ABSTRACT

of the thesis for the degree of Doctor of Philosophy (Ph.D.) in the educational program 8D07105 – «Chemical Technology of Organic Substances»

Shatabayeva Elvira

on the topic: «The development of new adhesive polymers based on gelatin for dosage forms»

The relevance of the work

The development of new adhesive polymers based on gelatin currently represents an important research direction in pharmaceutical science aimed at creating drug formulations with enhanced mucoadhesive properties. Effective adhesion of active substances to mucous membranes is a critical factor determining their bioavailability and therapeutic efficacy, particularly in cases where prolonged action on target tissues is required. Gelatin is a natural proteinous polymer derived through partial hydrolysis and heat denaturation from collagen, extracted from bones, cartilage, connective tissues, and skins of animals. Due to its distinctive physicochemical properties, including high biocompatibility, biodegradability, non-toxicity, and non-immunogenicity, gelatin is considered a highly favorable material for pharmaceutical applications. It has been classified as safe for use in accordance with the guidelines established by the U.S. Food and Drug Administration (FDA). Gelatin readily dissolves in warm water and has the ability to form physically crosslinked thermo-reversible hydrogels upon cooling and exhibits a melting point close to body temperature. These distinctive characteristics are the key factors driving its extensive applications in the pharmaceutical, food, and cosmetic industries.

Transmucosal drug delivery provides several important advantages, involving the ease of dosage form administration, non-invasive options, prolonged residence time of formulations, improved drug bioavailability, avoidance of hepatic first-pass metabolism, reduced dosing frequency, and possibility for quick termination of therapy when needed. Traditionally, gelatin is considered as a polymer with poor mucoadhesive properties. The adhesion of gelatin is attributed to its amphoteric nature, which results in the formation of weak electrostatic interactions with mucosal surfaces; however, these interactions are dependent on the pH of the mucosal environment. However, the presence of reactive sites within gelatin molecule, such as amine, carboxylic and hydroxyl groups, provides the opportunity for conjugation with functional groups leading to changes in its physical and chemical properties. Research indicates the potential benefits of modifying gelatin with various chemical compounds to improve its mucoadhesive properties. Such modified forms of gelatin can provide stronger adhesion to mucus by forming covalent bonds with cysteine-containing subdomains of mucin on mucosal surfaces. This opens new avenues for the application of gelatin in drug formulations that require prolonged contact with mucous membranes and contributes to improved bioavailability and efficacy of therapeutic agents by reducing dosage and increasing retention time on the mucosal surface. Furthermore, the potential utilization of these forms for transmucosal drug delivery may facilitate the development of new therapeutic methods, enhancing the transport of active substances and improving patient tolerance.

The Department of Chemistry and Technology of Organic Substances, Natural Compounds, and Polymers at Al-Farabi Kazakh National University has been successfully conducting research for several years on the development of drug formulations based on polymers with mucoadhesive properties, as evidenced by numerous publications in high-impact journals. Previously, the department has developed transmucosal drug formulations based on gellan gum, chitosan, and poly(2-ethyl-2-oxazoline), as well as iodine-containing compositions based on poly(2-oxazolines), which exhibit mucoadhesive properties.

This work represents a continuation of the aforementioned research and focuses on the development of novel adhesive polymers based on gelatin, as well as the formulation of dosage forms exhibiting mucoadhesive properties for drug delivery purposes.

The aim of the dissertation research is to the develop new modified polymers based on gelatin with enhanced mucoadhesive properties and the creation of dosage forms for use in otolaryngology and gynecology.

Tasks

1) Synthesis of gelatin derivatives with unsaturated anhydrides and investigation of their physicochemical and toxicological properties;

2) Preparation of microparticles based on gelatin and its methacryloylated, itaconoylated, and crotonoylated derivatives;

3) Development of film materials based on gelatin and its derivatives, as well as their blends with polyvinyl alcohol (PVA) containing metronidazole, and investigation of their physicochemical characteristics;

4) Study of the obtained materials as mucoadhesive forms for drug delivery;

5) Development of a technology for obtaining microparticles based on gelatin and its derivatives, calculation of the material balance of production, selection of main technological equipment, and economic justification.

Research methods

Fourier Transform Infrared Spectroscopy (FTIR), ^{1}H NMR Spectroscopy, Dynamic Light Scattering (DLS) method, Scanning Electron Microscopy (SEM), rheological studies, mechanical testing, fluorescence spectrophotometry, toxicological testing, in vitro studies on sheep and porcine vaginal and nasal tissues.

The main provisions submitted for defense

1. Introduction of unsaturated functional groups, namely crotonoyl, itaconoyl, and methacryloyl, significantly enhances the mucoadhesive properties in the following order: gelatin < gelatin-CA_6 < gelatin-IA_{10} < gelatin-MA_6.

2. The chemical modification of gelatin with unsaturated groups does not lead to an increase in the polymer's toxicity. The results obtained from the MTT assay indicate that cell viability remains high (>70%) after exposure to gelatin and its modified derivatives.

3. Films based on gelatin and its modified derivatives, exhibit rapid release of metronidazole (>80% saturation within 8 hours), films based on blend of PVA with gelatin and its modified derivatives demonstrate a more sustained release of metronidazole (>50%).

The main results of the study and the rationale for the novelty and

importance of the results obtained

1. For the first time, gelatin was modified with itaconic and crotonic anhydrides to enhance mucoadhesive properties, and their structures were studied using IR and NMR spectroscopy and TNBS analysis. The isoelectric points of itaconoylated and crotonoylated gelatin were determined for the first time through using DLS and viscometric methods. It was found that the IEPs of itaconoylated and crotonoylated gelatin derivatives were within the range of pH 2.5–3.8, which is lower than that of native gelatin (pH 7.0). The rheological properties of itaconolated, crotonoylated, and methacryloylated derivatives of gelatin were investigated for the first time. The study revealed that the melting temperature and gelation temperature of these derivatives are in the ranges of 23.9 to 27.6 °C and 5.7 to 15.6 °C, respectively. These values are lower than those of native gelatin, which exhibits a melting temperature of 30.4 ± 0.3 °C and a gelation temperature of 17.2 ± 1.2 °C during cycles of heating and cooling.

2. The MTT assay was used to assess the *in vitro* cytotoxicity of gelatin and its modified derivatives (gelatin-MA_6, gelatin-CA_6, gelatin-IA_{10}). The results indicated that cell viability remained high (>70%) after exposure to gelatin and its derivatives at concentrations of 1.3% and 5%. An *in vivo* mucosal irritation test was conducted for the first time, which involves mucus production (MP) upon contact with irritants. It was found that after exposure to gelatin, gelatin-MA_6, gelatin-CA_6, and gelatin-IA_{10} (pH 7.32–7.41), MP was 3 ± 1%, 2 ± 1%, 3 ± 1%, and 2 ± 1%, respectively, indicating a non-irritating effect.

3. Microparticles based on gelatin and its modified derivatives (gelatin-MA, gelatin-IA, gelatin-CA) were obtained using spray drying. It was found that the microparticles had a spherical shape with a wrinkled surface, free from inclusions, pores, and cracks. The average particle diameter was approximately 5 ± 1 μm.

4. Polymeric films based on gelatin and its modified derivatives (gelatin-MA, gelatin-IA, gelatin-CA), as well as their blends with PVA, were obtained. The investigation of the morphology of cross-sections of the samples using SEM methods confirmed the presence of a completely homogeneous structure of the films, with no signs of phase separation or phase boundaries. The mechanical properties of the obtained film materials were studied using a texture analyzer. It was found that an increase in the PVA content in the film samples led to a gradual increase in tensile strength and puncture strength.

5. The kinetics of metronidazole release from film materials based on gelatin and its modified derivatives (gelatin-MA, gelatin-IA, gelatin-CA), as well as their blends with PVA, were studied for the first time. It was found that films based on modified gelatin derivatives with PVA are capable of controlling the rate of metronidazole release, which can be important for achieving a prolonged therapeutic effect upon intravaginal administration. The addition of PVA as a plasticizer result in the formation of a denser and more stable polymer matrix, which attenuates the swelling of the film and the diffusion of metronidazole, thereby facilitating a prolonged release of the active ingredient.

6. The mucoadhesive properties of gelatin and its modified derivatives in various dosage forms (physical gel, microparticles, polymeric films) were studied for the first-time using flow analysis and *ex vivo* peel test. It was found that the

introduction of unsaturated functional groups (crotonoyl, itaconoyl, and methacryloyl) into the structure of gelatin significantly improved the mucoadhesive properties, resulting in more effective retention on nasal and vaginal mucosa compared to native gelatin.

7. A fundamental technological scheme for the production of microparticles based on modified gelatin was developed for the first time. The material balance of production for 1000 kg of product per year and the main economic indicators of production were calculated. It was found that the cost of the manufactured product, payback period, and profitability were 6,878.15 tenge, 3.47 years, and 28.81%, respectively.

Theoretical and practical significance of the results

The study has generated new insights into the structure and properties of modified gelatin derivatives, as well as the effects of chemical modification on their rheological and mucoadhesive characteristics. These findings constitute a significant advancement in the scientific foundation for the continued development of mucoadhesive drug formulations based on gelatin. The adhesive polymers and corresponding mucoadhesive drug forms developed in this research are recommended for applications in otolaryngology and gynecology, given their potential to enhance therapeutic efficacy. The enhanced mucoadhesive properties of these formulations promote improved interaction with mucosal surfaces, thereby increasing the bioavailability of active substances and facilitating deeper and more prolonged therapeutic effects. Consequently, investigations into gelatin modification reveal new avenues for creating more effective therapeutic strategies, which may substantially enhance clinical outcomes and patient quality of life. These findings underscore the significance and necessity of further exploration of modified gelatin derivatives in the context of innovative drug formulation development, potentially leading to meaningful advancements in pharmaceutical practice.

Compliance with the directions of development of science or government programs (projects)

The dissertation work was carried out within the framework of the grant funding program AP14869167 "Development of new drug forms with improved mucoadhesive properties for intravesical chemotherapy" by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan 2022-2024 year.

Publications

The dissertation work has resulted in the co-authorship of 4 scientific publications, comprising 1 article in the internationally peer-reviewed journal Biomacromolecules (Q1, percentile 92%), 1 review article in the journal «Chemical Bulletin of Kazakh National University» included in the list of journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Science and Higher Education of the Republicof Kazakhstan, as well as materials and abstracts of 2 reports at international scientific conferences.

Description of the contribution of the doctoral student to the preparation of each publication

The doctoral student was directly involved in obtaining experimental data, processing, and interpreting experimental results for the preparation of the article

«Enhancing Mucoadhesive Properties of Gelatin through Chemical Modification with Unsaturated Anhydrides» as the first author, published in the journal «Biomacromolecules» (American Chemical Society, 2024, Open Access. https://doi.org/10.1021/acs.biomac.3c01183, Q1, IF 6.2).

The doctoral student was directly involved in the collection of literature and data processing and the design of the review article «Gelatin: sources, preparation and application in food and biomedicine» as the first author, published in the journal «Chemical Bulletin of Kazakh National University» (98(3), 28-46, 2020, https://doi.org/https://doi.org/10.15328/cb1112).

The doctoral student was directly involved in obtaining experimental data, processing, and interpreting experimental results for the preparation of materials and abstracts of 2 reports at international scientific conferences.