Polymer Micelles as Building Blocks for Layer-by-Layer Assembly: An Approach for Incorporation and Controlled Release of Water-Insoluble Dyes

Ning Ma, Hongyu Zhang, Bo Song, Zhiqiang Wang, and Xi Zhang*

Key Lab of Organic Optoelectronics and Molecular Engineering, Department of Chemistry, Tsinghua University, Beijing, 100084, P. R. China and Key Lab for Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun, 130012, P. R. China

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We describe the use of block copolymer micelles as building blocks for incorporation of water-insoluble dyes and then fabricate multilayer films by alternating deposition of the polymer micelles of poly(styrene-*b*-acrylic acid) and poly(diallyl-dimethylammonium chloride) (PDDA). Pyrene, a small organic molecule that is difficult to assemble by the usual methods, was incorporated into the micelles and then assembled into multilayer film. The growth procedure of the multilayer films was monitored by UV−vis and fluorescence spectroscopies, and the results revealed that the multilayer films grew in a uniform way in every deposition cycle. In-situ and ex-situ atomic force microscopy (AFM) images proved the existence of block copolymer micelles in LbL films. Furthermore, pyrene molecules assembled can be released from the multilayer films by immersing the films into solutions of different ionic strength. Micelles with different core−shell structures exhibited different release rates of pyrene. These films have potential use as controlled assembly and release materials for some water-insoluble dyes.

Introduction

Layer-by-layer (LbL) assembly has been widely used as a versatile method for fabricating multilayer thin films with tailored structure and composition.[1] Numerous substances have been assembled successfully into the films via the LbL procedure, including oligo-charged dyes,[2] inorganic compounds,[3] polyelectrolytes,[4] photoreactive species,[5] thermoresponsive materials,[6] colloids,[7] biomacromolecules,[8] etc. Although some water-insoluble polymers can be assembled into multilayer thin films by LbL methods on the basis of hydrogen bond,[9] coordination bond,[10] or charge-transfer interaction,[11] there are many types of water-insoluble organic molecules that are difficult to assemble in this way.

It is well known that amphiphilic block copolymers can self-organize into core−shell micellar structures in selective solvents.[12] If the solvent is water, small water-insoluble molecules can enter the cores of micelles spontaneously and be released from the micelles under special conditions.[13] Therefore, micelles of block copolymers possess great potential for applications in biology and pharmacy for incorporation and release of some drugs.[14] More recently, there have been several reports about LbL assembly of micelles and vesicles.[15] For example, Kataoka and coworkers obtained a covalent-bonded layered thin film of block copolymer micelles.[15a] On the basis of these previous

* Corresponding author. Fax: 0086-10-62773155. E-mail: xi@mail.tsinghua.edu.cn.

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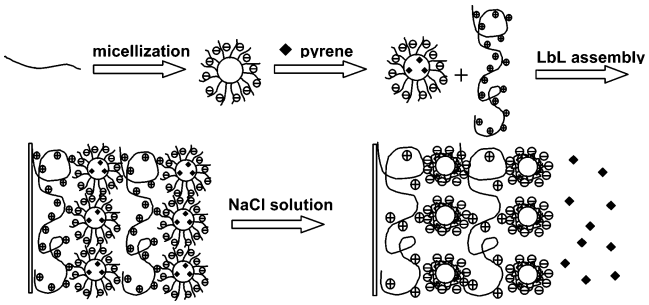
Scheme 1. Schematic Representation of Incorporation of Pyrene into Micelles, LbL Deposition of Micelles and PDDA, and Release of Pyrene from the Multilayer Film



works, in this paper we chose poly(styrene-*b*-acrylic acid), an amphiphilic block copolymer, as a model system and attempted to use the block copolymer micelles as matrixes for incorporation of small water-insoluble molecules for LbL assembly, as shown in Scheme 1. Moreover, we wondered if the incorporated molecules can be released from the multilayer films under some conditions and how micellar structures are related to the release rates.

Experimental Section

Materials. The two amphiphilic diblock copolymers used in this paper poly(styrene-*b*-acrylic acid), (M_n PS(5100)−PAA(3880), $M_w/M_n = 1.10$ and M_n PS(2200)−PAA(11300), $M_w/M_n = 1.08$), (PS_{49}−PAA_{54} and PS_{21}−PAA_{157} for short, respectively) were purchased from Polymer Source Inc. and used as received. Poly(diallyl-dimethylammonium chloride) (PDDA, M_w 400 000) and pyrene were obtained from Aldrich and used without further purification. *N*,*N*-Dimethylformamide (DMF), acetone, and sodium chloride are all analytical-grade products from Beijing Chemical Reagent Company.

Preparation of the Pyrene-Loaded Micelle Solution.[16] The block copolymer micelles were obtained by adding a suitable amount of water into the DMF solution of PS-*b*-PAA and then diluting to 0.2 mg/mL (contained only 4% DMF). Then 40 μL of a 0.5 mg/mL acetone solution of pyrene was injected into 5 mL of micelle solution. After sonication for 30 min the pyrene-loaded micelle solution was ready for LbL deposition.

Layer-by-Layer Deposition. The LbL film was assembled on a quartz slide, which was first cleaned by treatment on a hot piranha solution for 40 min (*caution: piranha solution is extremely corrosive*) and then thoroughly washed with pure water. A hydroxy-tailored quartz slide was first immersed into PDDA aqueous solution. In this way, the substrate was covered with a PDDA layer. After rinsing with pure water and drying under a nitrogen stream, the resulting substrate was transferred into a solution of pyrene-loaded PS-*b*-PAA micelles to add a micelle layer. The immersion time was 10 min in each step. By repeating the above two steps in a cyclic fashion, the LbL multilayer film was fabricated. All three polymers, PS_{49}−PAA_{54}, PS_{21}−PAA_{157} and, PDDA, were of the same concentration, 0.2 mg/mL in aqueous solution.

Instruments. UV−vis spectra were obtained on a Hitachi U-3010 spectrophotometer. Fluorescence emission spectra were recorded with a Perkin-Elmer LS 55 spectrofluorimeter. The release of pyrenes was carried out at a temperature of 293 K, and the amount remaining in the film was calculated by the fluorescence intensity of pyrene in the experiments. Atomic force microscopy (AFM) images were taken with a commercial instrument, Nanoscope IV. The tip involved was Si_3N_4 cantilever (Park Scientific, CA) with a nominal spring constant of 0.06 N/m for tapping mode. For in-situ AFM measurements polymer micelle solutions of appropriate concentrations were injected into the liquid cell and allowed to equilibrate for at least 1 h before imaging. Ex-situ AFM images of the polymer micelles were obtained with tapping in air.

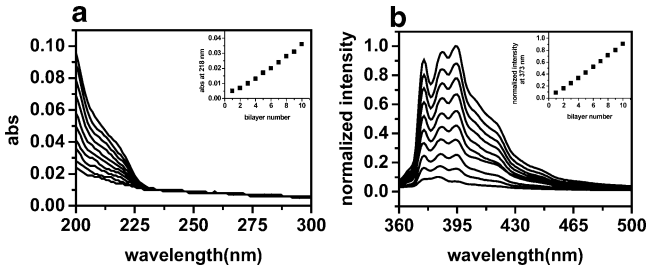


Figure 1. UV−vis (a) and fluorescence spectra (b) of a 10-bilayer film of PDDA and pyrene-incorporated micelles of PS_{49}−PAA_{54}. (inset) Absorbance or normalized intensity versus the number of bilayers.

Results and Discussion

Multilayer films were fabricated by alternating deposition of poly(diallyl-dimethylammonium chloride) (PDDA) and micelles of diblock copolymer PS_{49}−PAA_{54} on a quartz surface followed by UV−vis absorption. Figure 1a shows UV−vis spectra of a (PDDA/micelle)$_{10}$ multilayer film. The absorption band at ca. 218 nm is identified as the $\pi-\pi^*$ transition of the benzene ring in PS block. The inset of Figure 1a shows that the absorbance of quartz-supported multilayer films at 218 nm increases proportionally with the number of bilayers, which indicates that approximately an equal amount of micelles are assembled in each deposition cycle.

The polymer micelle formed by self-organization of PS-*b*-PAA block copolymer has a hydrophobic core and a hydrophilic shell, so it can be used to incorporate pyrene, which is a water-insoluble molecule. To investigate whether the pyrene-loaded polymer micelles can form LbL multilayer films, we used fluorescence spectra to observe the adsorption behavior of the pyrene-loaded micelles in multilayer fabrication. As seen in Figure 1b, the fluorescence intensity of pyrene in micelles increases in a linear fashion with respect to the increase of bilayer number. The fluorescence spectra indicate that the LbL films grow uniformly in every adsorption procedure, which agrees with the data of UV−vis spectra. We can also see that there are no pyrene excimers formed in the micellar cores by the absence of an emission band at ca. 475 nm. This result shows that self-quenching of pyrene is avoided under the conditions of our experiments, which may be ascribed to the relatively low concentration of pyrene and the "isolation effect" of micellar cores.

It is well known that the intensity ratio of the characteristic bands of pyrene's emission spectra can reflect the polarity of its microenvironment.[17] The fluorescence intensity ratio of the two peaks at 373 and 387 nm in the multilayer films is around 0.9. Such a low ratio indicates that pyrene molecules exist in a hydrophobic microenvironment, i.e., in the cores of polymer micelles.

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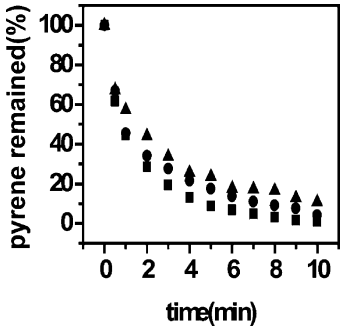

Figure 2. Release kinetics of pyrene from multilayer films of PDDA and $PS_{49}-PAA_{54}$ micelles: (■) 2.0 M NaCl solution, (●) 0.5 M NaCl solution, (▲) 0.1 M NaCl solution.

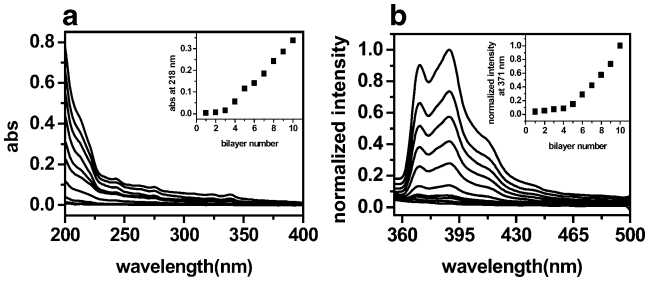

Figure 3. UV−vis (a) and fluorescence spectra (b) of a 10-bilayer film of PDDA and pyrene-incorporated micelles of $PS_{21}-PAA_{157}$. (inset) Absorbance or normalized intensity versus the number of bilayers.

Since the ionic strength of the aqueous solution can influence the structure of the polymer micelles,[18] we wondered if the incorporated pyrene could be released from the multilayer films depending on the ionic strength of the solution. The kinetics of uptake and release can be monitored by fluorescence spectra if the incorporated molecule is a fluorophore. For this purpose, the pyrene-loaded 8-bilayer films were immersed in NaCl aqueous solutions of different concentrations. Figure 2 shows the kinetics of pyrene release from the multilayer films. It is shown that the amount of pyrene remaining in the film decreases with increasing immersion time. Most pyrene molecules are released from the multilayer films in the first 10 min of immersion, but it needs about 30 min to fulfill a full release. It is easy to understand that in a solution of higher ionic strength the release rate of pyrene molecules from the multilayer film is larger than that in a solution of lower ionic strength. Therefore, we can tune the release rate of pyrene molecules by varying the ionic strength of the solution for immersion. We found that the NaCl aqueous solution in which the films were immersed had a weak fluorescence emission, suggesting that the pyrene molecules released from the multilayer films indeed went into the aqueous phase.

We also investigated the influence of different core−shell structures on the layered deposition and release of pyrene molecules. For comparison, another diblock copolymer with a higher ratio of PAA segment, $PS_{21}-PAA_{157}$, was used to form micelles and incorporate dyes for LbL deposition. The UV−vis and the fluorescence spectra of a 10-bilayer LbL film of PDDA and $PS_{21}-PAA_{157}$ micelles are shown in Figure 3. Similarly, there is linear growth for the multilayer fabrication except for the first three bilayers. Interestingly, it is found that the UV absorbance of $PS_{21}-PAA_{157}$ increased per bilayer is much greater than that of $PS_{49}-PAA_{54}$, though the micellar cores of the former are smaller in size than those of the latter. The reason that the micelles formed by $PS_{21}-PAA_{157}$ have a larger adsorption could be related to the more compact accumulation of the micelles in the LbL assembly. To verify our assumption, atomic force microscopy (AFM) was employed to observe the surface structure of the polymer micelles in multilayer films. As indicated by ex-situ AFM images in Figure 4c and 4d, we can see clearly that spheric micelles of $PS_{49}-PAA_{54}$ and $PS_{21}-PAA_{157}$ assembled in multilayer films are about 40 and 10 nm in diameter, respectively. Moreover, a multilayer film composed of $PS_{21}-PAA_{157}$ shows a greater density of adsorption on the substrate, which may result in the higher absorbance per bilayer than that of a multilayer film formed by $PS_{49}-PAA_{54}$.

Comparing Figure 4a with 4b, interestingly we find that the ex-situ AFM observation of the micellar structure of $PS_{49}-PAA_{54}$ in a 1-bilayer film shows a similar image to that observed in-situ conducted at the water/substrate interface, suggesting that the micellar structures can remain to some extent even in the dry state. This means that the polymer micelles are quite stable and of engineering robustness, allowing for layer-by-layer fabrication.

The release rate of pyrene molecules can also be influenced by the different core−shell structures. The micelles of $PS_{21}-PAA_{157}$ were incorporated with pyrene, and the resulting LbL films were immersed in NaCl aqueous solutions of different concentrations. The relationship of the percentage of pyrene remaining in the films versus time is shown in Figure 5. It is found that most of the pyrene can be released from the films within 1 min. Comparing Figure 5 and Figure 2 it is clearly demonstrated that the release rate of pyrene from micelles of $PS_{21}-PAA_{157}$ in the multilayer film is much faster than that from micelles of $PS_{49}-PAA_{54}$. In other words, the pyrene molecules in smaller micellar cores are easier to be released from the films than those in larger cores. For these two systems, however, the pyrene can be released faster with increasing ionic strength of solution.

There have been some reports about the change of micellar scales in solution by adding salts,[18] including shrinkage of the corona and looseness of the core. We were curious whether we could observe those changes on a solid/liquid interface. For this purpose, NaCl aqueous solution was injected onto a 1-bilayer PDDA/micelle film for in-situ AFM measurements. We observed that the micelles shrank from 34 to 22 nm in average diameter for the diminution of the interchain charge repulsion, as shown in Figure 6. Another influence to the micelles by adding salts is the "loosen effect" to the micellar cores. In normal PS-*b*-PAA micellar solution the micellar cores are quite compact and "frozen" and the exchange of substance between the cores and the aqueous phase is very slow. Previous results show pyrene can be released from the micelles into pure water[15b] as there is a concentration difference between micellar cores and water. When we add NaCl solution to our system, the micellar cores become looser than before and the process of this exchange dramatically speeds up, which is about several hundred times faster than that in water. It can be easily understood that

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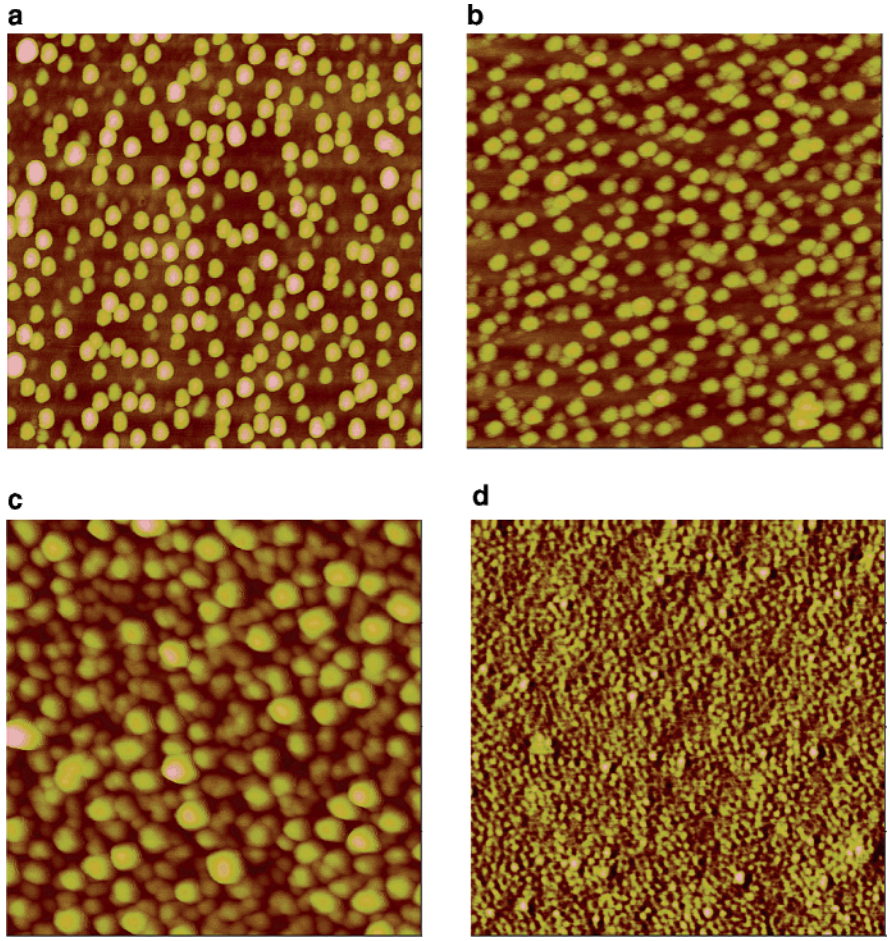

Figure 4. AFM images of PDDA/micelles multilayer films. (a) Ex-situ image of a 1-bilayer film composed of PDDA and $PS_{49}-PAA_{54}$ micelles. Scale: 1.0 μm × 1.0 μm. (b) In-situ image of a 1-bilayer film composed of PDDA and $PS_{49}-PAA_{54}$ micelles. Scale: 1.0 μm × 1.0 μm. (c) Ex-situ image of a 10-bilayer film composed of PDDA and $PS_{49}-PAA_{54}$ micelles. Scale: 1.0 μm × 1.0 μm. (d) Ex-situ image of a 10-bilayer film composed of PDDA and $PS_{21}-PAA_{157}$ micelles. Scale: 1.0 μm × 1.0 μm.

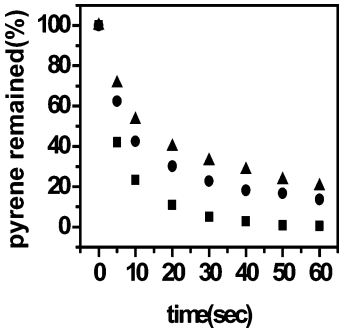

Figure 5. Release kinetics of pyrene from multilayer films of PDDA and $PS_{21}-PAA_{157}$ micelles: (■) 0.5 M NaCl solution, (●) 0.1 M NaCl solution, (▲) 0.01 M NaCl solution.

smaller micellar cores are more easily influenced by ionic strength than larger ones and exhibit a more rapid release from the multilayer films because the smaller cores are more sensitive to the "loosen effect" by NaCl solution than larger ones.

We also attempted to immerse the pyrene-released multilayer film into a saturated aqueous solution of pyrene and found that after a 72-h immersion there was no obvious increase of the fluorescence emission intensity. This may suggest that pyrene cannot go back to the micellar cores in multilayer film because of the robustness of the film in pure water. In pure water the film is quite stable and there are not many tunnels for exchange between micellar cores and the aqueous phase. However, the case is much different from the release process of pyrene. The addition of salts makes both the multilayer film and the micellar cores looser than before so as to make many more tunnels and speed up the release process of pyrene from the multilayer film. In addition, the release rate of the pyrene has no obvious difference for either PDDA or block copolymer micelle as the outermost layer.

Conclusion

We used a preassembly method to incorporate water-insoluble molecule of pyrene into micellar cores and then employed the pyrene-loaded polymer micelles as building blocks for LbL assembly. This process makes it possible to assemble water-insoluble molecules into polyelectrolyte multilayer films, which are not feasible by the normal LbL method. Moreover, we demonstrated that the loaded molecules can be released from the LbL films, which can be controlled by ionic strength as well as influenced by the core−shell structures of the block copolymer micelles. The compactness of micellar cores that change in the NaCl solution may be attributed to the different release rates influenced by ionic strength and core−shell structures.

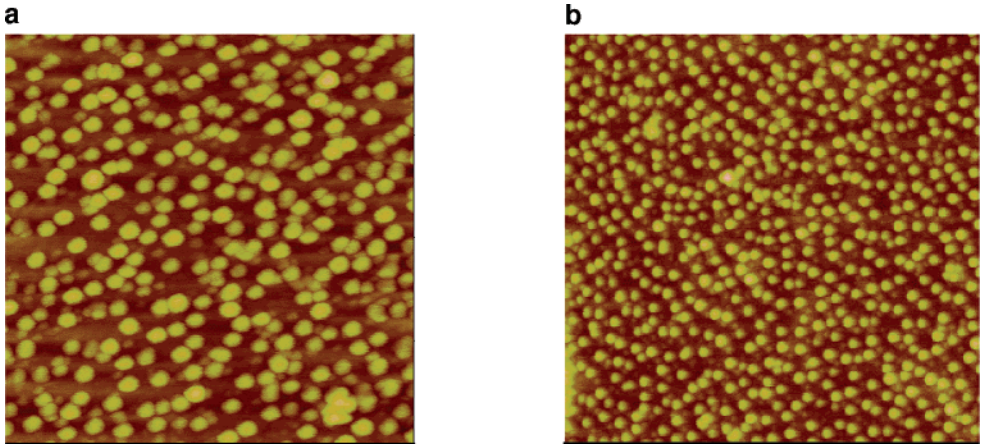

Figure 6. In-situ AFM images of 1-bilayer PDDA/micelles film before (a) and after (b) injection of 0.1 M NaCl aqueous solution into the liquid cell. Scale: 1.0 μm $\times$ 1.0 μm

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