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Synthesis and pH Responsive Self-Assembly of New Double Hydrophilic Block Copolymers

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ABSTRACT: The synthesis of a new class of double hydrophilic block copolymers, namely poly(*p*-hydroxystyrene-*b*-methacrylic acid) (PHOS−PMAA), by a combination of anionic polymerization high-vacuum techniques and postpolymerization acidic hydrolysis, is described. A series of PHOS−PMAA copolymers with different molecular weights and compositions have been synthesized, indicating the good control over the molecular characteristics of the final copolymers. The solution behavior of PHOS−PMAA is studied as a function of the solution pH. A number of characterization techniques, including potentiometric titrations, fluorescence and IR spectroscopy, dynamic and electrophoretic light scattering, and atomic force microscopy, are used in an effort to elucidate the structural changes of the block copolymers at different levels, under different pH environments. Experimental results prove the pH responsive self-assembling ability of PHOS−PMAA copolymers.

Introduction

Double hydrophilic block copolymers (DHBCs) have recently received the attention of many research groups due to their exceptional combination of the well-known block copolymer self-assembly behavior in solution, the water solubility, and the ability for controlled interactions with other nanosized building blocks.[1,2] The application spectrum of these copolymers involves drug and gene delivery systems,[3] synthesis of well-defined nanoparticles,[4] surface modification,[5] and so on. The fundamental aqueous solution behavior of DHBCs depends greatly on their detailed chemical structure and is manifested through their stimuli responsive character. In particular, most DHBCs show the unique feature to change their chain conformation and to self-assemble as a result of changes in solution pH, ionic strength, or temperature.[6−19] Moreover, interaction of DHBC with different (macro)molecules, like surfactants, polyelectrolytes, and biomolecules, through electrostatic interactions, hydrogen bonding, and other secondary interactions, opens new routes to the construction of interesting, novel, and functional supramolecular nanostructures.

Special emphasis has been given to DHBCs that respond to pH changes, creating a variety of self-organized supramolecular structures in solution. This ability has been observed for poly(2-vinylpyridine-*b*-ethylene oxide) copolymers.[6] Since the poly(2-vinylpyridine) block is water-soluble only when the pyridine group is protonated, i.e., for pH lower than 5, formation of micelles was observed when the solution pH became higher than 5, with the poly(2-vinylpyridine) block in the core. It is worth mentioning that supramolecular structures have been also observed even at pH 1 for such block copolymers.[6b] A similar behavior was observed for poly(hexaethylene glycol methacrylate-*b*-(dimethylamino)ethyl methacrylate) copolymers at low pH values.[6b]

Armes and co-workers have presented a series of DHBCs based mainly on methacrylic acid derivatives. It was found that poly((dimethylamino)ethyl methacrylate-*b*-(diethylamino)ethyl methacrylate) (PDMAEMA-*b*-PDEAEMA) copolymers form micellar aggregates, with PDEAEMA being the core forming block, in an alkaline environment.[8] The influence of others external stimuli, like salinity, has been also investigated for this system.[9] Moreover, functionalization of PDMAEMA-*b*-PDEAEMA, via a sulfobetainization reaction, has led to DHBCs sensitive to environmental changes.[10] The above systems have been studied in depth in terms of their self-organization under variation of the solution pH.

A number of pH responsive DHBCs consisting of poly(ethylene oxide) and of a methacrylic acid block, or an ester derivative of this block, have been reported in the literature. Poly(ethylene oxide-*b*-methacrylic acid) has been studied at different pHs. Four distinct solution behaviors have been observed at the respective pH regions.[11] Supramolecular structures were formed by the above system because of inter- and intramolecular hydrogen bonding, hydrophobic interactions, and electrostatic repulsion. A series of pH responsive DHBCs consisting of poly(ethylene oxide) as the nonionic water-soluble block have been also presented by Leroux and co-workers.[12]

A series of block copolymers consisting of poly(2-hydroxyethyl methacrylate) derivative with succinic anhydrite as the first block and various other methacrylic acid derivatives with tertiary amino groups as the second one have been studied at different pHs.[13] These copolymers has been found to create micelles at acidic and alkaline environments with different core-forming block each time, while at intermediate pH values the copolymers were either molecularly dissolved or precipitated out of the solution.

Formation of micelles, instead of insoluble complexes, at alkaline solution has been observed in the case of diblocks of poly(vinylpyridine) and poly(methacrylic acid) or poly(acrylic acid).[14,15] Furthermore, poly(sulfonated styrene-*b*-4-styrenecarboxylate) copolymers have been observed to create micelles at acidic solution with hydrated poly(4-styrenecarboxylate) cores.[16] Finally, the ability of poly(methacrylic acid-*b*-diethylacrylamide) to form supramolecular structures under changes in both temperature and solution pH has been investigated. The particular system has been found to have very complicated solution behavior, but the formation of spherical micelles at pH lower than 4 has been observed.[17]

DHBCs containing poly(amino acid) blocks have been recently synthesized and studied at different pH environments. The formation of two different types of vesicles at distinct pH

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regions has been observed for poly(glutamic acid-*b*-lysine) by means of NMR, dynamic light scattering, fluorescence, and neutron scattering.[18] Moreover, the synthesis and the pH responsive character of a series of DHBCs consisting of poly(ethylene oxide) and peptides with different amino acid sequences have been presented by Klok and co-workers. The above polymers have been found to aggregate upon changes in the solution pH.[19]

In the present paper we report the synthesis of novel well-defined DHBCs, namely poly(*p*-hydroxystyrene-*b*-methacrylic acid) (PHOS−PMAA) from poly(*p*-*tert*-butoxystyrene-*b*-*tert*-butyl methacrylate) (PtBOS−PtBMA) precursors, using anionic polymerization high-vacuum techniques and postpolymerization acidic hydrolysis. The precursor copolymers have been characterized in terms of their molecular weight and composition homogeneity. The aqueous solution behavior of the PHOS−PMAA copolymers has been studied as a function of pH, at functional groups, molecular and supramolecular level using potentiometric titrations, FT-IR and fluorescence spectroscopy, dynamic and electrophoretic light scattering, and atomic force microscopy. The presence of supramolecular structures has been identified upon changes in solution pH. The above copolymers are comprised of two anionic annealed polyelectrolyte blocks with different pK_a values, in addition to the combination of a highly hydrophobic block (poly(*p*-hydroxystyrene)) with a less hydrophobic block (namely poly(methacrylic acid)). Both characteristics influence their self-assembling behavior and solubility, as will be discussed in detail further on.

Experimental Section

Synthesis of the Precursor Block Copolymers. The synthesis of the precursor poly(*p*-*tert*-butoxystyrene-*b*-*tert*-butyl methacrylate) (PtBOS−PtBMA) copolymers has been realized by anionic polymerization high-vacuum techniques. All reagents were purified before their use. In particular, *p*-*tert*-butoxystyrene (tBOS, Aldrich) was stirred over CaH_2 overnight under vacuum, followed by distillation in an ampule equipped with a break-seal. The monomer was further purified by dibutylmagnesium for 30 min and distilled in another ampule under high vacuum. The purified monomer is easily thermopolymerized, even at low temperature; thus, the polymerization was performed the very next day of the purification procedure, while the monomer was stored at −80 °C. *tert*-Butyl methacrylate was purified in a similar way, using trioctylaluminum, instead of dibutylmagnesium, in the second step. The purified monomer was used immediately. All other reagents were purified using standard procedures.[20]

Polymerizations took place in THF at −78 °C. Initially, the addition of the first monomer, *tert*-butoxystyrene, was carried out followed by the addition of the initiator (*n*-BuLi as a solution in hexane). The polymerization of the first block was carried out for 1 h followed by the addition of diphenylethylene (DPE:Li = 2:1) and LiCl (LiCl:Li = 5:1) in order to reduce the reactivity of the living styrenic chain ends. Subsequently, the addition of the second monomer, *tert*-butyl methacrylate, was carried out by distillation into the polymerization reactor. The polymerization of the second monomer was continued for 30 min after distillation was completed. The living diblock copolymers were terminated using degassed methanol. The precursor copolymers were isolated by precipitation in a 10-fold excess of methanol/water (8/2 v/v) mixture. PtBOS−PtBMA samples were dried under vacuum.

Synthesis of the Double Hydrophilic Block Copolymers. Poly(*p*-hydroxystyrene-*b*-methacrylic acid) (PHOS−PMAA) was synthesized by acidic hydrolysis of the precursor diblock copolymers, PtBOS−PtBMA. The desired amount of polymer was dissolved in dioxane-1,4 in a round-bottom flask at a 5 wt % concentration. A stoichiometric excess of concentrated HCl was added, and the temperature was raised to 85 °C. Typically, a 5-fold molar excess of HCl with respect to the ether and ester oxygens was used. The solution was refluxed for 6 h. After the end of the reaction, the solution was cooled at room temperature and the final polymer was isolated by precipitation in 10-fold excess of hexane. PHOS−PMAA copolymers were dried under vacuum.

Characterization Techniques. The molecular weight and polydispersity of the precursor copolymers were estimated by size exclusion chromatography (SEC) using a system consisting of a Waters 1515 pump, three μ-Styragel separation columns with a continuous porosity of 10^2−10^5 Å, and a Waters 2414 differential refractive index detector. The solvent was tetrahydrofuran, and the flow rate was 1 mL/min. The calibration of the instrument was performed using narrow polystyrene standards (M_w varying from 2500 to 900 000). ^{1}H NMR spectra were recorded on a Bruker AC 300 instrument at 25 °C using $CDCl_3$ as the solvent. Mid-infrared spectra in the region 550−4000 cm^{-1} were obtained on a Fourier transform IR spectrometer (Equinox 55 from Bruker Optics) equipped with a single reflection diamond ATR accessory (DuraSamp1IR II by SensIR Technologies). An amount of the polymer was placed on the diamond element and held in contact by means of a press. Alternatively, a drop of a solution, adjusted at the desired pH value, was placed on the diamond followed by evaporation of the solvent in a steam of nitrogen before recording the spectrum. Typically, 100 scans were acquired at 4 cm^{-1} resolution.

Potentiometric titrations of PHOS−PMAA samples were performed with a PIONeer 65 portable pHmeter equipped with a PHC 5977 cartode comb pH electrode. During the titration, the fluorescence spectra of collected samples, containing pyrene as a probe, were obtained on a Fluorolog-3, model FL3-21, Jobin Yvon-Spex (excitation at 335 nm and recording of the emission spectra, in the 350−500 nm region). Solutions of PHOS−PMAA were obtained by direct dissolution of the powder polymer in an aqueous NaOH solutions with a pH value of 12 or higher, giving the sodium phenolate and sodium carboxylate form of the copolymers. In the above solutions, a small amount of pyrene, typically 6 μL of 1×10^{-3} M pyrene solution in acetone per 20 mL of polymer solution (final pyrene concentration 3×10^{-7} M), was added as the fluorescence probe. Adjustment of the solution pH was achieved using HCl standard solution (0.1 N).

Dynamic light scattering measurements on dilute aqueous solutions of PHOS−PMAA have been performed with an AXIOS-150/EX (Triton Hellas) light scattering photometer, equipped with a 30 mW laser source, operating at 658 nm, and an Avalanche photodiode detector at a scattering angle of 90°. The raw data were analyzed by the Contin and the cumulants algorithms. Apparent hydrodynamic radii, R_h, at different solution pH were calculated using the Stokes−Einstein equation. Electrophoretic light scattering experiments were performed with a Zeta-Plus analyzer (Brookhaven Instruments Corp.) equipped with a 35 mW solid-state laser at 660 nm. Solutions of the DHBCs at different pH values were prepared starting from a stock solution and subsequently adjusting the pH.

For the determination of critical micelle concentration, by fluorescence spectroscopy, a stock solution of each polymer was prepared. A predetermined amount of the powder polymer was dissolved in water at pH 12 or 13 (depending on the PHOS content). The above solution was titrated with HCl to pH 7 in order to prepare the stock solution. The above solutions were diluted and pyrene was added (pyrene concentration 3×10^{-7} M). The final solutions were allowed for equilibration overnight before performing the measurements.

For obtaining the atomic force microscopy (AFM) images a Quesant AFM Q-Scope 250 was used. The samples were prepared by dipping precleaned silicon wafers in aqueous solutions of the copolymers, at the appropriate pH, for 1 min. The concentration of the copolymers, typically 1×10^{-3} g/mL, was well above their critical micelle concentration.

Results and Discussion

Polymer Synthesis. The reaction steps for the synthesis of the new double hydrophilic block copolymers are shown

Scheme 1. Reaction Scheme Leading to the Synthesis of PHOS−PMAA Double Hydrophilic Block Copolymers

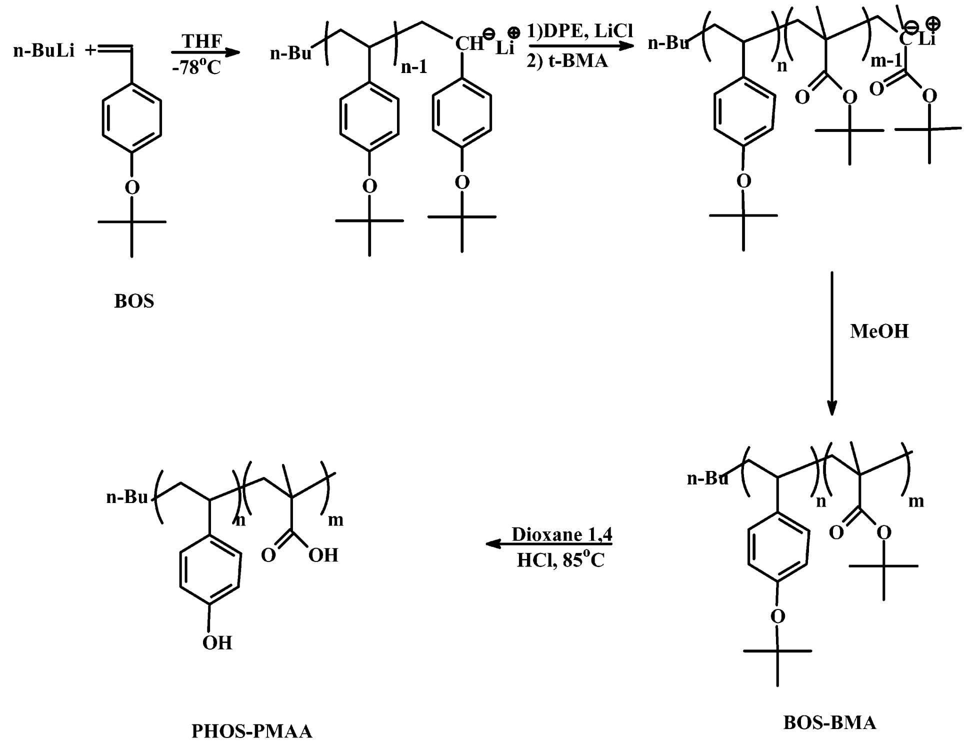

Table 1. Molecular Characteristics of the PtBOS−PtBMA Precursor Block Copolymers

sample	$M_w{}^a \times 10^{-4}$	$M_w/M_n{}^a$	wt % tBOS^b
PtBOS−PtBMA 1	2.46	1.10	27.7
PtBOS−PtBMA 2	2.35	1.05	68.2
PtBOS−PtBMA 3	2.57	1.15	84.6
PtBOS−PtBMA 4	1.50	1.11	67.8
PtBOS−PtBMA 5	3.90	1.08	75.8

[a] By SEC in THF at 40 °C. [b] By ^{1}H NMR in $CDCl_3$ at 25 °C.

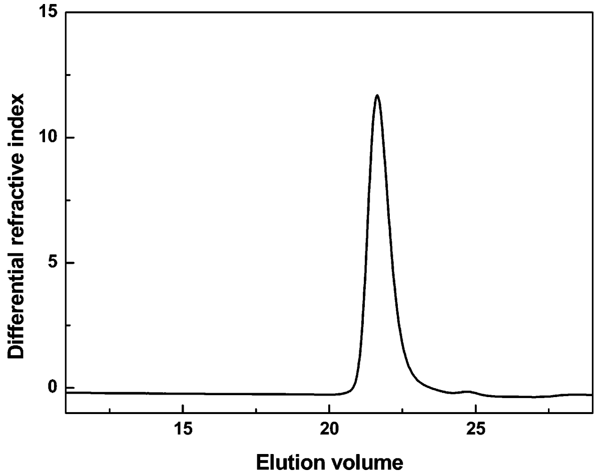

Figure 1. SEC chromatogram of sample PtBOS−PtBMA 5.

schematically in Scheme 1. The synthesis of the precursor block copolymers PtBOS−PtBMA has been realized by sequential addition of the monomers. Initially, the polymerization of tBOS was performed by addition of the initiator in a dilute THF solution of the monomer at −78 °C.[21] The color of the solution changed rapidly to orange, typical of the styrenic anions. The polymerization of BOS was continued for 1 h in order to ensure the complete polymerization of the monomer, although some preliminary experiments indicated that 15 min is enough for the polymerization of this monomer, as in the case of styrene. After the complete polymerization of the first block, the polymerization of the second monomer was performed, after capping the PtBOSLi living ends with diphenylethylene and in the presence of LiCl in order to ensure a well-defined character of the PtBMA block.[22]

The molecular characteristics of the precursor block copolymers, as they have been determined by SEC and NMR, are presented in Table 1. A characteristic chromatogram of the precursor polymer is shown in Figure 1. The presence of one, narrowly distributed, peak is observed, indicating the well-defined structure of the precursor block copolymers as well as the living nature of the system at all stages of the polymerization reaction. This is also corroborated by the fact that the observed molecular weights are close to the stoichiometric ones as well as the ability to synthesize a series of copolymers with desirable compositions.

The final double hydrophilic block copolymers were synthesized by acidic hydrolysis of PtBOS−PtBMA in order to remove the protective *tert*-butyl groups. The reaction was done at elevated temperature using an excess of hydrochloric acid. It is well established that the above conditions have no influence on the other molecular characteristics of the polymers but produce quantitative hydrolysis of *tert*-butyl group protected ester and ether functionalities.[21,22] The absence of the characteristic peaks at 1365 and 1390 cm^{-1}, attributed to the *tert*-butyl group, in the infrared spectra of PHOS−PMAA (Figure 2) indicates that the hydrolysis reaction is nearly quantitative. The molecular characteristics of the final double hydrophilic block copolymers are presented in Table 2, as they have been calculated from the data on the precursor copolymers, taking into account a complete hydrolysis of the ester and ether functionalities of the precursor block copolymers. Samples PHOS−PMAA 1, 2, and 3 have almost identical molecular weights and different content of PHOS block, where samples PHOS−PMAA 2, 4, and 5 have almost the same content of PHOS but different molecular weights. The experimental results verify that there is good control over the molecular characteristics of the block copolymers synthesized.

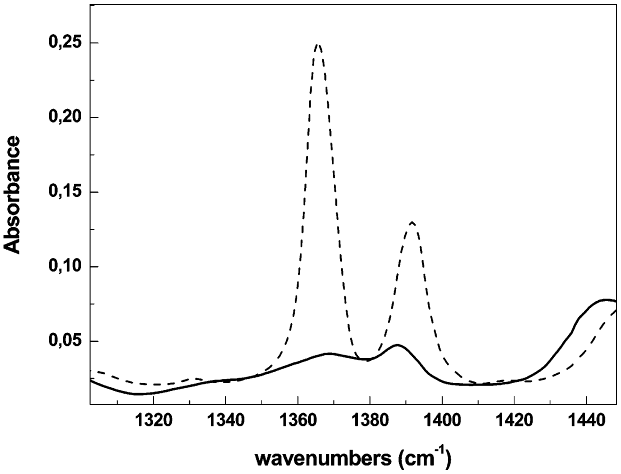

Figure 2. FT-IR spectra of PtBOS−PtBMA 1 (dashed line) and PHOS−PMAA 1 (solid line).

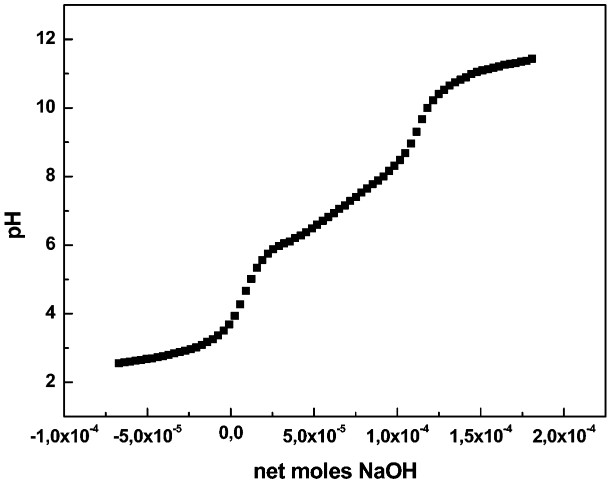

Figure 3. Potentiometric titration for sample PHOS−PMAA 1. pH vs net moles of NaOH (initial NaOH moles − added HCl moles) ($c_{\mathrm{pol}} = 1 \times 10^{-3}$ g/mL).

Table 2. Molecular Characteristics of the PHOS−PMAA Double Hydrophilic Block Copolymers

sample	$M_w \times 10^{-4}$	M_w/M_n	wt % HOS
PHOS−PMAA 1	1.54	1.10	30.1
PHOS−PMAA 2	1.55	1.05	70.7
PHOS−PMAA 3	1.72	1.15	86.1
PHOS−PMAA 4	0.98	1.11	70.3
PHOS−PMAA 5	2.56	1.08	77.9

Aqueous Solution Studies on PHOS−PMAA Copolymers. PHOS−PMAA is a DHBC consisting of two blocks having different functional groups with different pK_as. In particular, PHOS block has phenolic moieties with pK_a greater than the pK_a of the PMAA block, which has carboxylic acid groups. This expectation was verified by titration of alkaline copolymer solutions with hydrochloric acid. A representative result of these titrations is shown in Figure 3. The titration data indicate the existence of two equivalent points at pH ~9 and pH~4 which are attributed to protonation of NaPHOS and NaPMAA blocks, respectively. Moreover, a pH region ($4 < \mathrm{pH} < 9$) where only the PMAA block is disassociated could be observed.

The changes at the functional group level, as a function of pH, have been studied by means of FT-IR spectroscopy. Thin films of PHOS−PMAA have been produced by evaporation of the solvent from polymeric solutions with different pH values. The changes at the repeat units, because of the different pH, have been observed by recording FTIR-ATR spectra of the above thin films. In Figure 4 the changes at 1715 and 1248 cm^{-1} bands, attributed to carboxylic acid and phenolate groups, respectively, are shown. It is obvious that the intensity of the peak attributed to the carboxylic acid moieties is increased when the pH values are lower than 6 and reach its limiting values when the pH adopts values lower than 4. The above experimental result indicates that all the carboxylic acid moieties are in their unassociated form (which makes the PMAA block water-soluble) at alkaline environment, while protonation of the repeat units takes place at acidic environment, leading to precipitation of the PMAA block. Similar conclusions are obtained from the intensity increase of the peak at 1248 cm^{-1}, attributed to the hydroxyl groups of the PHOS block. In this case the lower the pH values, the higher the peak intensity; i.e., a decrease in pH leads to protonation of the repeat units in PHOS, which has as a result this block to become water-insoluble.

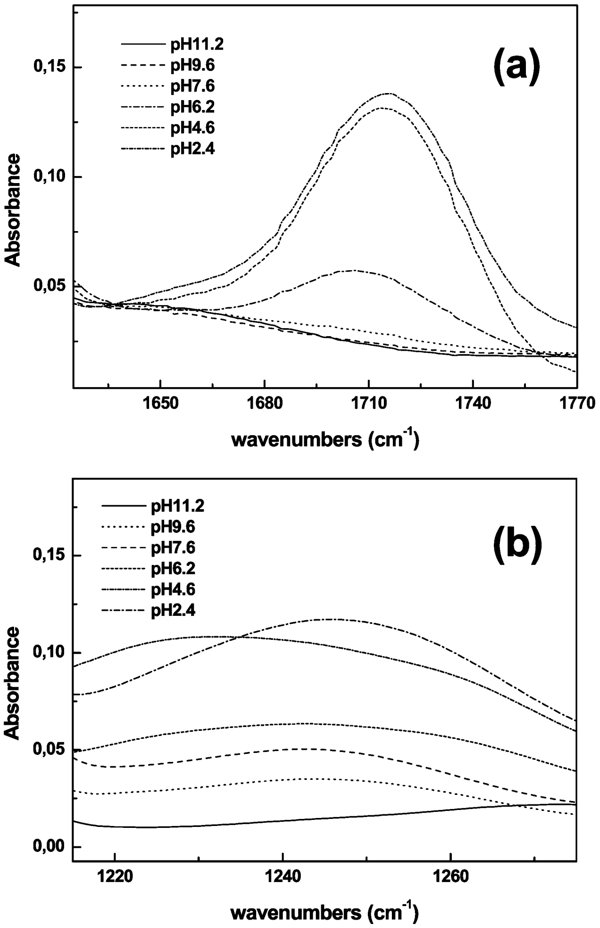

Figure 4. (a) Changes of the band at 1715 cm^{-1} in the IR spectra of PHOS−PMAA 1 at different pH values. (b) Changes of the band at 1248 cm^{-1} in the IR spectra of PHOS−PMAA 1 at different pH values.

When the solution pH is higher than 12, both of the blocks can be assumed to behave as annealed polyelectrolytes due to the full dissociation of the sodium phenolate and sodium carboxylate groups. When the solution pH values are between 4 and 9, only one of the blocks, namely the PMAA block, will be water-soluble, while the other block (PHOS) will form hydrophobic domains. Finally, for pH values lower than 4 both of the blocks will be water-insoluble, leading to the precipitation of the polymer from the solution. The existence of hydrophobic domains is confirmed by fluorescence spectroscopy, using pyrene molecules as probes.[23] The intensity ratio of the first over the third peak at the fluorescence spectra of pyrene, I_1/I_3, decreases with the appearance of hydrophobic areas in aqueous

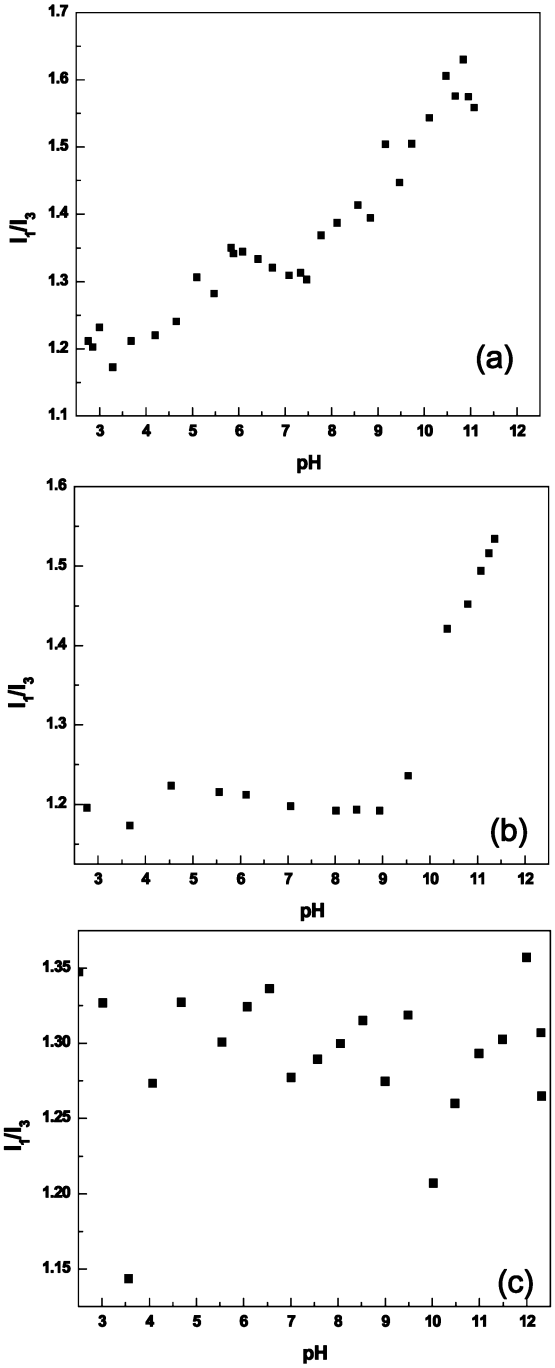

Figure 5. I_1/I_3 vs pH for samples PHOS−PMAA 1 (a), PHOS−PMAA 2 (b), and PHOS−PMAA 3 (c).

solutions. The dependence of I_1/I_3 ratio on the solution pH is shown in Figure 5 for three block copolymer samples with different compositions. In the case of PHOS−PMAA 1, i.e., the block copolymer with the lower PHOS content, the existence of two distinct regions where the I_1/I_3 ratio decreases rather sharply, with an intermediate plateau, are observed, which are attributed to the formation of hydrophobic domains due to protonation of PHOS (at the pH region 8.5−10) and PMAA blocks (at the pH region 3.5−5.5) (Figure 5a). It should be mentioned however that a completely hydrophobic character (i.e., hydrocarbon-like domains) is not observed in the first step (pH region 8.5−10), as indicated by the I_1/I_3 values in this region and the plateau, due to the fact that the hydroxyl groups of the PHOS blocks provide a relatively polar environment. Alternatively, the size of hydrophobic domains may not be sufficiently large in order to entrap the pyrene probes completely. In PHOS−PMAA 2 the content of PHOS is higher. The increased length of the PHOS block, which is insoluble at pH lower than 9, has as a consequence the creation of domains with a more intense hydrophobic character, leading to the decrease of I_1/I_3 ratio at its almost final values, just after the protonation of the PHOS block is completed (pH < 9, Figure 5b). Note also that the initial I_1/I_3 for this sample are lower than those for sample PHOS−PMAA 1, indicating an increase of the hydrophobic character in PHOS−PMAA 2 solutions even at high pH. The fluorescence data of PHOS−PMAA 3 sample are noticeable. This sample has the highest content of PHOS, and it has to be dissolved at pH 13 in order to be water-soluble. It is possible that even at this pH value some of the repeat units remain un-ionized, leading to the creation of hydrophobic domains by intra- or intermolecular assembly. These domains are possible to host the probe molecules, leading to a degreased I_1/I_3 ratio in the entire pH region under investigation (Figure 5c).

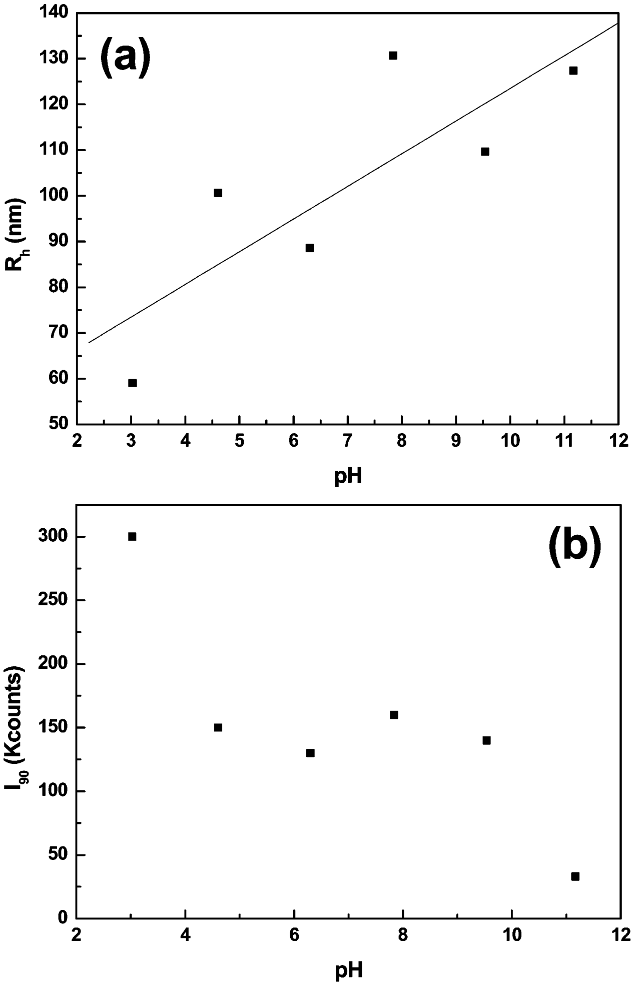

Figure 6. (a) Apparent hydrodynamic radius at 90°, R_h, vs pH for the sample PHOS−PMAA 1. The line is only a guide to the eye. (b) Light scattering intensity at 90°, I_{90}, vs pH for the same sample ($c_{pol} = 1 \times 10^{-3}$ g/mL).

Dynamic light scattering has been used in order to probe the formation of supramolecular structures at different pH values. It is expected that the existence of hydrophobic domains will lead to some kind of interchain aggregation and/or micelle formation as a function of pH. The light scattering data for PHOS−PMAA 1 are shown in Figure 6. Judging from the R_h values (Figure 6a) and the rather low molecular weight of the copolymer, the existence of supramolecular structures can be concluded, even at pH 12. The above observation is in agreement with the result from fluorescence spectroscopy that

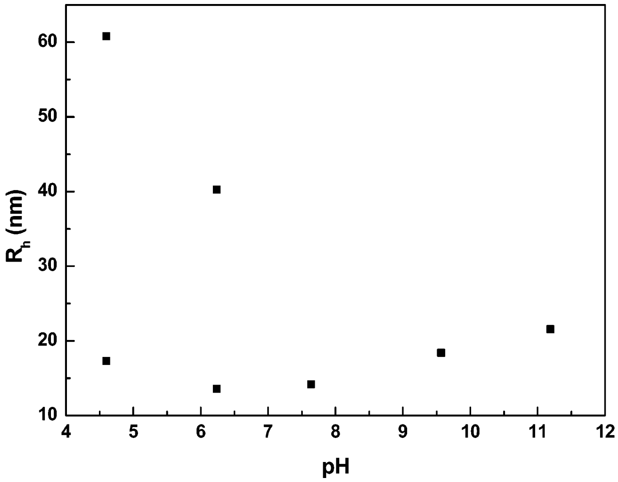

Figure 7. Apparent hydrodynamic radius vs pH for the sample PHOS−PMAA 2. Sample precipitates at pH lower than 4.

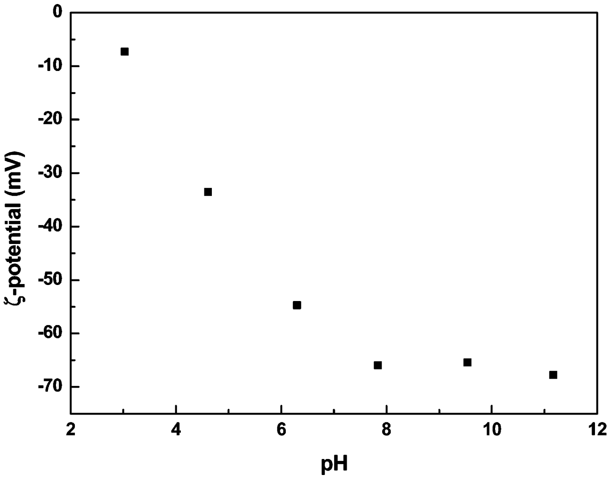

Figure 8. ζ-potential vs pH for the sample PHOS−PMAA 1($c_{\mathrm{pol}} = 1 \times 10^{-3}$ g/mL).

even at high pH values some un-ionized repeat units could be incorporated in the formation of hydrophobic domains which, in turn, could be correlated with interchain aggregates.[6b] Moreover, it is obvious, despite the large scattering of the experimental points, that the lower the solution pH, the smaller the supramolecular structures, because the number of the ionic sites is decreased by addition of HCl. Finally, at pH values lower than 4, the lowest R_h value is observed, even if precipitation is expected. The above observation could be probably explained by kinetic phenomena, considering that the macroscopically observed precipitation of the polymer is realized after standing for 24 h.

The creation of larger supramolecular structures at elevated pH values is rather surprising, considering that aggregation of the block copolymers is mainly expected by decreasing the solution pH. However, it has to be mentioned that the intensity of the scattered light is increasing during the addition of HCl in the solution (Figure 6b). The scattered light intensity at 90°, I_{90}, which is proportional to the mass of the aggregates, at pH ~11 is 33 kcounts and at $9 < \mathrm{pH} < 4.5$ is increased to ~140 kcounts, while at the more acidic pH region (pH ~ 3) the intensity value is more than 300 kcounts. Furthermore, a single peak is resolved by Contin analysis characterized by a polydispersity value of μ_2/Γ^2 from cumulants analysis larger than 0.1 at all pHs. The above results indicate that the aggregates have a loose structure at elevated pH values (large dimensions with low mass), while they become more compact upon acid addition (smaller dimensions and higher mass). The constancy of I_{90} values in the region $9 < \mathrm{pH} < 4.5$ may indicate the presence of micelles with a PHOS core and a PMAA corona. However, such micelles seem to be rather polydisperse in size. This polydispersity may be partly due to secondary interactions between the hydroxylated PHOS blocks and the carboxylated PMAA blocks of the same or different chains. Such interactions, in conjunction with hydrophobic interactions, can influence the way block copolymer chains are organized in a single micellar aggregate and the way separate aggregates interact with each other. Similar observations have been made in systems containing DHBC with marginally hydrophilic blocks or water-soluble chains with hydrophobic backbones.[1,6b]

Dynamic light scattering experiments on other samples of the series have led to similar results. Moreover, the existence of two populations, at low pH values, has been also observed in samples with high PHOS content, as shown for sample PHOS−PMAA 2 in Figure 7. The existence of two populations in this case at pH < 7 is probably related to the higher PHOS content and should be attributed to secondary interactions between aggregates as the solution pH decreases as discussed in the case of PHOS−PMAA 1. The population having the larger size must be a result of secondary aggregation between the initially formed aggregates at higher pH (characterized by the lower R_h values). The above experimental results are in agreement with macroscopic observations. PHOS−PMAA 1 precipitates after standing of the solution at pH 2 for at least 24 h while, in the case of samples with higher PHOS content, the precipitation of the polymer is observed immediately when the pH becomes lower than 4. The aforementioned macroscopic observations indicate the straightforward formation of large particles, which easily precipitate, at acidic conditions for samples with high PHOS content.

Further information on the solution behavior of the PHOS−PMAA copolymers was collected from ζ-potential measurements. Representative experimental results are shown in Figure 8. ζ-potential values remain negative at all pHs, indicating the presence of negatively charged particles in solution, as expected due to the presence of phenolate and carboxylate groups of the copolymers in the entire pH range. However, it is evident that the lower the pH values, the lower the absolute value of the ζ-potential; i.e., the system becomes more unstable as pH decreases. The decrease of the ζ-potential values can be explained by the protonation of phenolate and carboxylate groups during the titration. This behavior is common for all the copolymers under study. Moreover, it is worth noting that the higher the PHOS content among the polymers, the lower is the absolute value of the ζ-potential, at the same pH value. The results from ζ-potential measurements indicate, in agreement with the results from the other techniques, that the stability of the solutions is greatly influenced by the composition of the copolymer. In particular, increasing the PHOS content leads to more unstable copolymer solutions.

The existence of supramolecular structures even at elevated pH values has been also clearly observed by atomic force microscopy. AFM images (Figure 9) illustrate the existence of loose aggregates at the high pH values. Moreover, the formation of aggregates upon decreasing the solution pH could be also observed. In detail, at the alkaline pH region some loose isolated aggregates are observed with a height lower than 7 nm and width ~50 nm. At a first glance, this result is not in accordance with the data from dynamic light scattering experiments where particles with apparent diameters of more than 200 nm are

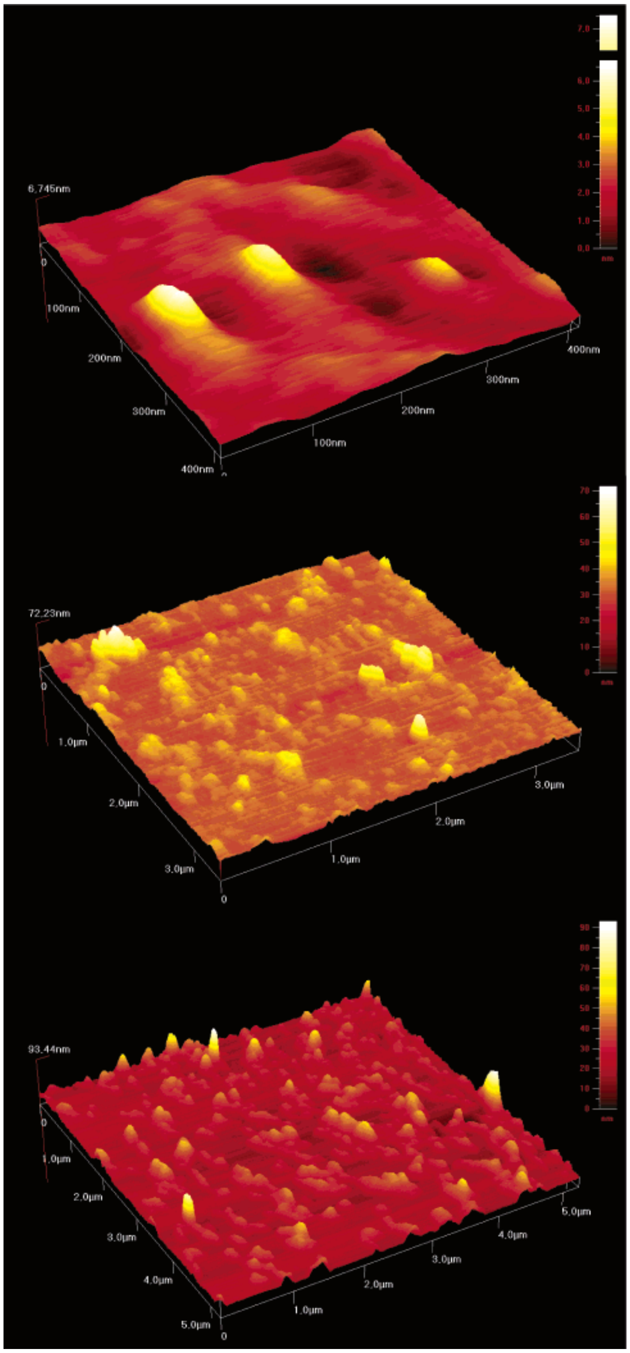

Figure 9. AFM images of PHOS−PMAA 1 aggregates at pH 11.4 (up), 7.0 (center), and 2.9 (bottom).

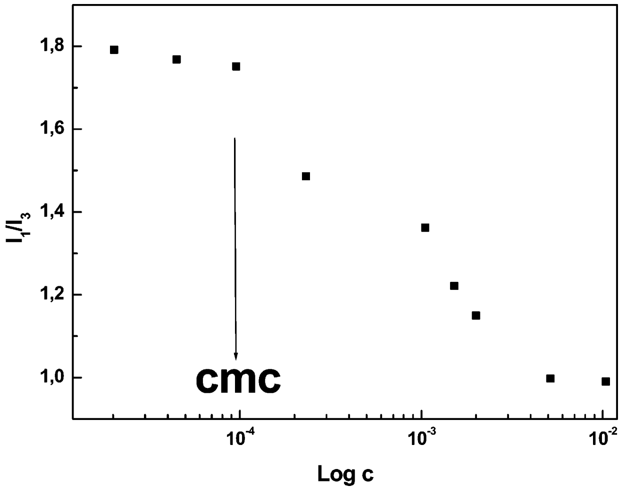

Figure 10. I_1/I_3 vs log c for the sample PHOS−PMAA 1.

observed. However, the explanation to this discrepancy should be the loose nature of the aggregates at these pH values. Loose aggregates can adopt a very flattened conformation on the solid surface and can show a very low profile and smaller dimensions in AFM measurements due to the difficulty in detecting the outermost, low-density regions of the aggregates. At lower pH aggregates show a higher profile and better defined spatial dimensions. Isolated micellar like aggregates can be observed in the region of intermediate pH (Figure 9, center) although some larger particles are also present, presumably from secondary aggregation of individual micelles. The calculated sizes and the observed polydispersity are in better agreement with the dynamic light scattering measurements. This secondary aggregation seems to take place to a larger extend at lower pH (Figure 9, bottom). This can be attributed to the more hydrophobic character of the supramolecular structures that are formed in this region.

The overall picture that emerges from the combined experimental results presented so far shows that loose aggregates are formed in aqueous solutions of PHOS−PMAA block copolymers at high pH (pH > 9) due to hydrophobic and secondary interactions originating from the hydrophobic character of the PHOS backbone and the phenolic/carboxylic acid groups present in the two blocks. As the solution pH decreases, the hydrophobic character of the PHOS blocks is increased, giving rise to the formation of micellar like aggregates at intermediate pH ($4 <$ pH < 9), with a PHOS core and a dissociated PMAA corona. Secondary interactions are still present in the system, favoring aggregation of primary aggregates (micelles) and resulting in a rather broad size distribution. These interactions may be facilitated from the rather hydrophilic character of the hydroxyl groups of the PHOS block that may still be able to interact with carboxylate groups of the PMAA chains belonging to the same or a different aggregate. At lower pH values (pH < 4) protonation of the PMAA chains renders the particles insoluble in water and leads to a precipitation of the copolymers.

To gain additional information on the supramolecular structures present in the intermediate pH range, the existence of a critical micelle concentration (cmc) for the samples synthesized has been investigated at pH 7, by aid of fluorescence spectroscopy. A representative plot of the I_1/I_3 ratio vs copolymer concentration is shown in Figure 10. The large I_1/I_3 values at low concentrations show that the aggregates present in the system at this pH dissociate if the solution is diluted sufficiently, indicating that an association mechanism similar to the one observed in equilibrium micelle formation takes place. The existence of a cmc indicates further that micellar like aggregates are formed at this pH. The experimentally determined cmc values are given in Table 3. The results show that the higher the PHOS content, i.e., the content of the hydrophobic block, the lower the cmc. Moreover, it is obvious that for samples with the same composition the higher the molecular weight of the copolymer, the lower the cmc. These variations are typical for block copolymer micelles in selective solvents.[24] It is worth

Table 3. Critical Micelle Concentrations for the PHOS−PMAA Samples Investigated

sample	PHOS−PMAA 1	PHOS−PMAA 2	PHOS−PMAA 3	PHOS−PMAA 4	PHOS−PMAA 5
cmc (g/mL)	9.03×10^{-5}	8.19×10^{-5}	7.07×10^{-5}	9.52×10^{-5}	6.40×10^{-5}

mentioning that for other amphiphilic block copolymers, like poly(styrene-*b*-methacrylic acid), the determined cmc values are at least 2 orders of magnitude lower.[25] The observed difference can be explained by the larger hydrophilic character of PHOS due to the presence of the hydroxyl groups.

Conclusions

Poly(*p*-hydroxystyrene-*b*-methacrylic acid) (PHOS−PMAA) double hydrophilic block copolymers were synthesized from poly(*p*-*tert*-butoxystyrene-*b*-*tert*-butyl methacrylate) precursors, prepared via anionic polymerization high-vacuum techniques, by postpolymerization hydrolysis reaction. The molecular characterization of the polymers indicates the well-defined structure of the copolymers as well as the convenient control over their molecular weight and composition. The pH responsive self-assembly behavior was studied in detail using a gamut of characterization techniques. Experimental results show a complex solution behavior, which depends on solution pH and the composition of the block copolymers. In summary, loose aggregates are formed at pH > 9, where phenolate and carboxylate groups are dissociated, as a result of hydrophobic interactions. Micellar like aggregates are present in solution at 4 < pH < 9 with a more compact structure having PHOS cores and PMAA coronas. Secondary interactions between aggregates seem to be present, leading to a broadening of size distribution. The copolymers precipitate from solutions at pH < 4, when PMAA chains become fully protonated. Finally, a typical variation of the cmc values of the micellar aggregates formed in the intermediate pH region was observed upon changes in molecular weight and composition. The above polymers have valuable potential in polymer synthesis. The presence of hydroxyl and carboxyl functional groups allows for the attachment of other macromolecular side chains and low molecular weight organic probes or drugs along the main block copolymer chain. Moreover, the pH responsive character of the supramolecular assemblies, formed by these novel block copolymers, is expected to find applications in water purification methodologies (if one considers the encapsulation of pyrene in their micelles, demonstrated in the course of the fluorescence spectroscopy experiments). The micelles can be used in order to encapsulate other hydrocarbon-based pollutants, especially those of phenolic type, due to their chemical similarity with the PHOS core forming block. The three “phase” states exhibited by the block copolymers in solution at different pHs (i.e., almost molecularly dissolved chains, micelles, and aggregates, precipitation) allow for a sequential encapsulation-separation process based on pH variation. Other nanotechnology-related applications may also be envisioned.

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References and Notes

(1) Colfen, H. *Macromol. Rapid Commun.* **2001**, *22*, 219−252.

(2) Butun, V.; Liu, S.; Weaver, J. V. M.; Bories-Azeau, X.; Cai, Y.; Armes, S. P. *React. Funct. Polym.* **2006**, *66*, 157−165.

(3) Nishiyama, N.; Kataoka, K. *Adv. Polym. Sci.* **2006**, *193*, 67−101.

(4) (a) Qi, L.; Colfen, H.; Antonietti, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 604−607. (b) Marentette, M.; Norwig, J.; Stockelmann, E.; Meyer, W. H.; Wegner, G. *Adv. Mater.* **1997**, *9*, 647−651. (c) Colfen, H.; Antonietti, M. *Langmuir* **1998**, *14*, 582−589. (d) Bronstein, L. M.; Sidorov, S. N.; Gourkova, A. Y.; Valetsky, P. M.; Hartmann, J.; Breulmann, M.; Colfen, H.; Antonietti, M. *Inorg. Chim. Acta* **1998**, *280*, 348−354. (e) Sidorov, S. N.; Bronstein, L. M.; Valetsky, P. M.; Hartmann, J.; Colfen, H.; Schnablegger, H.; Antonietti, M. *J. Colloid Interface Sci.* **1999**, *212*, 197−211.

(5) (a) Webber, G. B.; Wanless, E. J.; Armes, S. P.; Tang, Y.; Li, Y.; Biggs, S. *Adv. Mater.* **2004**, *16*, 1794−1798. (b) Mahlting, B.; Gohy, J. F.; Antoun, S.; Jerome, R.; Stamm, M. *Colloid Polym. Sci.* **2002**, *280*, 495−502. (c) Mahltig, B.; Gohy, J. F.; Jerome, R.; Stamm, M. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 709−718. (d) Hamley, W.; Connell, S. D.; Collins, S. *Macromolecules* **2004**, *37*, 5337−5351.

(6) (a) Martin, T. J.; Prochazka, K.; Munk, P.; Webber, S. E. *Macromolecules* **1996**, *29*, 6071−6073. (b) Vamvakaki, M.; Papoutsakis, L.; Katsamanis, V.; Afchoudia, T.; Fragouli, P. G.; Iatrou, H.; Hadjichristidis, N.; Armes, S. P.; Sidorov, S.; Zhirov, D.; Zhirov, V.; Kostylev, M.; Bronstein, L. M.; Anastasiadis, S. H. *Faraday Discuss.* **2005**, *128*, 129−147.

(7) Pispas, S. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 606−613.

(8) Butun, V.; Billingham, N. C.; Armes, S. P. *Chem. Commun.* **1997**, 671−672.

(9) Lee, S.; Gast, A. P.; Butun, V.; Armes, S. P. *Macromolecules* **1999**, *32*, 4302−4310.

(10) (a) Butun, V.; Bennett, C. E.; Vamvakaki, M.; Lowe, A. P.; Billingham, N. C.; Armes, S. P. *J. Mater. Chem.* **1997**, *7*, 1693−1695. (b) Webber, G. B.; Wanless, E. J.; Armes, S. P.; Biggs, S. *Faraday Discuss.* **2005**, *128*, 193−209.

(11) Holappa, S.; Kantonen, L.; Winnik, F. M.; Tenhu, H. *Macromolecules* **2004**, *37*, 7008−7018.

(12) Dufresne, M. H.; Garrec, D. L.; Sant, V.; Leroux, J. C.; Ranger, M. *Int. J. Pharm.* **2004**, *277*, 81−90.

(13) Azeau, X. B.; Armes, S. P.; van den Haak, H. J. W. *Macromolecules* **2004**, *37*, 2348−2352.

(14) Wang, C.; Ravi, P.; Tam, K. C.; Gan, L. H. *J. Phys. Chem. B* **2004**, *108*, 1621−1627.

(15) Creutz, S.; Jerome, R. *Langmuir* **1999**, *15*, 7145−7156.

(16) Gabaston, L. I.; Furlong, S. A.; Jackson, R. A.; Armes, S. P. *Polymer* **1999**, *40*, 4505−4514.

(17) Andre, X.; Zhang, M.; Muller, A. H. E. *Macromol. Rapid Commun.* **2005**, *26*, 558−563.

(18) Rodriguez-Hernadez, J.; Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026−2027.

(19) Vandermeulen, G. W. M.; Tziatzios, C.; Duncan, R.; Klok, H. A. *Macromolecules* **2005**, *38*, 761−769.

(20) (a) Hadjichrisitidis, N.; Iatrou, H.; Pispas, S.; Pitsikalis, M. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 3211−3234. (b) Uhrig, D.; Mays, J. W. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 6179−6222.

(21) (a) Jannasch, P. *Macromolecules* **2000**, *33*, 8604−8610. (b) Se, K.; Miyawaki, K.; Hirahara, K.; Takano, A.; Fujimoto, T. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 3021−3034. (c) Se, K. *Prog. Polym. Sci.* **2003**, *28*, 583−618.

(22) Baskaran, D. *Prog. Polym. Sci.* **2003**, *28*, 521−581.

(23) Szczubialka, K.; Ishikawa, K.; Morishima, Y. *Langmuir* **1999**, *15*, 454−462.

(24) (a) Hamley, I. A. *The Physics of Block Copolymers*; Oxford University Press: Oxford, 1998. (b) Hadjichristidis, N.; Pispas, S.; Floudas, G. *Block Copolymers: Synthetic Strategies, Physical Properties and Applications*; Wiley-Interscience: New York, 2002. (c) Alexandridis, P., Lindman, B., Eds.; *Amphiphilic Block Copolymers. Self-Assembly and Applications*; Elsevier: Amsterdam, 2000.

(25) Lysenko, E. A.; Bronich, T. K.; Slonkina, E. V.; Eisenberg, A.; Kabanov, V. A.; Kabanov, A. V. *Macromolecules* **2002**, *35*, 6351−6361.

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