

# Polyelectrolyte Multilayers Composed of Chitosan and Poly(acrylic acid)

## II. Sorption/release of methylene blue

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*Chitosan (CHI) and poly(acrylic acid) (PAA) polyelectrolyte multilayers were constructed onto silica microparticles with particle diameter of 40 – 60  $\mu\text{m}$  (Daisogel type). The multilayers were subject to two post-treatment strategies: (i) immersion in an aqueous solution with  $\text{pH} = 2.4$  for 5 min, followed by immersion for 1 min in water and thermal treatment at  $120^\circ\text{C}$  for 60 min and (ii) cross-linking with glutaraldehyde for 60 min. The sorption properties for a cationic dye were investigated taking methylene blue (MB) as a model. The reusability of  $(\text{CHI}/\text{PAA})_n$  multilayers deposited onto Daisogel microparticles in the consecutive sorption/desorption cycles of MB was investigated as a function of the number of double layers and the post-treatment strategy.*

*Keywords: polyelectrolyte multilayer; chitosan; poly(acrylic acid); methylene blue; silica.*

The layer-by-layer (LbL) assembly technique is a facile way to construct multifunctional thin films with properties controlled at nanometer scale [1]. The assembly process is mainly driven by the electrostatic interactions between the ionic groups of oppositely charged species, but other type of physical interactions, such as hydrogen bonds, hydrophobic interactions or van der Waals forces can play an important role [1-5]. The versatility of the assembly procedure and the large number of chemical species that can be used to create multilayer thin films allowed their utilization in various areas such as controlled release of drugs, genes or proteins [6-8], sensors [9,10], membranes for purification [11, 12] and enzymatic or non-enzymatic catalysis [13-15].

Among these applications, controlled release of bioactive compounds attracted an increased interest due to the possibility to control the release process by manipulating multilayers properties. Dyes were often used as model molecules to investigate the sorption/release capacity of polyelectrolyte multilayers [16-20]. Rubner and coworkers investigated the loading and release behaviour of poly(allylamine hydrochloride) (PAH)/poly(acrylic acid) (PAA) multilayer films using methylene blue (MB) as a model, showing that the release mechanism is  $\text{pH}$ -dependent, the release rate being higher in low  $\text{pH}$  media [16]. Aravind and coworkers deposited chitosan (CHI)/PAA and poly(ethylene imine) (PEI)/PAA polyelectrolyte multilayers onto polyamide microfiltration membranes and investigated the removal efficiency of these composite membranes using MB and Coomassie brilliant blue as model textile effluents [18]. They showed that the adsorption and desorption processes can be controlled by manipulating the molecular architecture of the multilayers, which in turn depends on a number of parameters such as polyelectrolyte type, number of bilayers,  $\text{pH}$  of polyelectrolyte or effluent solutions.

Chitosan (CHI), the copolymer of  $\beta$ -(1-4) linked 2-acetamido-2-deoxy-D-glycopyranose and 2-amino-2-deoxy-D-glycopyranose) produced by deacetylation of chitin from crustacean exoskeletons and fungal cell walls,

has often been used to construct polyelectrolyte multilayers due to its intrinsic antimicrobial, and antioxidant properties [21, 22].

The aim of the present work was to investigate the influence of deposition conditions and post-treatment strategies onto the sorption properties of CHI/PAA multilayers. Thus, CHI/PAA multilayers have been constructed onto Daisogel microparticles. As post-treatment strategies, the films were immersed: (i) in an aqueous solution with  $\text{pH} = 2.4$  for 5 min, followed by immersion for 1 min in water and thermal treatment at  $120^\circ\text{C}$  for 60 min and (ii) in a glutaraldehyde solution for 60 min. The sorption properties for cationic dyes were investigated taking methylene blue (MB) as a model. The stability and reusability of  $\text{Daisogel}/(\text{CHI}/\text{PAA})_n$  composite microparticles during the MB sorption/desorption cycles were investigated as a function of the applied post-treatments and the number of CHI/PAA layers deposited.

### Experimental part

#### Materials and methods

CHI was purchased from Sigma-Aldrich and used without further purification. The degree of deacetylation, established by infrared spectroscopy, was approximately 85%. The average molar mass of CHI was determined by viscometry was 467000 g/mol. PAA, with molar mass of approximately 58000 g/mol, was synthesized in our laboratory. MB from Sigma-Aldrich was used without purification. Glutaraldehyde (GA) (25% solution in water, w/v) was purchased from Sigma-Aldrich and used as received. NaCl analytical grade, purchased from Chimopar (Romania), was used without further purification. The chemical structures of the polyelectrolytes and MB are presented in figure 1.

As deposition substrate, silica microparticles (Daisogel type) from Daiso Co. (Japan) with a diameter of 40 – 60  $\mu\text{m}$  and pore size of about 100 nm were used. Prior to multilayers deposition, the Daisogel microparticles were activated with 1%  $\text{NaHCO}_3$  aqueous solution. For all

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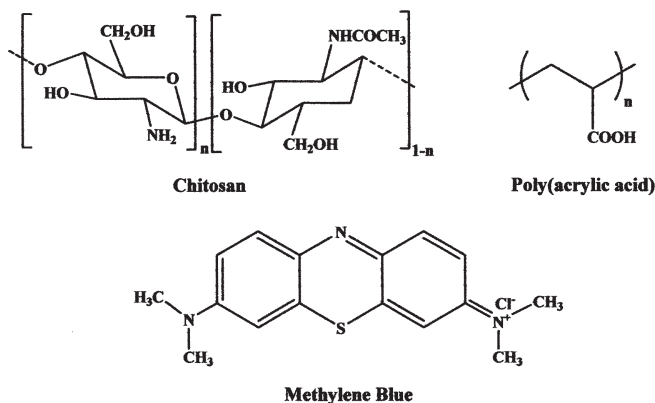


Fig. 1. Chemical structures of the main compounds utilized experiments Millipore grade water with a conductivity of  $0.055 \mu\text{S}/\text{m}$  was used.

### Construction of CHI/PAA polyelectrolyte multilayers

Multilayer films were constructed onto silica microparticles by alternated deposition of CHI and PAA from aqueous solutions with concentration of  $10^{-2} \text{ mol/L}$ , taking into account the molar mass of the polymer repeat unit. The deposition procedure was performed using PAA solution with  $\text{pH} = 3.5$  (obtained by dissolving the PAA in distilled water) and CHI solution with  $\text{pH} = 5.5$ . The CHI solution was prepared by dissolving the CHI powder in an aqueous solution with  $\text{pH} = 2$  (adjusted using  $0.1 \text{ mol/L}$  HCl solution). After CHI dissolution, the  $\text{pH}$  was adjusted to  $5.5$  using a  $2 \text{ mol/L}$  NaOH solution. For the deposition of CHI/PAA multilayers onto silica microparticles, a Daisogel sample ( $4 \text{ g}$ ) was suspended in  $200 \text{ mL}$  of CHI solution containing  $10^{-1} \text{ mol/L}$  NaCl. The adsorption time was  $30 \text{ min}$  and the suspension was gently shaken at room temperature. To remove the excess of polyelectrolyte, the silica/CHI composite microparticles were washed three times with water containing the same amount of NaCl as the deposition solutions. The composite microparticles were then suspended for  $30 \text{ min}$  in  $200 \text{ mL}$  of PAA solution containing  $10^{-1} \text{ mol/L}$  NaCl, and after that washed three times as previously shown. The entire procedure was repeated until the desired number of double layers was deposited.

### Post-treatment strategies

After the construction of CHI/PAA multilayers, two post-treatment strategies were applied in order to generate pores onto their surface. The first post-treatment ( $T_1$ ) consisted of immersing the multilayers in an aqueous solution with  $\text{pH} = 2.4$  for  $5 \text{ min}$ , followed by immersion for  $1 \text{ min}$  in water and thermal cross-linking at  $120 \text{ }^\circ\text{C}$  for  $60 \text{ min}$ . The second treatment ( $T_2$ ), consisted of immersing the Daisogel/(CHI/PAA) $_n$  composite microparticles in a GA solution for  $60 \text{ min}$  to cross-link CHI chains inside the LbL film.

### Sorption/desorption experiments

Sorption of the cationic dye MB onto the Daisogel/(CHI/PAA) $_n$  composite microparticles was carried out by a batch procedure. The influence of the contact time was investigated by immersion of  $50 \text{ mg}$  of composite microparticles in  $10 \text{ mL}$  dye solution with a concentration of  $5 \cdot 10^{-5} \text{ mol MB/L}$ , up to  $7 \text{ h}$ , and of the initial concentration of MB by immersion of  $50 \text{ mg}$  of composite microparticles, for  $2 \text{ h}$ , in  $10 \text{ mL}$  dye solution with different concentrations of MB. The adsorbed amount of MB ( $q_s$ ) was determined from the supernatant solution with a SPECORD 200 Analytic Jena UV-Vis spectrophotometer at  $665 \text{ nm}$  using the following equation:

$$q_s = \frac{(C_0 - C_e) \cdot M \cdot V}{m} \cdot 10^3, \text{ mg/g} \quad (1)$$

where  $C_0$  and  $C_e$  represent the concentration of MB in aqueous solution ( $\text{mol/L}$ ) before and after sorption on the composite microparticles,  $M$  is the molar mass of MB,  $V$  is the volume of the supernatant ( $\text{mL}$ ) and  $m$  is the amount of solid phase ( $\text{mg}$ ). After adsorption experiments, the composite microparticles were washed three times with water.

Desorption experiments were carried out by immersing the Daisogel/(CHI/PAA) $_n$  loaded with MB in  $20 \text{ mL}$   $0.1 \text{ mol/L}$  HCl aqueous solution for  $30 \text{ min}$ . After MB desorption, the composite microparticles were washed three times with water. The reusability of Daisogel/(CHI/PAA) $_n$  microparticles as sorbent for MB was investigated in three consecutive sorption/desorption cycles, the initial concentration of MB being  $5 \cdot 10^{-5} \text{ mol/L}$ .

### Results and discussions

The capacity of the Daisogel/(CHI/PAA) $_n$  composite particles to act as sorbent for cationic species was investigated taking MB as a model compound. Figure 2 shows the influence of MB concentration and adsorption time on the dye adsorbed amount onto Daisogel/(CHI/PAA) $_4$  before and after post-treatment  $T_1$ .

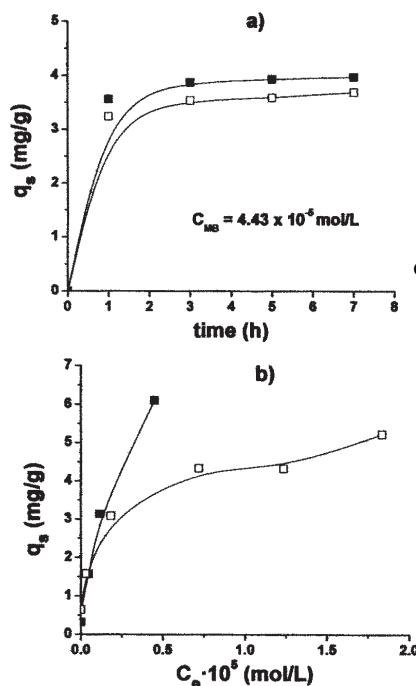


Fig. 2. Dependence of MB sorbed amount,  $q_s$ , onto Daisogel/(CHI/PAA) $_4$  before (■) and after (□) pH/thermal treatment on time (a) and equilibrium concentration,  $C_e$  (b)

The adsorption equilibrium was reached after a relatively short period of time ( $\sim 2 \text{ h}$ ) for both samples (fig. 2a). This could be attributed to the fact that the small molecule of MB dye was able to enter fast inside the multilayer. The amount of MB sorbed onto the Daisogel/(CHI/PAA) $_4$  hybrid particles increased with the increase of MB concentration both before, as well as after post-treatment  $T_1$  (fig. 2b). Also, it was noticed that the untreated Daisogel/(CHI/PAA) $_4$  composite microparticles sorbed a higher amount of MB than the post-treated sample, no plateau being reached. This suggests that the composite microparticles have a high affinity for the MB molecules, the adsorption being controlled by the electrostatic interaction between the PAA chains in the film and the dye molecules, as well as by the porous nature of the multilayer. After post-treatment, the amount of MB sorbed onto the Daisogel/(CHI/PAA) $_4$  hybrid particles reached a plateau (ca.  $4.5 \text{ mg/g}$ ).

Parameter	Daisogel//((CHI/PAA) <sub>4</sub> before pH/thermal treatment	Daisogel//((CHI/PAA) <sub>4</sub> after pH/thermal treatment
<i>Langmuir model</i>		
$C_s = \frac{C_{max} K_L C_e}{1 + K_L C_e}$		
R <sup>2</sup>	0.9997	0.9735
C <sub>max</sub> (mg/g)	9.31	4.61
K <sub>L</sub> (L/mg)	1.35	5.37
<i>Freundlich model</i>		
$C_s = K_F C_e^N$		
R <sup>2</sup>	0.9659	0.9602
K <sub>F</sub> (mg/g)	5.11	3.22
N	0.5937	0.2786

where: C<sub>max</sub> is the maximum sorbed amount;

K<sub>L</sub> is the Langmuir sorption constant;

K<sub>F</sub> is Freundlich constant;

N is a measure of the nature and strength of the adsorption process and of the distribution

of active sites.

g). The lower affinity of the treated multilayers for the sorption of MB can be explained by formation of some amide bonds inside the film between the carboxylic groups of PAA and amine groups of CHI, thus reducing the number of PAA ionic centers which are able to form electrostatic interactions with MB.

The experimental data of the MB sorption onto non-treated and post-treated Daisogel//((CHI/PAA)<sub>4</sub> composite particles were analyzed by the linear forms of the Langmuir [23] (eq. 2) and Freundlich [24] (eq. 3) isotherm models.

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L q_m C_e} \quad (2)$$

$$\log q_e = \log K_F + N \log C_e \quad (3)$$

where: q<sub>m</sub> is the saturated monolayer sorption capacity (mg/g), K<sub>L</sub> is the Langmuir constant (L/mg) related to the energy of adsorption, which reflects the affinity between the sorbent and sorbate, K<sub>F</sub>, the Freundlich constant, which predicts the quantity of metal ion per gram of composite at the equilibrium concentration (mg/g), and N, a measure of the nature and strength of the adsorption process and of the distribution of active sites.

The calculated parameters from the linear form of the Langmuir and Freundlich isotherms are listed in table 1.

According to the R<sup>2</sup> values, the Langmuir equation fits better the experimental data, in both cases. The feasibility of adsorption can be expressed by an equilibrium parameter, R<sub>L</sub> in a certain concentration range [25]:

$$R_L = \frac{1}{1 + K_L C_i} \quad (4)$$

where K<sub>L</sub> is the Langmuir constant, and C<sub>i</sub> is the highest initial concentration of MB (mg/L). From Table 1, the R<sub>L</sub> for MB sorption onto untreated and treated Daisogel//((CHI/PAA)<sub>4</sub> hybrid particles was 0.022 and 0.006, respectively.

**Table 1**  
LANGMUIR AND FREUNDLICH ISOTHERM PARAMETERS FOR THE MB SORPTION ON DAISOGEL//((CHI/PAA)<sub>4</sub> COMPOSITE MICROPARTICLES BEFORE AND AFTER pH/THERMAL TREATMENT

Based on R<sub>L</sub> values it can be concluded that MB sorption is favourable in both cases because 0 < R<sub>L</sub> < 1.

To study the reusability of the Daisogel//((CHI/PAA)<sub>n</sub> hybrid particles treated using both post-treatments, three consecutive sorption/desorption cycles were performed at 5 · 10<sup>-5</sup> mol/L initial concentration of MB, as can be seen in figures 3 and 4.

Each sorption and desorption process was studied as a function of the number of CHI/PAA double layers, n, deposited onto the surface of Daisogel microparticles. During the first sorption of MB onto the Daisogel//((CHI/PAA)<sub>n</sub> composite particles, the amount of sorbed dye increased with n for the samples pH/thermally post-treated and was constant for the same samples post-treated with GA solution. The amount of MB desorbed in the first desorption study was ~ 70% from the initial adsorbed amount, for both types of hybrid particles. The second sorption experiments revealed that the capacity of

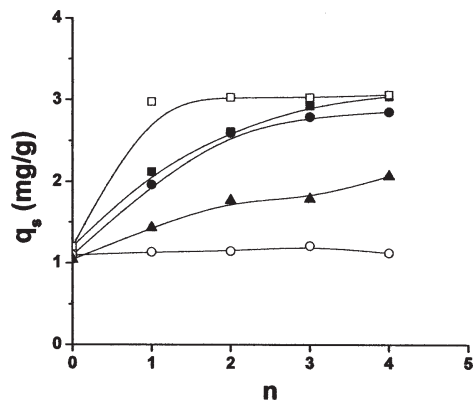


Fig. 3. The MB sorbed amount, q<sub>s</sub>, after first (squares), second (circles) and third (triangles) sorption cycles onto Daisogel//((CHI/PAA)<sub>n</sub> obtained after pH/thermal treatment (closed symbols) and chemical treatment (opened symbols), in function of the deposited number of double layers, n

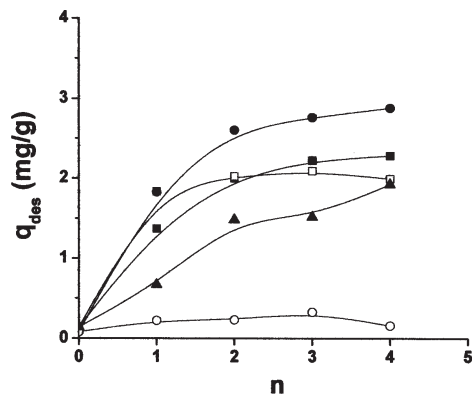


Fig. 4. The MB desorbed amount,  $q_{des}$ , after first (squares), second (circles) and third (triangles) desorption cycles from Daisogel// $(CHI/PAA)_n$  obtained after pH/thermal treatment (closed symbols) and chemical treatment (opened symbols), as a function of the deposited number of double layers,  $n$ .

Daisogel// $(CHI/PAA)_n$  composite particles post-treated with GA to sorb MB was lost. This was attributed to the fact that during the MB desorption in 0.1 mol/L HCl, the stability of  $(CHI/PAA)_n$  multilayers chemically post-treated was affected, the acidic media cleaving the imine bonds ( $-C=N$ ) formed between the aldehyde groups of GA and the amine groups of CHI. Thus, the GA post-treatment was not suitable to increase the stability of the Daisogel// $(CHI/PAA)_n$  hybrid particles for repetitive sorption/desorption cycles of MB. However, in the case of the Daisogel// $(CHI/PAA)_n$  composite particles pH/thermally post-treated, the sorption data in the second cycle showed the same variation for the MB sorbed amount as a function of  $n$ , as in the first sorption experiments. The stability of the thermally cross-linked multilayers was underlined by the third sorption data, when they still retained ~70% from the amount of MB retained in the previous two sorption experiments. From figure 3 and 4 it can be seen that the sorption capacity of Daisogel// $(CHI/PAA)_n$  obtained after the pH/thermally post-treatment remained approximately the same after three sorption/desorption cycles, thus demonstrating that this kind of post-treatment enhances the stability of the CHI/PAA multilayers under repetitive sorption/desorption conditions.

## Conclusions

The influence of two post-treatment strategies on the sorption properties of CHI/PAA polyelectrolyte multilayers was studied. The capacity of CHI/PAA multilayers deposited onto Daisogel microparticles to sorb/desorb MB was investigated as a function of number of double layers deposited and post-treatment type. It was demonstrated that, although pH/thermally post-treatment led to a slightly lower sorption capacity of MB onto multilayer, it enhanced the stability of the CHI/PAA multilayers under acidic

conditions, allowing the composite to be used in repetitive sorption/desorption cycles. This kind of porous polyelectrolyte multilayers might find potential applicability for the subsequent load of bioactive molecules, such as drugs, proteins or enzymes.

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