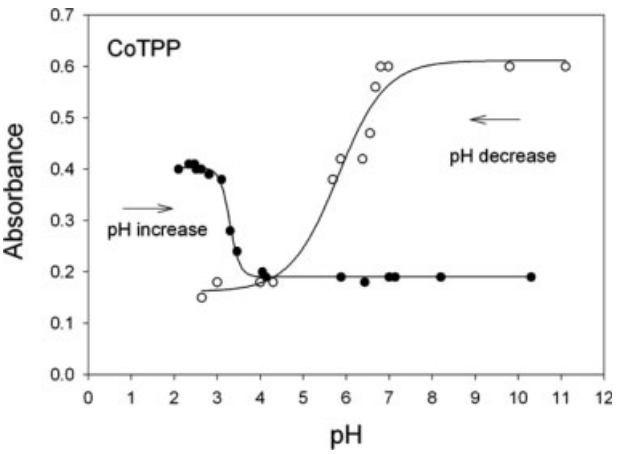

Figure 8. Changes in absorbance of CoTPP (412 nm) on pH decrease for CoTPP loaded in micelles formed at high pH, and on pH increase for CoTPP loaded in micelles formed at low pH.

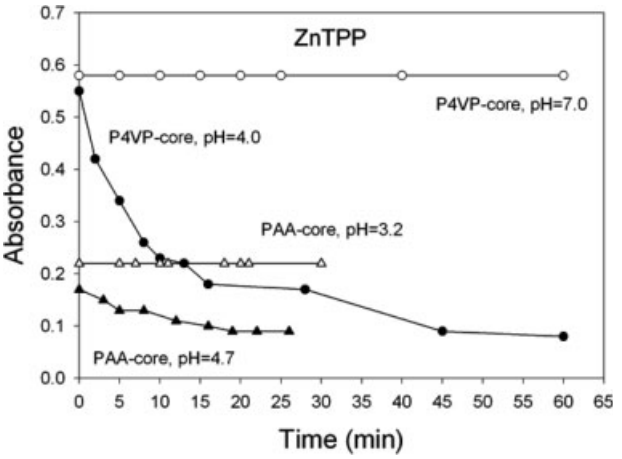

Figure 9. Changes in absorbance of ZnTPP (426 nm) as a function of time at fixed pH, for both micelles formed at high pH (P4VP core) and at low pH (PAA core), showing the two-way pH change-induced release of ZnTPP in time over the pH range between about 4 and 5.

verted to larger aggregates due to the complexation of the two blocks. This may especially be the case for ZnTPP coordinated with the P4VP core, for which the protonation of the pyridine group is required to detach ZnTPP from the polymer. In the case of CoTPP, Figure 8 shows also the two-way release. However, as compared to ZnTPP, on pH increase the release of CoTPP from the micelles with PAA core occurs at lower pH (>3), while on pH decrease the release takes place at higher pH (<6.5). The residual absorbance of CoTPP is also significantly higher than that of ZnTPP, suggesting a less thorough release from the polymer micelles. In addition to the difference in concentration of the micelles, which may be a factor accounting for the different behaviors, it can be speculated that the encapsulation stability and the release of different metalloporphyrins might not be the same due to different interactions with the polymer. We mention that separate sets of experiments were carried out, and similar results were obtained.

The release of metalloporphyrins as a function of time at a fixed pH was also investigated. Figure 9 shows an example of the results for ZnTPP entrapped by both types of micelles. For the micelles formed at pH = 10 with P4VP core, they remained stable after the pH was lowered to pH = 7. No change in absorbance of ZnTPP was observed over 1 h, indicating no release of ZnTPP into the aqueous solution. By contrast, when more HCl was added to reach pH = 4, the absorbance of ZnTPP was decreased quickly over the first 15 min, as a result of the dissociation of the micelles and the concomitant aggregation of ZnTPP in the aqueous solution. Similar observation can be made for ZnTPP entrapped by micelles formed at pH = 2 with PAA core. When pH was increased to pH = 3.2, no release took place, while at pH = 4.7, the release occurred as indicated by the decrease in absorbance of ZnTPP. Similar results were obtained for CoTPP.

For most pH-sensitive polymer micelles studied as potential nanocarriers for drug delivery applications, they are disrupted, thus triggering the release of the encapsulated compound, by lowering pH.[1a–1c,21] These systems are relevant to drug delivery targeting tumor tissues (pH ~ 6.8) or the endosomal and lysosomal compartments of cells (pH ~ 5–6), which have lower pH values than the physiological pH 7.4. However, for oral administration of drugs, the opposite pH sensitivity is needed. Polymer micelles should remain in the aggregated state at low pH (in the stomach), but be disrupted at higher pH (>5) in the small intestine for the release of encapsulated compound.[22] Even though the pH range over which the release of ZnTPP or CoTPP from PAA-*b*-P4VP micelles occurs is not suitable for pH change-controlled drug delivery, the present study suggests an attractive new possibility of exploring the micelles of double-hydrophilic BCPs for drug delivery applications. That is, the two-way pH sensitivity of this type of micelles could make it possible to design and develop polymers nanocarriers that are able to encapsulate both drugs for oral administration, whose release should take place on pH increase, and anticancer drugs for which the release should be triggered by pH decrease.

CONCLUSIONS

In this paper, we have reported the synthesis of a simple double-hydrophilic block copolymer, PAA-*b*-P4VP, and its formation of two types of micelles in aqueous solution, with P4VP core and PAA corona at high (basic) pH and with PAA core and P4VP corona at low (acidic) pH. We have investigated the encapsulation of two hydrophobic metalloporphyrins, ZnTPP and CoTPP, by these micelles. The results indicate that the micelles with P4VP core are able to load more ZnTPP or CoTPP than the micelles with PAA

core, which could be attributed to the axial coordination between the transition metals and the pyridine groups of the P4VP core. The study found, for the first time, that the metalloporphyrins encapsulated by the micelles with P4VP core could be released on pH decrease, while those loaded in the micelles with PAA core could be released on pH increase. The release is through a destruction-reorganization process of the micelles. This two-way pH-controlled release could be explored to make pH-sensitive polymer nanocarriers that are useful not only for anticancer drugs which need to be delivered on pH decrease, but also for orally administrated drugs which should be released on pH increase.

The authors thank Prof. Pierre Harvey for the use of spectrometer of fluorescence. Financial support from the Natural Sciences and Engineering Research Council of Canada and le Fonds québécois de la recherche sur la nature et les technologies of Québec are also acknowledged.

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