Loading and Release Behaviors of Compressed Polyelectrolyte Multilayers for Small Dye Molecules

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Loading and release behaviors of compressed polyelectrolyte multilayers composed of poly(styrenesulfonate sodium salt) and poly(diallyldimethylammonium chloride) were investigated using fluorescein and rhodamine 6G as indicators by confocal laser scanning microcopy, fluorescence spectroscopy, and UV−vis spectroscopy. Compression of the multilayers resulted in a more densely packed microstructure, leading to the decrease of fluorescence intensity of the incorporated probes to 80% of its initial value, and much slower releasing rate as well as smaller releasing amount regardless of the types of the probes and the presence of salt. Utilizing the difference of loading and release rates between the compressed and the uncompressed regions, arrays of dye reservoirs have been fabricated on a chemical homogeneous but physical heterogeneous multilayer film.

1. Introduction

Control over surface and interface properties is very important in many existing and emerging technologies, especially those with electroactive or bioactive materials. Subtle changes in organization and composition at a molecular level would often result in dramatic performance enhancement of surfaces or interfaces, which can be fulfilled by the layer-by-layer (LbL) technique, a method to build multilayers of polyelectrolytes.[1] In this process, when a surface is exposed alternately to polyelectrolytes of opposite charge, a polymer composite film of uniform thickness is fabricated. Combinations of polyelectrolytes with other charged materials, including small organic molecules or inorganic compounds, biomacromolecules such as proteins or DNAs, macromolecules such as dendrimers, and colloids or latex particles, have proven the flexibility and the promise of the LbL technique.[2]

A consistent picture of polyelectrolyte multilayer (PEMs) growth and structure has been rather difficult to obtain. However, the integration of the observations of layer interpenetration, individual layer profiles, charge stoichiometry, surface charge, and counterion content have conveyed a hint of the intrinsic structure of PEMs.[3] Moreover, the permeability and swelling−shrinking property of PEMs also exclusively correlate with their microstructure, such as the existence of defects, pores, and water content in the films.[4]

Recently, we have reported the irreversible compression of polyelectrolyte multilayers, particularly assembled with poly(diallyldimethylammonium chloride) (PDADMAC), by pressing a poly(dimethylsiloxane) (PDMS) stamp against the films. The compression extent varies from several to hundreds of nanometers following a positive correlation with the multilayer thickness, which can be normally tuned by layer number or salt concentration. Dependence of the compressibility on water content, molecular architecture, salt concentration, layer number, pressing time, pressure, surface morphology, and other factors has been evaluated. The extent of desiccation is the most crucial factor.[5]

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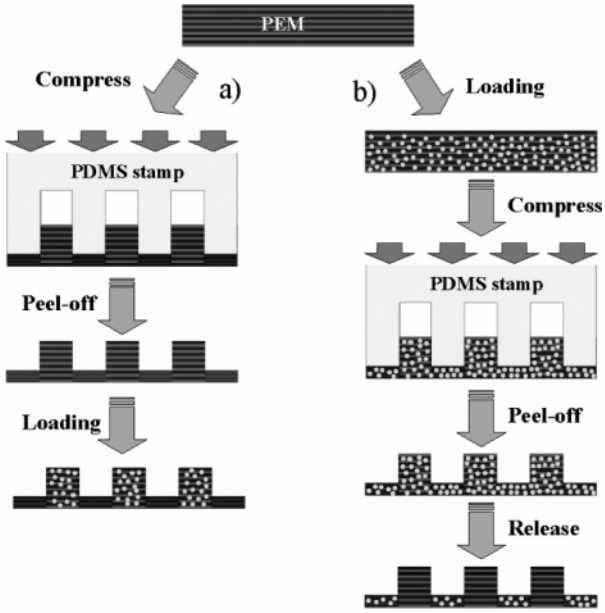

Figure 1. Schematic illustration to show the difference of loading and release behaviors for compressed and uncompressed PEMs. For details see text.

Herein we make further study on variation of loading and release behaviors of the compressed poly(styrenesulfonate sodium salt) (PSS)/PDADMAC multilayers for small dye molecules, which can be expected by the compact state of the layer structure after compression. Indeed, one of the most promising potential uses for PEMs is in the area of controlled release, with implications in drug delivery, personal care products, sensors, and filtration.[6] In this paper, we attempt to extend the understanding and applications of compressed multilayer films for controlled loading and release of small dye molecules. We show that both the loading and release behaviors vary dramatically after the compression (Figure 1). For this purpose, two protocols are adopted. In the first protocol (Figure 1a), PSS/PDADMAC multilayers are compressed first by using a patterned PDMS stamp to create alternate compressed and uncompressed regions on the same film. A subsequent incuba-

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tion of the film in dye solution will selectively load dyes in the uncompressed regions. In the second protocol (Figure 1b), loading of the dyes in the multilayers is performed before compression. A slower release rate in the compressed regions can then yield dye patterns.

2. Experimental Section

Polyethylenimine (PEI, M_n 60 000, M_w 750 000), PDADMAC (M_w 200 000−350 000), and PSS (M_w 70 000) were obtained from Aldrich to prepare 10^{-4} M aqueous solutions, supplemented with 1 M sodium chloride (99+%). PDMS prepolymers were obtained from Dow Corning, Sylgard 184. Fluorescein and rhodamine 6G were purchased from Aldrich-Sigma to prepare 1 mg/mL solution.

Quartz and silicon wafers were cleaned in 70% H_2SO_4 (concentrated)/30% H_2O_2(aq) ("piranha") and then in hot H_2O_2/ammonia/water, 1:1:5 (vol/vol), rinsed in water, and blown dried with a stream of N_2. To obtain an increased adsorption and a flat surface, a precursor layer of PEI was deposited on the treated substrata. Then sequential adsorption of the polyelectrolytes was performed by manually dipping. Between alternate exposures to two kinds of polymer solutions for 20 min, there were three rinses with 0.1 M NaCl solution for 3 min. At the last step, the films were rinsed with triple-distilled water for at least 5 min to eliminate the adsorbed salt and then dried at 10 °C in close chamber with 70% relative humidity for 12 h.

Patterned PDMS stamps, with microwells each measuring 30 μm in diameter, 30 μm in space, and 4 μm in deepness, were molded from lithographically prepared master.[7] The patterned or flat stamp was then pressed onto the PEMs on substrata for 30 min with a normalized pressure of 200 g/cm^2.

Films were immersed in fluorescein or rhodamine 6G solutions at room temperature for 2 h and then were rinsed with triple-distilled water for 1 min. The loading amount was quantified by fluorescence spectroscopy as well as UV−vis spectroscopy. Some samples were compressed with a flat stamp. The release of the dyes from the uncompressed or compressed films was tracked using fluorescence spectroscopy. Fluorescein and rhodamine 6G were exited at 494 and 510 nm to produce maximum emission wavelengths at 510 and 550 nm, respectively. The films were then immersed into a beaker containing 50 mL water or 2 M NaCl solution. The solution was replaced with fresh water or salt solution every 30 min to ensure accurate absorbance readings, so that the concentration of the dyes remained dilute enough to follow Beer's law.

Confocal laser scanning microscopy (CLSM) images were obtained using Bio-Rad Radiance 2100 confocal laser-scanning microscope. Topographic images were collected by atomic force microscope (AFM, SPI3800N, Seiko Instruments Inc.) in dynamic force mode. Fluorescence spectra were recorded on a fluorescence spectrophotometer (Hitachi F-4500). UV−vis spectra were obtained from a UV−vis spectrophotometer (Cary 100 BIO, America).

3. Results and Discussion

As PSS molecules shows a more severe adhesion to PDMS stamp which would destroy the uniformity of the multilayers during the compression process, the PEI(PSS/PDADMAC)$_7$ multilayer platform with PDADMAC as the outmost layer was selected for loading and releasing small dye molecules. The compression deepness was measured with AFM, and it was approximately 156 nm for PEI(PSS/PDADMAC)$_7$ multilayers, which is approximately 85% of the original thickness.[5]

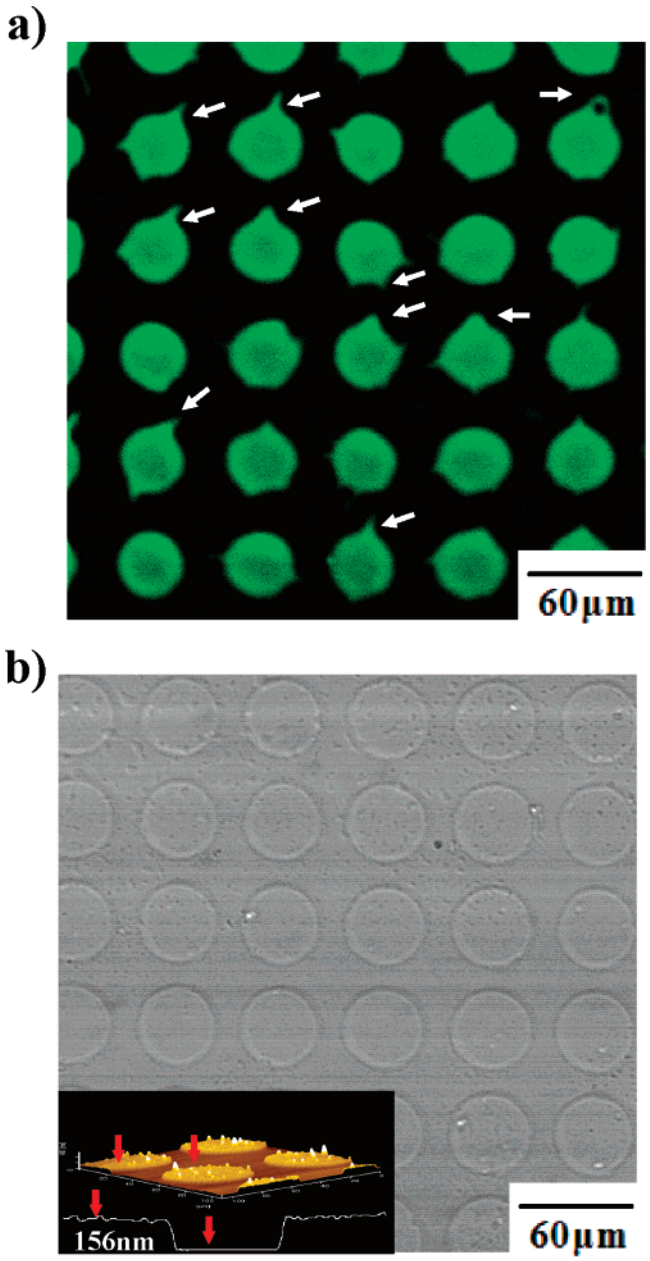

Figure 2. (a) CLSM image of patterned compressed PEI(PSS/PDADMAC)$_7$ multilayers after incubated in fluorescein solution and rinsed. Uncompressed regions show a green color due to the dye loading. (b) Transmission CLSM image of patterned multilayers. Inset: AFM image as a higher magnification of (b). Concave regions had been brought contact with stamp.

After compressed with a patterned stamp, the multilayers were immersed in fluorescein solution followed by rinsing with distilled water to remove the dye molecules that weakly adsorbed on the surface. Figure 2a shows that the dye molecules only permeated through the uncompressed regions (in green). For those compressed regions, it seems that, at least, the dye molecules did not diffuse into the film with sufficient amount that can be observed by CLSM (in black). By this way, fluorescein patterns were fabricated on a chemical homogeneous but physical heterogeneous surface. It could be also regarded as formation of arrays or microreservoirs for small molecules. Previous study[8] has shown that diffusion coefficient of small dye molecules (rhodamine) in the multilayers, which are prepared without additional salt, is higher by a factor of 2 than that of multilayers prepared with 1 M NaCl. The difference in

the permeability was explained by the size distribution of defects in the multilayers, from a few large defects at low ionic strength to many small ones at high ionic strength.[8] Apparently, the variation of loading behavior here is brought by the denser package after compression.

What is more interesting is that, although the round-shape patterns have been checked with CLSM transmission mode or AFM (Figure 2b), antenniform protuberances around the patterns as indicated by the arrows in Figure 2a were still visible. The formation of these protuberances is attributed to the stress variation at the pattern boundary when the stamp is peeled off from the multilayers, which usually does not cause the deformity of the patterns but variation of other modalities, permeability for example.

This result implies that to study the release behavior, the loading has to be performed in prior to the compression, since it is difficult for the dye molecules to permeate into the compressed films directly. We adopted here fluorescein sodium salt (negatively charged) and rhodamine 6G (positively charged) of similar size and hydrophilic/hydrophobic characters as typical probes. The red shift of maximum emissions for fluorescein and rhodamine 6G was observed upon incorporation in multilayers compared with free state in solutions (Figure 3, a and b, respectively, indicated by the arrows) probably due to a change in the environment.[6] After compression with a flat PDMS stamp, the maximum emission wavelength of the probes did not vary significantly, but the intensity of the emission was only 80% of the uncompressed ones. Characterized under UV−vis spectroscopy, a similar red shift was also observed for fluorescein, but the absorbance of the compressed and the uncompressed films was almost same (Figure 3c). Thus, it can be deducted that the dye molecules did not transfer to the stamp in the compression process. No detectable fluorescence on stamp after compression also confirmed this observation. The inconsistency between fluorescence spectrum and UV−vis spectrum could be explained by environmental variation of fluorescent molecules after compression.[9] Unfortunately, UV−vis spectroscopy was not sensitive enough to detect the absorbance of rhodamine 6G. Possibly it could be attributed to the molecular architecture resulting in weak absorbance. However, the influence of small amount of loading could not be excluded.

Mass amounts of dye released into solution were measured with a fluorescence spectrophotometer. The released profiles of fluorescein and rhodamine 6G from PEI(PSS/PDADMAC)$_7$ multilayers immersing in pure water or 2 M NaCl solution are presented in Figure 4. Basically the release rates were much higher from the uncompressed films than those from the compressed ones regardless of the types of the dye molecules and the salt concentration. From the uncompressed films in 2 M NaCl solution, the releasing rate was quite rapid; e.g., 80% of the total dyes was released within 25 min. Even in pure water, the half-life of the release was shorter than 50 min. By contrast, the releasing rates from the compressed films were much slower. Moreover, the total mass released from the compressed films in 6 h (<100 μg/cm^2 for fluorescein) was only about half of the uncompressed ones (>180 μg/cm^2 for fluorescein).

The concentration gradient between the multilayer film and its surrounding environment causes dye diffusion from the film. Salt here exhibits a positive effect to accelerate the release of the adsorbed dyes from the uncompressed multilayers, especially in the initial stage within 6 h. However, the releasing profiles from the compressed films were quite insensitive to the addition of salt. Theoretically, the total released amount should show a positive correlation to the loaded amount rather than to the state

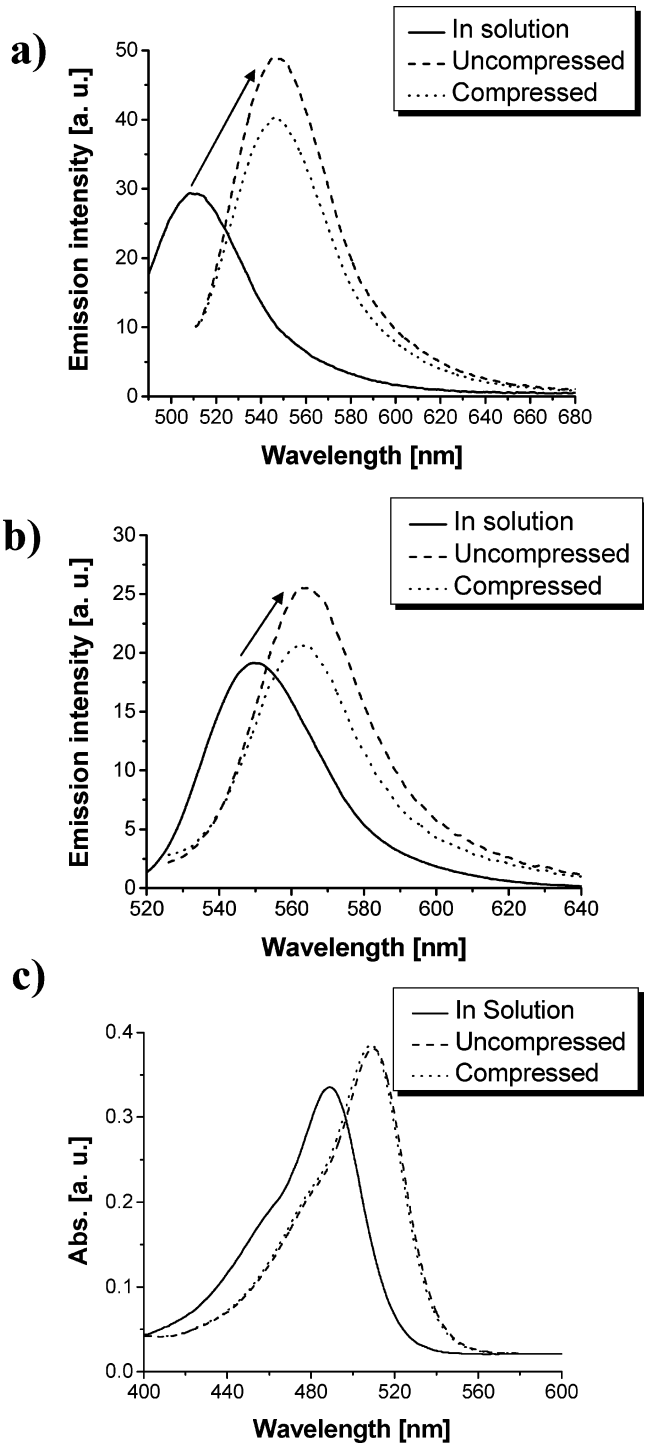

Figure 3. Fluorescence spectra of (a) fluorescein, and (b) rhodamine 6G in solution and in PEI(PSS/PDADMAC)$_7$ multilayers before and after compression. (c) UV−vis spectra of fluorescein in solution and in PEI(PSS/PDADMAC)$_7$ multilayers before and after compression.

of the multilayers. So the compressed films would be expected to release similar amount of dyes as the uncompressed ones but in a much longer period of time. Actually, dye molecules could be detected in the solution even after 1 week for the compressed films with continuous replacement of fresh water. As to the difference between rhodamine 6G and fluorescein, one could only measure half of the released amount of the former to the latter. This result is in consistence with the loaded amount and might be induced by the electrostatic repulsive force between the outmost layer (PDADMAC, positively charged)

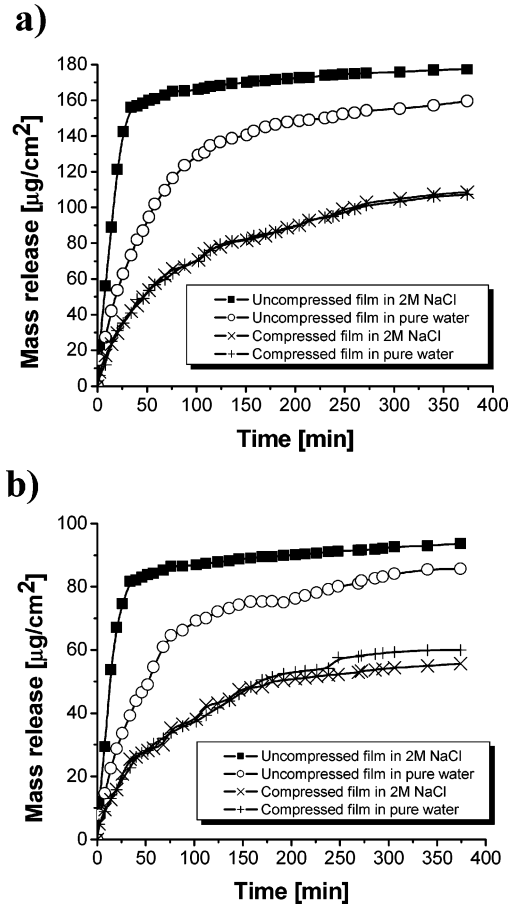

Figure 4. Release profiles of (a) fluorescein and (b) rhodamine 6G from compressed and uncompressed PEI(PSS/PDADMAC)$_7$ films in water or in 2 M NaCl solution.

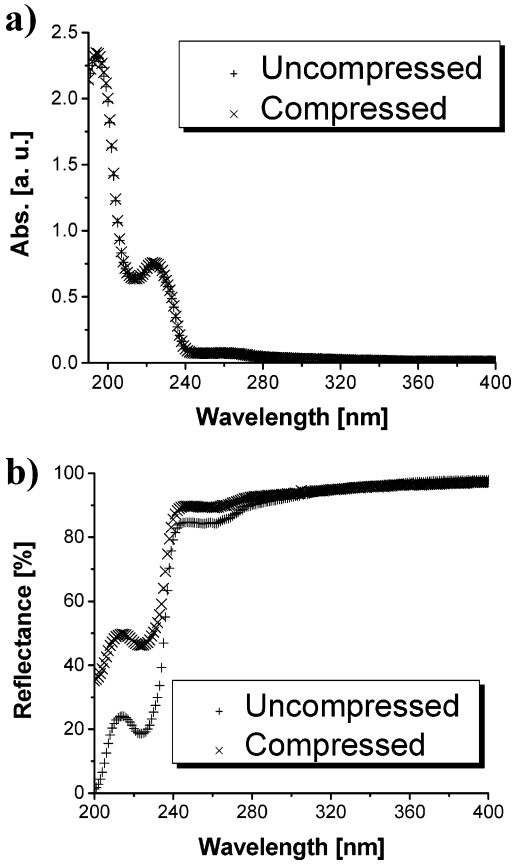

Figure 5. UV−vis (a) absorbance spectra and (b) reflectance spectra of uncompressed and compressed PEI(PSS/PDADMAC)$_7$ multilayers (bare quartz is defined as 100% reflectance).

and the rhodamine molecules (also positively charged), which may result in the difficulty of loading.[10]

The compression likely closes up voids and channels in the polymer matrix that facilitate small molecule movement. Also, the larger the free volume in the uncompressed film, the more likely small aggregates of dye molecules will escape in addition to the single molecules. Pore formation for macroscopic flat films composed of weak polyelectrolytes is recently detected in acids and salt solutions.[11] These pores have a radius of a order of 10^2 nm and a cross section of approximately 105 nm^2. We also have observed the micrometer scale pores on poly(acrylic acid) (PAA)/PDADMAC multilayers as well as the close of pores after compression by AFM (data not shown). Unfortunately, similar pores were not observed for PEI(PSS/PDADMAC)$_7$ multilayers. Nevertheless, that the detected surface roughness by AFM decreased from 18 to 6 nm after compression[5] could be regarded somewhat as a proof of channel close. Moreover, contrast of the uncompressed and the compressed films was investigated by UV−vis absorbance spectroscopy (Figure 5a) as well as reflectance spectroscopy (Figure 5b). The bands at 195 and 226 nm in the absorbance spectra originated from the aromatic chromophores of PSS did not vary with respect to both position and intensity, indicating again that no mass loss in the compression process. However, the relative reflectance below 280 nm was considerably enhanced after compression (Figure 5b), reflecting that the film was more densely packed with a smoother surface.

The other effect brought by the compression is that the positive and the negative charged polyelectrolyte chains can approach much closer to produce higher density of ion cross-linking, which in turn can effectively minimize film swelling caused by osmotic force in aqueous environment that is crucial for substance release.[6,11a] That roughness increases from 18 to 21 nm for the uncompressed films as a result of exposure to water for 6 h could be regarded as a proof of this swelling. By contrast, the roughness for the compressed films remains unchanged. The stronger electrostatic bindings between the polyelectrolytes cause again the difficulty for the probe molecules to diffuse through the network via transient bond breaking and reestablishment.[12] Even though the mechanism cannot be pointed out absolutely, we believe that all these factors contribute to the deceleration of release rate after compression jointly.

As to the influence of salt, small ions are known to weaken electrostatic bonds between the polyelectrolytes to induce swelling in the uncompressed multilayers.[13] Although direct measurement of the expansion of PEMs by salt is quite difficult,[13] increased permeability of multilayers under higher salt concentrations attributed to weakened electrostatic binding has been actually observed.[6a,12] X-ray reflectivity measurements on planar multilayer films also indicate that swelling occurs at salt concentrations larger than 10^{-2} M, caused by the shielding and breaking of ionic bonds between the polycations and the polyanions.[14] It has also been reported that the PSS/PDADMAC multilayers would swell to 130% of their original thickness in the presence of 2 M NaCl.[3f,14b] Because of the swelling effect, the release of the uncompressed films was accelerated in the presence of salt. As to the compressed film, the disability of swelling indeed causes the insensitivity of release behavior to the presence of salt, which is consistent with our previous study,[5] in which no recovery of multilayers' deformation could be

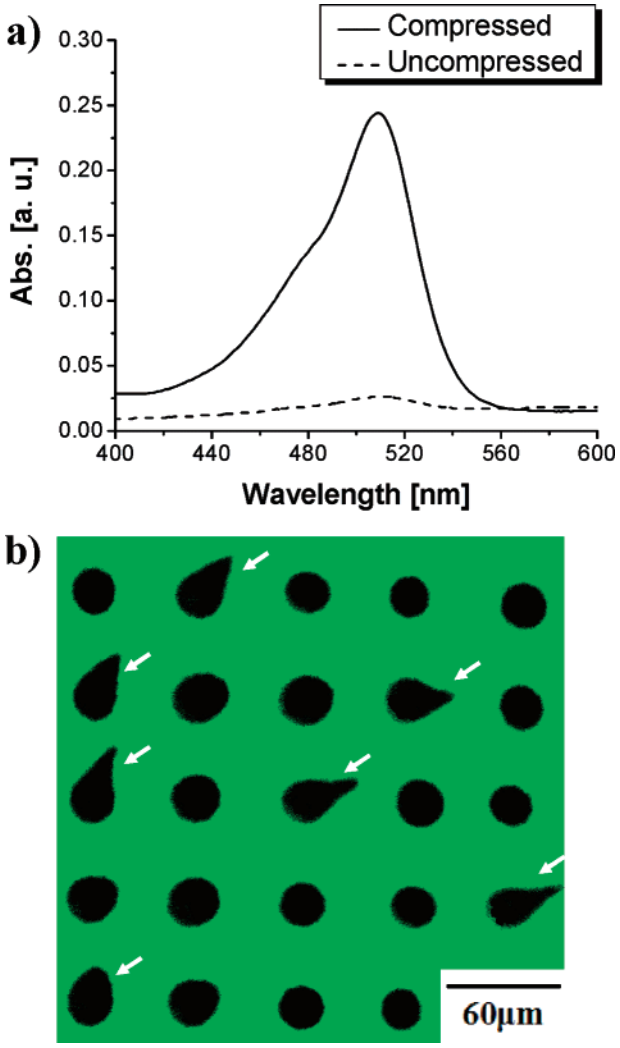

Figure 6. (a) UV−vis spectra of fluorescein in uncompressed and compressed multilayers after incubation in 2 M NaCl for 25 min. (b) CLSM image of patterned compressed multilayers loaded with fluorescein after incubated in 2 M NaCl solution for 25 min. Compressed regions show a green color due to the presence of dye molecules.

observed even immersed in 2 M NaCl for 8 h. It is worth mentioning that after incubation in 2 M NaCl for 6 h the roughness of the uncompressed films decreases from 18 to 10 nm, while that of the compressed ones remains constant (6 nm). Higher salt concentration is known to enhance the mobility of the charge-paired polyelectrolyte chains which smoothes the multilayer surface.[15] Compression presumably freezes the segments of the polymer chains, which retards the migration of the polyelectrolytes as well as the swelling capability.

We have noticed that in 2 M NaCl solution 90% fluorescein molecules had been released in 25 min for the uncompressed films while only less than 30% for the compressed ones. UV−vis spectroscopy also confirmed this observation (Figure 6a). After incubation in salt solution for 25 min, the absorbance at 510 nm decreased from 0.38 to 0.26 for the compressed film and further to 0.03 for the uncompressed films. Utilizing the difference of the release rate, the uncompressed regions on the patterned dye-loaded film appeared black under the observation of CLSM (Figure 6b), while the compressed regions remained in green after immersion in 2 M NaCl for 25 min. Antenniform protuberances around the patterns (indicated by the arrows) could also be observed as that of the loading (Figure 2a). This novel patterned release probably could find wide applications in biosensors, nanofiltration, and bioreactors.[16]

4. Conclusions

We have demonstrated here the distinctive loading and release behaviors of small dye molecules in the compressed polyelectrolyte multilayers. Much slower releasing rate and smaller releasing amount were found for the homogeneously compressed PSS/PDADMAC multilayers. The mechanism was investigated by surface topography and UV−vis spectroscopy. More densely packed microstructure after compression is the main reason for this variation of modalities. Utilizing the difference of loading and release rates between the compressed and the uncompressed regions, arrays of dye reservoirs have been fabricated on a chemical homogeneous but physical heterogeneous multilayer surface, which in turn may find wide applications in design of electronic and photonic devices as well as biosensors.

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