DETERMINATION OF THE OPTIMAL CONCENTRATIONS OF PECTIN AND CALCIUM CHLORIDE FOR THE SYNTHESIS OF CHITOSAN-PECTIN MICROPARTICLES

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ABSTRACT

The oral route of drug inclusion is the most convenient for the patient. In addition to ease of use, this method of drug inclusion has such advantages as non-invasiveness of inclusion, absence of complications during injection; comparative safety for the organism due to the passage of the active substance and auxiliary compounds through the gastrointestinal tract; the possibility of introducing larger doses of the drug at one time. However, despite the obvious advantages, the oral route of inclusion has a number of significant disadvantages that significantly limit its use for a number of drugs. Among them are: relatively slow therapeutic action of the drug with this route of inclusion; the aggressive effect of a number of drugs (for example, antibiotics) on the gastrointestinal tract; low bioavailability of a number of substances (especially high molecular weight hydrophilic compounds), caused by poor permeability of the intestinal epithelium for hydrophilic and large molecules, as well as enzymatic and chemical degradation of the active substance in the gastrointestinal tract.

There are various approaches used in the development of oral drug delivery systems. In particular, for the targeted delivery of drugs, it is proposed to use nano- and microcapsules with mucoadhesive properties. Among the polymers used for the synthesis of these microparticles, it is preferable to use pH-dependent, gelable biopolymers that change their structure depending on the acidity of the environment. Microcapsules obtained from compounds with the above properties are capable of protecting the active substance (or from the active substance) in the stomach environment and ensuring its release in the intestine. These properties are possessed by such polysaccharides as alginate, pectin, carrageenan, xylan, etc. The listed biopolymers are non-toxic, biocompatible, and biodegradable, which makes microparticles containing these polysaccharides promising as oral drug delivery systems. To impart mucoadhesive properties to nanoparticles, complexes of the listed polymers with chitosan are used.

In this research, pectin, a polysaccharide formed mainly by residues of galacturonic acid, was used as a structural polymer. The concentrations of substances in the initial solutions were selected that were optimal for the synthesis of microcapsules. The main parameters for evaluating the resulting microparticles were the size of the capsules (less than 1 μm for oral inclusion), the zeta-potential,

showing the tendency of the microparticles to stick together, and the completeness of the binding of the microparticles to chitosan.

It was found that the optimal solutions for the synthesis of microparticles are: 15.7 ml of a solution of pectin 0.093% by weight, 3.3 ml of a solution of chitosan 0.07% by weight and 1.0 ml of a solution of $CaCl_2$ 20 mM. The diameter of the microparticles obtained by this method was 700-800 nm, and the value of their zetta-potential, equal to - (34 ± 3) mV, does not cross the particle adhesion threshold. It was also found that the synthesis of microparticles at these concentrations of calcium chloride provides the most complete binding of chitosan to their surface, which increases the mucoadhesive properties of microparticles.

Keywords: *pectin, chitosan, microparticles, sorption capacity*

INTRODUCTION

The oral route of inclusion of drugs is the most convenient in therapy due to the ease of use for the patient, atraumatic introduction into the body and the possibility of taking large doses of the drug [1]. However, this method of drug inclusion also has a number of disadvantages that limit its use. Among them, one can single out low bioavailability for a number of active substances, the effect of aggressive drugs on the gastrointestinal tract (in particular, this item refers to various kinds of antibiotics) [1], the destruction of biological substances of a protein nature under the action of enzymes of the gastrointestinal tract and its aggressive environment [1], [2], low permeability of the intestinal mucosa for high molecular weight compounds [2] and a relatively slow therapeutic effect of the drug with this route of inclusion [1]. Modern advances in biotechnology have led to the possibility of industrial production of protein-based drugs and their widespread use in therapeutic practice [3]. These trends make the search for and development of new oral drug delivery systems a promising topical direction for almost two decades.

There are various approaches used in the development of oral drug delivery systems. In particular, for targeted delivery of drugs, it is proposed to use nano- and microcapsules with mucoadhesive properties [2], [3]. Among the polymers used for the synthesis of these microparticles, it is preferable to use pH-dependent, gelable biopolymers that change their structure depending on the acidity of the environment. Microcapsules obtained from compounds with the above properties are able to protect the active substance (or from the active substance) in the stomach environment and ensure its release in the intestine [3]. These properties are possessed by such polysaccharides as alginate, pectin, carrageenan, xylan, etc. [4]. The listed biopolymers are non-toxic, biocompatible, and biodegradable, which makes microparticles containing these polysaccharides promising as oral drug delivery systems [4]. To impart mucoadhesive properties to nanoparticles, complexes of the listed polymers with chitosan are used [4]. Chitosan is a biodegradable non-toxic polysaccharide consisting of randomly linked β- (1-4) D-glucosamine units and N-acetyl-D-glucosamines (with a predominance of residues of the first monomer in the composition). This biopolymer is capable of forming stable polyelectrolyte complexes with the aforementioned polysaccharides and, due to its mucoadhesive properties, is able to increase the residence time and the amount

of the active substance at the site of adsorption, creating a concentration gradient leading to the rapid absorption of protein molecules through the intestinal mucosa [3].

In this research, pectin, a polysaccharide formed mainly by residues of galacturonic acid, was used as a structural polymer. The scheme of the formation of a polyelectrolyte complex of chitosan-pectin microparticles is shown in Fig. 1-2

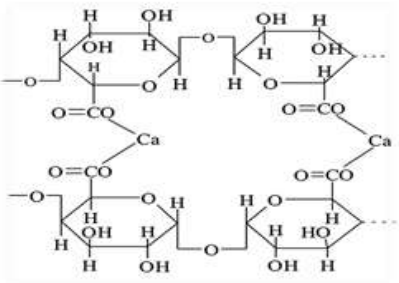

Fig. 1. *Scheme of the formation of calcium bridges between pectin molecules through carboxyl groups*

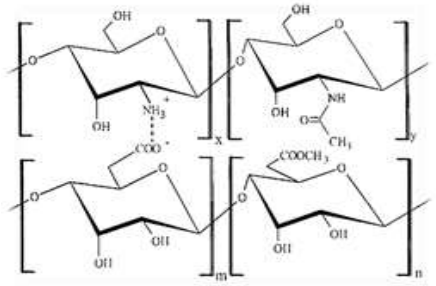

Fig. 2. *Scheme of the formation of the polyelectrolyte complex of chitosan with pectin*

The aim of this work is to determine the optimal ratio of the concentrations of the initial solutions of pectin, chitosan, and calcium chloride for the synthesis of chitosan-pectin microparticles that are effective when therapeutic molecules are included in them.

MATERIALS AND METHODS

The objects of this investigation were: low molecular weight chitosan (200 kDa) with a degree of diacetylation of 85%, manufactured by Sigma-Aldrich; apple pectin with a molecular weight of 12 kDa and a degree of metaxylation of 66% produced by Cargill (France); lyophilizate of doxorubicin hydrochloride for the preparation of a solution for intravascular and intravesical administration, produced by the pharmaceutical company "Teva".

To synthesize chitosan-pectin microparticles, 7.5 ml of a 22 mM calcium chloride solution was added to 117.5 ml of a 0.094% (mass) pectin solution (pH 4.3) dropwise at a rate of 0.125 ml/min using a peristaltic pump with constant stirring on a magnetic stirrer (stirring speed 800 rpm). At the end of the addition of the chitosan solution to stabilize the microparticles, the prepared suspension of microparticles was stirred on a magnetic stirrer for 30 minutes at a speed of 800 rpm. The separation of microparticles from the solution was carried out by centrifugation at a speed of 10,000 rpm for 30 minutes. To obtain microparticles loaded with a substance (in this investigation doxorubicin was used as an active compound), it was added to the initial pectin solution.

The size and zetta potential of the obtained microparticles were determined using a JEOL 1610LV scanning electron microscope with an SSD X-Max Inca

Energy energy dispersive spectrometer for electron probe microanalysis (JEOL, Japan; Oxford Instruments, Great Britain).

The completeness of the incorporation of chitosan into polyelectrolyte complexes was determined by the method of IR spectroscopy of the supernatant carried out on an IR Fourier spectrometer with an ATR attachment and additional equipment Nicolet 380 (Thermo Fisher Scientific Inc., USA) [5].

The capacity of chitosan-pectin microparticles for doxorubicin was determined by measuring the residual amount of doxorubicin in the supernatant obtained after centrifugation of the suspension. The concentration of doxorubicin was established by determining the optical density of the sample at a wavelength of 475 nm [6].

RESULTS AND DISCUSSION

The main criteria by which the primary assessment of the obtained chitosan-pectin microparticles was carried out were their size (the permitted size of the preparation for oral administration is up to 1000 microns) [7], the zetta-potential, which characterizes their tendency to sticking together (sticking threshold | 27-29 | mV), as well as the completeness of binding of chitosan to microparticles.

At the first stage, it was necessary to determine the optimal concentration of the initial solution of the structure-forming polysaccharide (pectin) for the synthesis of microparticles. For this purpose, a series of chitosan-pectin microcapsules were prepared by varying the initial concentration of the pectin solution at constant concentration values of solutions of calcium chloride (18 mM) and chitosan (0.07% by weight).

The size of the obtained microparticles and their zeta-potential were measured. The measurement results are presented in Table 1.

Table 1. *Characteristics of chitosan-pectin microparticles synthesized using solutions of pectin with various concentrations at constant concentrations of solutions of chitosan (0.07% by weight) and calcium chloride (18 mM)*

Pectin concentration,% mass	Microparticle diameter, nm	Zetta potential of microparticles, mV
0.042	500-600	-(33±2)
0.085	500-600	-(39±3)
0.093	600-700	-(32±2)
0.102	800-900	-(29±1)
0.111	1000-1100	-(22±1)

From the presented data, it can be seen that the maximum permissible sizes for oral administration of microparticles are reached at a concentration of a pectin solution of 0.102% by weight, however, these microparticles have a zetta potential close to the sticking threshold. Proceeding from this, the optimal concentration of the solution at which the synthesized polysaccharide microparticles have the

maximum allowable size and do not have a tendency to stick together was taken equal to 0.093%.

The next step was to determine the optimal concentration of the binder solution (calcium chloride) for the synthesis of microparticles. For this purpose, samples of chitosan-pectin microcapsules were prepared by varying the initial concentration of calcium chloride solution at constant concentration values of solutions of pectin (0.093 wt%) and chitosan (0.07 wt%). The size and zetta potential of the synthesized microparticles were measured. The measurement results are presented in Table 2.

The presented data show that an increase in the concentration of the initial solution of calcium chloride leads to an increase in the zetta potential of chitosan-pectin microparticles, that is, their tendency to stick together decreases. All synthesized microparticles have an acceptable range of zetta-potential values. The maximum size of microparticles is reached at solution concentrations of 20 and 22 mM. An increase in the concentration of the binder leads to a decrease in their size. It can be assumed that this is due to the fact that only a small part of chitosan was able to bind to a complex with microparticles.

Table 2. *Characteristics of chitosan-pectin microparticles synthesized using solutions of calcium chloride with various concentrations at constant concentrations of solutions of pectin (0.093% by weight) and chitosan (0.07% by weight)*

Concentration of $CaCl_2$, mM	Diameter of microparticles, nm	Zetta-potential of microparticles, mV
18	600-700	-(32±2)
20	700-800	-(34±3)
22	700-800	-(35±3)
24	500-600	-(36±3)
26	500-600	-(37±3)

The completeness of the inclusion of chitosan in the polyelectrolyte complex has already been mentioned as one of the key criteria for evaluating the synthesized microparticles. To determine it, the IR spectra of the supernatant liquid obtained after the separation of microparticles were recorded.

As a control, the IR spectrum of a solution containing: 25 ml of a 0.07% chitosan solution, 117.5 ml of a CH_3COOH solution (pH 4.3) and 7.5 ml of water was taken. The results are shown in Fig. 1-3.

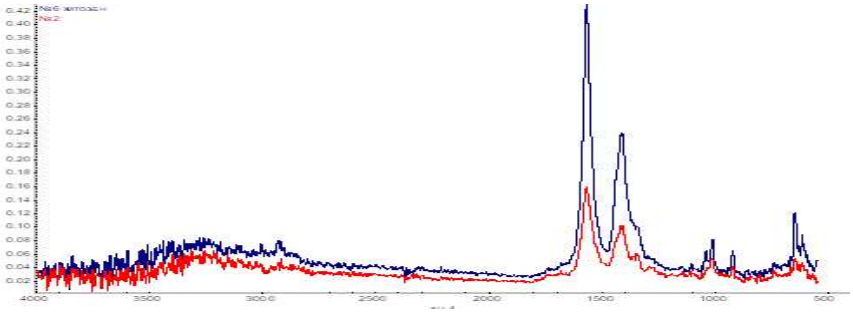

Fig. 1. *Spectrum of the supernatant liquid obtained after the separation of microparticles, in comparison with the chitosan solution with the initial concentration; concentration of solutions for synthesis: pectin 0.093% by weight, chitosan 0.07% by weight, $CaCl_2$ 20 mM*

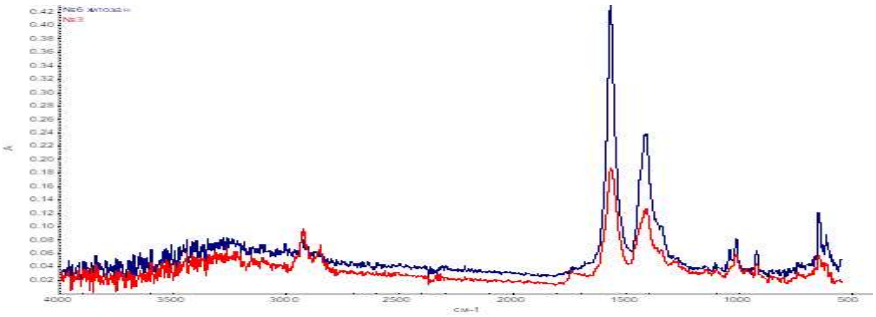

Fig. 2. *Spectrum of the supernatant liquid obtained after the separation of microparticles in comparison with the chitosan solution with the initial concentration; concentration of solutions for synthesis: concentration of solutions for synthesis: pectin 0.093% by weight, chitosan 0.07% by weight, $CaCl_2$ 22 mM*

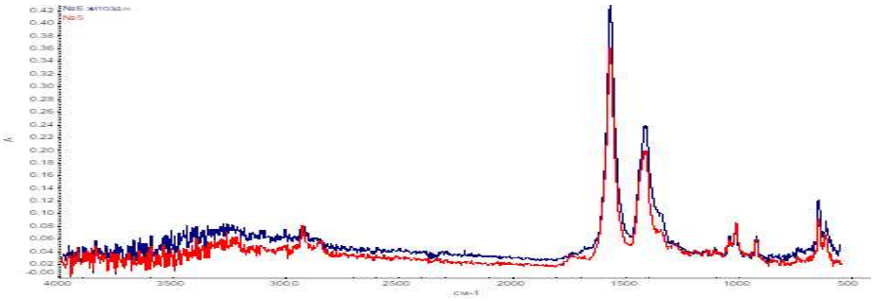

Fig. 3. *Spectrum of the supernatant liquid obtained after the separation of microparticles in comparison with the chitosan solution with the initial concentration; concentration of solutions for synthesis: pectin 0.093% by weight, chitosan 0.07% by weight, $CaCl_2$ 26 mM*

From the data obtained, it can be seen that the most complete binding of chitosan to microparticles is provided at a concentration of the initial calcium chloride solution of 20 mM. The spectrum taken from the supernatant obtained after the separation of microparticles synthesized using a 26 mM calcium chloride solution shows that chitosan, in fact, did not bind to microparticles and almost all remained in the supernatant.

Thus, according to the above criteria, the following initial solutions are optimal conditions for the synthesis of microparticles: 117.5 ml of a 0.093% mass pectin solution, 25 ml of a 0.07% mass chitosan solution and 7.5 ml of a 20 mM calcium chloride solution.

The next stage of the work is to determine the effectiveness of using these microparticles for the inclusion of therapeutic substances in them using doxorubicin as an example. First of all, their capacity for this connection was assessed.

To determine the capacity of microcapsules for doxorubicin when preparing a suspension of microcapsules, the studied antibiotic was added to the pectin solution, varying its concentration in the range of 0.021 - 0.13 mg/ml.

The concentration of the antibiotic not included in the microparticles in the supernatant was monitored by measuring the optical density at 475 nm. Separately, the background was measured - the supernatant obtained after the deposition of unloaded microparticles. The results on the capacity of microparticles for doxorubicin and the degree of its sorption are shown in Fig. 4-5.

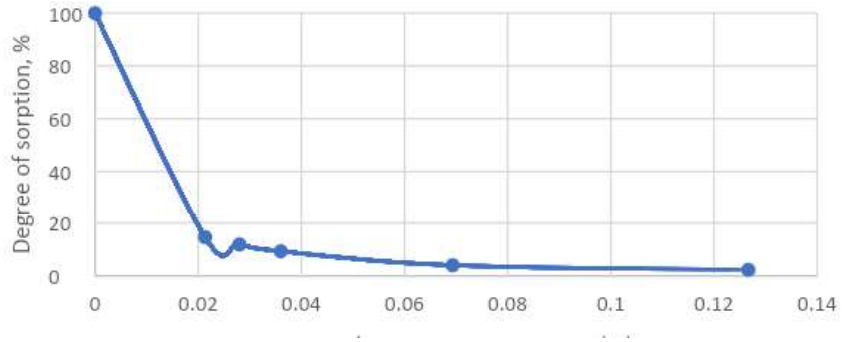

Fig. 4. *Dependence of the degree of sorption of doxorubicin into chitosan-pectin microparticles (synthesized using a 20 mM $CaCl_2$ solution) on its concentration in suspension*

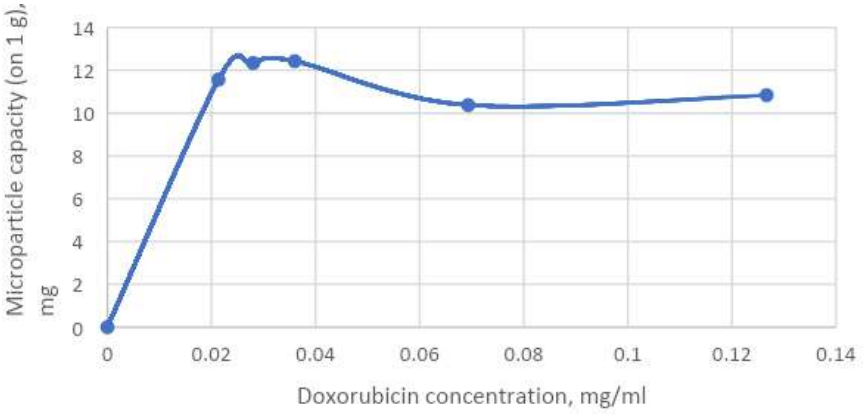

Fig. 5. *Dependence of the amount of doxorubicin included in chitosan-pectin microparticles (synthesized using a 20 mM $CaCl_2$ solution) on its concentration in suspension*

The graphs show that microparticles synthesized using a 20 mM calcium chloride solution have a low capacity for doxorubicin (12.34 mg doxorubicin / g microparticles) and a low degree of sorption of this antibiotic. Therefore, they are ineffective for the inclusion of low molecular weight therapeutic drugs.

For comparison, the capacity of chitosan-pectin microparticles synthesized using a 0.093 wt% pectin solution, a 0.07 wt% chitosan solution and a 22 mM calcium chloride solution was determined. The concentration of doxorubicin in the initial pectin solution was varied in the range of 0.0133 - 0.133 mg/ml. The results on the capacity of microparticles and the degree of sorption of doxorubicin in them are shown in Fig. 6-7.

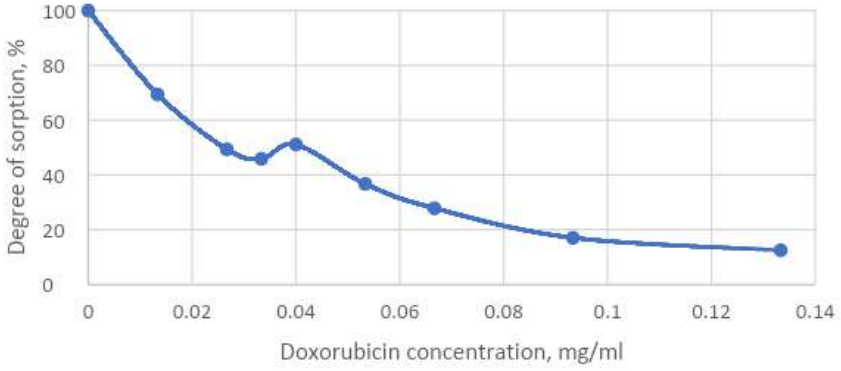

Fig. 6. *Dependence of the degree of sorption of doxorubicin into chitosan-pectin microparticles (synthesized using a 20 mM $CaCl_2$ solution) on its concentration in suspension*

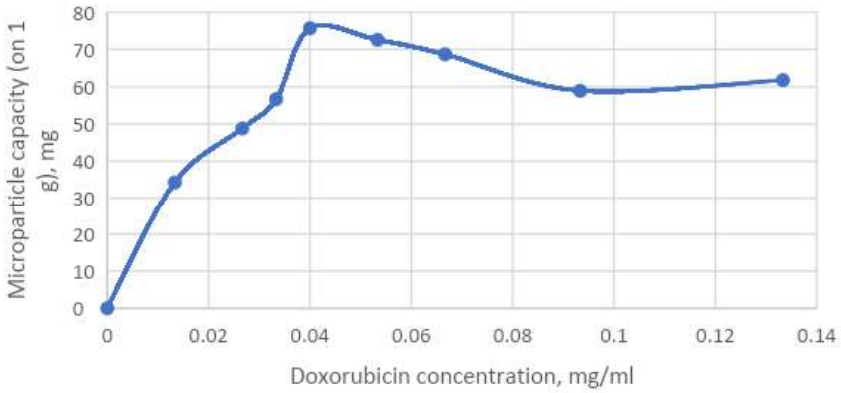

Fig. 7. *Dependence of the amount of doxorubicin included in chitosan-pectin microparticles (synthesized using a 20 mM $CaCl_2$ solution) on its concentration in suspension*

From the obtained dependences it can be seen that these microparticles, in comparison with the previous ones, are able to absorb a larger amount of doxorubicin (the maximum degree of sorption reached 69.4%) and have a higher capacity than the previous ones by 6.4 times (the degree of sorption reaches 77.28 mg of doxorubicin / g microparticles).

These results confirm the feasibility of using these chitosan-pectin microparticles for the inclusion of low-molecular-weight therapeutic drugs. In this regard, the following initial solutions are optimal conditions for the synthesis of microparticles: 117.5 ml of a 0.093% mass pectin solution, 25 ml of a 0.07% mass chitosan solution and 7.5 ml of a 22 mM calcium chloride solution.

CONCLUSION

Received chitosan-pectin microparticles, the diameter of which was 700-800 nm. The zetta potential of the microparticles was - (35 ± 3) mV, which indicates the absence of adhesion tendencies in microparticles. The spectrum of the supernatant obtained after the separation of microparticles confirms the inclusion of most of the chitosan in the polyelectrolyte complex.

The optimal ratio of the concentrations of structural components in the initial solutions for the synthesis of microparticles was selected: pectin solution - 0.093% by weight; chitosan solution - 0.07% by weight; CaCl2 solution - 22 mM. It was found that the initially established criteria for evaluating the optimal conditions for the synthesis of microparticles do not guarantee their effectiveness and high capacity for therapeutic drugs.

The optimal concentration of doxorubicin in the suspension (0.04 mg/ml), at which the maximum incorporation of this antibiotic into chitosan-pectin microparticles is achieved (degree of sorption is 52.11%), has been determined. Based on the data that the concentration of microparticles in the suspension is 6

mg/ml, and their dry weight is 4.5%, the doxorubicin capacity of microparticles is 77.28 mg/g of dry microparticles.

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