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DEVELOPMENT OF THE COMPOSITION AND TECHNOLOGY OF A WOUND-HEALING GEL FOR THE MILITARY

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Резюме. Лікування інфікованих ран залишається важливою проблемою у щоденній хірургічній практиці. Одним із напрямків пошуку ефективного способу лікування таких ран є розробка комбінованих лікарських препаратів мультифункціональної дії для зовнішнього застосування, що містять у своєму складі кілька діючих речовин, що мають комплексну терапевтичну активність щодо основних субстратів складної рани, яка довго не загоюється.

Нами розроблено фармацевтичну композицію на основі інноваційних субстанцій – комплекс хітозан-хімопсин для лікування інфікованих ран у лікарській формі гель для зовнішнього застосування. Комплекс хітозан-хімопсин забезпечує пролонговану протеолітичну дію ферменту, відновлює мікроциркуляцію в стінках рани, покращує обмінні процеси та знімає місцеве запалення.

Запропоновано склад гелю з композицією фармацевтичних субстанцій хітозану та хімопсину, який виявляє протеолітичну, осмотичну, антибактеріальну та ранозагоювальну активність. Проведено підбір оптимального гелеутворювача та допоміжних речовин, їх концентрації та сумісності.

Abstract. Treatment of infected wounds remains an important challenge in daily surgical practice. One of the directions in the search for an effective method of treating such wounds is the development of combined drugs of multifunctional action for external use, containing several active substances in their composition, which have complex therapeutic activity against the main substrates of a complex, long-unhealed wound.

We have developed a pharmaceutical composition based on innovative substances - a chitosan-chymopsin complex for the treatment of infected wounds in the medicinal form of a gel for external use. The chitosan-chymopsin complex provides a prolonged proteolytic effect of the enzyme, restores microcirculation in the wound walls, improves metabolic processes and relieves local inflammation.

A gel composition with a composition of pharmaceutical substances chitosan and chymopsin is proposed, which exhibits proteolytic, osmotic, antibacterial and wound-healing activity. Selection of the optimal gelling agent and auxiliary substances, their concentration and compatibility were carried out.

Keywords: gel, chitosan, chymopsin, quality indicators.

Introduction: Difficulties in wound healing remain a pressing problem in surgical practice. The widespread prevalence of wound pathology and associated complications, difficulties in timely diagnosis and treatment, and the resulting

economic damage are developing into a serious social problem. Long-term treatment of wounds in a hospital and its outpatient rehabilitation leads to significant material costs, thereby determining the significance of the problem [1, 2, 3].

The wound process is strictly cyclical, and each preliminary stage is preparatory for the next [4]. In the case of an open chronic wound, tissue regeneration slows down and the damage shows no or insufficient signs of healing, despite concomitant therapy and time. With prolonged adverse effects, the wound process becomes chronic: diabetic foot, bedsores, venous trophic ulcers of the leg, ischemic ulcers. Such pathological conditions are characterized by the presence of signs of all 3 phases of the wound process at once; therefore, the treatment of chronic wounds is an extremely complex clinical problem [3].

In recent decades, local enzyme therapy has been used in the arsenal of basic medicines to eliminate purulent-necrotic processes [5]. Trypsin, produced by the domestic industry and is a medicinal product, is widely used. This choice is due to several reasons. When trypsin comes into contact with a purulent-necrotic wound, it breaks down non-viable tissues that are usually removed during surgical treatment [6].

Chitin is the basis of the skeletal system of tissues in crustacean shells, and is also part of the cell wall of fungi and some bacteria. In crustacean organisms, chitin is present in the form of a chitin-carbonate complex, therefore, to isolate chitin from the main types of raw materials (crabs, krill, lobsters), technological operations such as demineralization and deproteinization are carried out, including the treatment of crushed shells with a solution of hydrochloric acid and sodium hydroxide with further washing water, removing coloring pigments using bleaching reagents and lipophilic substances, washing with alcohol and ether [7].

Numerous literature data indicate the promising use of chitin and their derivatives in medicine, biotechnology, pharmaceuticals, and the food industry [8]. Chitin has anti-inflammatory, antibacterial, immunostimulating and sorbing effects, so the creation of new drugs based on it that have a wound-healing effect is promising.

Aim: development of a gel composition with a pharmaceutical composition based on innovative substances - a chitosan-chymopsin complex, exhibiting proteolytic, osmotic, antibacterial and wound-healing effects

Materials and methods: The object of the study is the pharmaceutical substances chitosan and chymopsin, as well as the gel made on their basis.

Research methods. Information retrieval, information analytical, organoleptic, physicochemical, pharmacotechnological.

Results and discussion: The principle of “quality by design” (Quality-by-Design, QbD) aims to achieve the properties of the drug necessary for the patient by ensuring continuous compliance with the planned value of all parameters and characteristics of the technological process, functionally related to safety and effectiveness.

The goal of pharmaceutical development research is to design the product and its manufacturing process to achieve the intended effectiveness and expectations of patients, healthcare professionals and regulatory authorities.

Pharmaceutical technology requires multifactorial scientific research on the relationship with each other. One of its tasks is to define the design space - a

multifactorial combination and interaction of input variables (for example, material characteristics), as well as process parameters at which quality is proven to be ensured.

As part of the qualification work in accordance with international requirements and approaches [9], we studied the following elements of pharmaceutical development:

– active ingredients of the medicinal product: pharmaceutical substance;

– excipients;

– medicinal product: development of dosage form; physical and chemical properties (quality control); technological process development; packaging (closure) system; microbiological properties.

The selection of active ingredients was based on theoretical and experimental basis. Since the finished dosage form is a combined drug consisting of complexes of chymopsin and chitosan, it was necessary to study the compatibility of the active substances with each other in order to avoid the formation of new compounds that could distort the therapeutic effect or lead to the formation of a toxic product.

It was important not only to obtain a drug with high specific activity, but also to maintain it in accordance with the shelf life of the drug [10].

The substance under study is an innovative chitosan-chymopsin complex. The composition of the chitosan-chymopsin complex is presented in the table 1.

Table 1

Composition of the chitosan-chymopsin complex (n=5, P=95 %)

Compound	Quantity, g
Chymopsin	0.20
Chitosan	1.00
Purified water	to 100.00

Description - a lyophilized mass in the form of powder, lumps or plates of white or light-yellow color, with a faint odor of acetic acid, is a combined pharmaceutical substance of a proteolytic enzyme preparation - chymopsin and a high molecular weight polysaccharide of natural origin - chitosan acids.

Solubility. Forms colloidal systems with water; insoluble in ether, hexane, ethanol 95 % [11].

Content: from 99.0 to 101.0 %.

The choice of the chymopsin enzyme complex as an integral part of the chitosan-chymopsin proteolytic complex is explained by the fact that the use of a natural mixture of proteolytic enzymes (chymotrypsin and trypsin) increases the effectiveness of therapy by enhancing the therapeutic effect due to faster and more complete hydrolysis of peptide bonds [12].

The components of chymopsin cleave various peptide bonds in the protein molecule: trypsin hydrolyzes peptide bonds containing arginine and lysine residues; Chymotrypsin cleaves peptide bonds with residues of aromatic amino acids - tyrosine and tryptophan [13].

An artificial model compartment was developed and a surface was selected that imitated a wound defect in relation to the compartment. When selecting substances used in the development of the model compartment, the following criteria were taken into account:

– the rheological parameters of the model fluid should be standard and as close as possible to those characteristic of the wound;

– the substances necessary to create a model liquid must be relatively accessible (availability of chemicals on the domestic market, relative cheapness);

– substances must be safe;

– the process of creating a model detachable element must be reproducible (ease of preparation) [14, 15].

During the development process, solutions of the following substances were studied:

– sodium salt of polycarboxymethyl cellulose ether (NaCMC);

– methylhydroxyethylcellulose;

– HPMC.

For this purpose, aqueous solutions of the listed substances were prepared with different concentrations: NaCMC 1 – 2 %; methylhydroxyethylcellulose 1-2 % and HPMC from 0.5-6.0 %. The resulting solutions were examined for viscosity using a REOTEST-RN4.1 type viscometer in accordance with SPhU 2.1.

Based on the data obtained, the dependence of the viscosity of the solutions under study on their concentration was assessed (table 2).

Table 2

Viscosity of the studied cellulose derivative solutions (n=5, P=95 %)

Solutions of sodium salt of carboxymethylcellulose (NaCMC)							
Concentration, %	1.0 ± 0.1	2.0 ± 0.1	-	-	-	-	-
Viscosity, Pa·sec	0.4 ± 0.1	0.7 ± 0.1	-	-	-	-	-
Methylhydroxyethylcellulose solutions							
Concentration, %	1.0 ± 0.1	2.0 ± 0.1	-	-	-	-	-
Viscosity, Pa·sec	0.3 ± 0.1	0.4 ± 0.1	-	-	-	-	-
Hydroxypropyl methylcellulose (HPMC) solutions							
Concentration, %	0.5 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	3.0 ± 0.1	4.0 ± 0.1	5.0 ± 0.1	6.0 ± 0.1
Viscosity, Pa·sec	0.1 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	1.4 ± 0.2	1.7± 0.1	2.7 ± 0.1	3.2 ± 0.1

To study the compatibility of the active ingredients of the gel under development with bases, samples of bases were prepared and solutions of active ingredients were introduced individually into the base. The resulting samples were stable compositions with acceptable organoleptic characteristics.

During the development of the composition and technology of the gel base, 9 experimental samples were obtained.

The experimental compositions were a gel matrix multicomponent base of cellulose derivatives, chitosan and acrylic acid derivatives, presented in various concentrations.

In the course of the studies, a significant influence of viscosity modifiers on the biopharmaceutical characteristics was revealed. This is probably due to the different structure of cellulose derivatives, the length of the polymer chains and, as a consequence, the different density of the resulting polymer gel structures.

Despite the fact that chitosan is an integral part of developed pharmaceutical substances, it has been included in the composition of the bases for a number of experiments. The compositions of the samples are presented in the table 3.

Table 3

Compositions of the studied gel samples with the chitosan-chymopsin complex (n=5, P=95 %)

Sample	HPMC, g	PAA, g	Chitosan-chymopsin, g	Glycerin, g	Purified water, g
1	4.00	0.20	1.00	4.00	to 100.00
2	3.50	0.20	1.00	4.50	
3	3.00	0.20	1.00	5.00	
4	2.50	0.10	1.00	5.00	
5	3.50	0.20	2.00	4.50	
6	2.00	0.10	2.00	5.00	
7	1.00	0.10	1.00	5.00	
8	0.70	0.15	1.00	5.00	
9	0.50	0.10	1.00	5.00	

After storage at room temperature for 12 months, the organoleptic properties of gels with the chitosan-chymopsin combination remained unchanged. The tested gel samples were a transparent, milky jelly-like mass. The color of the gels did not change; signs of loss of homogeneity and recrystallization were not detected.

The introduction of lidocaine into the composition does not lead to a sharp change in the viscosity of the system. Lidocaine is a readily soluble active ingredient, and a slight drop in viscosity does not affect the rheological characteristics of the composition. These systems have thixotropic properties; they involuntarily recover from mechanical destruction under load, that is, they are able to almost completely restore their internal structure after removing the load.

Chymopsin is also a readily soluble substance and does not disrupt the structural conformation of carrier polymers. In addition, in all cases, the inclusion of the active substance in the polymer occurs at the level of physical interactions, without the formation of strong covalent bonds.

During the study of quality indicators, a study was carried out on the tightness of the packaging. When 10 tubes of each sample were kept on filter paper for 8 hours at 60 ± 3 C, no staining was observed. Table 4 describes the quality indicators of the selected gel samples with the chitosan-chymopsin combination. Considering the principle of action of the gel being developed, aimed at wound healing, one of the target quality profiles is the presence of high osmotic activity in the gel composition, which leads to rapid cleaning of the wound from necrotic tissue and wound contents.

Table 4

Quality indicators of developed gel samples with a chitosan-chymopsin combination (n=5, P=95 %)

Indicator	Determination methods	Result
Form	Visually	Transparent, jelly-like mass, containing no foreign impurities, milky in color
Identification	1. Reaction with a solution of sulfuric acid with iodine (chitosan) 2. Milk fermentation reaction (chymopsin)	1. The interaction of the aqueous extract of the gel with iodine and a weak solution of sulfuric acid forms the appearance of a purple color. 2. Milk fermentation occurs within a maximum of 50 seconds
pH water environment	Potentiometry	pH aqueous extract of gel 1:25 (by weight) is in the range of 6.5 ± 0.5
Package weight		The average weight of 10 packages must not be less than the specified weight, and the weight of the contents of each tube must not be less than 90 % of the specified weight
Tube tightness		When 10 tubes of each sample are kept for 8 hours at a temperature of 60 ± 3 °C, no spots are observed on the filter paper
Package		50 g each in aluminum tubes with an internal varnish coating and a polypropylene cap. 1 tube is placed in a cardboard pack with instructions
Marking		According to regulatory documentation
Shelf life		Keep out of reach of children, dry, protected from light at a temperature of 15–25 °C. Shelf life: 1 year

Conclusions. 1. The concentration of active ingredients of a combination of chitosan and chymopsin was selected, which would exhibit osmotic, wound-healing and anti-inflammatory activity in the developed gel for the treatment of purulent wound process.

2. Research has been carried out on the selection of excipients and their concentration. It was determined that the optimal gelling agent is polyacrylamide polymer and hydroxypropyl methylcellulose. Glycerin was chosen as excipients at a concentration of 5 %.

3. The quality of the developed soft dosage form of the gel was assessed according to the criteria of regulatory documentation.

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