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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

DEVELOPMENT AND INVESTIGATION OF CHITOSAN-BASED BIOCIDE POLYMER CARRIERS FOR PROLONGING THE EFFECT DURATION OF LEVOTHYROXINE PREPARATION

Specialty: 2304.01-Chemistry of macromolecules

Field of science: Chemistry

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GENERAL DESCRIPTION OF THE WORK

Relevance and degree of research of the topic. To extend the therapeutic effect of biologically active compounds or to maintain stable concentrations in blood circulation, complex compounds with organic or inorganic materials are widely used in pharmacology. However, such complexes do not fully resolve the issues of prolonged controlled release and targeted delivery of drugs to specific organs. Although these compounds may have high stability, they are susceptible to enzymatic and oxidative processes, leading to rapid structural degradation. Additionally, secondary or tertiary components used in these formulations sometimes generate toxicity during these processes, transforming into substances that are difficult to metabolize[1]. To address this issue, recent advancements have focused on immobilizing drugs not onto small molecules but onto polymers compatible with the body, particularly natural polyaminosaccharides and other gel matrices, creating new formulations with enhanced properties. Hydrophilic polymer and gel systems have structures that can respond to changes in pH and temperature by altering their aggregate state or volume. This adaptability allows for the prolonged transport and controlled release of drugs loaded into the matrix, thus addressing these challenges more effectively[2].

Among such natural polysaccharides, chitosan holds significant importance and is currently one of the foremost matrices in the field of pharmacology worldwide[3]. The multifunctionality of chitosan and its derivatives, their strong tendency to form complexes, and the absence of toxic by-products during biodegradation make chitosan an effective medium for transporting biologically active substances.

[1] Altuntaş, E., Biopolymer-Based Nanogel Approach in Drug Delivery: Basic Concept and Current Developments, / E.Altuntaş, B.Özkan, S.Güngör [et al.]// Pharmaceutics -2023, v.15(6),-p. 1644.

[2] Duceac, I.A., Coseri, S., Biopolymers and their derivatives: Key components of advanced biomedical technologies, -2022, v.61, -p. 108056.

[3] Elgadir, M.A., Impact of chitosan composites and chitosan nanoparticle composites on various drug delivery systems: a review / M.A.Elgadir, M.S.Uddin, S. Ferdosh, [et al.] // J. Food Drug Anal.,-2015, v.23, -p.619-629.

Additionally, it is known that thyroid dysfunction, leading to goiter, is increasingly prevalent in certain regions globally and within our country, with patient numbers steadily rising. The thyroid hormone L-thyroxine plays an essential role in the body, requiring a stable concentration level in blood circulation. However, in cases of thyroid disease or following surgical removal of the gland, the body's demand for this hormone must be met by taking its synthetic substitute, levothyroxine sodium pentahydrate.

It is essential to consider that, in cases of goiter, there is a need to design new pharmacological forms of the drug complex containing levothyroxine sodium pentahydrate, used as a hormone substitute, with an improved composition aimed at reducing side effects. Given the import of this drug into our country, it is deemed important to develop and apply a new formulation based on our own research and experiments. For this purpose, it is necessary to first immobilize the medically significant active ingredient on novel chitosan-based polymer gel systems as carriers. This approach would allow for the development of low-concentration nanogel-levothyroxine sodium pentahydrate systems that can enhance therapeutic effects and be applied in medical treatments.

Research Object and Subject. The object of the dissertation research is the synthesis of chitosan-based biocidal hydrogels, while the subject of the research focuses on the immobilization of the L-thyroxine drug within these hydrogels and the investigation of its controlled release.

Research Aim and Objectives. The primary aim of the research is to synthesize biocidal, hydrophilic, or "smart" nanogels based on the natural bioactive polyaminosaccharide chitosan. This approach is intended to reduce the side effects of the thyroid hormone replacement, levothyroxine sodium pentahydrate, and to maintain the daily required dose with minimal quantity. The research involves immobilizing the active substance into these gels using sorption methods and studying the kinetics of its controlled release in both in vitro and in vivo conditions.

To achieve the stated aim, the following tasks are planned to be addressed:

- Investigation of matrices obtained from quaternization of new alkyl derivatives of the natural polysaccharide chitosan with methyl iodide and evaluation of their biological activity after immobilizing levothyroxine sodium pentahydrate.

- Synthesis and study of Schiff base derivatives of chitosan containing benzyl and alkyl groups, followed by the loading of levothyroxine sodium pentahydrate to investigate the kinetics of its controlled release.

- Cross-linking of chitosan with N-vinylpyrrolidone to develop pH-sensitive hydrogels and examine their potential as biocidal materials for the transport of levothyroxine sodium pentahydrate.

- Synthesis of chitosan nanogels with other natural polysaccharides such as arabinogalactan and gum arabic, and analysis of the release of levothyroxine sodium pentahydrate from the immobilized matrix. The release mechanism will be determined by applying Korsmeyer-Peppas, Hixson-Crowell, Higuchi, zero-order, and Fickian kinetic models.

- Evaluation of the acute and chronic toxicity and bioactivity of levothyroxine sodium pentahydrate biocomplexes with the obtained chitosan-based nanogel samples in vivo on mice and rabbits.

Methods of research. In the dissertation work, physical and chemical properties and structures of the obtained products were investigated using analysis methods such as SEM, UV, FTIR, TGA, DTA, X-Ray, and elemental spectroscopy.

The main scientific theses for the defense. The results obtained from the research of the dissertation have been aligned with the theories explaining polymer-based hydrogel systems and the immobilization, transport, and controlled release of drug substances. The following theses have been presented for defense as the main conclusions:

- The investigation of the swelling rates, kinetics, degradation, and structures of nanogel systems obtained from the interaction of chitosan with acetate and benzaldehyde separately, their quaternization with methyl iodide, as well as the cross-linking of chitosan with N-vinylpyrrolidone and processing with arabinogalactan in water, buffer systems, and enzyme environments.

- The immobilization of levothyroxine sodium pentahydrate into the synthesized new chitosan-based biocidal nanogel matrices via sorption, the study of the chemical nature of the interaction between the gel and the drug substance, characterization of the release amount of the active substance from the carrier into the medium, and determining the release mechanism by applying the results to kinetic models.

- The preparation of medical formulations of various chemically structured and composed chitosan-based biocidal nanogel-levothyroxine sodium pentahydrate biocomplexes, and the results of their testing for acute and chronic toxicity in mice, which have been presented for defense. Additionally, the evaluation of the release rate and duration of the active substance in the bloodstream of rabbits, previously subjected to thyroidectomy, has been conducted as part of these investigations.

Scientific novelty of the research. Chitosan N,N-diethyl and N-methyl N-benzyl derivatives, as well as its cross-linked copolymer with N-vinylpyrrolidone, have been used to synthesize smart biocidal carriers. New soluble salt forms were created by quaternizing these carriers with methyl iodide, and their structure and properties were identified. Biological active drug formulations were developed by immobilizing levothyroxine sodium pentahydrate onto these samples using the sorption method. Furthermore, biosid nanogel samples capable of effectively transporting levothyroxine sodium pentahydrate were designed by processing chitosan with arabinogalactan and gum arabic at low temperatures. The mechanism of drug release from the matrix was determined to follow Fickian diffusion and non-Fickian mechanisms, depending on the mass fraction of polysaccharides in the composition.

Theoretical and practical significance of the research. Levothyroxine sodium pentahydrate biosystems immobilized on chitosan-based biosid nanogels, which are sensitive to environmental pH, ionic strength, and enzymatic effects but do not have toxic effects on living organisms, ensure effective transport and long-term controlled release in the bloodstream and tissue fluid environments across a wide pH range.

Compared to other polysaccharides, chitosan and arabinogalactan have biological advantages, such as the ability to sorb and eliminate toxic substances from the organism. Therefore, chitosan and its derivatives not only ensure targeted delivery and controlled release of drugs, but also actively participate in other physiological processes, possessing complex properties that make them effective as drug carriers. They can be applied as an effective depot for transporting not only levothyroxine sodium pentahydrate but also anthracycline antibiotics and alkaloids.

Based on the results obtained, it is possible to perform a Schiff reaction of chitosan with other alkyl and aromatic aldehydes, followed by quaternization with methyl iodide, as well as to cross-link chitosan with N-vinylpyrrolidone and process it with arabinogalactan, resulting in salts with different solubility degrees. With these salts, nanogel biosid carriers with different drug release rates can be created through the effective sorption of small-molecule drugs. Such nanogel-based matrices can be used not only for drug delivery but also for the creation of biosensors and catalytic systems in medicine and various fields of biotechnology.

Approbation and application. The results of the dissertation work have been presented at the following national and international scientific conferences:

Modern Achievements of Pharmaceutical Technology and Biotechnology Proceedings (Kharkiv, Ukrania 2019), International Conference «Chemistry of Organoelement Compounds And Polymers2019» (Moscow, Russian), Elmi Tədqiqat Beynəlxalq onlayn elmi jurnal II Respublika Elmi Konfransı, Bakı.11. 2023, Old And New Technologies Of Learning Development İn Modern Conditions (Berlin,Germany 2024), Proceedings of the 3rd International Scientific and Practical Conference «Innovative Development in the Global Science 2024» Boston, USA, II International Scientific and Practical Conference «Innovations in education: prospects and challenges of today», Sofia, Bulgaria 2024.

Some of the research related to the dissertation topic has been submitted to the grant project programs of the SOCAR Science Fund, the Azerbaijan Science Fund, and the Azerbaijan National Academy

of Sciences (ANAS) in priority areas, and successfully funded in the following projects:

- 2018-2019 - Joint Research and Development Project between ANAS and the National Research Council of Italy. “Encapsulation of L-thyroxine into N-trimethyl iodide derivative of chitosan and its biological study of controlled release”.

- 2018 – 33 LR-ANAS-SOCAR. “Synthesis of N-trimethyl iodide chitosan for L-thyroxine transport, rheological and spectral investigation”.

- 2018-2019 – “Synthesis and study of nitrogen and oxygen-containing hydrophobic and biocidal polymers for immobilization of medicinal preparations”, Azerbaijan Science Foundation.

- 2020-2021 – “Synthesis of nanoscale carriers based on chitosan and its derivatives and their long-term therapeutic application in the treatment of the thyroid gland”, Azerbaijan Science Foundation.

Name of the organization where the dissertation work was performed. The work was performed at the Laboratory “Nanostructured metal-polymer catalysts” of Institute of Catalysis and Inorganic Chemistry named after academician Murtuza Naghiyev of the Ministry of Science and Education of the Republic of Azerbaijan. Some experimental studies related to the dissertation work and the structural-compositional analysis of the obtained gels were conducted within the framework of grant projects at the Institute of Polymers, Composites, and Biomaterials (Italy) and at Azerbaijan Medical University. In addition, some spectral and elemental compositions of the samples were confirmed at the National Nuclear Research LLC under the Ministry of Communications and High Technologies of the Republic of Azerbaijan.

Scientific works published on the topic of the dissertation. The content of the dissertation work has been published in 13 scientific papers. Of these, 6 are articles published in international and national scientific journals, 6 are abstracts of presentations, and 1 is the Eurasian patent. Three of the articles have been published in journals indexed in scientific databases such as Scopus and CAS

(Asian Journal of Chemistry, International Journal of Innovation and Applied Study, Journal of Chemical Problems).

The structure and volume of the dissertation. The dissertation consists of an introduction (18661 characters) and three chapters (Chapter I: 40114 characters, Chapter II: 30839 characters and Chapter III: 102383 characters), followed by a conclusion (4064 characters), with a total of 177400 characters. The total length of the dissertation is 167 computer pages. The work includes 81 figures, 14 tables, 1 schematic diagram, and the reference list with 152 literatures.

MAIN CONTENT OF THE WORK

The introduction justifies the relevance of the dissertation, outlines the objectives of the work, its scientific novelty, practical significance, and presents the main propositions to be defended.

The first chapter provides a review, analysis, systematization, and comparative analysis of literature from the past 10-15 years on the synthesis of gel matrices for the delivery of drug compounds based on natural polysaccharides, as well as the immobilization of the synthetic thyroid hormone analogue levothyroxine-Na pentahydrate in chitosan (XZ) and other polysaccharide-based hydrogels. It also covers the investigation of controlled release kinetics in model systems. The literature review is comprehensive, highlighting the strengths and shortcomings of the conducted research, and specifically emphasizes the relevance of the study.

The second chapter is dedicated to the synthesis of chitosan-based alkyl and aromatic Schiff base derivatives, their copolymers with vinyl monomers, and the synthesis of chitosan-arabinogalactan (AQ) nanogels. It also covers the methodology for immobilizing levothyroxine-Na pentahydrate through adsorption. The research includes the study of the physical and chemical properties and structures of the obtained products using SEM, UV, FTIR, TGA, DTA, X-ray, and elemental spectroscopy analysis methods. Additionally, this chapter presents the application of kinetic models for the release of levothyroxine-Na pentahydrate from the synthesized chitosan-based nanogels, degradation methods of the

obtained chitosan-drug biocomplexes in enzymatic and pH environments, and *in vivo* experiments conducted on mice and rabbits.

The third chapter focuses on the synthesis of new chitosan-based biosid derivatives, as well as the preparation of their salts through quaternization, analysis of the stages of the process, and the results of infrared spectroscopy, X-ray, and thermogravimetric methods for the intermediate products. This chapter also confirms the structure of the nanogels. Furthermore, it presents the results of studying the optimal immobilization conditions for levothyroxine-Na pentahydrate adsorption in these synthesized nanogel systems and binary hydrogel systems obtained by modifying XZ with AQ. Kinetic studies of levothyroxine-Na pentahydrate immobilization in the synthesized XZ-based polysaccharide nanogels were conducted, and the results were applied to Freundlich and Langmuir kinetic models. The kinetic and thermodynamic parameters of the process were determined, allowing for the identification of the sorption mechanism and the nature of the interaction between levothyroxine-Na pentahydrate and the gel. Additionally, the results of *in vitro* and *in vivo* separation experiments of immobilized levothyroxine-Na pentahydrate from XZ-based nanogel samples under laboratory conditions are provided, and the mechanism of controlled release is demonstrated by applying the kinetic model values.

The synthesis and investigation of N,N-diethyl N-methyl derivatives of chitosan.

In the study, a new N,N-diethyl chitosan derivative, soluble in water, was synthesized for the transport of levothyroxine-Na pentahydrate, and N,N-diethyl N-methyl iodide chitosan was obtained by quaternizing it with methyl iodide. Based on the analysis, we proposed the alkylation of chitosan with acetic aldehyde via a Schiff reaction and also suggested the possible occurrence of intermediate stages in the process and their mechanism. The surface morphology and thermal stability of the obtained products were thoroughly analyzed, and the presence of intermediate products in the N,N-diethylation and NaBH4 reduction process, as well as in its

quaternization and alkylation reactions, was identified using various spectroscopy methods.

It is not always helpful to rotate the crank counterclockwise. Therefore, during the upward movement of the rod suspension point, negative deaxial pumping units are used to reduce its acceleration. At present, more and more positive deaxial sucker rod pumping units are used in foreign countries.

The choice of sucker rod pumping units also depends on the properties of the extracted liquid. If the distance between the plunger and the cylinder is too long, the fluid will leak downwards, reducing the efficiency of the pump, as the time for the rod to move upwards is too long. Conversely, if a large negative deaxial pumping units is adopted unreasonably, the probability of the rod column hanging in the tubes increases as the lowering speed of the rod suspension point moves downwards.

The synthesis and structural investigation of N-methyl, N-benzyl derivatives of chitosan.

For the immobilization of levothyroxine-Na pentahydrate, a derivative of chitosan (XZ) was synthesized in which both methyl and benzyl groups were introduced into the amino groups of the macromolecule. The incorporation of both alkyl and aromatic radicals into the structure is of significant interest regarding the properties of the newly obtained carrier, particularly in terms of its drug capacity, isotherms, thermodynamic parameters, and release characteristics in certain environments for levothyroxine-Na pentahydrate. The synthesis of N-methyl N-benzyl chitosan (MBXZ) in an acidic medium with formaldehyde and benzaldehyde was carried out, and the structure of the obtained product was studied using UV, FTIR, and X-ray analysis.

The synthesis of the primary matrix was performed according to the appropriate method, and the formation process of MBXZ was investigated depending on the molar ratio of the components, the concentration and nature of the aldehydes, the average molecular weight of the XZ, and the reaction time.

Initially, before alkylation, the SEM surface morphology of XZ was studied, as well as the surface morphology of the final product after reacting with formaldehyde and benzaldehyde (Figure 1).

As seen in the figure, the surface microstructure of the samples, when magnified at 200x, differs despite being the same 500 μm scale. The XZ surface appears smooth with micro-particles, while after alkylation, more wrinkles, folds, and roughness are observed. The bends and surface compressions suggest that the XZ surface has undergone chemical transformation [8].

Figure 1. SEM micrographs of chitosan (left) and N-methyl N-benzyl chitosan (right) [8].

During SEM analysis, the surface structure of the main product was also examined at 800x and 1500x magnifications, providing clearer images (Figure 2).

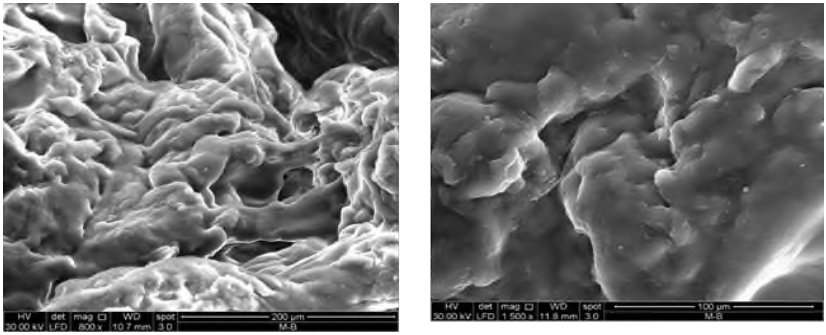

Figure 2. SEM micrographs of N-methyl N-benzyl chitosan at different magnifications [8].

As can be seen in the figure, a uniform microstructure has formed across the entire surface of the main product. This indicates

that alkylation occurred evenly throughout all fragments of the polysaccharide macromolecule. The alkylation of N-methyl N-benzyl chitosan with aldehydes covered the entire chain without causing degradation or fragmentation of the polymer chain into smaller particles.

Synthesis of a gel based on the graft copolymer of low molecular weight chitosan with N-vinylpyrrolidone.

To further enhance the functionality of chitosan (XZ), control its solubility and antibacterial properties, and transform it into an environment-sensitive carrier for drug delivery, chemical transformations are applied to the macromolecule structure, and graft copolymers with vinyl monomers are synthesized. Analyzing the literature reveals extensive studies on hydrogels obtained from radical graft copolymerization reactions of chitosan with both hydrophilic and hydrophobic vinyl monomers, focusing on drug loading and in vivo and ex vivo testing. In this research, a low to medium molecular weight chitosan matrix was synthesized with vinylpyrrolidone (VPr) for the immobilization of levothyroxine-Na (LTH-Na), and the potential of using the hydrogel obtained from its crosslinking as a carrier was investigated.

Study of the swelling degree of nanogels synthesized based on chitosan.

One of the main requirements for the immobilization of bioactive compounds is the swelling degree of the materials. From this perspective, graft copolymers of chitosan (XZ) synthesized with vinylpyrrolidone (VPr), arabinogalactan (AQ), and gum arabic (QA), as well as products derived from N,N-diethyl, N-methyl, and N-methyl N-benzyl derivatives, were crosslinked with N,N'-methylenebisacrylamide (MBAA), a bifunctional reagent. The swelling degrees of the gels were comparatively studied. The synthesis of graft copolymers with natural polymers was first carried out via radical initiation of the monomer, followed by crosslinking reactions to obtain the gel.

Despite being conducted under different conditions, the crosslinking process of XZ with VPr and natural polymers occurred through the same chemical mechanism. The crosslinking of XZ with

VPr was carried out under continuous ultraviolet (UV) irradiation for 4 hours. The synthesis and crosslinking of XZ with AQ and QA were performed in situ via a thermal method under synchronized conditions. Despite differing conditions, the resulting porous, three-dimensional crosslinked networks possessed a gel structure and exhibited swelling behavior in water.

It was determined that as the amount of MBAA increased during the crosslinking of XZ-based samples, both the gel fraction yield and the efficiency of the crosslinking reaction increased. Initially, as the MBAA content increased, the stiffness of the macromolecules in the products also increased, and the gel tended to aggregate into relatively small volumes during the precipitation of the reaction products from the mixture. This phenomenon is related to the densification of macromolecular chains, reduction in the size of the formed networks, and the creation of entangled chains as the amount of MBAA increased. The swelling degrees of the hydrogels obtained from the graft copolymers and alkyl-aromatic derivative samples, crosslinked with MBAA in distilled water, were studied based on the amount of crosslinking reagent, and the results of the study are presented in Table 1.

Table 1

Dependence of the swelling degree of gels synthesized from natural polymers in deionized water on the amount of the crosslinking reagent at T = 20°C, V_{wat} = 10 ml, t = 24 hours [10].

MBAA,% / Gellər	1	3	5	10	15	20	30
Xz-graft-VPr	175	197	151	138	103	74	48
Xz-graft-QA	187	203	227	135	109	86	59
Xz-graft-AQ	201	213	249	154	125	108	74
DEMX	178	159	117	82	56	35	19
MBXz	146	119	80	69	52	38	21
BXz	106	83	71	58	37	23	12

As seen in the table, the swelling degree decreases as the amount of MBAA in the composition increases in graft copolymers of XZ, whether obtained with other natural polymers or with synthetic vinyl monomers. This is due to an increase in the number of pores and a decrease in their size as the amount of cross-linking reagent in the composition rises. Additionally, as cross-linking increases, the macromolecule becomes more rigid, reducing its hydrophilicity, which is reflected in the swelling degree values. The results in Table 1, which show the dependency of swelling degree on the amount of cross-linking agent, are extensively discussed in the dissertation.

It is known that pH value is an important parameter in drug delivery. Therefore, it is necessary to study the dependency of the swelling degree of synthesized hydrogel samples on the pH of the medium. This dependency affects the ionization degree of functional groups in the gel, as well as the nature and degree of interaction between the gel and the immobilized drug. The dependency of swelling degree on pH in hydrogels obtained from the cross-linking of XZ with VPr, as well as with AQ and QA in graft copolymer form, was studied, and the results are presented in Figure 3.

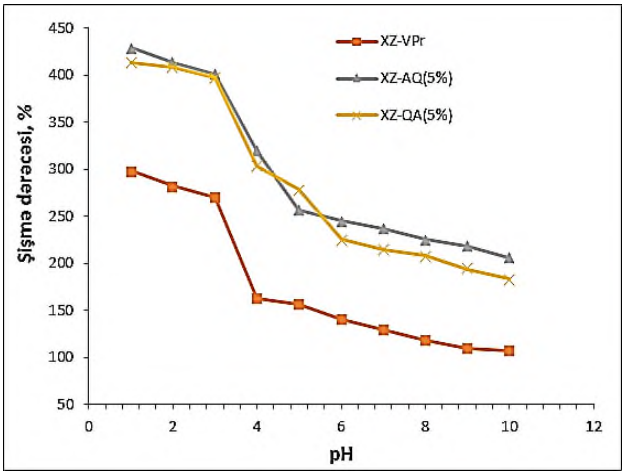

Figure 3. The dependency of the swelling degrees of gels based on chitosan graft copolymers with natural and synthetic polymers on the pH of the medium, T=24ºC, Vbuffer=10 ml, t=24 hours [10].

The gels based on XZ graft copolymers with AQ and QA contain a large number of ionizable groups, such as –OH, -COOH, -NH_2, and -CH_2OH, which increases their swelling degree. Specifically, gels obtained from grafting 5% by mass of chitosan with AQ or QA around pH=6 show a higher swelling degree (240-265%) compared to the gel with vinyl monomer. This is explained by the inclusion of AQ and QA fragments containing -COOH groups into the XZ structure.

In general, the swelling degrees of all obtained hydrogel samples range between 120-250% in the pH range of 6-8, which makes them suitable for transporting biologically active substances that can be active in these environments.

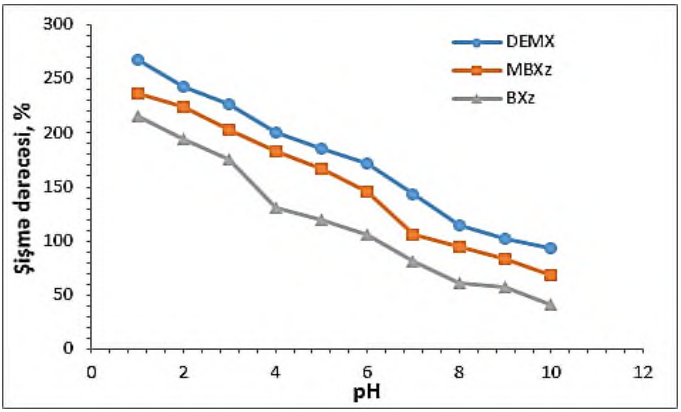

Figure 4. The dependency of the swelling degrees of N,N-diethyl, N-methyl, N-benzyl, and N-benzyl chitosan-based hydrogels on the pH of the medium, T=24°C, V_{buffer}=10 ml, t=24 hours [10].

However, the swelling behavior is different in hydrogels obtained from the cross-linking of XZ alkyl and aromatic derivatives with MBAA (Figure 4). Since the ethyl, methyl, and benzyl groups included in the composition are hydrophobic in nature, they cannot ionize, resulting in relatively lower swelling degrees compared to other hydrogel samples [10]. Furthermore, the most electronegative groups capable of ionization in XZ are the amino groups, whose protons have been replaced by alkyl or benzyl groups through the

Schiff reaction. Consequently, the nitrogen atoms are protected by methyl, ethyl, and benzyl groups, making protonation with H^+ ions in acidic environments spatially challenging. The maximum swelling degree is achieved when the cross-linker is at 1% by mass, which is attributed to the unmodified amino groups and free hydroxyl groups.

As shown in Figure 4, the swelling degrees of hydrogels obtained from the cross-linking of chitosan's alkyl and benzyl derivatives decrease monotonically with pH. This indicates that the composition has a homogeneous structure. In other words, the presence of only amino and hydroxyl groups capable of ionization depending on the acidity of the environment causes this effect. However, the fact that these samples exhibit swelling over a wide pH range allows them to be used as carriers for the release of drugs not only in acidic environments but also in pH=5-7 environments.

It is known that after drug immobilization, the release of drugs into the environment is directly related to the gel structure, the nature of the environment, and the kinetics of the gel's swelling degree under those conditions. The gradual increase in the swelling degree over prolonged release periods also contributes to controlled release. In this context, the swelling kinetics of the obtained XZ-based hydrogels have been studied at pH=3 (Figure 5) and pH=8 (Figure 6) at 24°C and under temperature conditions compatible with the body, taking into account the acidic nature of gastric juice and the neutral environment of the small intestine.

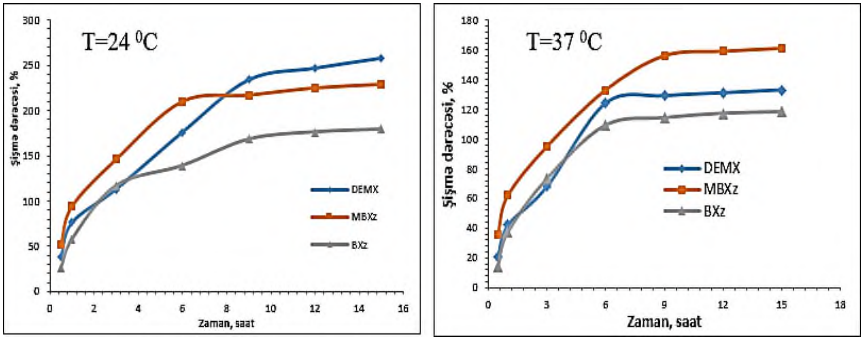

Figure 5. Swelling kinetics of chitosan-based biocidal polymer hydrogels at pH=3, at 24°C and 37°C, Vbuffer=10 ml [10].

As seen in Figure 5, increasing the temperature to 37°C leads to a reduction in the swelling degrees of the samples. Swelling equilibrium is reached after 8-9 hours at 24°C, while at 37°C it occurs after about 6 hours. In the benzyl chitosan (BXz)-based hydrogel sample, swelling equilibrium starts after 6 hours at both temperatures. However, up to 6 hours at 37°C, the dependency displays a sharply linear character, a behavior observed in all hydrogel samples.

The decrease in swelling degrees with rising temperature can be explained by the following factor:

- As temperature increases, the mobility of macromolecules rises, which spatially enhances the interaction between the gel's functional groups and water molecules. However, at 37°C, the desorption of protons that enable the ionization of amino groups occurs, reducing electrostatic interactions with molecules penetrating the hydrogel's pores.

At 24°C, the swelling degrees gradually increase over time, which is considered favorable for drug delivery systems. However, a high swelling degree may lead to the release of a larger quantity of the immobilized drug at the beginning of the process, which is undesirable. In contrast, a reduction in swelling degrees by approximately 1.2-1.4 times at 37°C is more suitable for these systems. These results suggest that the synthesized hydrogels, being sensitive to both pH and temperature, could be used as carriers for drug immobilization.

In studies on swelling degrees at pH=8 under similar temperatures, the results showed an opposite trend. Increasing the temperature to 37°C led to a relative increase in the swelling degrees of the hydrogels, with swelling equilibrium reached within the initial 3 hours. It is known that at pH=8, the ionizable functional groups in chitosan-based hydrogels do not polarize as much as in acidic environments. The abundance of hydrogen bonds among amino groups hinders ionization. However, the increase in temperature weakens these hydrogen bonds, freeing the functional groups, which allows ions or their hydrated forms that constitute the pH=8

environment to penetrate the internal pores of the gel more quickly and in larger quantities.

The swelling kinetics of hydrogel samples based on graft copolymers of XZ with VPr, AQ, and QA at pH=8 at various temperatures have been studied, and the results are presented below (Figure 6).

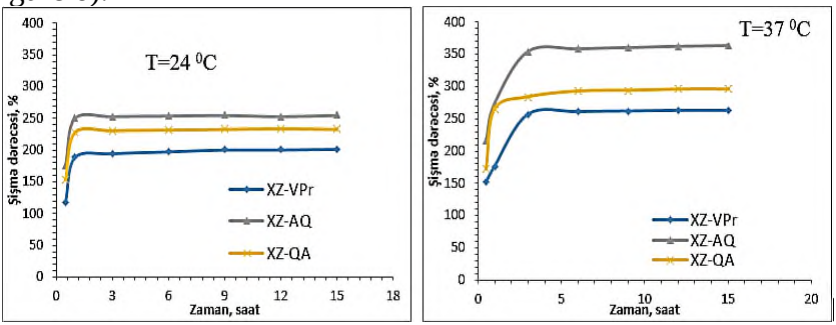

Figure 6. Swelling kinetics of chitosan-based biocidal polymer hydrogels with graft copolymers of VPr, AQ, and QA at pH=8, at 24°C and 37°C, Vbuffer=10 ml [10].

As seen in Figure 6, when polyar functional groups are incorporated into the structure of XZ, the swelling degree of the resulting hydrogel begins to increase with temperature at pH=8. Specifically, the XZ-VPr-based hydrogel reaches swelling equilibrium at 24^0C within 1 hour at pH=8, with a swelling degree of 170-180%. However, at 37^0C, the equilibrium is reached within 3 hours, characterized by a higher swelling degree of 240-250%. This can be attributed to the increase in the number of polyar functional groups in the composition with rising temperature, which enhances the freedom of macromolecular chains. As a result, the environment molecules or their ionized forms that penetrate the internal pores create more hydrogen bonds and electrostatic interactions with the gel's functional groups.

This increase in swelling degree helps control the amount of drug release when the immobilized drug is affected by the environment's irritants. Thus, all the gel samples obtained can be used as matrices for the transport of various drug substances, depending on the pH

and temperature of the environment in which the immobilized preparation remains active.

The kinetic release of levothyroxine-Na pentahydrate from the chitosan-arabinogalactan graft copolymer-based gel

The aim of the research is to synthesize a hydrogel from the chitosan (XZ)-arabinogalactan (AQ) mixture in various mass ratios at low temperatures and to immobilize levothyroxine-Na pentahydrate. The structure of the synthesized chitosan/arabinogalactan (XZ/AQ)-based gel was characterized by FTIR, SEM, X-Ray, and TGA. The optimal swelling degree of the gel was studied in phosphate buffer solution (PBS) at pH=7.4, deionized water, and physiological saline.

Furthermore, the release of immobilized levothyroxine-Na from the synthesized XZ/AQ hydrogel was investigated. The release kinetics of the bioactive compound from the gel was studied, and its conformity with various mathematical models was checked. Additionally, in vivo experiments were conducted to evaluate the toxicity of the immobilized drug in gel samples, using mice, and the biological activity was assessed by measuring the TSH levels in the blood of rabbits that underwent thyroid surgery.

Table 2.

Temperature dependence of particle sizes and zeta potential values in deionized water for lyophilized XZ/AQ and XZ/AQ-LTH-Na samples.

Formulas	Dimession, mkm		Zeta potensial, mV	
	20°C	37°C	20°C	37°C
Xz/AQ-5:1	2.38	3.76	-8.48	-10.34
Xz/AQ-5:1/LTH50	2.84	4.18	-10.62	-12.06
Xz/AQ-5:1/LTH100	3.12	4.85	-11.57	-13.18
Xz/AQ-5:1/LTH200	4.27	6.72	-13.46	-14.25
Xz/AQ-2:1	5.46	5.82	-5.28	-6.19
Xz/AQ-2:1/LTH50	5.63	6.38	-6.51	-6.97
Xz/AQ-2:1/LTH100	6.14	6.94	-7.36	-8.53
Xz/AQ-2:1/LTH200	6.88	7.25	-8.84	-9.35

Table 2. continued

Xz/AQ-1:1	11.27	13.73	-0.98	-2.78
Xz/AQ-1:1/LTH50	12.59	15.38	-3.14	-5.59
Xz/AQ-1:1/LTH100	13.65	16.17	-5.76	-7.83
Xz/AQ-1:1/LTH200	14.89	18.64	-7.37	-9.42

The determination of the immobilization and release mechanisms of drug compounds in polymer matrices is an important parameter, which includes the measurement of zeta potential and particle size. Zeta potential also influences the stability of particles in the liquid phase and their electrostatic attraction or repulsion. The zeta potential and particle size values for the obtained XZ/AQ gels were measured and are presented in Table 2.

As seen in Table 2, as the amount of AQ in the composition of XZ increases, both the particle size and the zeta potential value of the gel increase. After the maximum amount of LTH-Na was immobilized into each gel sample, the separation process of XZ/AQ-LTH-Na samples in deionized water was studied (Figure 7). The experiments were conducted on gel samples with a high mass fraction of XZ in the composition, such as XZ/AQ-5:1 and samples with a polysaccharide ratio of 1:1 (mass, %). The amount of LTH-Na immobilized in the gel matrices ranged from 132 to 175 μg.

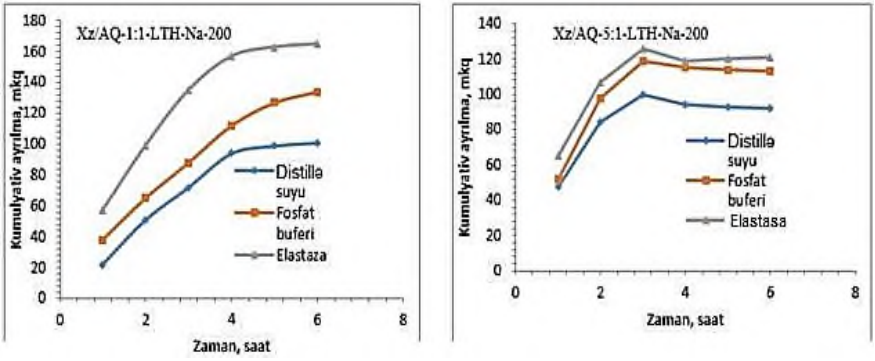

Figure 7. Kinetics of cumulative release of LTH-Na from XZ/AQ 1:1-LTH-Na-200 and XZ/AQ-5:1-LTH-Na-200

As seen in Figure 7, when the content of XZ is high, the release of LTH-Na increases during the first 3 hours, and then the release

stabilizes with a slight decrease. The main portion of LTH-Na (70-90%) is released during the initial 1-3 hours. The release in PBS and elastase environments is more abrupt, while in the water environment, it is observed to be relatively regular. In the XZ/AQ-1:1 sample, the release continues with increasing tempo up to 4 hours. This, of course, depends on the chemical composition of the gel and the interactions between the polysaccharide macromolecule and LTH-Na. In all cases, since the gel's enzymatic degradation is rapid, the active substance is released into the environment more quickly and in larger quantities. By applying various kinetic models to the release values, information can be obtained about the release mechanism. It has been determined that the regression coefficients vary for each gel sample (Table 3).

Table 3
Regression coefficients for the cumulative release of LTH-Na from XZ/AQ-LTH-Na-200 samples according to kinetic models.

Gel samples	Higuchi,R^2			Hixson-Crowell,R^2			Korsmeyer-Peppas,R^2		
	Distilled water	Phosphate buffer	Elastase	Distilled water	Phosphate buffer	Elastase	Distilled water	Phosphate buffer	Elastase
Xz/AQ-5:1/LTH-200	0.4638	0.5372	0.5366	0.6735	0.7824	0.6345	0.9245	0.9618	0.9743
Xz/AQ-2:1/LTH-200	0.8342	0.6481	0.7216	0.7953	0.8367	0.8572	0.8594	0.7683	0.6158
Xz/AQ-1:1/LTH-200	0.9016	0.9680	0.8681	0.8957	0.9156	0.8348	0.5674	0.4861	0.3956

As seen in Table 3, when the ratio of XZ to AQ is 5:1 (weight), the release of LTH-Na follows the Korsmeyer-Peppas model, while when the ratio of polysaccharides is 50:50 (%), it fits the Higuchi kinetic model. When the mass ratio of XZ to AQ is 2:1 (weight, %), the release of the active drug in water follows the Higuchi and Korsmeyer-Peppas models, and in the elastase medium, it best fits

the Hixson-Crowell kinetic model. The highest R^2 values for the release of LTH-Na are observed for the XZ/AG-1:1/LTH-Na-200 sample in PBS (Higuchi, R^2=0.9680), for XZ/AG-2:1-200 in elastase (Hixson-Crowell, R^2=0.8572), and in distilled water, Korsmeyer-Peppas (R^2=0.8594). For the XZ/AG-5:1-200 gel, the best fit is Korsmeyer-Peppas in all media (R^2>0.9245).

It is known that the Higuchi kinetic model describes the release of drugs from polymer matrix systems. According to the Hixson-Crowell kinetic model, the surface area and diameter of the drug matrix are proportional to the cube root of its volume. The Korsmeyer-Peppas model is also used to describe the drug release mechanism from polymer-based gel systems. In the system we studied, it was observed that in certain parts of the graphs, the release corresponds to zero and first-order kinetics, as well as the Fickian mechanism. According to the Fickian mechanism, the release is diffusion-controlled, involving the movement of drug molecules from regions of high concentration to lower concentration environments. According to the zero-order kinetic model, the release of the drug occurs at a constant rate over time. The first-order kinetic model shows exponential release, where the drug's time-dependent graph is linear. As seen in Figure 7, between 0-3 hours, the release of the drug from XZ/AG-5:1-LTH-Na-200 follows zero and first-order kinetic mechanisms. After 3 hours, the release nature changes and follows other kinetic models. The drug release from XZ/AG-1:1-LTH-Na-200 also fits zero and first-order kinetic models during the initial 3 hours. It was also determined that the release of LTH-Na from XZ/AG-5:1 gel in water and from XZ/AG-1:1 in PBS and elastase media follows Fickian diffusion kinetic models.

Sorption of levothyroxine-Na onto graft copolymers of chitosan with vinyl monomers, as well as onto pH-sensitive hydrogels with N-methyl N-benzyl derivatives.

Hydrogels were synthesized from the copolymer of chitosan with VPr and 4-vinylpyridine (4VP), as well as from samples obtained through the Schiff reaction with benzaldehyde and formaldehyde. The dependence of levothyroxine-Na (LTH-Na)

sorption on various parameters was systematically studied using these hydrogels.

The effect of pH on the sorption of LTH-Na was tested with an initial drug concentration of 50 mg/L, using 50 mg of gel at 24°C over a period of 30 minutes. Under these conditions, the pH of the medium was varied between 2 and 10 (Figure 8).

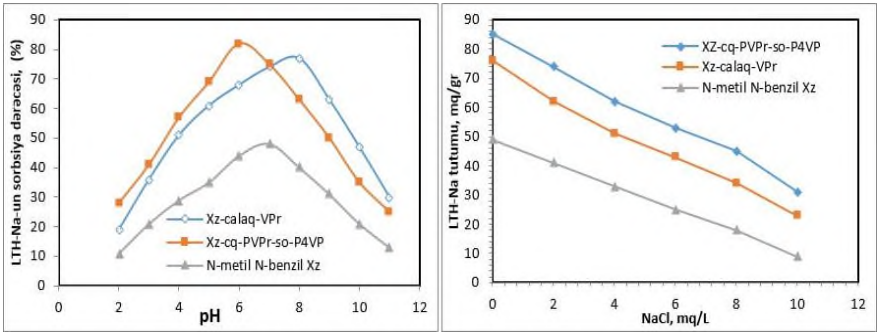

Figure 8. Dependence of LTH-Na sorption on the pH of the medium and on the concentration of NaCl at optimal pH levels [3].

As shown in Figure 8, at pH=2, the sorption of LTH-Na reaches 9–15% for Xz-PVPr and MBXz gels and 25–27% for Xz-PVPr-c-P4VP. This lower sorption in an acidic environment is due to the protonation of H^+ ions at the active centers of the gel and the amine groups in the drug molecule. The minimal sorption is related to the presence of acidic and Ar-OH groups in the LTH-Na molecule, which remain unbound in this medium and interact only slightly with the cationic form of the gel through charge interactions.

As observed, due to the different nature of the matrices, maximum sorption depends on the ionization degree of functional groups. Maximum sorption of LTH-Na is observed in Xz-PVPr-c-P4VP at pH = 6 with 84%, and in Xz-VPr at pH=8 with 73%. In this neutral environment, electrostatic interactions and π→π electron-donor-acceptor interactions between the gel and LTH-Na explain this maximum sorption.

It was determined that at the optimal pH for sorption, as the concentration of NaCl increases, the sorption capacity of LTH-Na in

gel samples decreases. This is attributed to the increased ionic strength of the medium, causing hydrated ions to surround the positively or negatively charged functional groups at the active centers. These blocked active centers are insufficient for chemical interactions with LTH-Na molecules at a stable concentration. Studies on LTH-Na sorption on modified chitosan matrices are further discussed in detail in the dissertation [3].

In vivo Testing of the Acute and Chronic Toxicity of Biologically Active Conjugates of Polymer Preparations Loaded with L-Thyroxine

In the studies, biocomplexes were created by incorporating L-thyroxine into polymer samples obtained in a homogeneous form, including both homopolymers and graft copolymers, as well as gel derivatives. The in vivo biological activity of these biocomplexes was tested using the Körber method on mice that had undergone thyroidectomy, at the Department of I Surgical Diseases of the Azerbaijan Medical University. The analysis of results and the related positive findings are extensively discussed in the dissertation.

RESULTS

1. pH-sensitive smart hydrogels were synthesized by crosslinking chitosan (XZ) derivatives, including N,N-diethyl, N-methyl N-benzyl, as well as graft copolymers with N-vinylpyrrolidone (NVPr), arabinogalactan (AG), and gum arabic (QA) using N,N'-methylenebisacrylamide. The swelling degrees of these hydrogels in water, buffer systems, 0.9% NaCl, and glucose solutions were studied at room temperature and 37°C, showing that the hydrogels reached swelling equilibrium (180–400%) within 4–6 hours. The resulting products have been shown to serve as effective matrices for the immobilization of biologically active compounds [2, 8, 9, 10].

2. To reduce the side effects of levothyroxine-Na pentahydrate, its sorption with the quaternized salt of a new N,N-diethyl N-methyl derivative of chitosan (XZ) was studied. The hydrogel's sorption

capacity was analyzed based on the pH of the medium, ionic strength, hydrogel amount, drug concentration, and temperature. It was shown that the effective sorption of levothyroxine with the XZ-based hydrogel occurs optimally at pH levels of 6–8.5, with a levothyroxine concentration of 50 mg/L, and 10–50 mg of hydrogel. After a temperature of 40°C, the sorption rate decreases, indicating the dual nature of the sorption process [1, 4].

3. Sorption studies of levothyroxine-Na pentahydrate onto synthesized XZ-based polysaccharide hydrogels were conducted. The results were applied to the Freundlich and Langmuir models to investigate the nature of the interaction between levothyroxine-Na pentahydrate and the gel. The sorption process is shown to primarily follow the Langmuir isotherm and, to some extent, the Freundlich equation [4].

4. The pharmacological properties of the L-thyroxine preparation complexed with XZ were studied, focusing on its toxicity and the feasibility of its use for the treatment of hypothyroidism. Initially, the toxicity of the L-thyroxine/polymer complex was examined at a dose of 5 μg/kg. Based on the results, the application of the L-thyroxine/polymer complex did not cause any sedation and was proven to be non-toxic. During the experiment, a solution of the L-thyroxine/polymer complex dissolved in 0.4 ml of gastric juice was administered into the abdominal cavity of animals. No severe negative reactions were observed within 5–10 minutes. It was shown that in the animals, sedation, aggregation, and locomotion were predominant behaviors [5].

5. pH-sensitive hydrogels based on chitosan (XZ) were synthesized using derivatives of VPr, 4-vinylpyridine (4VP), and N-methyl N-benzyl (MBXz) via the Schiff base method. The effective sorption of levothyroxine-Na (LTH-Na) from its aqueous solution onto the synthesized hydrogels was investigated. The effects of the medium's pH, ionic strength, contact time, hydrogel amount, initial concentration of LTH-Na, and temperature on the sorption of the biologically active substance were studied. The sorption kinetics and isotherms of the process on both the surface and volume of the gel were examined. It was determined that the maximum sorption

capacity follows the order: XZ-PVPr-so-P4VP >XZ-PVPr > MBXz [3].

6. A new hydrogel was synthesized by processing mixtures of chitosan (XZ) and arabinogalactan (AQ) at various mass ratios at low temperatures. The sorption of levothyroxine-Na pentahydrate and its release in different media were studied. The swelling kinetics and degradation of the hydrogel were also investigated. It was shown that in an oxidative environment, the gel degraded by 64% within 2 weeks, while in elastase and PBS (phosphate-buffered saline) environments, the degradation was approximately 19-22%. It was determined that the controlled release of levothyroxine-Na from the XZ/AQ-based hydrogel follows the Hixson-Crowell, Korsmeyer-Peppas, and Higuchi kinetic models, depending on the molar ratio of the polymers and the amount of immobilized sorbate [7, 11].

7. The structures of the synthesized modifiers based on XZ, as well as their complexes with levothyroxine-Na pentahydrate, were investigated using techniques such as SEM (Scanning Electron Microscopy), FTIR (Fourier Transform Infrared Spectroscopy), UV-Vis spectroscopy, TGA (Thermogravimetric Analysis), DTA (Differential Thermal Analysis), and X-ray phase analysis. The nature of the interactions in the complex formation was determined. It was shown that the interaction between these hydrogels and levothyroxine-Na pentahydrate occurs mainly through hydrogen bonding, electrostatic interactions, and orientation forces [13].

8. The acute and chronic toxicity of XZ-based polymer hydrogels and their immobilized levothyroxine was tested in vivo on mice and rabbits in the relevant laboratories of the Azerbaijan Medical University. Positive results regarding their bioactivity were confirmed in the provided reports [5, 13].

The main points of the dissertation are presented in the following articles:

1. Safaraliyeva, S.F., Zeynalov, N.A., Synthesis of Bioside Chitosan Derivatives As a Drug Carrier // Chemistry of Organoelement Compounds and polymers,-2019, -p.352.

2. Safaraliyeva, S.F., N- Methyl, N- Benzyl Derivatives of Chitosan As A Carrier of The Thyroid Preparation / S.Safaraliyeva, D.Tagiyev, N.Zeynalov[et al.] // Modern Achievements of Pharmaceutical Technology And Biotechnology /Ukrain,Kharkov,-2019,-p.33.

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Diverse Environmental Conditions // Asian Journal of Chemistry,- 2024, v.36, № 5, -p.1067-1071.

11. Safaraliyeva, S.F. / Preparation of Chitosan–Arabinogalactan Based Hydrogel/II International Scientific and Practical Conference «Innovations in education: prospects and challenges of today», Sofia, Bulgaria,- 2024,16-19 January, -p.43.

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13. Сафаралиева, С.Ф., Состав На Основе Хитозана Арабиногалактана Для Длительного Действия Пентагидрата Левотироксина Натрия / Евразийский Патент На Изобретение № 046579 / Гумматов, А.Ф., Зейналов, Н.А, Тапдыгов, Ш.З.–2024.

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