ABSTRACT
Of dissertation for the doctor of philosophy degree (PhD)
6D060600 – Chemistry

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Physical and chemical bases of obtaining antibacterial coatings for implantable products

The dissertation is devoted to establishing conditions for the production of antibacterial nanofilms based on polyelectrolytes and bioactive compounds for biomedical products. The use of chitosan, sodium carboxymethyl cellulose, polyacrylic acid as multilayers and chlorhexidine, triclosan and silver nanoparticles as promising antiseptics for polymers was justified. A technique was developed for conducting multilayer assembly using the LbL method and methods for incorporating antibacterial agents into the resulting films, a mechanism was proposed for the formation of multilayers, the effect of physicochemical parameters on the surface morphology of thin films was studied, and the possibility of using the studied materials as antibacterial coatings for implantable systems was shown. All research within the framework of the dissertation was carried out for the first time, the results are presented in the form of articles, including in a peer-reviewed journal, and abstracts of reports of international conferences, and an author's certificate for a utility model was obtained.

The relevance of the research topic. In practical medicine, an important problem today is infectious diseases associated with the consequences of biomedical products implanted in the body. The fixation of bacteria and the development of a biofilm on the surface of implantable biomedical devices very often lead to infections and inflammations, unfortunately, even to the need to remove them, which is accompanied by moral and material costs, and sometimes death of the patient. In this regard, the issue of inhibition of biofilm growth around the implant is acute. After the formation of a biofilm on the surface of the implant, the removal of the latter and the use of antibiotics are usually required. Implants treated with antimicrobial agents that prevent bacterial adhesion and biofilm formation can do without long-term ineffective systemic antibiotic therapy, reduce the risk of resistance and the need to remove the implant (plagiarism). Ultrathin multilayer films containing antibacterial agents that can be grown on the surface of objects introduced into a living organism can serve as such carriers.

One of the most scientifically and scientifically promising varieties of antibacterial coatings is thin polymer films that release biologically active substances and prevent infectious changes near the implant product.

Therefore, the development of conditions for the production of polyelectrolyte multilayers based on biocompatible, environmentally friendly and affordable polyelectrolytes with the subsequent application of antibacterial coatings on their surface for biomedical products is an urgent problem of modern practical medicine.

The **aim** of the dissertation work is the development conditions for the production of antibacterial films based on biocompatible, environmentally friendly polyelectrolytes for biomedical products.

For the achievement of the given goal the following tasks are introduced:

1 Quantum-chemical substantiation of the energy and structural characteristics of bioactive polysaccharides and antibacterial drugs;

2 Establishment of conditions for producing antibacterial thin films on the surface of medical implants using the Lbl method;

3 Determination of the features of changes in the morphology and growth of films depending on the pH of the system and the introduction of an antibacterial agent in the structure of the films;

4 Determination of the antibacterial activity of the obtained films;

5 Give recommendations on the production of antibacterial thin films based on polyelectrolytes on the surface of medical implants.

Objects of research: thin films based on polyelectrolytes on the surface of various substrates with antibacterial properties.

The subject of scientific research: applying films to the surface of various substrates by the LbL method; the dependence of the surface morphology of the films on physicochemical parameters; antibacterial properties of films based on polyelectrolytes.

Research methods. When performing research in the framework of the thesis, the following methods were used to obtain polyelectrolyte films and physicochemical studies: method of multilayer assembly LbL; IR spectroscopy; ellipsometry; atomic force and scanning electron microscopy; determination of the wetting angle by the method of a lying drop; determination of antibacterial activity by agar diffusion.

Source database and research materials comprise 190 sources of literature on the methods for producing antibacterial multilayers based on polyelectrolytes, film growth on the surface of medical implants, and also in other fields of natural science concerning the subject of this study.

Scientific novelty:

- For the first time, conditions have been developed for the production of polysaccharide-based nanofilms: chitosan- sodium carboxymethyl cellulose, chitosan-polyacrylic acid, and the physicochemical characteristics of the obtained multilayers have been investigated;

- the influence of the nature of polyelectrolytes and the pH of the medium on the mechanism of formation of nanofilms has been established, the dependence of the thickness of the films on the number of bilayers and the forms of occurrence of polyelectrolytes has been substantiated;

- two methods for producing silver nanoparticles and a mechanism for their introduction into multilayers have been developed;

- established antibacterial activity of the obtained nanofilms on the surface of titanium and steel implants.

The theoretical significance of the research. The experimentally found conditions for the production of thin films based on polyelectrolytes for coating the

surface of implantable systems, the proposed mechanism for the formation of nanofilms depending on the pH of the medium and the nature of the polymer matrices, the physicochemical parameters that affect the surface morphology of thin films, and the conditions for the introduction and release of an antibacterial agents are of significant theoretical interest.

The practical value. The value of the results obtained during the implementation of the dissertation thesis in the field of producing thin films based on polyelectrolytes lies in the possibility of their use for coating implantable systems to give them antibacterial properties; in addition, the results of a study of the dependence of the surface morphology of thin films on physicochemical parameters will find application in the future in the production of thin films; based on the results of the dissertation, recommendations will be issued on the preparation and application of thin films based on polyelectrolytes on various substrates, in particular on implantable materials to give them antimicrobial properties.

The main points for the defense of the thesis:

1 A quantum-chemical study of the structural and energy characteristics of chitosan, sodium carboxymethylcellulose, chlorhexidine and triclosan from the point of view of potential active centers of electrostatic interaction in polymer matrices and bioactive reagents makes it possible to substantiate the use of the above substances as polymer matrices for the production of bioactive nanofilms with antibacterial properties;

2 Using the semi-automatic immersion method, a modification of the LbL multilayer assembly method, allows one to grow monotonously a film based on chitosan, PAA and CMC, on the surface of solid substrates. The initial concentration of solutions of polyelectrolytes and their pH affects the thickness, roughness, and morphology of the films of Chi / PAA and Chi / CMC.

3 Films with Chi show antibacterial activity against S. aureus, as well as against the museum strain of E. coli ATCC25922 and wild strain P. Aeruginosa. The zone of inhibition of films $(Chi / CMC)_{40}$ with integrated Chi is 19 mm and 7 mm, against E. coli ATCC25922 and P. Aeruginosa, respectively, and films containing silver nanoparticles showed good antibacterial activity against museum strains Staphylococcus aureus. The oppression zone for the Chi / CMC sample with integrated AgNP is 7.1 ± 0.03 mm.

Main results of the research are published in 12 scientific papers, including:

- in one article published in an international scientific journal that indexed by Scopus database;
- in one article published in Proceedings, indexed by the Scopus database;
- in four articles published in journals recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan;
- in five theses of reports at foreign and republican international conferences and symposia.

In addition, a positive decision was received on the issuance of a patent of the Republic of Kazakhstan for the utility model “A method for producing an

antibacterial coating on the surface of titanium and steel implants based on chlorhexidine” («Sposob polucheniya antibakterial'nogo pokrytiya na poverkhnosti titanovykh i stal'nykh implantatov na osnove khlorgeksidina») (In Russian).

The structure and scope of the thesis.

The thesis consists of an introduction, three sections, a conclusion, as well as a list of references of 180 titles. The work is presented in 124 pages, contains 52 figures and 14 tables.

The following conclusions can be made from **the results of this thesis:**

1 Based on quantum-chemical calculations of structural and energy characteristics, a scientifically-based choice of polymer matrices: chitosan, sodium carboxymethyl cellulose, alginate and antibacterial agents from promising antiseptics, such as triclosan, chlorhexidine, was made. The results of these studies made it possible to theoretically predict and justify the nature of the active centers of the electrostatic interaction of the selected polyelectrolytes and antibacterial agents: chlorhexidine and triclosan with polymer matrices of the proposed multilayers.

2 Conditions have been developed for cleaning, preparing and activating the surface of standard plates (silicon and glass) and orthopedic implants. Based on the physicochemical methods, the morphology and hydrophilicity of the surfaces were investigated and it was established:

- etching the surface of titanium, steel implants with organic solvents, followed by treatment in an ultrasonic bath and a piranha solution significantly reduces the contact angle to 62 ° and ensures hydrophilicity of the surface of the media;

- for silicon and glass carriers, the most suitable treatment with a solution of sulfuric acid with the addition of hydrogen peroxide. Numerous microcracks obtained as a result of processing are active centers and, according to Taylor's theory, promote good and uniform adsorption of functional groups of polyelectrolytes.

3 The conditions for obtaining high-quality nanocoatings on the surface of solid substrates are established. The conditions of the “immersion” method are characterized: a) automatic immersion and removal at a given speed (0.001 m / s) without stirring; b) manual immersion of the substrate in the solution with stirring. It was found that the use of a semi-automatic “dip coater” allows one to produce a monotonically growing film on both sides of a solid substrate, while manual immersion of a substrate in a solution with stirring leads to a chaotic superposition of the nanofilm layers and the formation of defects as a result of random diffusion. The study of the influence of the initial concentration of polyelectrolytes on the film quality showed that in the range of parameters studied, the optimal concentration of polyelectrolytes to obtain a smoother film is 0.01 M.

The effect of the pH of solutions of the applied polyelectrolytes on the thickness, roughness, and morphology of the films of Chi / PAA and Chi / CMC is shown. It was revealed that the film thickness depends linearly on the number of bilayers, the degree of ionization and conformation of polyelectrolytes caused by a change in the pH of the system is a criterion for the formation of a thin transparent

and thick opaque film. To fully characterize the morphological properties of the coatings obtained in the pH range of 3-6, the mean square roughness (R_q) was determined using the AFM semi-contact mode. A relatively large R_q value of 15.3 ± 1.4 nm was obtained for the sample at pH-5, while a change in pH by one leads to a decrease in the R_q value to 4.1 ± 0.1. The refractive index (n), which is practical for all film samples at λ = 632.8 nm, corresponds to a value in the range 1.4–1.55, which is characteristic of transparent polymer films. The results of the ellipsometric analysis showed that the obtained films are well described by the Cauchy dispersion model in the framework of the model of a single-layer reflecting system.

4 Two methods of applying an antibacterial agent - chlorhexidine to multilayer films are justified: 1) direct deposition of CX molecules by association with one of the polyelectrolytes, taking it as one of the multilayer components; the second is the immersion and impregnation of pre-assembled films in an antiseptic solution. For each approach, a “reservoir” was chosen for the delivery of an antibacterial agent. A scheme was developed for the formation of an antibacterial coating in films, the so-called “underlying and upper lying parts”. The kinetics of CX release from the film $(CX / PAA)_{10}$ - $(CX / PAA)_{40.5}$ was established, according to which 0.45 mg/ml is released in the first 24 hours, the largest dose of antiseptic, and a slower release in the following hours. It was revealed that the samples obtained by two methods showed antibacterial activity against S.aureus (they are the most common microorganisms involved in infection associated with orthopedic implantations), as well as against the museum strain E. coli ATCC25922 and wild strain P. Aeruginosa. It was established that coatings $(CX / CMC)_{40}$ with CX showed 19 mm and 7 mm of the oppression zone against E. coli ATCC25922 and P. Aeruginosa.

5 Two methods (approaches) for the production of polyelectrolyte multilayers containing silver nanoparticles (AgNP) have been developed. In the first approach, based on the phased incorporation of AgNP into one of the polymer matrices, the influence of the initial concentration of $AgNO_3$ on the particle size was established. The formation of AgNP of three types was revealed: such as dendrites, spherical and aggregates. In the second approach, when obtaining silver nanoparticles, the mechanism of in situ chemical reduction of silver ions to nanoparticles is justified, which is based on the fact that the films are immersed in a solution of ascorbic acid to reduce silver ions to metal, and then the released carboxyl groups are regenerated and ready again for re-bonding the cation. Based on experimental data, a general scheme for the formation of such films is proposed. The size of nanoparticles in multilayers and their distribution over the surface can be controlled by choosing the initial concentration of $AgNO_3$ and the optimal concentration of silver ions to obtain nanoscale spherical particles is 0.001 M.

The obtained nanofilms containing silver nanoparticles in their composition showed good antibacterial activity against museum strains of the bacterium Staphylococcus aureus. The oppression zone for the Chi / CMC sample with AgNP is 7.1 ± 0.03 mm.

Evaluation of completeness of the solutions for the given tasks. All the tasks set for solving the purpose of this dissertation are solved in full. Quantum-chemical substantiates the use of chitosan, sodium carboxymethyl cellulose, polyacrylic acid as polymer matrices for multilayers, and chlorhexidine, triclosan, and silver nanoparticles as promising antiseptics. A technique was developed for conducting multilayer assembly using the LbL method and methods for incorporating antibacterial agents into the resulting films.

Thus, the objectives of the dissertation research have been achieved - conditions have been developed for obtaining antibacterial films based on biocompatible, environmentally friendly polyelectrolytes for biomedical products, and their antibacterial conditions have been investigated.

Evaluation of technical and economic efficiency of the proposed solutions in the thesis. The solutions proposed in the framework of this dissertation can form the basis for the production of thin antibacterial films based on polyelectrolytes on the surface of medical implants.