

Photoresponsive Multilayer Films of Chitosan and an Azopolymer

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ABSTRACT: The main goal of this work was the development of photoactive multilayer films. Taking into account that chitosan is a biodegradable, biocompatible, nontoxic, and antibacterial biopolymer, the fabrication of chitosan-based multilayer films can be an interesting pathway to obtain biopolymer-based films in which properties can be combined with a light-responsive material, such as an azobenzene-containing polymer. In particular, the layer-by-layer technique was used for the fabrication of the optically active films consisting of alternating layers of chitosan and an azopolymer. The influence of the pH and the number of bilayers on the structure and properties of the films was investigated by different experimental techniques and it was found that the roughness and elastic modulus of the multilayer films increased with the decrease in chitosan solution pH. Besides, induced birefringence measurements revealed that a higher level of photoorientation was attained with the decrease in pH and the increase in bilayer number of the developed films.

KEYWORDS: Azopolymer, birefringence, chitosan, layer-by-layer, polyelectrolyte

1 INTRODUCTION

Chitin [(1 → 4)-2-acetamido-2-deoxy-β-D-glucan] is a biomaterial encountered in the exoskeleton of shrimps, crabs and other arthropods, as well as in the cell walls of fungi, and is the most abundant natural polymer in the world after cellulose [1]. In addition, it is a white, hard, inelastic, nitrogenous polysaccharide, which can be degraded by chitinase, and its immunogenicity is exceptionally low, in spite of the presence of nitrogen [2]. However, the applications of this naturally abundant material are limited because of its poor solubility.

When the degree of deacetylation of chitin reaches around 50%, it becomes soluble in aqueous acidic media and is called chitosan (CH) [(1 → 4)-2-amino-2-deoxy-β-D-glucan]. The solubilization occurs by protonation of the -NH₂ group on the C-2 position of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in acid media [1].

Thus, chitosan, with a pKa of around 6.5, behaves as a polycation in acid media and it finds applications in many different areas. In fact, chitosan is a biodegradable, biocompatible, nontoxic, and antibacterial biopolymer that has simple processing properties that make it a promising candidate for varied purposes, including food preservation and packaging, crop protection, cosmetics, and biomedical and pharmaceutical applications [2,3]. Figure 1 shows the chemical structures of chitin and chitosan.

Layer-by-layer (LbL) adsorption of oppositely charged polyelectrolytes on solid substrates has been shown to be a versatile technique for the development of polyelectrolyte multilayer (PEM) films [4–6], where not only thickness and composition can be controlled, but also their properties may be tuned synergistically with a combination of distinct materials in the same film [4,7].

The fabrication of chitosan-based PEM films can be an interesting pathway to obtain high quality films in which properties can be manipulated or improved by combination with other polymers [8–10]. For instance, in combination with photoresponsive polyelectrolytes, light can be used to control PEM films' properties [5]. Azopolymers offer advantages over other

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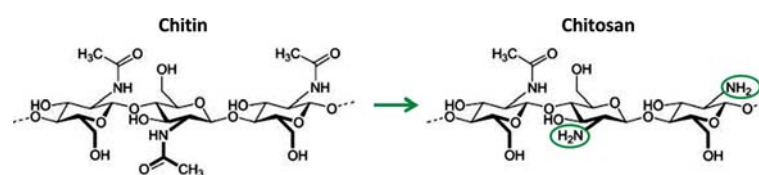


Figure 1 Chemical structures of chitin and chitosan.

stimuli-responsive materials due to the fast, reversible and innocuous trans-cis isomerization of azobenzene molecules upon light exposure, which leads to interesting applications ranging from optical storage to photomechanical bending and, recently, to different biomedical uses [11–21].

In this work, PEM films of chitosan, as polycation, and a nontoxic azopolymer, poly[1-[4-(3-carboxy-4-hydroxyphenylazo)benzenesulfonamido]-1,2-ethanediyl, sodium salt] (PCBS), as polyanion, were developed and characterized. Atomic force microscopy (AFM) equipped with PeakForce QNM (Quantitative Nanomechanical Property Mapping) is a powerful tool for studying local mechanical properties of materials and was used simultaneously to obtain the Young modulus and the topography of the multilayer films. Additionally, induced birefringence measurements were carried out using an optical setup described elsewhere [22].

2 EXPERIMENTAL

2.1 Materials

The azopolymer, poly[1-[4-(3-carboxy-4-hydroxyphenylazo)benzenesulfonamido]-1,2-ethanediyl, sodium salt], was supplied by Sigma-Aldrich and used as received. Medical-grade chitosan (degree of deacetylation of 96% and molecular weight of 300000 g/mol, measured by viscosity) was purchased from Mahtani Chitosan PVT. Ltd. (India). CH was purified before used by a precipitation method [23]. Briefly, CH was dissolved in 1% (v/v) aqueous acetic acid solution, filtered and precipitated by neutralizing with sodium hydroxide up to a pH = 8.5. The ensuing precipitate sample was washed with distilled water until a neutral pH and air-dried.

2.2 Polyelectrolyte Multilayer Films Preparation

Multilayer films were obtained by LbL self-assembly of CH, with a pKa value of about 6.5, and PCBS that contains a carboxylic acid which has a pKa value of around 4–5. Aqueous solutions of both polyelectrolytes were prepared in concentrations of 5 g/L. The pH of

PCBS solution was 9 in all experiments. In the case of CH, the pH of the solutions was adjusted within the range from 2 to 5. Washing solutions were Milli-Q water with the same pH as CH solution.

The PEM films were deposited onto glass slides at room temperature. Firstly, glass substrates were placed in a ‘piranha’ solution (70% sulfuric acid / 30% hydrogen peroxide) for 30 min to clean and render a negative surface charge. The cleaned glass slides were then rinsed thoroughly with Milli-Q water and dipped alternately in the CH and PCBS solutions for 60 s up to different number of layers, as represented in Figure 2. Although it may seem surprising that such a short period of time is sufficient for polymer adsorption, it has been shown that the first stage of adsorption, which may represent a considerable amount of material adsorbed, occurs within 5–10 s [24]. In all cases, the last layer was of PCBS.

2.3 Polyelectrolyte Multilayer Films Characterization

Ultraviolet-visible (UV-Vis) absorption spectra of all PEM films deposited onto clean glass slides were recorded with a UV-3600 UV-Vis-NIR spectrophotometer from Shimadzu.

The film thickness was determined by partly scratching the film off the glass slide surface and measuring the step height between the uncovered glass slide and the film by atomic force microscopy. The AFM images were obtained with a Nanoscope IIIa scanning probe microscope (Multimode™, Digital Instruments) under ambient conditions. Tapping mode in air was employed using an integrated tip/cantilever (125 μm in length with ca 300 kHz resonant frequency and spring constant of ca 40 N/m), with typical scan rates during recording of 0.7–1 line/s. Morphological and quantitative nanomechanical properties of the developed PEM films were also investigated by AFM. In this case, measurements were performed using a Bruker Dimension Icon AFM operated under PeakForce mode in order to obtain high-resolution elastic modulus mapping under ambient conditions, with an integrated TAP150A tip having a resonance frequency of 142–162 kHz, spring constant of ca 3.6 N/m and estimated tip radius of 40 nm.

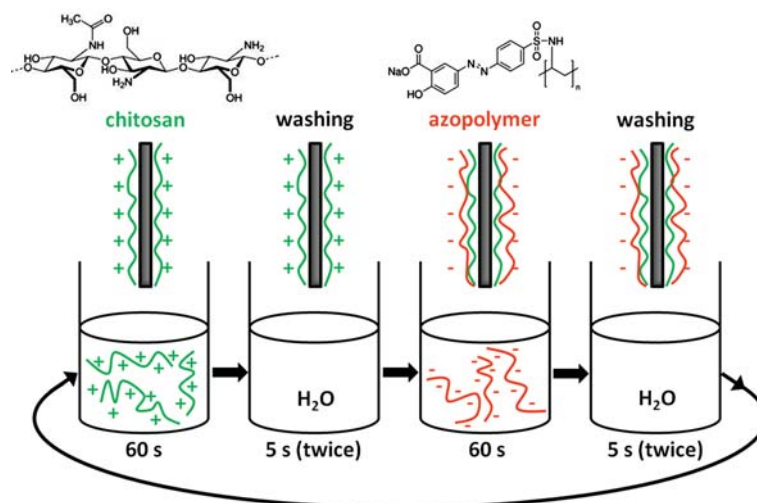


Figure 2 Schematic illustration of the layer-by-layer process and chemical structures of chitosan and the azopolymer.

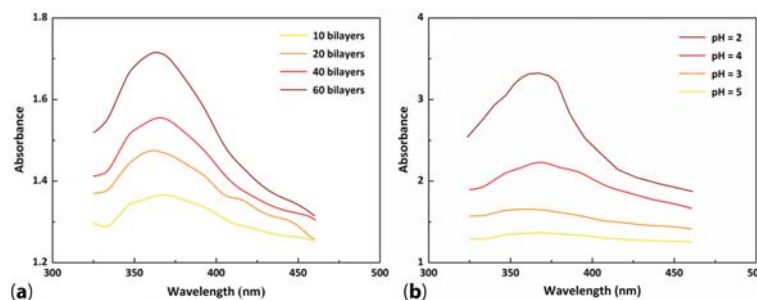


Figure 3 UV-Vis spectra as a function of (a) bilayer number for PEM films obtained at pH = 5, and (b) pH for PEM films with 10 bilayers.

Birefringence was induced in PEM films under ambient conditions using a linearly polarized argon laser operating at 488 nm (pump beam) with a polarization angle of 45° with respect to the polarization direction of a low power He-Ne laser operating at 632.8 nm (probe beam), as described elsewhere [22]. The power of the pump beam used in the experiments was varied between 6 and 20 mW on a spot of 0.4 mm^2 and the change in the transmission of the probe beam, which passed through the sample between two crossed polarizers, was measured with a photodiode.

3 RESULTS AND DISCUSSION

Both CH and PCBS are weak polyelectrolytes. Therefore, the degree of ionization varies significantly with the change in pH, leading to variations in the PEM films' features. In order to study this effect, PEM films were fabricated under different pH conditions and the number of CH/PCBS bilayers was also modified.

The UV-Vis absorbance spectra of the different PEM films as a function of bilayer number and pH are shown in Figure 3. An absorption maximum appears at around 365 nm, which corresponds to the electronic transition of azobenzene groups in PCBS. The optical absorbance of the films increased with the number of bilayers deposited, indicating a uniform absorption at each deposition step. However, on increasing the pH of CH solution the UV-Vis absorbance of PEM films decreased. This could be related to the fact that polyelectrolytes can change their conformation and interactions between them under different pH conditions [25]. In each CH/PCBS bilayer interface, acid-base reactions between the amino and carboxylic groups take place, which result in proton transfer from CH to PCBS. Here, PCBS solution was maintained at pH = 9 and CH solution was varied within the range of pH = 2–5. Hence, the azo-containing polyanion solution was at a high constant pH and, therefore, polymeric chains might be completely charged and adopted an expanded conformation due to high electrostatic repulsions between polymer segments. Under lower

pH conditions, less charged polymeric chains would adopt more globular conformations. In the case of CH solution, with increasing pH from 2 to 5, less polymeric segments became charged. In the deposition process, PCBS will interact with CH chains in an attempt to neutralize the positively charged surface. If the pH of CH solution is very low, more polymeric segments can become charged and, thus, a higher amount of PCBS chains would be able to interact with CH layer. Conversely, if the pH of CH solution is higher, less polymeric segments can become charged and a lower amount of PCBS would be absorbed onto the previous CH layer.

In this case, the maximum UV-Vis absorbance was achieved for films fabricated under the lowest pH conditions (pH = 2). This result is in good agreement with the above assumption. At lower pH conditions, a higher amount of the photoactive polyanion was absorbed in the deposition process, leading to a higher UV-Vis absorbance. The only exception occurred at pH = 3, a condition for which the films showed an absorbance lower than expected. This fact was also observed by other authors [24,26,27]. A possible explanation for

that behavior might be associated with a distinct coiling of CH at pH = 3 [24].

Figure 4 shows topographic images of the multilayer films obtained by AFM. The PEM films morphology changed as a function of the pH of CH solution. On decreasing the pH, the number of surface bumps decreased and they became higher and wider, due to a coalescence process and a consequent radial growth favored by electrostatic interactions. The increase of roughness during the coalescence process confirmed that the growth of surface bumps was also vertical. Cross-section profiles of elastic modulus mapping images obtained from AFM of PEM films are also shown in Figure 4. The elastic modulus increased from around 106 to 178 MPa on decreasing the CH solution pH from 5 to 2. It should be taken into account that the buildup of these multilayers results from the formation of interactions between the cationic and anionic groups of CH and PCBS, respectively; and, as a function of pH, the number of ionized groups and interactions between layers change. The degree of ionization of PCBS chains in solution is very high during all the deposition process since the

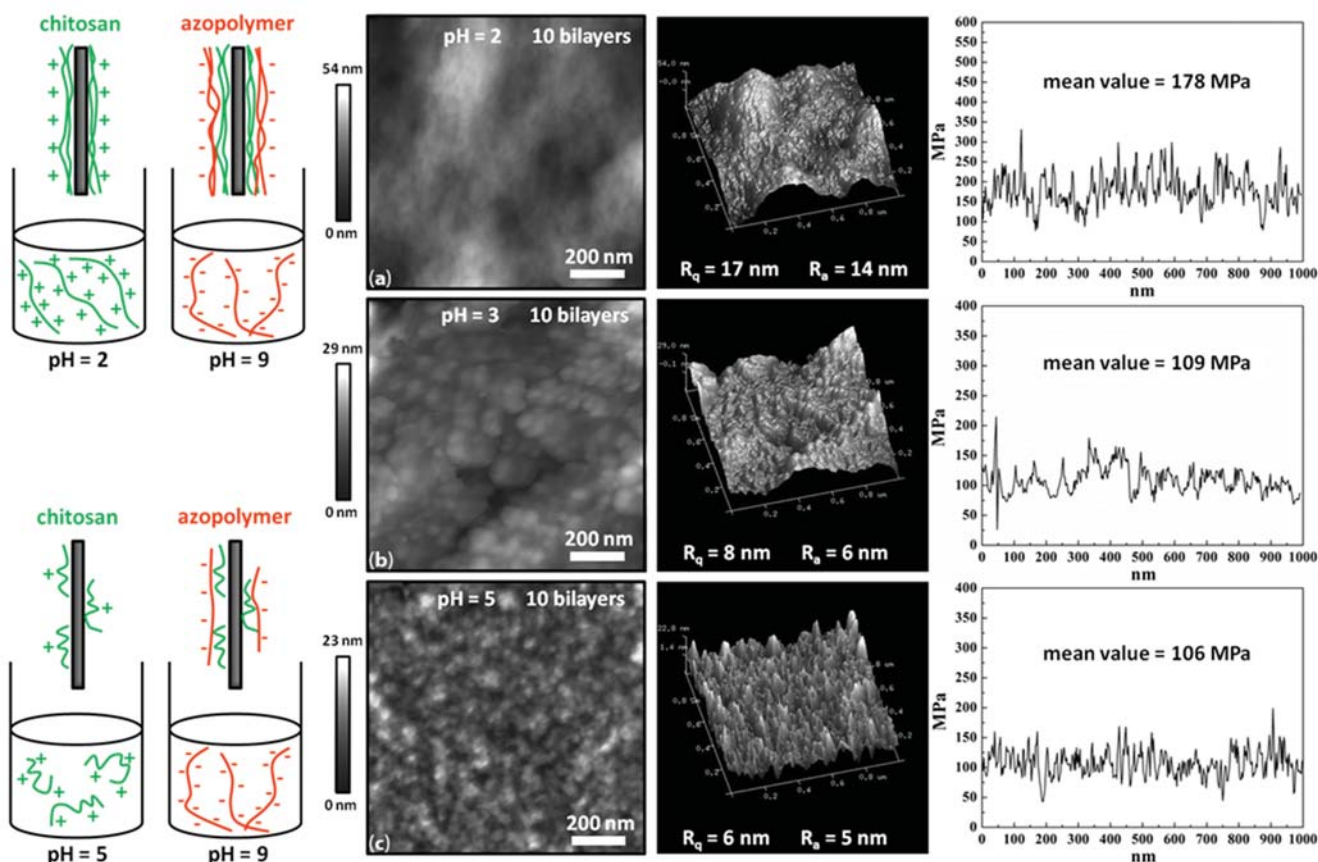


Figure 4 AFM topographic images ($1 \mu\text{m} \times 1 \mu\text{m}$) and cross-section profiles of multilayer films obtained from PeakForce QNM as a function of pH, and schematic illustration of the films formation process depending on pH.

pH is kept at a high constant value of 9. In the case of CH, by decreasing the pH solution its charge density increased. The increase in the charge density of the CH implies a higher amount of ionic interactions with the PCBS and a higher quantity of crosslinks between layers. Assuming that the elastic modulus of the multilayer films is directly proportional to the density of ionic crosslinks [28], the modulus increased while decreasing the pH of the CH solution due to the increment of ionic interactions among layers. It should also be noted that lower pH conditions led to a higher amount of PCBS absorbed in the films. As a result, the increase in elastic modulus might also be related to the increase in the azopolymer quantity in the PEM films.

The optical properties of the designed PEM films containing chitosan and an azopolymer were also investigated. Azobenzene molecules can be oriented by irradiation with linearly polarized light of appropriate wavelength. Upon absorbing this light, azobenzene groups undergo a series of *trans-cis* isomerization cycles. Those molecules with dipole moment perpendicular to the polarization direction of the light electric field do not absorb light to undergo further isomerizations. At the end of several cycles, a net population of azobenzene groups is oriented in this perpendicular direction, giving rise to a birefringence in the film structure. The birefringence formation can be inferred by the change in transmittance of a probe beam that passes through the sample between crossed polarizers. To investigate the kinetics of the azobenzene groups' orientation in the developed PEM films, the creation of birefringence was followed over time through three different irradiation regimes (Figure 5): when the linearly polarized orienting beam was turned on (A), after it was turned off (B) and, finally, when irradiated with

circularly polarized light to randomize the induced orientation (C). The transmitted signal was normalized between 0 and 1.

At the beginning of the experiment there was no transmission of the probe beam, since the azobenzene molecules were randomly distributed. At point A, the pump beam was turned *on* and the probe beam was transmitted through the polarizer-sample-polarizer system due to the birefringence induced in the film. When the pump beam was turned *off* at point B, molecular relaxation took place, but a considerable number of azobenzene groups remained oriented, thus leading to a stable birefringence pattern. Finally, at point C, in order to remove the remaining birefringence, circularly polarized light was introduced, which completely randomized the induced azobenzene molecules orientation. This optical experiment was carried out with all PEM films developed. Nevertheless, for multilayer films obtained with pH = 5 the transmitted signal was too low to achieve clear plots, due to the low quantity of PAZO adsorbed. Consequently, our investigation was focused on the analysis of the optical behavior of PEM films obtained with pH = 2, 3 and 4.

Figure 6 shows the maximum and remaining birefringence normalized with respect to the absorbance of PEM films with 20 bilayers depending on pH, and PEM films at pH = 4 depending on bilayer number.

Generally, the capability of an azobenzene molecule to orientate depends on irradiation wavelength, quantum yields, free volume available for the molecules to orient, local environment around them and polymer chain mobility; whereas the orientation relaxation when turning *off* the pump beam is only attributed to the free volume, the local environment around the azobenzene groups and the polymer chain mobility [29]. Thus, in order to develop a

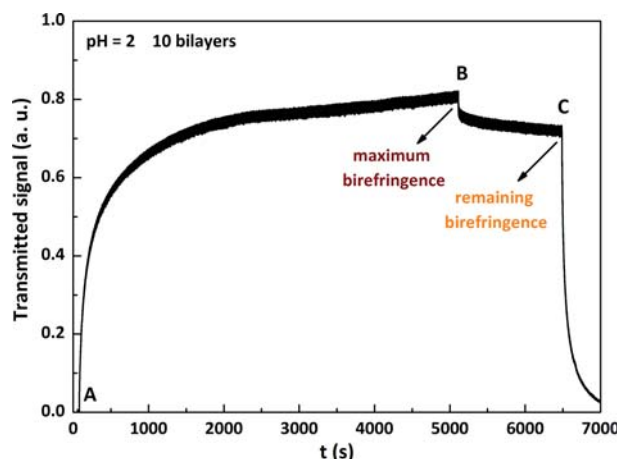


Figure 5 Induced birefringence and relaxation as a function of time for a film of 10 bilayers obtained at pH = 2.

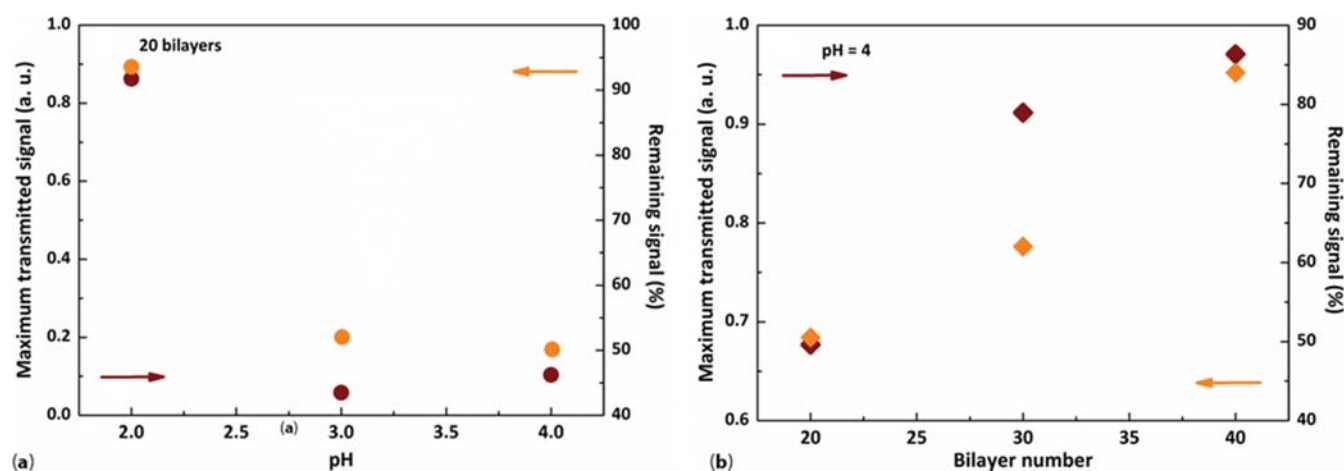


Figure 6 Maximum and remaining birefringence normalized with respect to the absorbance of (a) films with 20 bilayers depending on pH, and (b) films at pH = 4 depending on bilayer number.

material with a maximal level of induced orientation, equilibrium must be reached where the azobenzene molecules have enough freedom for isomerization and orientation, while also being in a polymer matrix that is stable enough that subsequent relaxation is minimized.

On the one hand, both maximum and remaining values increased very significantly for the lowest pH employed (pH = 2). As discussed before, lower pH conditions led to a higher amount of azopolymer adsorbed in the deposition process. Since the transmitted signal is the result of the photoinduced orientation of the azobenzene groups, a larger number of photoactive units in the films generated a higher birefringence and, accordingly, a higher signal transmission. In addition, the increase in cooperative interactions among azobenzene molecules, as a consequence of the increase in azopolymer concentration in the PEM films at low pH, could also help to stabilize the induced orientation [30]. Indeed, the remaining photoorientation for PEM films obtained at pH = 2 was higher than 90%, while for multilayer films fabricated at pH = 3 and 4, around half of the induced orientation was lost after turning off the pump beam. Besides, as found by PeakForce quantitative nanomechanical mapping, the elastic modulus of the developed PEM films increased with the decrease in pH. This fact might also contribute to the stabilization of the optically induced orientation. On the other hand, when increasing the number of bilayers, both the maximum and remaining birefringence increased. Higher bilayer number implies more amount of azopolymer adsorbed, as well as higher cooperative interactions between azobenzene molecules. These effects may contribute to the orientation creation and its stabilization.

4 CONCLUSIONS

Light-responsive PEM films consisting of alternating layers of chitosan and an azopolymer were fabricated and characterized. It was found that the properties of the designed multilayer films can be controlled by adjusting the pH conditions and the number of bilayers. In particular, the roughness and elastic modulus of these photoactive films increased with the decrease in CH solution pH. Additionally, induced birefringence measurements revealed that higher orientation was achieved with the pH decrease and the increase in bilayer number.

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