A facile method for the fabrication of covalently linked PAH/PSS layer-by-layer films†

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We report a facile method for the fabrication of robust polyelectrolyte multilayers including strong polyanions. Covalently cross-linked PAH/PSS [PAH: poly(allylamine hydrochloride); PSS: poly(sodium-p-styrene sulfonate)] multilayered films are fabricated using a traditional layer-by-layer technique, followed by post-infiltration of a bifunctional photoactive molecule 4,4′-diazido-2,2′-stilbenedisulfonic acid disodium (DAS) and subsequent photochemical cross-linking. The stability of the cross-linked multilayers was enhanced significantly. Over 90% of the cross-linked multilayers were preserved in a basic solution or good solvent of the polyelectrolyte, forming a clear comparison with the un-crosslinked multilayers, which almost completely decomposed under the circumstances mentioned above.

Introduction

The layer-by-layer (LBL) technique has been widely applied to construct various composite nano-/micro-structures and functional devices. It bears merits such as (i) being facilely constructed, (ii) being compatible with various functional components including functional organic molecules, proteins, and nanoparticles, and (iii) the nanostructure of the targeted multilayers can be controlled by adjusting the experimental parameters, among others. Not only in cutting-edge research, but also in commercial products, the LBL technique has been widely employed. However, LBL multilayers were traditionally fabricated using oppositely charged polyelectrolytes, and the driving force for construction was electrostatic attraction that was unstable in certain situations, such as in solutions with high/low pH values, in solutions of high ionic strength, in good solvents of the employed polyelectrolytes, or in solutions with disturbances, like under sonication.† The insufficient stability of the traditional LBL multilayers posed pressing challenges for the application of LBL multilayers. To address this challenge, chemists have developed strategies to convert the interlayer forces from weak supramolecular interactions to strong covalent bonds. Generally, two strategies have been developed: one is to fabricate multilayers using chemical reactions as driving forces;‡ the other is post-cross-linking of the as-prepared self-assembled films.† For example, for the first strategy, 3,4,9,10-perylene-tetracarboxylic dianhydride and glutaraldehyde were employed not only as building blocks but also as cross-linking agents for the fabrication of the multilayers;* 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was used in an LBL process to activate the carboxylic groups to construct covalently linked multilayers;‡ terephthaloyl chloride and glutaraldehyde were employed as reactive building blocks;‡ poly (acrylic acid) (PAA) grafted with either azido groups or alkyne groups were used as reactive building blocks, among others. For the second strategy, photochemical reactions were proven to be an efficient method to post-cross-link the constructed multilayers: polyelectrolyte with diazonium groups and vinylbenzyl groups served as the cross-linkable component in the construction of photochemically linked multilayers;†‡* post-cross-linking of the multilayers using thermo reactions†* and redox reactions†‡ were also reported. However, most of the above strategies rely on building blocks prepared by tedious synthesis, which bear the specific functional groups for a certain reaction.

Strong polyanions are widely used in the construction of multilayers using the technique of layer-by-layer (LBL) deposition, but the obtained multilayers can be disassembled in highly acid or basic conditions or in a good solvent of the polyelectrolyte. Since it’s difficult to modify most strong polyanions with reactive functional groups, it has been a challenge for chemists to covalently cross-link the multilayers containing strong polyanions. Herein, taking PSS [poly(sodium-p-styrene sulfonate)] as an example, we report a facile method for the construction of crosslinked polyelectrolyte multilayers including polyanions. We stabilized the PSS/PAH multilayers through the post-infiltration and subsequent photochemical cross-linking strategy: PAH/PSS multilayers were constructed using traditional procedures. Then, a bifunctional photoactive small molecule 4,4′-diazido-2,2′-stilbenedisulfonic acid disodium (DAS) was infiltrated into the multilayers. Subsequently,
UV irradiation was employed to initiate the photochemical reaction between DAS and its adjacent C–H or N–H functional groups to cross-link the multilayers. The results showed that, after photochemical cross-linking, the stability of the multilayers had been enhanced significantly. Before UV irradiation, the un-cross-linked multilayer could be disassembled thoroughly in an alkaline solution with a pH value larger than 12; after photochemical cross-linking, 90% of the original amount of the cross-linked multilayers was retained under an identical basic treatment. Therefore, this method proves to be an efficient strategy to covalently stabilize PAH/PSS multilayers.

**Experimental**

### 2.1 Materials and instruments

The following chemicals were used as supplied: hydrogen peroxide (30 wt%), sulphuric acid, and sodium dodecyl sulfate (SDS) were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Poly(allylamine hydrochloride) (PAH) (M<sub>w</sub> = 15,000), poly(sodium-p-styrene sulfonate) (PSS) and (3-mercaptopropyl)-trimethoxysilane (MPTS) were purchased from Sigma-Aldrich. 4,4′-diazido-2,2′-stilbenedisulfonic acid disodium (DAS) was purchased from TCI.

UV-visible spectra were obtained on a Hitachi U-3900H spectrophotometer. The surface morphology of the polyelectrolyte multilayers was characterized with atomic force microscopy (AFM, Dimension 3100) from Veeco, U.S.A. Photochemical cross-linking was conducted employing a commercially available 400 W high-pressure mercury lamp. An ultrasonic field was applied with an ultrasonic cleaner with a power of 150 W and a frequency of 40 kHz.

### 2.2 PAH/PSS multilayers

#### 2.2.1 The modification of sulphonic groups on the substrate. The self-assembled monolayer of sulphonic groups was formed through the following procedure: first, the substrate (quartz substrate or silicon wafer) was treated with a piranha solution (H<sub>2</sub>SO<sub>4</sub>:H<sub>2</sub>O<sub>2</sub> (quartz substrate or silicon wafer) was treated with a piranha solution is very corrosive and should be handled with caution) and washed with copious amounts of water; secondly, the as-treated substrate was immersed in a toluene solution of MPTS (1 × 10<sup>−5</sup> M) for 12 h, to produce a monolayer of mercapto groups;thirdly, the substrate modified with mercapto groups was treated with a mixed solution of 30% H<sub>2</sub>O<sub>2</sub>-acetic acid (v/v = 1 : 5) at 50 °C for 1 h to oxidize the mercapto groups to sulphones.

#### 2.2.2 Formation of PAH/PSS multilayered films. The LbL assembly process of PAH/PSS multilayers was carried out as follows (Scheme 1): first, the quartz substrate modified with sulphonic groups was immersed in PAH (aq., 1 mg mL<sup>−1</sup>, pH = 9.4) for 20 min, washed with pure water and dried under a nitrogen flow; second, the quartz was transferred to PSS (aq., 1 mg mL<sup>−1</sup>, pH = 6) for 20 min, washed with pure water and dried under a nitrogen flow; third, the above two steps were alternatively deposited in cycles until the desired bilayers were obtained. The assembled multilayer is noted as (PAH/PSS)<sub>7.5</sub> when the outer layer is PAH.

#### 2.2.3 Photochemical cross-linking of the multilayers and the methods employed to investigate their stability. We immersed the substrate with (PAH/PSS)<sub>7.5</sub> multilayers into an aqueous solution of DAS (5 mg mL<sup>−1</sup>) with a pH value of 3.8 for 80 min to reach its equilibrium distribution. The (PAH/PSS)<sub>7.5</sub> multilayers were irradiated with a 400 W high-pressure mercury lamp from a distance of 20 cm with an intensity of 2.5 mW cm<sup>−2</sup> for 120 s without any filters.

To evaluate the stability of the films, we treated the (PAH/PSS)<sub>7.5</sub> multilayers by immersion of the substrate into aqueous basic solutions for 30 min. The pH was adjusted using NaOH to the desired values. We also used saturated SDS solution to evaluate the stability of the multilayers, which served as a good solvent of the polyelectrolytes. In such evaluations the substrates were sonicated in aqueous saturated SDS solution for 10 min.

**Results and discussion**

Because PAH and PSS are widely used in the construction of multilayers using the technique of layer-by-layer (LbL) deposition, and the as-constructed multilayers could be disassembled in basic conditions (see below), we aim to increase the stability of the PAH/PSS multilayers by photochemical cross-linking with difunctional photoactive small molecules. The method we employed was described as “post-infiltration and subsequent photochemical cross-linking”. A UV-visible spectrum was used to track the LbL deposition process of PAH and PSS. As shown in Fig. 1a, PSS featured a peak at 225 nm and the number of bilayers. The linear increase suggested that the content of building blocks absorbed within every bilayer was almost identical.

The driving force for the PAH/PSS multilayers was electrostatic interaction. Because the ionization of PAH would be inhibited under basic conditions, the interaction between PAH
and PSS would be destroyed, leading to the entire disassembly of the multilayer. As shown in Fig. 1b, the (PAH/PSS)_{7.5} multilayer (black line) exhibited an absorbance value of 0.35 at 225 nm and after immersing in a basic solution (pH = 14) for 30 min, the typical absorbance of PSS disappeared and the absorbance of the substrate returned to the baseline (red line). This change of absorbance demonstrated that the PAH/PSS multilayers decomposed from the substrate during immersion, highlighting the necessity to enhance their stability under similar circumstances.

To increase the stability of the multilayers, the post-infiltration and subsequent photochemical cross-linking of a bifunctional photoactive molecule DAS was applied. DAS carries two sulfonic groups and two azido groups. The sulfonic groups brought negative charges to DAS, which could facilitate the diffusion of DAS into the multilayers by the electrostatic attraction provided by the positively charged PAH; meanwhile, the azido groups were able to generate a highly reactive nitrene under UV irradiation, which was expected to insert into the adjacent C(sp^3)-H, C(sp^3)-H, N-H bonds non-selectively, or undergo rearrangement followed by addition from a nucleophilic reagent like amine (Scheme 2). Therefore, after the construction of the (PAH/PSS)_{7.5} multilayer, the substrate was immersed into an aqueous solution of DAS (5 mg mL^{-1}, pH = 3.8) and characterized by UV-visible spectroscopy. In Fig. 2a, the typical absorbance of DAS at 340 nm in the multilayers increased with immersion time. The diffusion process was rapid at the early stage but slowed down gradually after 5 min, and reached equilibrium within 20 min (Fig. 2a inset). The maximum loading amount of DAS molecules in a (PAH/PSS)_{7.5} multilayer was calculated to be 0.3099 μg cm^{-2} per layer.

In order to clarify that the adsorption of DAS occurred not only on the outmost layer of PAH but also within the multilayer, we replaced the outmost layer from PAH to PSS by changing the (PAH/PSS)_{7.5} multilayer to (PAH/PSS)_{8} multilayer and investigated the adsorption kinetics of DAS (Fig. 2b). Due to the electrostatic repulsion of sulfonic groups between DAS and PSS at the outmost layer, the loading rate was decreased. The time to reach equilibrium increased from 20 min to 80 min, while the saturated adsorption value declined from 0.57 to 0.35, resulting in a maximum loading amount of DAS molecules of 0.1903 μg cm^{-2} per layer. This phenomenon demonstrated that the strong polyelectrolyte did affect the loading of DAS through electrostatic repulsion and the DAS molecules infiltrated into the interior of the multilayer instead of surface adsorption only.

After saturated adsorption of DAS, the multilayers were subjected to UV irradiation (400 W, from a distance of 20 cm) for 2 min. The absorbance of the multilayers at 340 nm decreased with the elongation of the duration of irradiation and the absorbance around 210–300 nm, and around 400 nm increased. These phenomena were consistent with the hypothesized process that DAS was photochemically decomposed to generate reactive nitrenes and then reacted with adjacent C-H or N-H functional groups. The photoreaction of DAS can be modelled as an approximately first-order reaction. For peak absorbance at 340 nm, a linear fit of ln([A_0 - A_{min}]/(A_{0} - A_{min})) versus time gave a rate constant (k) of 0.63 s^{-1} for the photochemical reaction (Fig. 2c), where A_{min} is the minimum invariable absorbance after UV irradiation for 120 s and A_0 and A_t represent the absorbance at time zero and time t, respectively.

To investigate the stability of the multilayers after photochemical cross-linking, we compared the multilayers with or without photochemical cross-linking by treating them with different solutions. After being subjected to a basic solution (pH = 14) for 30 min, the absorbance of the photo-cross-linked multilayers barely changed (Fig. 3a), forming a clear comparison with un-crosslinked multilayers as described above (Fig. 1b). In addition, we further observed that with the loading amount of DAS at 25%, 45%, and 50% of the saturated value, the residual amount in the multilayer was 34.5%, 93.53%, and 93, 76%, detected by UV-vis spectroscopy. A control experiment also indicated that UV irradiation itself didn’t increase the durability of the multilayer, since multilayers without infiltration by DAS but irradiated under UV light also disassembled upon washing in basic solution (Fig. S1†). To demonstrate the comparisons more clearly in stability before and after the “infiltration and subsequent photochemical cross-linking” process, the stability of the multilayers was evaluated at different pH values (Fig. 3b). The un-crosslinked multilayers presented partial deterioration in the pH range 10.5–11, and were completely decomposed at pH values above 12. However, in a clear comparison, the cross-linked multilayers demonstrated significantly enhanced stability in all the basic solutions (pH ranges from 10 to 14), and over 90% of the multilayers
remained as previously indicated by UV-vis absorbance measurements. Moreover, we tested the stability of the multilayers in a good solvent of polyelectrolytes. A film being ultrasonicated in a saturated SDS solution for 10 min, the un-crosslinked multilayers almost completely decomposed and the absorbance returned to the baseline. In comparison, the cross-linked multilayers presented satisfactory stability and 90% of the absorbance was preserved (Fig. 3c). These results demonstrated that the post-infiltration and subsequent photochemical cross-linking with DAS could improve the stability of the PAH/PSS multilayers significantly.

During the stabilization, AFM was used to characterize the surface morphology of the multilayers. Before infiltration of DAS, the as-prepared multilayer of (PAH/PSS)$_{7.5}$ showed a smooth surface with a roughness of 1.2 nm (Fig. 4a). After loading with DAS, the surface morphology changed little and had a similar surface roughness, which was almost identical to the conditions after the photochemical cross-linking process. This phenomenon indicated that the multilayers were not disturbed during the infiltration process or by the photochemical reaction (Fig. 4). The contact angle on the surface of the multilayers changed from 60.1° (before the infiltration of DAS) to 47.8° (after photo-crosslinking) (Fig S2†). Compared with other stabilizing approaches such as the diazo-resins system, the post-infiltration and subsequent photochemical cross-linking strategy does not change or damage the surface morphology and structures and also retains its surface charge while increasing the stability remarkably. Therefore, the cross-linked multilayer can be used as a substrate to reassemble the same or different kinds of polyelectrolyte multilayers. To demonstrate this concept, we reassembled the PSS/PAH multilayer on the stabilized (PAH/PSS)$_{7.5}$ film and observed that the subsequent PSS/PAH presented a linear increase, as shown in Fig. 5. The results demonstrated that the cross-linked multilayer retained its surface charge and thus provided promise for applications in the fabrication of sandwiched films with various composites of different cross-linking degrees.

With the widespread application of the LbL technique, the need to enhance the stability of the as-constructed multilayers became a pressing challenge. First reported by us, the “post-infiltration and subsequent photochemical cross-linking” strategy proved a promising solution to the challenge. However, until now, only multilayers constructed by PAH/PAA polyelectrolyte pairs and PAH/iron oxide nanoparticles could be significantly stabilized. This report demonstrated for the first time the applicability of the strategy to PSS/PAH multilayers. PSS, as a strong electrolyte, displayed different physical chemical properties to PAH. The applicability of the strategy demonstrated with multilayers comprising weak electrolytes in systems including a strong polyelectrolyte should not be taken for granted. For example, the dense negative charges on PSS would possibly impede the infiltration of the negatively charged DAS into the multilayers. Fortunately, the experimental results demonstrated that DAS could still diffuse into the multilayers, possibly facilitated by the positively charged PAH. Considering the universality of the application of PSS in the construction of polyelectrolyte multilayers, it’s expected that the report would facilitate research based on PSS/PAH multilayers, possibly

![Fig. 2](image-url) **Fig. 2** UV–vis spectra and absorption kinetics of DAS in (a) (PAH/PSS)$_{7.5}$ multilayers and (b) (PAH/PSS)$_{8}$ multilayers; (c) kinetic decomposition of DAS monitored by UV–vis spectra.

![Fig. 3](image-url) **Fig. 3** (a) UV-vis spectra of cross-linked (PAH/PSS)$_{7.5}$ multilayers before (black) and after (red) immersion into a basic solution (pH = 14) for 30 min. (b) Fraction retained for the cross-linked (red) and un-crosslinked (black) (PAH/PSS)$_{7.5}$ multilayers upon immersion into basic solutions of different pH values. (c) UV-visible spectra of multilayers: before (Line 1) and after (Line 3) UV irradiation, ultrasonicated in SDS for un-crosslinked (Line 2) and cross-linked (Line 4) films.
including various protecting layers constructed via the LbL technique; nano-/micro-vesicles constructed via the LbL technique and numerous biomedical-related retarded-release or targeted-delivery systems.

Conclusions

In conclusion, we demonstrated that “post-infiltration and subsequent photochemical cross-linking” was a facile and efficient strategy to significantly stabilize PSS/PAH multilayers. After multilayers of PSS/PAH were constructed using a conventional LbL technique, a bifunctional photocative molecule DAS was infiltrated into the multilayers. Subsequently, photochemical cross-linking was carried out by UV irradiation. In a clear comparison to the un-crosslinked PSS/PAH multilayers, which decomposed completely under basic solution (pH range from 12 to 14) or in a good solvent of the polyelectrolyte, the cross-linked multilayers demonstrated satisfactory stability with over 90% of the absorbance retained. Considering the widespread application of LbL techniques, this report is anticipated to benefit research based on PSS/PAH multilayers, and has promising applications in various related functional systems.

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Notes and references


