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PROLONGED RELEASE OF ANTICANCER DRUG CYTOXSANE FROM ALGINATE/CHITOSAN MICROPARTICLES

Abstract. Release of anticancer drug cytoxsane from alginate/chitosan microparticles has been studied. Modified microparticles were obtained in the following way: at the first stage sodium alginate was dissolved in distilled water at 2.5 wt.%. Cytoxsane as model antitumor drug was added to alginate solution and dissolved completely with stirring. The resulting solution was filtered and syringed dropwise into chitosan solution in calcium chloride at a constant dropping rate from the needle tip. As a result gel microparticles of calcium alginate were obtained, containing immobilized drug and a surface layer of chitosan. The release of cytoxsane from alginate gel particles into a physiological solution with different thickness of chitosan coating were studied. It was shown that the characteristic maximum on the curve of release observe after 120 min for the samples with the coating thickness 150 mcm. The data obtained shown a possibility of the regulation of the rate of cytoxsane release from the alginate/chitosan microparticles through thickness of the chitosan coating.

Key words: release, cytoxsane, alginate, chitosan, microparticles.

Introduction. Cancer diseases are currently one of the most common pathologies, and every year the number of people suffering from malignant neoplasms is steadily growing. The most widely used method of treating patients with cancer is chemotherapy, the principle of which is based on the introduction of powerful drugs into the body, that destroy cancer cells or inhibit their development. One of the main limitations of chemotherapies is their high toxicity which could lead to serious side effects, reducing the administrable and the therapeutic effect. To address this issue, it is essential to transport the antitumor drugs mainly to the target where it is needed and at the required time and level [1].

This effect could be achieved by embedding the drugs into nontoxic and biodegradable polymeric microcapsules from which the drug will be released in a sustained manner. Furthermore, the encapsulation of the drugs can improve drug solubility and stability as well as reduce other disadvantages of cancer chemotherapy including toxicity, pain management, short in vivo half-lives and repeated after administrations [2]

Among polymeric carriers, applied in pharmaceutical practice with the purposes of immobilization the alginic acid is of particular interest. This polymer is 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. In the presence of two-valent 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 [3].

Chitosan widely used as cationic polymer for the preparation drug-loaded alginate/chitosan microparticles. Chitosan is biodegradable, readily available and has mucoadhesive properties [4]. Numerous results have been reported about the formation of polyelectrolyte complexes of alginate with chitosan under different conditions.

Novel composite microparticles of alginate coated with chitosan were prepared via ionotropic/external gelation technique for controlled release and protection of ascorbic acid, a strong antioxidant. The microparticles were characterized by Fourier transform infrared, X-ray diffraction, and scanning electron microscopy. The encapsulation efficiency and loading capacity were 89,94±5.62% and 37.01±1.83%, respectively. The degree of swelling of the microparticles at pH 7 was much higher than that at pH 2.0. The release profiles showed that ascorbic acid could be slowly released from microparticles and polymeric relaxation was the prevailing release mechanism. The microparticles exhibited protective effect on ascorbic acid against light and heat exposure degradation. After 7 days of ultraviolet exposure and 80°C heating, the residual rates of ascorbic acid in microparticles were 90.31%. The results suggested that the microparticles can be used as a reliable delivery system for ascorbic acid [5].

Alginate/chitosan nanoparticles were used for controlled release of vitamin B2. Ionotropic polyelectrolyte gelation was used as production method being chitosan and alginate used as main materials. Nanoparticles were characterized in terms of average size, polydispersity index, zeta potential and vitamin entrapment efficiency. The average size for alginate/chitosan nanoparticles was 119.5±49.9nm for samples without vitamin B2 and 104.0±67.2nm with the encapsulation of vitamin B2, presenting a polydispersity index of 0.454±0.066 and 0.319±0.068, respectively. The nanoparticles showed encapsulation efficiency and loading capacity values of 55.9±5.6% and 2.2±0.6%, respectively. Release profiles were evaluated at different conditions showing that the polymeric relaxation was the most influent phenomenon in vitamin B2 release. In order to study their stability nanoparticles were stored at 4°C being particles sizes and PDI evaluated during 5 months showing the results that vitamin B2-loaded nanoparticles are more stable than nanoparticles without drug [6].

The objective of the present work was the preparation of calcium alginate microparticles modified by a surface layer of chitosan and entrapped antitumor drug cytoxsane. The dependence of drug release from alginate/chitosan microparticles on the thickness of chitosan coating was examined. The article also was described the study of interaction between sodium alginate and chotosan with interpolymer complex formation.

EXPERIMENTAL PART

Sodium alginate and chitosan were purchased from Sigma Chemicals, St.louis. The medium viscosity sodium alginate, isolated from *Macrocystis pyrifera*, had a molecular weight between 75000 and 100000 Dalton, and viscosity of 2% solution (25°C) approximate 3500 cps. The practical grade chitosan from Crab Shells had acetylated degree 15% and molecular weight 600000 Dalton. Cytoxsane from Bristol-Myers Squibb, Germany was used pharmaceutical grade.

Modified microparticles were obtained in the following way: at the first stage sodium alginate was dissolved in distilled water at 2.5 wt.%. Cycoxane (0.01 mg) as model antitumor drug was added to 20 mL of the resulting alginate solution and dissolved completely with stirring. The resulting solution was filtered and syringed dropwise into 100 mL of 0.5%,1.0 and 1.5% chitosan solution in 0.1 *M* calcium chloride at a constant dropping rate of 1.0 mL/min from the needle tip (0.65×25 mm). The obtained modified microparticles of calcium alginate with average diameter of 1.0±0.5 mm were treated by the solution of calcium chloride for 30 min, washed with 100 mL of distilled water and 50 mL of physiological solution. As a result gel microparticles of calcium alginate were obtained, containing immobilized drug and a surface layer of chitosan [7].

The surface of the alginate/chitosan microparticles was examined using scanning electron microscopy Superprobe733, Russia, equipped energy dispersion spectrometer Inga Energy. Dry loaded microparticles were sputter coated with gold using Fine Coat equipment and imaged at 20 kV at regime of secondary electrons.

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 37^0C.

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 [8].

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».

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. 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 1.

Figure 1 – SEM micrographs of the cytoxsane-loaded alginate/chitosan microparticles

The interaction between sodium alginate and chitosan in solution has been studied. 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. 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 (figure 2).

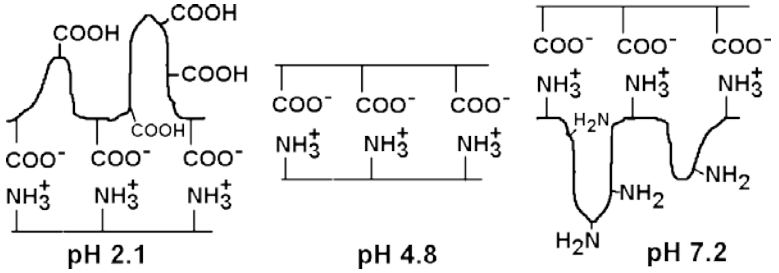

Figure 2 – Schematic representation of polyelectrolyte complexes between alginate and chitosan at certain pH

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. Table present kinetics of cytoxsane release from the modified alginate gel particles into a physiological solution with different thickness of a chitosan coating. It has been shown that with

Kinetics of cytoxsane release from the modified alginate gel microparticles into a physiological solution with different thickness of a chitosan coating

Thickness of chitosan, mcm	Released cytoxsane, %				
	30 min	60 min	120 min	180 min	240 min
25	12	26	35	44	59
50	10	24	33	41	55
100	9	22	31	39	51

an increase in thickness of a cover the release of drug decreases significantly. Thus, in the absence of a chitosan coating cytoxsane 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 cytoxsane 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 cytoxsane 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 h, their complete destruction is observed, stipulated by the substitution of calcium ions for sodium ions.

Conclusion. The possibility of the regulation of the rate of cytoxsane release from the modified alginate particles by way of alternation of thickness of the chitosan coating has been shown. The use of alginate/chitosan microparticles makes it possible to develop the systems with a regulate delivery of drugs to the organism in accordance with the pre-set time program.

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Резюме

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ІСІККЕ ҚАРСЫ ПРЕПАРАТ- ЦИТОКСАННЫҢ, АЛЬГИНАТ/ХИТОЗАН МИКРОБӨЛШЕКТЕРІНЕН БОСАТЫЛУ МЕРІЗІМІН ҰЗАРТУ

Ісікке қарсы препарат цитоксанның, альгинат/хитозан құрамынан жеке бөлініп, оның шағын бөліктерінен босап шығуы зерттелді. Модификацияланған микробөлшектер кальций хлоридіндегі хитозан ерітіндісіне натрий альгинатының құрамында

цитоксан бар ерітіндісін тамшылар бойынша шприцтеу жолымен алынған. Иммобилизацияланған цитоксаннан тұратын альгинат микро бөлшектері хитозанның үстіңгі қабатымен жабылған. Цитоксанның альгинатты гель бөлшектерінен хитозанды жабындысының қалыңдығы әртүрлі физиологиялық ерітіндіге бөлінуі зерттелді. Бөлу қисығына тән максимум қалыңдығы 150 мкм үлгілер үшін 120 минуттан кейін байқалады. Алынған мәліметтер хитозанды жабынның қалыңдығы арқылы альгинат/хитозан шағын бөліктерінен цитоксанның босатылу жылдамдығын реттеу мүмкіндігін көрсетті.

Түйін сөздер: препарат, босатылу, цитоксан, альгинат, хитозан, микробөлшектер.

Резюме

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ПРОЛОНГИРОВАННОЕ ВЫСВОБОЖДЕНИЕ ПРОТИВОРАКОВОГО ПРЕПАРАТА ЦИТОКСАНА ИЗ МИКРОЧАСТИЦ АЛЬГИНАТА/ХИТОЗАНА

Изучено высвобождение противоопухолевого препарата цитоксана из микрочастиц альгината/хитозана. Модифицированные микрочастицы получали путем шприцевания по каплям цитоксан-содержащего раствора альгината натрия в раствор хитозана в хлориде кальция. Полученные микрочастицы альгината, состоящие из иммобилизованного цитоксана, были покрыты поверхностным слоем хитозана. Исследовано выделение цитоксана из частиц альгинатного геля в физиологический раствор с различной толщиной хитозанового покрытия. Показано, что характерный максимум на кривой выделения наблюдается через 120 мин для образцов с толщиной покрытия 150 мкм. Полученные данные показали возможность регулирования скорости высвобождения цитоксана из микрочастиц альгината/хитозана через толщину хитозанового покрытия.

Ключевые слова: высвобождение, цитоксан, альгинат, хитозан, микрочастицы.