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CHITOSAN/ALGINATE POLYELECTROLYTE MULTILAYERS: EFFECT OF pH AND IONIC STRENGTH ON FILM GROWTH AND MORPHOLOGY

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ABSTRACT

The growth process of chitosan/alginate polyelectrolyte films deposited by layer-by-layer assembly at different pH and ionic strength was studied in this work. The films were investigated using surface plasmon resonance (SPR) to examine the build-up kinetics, and atomic force microscopy (AFM) to visualize the surface topography. Each deposition step resulted in a progressive increase in mass. The film growth and thickness strongly depended on the polyelectrolyte charge density and complexation between the used polyelectrolytes. The film surface changed from smooth to rough when the pH increased. The results achieved could be used in estimating the potential of the multilayers to be applied as drug delivery systems.

Keywords: surface plasmon resonance, polyelectrolyte multilayers, chitosan, alginate.

INTRODUCTION

Application of multilayer structures, made from different polyelectrolytes, for controlled drug delivery is widely spread. Polysaccharides, such as chitosan and alginate, are often used for the creation of such multilayers, as they possess many beneficial properties [1] and their properties can be tailored by controlling the pH and ionic strength of their solutions [2]. Many studies have demonstrated that chitosan/alginate multilayers can be employed as systems for controlled release of bioactive materials [3 - 5]. However, one of the things that prevent the creation of better drug delivery systems is the lack of understanding of the exact process of layer creation in the multilayer structures. One of the technologies that can be used to better understand this process is surface plasmon resonance or SPR. The benefit of this technique is that it can investigate the process of layer deposition in real time and provide data on the properties of the deposited layer. This is often shown in biosensor studies, where the detection of the desired biomarker can be done with remarkable precision [6, 7]. In fact, SPR has been used for monitoring of layer-by-layer deposition of chitosan and alginate multilayers but the effects of different variables on the multilayer properties have not yet been sufficiently investigated. In our paper we aim to investigate the effects of different pH and ionic strengths of both polysaccharide solutions on the multilayer build-up process [8 - 10].

EXPERIMENTAL

Materials

High molecular mass chitosan (Chit), Sodium alginate (Alg) and Polyethyleneimine (PEI) were bought from Sigma Aldrich. All other used chemicals were with analytical grade. The reported by the producer value for the degree of deacetylation of chitosan is 75 %.

Methods

Atomic force microscopy

Atomic force microscopy (AFM) was used to investigate the PEMs surface morphology. The measurements were performed by AFM NANOSURF FLEX AFM (SWITZERLAND), in tapping mode with standard cantilever Tap190Al-G with 10 nm tip radius. The viewing field consisted of 256×256 pixels, revealing the morphology of 10 μm x 10 μm area from the sample surface. The line scan time was 1 s. Based on AFM images, the root mean square roughness Sq was calculated.

SPR methodology

Chitosan and alginate were dissolved in acetate buffer with chosen pH and ionic strength at concentration 2 mg mL^{-1}. Both solutions were filtered through a 0.45 μm syringe filter before being loaded into the SPR apparatus. Polyethyleneimine (PEI) was also dissolved in the same acetate buffer at concentration of 0.1 mg mL^{-1} and was also placed in the SPR. A clean gold sensor was inserted into the apparatus and was primed with buffer before any layer deposition. After the initial priming a buffer flow of 50 mL min^{-1} was set and the measurements were initialized. The flow rate of the buffer was maintained for the initial 10 min of the measurement so that a stable baseline could be obtained. An initial layer of PEI was deposited on the surface of the gold sensor to create an initial active layer. This was followed by alternating layers of alginate and chitosan until 16 layers were achieved on the surface of the sensor. All layers were deposited at a flow rate of 50 mL min^{-1}, with a 5 min injection delay after each layer. The total sample volume of each layer was set to 340 μL and the injection length was set to 4 min. All measurements were done with three wavelengths.

RESULTS AND DISCUSSION

Alginate and chitosan, used in this study, are both weak polyelectrolytes and their degree of ionization greatly depends on the pH and ionic strength of the dissolution media. The pK_a of the primary amine of chitosan is ~ 6.5, depending on the degree of N-deacetylation [11]. The pK_a values of mannuronic and guluronic acid residues of alginate are measured to be 3.38 and 3.65, respectively [12]. With these values in mind, the pH at which the layers are deposited is chosen in such a way that both polyelectrolytes are ionized. Taking into account the degree of deacetylation of chitosan, according to the Katchalsky's theory [13], the following values are calculated for the Chitosan/Alginate ratio at charge balance at the corresponding pH (Table 1).

Polyethyleneimine (PEI) can both crosslink alginate networks and under appropriate conditions, bind to a gold surface with a high affinity. Therefore, to study alginate and chitosan adsorption by surface plasmon resonance, the gold surface of a surface plasmon resonance sensor was initially coated with PEI to form a positively charged base layer. After the initial deposition of PEI, the surface was rinsed with acetate buffer with the same pH and ionic strength as the deposited layers.

The surface morphology of PEMs was investigated by atomic force microscopy. The surface morphology of PEMs deposited on PEI modified gold slide sensor at different pH (3.5, 4.5 and 5.5) and ionic strength 100 mM are presented in Fig. 1.

The root means square roughness and the size of grains of all investigated PEMs were calculated. The values of the root mean square roughness for different types of PEMs are presented in Table 2.

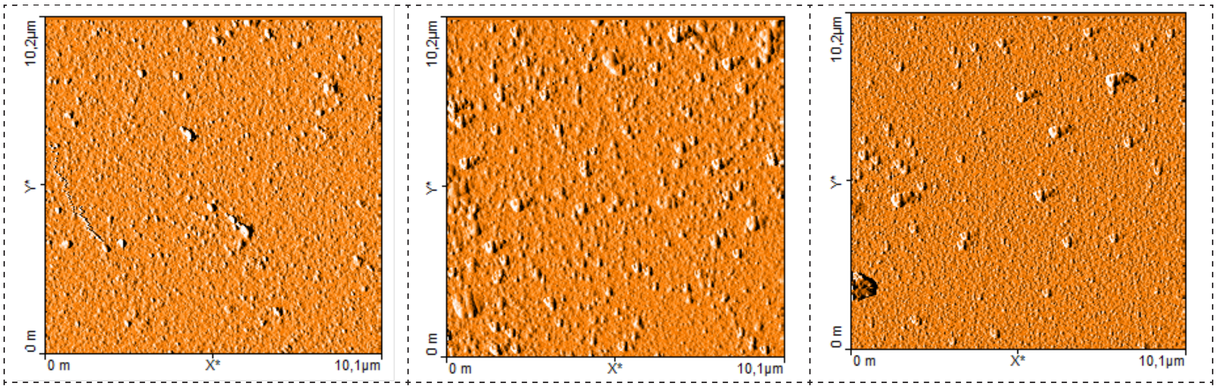
The results presented in Fig. 1 and Table 2 show that the values of root mean square roughness and size of grains increase with increasing of pH of the starting solution of chitosan and alginate. Similar behaviour was observed for chitosan casein multilayers formulated at pH 3, pH 4, and pH 5 on preliminary corona charged poly (DL-lactic acid) substrates [14]. The observed

Table 1. Chitosan/Alginate ratio at charge balance.

pH	3.5	4.5	5.5
Chitosan/Alginate ratio	0.5	0.9	1.1

Table 2. Values of the root mean square roughness and grains size for all investigated PEMs.

Type of PEMs	S_q, nm	Grains size, nm
pH 3.5, 100 mM	3.12	200
pH 4.5, 100 mM	3.23	350
pH 5.5, 100 mM	3.76	400



pH 3.5 pH 4.5 pH 5.5

Fig. 1. Surface morphology of PEMs obtained at different pH (3.5, 4.5, and 5.5) and ionic strength 100 mM.

dependence can be explained by a different conformation of the polyelectrolytes included in the layers. At pH 5.5, chitosan is less charged, hence it is deposited in a coiled conformation, while at pH 3 its charge density is higher, thus being deposited in a stretched conformation. Such conclusions were reached also by Ghiorghita Dragan during the study of polyelectrolyte multilayer thin films assembled using poly (*N, N*-dimethylaminoethyl methacrylate) and polysaccharides [15].

A multilayer in the SPR instrument was fabricated by sequential deposition of alginate and chitosan on preliminary activated gold sensor. A continues increase in the sensor response (sensogram) was observed, indicating irreversible binding of the polyelectrolytes. After biopolymer addition, the response quickly reaches a plateau, and therefore it could be assumed that the interactions between chitosan and alginate is characterized with high binding affinity.

The SPR data were fitted by stretched exponential function [16], given by Eq. 1:

$$\Delta R(t) = \Delta R_{\infty}\left(1 - exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right)\right) \qquad (1)$$

where $\Delta R(t)$ is the response change at time t, ΔR_{∞} is the response change at steady state (the observed plateau after the polyelectrolyte injection, τ - the characteristic increasing time, characterizing the binding rate, and β - stretching factor, indicating the homogeneity of binding. For $\beta = 1$ the model describes a single exponential behavior (pseudo first order), while for $\beta < 1$ it accounts for hindered sorption processes. This delay may be caused by various factors, such as an overlaying layer through which the desorbing object has to diffuse or a substrate that is not homogenous in terms of morphology or chemical composition.

The values of ΔR_{∞} for multilayers deposited at different pH and ionic strength are presented in Fig. 2.

Based on the calculated values of the ΔR_{∞} it could be notices that the plateau values for the first three bilayers at all investigated deposition conditions are lower than the values of the upper layers. After six switches, steady values of ΔR_{∞} are riched, which lead to linear increase in the multilayer thickness. Similar results were already reported by other authors [17]. Probably, the smaller deposited amount of polyelectrolytes in the first layers is due to island trapping on the substrate and subsequent inhomogeneities [18].

The response change is higher for chitosan layers than those of alginate. It could be due to higher charge density and bigger bound amount.

The response change is bigger at low ionic strength (10 mM). In this case the screening effect is smaller and the electrostatic interaction between the positively charged chitosan and negatively charged alginate increases. It leads to higher deposited amount of polyelectrolytes [19].

The response change is bigger at low pH (pH = 3.5). At these conditions the ionisation degree of chitosan is almost 100 % and the bound amount is the highest.

The values for the stretching factor β for deposition of alginate and chitosan layers at different pH and ionic strength are presented in Table 3 and Table 4 respectively. It is noteworthy that at pH 3.5, except for the first layer, β has values very close to 1. This observation can be related to a homogeneous distribution of macromolecules in the layers. As the pH increases, the charge density of chitosan decreases, resulting in looser and disordered structures leading to inhomogeneities [20].

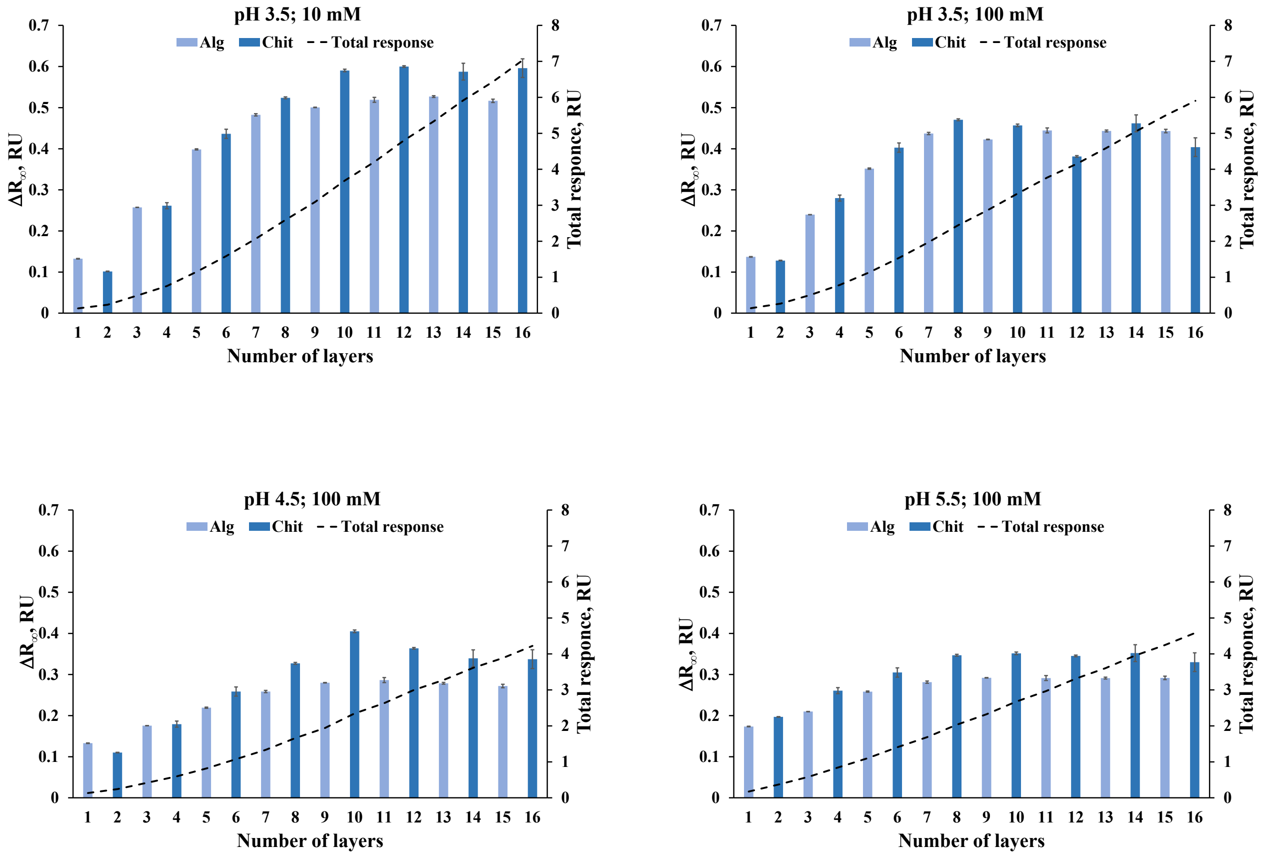


Fig. 2. Response change at steady state for multilayers, deposited at different pH and ionic strength.

Table 3. Stretching factor β for deposition of alginate layers at different pH and ionic strength.

Alginate layer	pH 3.5; 10 mM	pH 3.5; 100 mM	pH 4.5; 100 mM	pH 5.5; 100 mM
1	1.000 ± 0.069	0.410 ± 0.026	0.608 ± 0.012	0.649 ± 0.028
2	1.000 ± 0.092	0.737 ± 0.030	0.695 ± 0.053	0.441 ± 0.009
3	1.000 ± 0.084	0.733 ± 0.194	0.416 ± 0.011	0.339 ± 0.031
4	1.000 ± 0.116	1.000 ± 0.259	0.471 ± 0.009	0.645 ± 0.037
5	1.000 ± 0.064	0.779 ± 0.031	0.803 ± 0.054	0.763 ± 0.052
6	1.000 ± 0.216	1.000 ± 0.304	0.680 ± 0.031	0.624 ± 0.031
7	1.000 ± 0.11	1.000 ± 0.214	0.671 ± 0.024	0.601 ± 0.028
8	1.000 ± 0.115	1.000 ± 0.254	0.541 ± 0.012	0.438 ± 0.008

Table 4. Stretching factorb β for deposition of chitosan layers at different pH and ionic strength.

Chitosan layer	pH 3.5; 10 mM	pH 3.5; 100 mM	pH 4.5; 100 mM	pH 5.5; 100 mM
1	0.637 ± 0.024	0.514 ± 0.021	0.295 ± 0.014	1.000 ± 0.245
2	1.000 ± 0.041	1.000 ± 0.176	0.259 ± 0.012	0.344 ± 0.01
3	1.000 ± 0.044	1.000 ± 0.183	0.801 ± 0.026	0.792 ± 0.016
4	0.999 ± 0.04	0.484 ± 0.01	0.485 ± 0.046	0.395 ± 0.009
5	1.000 ± 0.036	0.974 ± 0.031	0.223 ± 0.008	0.451 ± 0.048
6	0.999 ± 0.06	0.526 ± 0.009	0.336 ± 0.017	0.557 ± 0.058
7	1.000 ± 0.053	1.000 ± 0.136	0.666 ± 0.039	0.447 ± 0.015
8	0.999 ± 0.044	1.000 ± 0.147	0.458 ± 0.029	0.521 ± 0.02

The assumptions made also explain the type of surface topography of the layers observed by AFM. As the pH increases, the roughness of the structures increases, and the size of the formed grains also increases.

CONCLUSIONS

The alternating deposition of chitosan and alginate on activated gold surface results in the formation of a multilayer structure. Multilayer formation was observed at a pH when both polymers carried a charge. Increasing pH, resulting in suppression of charge on the chitosan, resulted in decrease of deposited amount, highlighting the importance of electrostatic interactions in multilayer formation and stability. Deposition was irreversible over practical timescales and the thickness of an individual layer showed a dependence on polyelectrolyte charge density. The most stable and uniform multilayers were formed at pH 3.5 and ionic strength 10 mM.

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