

LOADING AND RELEASE OF A MODEL CATIONIC DYE ONTO/FROM CHITOSAN/POLY(ACRYLIC ACID) POLYELECTROLYTE MULTILAYERS

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Polyelectrolyte multilayers of chitosan (CS) and poly(acrylic acid) (PAA) were constructed onto glass slides by the deposition from aqueous solution containing NaCl as support electrolyte. After construction, the CS/PAA multilayers were post-treated, changing the water pH and the ionic strength in order to generate pores inside the polymer matrix. A thermal treatment was further used to stabilize the film morphology. The morphological modifications of the multilayers surface, before and after the post-treatments, were monitored by atomic force microscopy as a function of the number of double layers deposited. The capacity of the CS/PAA multilayer thin films to sorb/release cationic species was evaluated using Toluidine Blue O as a model dye. The stability of the post-treated multilayer thin films in successive sorption/desorption cycles was explained by the formation of amide bonds between ionic groups of CS and PAA.

Keywords: polyelectrolyte multilayer, chitosan, poly(acrylic acid), Toluidine Blue O, loading/release

INTRODUCTION

Polyelectrolyte multilayers are thin films obtained by alternate deposition of different molecular species onto solid substrates. Since their introduction by Decher and coworkers, polyelectrolyte multilayers have attracted considerable interest due to the construction simplicity, low cost and mildness of assembly procedure.¹ The deposition conditions (polymer concentration, pH, ionic strength, charge density, temperature), as well as the nature of the building blocks (synthetic/natural polyelectrolytes, inorganic nanoparticles, biomacromolecules, dyes) have an important role on the driving forces (electrostatics, hydrogen bonds, hydrophobic interactions, specific biointeractions), which lead to the formation of the multilayers.²⁻⁵ Polyelectrolyte multilayers have shown potential applications in various areas like separation membranes,⁶⁻⁸ controlled release of bioactive species (drugs, proteins or enzymes)⁹⁻¹¹ or biosensors.¹²⁻¹⁵ Among these applications, loading/release capacity of bioactive compounds has been one of the main areas of interest in

recent years due to the possibility of tuning the multilayer properties.

Dyes are often used to investigate the loading and release capacity of polymeric matrices due to the structural resemblance with many low molecular drugs.¹⁶⁻²¹ Ding *et al.* showed that the loading and release of methylene blue (MB) from polyelectrolyte multilayers constructed using poly(diallyldimethylammonium chloride) and poly(acrylic acid) (PAA) strongly depended on the pH and ionic strength of the external media.²⁰ Chung and Rubner showed that the loading and release of MB into/from multilayers based on poly(allylamine hydrochloride) (PAH) and PAA has been also influenced by the pH conditions used for the construction of the films.¹⁶

One of the main building blocks for the construction of polyelectrolyte multilayers is chitosan (CS), a biocompatible polysaccharide with amine and hydroxyl functionalities. In literature, a considerable number of studies is dedicated to the use of CS for dye removal from aqueous solutions.^{19,22}

In this work, we investigate the formation of CS/PAA polyelectrolyte multilayers using deposition solutions of $C_{\text{polymer}} = 10^{-2}$ mol/L and $C_{\text{NaCl}} = 10^{-1}$ mol/L. The morphological modifications of CS/PAA multilayers upon applying a post-treatment involving immersion for a short time in an aqueous solution with $\text{pH} = 2.4$ and thermal treating for 60 min at 120°C , were monitored by atomic force microscopy (AFM). The loading and release capacity of the multilayers towards a model cationic dye were investigated as a function of the number of double layers deposited.

EXPERIMENTAL

Materials

CS was purchased from Sigma-Aldrich and used without further purification. The degree of

deacetylation, established by infrared spectroscopy, was approximately 85%.²³ The average molar mass of CS was determined by viscometry. The intrinsic viscosity of CS solved in the mixture of 0.3 M acetic acid and 0.2 M sodium acetate (1:1, v/v) was measured with an Ubbelohde viscometer at $25 \pm 0.1^\circ\text{C}$. The viscometric average molar mass (M_v) of CS was estimated using Equation 1:²⁴

$$[\eta] = 1.38 \times 10^{-4} M_v^{0.85} \quad (1)$$

The M_v of CS used in this study thus determined was 467000 g/mol.

PAA, with a molar mass of approximately 58000 g/mol, was synthesized in our laboratory.²⁵ Toluidine Blue O (TBO), purchased from Sigma-Aldrich, was used as model dye. Analytical grade NaCl, purchased from Chimopar (Romania), was used as received. The chemical structures of polyelectrolytes and model dye are presented in Figure 1.

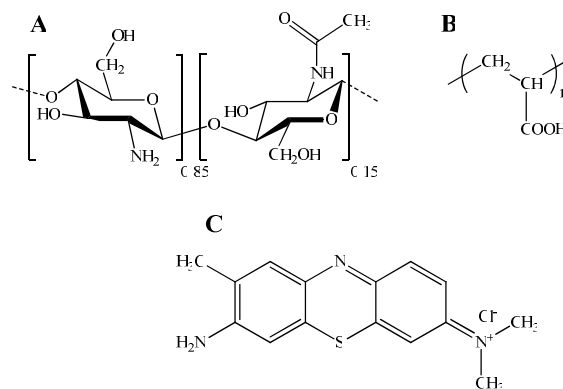


Figure 1: Structures of (A) chitosan, (B) poly(acrylic acid) and (C) Toluidine Blue O

Glass slides with dimensions of 76 mm x 26 mm x 1 mm were used as deposition substrates. Prior to multilayer deposition, the substrates were carefully cleaned according to the following procedure: (i) immersion for 20 min in Piranha solution (70:30 v/v $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ mixture), followed by intensive rinsing with water, and (ii) immersion in a mixture of $\text{NH}_4\text{OH}/\text{H}_2\text{O}_2/\text{H}_2\text{O}$ (1:1:1) and sonication at 70°C for 60 min, and followed by intensive rinsing with water. For all experiments, Millipore grade water with a conductivity of $0.055 \mu\text{S}/\text{m}$ was used.

Methods

Multilayer films were prepared by alternated deposition of CS and PAA onto glass slides. The concentration of both polyelectrolytes was 10^{-2} mol/L. The deposition procedure was performed using PAA solution with $\text{pH} = 3.5$ (obtained by dissolving the PAA in distilled water), and CS solution with $\text{pH} = 5.5$. The CS solution was prepared using the following procedure: (i) the CS powder was dissolved in an aqueous solution with $\text{pH} = 2$ (adjusted using a 0.1

mol/L HCl solution); (ii) after CS dissolution, the pH was adjusted to 5.5 using 2 M NaOH solution. The ionic strength of CS and PAA solutions was adjusted at 10^{-1} mol/L by adding appropriate amounts of NaCl (it is noteworthy that the CS solutions already contained a certain amount of NaCl resulted from the pH adjustment procedure).

The deposition procedure was performed at room temperature in open beakers. The substrates were first immersed in the CS solution for 20 min, followed by three rinsing steps for 1 min each. Then, the substrates were immersed in PAA solution for 20 min, followed by a similar rinsing cycle. The water used in the rinsing cycles contained the same amount of NaCl as the deposition solutions. This procedure led to the assembly of one double layer. The entire process was repeated until multilayers with the desired number of double layers were obtained. In all experiments, two glass slides were modified with the same number of double layers. After construction, one modified glass slide was kept as obtained, while the other was subjected to the following post-treatment: immersion

for 5 min in an aqueous solution with pH = 2.4, immersion in water for 1 min and thermal treatment at 120 °C for 60 min.

The morphology of CS/PAA thin films with 2.5, 4.5 and 6.5 double layers, before and after post-treatment, was investigated by AFM as a function of the number of double layers deposited, using an SPM Solver Pro-M (NT-MTD Co. Zelenograd, Moscow, Russia) device. All images were acquired in air, at room temperature, using a high-resolution no contact "Golden" silicon NSG10/Au/50 cantilever, with Au conductive coating. Surface parameters were obtained using WSxM 5.0 Develop 4.1 software.²⁶ The loading and release of TBO from CS/PAA multilayers was investigated by UV-vis spectroscopy with a SPECORD 200 Analytic Jena spectrophotometer. FTIR spectra of the dry multilayers removed from the surface of the glass slides were recorded with a Bruker Vertex FTIR spectrometer, in the range of 4000-400 cm⁻¹ by the KBr pellet technique.

For dye loading studies, the glass substrates modified with CS/PAA thin films having 2, 3, 4 and 5 double layers deposited on both faces of the slides were immersed in 45 mL TBO solution ($C = 2.5 \times 10^{-5}$ mol/L). The glass substrates were taken out after 60 min, then rinsed with water and dried in air. To determine the adsorbed amount of dye, the calibration curve method based on a series of standard solutions of TBO was used. The adsorbed amount, q_s , was calculated using Equation 2:

$$q_s = \frac{(C_0 - C) \cdot M \cdot V}{S}, \text{mg/m}^2 \quad (2)$$

where C_0 and C represent the concentrations of dye in solution (mmol/L), before and after interaction with the multilayers, M is the molar mass of TBO, V is the volume of the solution used for the sorption experiments and S represents the surface area of a glass slide.

Desorption experiments were carried out by immersing the glass substrates modified with the CS/PAA multilayers into 45 mL 0.1 mol/L HCl aqueous solution, for 20 min. The desorbed dye amount, q_{des} , was determined using Equation 3:

$$q_{des} = \frac{C \cdot M \cdot V}{S}, \text{mg/m}^2 \quad (3)$$

After desorption experiments, the glass slides modified with the CS/PAA multilayers were immersed for 5 min in 0.1 M NaOH to regenerate the $-\text{COO}^-$ groups. Further, to investigate the reusability of the multilayers for the loading of TBO, the glass slides were re-immersed in the dye solution with $C = 2.5 \times 10^{-5}$ mol/L.

RESULTS AND DISCUSSION

Morphological characterization of CS/PAA multilayers

The morphology of CS/PAA multilayers deposited onto glass slides was monitored by AFM. Figure 2 shows the AFM images, obtained in amplitude mode, of CS/PAA multilayers having 2.5, 4.5 and 6.5 double layers, before and after post-treatment. The number of double layers (2.5, 4.5 and 6.5) denotes multilayers containing 2, 4 or 6 layer pairs of CS and PAA onto which another layer of CS was deposited.

As AFM images show, the surface of CS/PAA multilayers before post-treatment was well-organized and the values of root-mean-square roughness (RMS) increased almost regularly with the increase in the number of double layers deposited. After post-treatment, the morphology of the films changed significantly, some cracks being visible on the surface. These morphological modifications were accompanied by a large increase of the RMS values. Similar results were reported by Mendelsohn *et al.* for PAH/PAA multilayers, constructed using a PAH solution with pH = 7.5 and a PAA solution with pH = 3.5.²⁷ The morphology of PAH/PAA multilayers was significantly altered upon immersion into an aqueous solution with pH = 2.4, a highly interconnected porous microstructure being visible on their surface. These morphological changes occurred because in acidic pH a large number of $-\text{COO}^-$ groups are protonated to $-\text{COOH}$, thus cleaving the interchain electrostatic interactions, a spinodal decomposition process being enabled.

Loading and release of TBO by CS/PAA multilayers

TBO is a cationic dye which has a maximum absorption wavelength (λ_{max}) in aqueous solution at 634 nm, with an extinction coefficient larger than 40000 L mol⁻¹ cm⁻¹. During the loading process, λ_{max} of TBO showed a hypsochromic shift to 564 nm, due to the formation of molecular aggregates inside the CS/PAA multilayers. Figure 3 shows the absorbance at 564 nm of the CS/PAA multilayer thin films loaded with TBO, before and after post-treatment, as a function of the number of double layers deposited, PAA being always the last layer deposited.

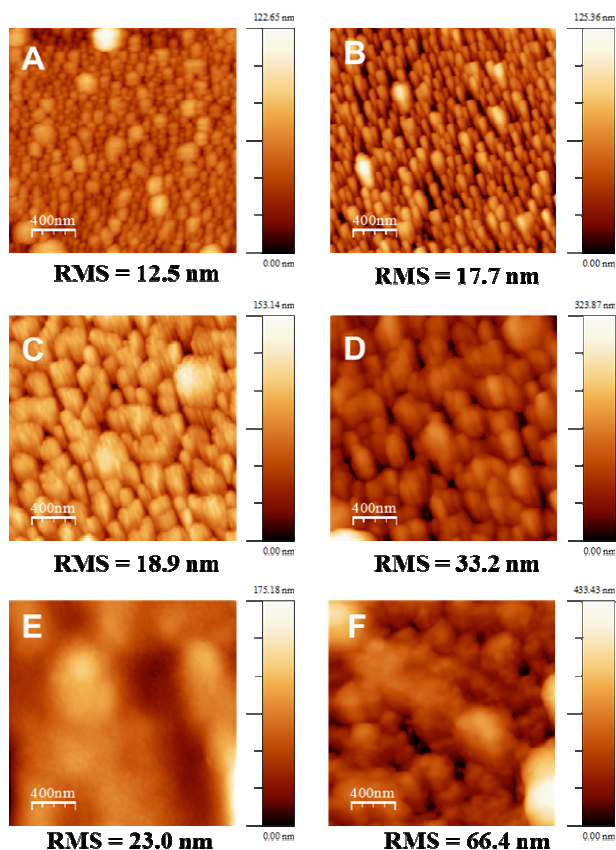


Figure 2: AFM images ($2 \times 2 \mu\text{m}^2$) of $(\text{CS/PAA})_n$ [$n = 2.5$ (A and B), 4.5 (C and D) and 6.5 (E and F)] multilayers, deposited from solutions of $C_{\text{polymer}} = 10^{-2} \text{ mol/L}$ and $C_{\text{NaCl}} = 10^{-1} \text{ mol/L}$, before (A, C and E) and after (B, D and F) post-treatment

As can be seen, before post-treatment, the absorbance at 564 nm increased almost linearly with increasing the number of double layers deposited, indicating that the TBO molecules diffused inside the inner layers of CS/PAA multilayers. After post-treatment, the absorbance was much lower than before the treatment, being approximately constant up to four double layers and increasing afterwards. The salt present in the deposition solutions screened the ionic groups of the polyelectrolytes, making them adopt a coiled conformation, which favored the adsorption of a high amount of dye. When the multilayers were immersed in the post-treatment solutions (5 min in aqueous solution with $\text{pH} = 2.4$ and 1 min in pure water) the Na^+ and Cl^- ions diffused out of the multilayer matrix, thus regenerating the electrostatic interactions between the ionic groups of the polyelectrolytes. Thus, the lower adsorption of TBO by the treated multilayers can be explained by the post-treatment strategy used,

which reduced the number of COO^- groups available for the interaction with TBO molecules.

In order to quantify the adsorbed amount of TBO by the CS/PAA multilayers, before and after post-treatment, the spectroscopic analysis at $\lambda_{\text{max}} = 634 \text{ nm}$ of the dye solution was performed (Figure 4).

As Figure 4 shows, the untreated multilayers adsorbed a higher amount of the dye than the treated ones. The results obtained confirm the evolution of absorbance values presented in Figure 3. The amount of TBO retained by the CS/PAA multilayers, q_s , increased slightly from two to four double layers, but a large increase of q_s was observed for the films with five double layers. As shown by Guzman *et al.*, the thickness of CS/PAA multilayers grows in a linear regime at a low number of double layers, while after a certain number of double layers it begins to grow exponentially.²⁸ In our case, it is possible that the threshold between linearly and exponentially

growing regimes is located between four and five double layers. Thus, the retention of a high amount of TBO by the untreated (CS/PAA)₅ multilayer, of approximately 37 mg/m², could be explained by the larger thickness of this multilayer. Comparing Figure 3 and Figure 4, it can be seen that the high increase of q_s is not in accordance with the absorbance measured on the modified glass slides. This difference might be caused by the removal of excess TBO during the washing step performed after dye sorption.

After the TBO adsorption experiments, all samples were treated with 0.1 M HCl to release the TBO from the multilayers. Figure 5 presents the dependence of the desorbed dye amount, q_{des} , on the number of CS/PAA double layers.

Figure 5 shows that the amount of TBO released from the untreated multilayers was higher than the amount desorbed from the treated ones, which confirms the results obtained in the sorption experiments (Figure 4). Moreover, it can be seen that the untreated multilayers released a lower amount of dye than the amount that was retained, while the treated multilayers released all the dye loaded. This indicates that TBO desorption from the untreated multilayers is incomplete, probably because some TBO molecules are retained by other types of interactions than electrostatics, such as hydrogen bonds or hydrophobic interactions.

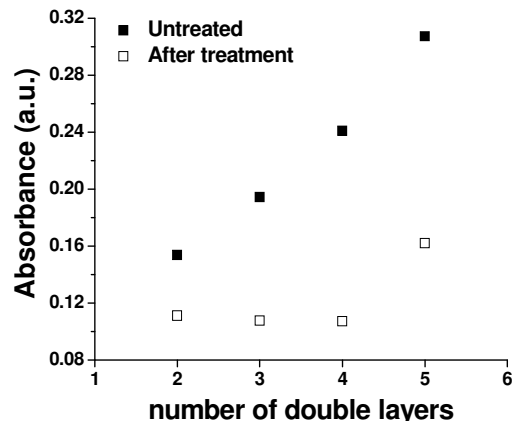


Figure 3: Absorbance at 564 nm of CS/PAA multilayers after sorption of TBO, before and after treatment, as a function of the number of double layers deposited

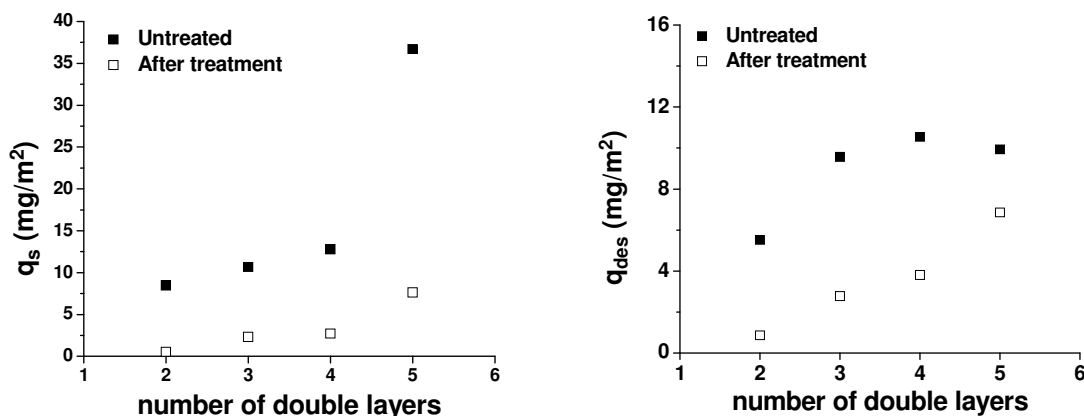


Figure 4: Amount of TBO sorbed, q_s , by CS/PAA multilayers, before and after post-treatment, as a function of the number of double layers deposited

Figure 5: Dependence of the desorbed amount of TBO, q_{des} , from the CS/PAA multilayers, before and after post-treatment, as a function of the number of double layers deposited

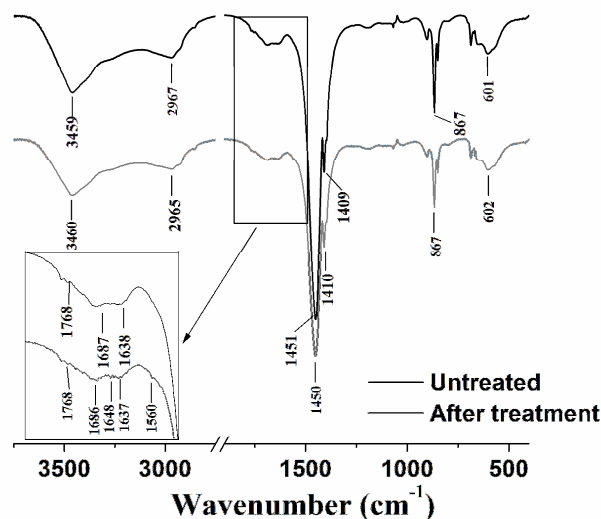


Figure 6: FTIR spectra of CS/PAA multilayers, before and after post-treatment

Further investigation on the reusability of CS/PAA multilayers in the sorption process of TBO showed that only the treated films were able to sorb again dye molecules. This indicates that the post-treated multilayers were more stable on the solid surface after desorption/regeneration experiments. In order to investigate the origin of this stability, FTIR spectra of CS/PAA multilayers, which were previously removed from the surface of the glass slides, were recorded (Figure 6).

As Figure 6 shows, the post-treatment led to the appearance of some small signals at 1648 cm^{-1} and 1560 cm^{-1} , corresponding to amide I and amide II bands, respectively. This indicates the formation of some amide bonds between carboxylic groups of PAA and amino groups of CS, which explains the increased stability of the treated CS/PAA multilayers.

CONCLUSION

The results obtained in this work provide useful information on the morphology and interaction between dyes and CS/PAA multilayers deposited on glass substrates from solutions having polymer and salt concentrations of 10^{-2} mol/L and 10^{-1} mol/L , respectively. It was shown that the morphology of the CS/PAA multilayers was influenced significantly by the number of double layers deposited and by the post-treatment. The potential application of CS/PAA multilayers as a drug delivery matrix was proved by the loading and release studies of TBO. As shown,

the loaded amount of dye increased with increasing the number of double layers deposited, after post-treatment, the amount of dye retained by the multilayers being lower. The desorption experiments showed that TBO was not completely released in acid media by the untreated multilayers, while the treated ones completely released the dye. Moreover, the reusability studies showed that only the treated multilayers were stable and able to sorb again TBO molecules, which was attributed to the formation of some amide bonds between carboxylic groups of PAA and amine groups of CS during post-treatment.

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