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Encapsulation of Insulin in Biodegradable Polymers

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Abstract

Encapsulation of insulin into alginate particles was carried out by the method of ionotropic gelation. To protect against the acidic, alkaline environment of the gastrointestinal tract, alginate particles were coated with gelatin. The optimal concentration of the solution of the crosslinking agent – $CaCl_2$ was determined during the optimization of the particle preparation method. The mechanism of interaction between alginate and gelatin was investigated using FTIR spectroscopy, FTIR spectra data confirm the formation of a polyelectrolyte complex between alginate an-d gelatin. The roughness and morphology of samples were determined by atomic force microscopy. The swelling of particles under simulated pH conditions of various parts of the human gastrointestinal tract was studied. The release of insulin from the particles was evaluated using UV spectroscopy, at pH 6.86; 9.18 the release of insulin reached 50%; 83% relatively.

1. Introduction

Diabetes is considered one of the most dangerous diseases in terms of the rate of spread and the side effects it has on the body. According to the World Health Organization, about three million people a year in the world die due to diabetes [1]. The number of diabetic patients worldwide may exceed 643 million by 2030 [2]. An effective and frequently used drug in diabetes is insulin [3]. Approximately 20–30% of diabetic patients take insulin injections daily to maintain normal glucose levels [4]. There are some difficulties when using insulin in the form of injections: physiological stress, the need for injections, deviation of insulin concentration from the physiologically necessary norm, and infection [5]. In the initial stage of diabetes mellitus, one of the main obstacles to the use of insulin is associated with the inconvenience of the medication being administered, as well as the inability of patients to take it. These barriers may be removed when the oral form of insulin becomes available [6].

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Oral administration of peptide and protein drugs requires their protection from dissolution in the gastrointestinal tract [7]. To protect insulin from changes in the acidic environment (denaturation/degradation), pH-sensitive polymers were used as a convenient carrier [8]. Several studies have been conducted, such as the use of liposomes, microemulsions, microspheres, and nanoparticles to eliminate gastrointestinal barriers during oral insulin delivery [9]. In the study [10], lipid nanoparticles were prepared for the oral delivery of insulin and insulin analogs. Ex vivo and in vivo studies were performed with fluorescently labeled peptides. The results of in vivo studies showed the absolute bioavailability of the drug substance. According to ex vivo results, penetration has reached up to 30% dose/ml. The possible use of magnetosomes in the treatment of diabetes was studied in the work [11]. Over a long period, magnetosomes have shown efficacy in controlling blood glucose levels in diabetic rats and protecting insulin from acidic environments. For the treatment of insulin resistance in the study [12] a system of self-assembling micelles from a complex of polygalacturonic and oleanolic acids was developed. The complex

is of plant origin, overcomes gastrointestinal barriers, and maintains the concentration of the medicinal substance and the level of glucose in the blood. It is a simple, effective strategy for insulin-resistant treatment. Zwitterionic micelles with an ultra-low micelle concentration were used for oral insulin delivery in the study [13]. The micellar platform provided efficient epithelial absorption and penetration of the drug through the mucus. The bioavailability of insulin has reached >40%.

Microsized devices such as micro containers were used for the oral delivery of insulin. When loading peptides into micro-sized devices, the absorption area is limited due to unidirectional release and thereby absorption is enhanced. Despite the absence of cytotoxicity when using micro containers, there was a deterioration in the condition of cells when evaluating cell monolayers [14].

Recently, the use of natural polymers, especially polysaccharides, in the transportation of medicines has been widely studied [15]. Natural polymer hydrogels are biologically safe, do not require organic solutions, and are inert to drugs [16]. Polysaccharides such as alginate, chitosan, and pectin due to their long and persistent therapeutic effect can be used to transport many drugs Chitosan and alginate have mucoadhesive properties due to their high charge density, thus they can extend the residence time of the capsule in the release zone. This property explains another reason for their use in the transportation of medicines [17, 18].

The ability of alginate to constrict at low pH (gastric environment) may be useful in the development of an oral drug delivery system, as the release of encapsulated drugs is significantly reduced in low pH solutions [19].

Chitosan is a cationic polysaccharide. It is considered a safe and effective enhancer of intestinal absorption of therapeutic macromolecules [20]. Chitosan is also known as an antimicrobial agent against *Escherichia coli* bacteria [21]. For the treatment of diabetes, insulin–loaded nanoparticles were prepared by self-gelation based on mucin-chitosan complexes. The results of toxicity studies showed no signs of toxicity regarding the viability of the liver and cells, presenting a safe, biocompatible composition of nanoparticles. The efficiency of insulin encapsulation reached 89–93%, and the release of insulin was observed for 8 h. The results of in vivo and in vitro studies showed a pronounced hypoglycemic effect in diabetic rats [22].

In the work of S. Sajeesh and C. Vauthier et al. hydrogel microparticles based on thiol-functionalized polymethacrylic acid, a copolymer of polyethylene glycol and chitosan were used to develop an oral insulin delivery system. The results of insulin encapsulation efficiency and swelling of thiolated hydrogel microparticles showed lower values compared to unmodified particles. In turn, the lower swelling of thiolated particles than the swelling of non-thiolated particles may affect the efficiency of drug administration into microparticles [23]. In the study [24], thiolated chitosan was synthesized and nanoparticles with sodium alginate were prepared for the delivery of eye preparations. The results of the study indicate that due to the higher positive charge and mucoadhesive properties, the stability and effectiveness of thiolated chitosan-alginate nanoparticles are higher than chitosan particles. It should be emphasized that thiolated chitosan derivatives are synthesized using some toxic reagents, such as mercapto carboxylic acids and carbodiimide. In the study [25], the synthesis of thiolated chitosan nanoparticles includes two reaction stages. The first stage proceeds with the formation of mercapto carboxylic acid ester, which further interacts with the amino groups of chitosan.

Solubility of chitosan only at acidic pH (pH <6), and mucoadhesiveness only at limited pH values are problems of chitosan in drug delivery through mucous membranes [26]. Since many proteins and peptide-based drugs are unstable at a low pH value, decreasing the pH of chitosan carriers limits their use in drug delivery [27]. The high solubility of chitosan in gastric fluids leads to a rapid release of the drug in the stomach, limiting chitosan in targeting drugs to the intestine. The solubility of chitosan in an acidic environment can be reduced ed by the chemical crosslinking of microspheres with aldehydes, but it is ineffective in preventing the release of encapsulated drugs [28].

Biopolymers are a good substitute for synthetic polymers, many of the side effects associated with synthetic polymers can be improved by using natural polymers. The development of multifunctional release systems based on biopolymers can support the functionality of biological molecules. An advantage of biopolymer composites for medical applications is the similarity between the human body and polymer composites [29]. Multiple emulsions stabilized with albumin, together with a natural permeability enhancer – piperine can be a potentially significant system for the oral administration of insulin [30]. Insulin was encapsulated in the internal dispersed phase of the emulsion, as it has a hydrophilic nature. Albumin can be used as a

substrate for degrading enzymes and will improve the hypoglycemic effect. The lipophilic part of the emulsifier works as a protective layer against proteolytic enzymes.

In the study [31], composites of sodium alginate and gelatin were used to obtain micron-microparticles with the dispersion of the microbial fungicide Bacillus subtilis SL-13 by the emulsification/internal gelation method. With the addition of gelatin to sodium alginate, the swelling degree, biodegradation, and the size of microcapsules increased. Gelatin was used to encapsulate curcumin and stabilize the silver nanoparticles to form the therapeutic composite GelCurAg. The results of the research showed that the composite synthesized from a 1% gelatin solution has strong bactericidal and antioxidant properties [32]. In the study [33], the aromatic powder was encapsulated in multi-core capsules using gelatin, and gum arabic. The results of the study showed that gelatin binds to the aroma of pandanus better than gum arabic. In general, encapsulation with gelatin significantly improves the stability of the aromatic powder.

Gelatin is a soluble protein compound obtained from collagen by hydrolysis. Due to its biocompatibility and biodegradability in the physiological environment, gelatin is widely used in medicine and pharmaceuticals as a gel-forming agent [34, 35]. Gelatin shows good biocompatibility, and matrix metalloproteinase degradation in tissue engineering [36]. Gelatin peptide sequences facilitate cell adhesion and enzymatic degradation. Safety in medicine as a blood volume expander, lack of immunogenicity, and low cost allows the use of gelatin for cell delivery [37]. The presence of arginyl glycyl aspartic acid (RGD sequence) in the gelatin structure distinguishes the biological characteristic of gelatin from other synthetic polymers since these amino acid sequences can recognize cells and modulate cell adhesion [38]. Encapsulation of drugs in gelatin increases stability, ensures the delivery of the drug to certain places, and controls its release [39]. Gelatin is also used to improve the properties of alginate for controlled drug release. The addition of gelatin to the alginate film in the study [40] contributed to the homogeneous dispersion of the drug.

This study aims to obtain particles for creating an oral form of insulin based on widely distributed, biodegradable polymers such as alginate and gelatin. The composition of the particle should protect insulin from the aggressive environment of the stomach and ensure it enters the intestines. For this purpose, the effect of gelatin on the stability of alginate particles in acidic and alkaline environments was investigated. To evaluate the behavior of the obtained particles in various parts of the gastrointestinal tract, the degree of swelling of the particles was investigated. The release of insulin from particles was evaluated in simulated gastrointestinal solutions to control the bioavailability of the drug. The mechanism of interaction between alginate and gelatin was investigated using FTIR spectroscopy. The roughness and morphology of the samples were determined by atomic force microscopy to confirm the appropriate morphology of the particles for the release of insulin.

2. Experimental

2.1. Materials

Sodium alginate (19-40 kDa, Sisco Research Laboratories, Turkey), gelatin (GOST 11293-89, Russia), calcium chloride (Sinopharm Chemical Reagent Co., Ltd, China), insulin-isophane (human genetically engineered, Novo Nordisk A/S, Denmark).

2.2. Preparation of sodium alginate particles by ionotropic gelation

To obtain alginate particles by ionotropic gelation, 1% aqueous solution of sodium alginate is prepared. The sodium alginate solution is dropped from a height of about 15 cm through a TopPette pipette (DLAB Scientific Co., Ltd., China) into the crosslinker solution. $CaCl_2$ solution was used as a crosslinking agent. To optimize the method of particles preparation, various concentrations (0.5%; 1.0%; 1.5%; 2.0%) of the cross-linking agent $CaCl_2$ were prepared and studied. The sodium alginate particles are kept in the crosslinker solution for 30 min, after 30 min of polymerization, the formed particles are collected by filtration and washed with deionized water.

2.3. Swelling under simulated gastrointestinal conditions

The kinetics of particle swelling was determined by the rate of increase in their weight over time. The particles were placed in simulated gastric fluids and the weight of the particles was measured every 30 min for 180 min. Simulated gastric fluids and their pH values used during the experiment are shown in Table 1.

Table 1
Simulated gastric fluids and their pH values

#	Simulated gastric fluids	pH value
1	0.1 N HCl solution	1.0
2	Saturated solution of potassium hydrogen phthalate at 25 °C	4.01
3	Phosphate buffer	6.86
4	0.01 mol/kg of sodium tetraborate solution	9.18

The degree of swelling was calculated by the equation:

$$K = \frac{m_t}{m_0} \quad (1)$$

where m_t – is the weight of particles at time t, mg; m_0 – is the weight of particles at time 0, mg.

2.4. Coating alginate particles with gelatin

To cover the particles with a protective membrane of gelatin, 8% aqueous solution of gelatin is prepared. The obtained alginate particles are added to the gelatin solution and kept for 30 min. After 30 min, the particles are removed from the gelatin solution by filtration and washed with deionized water.

2.5. Immobilization of insulin in gelatin-coated alginate particles

To obtain particles with insulin, 50 ml of 1% sodium alginate solution containing 0.13 mg/ml insulin was added drop by drop into 1.5% solution of $CaCl_2$. After 30 min, the formed particles were collected by filtration, washed with deionized water, and covered with a gelatin membrane.

2.6. FTIR spectroscopy

IR spectra of samples (sodium alginate, gelatin, insulin) were recorded on the Cary 660 Agilent IR Fourier spectrometer (Agilent Technologies, USA), spectral range 7900-375 cm^{-1}, signal-to-noise ratio 10000:1

2.7. Atomic force microscopy

AFM images were taken using the atomic force microscope Ntegra Therma (NT-MDT, Russia) in semi-contact mode.

2.8. Release of insulin from particles

Insulin release was assessed using a UV-spectrophotometer UV-7504 (Shanghai Hansom Technology & Sales Limited, China), range 200–1000 nm, at a wavelength of 280 nm.

Particles loaded with insulin were placed in 20 ml buffers with different pH values (1.0; 4.01; 6.86; 9.18), simulating the conditions of various parts of the gastrointestinal tract, and incubated at 37 °C with stirring (100 rpm). Aliquots of the buffer solution were taken at a certain time and the concentration of insulin was analyzed. The volume of aliquots was compensated by the same volume of fresh buffer.

3. Results and discussion

The most important property of alginate is the ability to form gels when interacting with divalent cations such as Ca^{2+}. Alginate particles are obtained by adding an alginate solution drop by drop into a crosslinking agent solution [41]. Ionic crosslinking of alginate with counterions leads to the formation of alginate beads cured with Ca^{2+} ions [42].

When taken orally, alginate is non-toxic and has a protective effect on the mucous membranes of the upper gastrointestinal tract. Alginate-based particles can be used for a controlled drug release system since dried alginate particles have the property of re-swelling [43]. Therefore, sodium alginate was chosen as a matrix for the immobilization of insulin.

To optimize the method of obtaining particles by ionotropic gelation, solutions of various concentrations of the crosslinking agent $CaCl_2$ were used (0.5%; 1.0 %; 1.5%; 2.0%). The effect of Ca^{2+} ions on sodium alginate particles was significant. With an increase in the concentration of Ca^{2+} ions, particles began to form stable aggregates. Particles with high stability were obtained using solutions of 1.5%; 2% cross-linking agent $CaCl_2$.

With homogeneous dispersion of the drug into a polymer matrix, the final product can be in the form of swelling microspheres or conventional tablets. The release of the drug from such systems occurs by diffusion through swelling and dissolution of the matrix [44]. Since alginate is a hydrophilic polymer, calcium alginate gel swells and dissolves in an aqueous medium [45].

The degree of swelling of alginate particles obtained by ionotropic gelation was studied to observe their changes in the gastrointestinal tract.

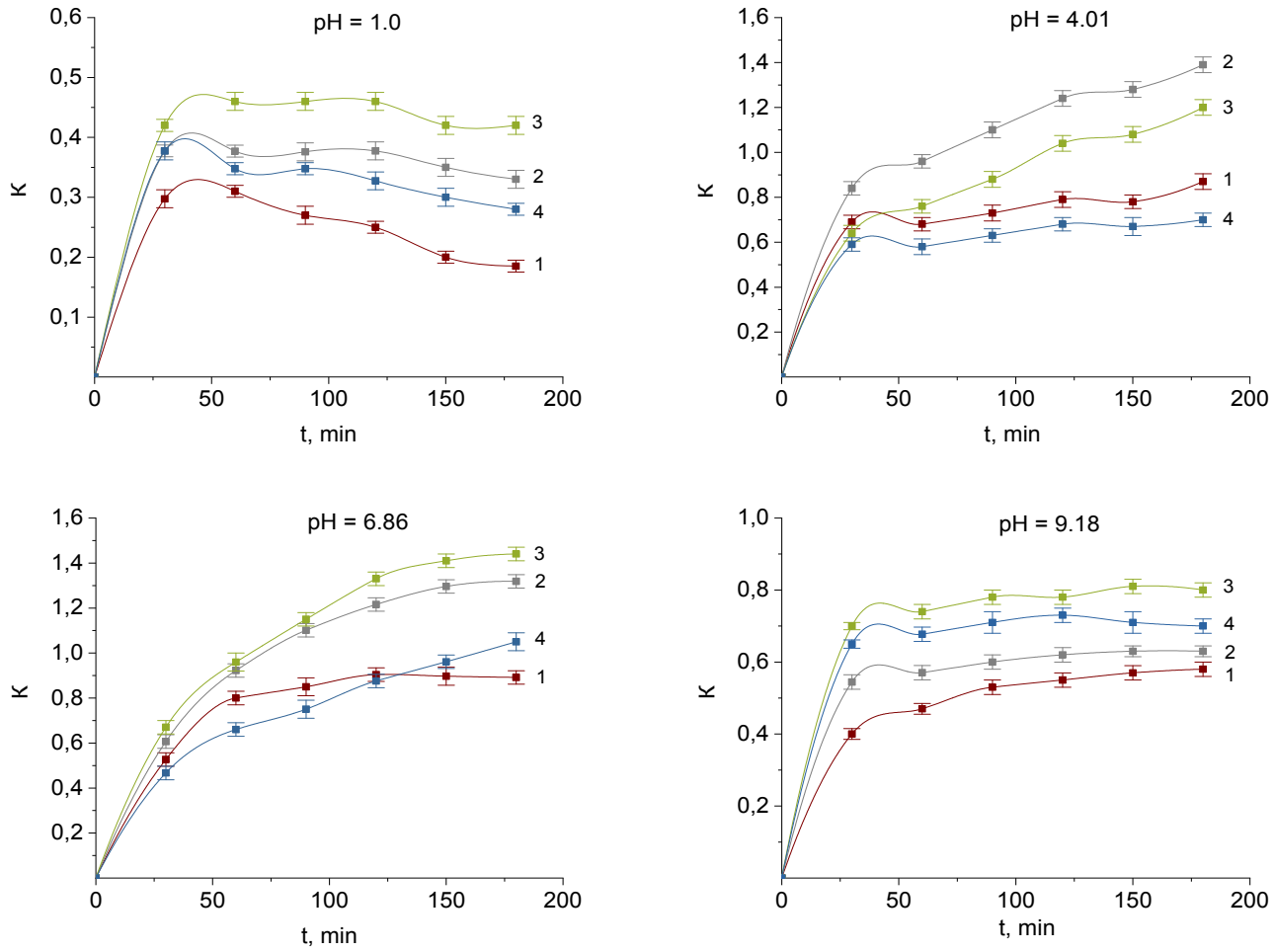

Fig. 1. Swelling of alginate particles under simulated gastrointestinal conditions: 1 – $CaCl_2$ (0.5 %); 2 – $CaCl_2$ (1%); 3 – $CaCl_2$ (1.5%); 4 – $CaCl_2$ (2%).

Various concentrations (0.5%; 1.0%; 1.5%; 2.0%) of the crosslinking agent $CaCl_2$ were taken and their effect on the degree of swelling of the alginate particle was evaluated.

The degree of swelling (K) of particles based on sodium alginate at pH values (1.0; 4.01; 6.86; 9.18) of the simulated gastrointestinal tract is shown in Fig. 1.

The data in Fig. 1 show that at pH=1.0, the alginate particles swelled for 60 min, then the degree of swelling decreased, indicating the partial dissolution of the particles. When using a 1.5% solution of $CaCl_2$, an increase in swelling is observed within 120 min, then a decrease in the degree of swelling occurs, which is associated with the onset of dissolution of the particles.

When the particles were exposed to solutions at pH=4.01 and pH=6.86, the alginate particles continued to swell for 180 minutes at all $CaCl_2$ concentrations, but the degree of swelling of the alginate particles when using 1.0%; 1.5% $CaCl_2$ was 0.33–0.69 conventional units higher at pH=4.01; at pH=6.86, the degree of swelling showed 0.26–0.55 conventional units higher than that of alginate particles when using 0.5%; 2.0% $CaCl_2$ solution.

Compared with pH=4.01 and pH=6.86, particles at pH=9.18 had a low degree of swelling, 0.29–0.76 conventional units lower. This confirms that the particles should begin to dissolve when they enter the small intestine.

Since, when alginate particles were obtained, the degree of swelling of the particles using 1.5% $CaCl_2$ solution was mainly higher compared to other concentrations of the crosslinking solution, 1.5% $CaCl_2$ was chosen as the optimal crosslinking solution for obtaining alginate particles.

Polyelectrolyte gels can swell intensely in an aqueous environment [46] and their ability to reabsorb water is used in pharmaceuticals and cosmetics [47]. Cross-linked polymer gels are more swellable than neutral non-ionic polymer gels. They are also subjected to intermittent volumetric phase transi-

tions this ability is of particular interest in theoretical and applied research. Such phase transitions in polyelectrolyte gels are used to transport drugs and genes into the cells of living organisms [48].

In many works, chitosan has been studied for biomedical applications [7, 15, 49–56]. Chitosan is obtained by the deacetylation of chitin in an alkaline medium, therefore chitosan is a semi-synthetic natural polysaccharide that is soluble in dilute acetic acid [57]. Although chitosan is approved for external use as one of the components of wound coatings and is known as a non-toxic polymer [58], in a study [59] chitosans with different characteristics showed low cytotoxicity against CCRF cells – CEM (leukemic lymphoblasts) and L132 (human lung epithelial cell line).

One of the disadvantages of chitosan is its low solubility at pH 5.5–7.4. Chemical modification of chitosan aimed at increasing solubility may affect the toxicity of the molecule, which will be an obstacle to its use in medicine. Therefore, natural polymers such as gelatin, cellulose, agar-agar, pectin, starch, and others are considered promising for use in biomedicine, due to their ability to biodegradation and biocompatibility.

To increase the stability of alginate particles in acidic and alkaline environments, the particles were covered with a gelatin membrane (2%; 5%; 8% gelatin solutions) and the degree of their swelling was studied (Fig. 2).

According to the results of the study, the degree of swelling of gelatin (8%) – coated alginate particles was higher by 0.52 units at pH=1.0; 0.74 units at pH=4.01; 1.66 units at pH=6.86; 1.38 units at pH=9.18 than the degree of swelling of alginate particles. At pH=1.0, the swelling of the particles was less, the reason for this may be that alginate has an acid-gel character ($pK_a \approx 3.5$), and the strength of the acid gel increases with increasing proton concentration [60].

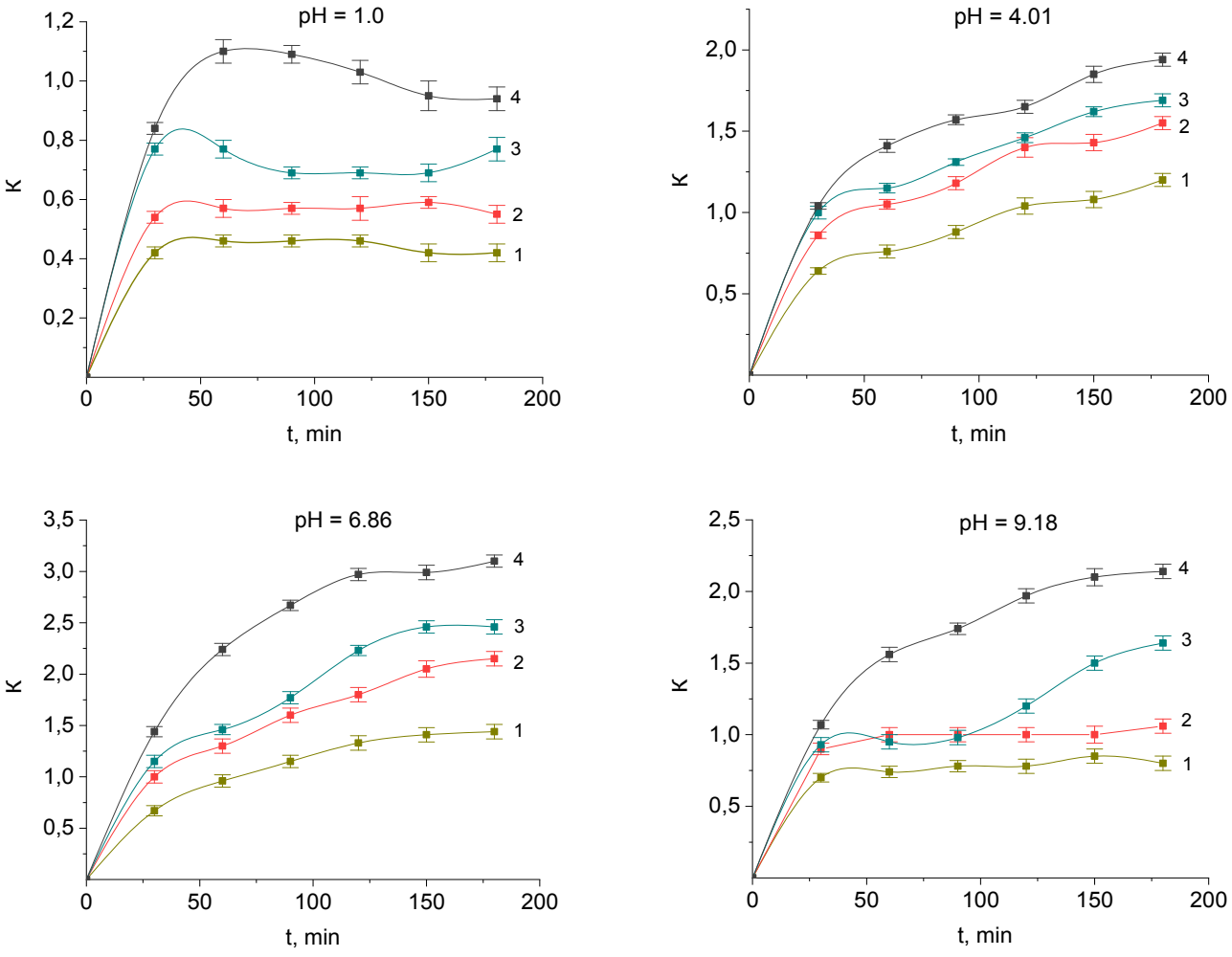

Fig. 2. Swelling of gelatin-coated alginate particles under simulated gastrointestinal conditions: 1 – alginate (1%); 2 – alginate-gelatin (2%); 3 – alginate-gelatin (5%); 4 – alginate-gelatin (8%).

The particles continued to swell for 180 min at pH=4.01 and pH=6.86. At pH=9.18, the particles had a low degree of swelling compared to pH=4.01 and pH=6.86 by 0.05–1.84 conventional units lower. The decrease in the degree of swelling of the gel alginate-gelatin in an acidic medium is due to the suppression of the dissociation of carboxyl groups of alginate macromolecules in the presence of H^+ ions.

The decrease in the swelling coefficient in the area of pH=9.18 is associated with the suppression of dissociation –COONa groups of sodium alginate in the presence of Na^+ ions of sodium hydroxide added to the medium to increase pH.

These data may be confirmation that the particles will begin to dissolve when they enter the small intestine. The reason for the dissolution of particles may be the competition of monovalent salts in gastric juice with calcium binding to carbon groups inside the particle [61]. The particles must be stable, and insoluble in an acidic environment. Because this is a guarantee that they will not release immobilized insulin in the acidic environment of the stomach at an early stage. Consequently, gelatin-coating reduces the solubility of alginate particles in an acidic environment and increases resistance to the aggressive environment of the gastrointestinal tract.

It is important to understand the release characteristics of drugs to select the optimal dose of the active substance and ensure the safety and efficacy of these drugs.

The release of the drug can be achieved due to degradation and swelling of the polymer or diffusion of the drug. Degradation and swelling of the polymer are the main mechanisms of drug delivery based on the polymer matrix [62].

Particles during passage through the gastrointestinal tract are first exposed to a low pH value in the stomach, then when they enter, the intestine is exposed to a higher pH value. The release of insulin has been studied to determine the bioavailability of insulin in various parts of the gastrointestinal tract.

The release of insulin was determined by the rate of change in its concentration in the solution:

$$K = \frac{C_t}{C_\infty} \times 100\% \qquad (2)$$

where C_t – is the concentration of insulin released at time t; C_∞ – the maximum possible concentration of insulin.

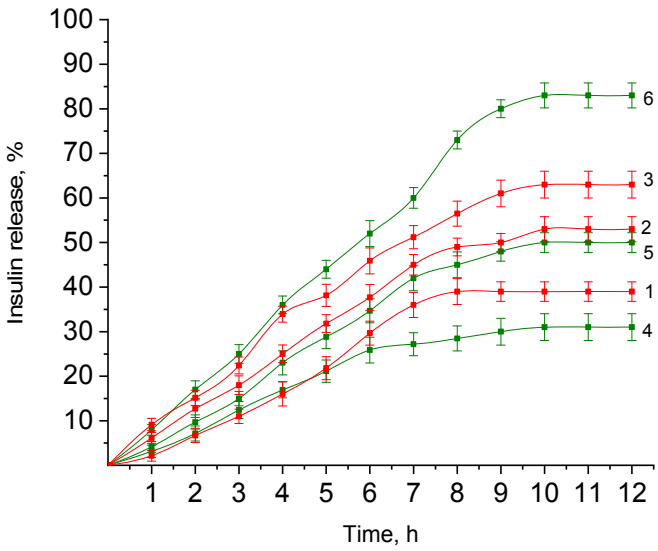

Fig. 3. Insulin release from alginate and gelatin-coated alginate particles under simulated gastrointestinal conditions: 1 – pH=4.01; 2 – pH=6.86; 3 – pH=9.18 for alginate particles; 4 – pH=4.01; 5 – pH=6.86; 6 – pH=9.18 for gelatin-coated alginate particles.

The release of insulin at pH=6.86 reached 53% for alginate particles, 50% for alginate-chitosan particles, at pH=9.18 it reached 63% for alginate particles, 83% for alginate-chitosan particles (Fig. 3). In a subacid medium pH=4.01, the insulin release was 39% for alginate particles, 31% for alginate-chitosan particles. No insulin release was observed at pH=1.0. The increase in insulin release in an alkaline medium for alginate-chitosan particles can be explained by the assumption that at pH>6 the polyelectrolyte complex of alginate and gelatin is destabilized by deprotonation of gelatin and thereby increases the release of insulin. The particles must be stable at pH=1.0, and pH=4.01 without dissolving in the stomach, releasing insulin early. Compared to alginate-gelatin particles, alginate particles cause a sudden release of insulin into an acidic environment before entering the intestinal solution. The gelatin-coated alginate particles produced more controlled insulin release during exposure to a pH 9.18 solution compared to alginate particles. The reason for this may be the reduction in the dissolution time of alginate when coated with gelatin.

Polymers with oppositely charged groups form polyelectrolyte complexes upon interaction, which are the basis for drug delivery [63, 64] proteins [65] and genes [66]. Encapsulation of proteins and peptides in polyelectrolyte complexes is protection against their degradation [67]. In this regard, to

increase the stability of alginate particles, gelatin was introduced into their composition. As a result of the electrostatic interaction of the amino groups of gelatin and carboxyl groups of alginate, polyelectrolyte complexes can be formed.

The data obtained during the experiment indicate an increase in the turbidity of solutions in the pH range of 2–7, which indicates the presence of polyelectrolyte complexes that can be formed because of the electrostatic interaction of gelatin amino groups and alginate carboxyl groups.

This, in turn, plays one of the key roles in the preparation of particles since the interaction of alginate and gelatin must occur from the moment of passage through the stomach (pH 2–7).

The FTIR spectra of the initial polymers were studied: alginate, gelatin, and the spectrum of the gelatin-coated alginate particle (Fig. 4).

The spectrum of sodium alginate showed important absorption bands concerning carboxyl functional groups. Asymmetric and symmetric stretching vibrations of carboxylate salt groups (-COONa) were observed at 1603 cm^{-1} and 1412 cm^{-1} [68]. Bands between 1122 and 949 cm^{-1} were attributed to C-O the stretching vibration of the pyranose ring, as well as C-O stretching with contributions from C-C-H and C-O-H deformation [69]. Vibrations at 1086 cm^{-1} and 1030 cm^{-1} can be attributed to glycosidic bonds (C-O-C stretching) which explain its saccharide structure [70].

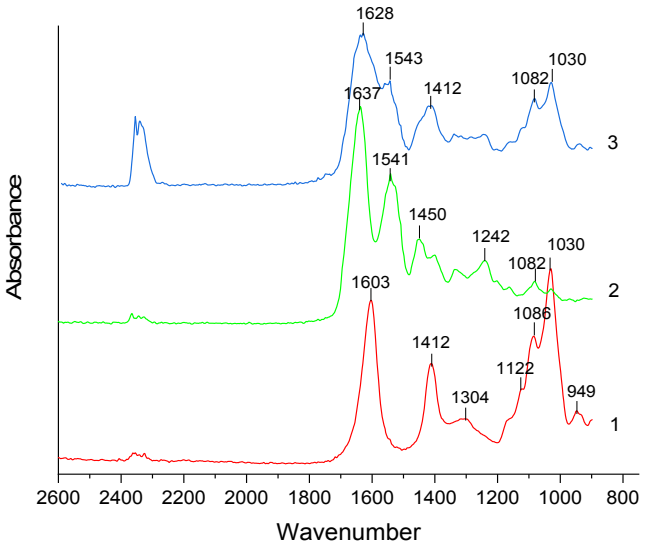

Fig. 4. FTIR spectrum of sodium alginate (1), gelatin (2), gelatin-coated alginate particle (3).

The spectrum of gelatin showed characteristic peaks in three regions: at 1637 cm^{-1} (amide I), 1541–1450 cm^{-1} (amide II), and 1242–1030 cm^{-1} (amide III) [71]. Absorption of amide I occur due to stretching of the carbonyl C=O (peptide) bond. The absorption of amide II occurs due to the bending mode of the N-H bond and the valence vibration of the C-N bond. C-N stretching vibrations combined with N-H planar bending vibrations from C-C and C=O bond stretching at planar bending cause absorption of amide III [72].

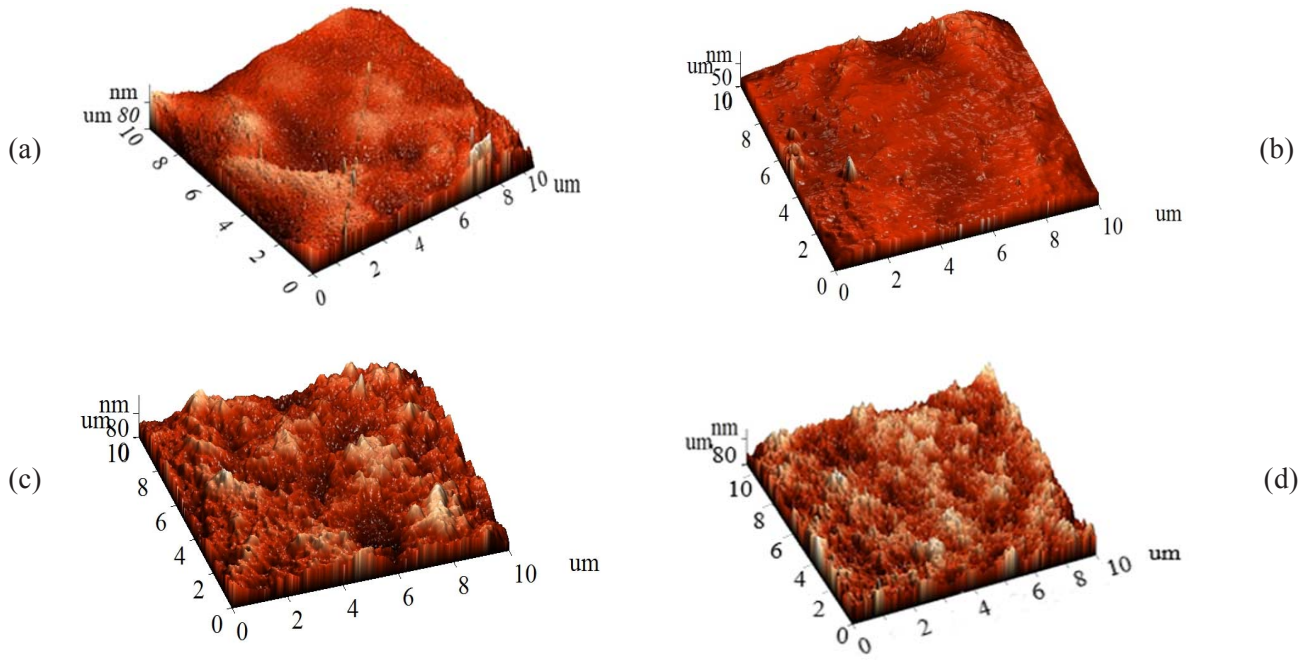

Fig. 5. AFM images of gelatin-coated alginate sample: alginate (a); alginate-gelatin (b); alginate-gelatin pH=6.86 (c); alginate-gelatin pH=9.18 (d).

The FTIR spectrum of the gelatin-coated alginate particle showed vibration at 1082 cm^{-1} and 1030 cm^{-1}, which are attributed to glycosidic bonds in the polysaccharide (C-O-C stretch). At 1412 cm^{-1}, vibrations of carboxylate salt groups (-COONa) were observed, which are characteristic of the spectrum of sodium alginate. Due to the stretching vibration of the C-N bond, absorption of amide II near the 1543 cm^{-1} band was obtained, which was also present in the FTIR spectrum of gelatin. The peak at 1628 cm^{-1} is attributed to the absorption of amide I, which occurs due to the stretching vibration of CO and relates to the spectrum of the protein.

After analyzing the FTIR spectra of alginate, gelatin, and gelatin-coated alginate particle, it can be confirmed that when the alginate particle is coated with gelatin, a polyelectrolyte complex is formed, which is the basis for insulin immobilization.

The release of insulin from the gelatin-coated alginate particle at pH=6.86 and pH=9.18 was clarified in the following study with the determination of the roughness and morphology of the gelatin-coated alginate sample by AFM. There is a difference in the surface morphology of the samples, which appears on the AFM images (Fig. 5).

The sodium alginate sample is smoother than the alginate-gelatin sample. Globules located in the bulk of the sample are visible on the surface. The size of globular particles is about 100 nm. The surface of the gelatin-coated alginate samples at pH=6.86 and pH=9.18 is very rough compared to the initial sample, which indicates the dissolution of the particle. On the AFM image of the gelatin-coated alginate sample (b), the surface roughness is about 100–130 nm, while on the gelatin-coated alginate sample at pH=6.86 (c) the roughness is about 150 nm, on the gelatin-coated alginate sample at pH=9.18 (d) is about 200 nm. On the AFM image of the gelatin-coated alginate sample, globular formations can be observed, which are in the volume of the sample. On the images of samples of gelatin-coated alginate at pH=6.86 and pH=9.18, granules are observed, the sizes of which are from 80–100 nm and above. Porosity (porous structure) is also evident in both samples, but in comparison with pH=6.86, smaller porous formations can be observed at pH=9.18. Porosity increases the sample, which eventually leads to its destruction in the intestinal phase. Determination of the roughness and morphology of the samples by atomic force microscopy confirms the appropriate morphology of the particles for the release of insulin.

4. Conclusions

As a result of the studies, gelatin-coated alginate particles loaded with insulin were obtained by the ionotropic gelation method, and their dependence under simulated gastrointestinal conditions was studied. Particles with high stability were obtained using 1.5%; 2% solutions of cross-linking agent $CaCl_2$. Since, when alginate particles were obtained, the degree of swelling of the particles using 1.5% $CaCl_2$ solution was mainly higher compared to other concentrations of the crosslinking solution, 1.5% $CaCl_2$ was chosen as the optimal crosslinking solution for obtaining alginate particles.

According to the results of the experiment, the degree of swelling of gelatin-coated alginate particles was 0.52; 0.74; 1.66; 1.38 units higher than the degree of swelling of alginate particles. Consequently, gelatin-coating decreases the solubility of alginate particles in an acidic environment.

The mechanism of interaction between alginate and gelatin was investigated using FTIR spectroscopy, and the data of FTIR spectra confirm the formation of a polyelectrolyte complex between alginate and gelatin.

When studying the release of insulin from gelatin-coated alginate particles, the release of insulin reached 50%; 83% at pH values of 6.86; 9.18 respectively.

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