MOLECULAR STRUCTURE AND FORMATION OF CHITOSAN AND PECTIN BASED THIN FILMS

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Abstract

In this study using methods of Atomic force microscopy (AFM) and Quartz crystal microbalance with dissipation monitoring (QCM-D) the special characteristics of formation and architecture of thin films coatings based on natural polysaccharides chitosan and pectin were investigated. A layer-by-layer (LbL) deposition technique for assembling of oppositely charged layers was used. The main factors, which have an influence on the process of thin film formation and molecular structure of these coatings, were investigated.

Key words: *QCM-D, chitosan, pectin, layer-by-layer (LbL), Atomic force microscopy (AFM), thin layers*

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1. Introduction

In recent years, functional coatings based on thin films with controlled parameters cause an increased interest of researchers. This is due to wide possibilities of their use in various fields [1-3]. Moreover nanostructured materials with predefined structure and predict surface properties are more effective than conventional materials [4]. For the effective application of such coatings it requires the understanding of the mechanisms of their formation and organization at the molecular level.

There are different ways to produce thin films, but it has been paid special attention to the layer-by-layer deposition techniques for assembling of oppositely charged layers [5, 6], which is based on alternately deposition of positive and negative charged solutions of polyelectrolyte onto a substrate. This method is simple, easily reproducible and allows to create thin films of the necessary thickness and to maintain layer main parameters to be controlled as required.

Thin films were formed on the basis of natural biocompatible, biodegradable pectin and chitosan polysaccharides. Functional groups of these polysaccharides have an opposite charges, and can be used to design coatings by LbL deposition technique.

The morphology of coatings was studied by atomic force microscopy (AFM) that allows investigating the thickness of films, the surface topography, the sizes of aggregates on the surface of oppositely charged layers, the growth characteristics of layers.

The process of film growth was also described using the method of Quartz crystal microbalance with dissipation monitoring (QCM-D). This technique based on an analysis of changes in frequency and dissipation of oscillations of the quartz resonator, which consists of a quartz disc placed between two gold electrodes. When AC electric field is using, then in the crystal oscillations are generated with a characteristic frequency *f*. QCM-D allows analyzing the characteristics of adsorbed layers at all stages of films assembling [7].

The special characteristics of formation of thin film polymer structures depending on various conditions were studied. Their influence on the final parameters of the films was presented.

2. Materials and methods

2.1. Materials

Crab chitosan was used for the films formation. Molecular mass was determined by viscometric measurement and was found to be 200, 80 and 18 kDa; the degree of deacetylation was about 87% ("Bioprogress", Moscow region, Russia).

As oppositely charged polyelectrolyte was used food citrus pectin (MM=170 kDa, degree of methoxylation was about 10%).

2.2 Methods

2.2.1 Purification of chitosan

We have prepared 1% solution of chitosan in 1% acetic acid, and then this solution was centrifuged at 5000 rpm for 10 min to separate undissolved precipitate followed by adding of 12% solution of NH_4OH to pH value equal 8.5. The solution was centrifuged at 6000 rpm for 15 min, and the precipitate, washed twice with distilled water, was dialyzed for 3 days and freeze-dried after then.

2.2.2 Preparation of polysaccharides solutions

For the films formation aqueous solutions of pectin with different concentrations (0,01%; 0,1%; 1%) were prepared.

Chitosan was dissolved in acetate buffer solution with different pH (3,0; 3,9; 4,6; 5,9) and in water with the addition of 6N HCl (to pH = 3,0). Prepared solutions with different concentrations (0,02%; 0,04%; 0,08%; 0,2%) were treated with ultrasound for 15 minutes.

2.2.2 Formation of films by LbL deposition techniques

Thin films were formed by immersing substrates into aqueous solutions of polyelectrolyte for 1 minute, washing with water and drying in air. Each new layer was deposited after drying the previous one.

2.2.3 Atomic force microscopy

The films surface images were obtained by Atomic force microscope NTEGRA Prima (NT-MDT, Zelenograd, Russia). Scanning was performed in contact mode and tapping mode in air using silicon cantilevers Etalon HA_NC (Tip curvature radius - less than 10 nm, Force constant - 3,5 N/m), Golden NSG01 (Tip curvature radius - 10 nm, Force constant - 5,1 N/m), CSG01 (Tip curvature radius - 10 nm, Force constant - 0,03 N/m).

Image processing was carried out in programs Nova and Image Analysis P9 (NT-MDT, Zelenograd, Russia).

2.2.4 Quartz crystal microbalance with dissipation monitoring

The measurements were carried out on a Q-sense E1 system (Biolin Scientific, Sweden) using a flow cell, in which a golden resonator was placed. Before use, resonators were cleaned according to standard methods: ozonation for 10 minutes, treatment with a mixture of Milli-Q water/NH_4OH/H_2O_2 in a ratio of 5:1:1 at a temperature of 75 °C, washing with Milli-Q water, drying by air flow, ozonation for 10 minutes [8].

Data analysis was performed using Q-Tools software supplied with the QCM-D equipment.

3. Results and discussion

3.1 Special characteristics of the thin films formation on the substrate surface

The growth of thin films on a surface depends critically on the interaction strength between adsorbed molecules and the surface. There are various ways of thin films growth: 1) layer-by-layer growth mechanism according the Frank-Van der Merwe model, when the adsorbate-surface and adsorbate-adsorbate interactions are balanced. It is an "ideal" growth mechanism. 2) Layer-plus-island growth mechanism following the Stransky-Krastanov model. It is an intermediary process characterized by both layer and island growth. Transition from the layer-by-layer to island-based growth occurs at a critical layer thickness which is highly dependent on the chemical and physical properties. 3) Island growth mechanism following the Volmer-Weber model. Islands are formed right away, because the adsorbate-adsorbate interactions are stronger than adsorbate-surface interactions [9]. We studied the formation mechanisms of pectin and chitosan coatings on the mica surface using AFM.

Pectin forms a fairly uniform layer at different concentrations of the solution, and while a concentration grows the filling degree is nearing to 100% (Fig. 1). On the basis of obtained results it can be assumed that in this case there is a layer-by-layer growth following the Frank-Van der Merwe mechanism, namely, the growth of the next layer doesn't begin until the formation of the previous one will end. Wherein, the surface tension of the film is less than the sum of the surface tension of a film and the interface between a film and a substrate.

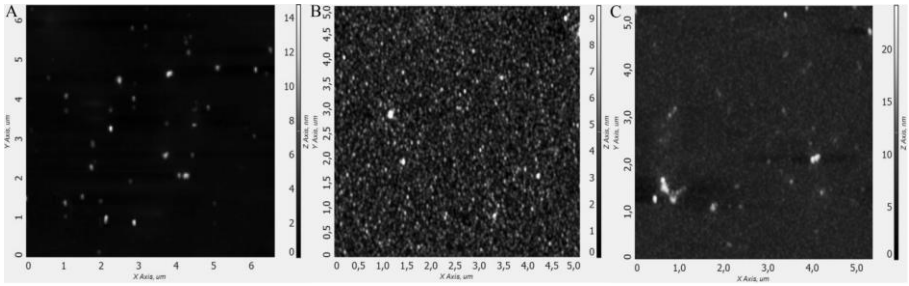

Figure1. AFM- images demonstrated a layer-by-layer growth model with an increasing of concentration of pectin solution

Chitosan (MM=200 kDa) forms heterogeneous structures with a lower degree of surface filling, and the generation of this patterns passes through three main stages. At the first stage the single molecules give a coating on the substrate. They are regular spaced on the surface; however, there are areas with a more high concentration (Fig. 2A). With the growth of layers number new chitosan molecules deposit onto already adsorbed ones. During this process while the modified surface was not fully filled, it could be observed the formation of globular structures: the single structures at the beginning (Fig. 2B), and then chitosan, as a whole, starts adsorbing on a substrate in the form of such particles (Fig. 2C). With the further increase of layers number it observed the growth not only the number of particles, but also increasing of their size. At the third phase, spherical particles generate large aggregates, which are nonregular spaced, but they not fill full surface of the substrate (Fig. 2D). It can be assumed that the growth of films, based on chitosan, shows layer-plus-island the Stransky-Krastanov growth model.

Similarly, we have studied the samples of chitosan with molecular mass of 18 kDa (Fig. 3A), 80 kDa (Fig. 3B), 200 kDa (Fig. 3C). In all cases particles and aggregates were formed on the substrate surface. The average size of patterns increased with the molecular weight growth, this may be associated with an acceleration of described above three stages due to the substance accumulation.

In this study it was also been examined the influence of chitosan solutions pH on the process of films formation. It was revealed that the pH optimum depends on the substrate properties on which the film is formed. The best chitosan adsorption on mica, which has a negative charge, is observed at low pH values (≤ 4), and on inert and hydrophobic HOPG (highly oriented pyrolytic graphite**)** and on gold substrate - at pH of 5.9. It is associated with a decrease of protonation degree of chitosan amino groups. The pH optimum for a pectin solution is 3.9.

Thus, it was considered the influence of various factors on the process of film formation. It is shown, that varying the initial conditions of the coatings formation (solution concentration, pH) we can get as a result patterns with different characteristics from the same polysaccharide.

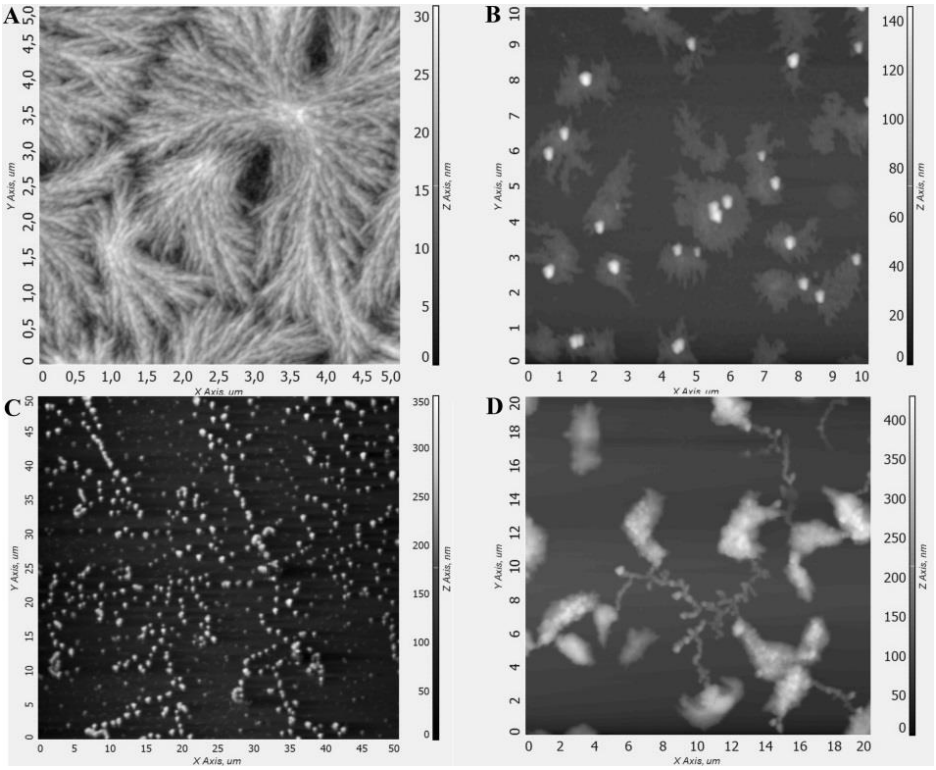

Figure 2. AFM images of chitosan based coating formation

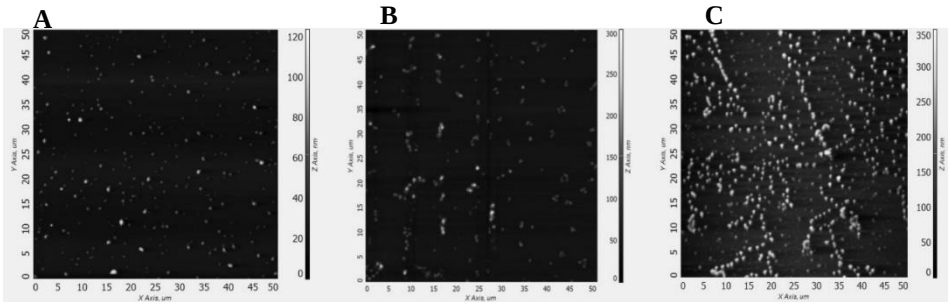

Figure 3. AFM images of coatings formed on the base of chitosan with different molecular mass

3.2 Studying of morphology of chitosan and pectin based bilayers

With AFM it was studied molecular structure and main properties of bilayers based on chitosan and pectin**.**

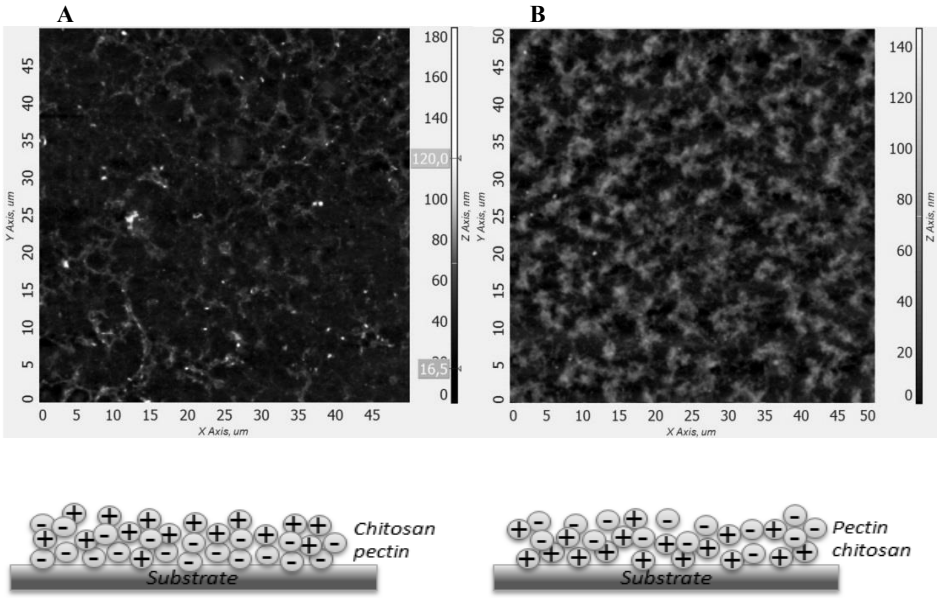

Figure 4. AFM-images of surface morphology of pectin-chitosan coatings

Chitosan-pectin and pectin-chitosan layers have similar morphology and differ from the coatings on the basis of a single polymer layer (Fig.4). Basic topographic parameters (Maximum peak height, Ten point height, Root mean square) and the magnitude of the strength of the adhesion for bilayers have similar values (Table 1). This suggests that during the formation of bilayer not only the deposition of one polymer to another occurs due to electrostatic interactions. It also takes place the penetration, diffusion of free polyelectrolyte chains of both polymers into each other during adsorption. Thus, at the interface of two polymers the new type of structure is formed, which does not depend on the order of polymers deposition.

Table 1. Characteristics of pectin and chitosan based mono- and bilayers (scan area size equal to 2,5x2,5 μm).

Surface	Maximum peak height (Rmax), nm	Ten Point Height (S10z), nm	Root Mean Square (Sq), nm	Adhesion, nN
Chitosan	35,5±5,1	31,1±4,8	4,8±0,9	4,1±0,7
Pectin	13,1±4,0	11,4±3,2	1,5±0,2	3,2±0,2
Chitosan-pectin	59,7±10,9	39,6±7,8	10,3±2,8	4,5±0,6
Pectin-chitosan	40,9±8,6	37,1±7,6	8,5±2,7	4,6±0,2

3.3 Formation of multilayer coatings

3.3.1 Investigation of the films growth using QCM-D

The film, consisting of 4 chitosan and pectin bilayers, was studied using the method of QCM-D (Fig.5). The first layer was formed by chitosan at pH= 5.9 and then pH was changed to a value of 3.9. There has been a steady increase in the amount of substance–with the

growth of layers number due to the electrostatic interactions. Chitosan is deposited in a smaller amount than pectin, forms a more rigid coating and both polymers are firmly adsorbed on the initial surface. The results shown in Figure 6 demonstrate that with the growth of layers number, the film becomes more flaking and soft.

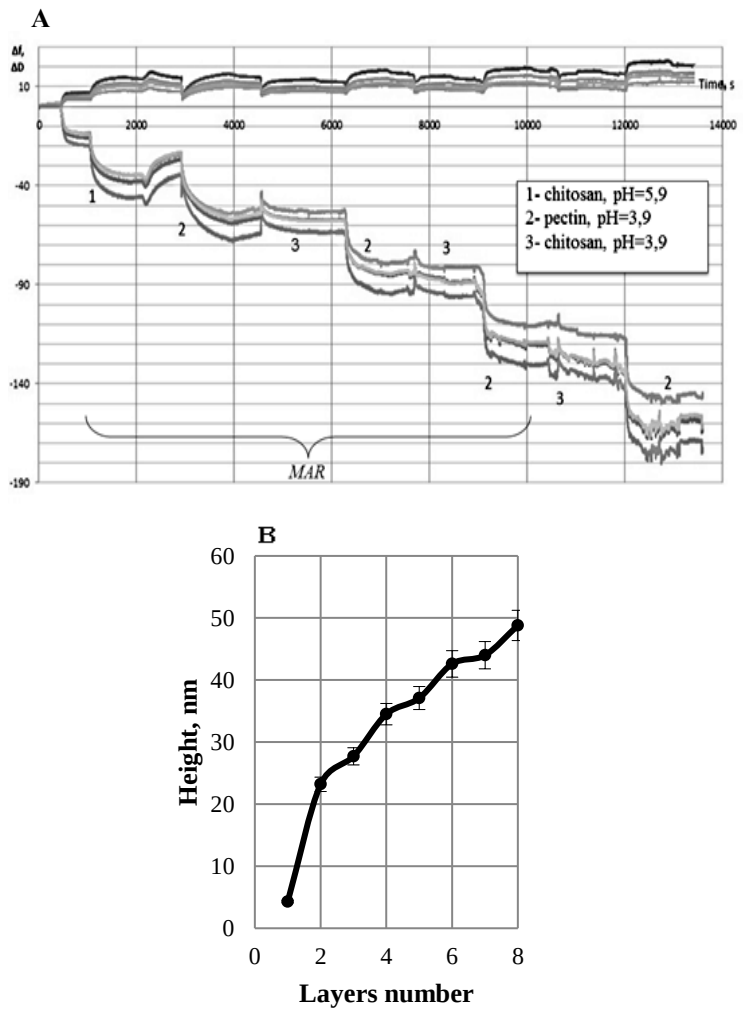

Figure 5. Changes in frequency and dissipation (A), and in coating height (B) during the build-up of chitosan-pectin film

3.3.2 Atomic force microscopy of multilayer films

Characteristics of film growth with increasing of layers number were examined using AFM. There are two main types of growth of thin films based on polymers – linear and exponential. A linear dependence between thickness and number of layers is explained by the same fixed amount of polyelectrolyte, which adsorbed at each assembly stage [10]. However, from Figure 6A it is seen that in the case of the formation of pectin-chitosan film this dependence is non-linear.

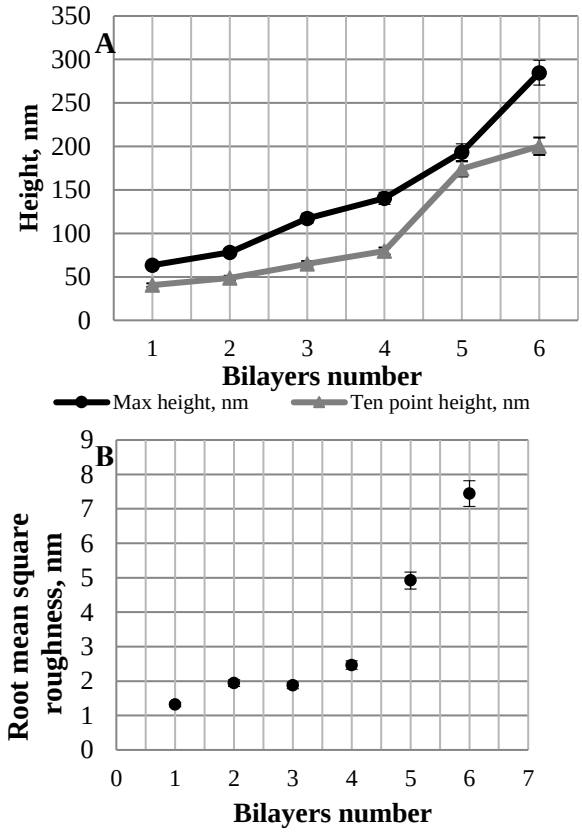

Figure 6. Changes in height (**A**) and Root Mean Square roughness (**B**) with the growth of layers number

The analysis of this character of growth shows that it could be connected with the increase of root mean square roughness when the layers number grows (Fig. 6B). It is indicates the decreasing of the degree of coating uniformity. Macromolecules that adsorbed in a number of initial coating layers are strongly attracted by the surface. They took a flat configuration due to the influence of the substrate. This effect is reduced, while a number of layers grow. The polymer molecules start to be adsorbed in convolute structures form. During this process the film surface area is increased. As a result any more amount of substance can be adsorbed on the next step.

Besides this, at a certain coating thickness, it is really due to the increase of the film roughness. Addition of other bilayer with the aim of changing the surface morphology will not be properly modify the surface properties. Thus, it is more efficient to manipulate a small number of bilayers, when we can really influence on the film structure. It could be confirmed also by the results of QCM-D (Fig.5). MAR (molecular architecture range) = 1÷6 is the number of layers of multilayer film that allows the most efficiently control the surface properties of the films. Subsequently, it allows to receive based on them coating with predetermined parameters.

4. Conclusions

Thus, the article considers the main approaches to the formation of coatings based on chitosan and pectin that allows you to manipulate the microstructure of thin films for obtaining predetermined result. Using AFM and QCM-D it was studied the morphology and physical properties of mono-, bi-, and multilayers, formed by LbL deposition method and the influence of some initial parameters (concentration, pH of the polymer solution, properties of the substrate) on the process of film formation. The optimal number of layers (≤ 6) has also defined, which will allow designing multilayer coatings with desired final molecular architecture by the most effective manner.

5. Acknowledgments

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6. References

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