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IMMOBILIZATION OF TILOZINE ON ALGINATE MICROPARTICLES

Abstract. The immobilization of antibacterial drug tilozine on the alginate microparticles has been carried out. The dynamic of drug release from polymeric microparticles was investigated. The possobility of the use of alginate spheregels for the development of polymeric drug delivery systems with prolonged release of tilozine is shown.

Key words: immobilization, tilozine, alginate, microparticles, drug, release.

Introduction. One of the actual problems of modern chemistry and technology is the development of new polymer materials, intended for immobilization of different compounds. Immobilized materials are widely used in different branches of science and technique, biotechnology, chromatography, biochemical diagnostics, clinical medicine. On the basis of immobilized preparations sensitive electrochemical sensors, materials for internal organs, artificial biocatalysts, stable medicinal forms of hormones, steroids, vitamins, enzymes, antibiotics have been developed [1].

Among polymeric carriers, applied in pharmaceutical practice with the purposes of immobilization the alginic acid and its derivatives are of particular interest. These polymers are practically harmless, hydrophobic, able to form viscous water solutions and pastes, possess homogenizing, loosening and emulsifying properties. Alginic acid presents a polysaccharide, obtained from seaweed (laminaria) and consisting of repeated links of β-D-mannuronic and α-L-guluronic acids, interconnected with glucosidic bonds [2].

Alginic acid and its sodium and potassium salts have already found a wide application in pharmaceutical practice as loosening and binding remedies upon the production of tablets and preparation of ointments and pastes. In the presence of two-valency cations alginic acid forms gels, built from guluronic acid with the participation of a cation. A strong structure of alginate gel and big sizes of pores allow one to easily immobilize large quantities of different physiologically active compounds, proteins, enzymes and even cells [3-7].

The purpose of present work is development of polymeric medicinal forms with the prolonged action by immobilization of the antibacterial drug tilozine on alginate microparticles.

EXPERIMENTAL PART

The antibacterial drug tilozine was used pharmaceutical grade. Sodium alginate, molecular weight 75-100 000 from *Macrocystis pyrifera* were purchased from Sigma Chemicals, USA.

Microparticles were obtained in the following way: at the first stage a solution of drug in 2,5% solution of sodium alginate was prepared, then obtained solution was filtered and syringed dropwise into a mixed solution of chitosan in 0,1 *M* of calcium chloride, at a constant dropping rate of 1,0 ml/min. The obtained modified microparticles of calcium alginate were treated by the solution of calcium chloride for 30 min, washed with distilled water and physiological solution. As a result gel microparticles of calcium alginate were obtained, containing immobilized drug and a surface layer of chitosan [8].

Thickness of a microparticles coating was determined with the help of a light optic microscope of the mark «Leica Eclipse TE300». Samples of alginate microparticles were preliminary frozen in liquid nitrogen and cross-cut with a scalpel just before measuring. Electronic absorption spectra of drugs in the field of 350–800 nm were detected on a spectrophotometer Jasco UV/VIS 7850 (Japan) in glass cuvettes with the thickness of 10 mm at 37°C.

The release of drug was studied under conditions *in vitro* at 37°C. With this purpose a definite quantity of alginate particles was placed in a metallic basket, immersed in 300 ml of physiological solutions at pH 7,1. A constant rate of mixing of the released medium was ensured with the help of a magnetic mixer. After definite time intervals 2 ml of the solution was selected for the determination of the content of drug with the help of the visible or ultraviolet spectroscopy. Release of drug was controlled each minute for the first 10 minutes, each 2 min for the following 100 minutes.

RESULTS AND DISCUSSION

Alginic acid in the presence of divalent cations such as calcium ions forms gels as result from specific and strong interaction between cation and guluronic acid resudes. At formation of alginate gels the dominant role play blocks of guluronic acids where everyone calcium cation coordinates with 10 oxygen atoms of four residues of L-guluronate. Thus a polymeric chain get the original cellular-extended form in which each cell has a certain orientation of atoms of oxygen to calcium ions, forming conformation-correct links. The model of this coordination is popularly known as the «eggs in box». The scheme of coordination of calcium ion with blocks of guluronic acids at formation of «eggs in box» model in alginate gels is presented in figure 1.

One of the effective antibacterial drugs widely used at veterinary is tilozine. However along with many advantages this drug possesses short-term pharmaceutical action. Therefore in the given work the researches for development of new drug delivery system based by immobilization of tilozine on alginate microparticles are conducted.

With the purpose of prevention of destruction and erosion of calcium alginate microparticles their surface modification by a natural polymer chitosan has been carried out. An influence of the concentration of a polymer and the time of modification upon thickness of a surface layer of alginate gels has been studied.

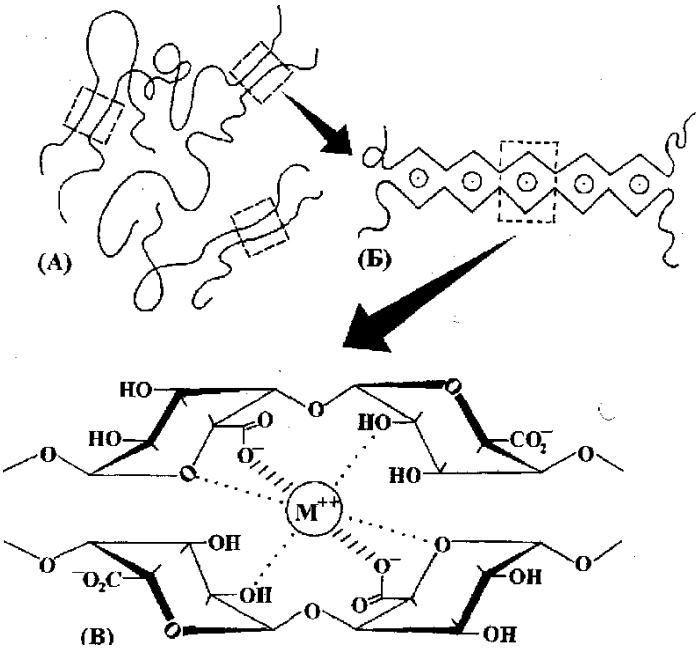

Figure 1 – Scheme of calcium ion coordination of the «egg box model» in alginate gel

For the determination of surface thickness a red congo dye has been used, able to form a complex with chitosan. It has been established that with an increase in the concentration of a polymer from 0,3 up to 1,5 mass % thickness of the modified layer increases from 5 up to 60 micron, and an increase of the time of gel exposition in a 2,5% solution of chitosan from 30 min to 24 h results in an increase in thickness of a layer from 5 up to 20 micron respectively. However, upon the exposition of microparticles a strong compression of an alginate sphere has been observed, stipulated by osmotic pressure of a polymer solution, resulting in the forcing out of water molecules from alginate microparticles. The conducted studies have made it possible to determine an optimal duration of modification of a surface layer of calcium alginate gel microparticles.

Calcium-alginate gel microparticles had a perfect spherical shape with regular smooth surfaces suitable for drug immobilization. Scanning electron micrographs of air dried alginate microparticles were illustrated in figure 2. Apparently from drawing the microparticle has the correct spherical form in diameter 400-600 microns, the surface of microparticles has friable fibrous structure. The surface morphology of Ca-Alg microparticles improved by increasing alginate concentration whereas increasing alginate concentration above 3% made preparation of the beads difficult.

The presence of aminogroup in the structure of chitosan, and of carboxylic group in the structure of alginic acid testifies to possibility of their interaction in solution due to electrostatic forces. That is why upon the obtaining of a modified

Figure 2 – SEM micrographs of the tilozine-loaded alginate microparticles

cover of alginate microparticles a formation of the polyelectrolyte complex between chitosan polycation and alginate polyanion. The interaction between sodium alginate and chitosan in solution has been studied with the help of conductometric and turbidimetric titration. It has been shown that upon the gradual addition of a sodium alginate solution to chitosan solution the decrease in electroconductivity and running of the solution takes place, accompanied by turbidity of the system. At the ratio of alginate:chitosan, equal to 1:1 a formation of insoluble precipitate is observed, which testifies to the formation of polyelectrolytic complex between the components. Upon the further addition of an alginate solution a precipitate dissolves and the characteristics of electroconductivity and running increase.

The composition of the polycomplex depends on pH of the medium. With low pH values a non-stechiometric complex, enriched by the links of alginic acid, is formed. In the neutral medium with pH of 7,2 aminogroups of chitosan are mainly in the non-ionized state and possess form of compact tuber, and sodium alginate macrochain is in the unfolded state. In the given case carboxylate anions interact only with the available protonated aminogroups of chitosan, located on the surface of chitosan tubers, and the formed polycomplex is enriched by the links of a polybase. In a weakly acidic medium with pH of 4,8 the formation of polyelectrolyte complex of an equimolar composition takes place, which is testified by wider range of minimum value of running through of a solution with the content of alginate of 40–60 %.

One of the most important characteristics of macromolecular therapeutic systems is a program of drug release to the organism. Figure 3 presents kinetics of

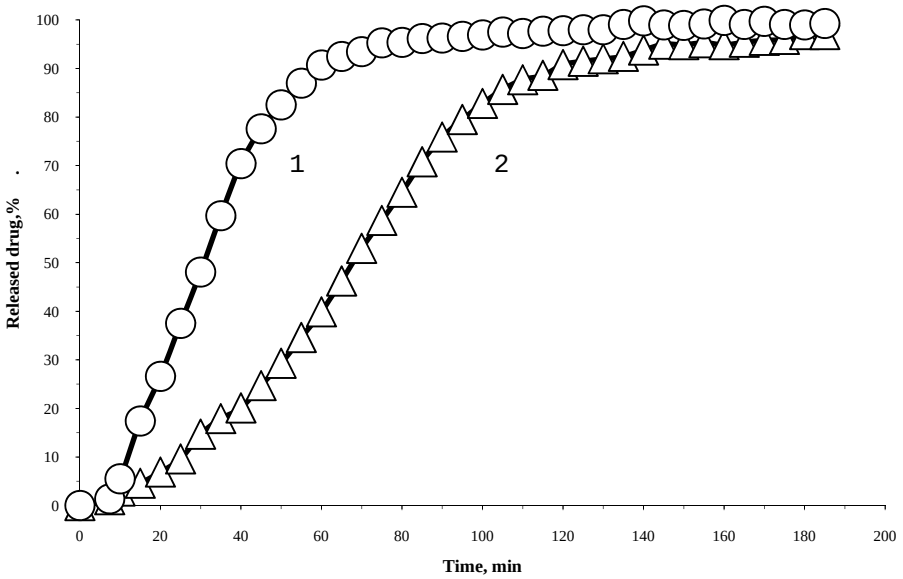

Figure 3 – Kinetics of tilozine release from the modified alginate gel microparticles into a physiological solution with different thickness of a chitosan coating: 1 – 50 mcm; 2 – 100 mcm

tilozine release from the modified alginate gel particles into a physiological solution with different thickness of a chitosan coating. It has been shown that with an increase in thickness of a cover the release of drug decreases significantly. Thus, in the absence of a chitosan coating tilozine diffuses from microparticles practically by 95–100% for 40–50 min. Upon the use of alginate particles with the coating thickness of 50 and 100 mcm the same quantity of the preparation is released for 180 and 240 min respectively. Maximal thickness of a chitosan coating provides a diffusion of the tilozine by 50–60% for 240–250 min. Noteworthy is the presence of an induction period of 10–20 min, when no release of the drug from the modified microparticles is observed. This time interval seems to be necessary for the passing of tilozine molecules through a layer of a chitosan cover, followed by a diffusion into the volume of the solution. After alginate microparticles are in the physiological solution for 4 hours, their complete destruction is observed, stipulated by the substitution of calcium ions for sodium ions.

Thus, the possibility of the regulation of the rate of drug release from the modified alginate particles by way of alternation of thickness of the chitosan coating has been shown. The use of such systems makes it possible to developed the constructions with a regulated and controlled delivery of drugs to the organism in accordance with the pre-set time program.

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Резюме

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АЛЬГИНАТ МИКРОБӨЛШЕКТЕРІНЕ ТИЛОЗИН ИММОБИЛИЗАЦИЯЛАУ

Альгинатты гелінің микробөлшектеріне бактерияға қарсы дәрі тилозин препаратын иммобилизациялау жүргізілді. Полимерлі микробөлшектерден дәрілік препараттарын босатылу динамикасы зерттелді. Альгинат сферогельдерін тилозиннің ұзақ әсерлі босату жуйесін жасауға қолдану мумкіндігі корсетілген.

Түйін сөздер: иммобилизациялау, тилозин, альгинат, микробөлшектер, дәрілік босатылу.

Резюме

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ИММОБИЛИЗЦИЯ ТИЛОЗИНА НА АЛЬГИНАТНЫХ МИКРОЧАСТИЦАХ

Проведена иммобилизация антибактериального препарата тилозина на альгинатных микрочастицах. Исследована динамика высвобождения лекарственного вещества из полимерных микрочастиц. Показана возможность использования сферогелей альгината для создания систем с пролонгированным высвобождением тилозина.

Ключевые слова: иммобилизация, тилозин, альгинат, микрочастицы, высвобождение лекарства.