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Synthesis of glucose-sensitive self-assembled films and their application in controlled drug delivery

Zhibo Ding [a], Ying Guan [a], Yongjun Zhang [a,*], X.X. Zhu [b]

[a] Key Laboratory of Functional Polymer Materials, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China
[b] Department of Chemistry, Universitéde Montréal, C.P. 6128, Succursale Centre-ville, Montreal, QC H3C 3J7, Canada

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ABSTRACT

Hydrogen-bonded layer-by-layer assembled films from poly(vinyl pyrrolidone) and poly(acrylic acid) were crosslinked with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and ethylenediamine, and modified with 3-aminophenylboronic acid and isopropylamine simultaneously. The resultant films present pH- and thermosensitive swelling behaviors. Furthermore, the swelling of the films is enhanced when saccharides, such as glucose or fructose, is present in the solution. Alizarin Red S (ARS) was used as a model drug to study the drug release behavior of the films. The loading amount of ARS increases with increasing ARS concentration, which binds covalently with the phenylboronic acid groups in the film. The release of ARS is faster in the presence of glucose, which competes with ARS for binding sites in the film. The novel multistimuli-sensitive films may find applications in self-regulated insulin delivery.

1. Introduction

Layer-by-layer (LBL) assembly is a method to fabricate multilayer films by sequential adsorption of polymer pairs with complementary functional groups onto various substrates [1]. It allows for fine control of the thickness, structure and composition of the resultant films. Common driving force for the film buildup is electrostatic interaction [1], however, other driving forces, such as hydrogen bonding [2], covalent bonding and charge-transfer interaction [3], have also been exploited. Up to now, a large number of thin films have been fabricated using this facile and versatile method. They have also found a broad range of applications [4]. Recently the applications of LBL films in biomedical areas, especially as drug delivery vehicles, drew attention of a lot of researchers [4]. For this application, one common strategy is the encapsulation of drugs by LBL coatings, where the LBL films act as diffusion barriers [5]. In a second strategy, drugs are integrated into macroscopic planar LBL films during or after the film fabrication. For example, polyionic drugs as one of the building blocks were used to fabricate LBL films with a degradable polymer [6,7]. The drugs are released as the films are eroded gradually in water. Recently, films from block copolymer micelles were used for the incorporation and release of small, uncharged, and hydrophobic therapeutics [8,9]. Compared with the conventional methods, the LBL method allows for modifying medical devices of complex shapes, such as implants or stents, with therapeutic coatings. It also allows for fine control of the amount and position of the therapeutics.

The polymeric LBL films can be regarded as thin layers of hydrogel in nature. Bulky hydrogels have been widely used for drug delivery [10]. Smart hydrogels which are sensitive to external stimuli, for example temperature, pH or ionic strength, are particularly desirable, because their environmental sensitivity can be explored for controlled drug delivery [10,11]. Some LBL films that can respond to external stimuli, such as temperature [12,13] and pH, have been fabricated. Temperature [14] or pH [15] -regulated release of dyes or drugs has been reported.

For the delivery of insulin, a self-regulated system is the most suitable because it is capable of adjusting the release rate of insulin automatically according to the blood glucose level, just like what pancreas does. To this end, a lot of glucose-sensitive hydrogels were synthesized. For example, Kataoka et al. synthesized a glucose-sensitive hydrogel composed of poly(*N*-isopropylacrylamide) with phenylboronic acid (PBA) groups [16], which act as glucose-sensitive moieties. On–off regulation of insulin release from this gel was achieved. For faster response, glucose-sensitive microgels with similar composition were synthesized by us and others [17–19]. We recently confirmed that the permeability of the microgel can be controlled by glucose concentration [20].

* Corresponding author. Tel.: +86 22 23501657; fax: +86 22 23503510.
E-mail address: yongjunzhang@nankai.edu.cn (Y. Zhang).

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Thin glucose-sensitive films may play a key role in the development of self-regulated insulin delivery systems. They may be used as a diffusion barrier to regulate the release rate of insulin from a drug reservoir. For thicker films, they may act as drug reservoir, and control the release rate of drug as well. Glucose-sensitive films may also find applications in the development of new glucose biosensors. In this work, glucose-sensitive LBL films were synthesized by crosslinking and modifying the poly(vinyl pyrrolidone)/poly(acrylic acid) LBL films with PBA groups. The influence of saccharide on their swelling behavior was studied. The loading and release of Alizarin Red S, as a model drug, were examined. Since LBL assembly can be carried out on substrate of any shape and size, it should be more feasible for the LBL films to meet the needs in biomedical areas. It is noteworthy that a glucose-sensitive LBL film from Concanavalin A and glycogen [21] and a hollow capsule containing PBA groups [22] have been reported, but their application for controlled drug release has not been presented.

2. Experimental section

2.1. Materials and apparatus

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 3-aminophenylboronic acid hemisulfate (APBA) and (3-aminopropyl)triethoxysilane were purchased from Alfa Aesar. Poly(acrylic acid) (PAA) (35 wt%, $M_w \approx 100{,}000$) was purchased from Aldrich. Poly(vinyl pyrrolidone) (PVP, K30), isopropylamine (IPA), Alizarin Red S (ARS), D-fructose, D-glucose and ethylenediamine were purchased from local providers. They were of analytical grade and used without further purification.

UV–vis absorption spectra were measured on a TU 1810PC UV–vis spectrophotometer (Purkinje General, China). Fourier transform infrared (FTIR) spectra were measured on a Bio-Rad FTS-6000 spectrometer. AFM images were recorded on a Benyuan CSPM 5500 Scanning Probe Microscope in tapping mode under ambient conditions.

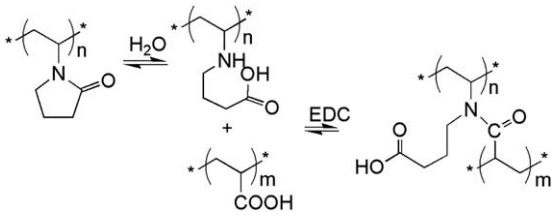
2.2. Fabrication of PVP/PAA films

LBL films for FTIR and AFM characterization were fabricated on silicon wafers. Other LBL films were all fabricated on quartz slides with a size of 44 mm × 10 mm × 1 mm. Before use, the substrates (quartz slides or silicon wafers) were cleaned in boiling piranha solution (3:7 v/v H_2O_2–H_2SO_4 mixture) (*caution*: *this solution is extremely corrosive!*), rinsed thoroughly with deionized (DI) water and dried. The substrates were modified with amino groups by immersing in 1 wt% toluene solution of (3-aminopropyl)-triethoxysilane overnight, followed by ultrasonic washing in toluene twice and dried at 100 °C in oven.

PVP/PAA LBL films were fabricated according to our previous report [23]. Because the substrates were modified with amino groups, the LBL assembly began with PAA. The concentration of the assembly solutions was 0.1 wt%. pH was adjusted to 3.0 by adding 0.1 M HCl. The substrates were immersed in the assembly solutions alternately, each for 4 min, intermediated by washing in 10^{-3} M HCl for 1 min. Totally 30 bilayers of PVP/PAA were fabricated. The films were dried in air and weighed. The net weights of the film materials were calculated by subtracting the weight of the bare substrate. The average net weight of a 30-bilayer PVP/PAA film fabricated on the quartz substrates is about 2.2 mg.

2.3. Crosslinking and modification of PVP/PAA films

The PVP/PAA films were soaked in 25 mM EDC with or without ethylenediamine at room temperature to crosslink them. pH of the crosslinking solution was adjusted to 5.0 by HCl. After 24 h, the films were withdrawn and washed with DI water. The crosslinked films were dried in air and weighed.



Scheme 1. Crosslinking of PVP and PAA in the presence of EDC.

In a second set of experiments, the PVP/PAA films were treated with an aqueous solution containing EDC (25 mM), APBA (2.5 mM), isopropylamine (21.25 mM), and ethylenediamine (0.625 mM) at 5 °C to crosslink and modify them simultaneously. pH of the mixture was adjusted to about 5.0 by HCl. After 24 h, the films were withdrawn and washed with DI water. The PBA-modified films (denoted as B-films hereafter) were dried in air and weighed.

The crosslinked/modified films were immersed in phosphate buffered saline (PBS) (50 mM, pH 7.5) to remove any uncrosslinked polymers. The weights of the net film material were calculated by subtracting the weight of the bare substrate.

2.4. Determination of the swelling degree of the B-films

The B-films (after removing uncrosslinked polymers) were immersed to swell in excess PBS. At predetermined time intervals the swollen films were taken out. They were blotted with tissue paper to remove adhering water and weighed. The isothermal swelling degree (SD) was calculated according to the following equation:

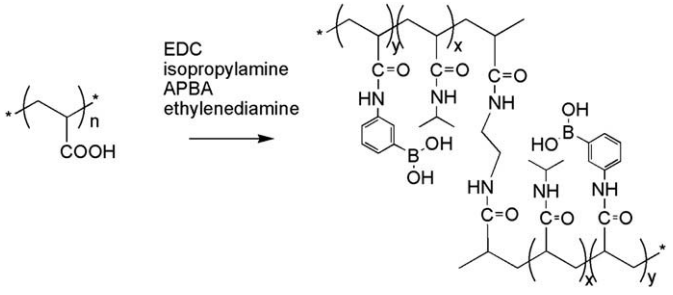
$$\mathrm{SD} = \frac{m_2 - m_0}{m_1 - m_0}$$

where m_0 is the weight of the bare substrate, m_1 and m_2 the weight of the dried and swollen films (with the substrate), respectively. The net weight of the dried films, $m_1 - m_0$, ranges from 2.5 to 3.3 mg, while that of the swollen ones, $m_2 - m_0$, ranges from 21.5 to 96.0 mg, depending on the swelling media and temperature.

The equilibrium swelling degree (SD_e), SD of the swollen film at equilibrium, was measured when the sample attained a constant mass. For each sample at least three swelling measurements were performed and the average values are reported.

2.5. Loading and release of ARS

The B-films (after removing uncrosslinked polymers) were immersed in ARS solution in PBS (50 mM, pH 8.5). At proper



Scheme 2. Simultaneous crosslinking and modification of PVP/PAA films.

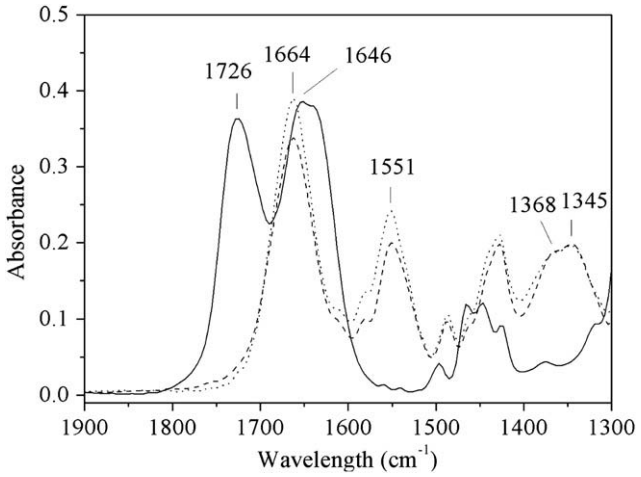


Fig. 1. FTIR spectra of an as-prepared $(PVP/PAA)_{30}$ film (solid line) and the same film after crosslinking and PBA-modification (B-film) (dash line), followed by immersing in PBS (50 mM, pH 7.5) for 24 h (dot line). The film was fabricated on silicon wafer.

intervals, the films were taken out, soaked in DI water briefly and dried. The loading amount of ARS was determined by UV–vis spectroscopy.

To measure the release kinetics of ARS, the B-films were first loaded with ARS by immersing in a 10 mM ARS solution in PBS (50 mM, pH 8.5) for 24 h, soaked in DI water briefly and dried at room temperature. They were soaked in 40 mL of release media with various compositions. At predetermined intervals the concentration of ARS in the solution was measured by UV–vis spectroscopy.

3. Results and discussion

3.1. Crosslinking and modification of the PVP/PAA LBL films

The PVP/PAA films were fabricated according to a method reported previously [23]. The driving force is the hydrogen bonding between PVP, as hydrogen acceptor, and PAA, as hydrogen donor. This system was chosen because it is easy to fabricate a thick PVP/PAA film, which allows for loading a large amount of drug as well as for the measurement of their swelling degree by weighing with an acceptable precision. It is well-known that hydrogen-bonded LBL films using polycarboxylic acids as hydrogen donor disintegrate at high pH [24]. In a first attempt to improve their stability, the PVP/PAA films were treated with EDC for 24 h at room temperature. 62.0 wt% of the original film material remained after the EDC treatment. Sequential treatment in 50 mM pH 7.5 PBS removed the uncrosslinked polymers and left 16 wt% of the original film material. The result indicates that the films are partially crosslinked by EDC. The result can be explained by the opening of the pyrrolidone rings in PVP [25] and the sequential formation of amide bonds between PVP and PAA under the catalysis of EDC (Scheme 1). As a second explanation, PVP involved in the hydrogen-bonded films may have been partially hydrolyzed before EDC treatment, which acts as crosslinker during the treatment. It is not clear at this stage whether hydrolysis of PVP takes place before or during EDC treatment.

In a second attempt, the PVP/PAA films were treated with EDC and ethylenediamine for 24 h at room temperature (pH = 5.0), where ethylenediamine acted as an additional crosslinker [26]. After the treatment, about 80.0% of the original film material remained. In the sequential treatment in pH 7.5 PBS, no more film materials were removed, indicating improved crosslinking efficiency.

In a third attempt, the PVP/PAA films were treated with a mixture solution containing EDC, APBA, isopropylamine and ethylenediamine, to crosslink and modify the film simultaneously. Again, ethylenediamine was used as crosslinker. The modification reaction is shown in Scheme 2. It is noteworthy that PVP also involves in the crosslinking of the film. The film weight increased by about 27.0% after modification. No significant weight loss was found after the sequential treatment in pH 7.5 PBS buffers.

The change of the film structure during modification and PBS treatment was studied by FTIR (Fig. 1). For the as-prepared PVP/PAA film, the stretching band of the carbonyl group in PAA moves from 1710 to 1726 cm^{-1}, while the stretching band of the carbonyl group in PVP moves from 1680 to 1646 cm^{-1}, indicating strong hydrogen-bonds form between the two polymers in the film [23]. After treatment with the EDC/amine mixture, the band of carboxylic acid in PAA at 1726 cm^{-1} disappeared completely, while a new band appeared at 1551 cm^{-1}, which is assigned to the stretch vibration of N–C═O. The result indicates that all carboxylic acid groups in PAA were amidated, as shown in Scheme 2. The band of carbonyl stretching in PVP shifts to 1664 cm^{-1}, indicating that PVP is no longer hydrogen-bonded with PAA, as a result of the conversion of PAA to polyacrylamides. The pair of bands for the gem di-methyl groups appears at 1368 and 1345 cm^{-1}, indicating the successful amidation of carboxylic acid groups with isopropylamine. After treatment in PBS, no significant change in FTIR spectra was found confirming again that the modified film was crosslinked effectively.

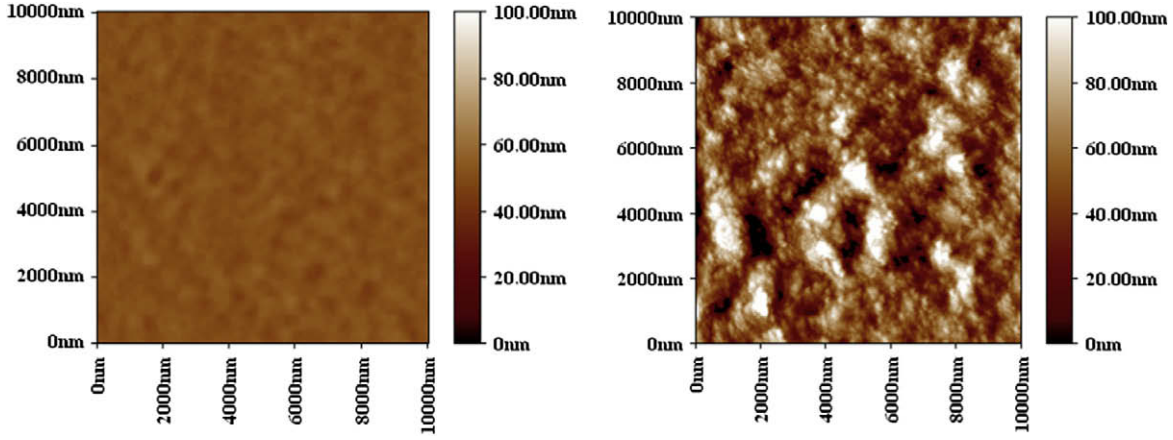


Fig. 2. AFM height images of a $(PVP/PAA)_{10}$ film before (left) and after crosslinking/modification (right).

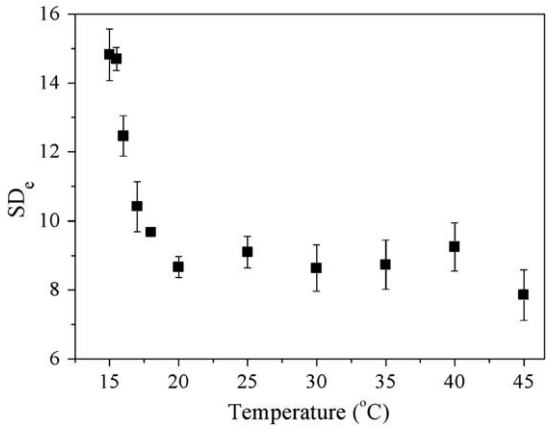
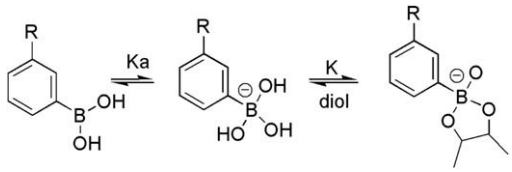


Fig. 3. SD$_e$ of a B-film in PBS (50 mM, pH 8.5) at various temperatures.

The change in surface morphology was studied using AFM. As shown in Fig. 2 the as-prepared $(PVP/PAA)_{10}$ film is highly smooth. The calculated RMS roughness is only 1.87 nm for the measured 10 μm × 10 μm area. This observation is in accord with our previous results [23]. After treated in the EDC/amine mixture, the film becomes rather rough with a RMS roughness of 21.8 nm. The film thickness was also measured by scratching part of the film. Before modification, the 10-bilayer film is about 250 nm in thickness. After modification, the film thickness is reduced to about 200 nm. (see Supporting information for details) The reduced film thickness may suggest that some film materials were lost during the crosslinking/modification process.

3.2. Swelling behaviors of the PBA-modified B-films

To study the effect of temperature on the swelling of the PBA-modified B-film, its equilibrium swelling degree (SD$_e$) was measured at various temperatures. As shown in Fig. 3, at $T > 20$ °C, SD$_e$ did not change with temperature, however, a significant increase in SD$_e$ was found when temperature was below 20 °C. At $T < 15$ °C partial film detachment occurred because the films were highly swollen, which prevented the accurate measurement of SD by weighing. From Scheme 2, the B-films are in nature a thin crosslinked hydrogel of poly(*N*-isopropylacrylamide-co-3-acrylamidophenyl boronic acid) (P(NIPAM-PBA)). PNIPAM, as a well-known thermosensitive polymer, has a lower critical solution temperature (LCST) of about 32 °C, above which it becomes hydrophobic and precipitates from an aqueous solution [27]. For PNIPAM hydrogels, a volume phase transition will occur at the same temperature [28]. The higher SD$_e$ of the B-films at low temperatures should be attributed to PNIPAM involved in the films. From Fig. 3, the volume phase transition temperature (VPTT) of the B-films can be estimated to be about 15 °C. The much low VPTT compared with that of PNIPMA hydrogels should be attributed to the hydrophobic "comonomer" 3-acrylamidophenylboronic acid. Assuming a similar reactivity of isopropylamine and APBA with PAA under EDC catalysis, the molar ratio of NIPAM/PBA in the film should be the same with that in the modification mixture, i.e., 8.5:1. The VPTT of the B-films is comparable to that of P(NIPAM-PBA) microgel with a similar composition [17].



Scheme 3. Complexation equilibrium between PBA derivative and 1,2-diol.

The synthesis of thermosensitive LBL films by incorporating the thermosensitive PNIPAM polymer into LBL films has been the subject of several previous efforts. Caruso et al. fabricated multilayer films from PNIPAM and PAA using hydrogen bonding as the driving force [14]. In other approaches charged derivatives of PNIPAM were synthesized and incorporated within polyelectrolyte multilayers [29–32]. However, all these films or capsules showed a very limited thermosensitivity, as compared with linear PNIPAM or PNIPAM hydrogels. In addition, their thermo-response was either irreversible or only partially reversible, which is quite different from their parent polymers. The poor thermosensitivity has been attributed to the strong spatial confinement of the PNIPAM chain in the LBL films [33].

This work presents a new approach to incorporate thermosensitive PNIPAM into LBL films. Instead of direct assembly of PNIPAM with a complementary polymer, PNIPAM is synthesized by the modification of a precursor polymer PAA. PAA has been widely used to fabricate LBL films, via either electrostatic interaction or hydrogen bonding, with a wide range of complementary polymers. Therefore this new method may provide an opportunity to synthesize thermosensitive LBL films with various compositions and properties. A second advantage of the present system is that

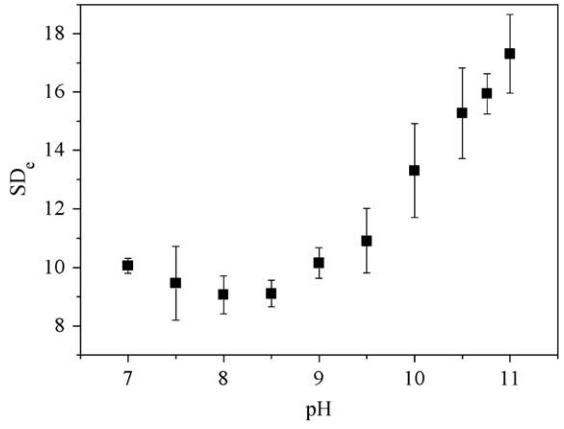


Fig. 4. SD$_e$ of a B-film in 50 mM PBS of various pH at 25 °C.

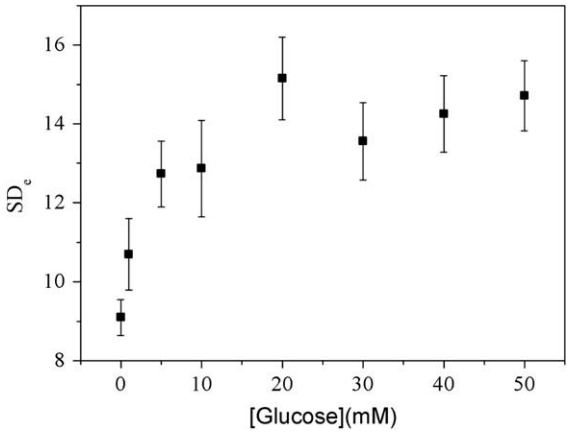
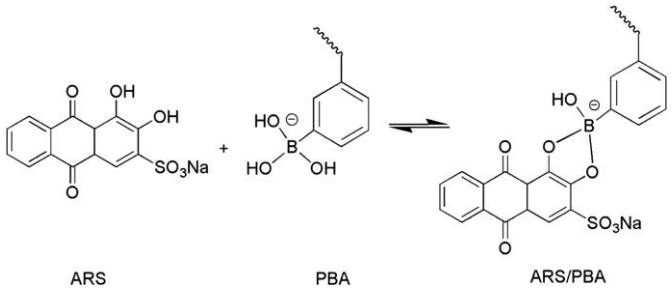


Fig. 5. SD$_e$ of a B-film in PBS (50 mM, pH 8.5) containing various concentrations of glucose. $T = 25$ °C.



Scheme 4. Structure of ARS and its binding with PBA group.

the PNIPAM chains in the resultant films are no longer hydrogen- or ionically bonded with a second polymer, as other LBL films containing PNIPAM [14,29–32]. The disappearance of restriction on PNIPAM chains significantly improves the thermosensitivity of the resultant films (Fig. 3).

Fig. 4 illustrates the effect of pH on the swelling of the B-films in 50 mM PBS at 25 °C. The SD_e of the films does not change in the pH range of 7.0–9.0. Significant increase in SD_e was observed when pH is higher than 9.5. PBA, as a weak acid, exists as the uncharged trigonal form and the charged tetrahedral phenylboronate in aqueous solutions (Scheme 3). The pK_a of the PBA unit in 3-(propionamido)phenylboronic acid was reported to be 8.6 [34]. With increasing pH, more PBA groups transform from the hydrophobic uncharged form to hydrophilic charged form. The increase in osmotic pressure in the film and Coulombic repulsion among the charged groups drive the film to swell to a larger degree at high pH.

The SD_e of the B-films in the presence of glucose were measured. As shown in Fig. 5, SD_e increases with increasing glucose concentration in the media and gradually level off when glucose concentration is higher than 10 mM. This result clearly shows that the B-films are glucose-sensitive. Obviously the PBA groups in the films act as glucose-sensitive moieties. As shown in Scheme 3, PBA is in equilibrium between the undissociated and the dissociated form in aqueous solutions. The binding of PBA with diols, such as glucose, causes the charged form thermodynamically more favorable. As a result, the dissociation equilibrium of PBA moves to the right and the effective pK_a of the PBA group decreases. For a hydrogel modified with PBA group, the binding of diols increases the degree of ionization on the hydrogel and builds up a Donnan potential for the hydrogel swelling [16]. As mentioned above, the B-films are thin hydrogels in nature. The binding of glucose with PBA groups in the films increases their degree of ionization, which in turn causes a more extensive swelling of the films.

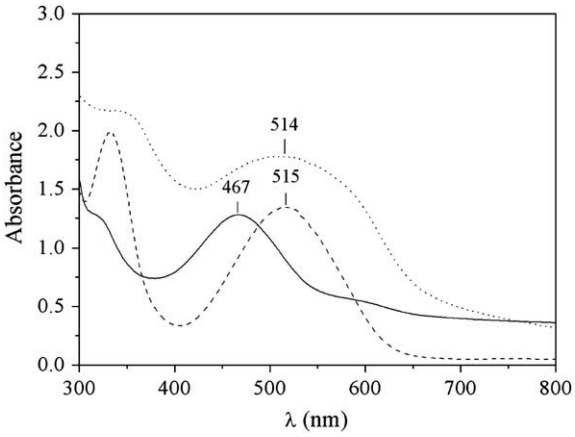


Fig. 7. Absorption spectra of (solid line) a B-film loaded with ARS, (dash line) ARS in 50 mM pH 8.5 PBS buffer and (dot line) a cast film from the ARS/PBS solution.

The presence of other diols, such as fructose, mannitol and galactose, also results in an enhanced swelling of the B-films. Among them, fructose was found to show the largest effect, which is in agreement with other groups' observation that fructose has a higher binding constant with PBA groups than other saccharides [35]. pH has a significant effect on saccharide-sensitivity of the films. For example, the films show high sensitivity to the presence of fructose at pH 8.5 and 9.0, however, only a slight fructose-induced increase in SD_e of the film was observed at pH 7.5 and 8.0. Further efforts are needed to achieve saccharide-sensitivity at physiological pH by modifying the structure of the PBA moiety [36] or other methods.

3.3. Loading and release of ARS

ARS, a dye with 1,2-diol structure, was selected as a model drug to study the loading and release behavior of the B-films. Its chemical structure is shown in Scheme 4. The B-films were immersed in ARS solution in PBS and the loading of ARS was traced by UV–vis spectra. As shown in Fig. 6A, the load of ARS is quick at first and slows down gradually. It reaches equilibrium in 500 min regardless of the ARS concentration in the loading solutions. Fig. 6B illustrates the equilibrium loading amount of ARS as a function of ARS concentration in the loading solution. As can been seen, the

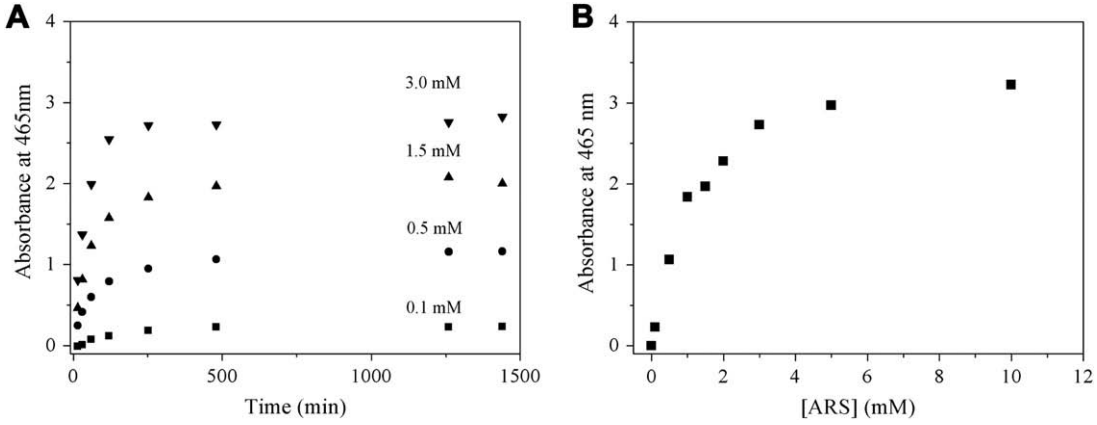


Fig. 6. (A) Loading kinetics of ARS from ARS solutions with various concentrations: (■) 0.1, (●) 0.5, (▲) 1.5 and (▼) 3.0 mM. The loading amount of ARS was represented by the film absorption at 465 nm. ARS solutions were prepared using 50 mM pH 8.5 PBS. $T = 25$ °C. (B) The equilibrium loading amount of ARS as a function of ARS concentration in the loading mixture.

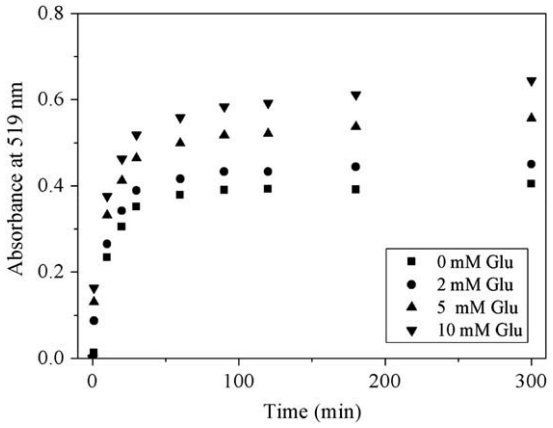


Fig. 8. Release of ARS in PBS (50 mM, pH 8.5) containing various concentrations of glucose. Release amount of ARS was represented by the absorption of the media at 519 nm. $T = 25\ ^{\circ}C$.

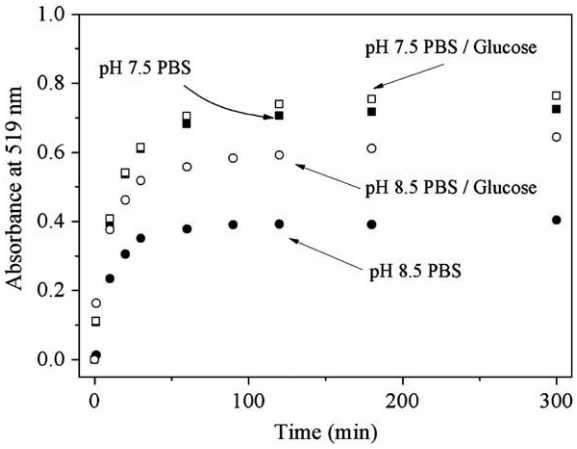


Fig. 9. Release of ARS in (■) PBS (50 mM, pH 7.5), (□) PBS (50 mM, pH 7.5) containing glucose (10 mM), (●) PBS (50 mM, pH 8.5), and (○) PBS (50 mM, pH 8.5) containing glucose (10 mM). Release amount of ARS was represented by the absorption of the media at 519 nm. $T = 25\ ^{\circ}C$.

equilibrium loading amount first increases linearly with increasing ARS concentration in the loading solution, but levels off at higher ARS concentrations, which can be explained by the saturation of ARS adsorption due to the limited binding sites in the B-film. Fig. 7 shows the absorption spectra of a B-film loaded with ARS. For comparison, the spectra of ARS in PBS buffer and a cast film from the ARS/PBS solution are also presented. Compared with ARS in solution, when loaded in the film, the absorption band of ARS shifts from 515 nm to 467 nm, indicating that most of ARS in the B-film are bound to the PBA groups, as shown in Scheme 4. Similar spectral change of ARS upon complexation with PBA group has been reported in the literature [37,38].

The B-films loaded with ARS were soaked in PBS and the release of ARS was followed spectroscopically. The release kinetics of ARS in pH 8.5 PBS contain various concentrations of glucose were studied. For all cases, an initial quick release of ARS was observed (Fig. 8). The release of ARS gradually slows down and finally stops in about 100 min, where the equilibrium of release and adsorption reaches. The initial release rate of ARS increases with increasing glucose concentration in the release media. The total released amount of ARS follows the same order.

The release of ARS in PBS can be regarded as a process to re-establish the equilibrium among the free ARS, the bound ARS (ARS/PBA) and the free PBA groups in the films as shown in Scheme 4. The presence of other diols, for example, glucose, will partially consume free PBA groups, thus shift the equilibrium to the left hand. In other words, glucose will compete with ARS for PBA binding sites and result in an increased release of ARS. Apparently, the more glucose is present in the media, the more ARS will release from the B-film. The competition of glucose with ARS for PBA binding sites is schematically illustrated in Scheme 5.

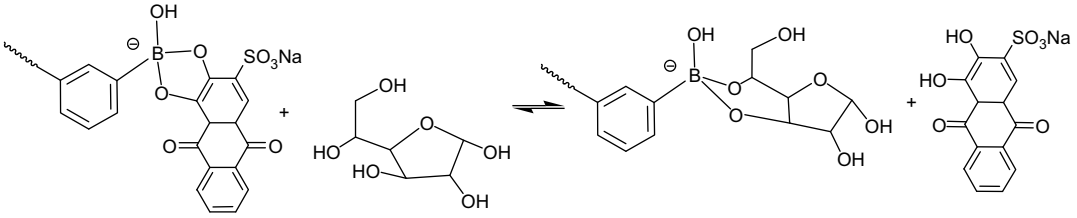
The flux of a material across a given plane is proportional to the concentration gradient across the plane, as described by Fick's first law of diffusion:

$$J = -D(\partial C(x,t)/\partial x)$$

where J is the flux, D the diffusion constant of the material, and $\partial C(x,t)/\partial x$ the concentration gradient. Although Fick's Law is too simple to describe the release of ARS from the film, it may explain the increased initial release rate in the presence of glucose. On one hand, the shift of equilibrium shown in Scheme 4 suggests a higher effective concentration gradient of ARS in the presence of glucose, which is the driving force for the release. On the other hand, the enhanced swelling of the films in the presence of glucose (Fig. 5) provides larger pores for the dye to diffuse through, i.e., ARS may have a higher diffusion constant. Both factors may contribute to the increased initial release rate in the presence of glucose.

A close examination of the release kinetics curve shown in Fig. 8 reveals that after the initial quick release, the release of ARS in pure PBS actually stops after a certain time, while a slow release is still proceeding in the presence of glucose. The result may suggest that the replacement of ARS by glucose is slower than the diffusion of ARS. At the initial stage, because of the high ARS concentration gradient, the release is mainly controlled by the diffusion of ARS, however, as the ARS concentration in the media gradually approaches that in the film, the replacement of ARS by glucose becomes the rate-limiting step.

The following set of experiments examined the effect of pH of the media on the release of ARS. As shown in Fig. 9, both the initial release rate and the total amount of released ARS in release media of pH 7.5 are higher than that in release media of pH 8.5. This result is not surprising because ARS and PBA have a lower association constant at pH 7.5 than at pH 8.5. At pH 7.5, more ARS/PBA will disassociate and more free ARS will release from the film. The presence of glucose in pH 8.5 release media significantly increases both the initial release rate and the total released amount, however, a much smaller effect was observed in the release media of pH 7.5.



Scheme 5. The competition of glucose with ARS for PBA binding sites in the film. Note that besides the mode shown here, glucose may also bind with PBS in other modes.

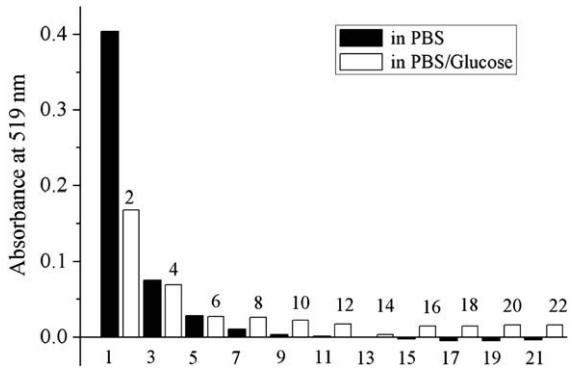


Fig. 10. Release amount of ARS as represented by the absorption of the media at 519 nm when immersed alternately in 50 mL of fresh PBS (50 mM, pH 8.5) and 50 mL of fresh PBS (50 mM, pH 8.5) containing 10.0 mM glucose, each for 10 min. $T = 25\ ^{\circ}C$.

This is in agreement with the observation that the film is more sensitive to saccharide at pH 8.5 than at pH 7.5.

A film loaded with ARS was immersed alternately in 50 mL of fresh PBS and PBS containing 10 mM glucose, each for 10 min. The released ARS, as indicated by the absorbance of the media at 519 nm, is plotted in Fig. 10. The release of ARS in pure PBS actually stops after changing the media for 6 times, but continues in PBS containing glucose. The result confirms again that at the late stage the release of ARS is mainly controlled by the replacement of ARS by glucose.

4. Conclusions

Hydrogen-bonded layer-by-layer assembled films from poly(vinyl pyrrolidone) and poly(acrylic acid) were crosslinked with EDC and ethylenediamine, and modified with 3-aminophenylboronic acid and isopropylamine simultaneously. The swelling of the resultant films present a pH-, thermo- and saccharide-sensitive behavior. ARS, as a model drug, was loaded in the films, which bound covalently with PBA groups in the films. The release of ARS is faster in the presence of glucose, which competes with ARS for binding sites. We expect that drugs containing diol structure, especially the glycosylated insulin [39], may bind to and release from the film in a similar way as ARS. The novel multi-stimuli-sensitive films may find applications in self-regulated insulin delivery.

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Appendix. Supporting information

The supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.polymer.2009.07.001.

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