Annotation

Dissertation for the degree of Doctor of Philosophy (PhD)
in the specialty "8D05301 - Chemistry"
Yerlan Gulzhan Yerlankyzy
on the topic: "Immobilization of insulin in polysaccharide gels"

Summary of work. The research work is devoted to obtaining an oral form of insulin by immobilizing insulin in polysaccharide gels; controlling the release of insulin by optimizing the composition and conditions of cross-linking of the polysaccharide matrix.

Relevance of the research topic. Diabetes mellitus is the third most common disease worldwide after heart disease and cancer. Currently, the problem of the diabetes epidemic is rapidly increasing. It is estimated that in 2030, the number of patients with diabetes may increase to 643 million, and in 2045 to 783 million.

The main goal of diabetes therapy is to improve glycemic control through the normalization of glucose levels in the blood. In patients with type 1 diabetes, and in cases where it is impossible to control the amount of glucose in the blood with oral drugs, physical and dietary supplements, the main treatment for type 2 diabetes is the use of insulin.

Insulin is a peptide-based hormone that regulates the absorption of glucose by cells.

Patients with diabetes must constantly monitor their blood insulin levels, which in turn can lead to such undesirable results as hyperinsulinemia and lipoatrophy. Non-compliance with the injectable insulin therapy regimen is often found in patients with diabetes mellitus, the cause of which is often associated with difficulties with injections.

Alternative forms of insulin delivery have been studied, many ideas and methods for their development have been proposed, and despite the availability of drugs that have passed various phases of clinical trials, only the injectable form of insulin is currently used on the market.

However, the natural and preferred way of administering insulin would be the oral form. This is because with oral delivery there is the possibility of controlling insulin secretion compared to other alternative forms, and the oral form is more suitable for patients than injections, or inhalers. It is possible to prevent the hypoglycemic effect by oral delivery of insulin, while insulin is directed directly from the intestine to the liver.

Disadvantages such as the use of organic solutions, surfactants, and harsh encapsulation conditions in such methods of obtaining protein preparations as liposome, microemulsion, emulsion, and production in the form of mixed micelles can lead to a decrease in insulin activity. In this regard, one of the urgent issues is the production of polyelectrolyte structure carriers that retain high insulin activity.

The aim of the dissertation: change the composition of the polysaccharide matrix used for insulin immobilization and to determine the molecular environment favorable for insulin stability using cross-linking agents.

To achieve this goal, the following tasks have been set:

1. Obtaining alginate-based particles by ionotropic gelation for the preparation of an oral form of insulin and coating with gelatin to increase the stability of the particles in the acidic and alkaline environment of the gastrointestinal tract;
2. Development of gelatin compositions to improve their structural and mechanical properties and study their dependence on various pH values of model solutions of the gastrointestinal tract;
3. Development of matrix design for hybrid transport systems and optimization of methods for obtaining matrices for insulin immobilization.
4. Evaluation of the effect of the enzyme on the kinetics of release and the diffusion coefficient of insulin from the hybrid matrix.

Objects of research: alginate and gelatin-based particles, crosslinking agents, insulin, enzyme, L-glutamic and citric acids, chitosan.

Subjects of research: The processes of immobilization of insulin in ionotropic polysaccharide gels.

Research methods: ionotropic gelation method; swelling kinetics; insulin immobilization; UV spectroscopy (insulin release kinetics); infrared spectroscopy; atomic force microscopy; rheology of films obtained based on gelatin compositions (MT 150 machine); stereomicroscopy; scanning electron microscopy, molecular dynamics modeling.

Main points to be defended:

1. Coating of alginate particles loaded with insulin with gelatin reduces the degree of insulin release at pH = 4.01 from 39% to 31% and increases it at pH = 9.18 from 63% to 83%.
2. The natural (α-helical) conformation of insulin is preserved by adding gelatin to the polysaccharide matrix and the process of its fibrinogenesis is inhibited.
3. Dipole-dipole interactions are the main mechanism of insulin binding in the hybrid protein-polysaccharide matrix.

The scientific novelty of the work:

1. The kinetics of insulin release from gelatin-coated alginate particles obtained by ionotropic gelation at pH = 6.86; 9.18 was calculated; the release values were 50% and 83%, respectively. The swelling kinetics of alginate particles coated with gelatin at pH = 1.0; 4.01; 6.86; 9.18 compared to alginate particles was higher by 0.52; 0.74; 1.66; 1.38 units.
2. To increase the resistance of alginate particles to the aggressive environment of the stomach, the structural and mechanical properties of gelatin compositions with

chitosan, citric, and L-glutamic acids were determined. It has been established that a biocompatible composition of gelatin with L-glutamic acid, due to an increase in the number of intermolecular hydrogen bonds and the formation of associative structures, forms a strong structure at low pH values and withstands the conditions of the acidic environment of the stomach, and in an alkaline environment, the structural and mechanical characteristics of this system are reduced, creating favorable conditions for the release of encapsulated insulin in the intestinal phase.

3. The matrix design of hybrid transport systems was developed and the effect of cross-linking agents ($CaCl_2$, GA; $CaCl_2$ – GA) on the hybrid matrix was studied. The addition of gelatin to alginate during gelation slows down the fibrillation process, which reduces the effect of insulin on the body. Structures with different surface morphology, internal structure, and swelling kinetics were obtained using cross-linking agents.

4. For the first time, insulin was immobilized on a created hybrid protein-polysaccharide matrix, and the effect of the biological catalyst pepsin on the kinetics of its release and diffusion coefficient was studied. Insulin release in the presence of pepsin from ALG; ALG – Gel particles were 57.7% and 61.4%, respectively. Since the presence of two crosslinking agents, Ca^{2+} and GA, has a positive effect on strengthening the properties of the polymer network, it was found that the matrix based on ALG – Gel ($CaCl_2$ - GA) is stable during insulin transportation. The potential energy of polymer matrices and mechanisms of intermolecular interactions were modeled using the Molecular Mechanics 2 (MM2) program. It was found that dipole-dipole interactions predominate during insulin immobilization.

Theoretical and practical significance of the research results. Encapsulation of insulin using protein-polysaccharide hybrid polymer matrices allows obtaining a convenient oral form of insulin, a hormone used in the treatment of diabetes, and developing an alternative to injectable insulin.

Relation of the work with state scientific programs

The dissertation work was carried out within the framework of the following research project:

- AP19677207 Design of nanostructured bionanocomposites based on natural raw materials.

Publications

9 scientific papers have been published on the topic of the dissertation, 1 article in the journal "Molecules" (Q1, 83%), 1 article in the journal "Eurasian Chemical-Technological Journal" (Q3, 29%) included in the Scopus database, 2 articles in the journal "Bulletin of KazNU" series "Chemistry" included in the list recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, as well as materials and abstracts of 5 reports at international scientific and practical conferences.

Findings of these dissertations were presented at the following international conferences and forums: International Conference of Students and Young Scientists "Farabi Alemi" (Almaty, 2021); International Scientific and Practical Conference "Trends, prospects and innovative approaches to the development of chemical science, production and education in the context of globalization" (Almaty, 2021); International Scientific and Practical Conference "7th International Conference on New Trends in Chemistry" (Turkey, 2021); "Promising directions for the development of chemical science, technology and ecology" (Almaty, 2022); "The VI International Conference on Colloidal Chemistry and Physico–Chemical Mechanics (IC CCPCM) is dedicated to the 125th anniversary of the birth of P. A. Rebinder" (Kazan, 2023).

Description of the doctoral student's contribution to the preparation of each publication:

Yerlan G.Ye., Tyusyupova B.B., Tazhibayeva S.M., Musabekov K.B., Balabushevich N.G. Structural and mechanical properties of gelatin composite films. Bulletin of KazNU, Chemistry series. No. 4 (107) 2022 - conducting experimental work, collecting data, processing and analyzing the results obtained during the research work, and preparing the results for publication and publication were carried out personally by the dissertation author.

The author of dissertation is the first and corresponding author of the published work.

2. G. Yerlan, B. Tyussyupova, S. Tazhibayeva, K. Musabekov, N. Balabushevich, and A. Kokanbayev, “Encapsulation of Insulin in Biodegradable Polymers”, Eurasian Chem.-Technol. J., Scopus, Q3, 29%, vol. 24, no. 4, p. 351–361, Dec. 2022. - conducting experimental work, collecting data, processing and analyzing the results obtained during the research work, and preparing the results for publication and publication were carried out personally by the dissertation author.

The author of dissertation is the first and corresponding author of the published work.

3. Yerlan G.Ye., Tyusyupova B.B., Tazhibayeva S.M., Musabekov K.B. Polymer systems for oral delivery of insulin. Bulletin of KazNU, Chemistry series. № 1 (108) 2023. – P. 12-29. - conducting experimental work, collecting data, processing and analyzing the results obtained during the research work, and preparing the results for publication and publication were carried out personally by the dissertation author.

Th author of dissertation is the first and corresponding author of the published work.

4. Yerlan, G.Y.; Shen, M.; Tyussyupova, B.B.; Tazhibayeva, S.M.; Musabekov, K.; Takhistov, P. Insulin Conformation Changes in Hybrid Alginate–Gelatin Hydrogel Particles. Molecules 2024, 29, 1254. Scopus, Q1, 83% - conducting experimental work, collecting data, processing and analyzing the results obtained during the research work, and preparing the results for publication were carried out personally by the dissertation author.

The author of the dissertation is the first author of the published work.

5. Materials and abstracts of 5 reports at international scientific and practical conferences - conducting experimental work, collecting data, processing and analyzing the results obtained during the research work, and preparing the results for publication were carried out personally by the dissertation author.

Dissertation structure and volume

The dissertation work consists of an introduction, a literature review, a practical part, research results, a conclusion, a list of references and appendices. The dissertation consists of 112 pages, 299 references, 30 figures and 4 tables.