

## Layer-by-Layer Assembly of Poly(allylamine hydrochloride)/Polyurethane and Its Loading and Release Behavior for Methylene Orange

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**ABSTRACT:** Here, layer-by-layer technique was used for sequential adsorption of oppositely charged polymer poly(allylamine hydrochloride) (PAH) and polyurethane (PU) through electrostatic interaction. 10 and 10.5 bilayer films were prepared separately, methylene orange (MO) was used as a model drug to evaluate the potential ability of this multilayer film used in drug delivery system. Experimental results showed the ability of loading and release of MO from the film was significantly influenced by pH and salt concentration, the loading rate of MO was faster and larger with increasing salt concentration or decreasing pH of MO solution, the release rate of MO was faster at higher salt concentration or in alkali solution. The result also indicated that the film had a good reversibility of loading and release. PAH/PU film could be a promising drug delivery system because of its biocompatibility, biodegradation and above properties. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 129: 2070–2075, 2013

**KEYWORDS:** drug delivery systems; films; stimuli-sensitive polymers; biocompatibility; polyurethanes

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### INTRODUCTION

Compared with traditional method, the layer-by-layer (LbL) not only had many remarkable highlights, such as the thickness, shape and permeability of the LbL film can be fine controlled by adjusting the number of layers, deposition time, and environmental conditions, including temperature, ionic strength, sugar and the pH value of the solutions, and so forth,<sup>1–6</sup> but also its instrument is very simple, low cost, and manufacturing conditions are milder. Since Decher et al.<sup>7–9</sup> adapted Iler's idea and made breakthrough of LbL assembly technique, not only several charged polymers have been successfully assembled into thin films, but also it has greatly improved, constitutes a powerful tool ranging from thin films to biocatalysis.<sup>10–13</sup>

Films fabricated by LbL method exhibited excellent performance, provided a very promising way to fabrication of carrier systems for dyes, enzymes, drugs, and cell,<sup>14–18</sup> especially usage for drug delivery system has attracted much attention. For example, Zan and Su<sup>19</sup> prepared antibacterial efficacy films that were composed by PDDA/PSS and silver nanoparticles. Fu et al.<sup>20</sup> constructed heparin/chitosan multilayered thin films, it exhibit strong anticoagulant and antibacterial activities. Smith et al.<sup>21</sup> used poly( $\beta$ -aminoesters) and poly(carboxymethyl- $\beta$ -

cyclodextrin) which was complex with a small molecule drug as the construct of film, this film has good performance to delivery small molecule drug, and had been developed to a custom coatings for small molecule drug applications. Among drug delivery system, there is an increasing interest in the field of the development of "smart" devices which is capable of actively controlling the loading and release of drug in response to external stimuli<sup>22–24</sup>; and, now the major challenge in drug delivery is to produce controlled, sustained and triggered release systems for small encapsulated drug molecules.<sup>25</sup>

Poly(allylamine hydrochloride) (PAH) is a biodegradable weak cationic synthetic polyelectrolyte.<sup>26,27</sup> The multilayer film constituted by PAH is very attractive for biomedical and pharmaceutical applications.<sup>28–30</sup> Polyurethanes (PUs) have been widely used as implantable medical devices for its flexibility, biostability and biocompatibility. Recent reports showed that PU represented as an important new component of the LbL technique with a variety of potential research value.<sup>31–33</sup> So, the multilayer film constituted by PAH and PU had potential application in drug delivery system. However, to the best of our knowledge, LbL films constituted by PAH and PU have not been prepared and studied.

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In this study, PAH /PU bilayer films were fabricated using LbL technique, and methylene orange (MO) had been used as a model drug to evaluate the potential ability of those multilayer films used in drug delivery system. The loading and release behavior of films were investigated under various conditions, including number of bilayers, pH value and ionic strength,

## EXPERIMENTAL

### Materials

PAH ( $M_w$  120,000–200,000) was purchased from Alfa Aesar China (Tianjin). Anionic PU ( $M_w \approx 280,000$ ; wt  $\approx 20\%$ ) was obtained from TFL (Germany), MO was bought from Tianxin Chemical Company (Tianjin, China). Sodium chloride was purchased from Beijing Chemical Reagent Company (Beijing, China). The pH value of MO and NaCl solution was adjusted using either the dilute HCl or NaOH solution; all commercial polyelectrolyte were used as received without further purification. Deionized water (18.2 M, Milli-Q Ultrapure Water System, Millipore) was used in experiment. All the experiment were carried out at room temperature (*ca.*, 25°C)

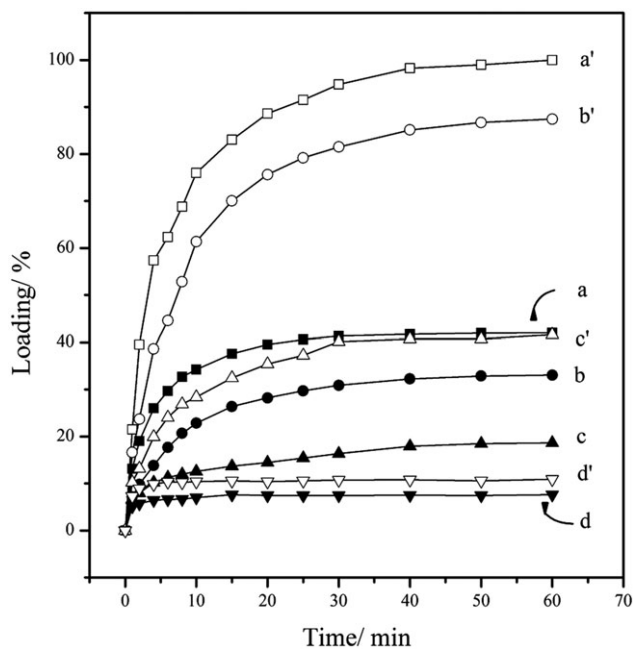
### Fabrication of LbL films

The LbL film was assembled on glass slides, which was first cleaned with fresh piranha solution (3 : 1 v/v mixture of 98%  $H_2SO_4$  and 30%  $H_2O_2$ ) for 40 min (Attention! Piranha solution is extremely dangerous and should be very carefully handled), followed thoroughly washed with deionized water and finally dried under air flow. The piranha treatment allows removal of residues of organic impurities from the substrates and makes the slides completely hydrophilic at the same time.

PAH/PU multilayer film was assembled according to the following procedure: the slide was first immersed in the PAH solution (wt 1%, pH 9.0) for 2 min to obtain a positively charged surface, then the glass slide was rinsed in two separate beakers of deionized water for 1 and 1 min, respectively, and dried by air flow. The PAH-deposited slide was then immersed in the PU solution (wt 1%, pH 9.0) for 2 min and followed by the same rinse cycle. Process was alternately repeated until designed number of  $(PAH/PU)_n$  bilayer films were deposited. Effect of deposition layer number to the loading amount was investigated at first. It was found that loading amount was quite small when the film deposition layer number was five to nine bilayers, 10 bilayers was choose as the basic deposition number, also this number of bilayer are also commonly used LbL field. The first layer was always PAH layer and each bilayer consists of PAH layer and PU layer. For example, a  $(PAH/PU)_{10}$  multilayer film contains 10 bilayers of PAH/PU, and  $(PAH/PU)_{10.5}$  contains 10.5 layer of PAH and 10 layer of PU (Supporting Information, S1). The effect of pH value of polyelectrolyte solution was also investigated, but it was found that the biggest loading amount could be reached at pH 9.0, so the optimized pH value to assemble films was set as 9.0 (Experimental data was not show).

### Loading and Release of MO

For the loading of MO, the LbL film-coated glass slides were immersed into 0.3 mg/mL of MO solutions containing 150 mM NaCl at different pH values. The films were taken out after a given time, then rinsed with deionized water to remove excess



**Figure 1.** The effect of pH values on the loading percentage of MO into  $(PAH/PU)_{10}$  and  $(PAH/PU)_{10.5}$  multilayer films. The films were immersed in 0.3 mg/mL of MO solutions containing 150 mM NaCl at pH 4.0 (a), 5.0 (b), 7.0 (c) and 9.0 (d), respectively (a for  $(PAH/PU)_{10}$ , a' for  $(PAH/PU)_{10.5}$ , by parity of reasoning).

MO molecules, and dried with air flow. The loading process of MO in  $(PAH/PU)_n$  films were monitored by UV-vis absorption spectra with a UV 2550 spectrophotometer (Shimadzu, Japan). Film size was controlled be  $5.5 \times 2.5 \text{ cm}^2$ , and the loading amount was calculation through calibration curve.

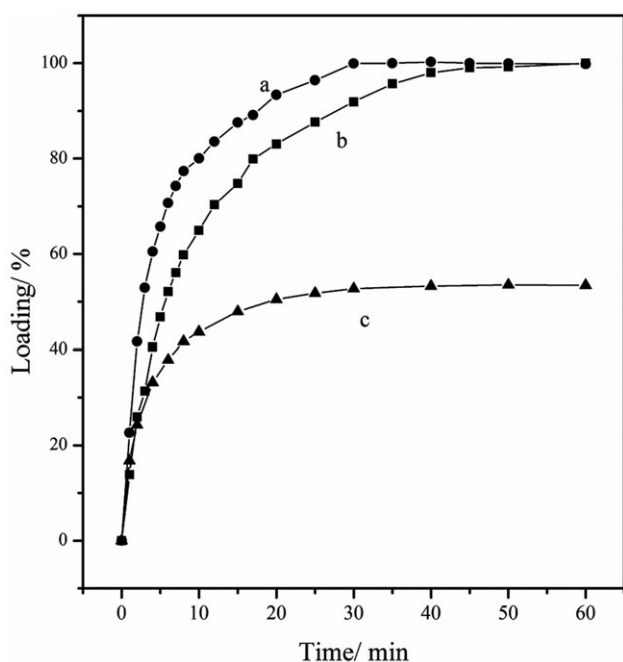
For the release study, the glass slides coated with MO loaded films were immersed into aqueous solutions with various NaCl concentrations and various pH values, which were mechanically stirred during the release process. The films were removed at regular intervals, and the amounts of MO released from the films were determined by UV-vis spectroscopy using calibration curve method at maximum absorption wavelength ( $\lambda_{max}$ ) of 463 nm. The immersion solutions were frequently replaced by a fresh one to ensure accurate absorbance reading. All measurements were carried out at room temperature (*ca.*, 25°C).

## RESULTS AND DISCUSSION

### Loading of MO

For the electrostatics-bounded weak polyelectrolyte film, the loading amount was always influenced by pH value and salt concentration, and the pH value is the dominant factor, so the loading process was conducted at first. The maximum absorbance ( $\lambda_{max}$ ) of MO in the film was blue-shifted to 426–380 nm as compared to the  $\lambda_{max}$  in aqueous solution (463 nm) (Supporting Information, S2). This result suggested that most of MO within the film formed molecular aggregates.<sup>34–36</sup>

The loading percentage of MO increased when the pH decreased from 9.0 to 4.0 (Figure 1). However, when pH value decreased to 3.0, the film was no longer stable and decomposed.



**Figure 2.** The effect of layer numbers on the loading percentage of MO into films. The films were immersed in 0.3 mg/mL of MO solutions containing 150 mM NaCl at pH 4.0. (PAH/PU)<sub>10.5</sub> (a), (PAH/PU)<sub>11</sub> (b), and (PAH/PU)<sub>10</sub> (c), respectively.

The reason was that at pH 3.0 the charge balance between PAH and PU was destroyed. The PAH/PU film showed a faster loading percentage and higher loading capacity at pH 4.0, and the saturation point was reached within 30 min. when the film was placed in pH 9.0 buffers, the loading amount became quite small, even could hardly be observed. That is reasonable, the PAH/PU film was deposited at pH 9.0, the  $-\text{NH}_2$  groups of PAH are partially ionized (the pKa of PAH is at about 8.5),<sup>37</sup> the fraction of protonized amino groups in PAH chain is limited and balanced with the negative charges of PU, there was few free ionized  $-\text{NH}_3^+$  groups to bind MO, as the result, the loading amount of MO was quite small. But, the decrease of the pH will leads to a larger charge density in the PAH chains, thus making the interaction with the charged surface more favorable and accelerate the adsorption process, when the pH decreased to 4.0, the electrostatic attractive forces between negative MO and  $\text{NH}_3^+$  were promoted, the loading amount of MO reached the maximum. Typically, there are two types of mechanism for pH-sensitive permeation behavior of polyelectrolyte films toward probes, one is pH-responsive structure change of the film, and another one is to the electrostatic interaction between probes and films.<sup>38</sup> Based on above explication it can be deduced that PAH/PU film suit to the second types of mechanism.

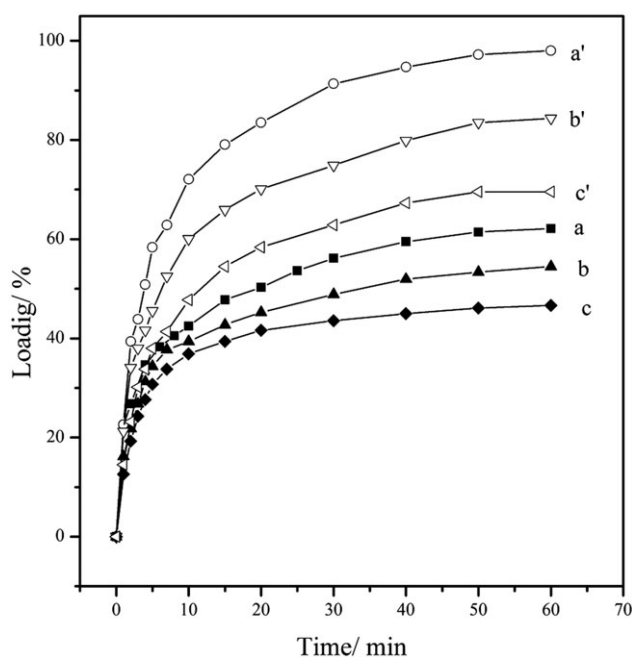
From Figure 1, it can be clearly seen that the loading percentage of (PAH/PU)<sub>10.5</sub> film was largely than (PAH/PU)<sub>10</sub> at the same pH value. When film size was controlled to be  $5 \times 2.5 \text{ cm}^2$ , combine with calibration curve (Supporting Information, S3) and volume (40 mL), the loading amount of MO in

(PAH/PU)<sub>10.5</sub> was  $10.6 \mu\text{g}/\text{cm}^2$  which was as twice as much as that of (PAH/PU)<sub>10</sub> ( $5.0 \mu\text{g}/\text{cm}^2$ ) at pH 4.0. We speculated that the increment of MO in (PAH/PU)<sub>10.5</sub> film was attributed to the more PAH ( $-\text{NH}_2$  groups) in films. The (PAH/PU)<sub>11</sub> film was assembled to confirmation this hypothesis. From Figure 2, it can be seen that the loading amount of MO in (PAH/PU)<sub>11</sub> was lower than (PAH/PU)<sub>10.5</sub> at the beginning of the loading processes, but the equilibrium point of it was as same as (PAH/PU)<sub>10.5</sub>. This phenomenon can be explained by the differences of the out layer between two films. As the amount of PAH was not changed in (PAH/PU)<sub>10.5</sub> and (PAH/PU)<sub>11</sub> films, so the equilibrium points to MO was alike.

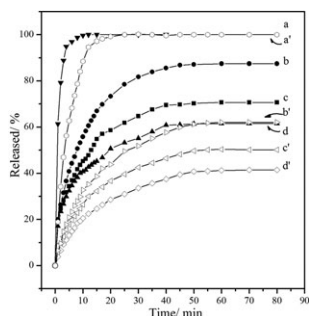
Beside pH value, the effect of salt concentrations also showed big effect on loading behavior of films. To investigate the effect of ionic strength, different MO solutions were prepared with NaCl concentrations varied from 0 to 150 mM. The results were illustrated in Figure 3. It can be seen that the adsorption rates were fast and the loading amounts increase with enhanced of salt concentrations. One possible explanation for this phenomenon is an electric-shielding effect.<sup>39,40</sup> Salt additives would screen the electrostatic interactions between PAH (positive charge) and PU (negative charge), the film construction became looser, which means that there was more free volume for the drug molecules to enter, so the amount of loading MO in films were raised.

#### Release of MO

Compared with loading, the release rate was very important for drug delivery system too. At first, all of the films were immersed in 0.3 mg/mL of MO solutions at pH 4.0 with 150 mM NaCl



**Figure 3.** The effect of salt strength on the loading percentage of MO into (PAH/PU)<sub>10</sub> and (PAH/PU)<sub>10.5</sub> multilayer films. The films were immersed in 0.3 mg/mL of MO solutions (pH 7.0) containing 150 (a), 75 (b), 0 mM NaCl (c), respectively. (a for (PAH/PU)<sub>10</sub>, a' for (PAH/PU)<sub>10.5</sub>, by parity of reasoning).



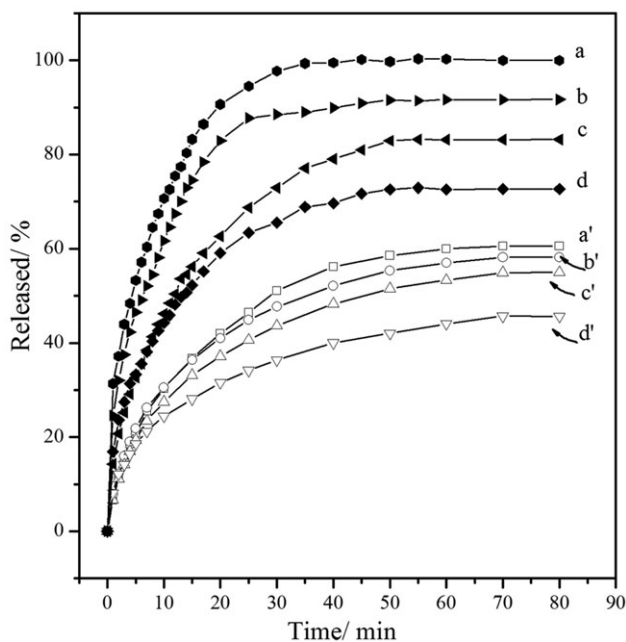
**Figure 4.** The release percentage of MO from (PAH/PU)<sub>10</sub> and (PAH/PU)<sub>10.5</sub> multilayer films in different pH value. MO was released in 150 mM NaCl solutions at pH 9.0 (a), 7.0 (b), 5.0 (c) and 4.0 (d), respectively (a for (PAH/PU)<sub>10</sub>, a' for (PAH/PU)<sub>10.5</sub>, by parity of reasoning).

for 30 min to reach adsorption equilibrium, then, the MO-loaded multilayer films were immersed in various pH values and NaCl concentrations solutions. At each predetermined time interval, the releasing solutions were monitored at 463 nm of UV absorbance.

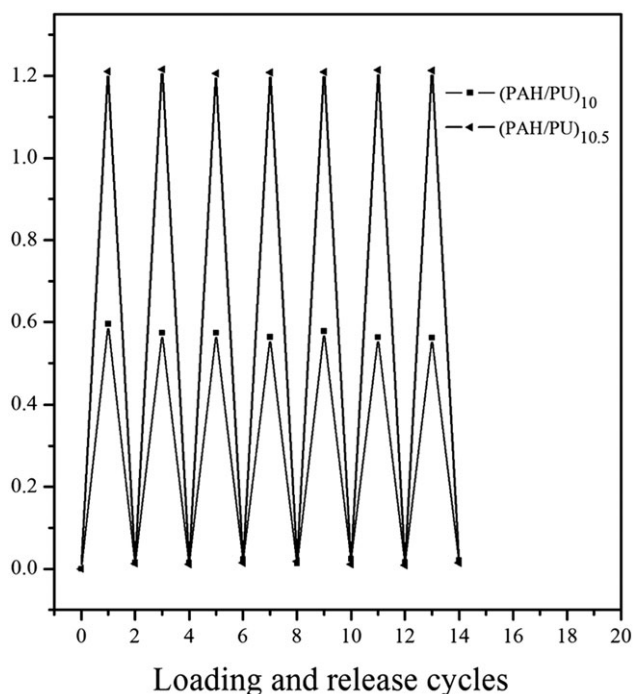
From Figure 4, it can be seen that the release rate of MO was higher and faster with the increase of pH value of solutions, and at pH 9.0 burst releasing happened. Petro et al reported that NH<sub>2</sub> groups of the PAH chains were high degree ionized at pH 4.5,<sup>37</sup> so at pH 4.0, almost all the NH<sub>2</sub> group of PAH were protonized and converted into NH<sub>3</sub><sup>+</sup>, which can strongly attracted with negative charged MO molecules. Thus, with increased pH value of immersion solutions, the NH<sub>3</sub><sup>+</sup> would de-protonized slowly, and there will be less and less PAH to bind MO molecules, it was the reason that the release rate

increased with the pH value high. However, when the pH value reached at pH 9.0, there was no free PAH to combined with negatively charged MO molecules, even electrostatic repulsive forces was emerged and became dominant in the release process, so the burst release happened. Compared with sustained drug release, the burst release may be a good candidate in some fields, such as site specific or targeted drug delivery system.<sup>41,42</sup> The trend of the release of MO from (PAH/PU)<sub>10.5</sub> and (PAH/PU)<sub>10</sub> was similar, but compared with (PAH/PU)<sub>10</sub>, the drugs released rate of (PAH/PU)<sub>10.5</sub> was much lower at same pH (pH 7.0, 90% vs. 60%; pH 5.0, 70% vs. 50%; pH 3.0, 70% vs. 40%). Since in the loading process, the loading amount of MO in (PAH/PU)<sub>10.5</sub> is as twice times as that of the (PAH/PU)<sub>10</sub>, which is suggested that the type of outmost layer played a key factor in drug release and loading procedures.

As shown in Figure 5, the release rate of MO in the (PAH/PU)<sub>10</sub> and (PAH/PU)<sub>10.5</sub> film can be well tuned by modifying the ionic strength of solution. The rate of MO release became faster with the increasing of NaCl concentration, which suggested a significant effect of ionic strength on the release rate. Such phenomenon could explained by the reason that the electrostatic interactions between PAH and PU in the film were weakened due to the electrostatic screening of salt, which lead to the swelling and permeability of the film increased,<sup>43</sup> and meanwhile, the electrostatic interaction between MO and binding sites in the films are weakened, all those factors led to a increase release rate in NaCl solution. Similar effects of ionic strength on the release rates of drugs in LbL films have been reported.<sup>44</sup>

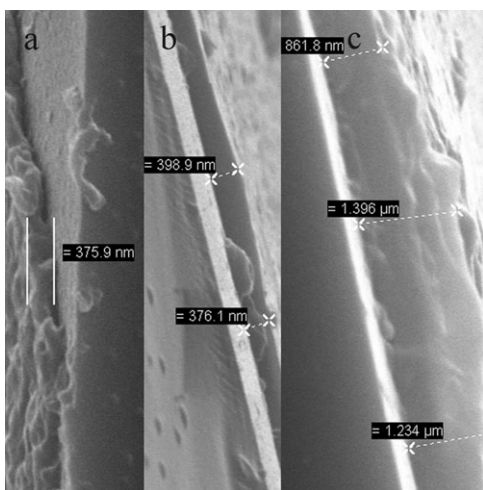


**Figure 5.** The release percentage of MO from (PAH/PU)<sub>10</sub> and (PAH/PU)<sub>10.5</sub> multilayer in different ionic strength. The immersion solutions contained 500 (a), 300 (b), 150 (c), and 0 mM NaCl (d), respectively (a for (PAH/PU)<sub>10</sub>, a' for (PAH/PU)<sub>10.5</sub>, by parity of reasoning).



**Figure 6.** The cyclic loading and release of the film with MO from (PAH/PU)<sub>10</sub> and (PAH/PU)<sub>10.5</sub>.





**Figure 7.** SEM image of absolute size of the film's. Five bilayer film (a), 10.5 bilayer film (b), after 14 times loading and release of 10.5 bilayer (c).

### Reversible Loading and Release of MO

To evaluate the stability of the films, the reversible property of (PAH/PU)<sub>10</sub> and (PAH/PU)<sub>10.5</sub> towards loading and release of MO was investigated. Totally 14 cycles of loading in pH 4.0 of MO solution and released in pH 9.0 solutions were performed, results were shown in Figure 6. It can be seen that the absorbance fluctuated regularly with the successive loading and release of MO molecules in the PAH/PU film, which means that the absorption efficiency of the PAH/PU film was almost unchanged after 14 times of loading and release. SEM technique was employed to measure the absolute size from transverse section of the film, to gain the further insight changes of the film structure. Figure 7(a) and b shows 10.5-bilayer's absolute size was larger than 5-bilayer, Figure 7(b,c) shows that the film's size was obviously swelled from 388 nm to 1.2 μm since many times loading and release process. Combined Figures 6 and 7, although the film's size was swelled, but the loading amount was almost no changed, all those image provided strong evidence that MO can migrate across the outside layers and then electrostatic interaction with PAH and the interaction plays a leading role on the loading and release process, the (PAH/PU)<sub>n</sub> film possess good stability in external environments.

Pavluhina et al.<sup>24</sup> had studied the release process, explained the release mechanisms in terms of three different processes, which is diffusion-controlled, film degradation and triggered control release. Immersion concentration can largely influenced the diffusion-controlled mechanisms, in the film degradation mechanisms, film will be destroyed in release processes. It is not only the immersion pH value that can fine influence loading and release behavior but also the PAH/PU films were very stable in a large pH range, it seems that our experimental results suited to the triggered released mechanisms. A fine pH-triggered reversible loading and release system was successfully constructed.

### CONCLUSIONS

PAH and PU had been successfully assembled by electrostatic interaction between them at pH 9.0 through LbL method. MO,

as a model drug, was loaded in the films, which bound covalently with PAH groups in the films. The number of assembled layer, pH value and salt concentration of immersion solution had show a significant effect on the loading and release. Compared with 10 and 10.5 bilayer films the larger number of deposition layer (11 bilayer) revealed a larger loading amount and sustained release behavior; the loading rate was faster and higher in the low pH values or high salt concentration, but the release rate was contrary. This biocompatibility and biodegradable film would be an excellent functional material, and to be a promising candidate for applications in triggered control release system which the release rate can be changed by pH and ionic strength.

### ACKNOWLEDGMENTS

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