ABSTRACT

on dissertation for the degree of Doctor of Philosophy (PhD)
6D072100 – “Chemical Technology of Organic Substances”

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Developing technology of hydrogel formulations and dressings

This dissertation is devoted to the preparation of novel hydrogel dosage forms, study of their physicochemical properties, establishing the patterns of intermolecular interactions of chitosan with poly(2-ethyl-2-oxazoline), as well as development of the technological scheme for preparation of polymeric films and wound dressings based on chitosan.

All studies were conducted for the first time, the results are presented as in the form of the article in peer-reviewed journals with a high impact factor according to the Thomson Reuters and Scopus databases and three articles in journals recommended by the Committee for the Control of Education and Science, Ministry of Education and Science of the Republic of Kazakhstan.

Relevance of the research topic.

In many cases, the treatment of various skin injuries or burns, inflammatory skin diseases, chronic wounds and the prevention of infectious diseases upon skin injuries continue to be a complex and intractable issues due to the resistance of various types of microorganisms to existing antimicrobial agents. The growing demand for novel effective dressings, the inefficiency of cotton-gauze dressings for the treatment of exudative wounds and high cost of dressings of foreign manufacturers that significantly limiting their use in domestic health care, put a new task – the development of a wound dressing that allows effectively, with prolonged action (up to several days), and thoroughly absorb wound discharge, therefore providing only “vertical drainage” and therapeutic effect.

Another important task of modern chemistry of biomedical polymers is the development of novel efficient dosage forms that could provide sustained and controlled release of drugs and their targeted delivery to the site of the pathological process. The prospect of preparing polymer carriers with the prolonged action of drugs is due to the fact that most drugs used in practical medicine have a short-term pharmacological effect with the manifestation of side effects on the body. In this regard, the development of dosage forms (films, gels) based on water-soluble and cross-linked polymers is considered as the most promising solution to this issue and represents the undoubted importance and relevance of the research topic. The application of polymeric excipients as drugs carriers creates certain advantages over conventional dosage forms, namely, it allows to obtain a prolonged therapeutic effect providing a uniform concentration of the drug in the body during an extended time period, reduce side effects and decrease the overall consumption of the drug in the treatment.

Previously, at the Department of Chemistry and Technology of Organic Substances, Natural Compounds and Polymers, Al-Farabi KazNU, film dosage forms with prolonged action of drugs with different biological activity; hydrogel wound dressings based on PVP/PEG/agar-agar structured with silver nanoparticles (NPs) as well as wound dressings containing a phytopreparation “Alkhidin” and an anaesthetic “Rikhlokain” were developed and their physico-chemical and therapeutic properties were studied.

This work undertaken is a continuation of these studies and is devoted to the development of the technology for the preparation of hydrogel materials with antimicrobial activity as wound dressings and films for biomedical applications.

The objective of the dissertation is the scientific interpretation of the principles of preparation of novel hydrogel wound dressings to treat wounds of various etiologies and mucoadhesive dosage films for application in ophthalmology, otolaryngology, gynecology, as well as the characterization of the physicochemical and biomedical properties of the developed formulations, as well as the development of the technological scheme for the preparation of film materials and wound dressings based on chitosan.

In accordance with above objective, the work included the solution of the following **tasks:**

- Synthesis and establishing the basic laws of the formation of hydrogels based on chitosan using radiation crosslinking;
- Study of the physicochemical, mechanical, antibacterial and adhesive properties of the obtained hydrogel wound dressings based on chitosan;
- Establishing the patterns of intermolecular interactions of chitosan with poly(2-ethyl-2-oxazoline) using FTIR spectroscopy, thermoanalytical, X-ray diffraction analysis, scanning electron microscopy techniques;
- Establishing the optimal conditions of preparation of films based on chitosan and poly(2-ethyl-2-oxazoline) using film casting technique and thermal cross-linking; the study of their mucoadhesive properties and the release of drugs from polymer matrices using *in vitro* and *in vivo* methods, conducting the microbiological studies of polymeric films;
- Development of the preparation technology of hydrogels and film dosage forms based on chitosan (calculation of the material balance, a functional diagram of automation, feasibility study).

Objects of study: hydrogel wound dressings based on chitosan (CHI) and chitosan with silver nanoparticles (CHI/Ag), films based on chitosan and poly(2-ethyl-2-oxazoline) (CHI/POZ), cross-linked films based on CHI/POZ.

Subject of scientific research: radiation cross-linking and the basic laws of the formation of hydrogel based on CHI and CHI/Ag, the physicochemical properties of hydrogels based on them, the intermolecular interactions of CHI with POZ, the physicochemical properties and microbiological studies of film dosage forms based on chitosan and poly(2-ethyl 2-oxazoline).

Methods. A set of state-of-the-art research and analysis techniques were used in this work: Fourier transform infra-red spectroscopy, thermogravimetric analysis (TGA),

differential scanning calorimetry (DSC), wide-angle X-ray diffraction (WAXD), scanning electron microscopy (SEM), gravimetry, dialysis, mechanical tests (Texture Analyzer), fluorescence spectrometry, UV/Vis spectroscopy, microbiological tests, studies of *in vivo* and *in vitro* adhesive and mucoadhesive properties of hydrogels and films.

The source base and research materials are of 202 sources of literature on the radiation-induced hydrogel crosslinking, the patterns of silver nanoparticle formation, intermolecular interactions of various polymers in the mixtures, investigation and preparation of mucoadhesive dosage forms, as well as other fields of natural science that related to the topic of this study.

Scientific novelty:

The basic laws of the formation of hydrogels based on poly(vinylpyrrolidone) and chitosan in the process of radiation-induced crosslinking were established. It was shown that the use of chitosan allows to obtain hydrogel dressings containing a smaller percentage of agar-agar without deterioration of mechanical performance and operational characteristics compared to commercially available AQUA DRESS® dressings. It was found that the gelation process is significantly affected by the content of chitosan in the hydrogel, its molecular weight and the radiation dose of the reaction mixture.

For the first time, the compatibility of chitosan with poly(2-ethyl-2-oxazoline) were studied using scanning electron microscopy, thermogravimetric analysis and wide-angle X-ray diffraction techniques. It was shown that chitosan with poly(2-ethyl-2-oxazoline) forms intermolecular hydrogen bonds between the hydroxyl and amide groups of chitosan and the carbonyl group of poly(2-ethyl-2-oxazoline).

For the first time, soluble and cross-linked films based on CHI/POZ were prepared. The formulations for the preparation of cross-linked films were developed and optimized, the basic laws and conditions of thermal cross-linking were established. It was found that the swelling ability of CHI/POZ film samples decreases with an increase in the temperature of thermal cross-linking from 100 to 110 °C and does not depend on the heat treatment time during 4-8 hours.

Experimental film samples based on CHI/POZ prepared for the first time were studied as the polymer matrix for the encapsulation of drugs. The kinetics of drug release from films based on linear and cross-linked CHI/POZ polymers was studied. It was established that the presence of chitosan in the film provides the prolongation of the drug.

For the first time, the antibacterial activity of CHI/POZ based films against *Escherichia coli* was studied. It was established that bactericidal activity is due to the presence of POZ in the film. The bactericidal properties provide the possibility of using these films as potential dosage forms for targeted drug delivery in ophthalmology and gynecology.

For the first time, the mucoadhesive properties of films based on CHI/POZ were established using *in vitro* and *in vivo* tests as well as cutting-edge methodologies, such as determination of the retention time of the film on a freshly excised bovine cornea *in vitro*; determination of the retention time of films on the

eyes of chinchilla rabbits *in vivo*; determination of the mucoadhesive detachment force from sheep vaginal mucosa. It was established that with an increase in the content of CHI in the films, it exhibits mucoadhesive properties due to the formation of hydrogen bonds and electrostatic interactions between the functional groups of chitosan and mucin (a component of the mucous membrane).

The theoretical significance of the work.

Establishing of the basic laws of the formation of hydrogels based on CHI and CHI/Ag using radiation cross-linking provides a significant contribution to the development of theoretical knowledge about the methodologies for the preparation of hydrogel wound dressings and their physicochemical properties.

The results of the study of intermolecular interactions between chitosan and poly(2-ethyl-2-oxazoline) could be used in research practice in the development of other highly effective systems with controlled delivery of formulations.

The practical significance of the study.

In the course of the study, novel mucoadhesive dosage forms based on chitosan and poly(2-ethyl-2-oxazoline) were developed containing an antibiotic ciprofloxacin and an anaesthetic lidocaine hydrochloride as well as hydrogel dressings based on chitosan/silver with antimicrobial activity, which could be used in postoperative care, in the treatment of purulent diseases and sores. The technological scheme and the functional scheme of automation of the preparation processes of polymer films and wound dressings based on CHI were developed.

The main provisions for the defense:

- 1. The gel formation in the process of radiation crosslinking is significantly affected by the content of chitosan in the initial reaction mixture, its molecular weight and radiation doses of the reaction mixture
- 2. The physico-chemical and physico-mechanical characteristics of the obtained hydrogel dressings substantially depend on the content of CHI and silver nanoparticles in hydrogels;
- 3. High compatibility of mixtures based on chitosan and poly(2-ethyl-2-oxazoline) is due to the formation of hydrogen bonds between the functional groups of these polymers;
- 4. The mucoadhesive properties of the resulting film materials are largely determined by the presence of chitosan in their composition;
- 5. The use of CHI/POZ polymer blends makes it possible to obtain mucoadhesive film materials with prolonged release of drugs.

The main results of the dissertation research were published in 9 scientific papers, including:

- an article published in a peer-reviewed international scientific journal with high impact factor according to Scopus and Thomson Reuters databases;
- three articles published in journals recommended by the Committee for the Control of Education and Science, Ministry of Education and Science of the Republic of Kazakhstan;
- five abstracts of reports in foreign and republican international conferences, seminars and symposia.

The structure and volume of the dissertation.

The dissertation includes an introduction, four sections, a conclusion, as well as a list of 202 references. The work is presented on 135 pages, contains 54 figures, 29 tables.

Based on the results of the dissertation research, the following conclusions are made:

1. Novel hydrogels based on chitosan were prepared using radiation crosslinking technique, which exhibit higher sorption characteristics and adhesive properties compared to commercially available AQUA DRESS® dressings. It was found that the use of chitosan allows to obtain wound dressings containing a smaller percentage of agar-agar without deterioration of mechanical performance and operational characteristics. Moreover, an increase in chitosan content led to the formation of polymer network with greater porosity, which contributes to the increase in the swelling ability of the hydrogel.

2. For the first, the mechanical, adhesive and antimicrobial properties of novel hydrogel materials based on chitosan were systematically investigated. It was shown that an increase in the radiation dose for obtained hydrogels resulted an increase in their elastic modulus and a decrease in the relative elongation at break, which is due to the formation of network structure with a large number of cross-links. Moreover, the presence of silver nanoparticles (NPs) in the composition of the polymer hydrogels led to their improved mechanical characteristics. It was established that the obtained hydrogels have a higher adhesive ability to the skin compared to AQUA DRESS® dressings, which is obviously due to the presence of chitosan in their composition. The presence of a synergistic effect with respect to antibacterial properties of dressings containing chitosan and silver nanoparticles was revealed.

3. For the first time, the intermolecular interaction between chitosan and poly(2-ethyl-2-oxazoline) was studied using sophisticated research techniques. IR-spectroscopy data, the presence of a single glass transition temperature on DSC thermograms of blends, a shift of a wide diffraction peak from 23.3° to 19.2° in WAXD diffraction patterns indicate the formation of hydrogen bonds between the hydroxyl and amide groups of CHI with the carbonyl groups of POZ, therefore demonstrating the high compatibility of chitosan and poly(2-ethyl-2-oxazoline) blends.

4. For the first time, dosage forms in the form of water-soluble and cross-linked films based on chitosan and poly(2-ethyl-2-oxazoline) of various compositions have been developed. The resulting polymer films were investigated and evaluated as mucoadhesive dosage forms using *in vitro* and *in vivo* tests. It was shown that the mucoadhesive properties of the films are due to the presence of chitosan in their composition, the amino groups of which through electrostatic interactions, bind to the negatively charged component of the mucous membranes – mucins. Sustained release of drugs (fluorescein sodium, ciprofloxacin, lidocaine monohydrochloride) from CHI/POZ polymer films was established, and the possibility of obtaining mucoadhesive dosage forms based on these polymers as ocular, vaginal and buccal films with controlled release was shown. The results of microbiological testing of films with the drug substance (ciprofloxacin) indicate a high antibacterial activity of

these samples against *Staphylococcus aureus* and *Escherichia coli.*

5. Technological schemes for preparation of hydrogel dosage forms and dressings based on chitosan were developed. The basic material flows are calculated, a consolidated material balance is drawn up. A functional diagram of the automation of a unit for production of the polymeric film, in particular a block for preparing the molding solution was developed, and the main technical and economic indicators for the production of the polymeric film were calculated.

Assessment of the completeness of the solution of tasks. The completeness of the solution of the tasks was achieved using radiation synthesis of hydrogels, the formation of films from polymer aqueous solutions and the study of physicochemical, microbiological, mucoadhesive properties using modern widely tested research methodologies (Fourier-transform infrared spectroscopy, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), gravimetry, mechanical testing of polymeric films and hydrogels, scanning electron microscopy (SEM), mucoadhesion study using *in vivo* and *in vitro* tests. The tasks are completely solved.

Assessment of technical and economic efficiency proposed in the dissertation. The results are characterized with a high scientific and practical level. Novel wound dressings synthesized in this work expand the range of domestic wound dressings with antimicrobial activity. The developed film dosage forms based on water-soluble and cross-linked (water-swelling) polymers have significant advantages. Their application allows to increase the duration of the therapy, reduce the toxic and side effects of drugs due to the prolonged action, thereby reducing the number of doses of the drug intake and decrease the overall consumption of drugs in the treatment.