

Thermoresponsive Reversible Behavior of Multistimuli Pluronic-Based Pentablock Copolymer at the Air–Water Interface[†]

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Surface behavior of the pH- and thermoresponsive amphiphilic ABCBA pentablock copolymer has been studied with respect to the environmental conditions. We demonstrate that the pentablock copolymer poly((diethylaminoethyl methacrylate)-*b*-(ethylene oxide)-*b*-(propylene oxide)-*b*-(ethylene oxide)-*b*-(diethylaminoethyl methacrylate)) possesses reversible temperature changes at the air–water interface in a narrow pH range of the water subphase. Significant diversity in the surface morphology of pentablock copolymer monolayers at different pH and temperatures observed were related to the corresponding reorganization of central and terminal blocks. Remarkable reversible variations of the surface pressure observed for the Langmuir monolayers at pH 7.4 in the course of heating and cooling between 27 and 50 °C is associated with conformational transformations of terminal blocks crossing the phase line in the vicinity of the lower critical solution temperature point. The observed thermoresponsive surface behavior can be exploited for modeling of the corresponding behavior of pentablock copolymers adsorbed onto various biointerfaces for intracellular delivery for deeper understanding of stimuli-responsive transformations relevant to controlled drug and biomolecules release and retention.

Introduction

Amphiphilic copolymers with different architectures and chemical compositions have been widely studied recently for their adaptive properties related to responses to various environmental conditions.^{1–7} They have been recognized as versatile materials for a large number of applications, including drug delivery systems, gene therapy, adaptive lubricants, and other “smart” surface coatings.^{8–12} Photoresponsive surface layers with incorporated photochromic groups reversibly responsive to the particular light illumination and binary brush layers with the ability to respond to the quality of solvent have been very recently demonstrated.^{13–16} Linear block copolymers are an attractive class of polymers for these applications due to their relatively

simple synthesis, their ability to form micelle structures in aqueous solution, and their wide availability through commercial production.^{17–21}

One of the most interesting applications in the biorelease-related field were found for responsive materials based on the amphiphilic triblock copolymer poly((ethylene oxide)-*b*-(propylene oxide)-*b*-(ethylene oxide)) (PEO–PPO–PEO) commercially known as Pluronic or Poloxamer. The PEO–PPO–PEO chain can be modified by varying the length of each block so that the final chain exhibits the desired thermoresponsive gelation properties at physiological conditions for injectable delivery.^{17,22} These gels can then dissolve slowly to release polymer micelles loaded with drugs. This base system can then be used as the central building block for further modification by adding various functional groups to both ends of the chain. To

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(1) (a) Luzinov, I.; Minko, S.; Tsukruk, V. V. *Prog. Polym. Sci.* **2004**, *29*, 635–698. (b) Tsukruk, V. V. *Prog. Polym. Sci.* **1997**, *22*, 247–269.

(2) *Responsive Polymer Materials: Design and Applications*; Minko, S., Ed.; Blackwell Publishing: Ames, IA, 2006.

(3) Tsitsilianis, C. In *Responsive Polymer Materials: Design and Applications*; Minko, S., Ed.; Blackwell Publishing: Ames, IA, 2006; pp 27–49.

(4) Verdonck, B.; Gohy, J. F.; Khoussakoun, E.; Jérôme, R.; Du Prez, F. *Polymer* **2005**, *46*, 9899–9907.

(5) Xiong, X. Y.; Tam, K. C.; Gan, L. H. *Polymer* **2005**, *46*, 1841–1850.

(6) Xu, C.; Fu, X.; Fryd, M.; Xu, S.; Wayland, B. B.; Winey, K. I.; Composto, R. J. *Nano Lett.* **2006**, *6*, 282–287.

(7) Sfika, V.; Tsitsilianis, C. *Macromolecules* **2004**, *37*, 9551–9560.

(8) Tan, J. F.; Ravi, P.; Too, H. P.; Hatton, T. A.; Tam, K. C. *Biomacromolecules* **2005**, *6*, 498–506.

(9) Tsukruk, V. V. *Adv. Mater.* **2001**, *13*, 95–108.

(10) Santer, S.; Kopyshin, A.; Donges, J.; Yang, H.-K.; Rühle, J. *Macromolecules* **2006**, *22*, 4660–4667.

(11) Liu, Y.; Klep, V.; Zdyrko, B.; Luzinov, I. *Langmuir* **2005**, *21*, 11806–11813.

(12) Kim, Y.; Pyun, J.; Frechet, J. M. J.; Hawker, C. J.; Frank, C. *Langmuir* **2005**, *21*, 10444–10458.

(13) (a) Genson, K. L.; Holzmüller, J.; Villacencio, O. F.; McGrath, D. V.; Vaknin, D.; Tsukruk, V. V. *J. Phys. Chem. B* **2005**, *109*, 20393–20402. (b) Genson, K. L.; Vaknin, D.; Villavicencio, O. F.; Holzmüller, J.; McGrath, D. V.; Tsukruk, V. V. *Thin Solid Films* **2005**, *493*, 237–248. (c) Sidorenko, A.; Houphouët-Boigny, C.; Villavicencio, O.; McGrath, D. V.; Tsukruk, V. V. *Thin Solid Films* **2002**, *410*, 147–154. (d) Peleshanko, S.; Sidorenko, A.; Larson, K.; Villavicencio, O.; Ornatska, M.; McGrath, D. V.; Tsukruk, V. V. *Thin Solid Films* **2002**, *406*, 233–240. (e) Tsukruk, V. V.; Luzinov, I.; Larson, K.; Li, S.; McGrath, D. V. *J. Mater. Sci. Lett.* **2001**, *20*, 873–876.

(14) (a) Lemieux, M.; Usov, D.; Stamm, M.; Shulha, H.; Tsukruk, V. V. *Macromolecules* **2003**, *36*, 7244–7255. (b) Lemieux, M. C.; Julthongpipit, D.; Duc Cuong, P.; Ahn, H.-S.; Lin, Y.-H.; Tsukruk, V. V. *Langmuir* **2004**, *20*, 10046–10054.

(15) Julthongpipit, D.; Lin, Y.-H.; Teng, J.; Zubarev, E. R.; Tsukruk, V. V. *J. Am. Chem. Soc.* **2003**, *125*, 15912–15921.

(16) Lin, Y.-H.; Teng, J.; Zubarev, E. R.; Shulha, H.; Tsukruk, V. V. *Nano Lett.* **2005**, *5*, 491–495.

(17) Alexandridis, P.; Hatton, T. A. *Colloids Surf., A* **1994**, *96*, 1–46.

(18) Mertoglu, M.; Garnier, S.; Laschewsky, A.; Skrabania, K.; Storsberg, J. *Polymer* **2005**, *46*, 7726–7740.

(19) Jankova, K.; Chen, X.; Kops, J.; Batsberg, W. *Macromolecules* **1998**, *31*, 538–541.

(20) Patten, T. E.; Matyjaszewski, K. *Adv. Mater.* **1998**, *10*, 901–915.

(21) Bernaerts, K. V.; Willet, N.; Van Camp, W.; Jérôme, R.; Du Prez, F. *Macromolecules* **2006**, *39*, 3760–3769.

(22) Liang, X.; Mao, G.; Ng, K. Y. S. *J. Colloid Interface Sci.* **2004**, *285*, 360–372.

Table 1. Molecular Characteristics of the Polymers

name	sample	M_n^{NMR}	PDI ^{GPC}
PI-Br (Pluronic-Br)	Br-PEO ₁₀₀ -PPO ₆₅ -PEO ₁₀₀ -Br	13 640	1.18
pentablock, PB	PDEAEM ₂₅ -PEO ₁₀₀ -PPO ₆₅ -PEO ₁₀₀ -PDEAEM ₂₅	22 000	1.34

make the thermoreversible gels effective for stimuli-sensitive drug delivery, the system must be responsive to both temperature and another stimulus such as pH. The pH response of these copolymers is dependent on the terminal blocks that this system is modified with, an example being a polyelectrolyte. The pentablock copolymer that results at the end of synthesis can be used in specific applications of targeted stimuli-sensitive drug delivery of insulin via aqueous solution, as well as controlled gene therapy through complexation with DNA.^{23,8,27} The resulting pentablock copolymer will show a change to environmental stimuli by varying its micelle structure in response to both pH and temperature.²⁴ This dual response is characteristic of the lower critical solution temperature (LCST) of PEO and PPO and the polyelectrolyte properties of the terminal blocks.²⁴

Surface interactions are important in drug delivery since it is hoped that a single injection of the carrier-drug system can be made so that it can then be carried to the site by the body. The carrier will need to be able to leave the blood stream and enter an affected cell through surface interaction where the drug is released by a specific temperature or pH condition.²⁵ When looking at the hydrophobic-hydrophilic balance for the Pluronic, a difference in the solubility of the PEO and the PPO plays a critical role. The PEO block is fully soluble in water at room temperature, where PPO is only partially soluble. This variation in solubility will have an effect on the micelle structure at the particular solution concentration.²⁶ It has also been observed that the drug release rate from the pentablock copolymer can be controlled on the basis of environmental conditions since the hydrophobicity of the polyelectrolyte blocks can change with pH.²⁵ It is also this hydrophobicity that determines how the micelles will be formed in the solution since it has an impact on the concentration of polymer in the solution, as more of the PEO is soluble than PPO.²⁶

Characterization of the system that is responsive to pH and temperature spread at the air-water interface and which can be used in drug delivery systems is a primary focus of the study reported here. To address this task, Pluronic was modified by attaching a functional block, poly(diethylaminoethyl methacrylate) (PDEAEM), to the ends of the central triblock copolymer to form the amphiphilic pentablock copolymer poly((diethylaminoethyl methacrylate)-*b*-(ethylene oxide)-*b*-(propylene oxide)-*b*-(ethylene oxide)-*b*-(diethylaminoethyl methacrylate)) that exhibits a dual response to temperature and pH by forming micelles.²⁴ The PDEAEM which was used to construct the pentablock copolymer can be attached to the Pluronic by atom transfer radical polymerization (ATRP) with Br-terminated Pluronic used as the macroinitiator according to the complete reaction routine described previously.²⁷ In addition, PDEAEM block also exhibits a LCST in water. While the stimuli-responsive behavior of these pentablock copolymers and their self-assembly

properties have been investigated in solutions, the surface studies presented here will provide an important evidence as to how the pentablock copolymers will behave when they interact with various interfaces in the body during drug delivery and how the structure will change when the carrier reaches the target site and changes its structure to release the drug. Although a significant fraction of events in these cases occurs at the liquid-liquid and liquid-solid interfaces, the presence of the micro and nano air bubbles in the complex aqueous media can play significant role in the behavior of stimuli-responsive materials used for drug delivery placing these materials at the gas-water interfaces. This is even more important in the case when these materials are used for external delivery via skin or mucosal delivery or in the form of nanocapsules where air-water interfaces play an important role. On the other hand, as a first level of approximation, the air-water interface can be considered as a model interface between good and bad solvents for particular blocks (e.g., oil-water) in which air can be considered as an extreme example of a "bad solvent" for polymers.

This modification to the Pluronic material allows for the molecule to be used as a carrier for a drug that can be tuned for controlled release. When the pentablock copolymer carrier reacts to the change in temperature and pH, it will change its micellar structure, allowing the drug to be released at a specific site and eliminating the need for removal of the drug vector since the pentablock copolymer is water soluble and can be excreted from the body.^{23,27,25} For this delivery technique to be successful, the interactions at the interface boundaries must be understood and characterized in detail. This full understanding of the surface interactions and phase transformations of the pentablock copolymer under variable environmental conditions is essential for the implementation of this material as a viable drug delivery material. However, unambiguous characterization of molecular transformations, micellar restructuring, and the corresponding alternation of the surface properties in response to external stimuli is a tremendously challenging task which was completed only for a few systems.

Our study focuses on this task by beginning with the deposition and collection of surface isotherm data of the described pentablock copolymer on clean silicon wafers using known Langmuir-Blodgett (LB) technique.²⁸ This was done at different temperature and pH values so that it could be seen how the molecule behavior changed under these different external conditions. These samples were characterized by ellipsometry to measure the thicknesses of the LB monolayers and by atomic force microscopy (AFM) to visualize the morphology and microstructure of the surfaces. This paper address the kinds of changes that occur in the micelle structure of the pentablock copolymers under different temperature and pH conditions and how these structural reorganizations affect the copolymer behavior at the air-water interface.

Experimental Section

Materials. The pentablock copolymer poly((diethylaminoethyl methacrylate)-*b*-(ethylene oxide)-*b*-(propylene oxide)-*b*-(ethylene oxide)-*b*-(diethylaminoethyl methacrylate)) (PDEAEM₂₅-PEO₁₀₀-PPO₆₅-PEO₁₀₀-PDEAEM₂₅) was synthesized by ATRP from Br-terminated Pluronic macroinitiator. Details of the synthesis and

(23) Vamvakaki, M.; Papoutsakis, L.; Katsamanis, V.; Afchoudia, T.; Fragouli, P.; Iatrou, H.; Hadjihristidis, N.; Armes, S. P.; Sidorov, S.; Zhurov, D.; Zhurov, V.; Kostylev, M.; Bronstein, L.; Anastasiadis, S. H. *J. R. Soc. Chem.* **2004**, *128*, 129-147.

(24) Determan, M. D.; Guo, L.; Thiyagarajan, P.; Mallapragada, S. K. *Langmuir* **2006**, *22*, 1469-1473.

(25) Determan, M. D.; Cox, J. P.; Mallapragada, S. K. *J. Biomed. Mater. Res.* **2006**, in press.

(26) Munoz, M. G.; Monroy, F.; Ortega, F.; Rubio, R. G.; Langevin, D. *Langmuir* **2000**, *16*, 1083-1093.

(27) Determan, M. D.; Cox, J. P.; Seifert, S.; Thiyagarajan, P.; Mallapragada, S. K. *Polymer* **2005**, *46*, 6933-6946.

(28) Ulman, A. *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self-Assembly*; Academic Press: Boston, 1991.

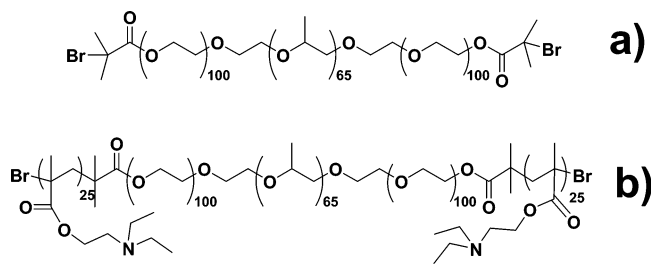


Figure 1. Chemical formulas of the initial ATRP macroinitiator **PI-Br**(a) (Pluronic-Br) and corresponding **PDEAEM₂₅-PEO₁₀₀-PPO₆₅-PEO₁₀₀-PDEAEM₂₅** pentablock (**PB**) copolymer (b).

characterization of these block copolymers were described previously.²⁷ The molecular weight, polydispersity, and chemical composition were verified using the combination of GPC and ¹H NMR, as represented in Figure 1 and Table 1. For further discussion, the notation **PI-Br** will be used to refer to Br-terminated Pluronic polymers and pentablock (**PB**) to amphiphilic **PDEAEM₂₅-PEO₁₀₀-PPO₆₅-PEO₁₀₀-PDEAEM₂₅** copolymer studied here (Table 1).

Monolayer Fabrication. Langmuir isotherms at the air–water interface and LB deposition onto a silicon substrate were conducted at different temperatures using a KSV 2000 mini-trough assembled with water bath temperature controller according to the usual procedure.²⁸ Dilute polymer solution (50–60 μ L, concentration less than 0.05 mg/mL) in chloroform (HPLC grade) was deposited in 12–15 drops uniformly distributed onto the water surface (Nanopure, 18 M Ω cm) and left to evaporate and spread evenly over a period of 30 min. The pH of the water subphase was adjusted by adding solution of 0.1 M HCl or 0.1 M NaOH. The surface molecular area, A_1 , was calculated as the point of initial appearance of a steep rise in the surface pressure. Highly polished [100] silicon wafers (Semiconductor Processing Co.) were cut into rectangular pieces (2 \times 1.5 cm²) and sonicated in Nanopure water for 10 min to remove silicon dust. The wafers were then chemically treated with “piranha solution” (30% concentrated hydrogen peroxide, 70% concentrated sulfuric acid, *hazardous solution!*) for 1 h to remove organic and inorganic contaminants and to strip the original silicon oxide surface layer and thermally grow a new fresh layer.²⁹ Finally, wafers were abundantly rinsed with Nanopure water and dried with dry nitrogen. During LB deposition, the surface pressure was held constant as the submerged substrate was slowly lifted from the trough at a rate of 3 mm/min. The temperature variation of the surface pressure of the Langmuir monolayer was tested by keeping the molecular area constant while slowly varying the temperature of the water subphase from 27 $^{\circ}$ C to about 50 $^{\circ}$ C and back and monitoring the surface pressure (about 40 min for a complete heating–cooling cycle).

Monolayer Characterization. The effective thickness of the LB monolayers was measured with a COMPEL automatic ellipsometer (InOmTech, Inc.) at an incident angle of 70 $^{\circ}$ and a wavelength of 634 nm according to the well-known procedure.³⁰ The refractive index of monolayers was taken as 1.45, as was suggested earlier for similar block copolymers.³¹ A possible variation of the composite refractive index because of the presence of the terminal blocks and partial swelling was estimated introducing error within ± 0.1 nm in most cases thus insignificantly affecting the thickness evaluation. The LB monolayers on the silicon substrates were studied with a Dimension-3000 AFM microscope in the “light” tapping mode in accordance to the usual procedure adapted in our lab.³² An amplitude ratio of 0.95 and higher was employed to avoid monolayer damage.³³ AFM characterization of the deposited LB monolayers was done

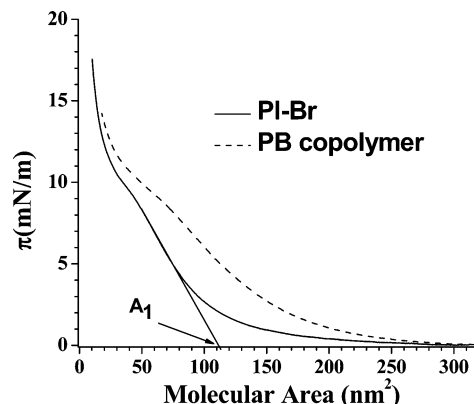


Figure 2. Pressure–area (π – A) isotherms of the **PI-Br** and amphiphilic **PB** copolymer at pH 7.4 and temperature 25 $^{\circ}$ C.

after drying in a desiccator for 24 h. The AFM scans were conducted at 0.5–1.5 Hz scanning rate for surface areas ranging from 20 \times 20 to 1 \times 1 μ m² at several randomly selected locations with at least 20 different images collected for each specimen. The AFM tip radii were between 20 and 35 nm and the spring constants of these cantilevers were in the range of 40–60 N/m.

Results & Discussion

Chemical Composition. The chemical structures of the brominated Pluronic macroinitiator (**PI-Br**) and the subsequent pentablock copolymer (**PB**) produced by ATRP synthesis are shown in Figure 1. Relatively narrow PDI values were obtained for the **PB** copolymer produced by this route (Table 1). The molecular weights of the copolymers were maintained below 25 000 to allow for renal excretion in a drug delivery application.

General Surface Behavior at the Air–Water Interface. Both copolymers studied here formed stable Langmuir monolayers at the air–water interface, indicating proper amphiphilic balance (see π – A isotherms in Figure 2). The monolayers are stable up to 20 mN/m, and the surface molecular area reaches 20 nm² in the precollapsed state. Considering that this type of block copolymer is prone to nonequilibrium behavior at the air–water interface,^{31,34} we conducted preliminary study of conditions under which we can obtain virtually reversible and repeatable behavior indicating close to equilibrium state. The surface-pressure isotherms have been collected at several different barrier speeds and waiting times, as well as at multiple compression–relaxation cycles, all of which generated very similar shapes and parameters. The reversibility of the Langmuir monolayers was examined by repeating cycles of compression and expansion within the low-pressure (<5 mN/m) regime. A minor hysteresis observed (10–15% surface area) in several particular cases for the **PI-Br** at pH 7.4 and **PB** at pH 7.4 and 10 indicated partially irreversible behavior due to the presence of a larger fraction of hydrophobic phase aggregated at the higher surface pressure which is not included in current report (not shown). In this state, a random corrugation of the LB monolayer with submicrometer island formation was observed which is very different from surface morphologies discussed below. Moreover, as will be demonstrated below, completely reversible temperature–pressure cycles can be obtained for these monolayers with minor creep related to the water level drift indicating predominantly reversible character of the molecular reorganization within Langmuir monolayers under conditions exploited here.

The increase in molecular weight of the pentablock copolymer **PB** (Table 1) is translated into overall shift of the isotherm toward

(29) Tsukruk, V. V.; Bliznyuk, V. N. *Langmuir* **1998**, *14*, 446–455.

(30) Azzam, R. M. A.; Bashara, N. M. *Ellipsometry and Polarized Light*; North-Holland Publishing Co.: New York, 1977.

(31) Muñoz, M. G.; Monroy, F.; Ortega, F.; Rubio, R. G.; Langevin, D. *Langmuir* **2000**, *16*, 1083–1093.

(32) (a) Tsukruk, V. V.; Reneker, D. H. *Polymer* **1995**, *36*, 1791–1808. (b) Tsukruk, V. V. *Rubber Chem. Technol.* **1997**, *70*, 430–467.

(33) Magonov, S. N.; Elings, V.; Whangbo, M.-H. *Surf. Sci.* **1997**, *375*, L385–L391.

(34) Baker, S. M.; Leach, K. A.; Devereaux, C. E.; Gragson, D. E. *Macromolecules* **2000**, *33*, 5432–5436.

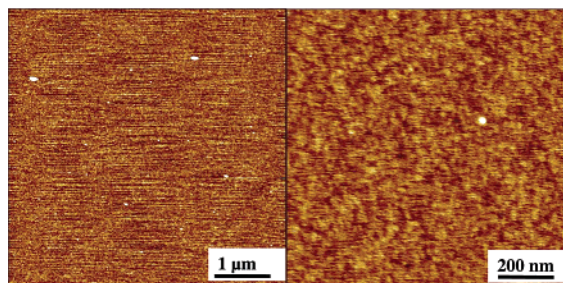


Figure 3. AFM topography of macroinitiator **PI-Br** monolayers deposited at surface pressure $\pi = 5$ mN/m and 25 °C. Z-scale is 2 nm.

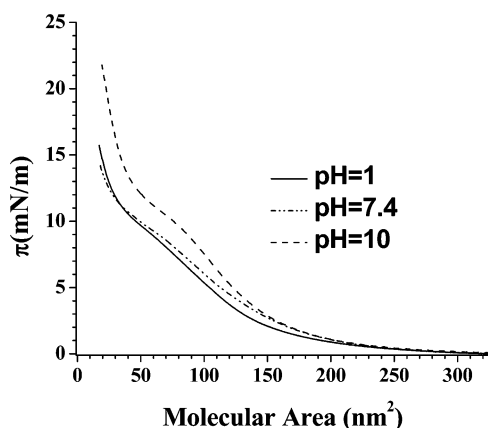


Figure 4. Pressure–area (π – A) isotherms of the **PB** copolymer at different pH (25 °C).

Table 2. Physical Characteristics of Monolayer of Amphiphilic Copolymers

sample and conditions	area per molecule A_1 , nm ²	thickness at $\pi = 5$ mN/m nm	microroughness nm
PI-Br , pH 7.4 25°C	110	0.42	0.2
PB , pH 1 25°C	155	0.56	0.4
PB , pH 7.4 25°C	174	1.46	0.3
PB , pH 7.4 50°C	188	0.75	0.2
PB , pH 10 25°C	170	0.54	0.4

larger surface area (Figure 2) compared to the **PI-Br**. The surface molecular area, A_1 , of the monolayer in the condensed state increases by 58%, which is only slightly below the increase in molecular weight by 60% (Tables 2 and 1). This insignificant difference indicates that the terminal **PDEAEM** blocks are completely spread at the air–water interface due to the insufficient ionization to submerge into the water subphase at pH 7.4 (Table 2).

The LB monolayer from **PI-Br** shows smooth surface morphology with the surface microroughness about 0.2 nm (calculated within $1 \times 1 \mu\text{m}^2$) and the fine texture with domain dimensions below 50 nm (Figure 3). The effective thickness of the LB monolayer at low surface pressure is 0.42 nm, and that points to complete spreading of macromolecular backbones on a hydrophilic silicon surface.

Surface Behavior at Different pH. The variation of the subphase pH resulted in some shifts of the surface isotherms for **PB** with overall preservation of their shapes (Figure 4). These differences indicate minor changes in the amphiphilic balance between different blocks without dramatic changes of the overall monolayer structure. Decreasing pH from 10 to 1 resulted in the formation of slightly more dense Langmuir monolayers, indicating more collapsed structure of the blocks situated at the air–water interface (Figure 4). The surface molecular area, A_1 , is the lowest

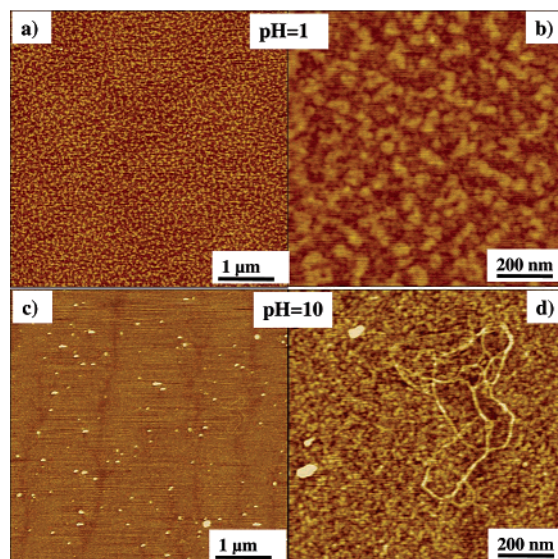


Figure 5. AFM topography of **PB** monolayers deposited at the surface pressure $\pi = 5$ mN/m and pH 1 (a and b) and pH 10 (c and d) at 25 °C. Z-scales are 5 (a–c) and 2 nm (d).

for pH 1 (10% lower than for higher pH, Table 2) indicating that the degree of the protonation of the terminal **PDEAEM** blocks affects their state at the air–water interface and their ability to submerge into the water subphase. The critical role of end-groups on surface behavior of block copolymers was demonstrated in our earlier studies.³⁵

The LB monolayers deposited at two limiting values of pH (1 and 10) showed distinctly different surface morphologies (Figure 5). First, LB monolayer fabricated at pH 1 showed very fine surface texture with clearly visible domains (Figure 5a). The lateral dimensions of these domain structures do not exceed 80 nm. The overall texture is much coarser and lateral dimensions of domains are slightly higher than that observed for **PI-Br** monolayer. In addition, all LB monolayers fabricated from **PB** are slightly (20%) thicker than the LB monolayers from **PI-Br** (Table 2). All these differences are apparently caused by the presence of additional terminal hydrophobic blocks. Finally, the LB monolayer fabricated at pH 10 showed a more uniform surface with occasionally visible threadlike structures and a number of collapsed aggregates which we suggest are collapsed and aggregated fibrils (Figure 5b). The diameter of these fibrillar structures in a dry state is between 1 and 10 nm, and their length can reach several hundred nanometers.

Surface Behavior at Different Temperatures. The variation of temperature of the water subphase played an insignificant role in the surface behavior of **PB** copolymer at very acidic or basic conditions. In both cases, the surface–pressure isotherms at 25 and 50 °C were virtually identical except for some minor deviations at very high surface pressures preceding the monolayer collapse indicating very minor temperature-dependence of block copolymer conformation under given protonation conditions (Figure 6a). However, at the physiological pH of 7.4, which is of interest in drug delivery applications, a significant shift to the higher surface pressures was observed at elevated temperatures (Figure 6b). The surface area per molecule at a constant pressure increased by 10–15% and the surface pressure at a constant molecular area increased by 15–40%, indicating significant molecular reorganization accompanying temperature variation under given ionic conditions. At a pH of 1, the **PDEAEM** blocks

(35) Gunawidjaja, R.; Peleshanko, S.; Tsukruk, V. V. *Macromolecules* **2005**, *38*, 8765–8774.

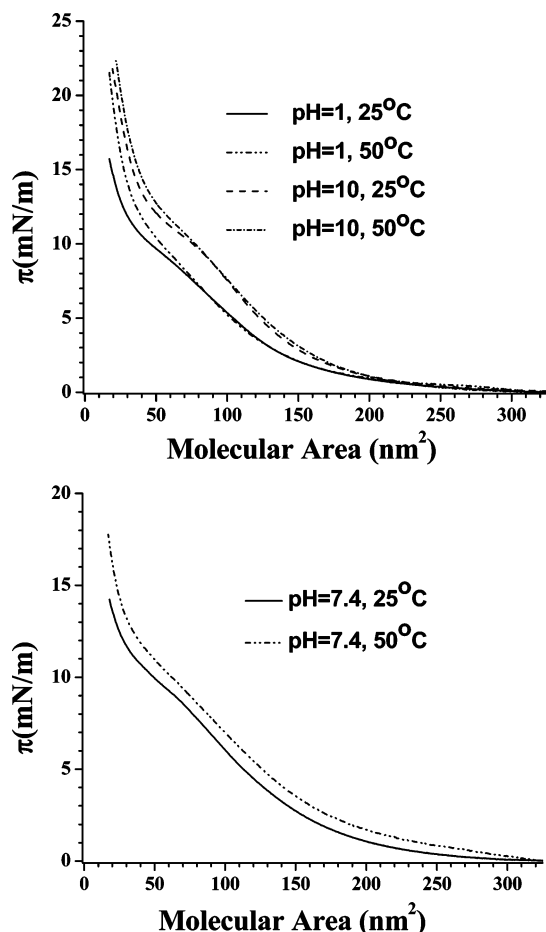


Figure 6. Pressure–area (π – A) isotherms of the **PB** copolymer at different pH and temperatures.

are fully protonated, while at a pH of 10, the **PDEAM** blocks are fully deprotonated.¹⁶ However, in the pH range of 7.4, which is close to the pK_a of **PDEAEM**, these blocks are partially ionized and the interplay between concurrent responses to pH and temperature is most evident in this regime.

AFM imaging of LB monolayers of **PB** deposited at different temperatures showed that relatively smooth surface morphology of the monolayer deposited at 25 °C is transformed to more heterogeneous morphology with clearly visible surface aggregates and very fine domains clearly visible for the monolayer deposited at 50 °C (Figure 7). Moreover, the monolayer thickness reduced dramatically from 1.46 to 0.75 nm, indicating significant reorganization and segregation of **PB** copolymers in both lateral and vertical directions (Table 2). The much lower (twice) monolayer thickness at 50 °C can be caused by the collapse of the **PDEAEM** blocks expected above LCST and the trend of these collapsed blocks to segregate laterally from the central hydrophilic blocks leading to more spread morphology with the reduced effective thickness of the monolayer.

Considering these significant and easily detectable changes in the surface properties, we conducted additional experiments to reveal the reversible character of the temperature-induced transformation of pentablock studied here. For this experiment, we kept constant either the surface pressure or the surface area per molecule and monitored the variation of the surface area or the surface pressure, respectively, while gradually changing the temperature of the water subphase from 27 to 50 °C in a cyclic manner (Figure 8). In these experiments, we observed both reversible variation of surface pressure and the surface molecular area, with the former being much more pronounced and thus

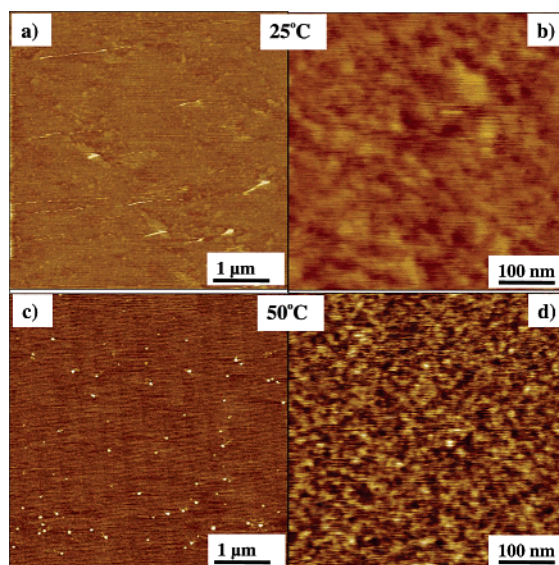


Figure 7. AFM topography of **PB** monolayers deposited at the surface pressure $\pi = 5$ mN/m, pH 7.4 and different temperatures: 25 (a and b) and 50 °C (c and d). Z-scales are 5 (a–c) and 2 nm (d).

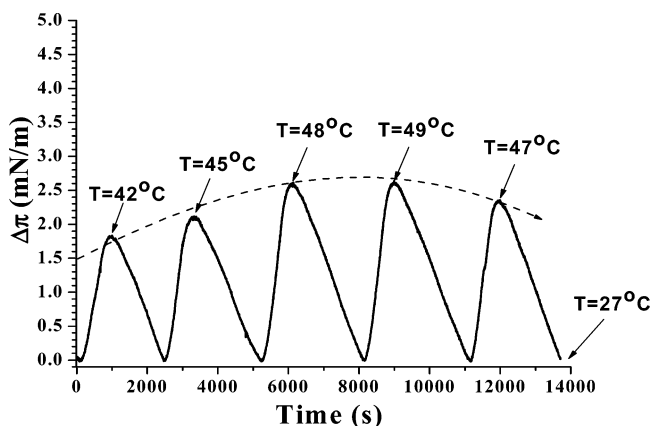


Figure 8. Temperature variation of the surface pressure of the **PB** copolymer monolayer at pH 7.4 at the constant surface molecular area of 120 nm² and temperature cycling between 27 °C and 42–49 °C.

presented here. The corresponding variation of the surface pressure (a value at 27 °C was taken as a zero base) at constant surface molecular area (selected at 140 nm² in this example) was remarkably cyclical following closely the temperature variation (Figure 8). The surface pressure increased by 1.5–2.5 mN/m at the elevated temperature, which indicates the formation of more dense monolayer due to stronger tendency to intramonolayer segregation induced by the collapse of the terminal **PDEAEM** blocks in the course of phase transition around LCST = 35 °C. Moreover, the minor variation of the temperature interval (increase in the higher temperature limit) clearly modifies thermoresponsive behavior, resulting in a temperature-controlled pressure variation window (see envelope curve in Figure 8). The reversibility of the polymer response over many cycles similar to that observed here is a key feature for use in drug delivery applications.¹⁷

General Discussion and Conclusions

The results obtained in this study help elucidate the surface properties of these multi-stimuli-sensitive polymers at the air–water interface under various environmental conditions and provide good comparison with the bulk behavior of these polymers

in aqueous solutions. This study clearly shows that the hydrophobic balance among the various blocks in the **PB** copolymer can be tuned by varying temperature and/or pH. Increasing temperature promotes the hydrophobicity of the terminal blocks due to LCST behavior. In the fully protonated state at low pH, the **PDEAEM** blocks are partially hydrophilic, while they are hydrophobic in the unprotonated state at high pH values. The surface studies presented here provide a facile way of gauging the amphiphilicity and the surface structure of the copolymer under various conditions, compared to small-angle X-ray or neutron scattering techniques for the bulk solutions that are time-consuming and relatively more complex to analyze.²⁴ The surface area per molecule measurements obtained here correlate well with the overall hydrophobic balance of the **PB** copolymers under various pH and temperature conditions. At lower pH values, due to the increase in the degree of protonation, more collapsed surface structures are seen. At high pH values of 10, collapsed aggregates and fibrils seen in the LB monolayers correlate well with the cylindrical micellar structures with similar dimensions ($R_g \approx 4.4$ nm) suggested for bulk solutions from small angle neutron scattering,²⁴ and the sizes of the molecular aggregates seen at pH 7.4 are between those seen at pH 1 and 10, as expected from earlier solution studies.²⁴

Then, as was observed here under extremely basic or acidic pH conditions, the effect of increasing temperature from 25 to 50 °C is not very pronounced. Apparently, this is associated with the changes in relative hydrophobicities of the **PDEAEM** blocks which are strongly correlated to pH and not temperature in this regime. However, at the physiologically relevant pH of 7.4, which is close to the pK_a of the **PB** copolymer where the **PDEAEM** blocks are partially ionized, the copolymer shows the greatest sensitivity to changes in temperature. Significant increase in the surface area (and pressure) is observed as the temperature is increased from 25 to 50 °C. Because the upper temperature is above the LCST temperature of **PDEAEM**, this increase leads to the shifting hydrophobic balance. Obviously, this shift in hydrophobic balance naturally leads to the increase in the surface area, as well as the increase in the aggregate formation.

In conclusion, we found an interesting reversible thermoresponsive surface behavior of the **PDEAEM**₂₅–**PEO**₁₀₀–**PPO**₆₅–**PEO**₁₀₀–**PDEAEM**₂₅ pentablock copolymer designed to be a multistimuli responsive copolymer with both pH and temperature responsive behavior. In fact, we found that by varying pH, we can trigger thermoresponsive behavior of this copolymer at the air–water interface similar to that observed and studied for micellar solutions. Moreover, we found that the temperature-driven conformational changes result in the dramatic reorganiza-

tion of both vertical and lateral segregation of pentablock copolymers confined into a single molecular layer. Remarkably, unlike conventional thermally responsive polymers, for our pentablock copolymer, the changes induced by temperature-triggered intramolecular transformations are fully controlled by the pH environment and can be enhanced or suppressed on demand. Importantly, we demonstrated that fully reversible, multiple, and robust variation of the surface pressure accompanies the temperature-induced phase transformation around LCST. The reversibility of the copolymer behavior at the interface with respect to temperature unambiguously demonstrated here is crucial for drug delivery applications which rely on nonmonotonic and precisely tuned character of the interfacial interactions. Moreover, the approach explored here can be applied in future studies for an interesting and very relevant example of stimuli-responsive block copolymers interacting with lipid bilayers of cell membranes and affecting cross-membrane transport of small biological molecules. For these model systems, questions such as the role of the block copolymer conformation in intracellular delivery can be addressed by fabricating mixed monolayers of lipids and the block copolymer and changing surface tension and interfacial state by adding biological molecules into the aqueous subphase. It is worth noting that our preliminary experiments indicated that even very minor presence of DNA molecules in the aqueous subphase can dramatically change the interfacial behavior of these block copolymers and its ability to reversible reorganization which is a subject of further studies.

The surface studies described here provide a powerful method to analyze the response of multi-stimuli-sensitive polymers to various environmental conditions and provide a fast method for complementary investigation of the bulk solution behavior of responsive polymers. We suggest that the observed thermoresponsive surface behavior can be exploited for modeling of the corresponding behavior of pentablock copolymers adsorbed onto various interfaces (e.g., biomaterials or tissues). Detailed studies of this behavior and the role of water-soluble small molecules on kinetics of surface transformations might enhance our understanding of molecular transformations under variable environmental stimuli relevant to controlled drug and biomolecule release and retention.

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