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Research Article

DEVELOPMENT OF COMPOSITION AND MANUFACTURINGMETHOD FOR COMBINATION DRUG PRODUCT BASED ONCHITOSAN-CONTAINING PHARMACEUTICAL SUBSTANCES

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ABSTRACT

The composition described in current article is based on derivatives of glucosamine and acrylate polymers and is intended for treatment of various infected wounds. A semi-transparent gel demonstrates complex therapeutic activity due to several active pharmaceutical ingredients (AFIs): chitosan, chymopsin, miramistin, and lidocaine hydrochloride. Mechanism of action of the developed drug is complex and includes several therapeutic effects: enzymatic biochemical wound debridement due to lysis of denaturated proteins (without healthy tissues damaging); indirect antimicrobial activity due to chymopsin that promotes lysis of microbial growth medium; direct antimicrobial effect is provided by miramistine; and the pain is reduced by lydocaine and intrinsic cooling effect of gel dosage form. Generalizing the literature data about the products used in the infected wounds treatment, the following AFIs were chosen for the development of the topical gel: complex of proteolytic agent chymopsin and chitosan, chitosan-miramistin complex, and lidocaine anesthetic. Hydroxypropyl methylcellulose, polyacrylamide, and glycerol were utilized as excipients. Proper development of vehicles for gels used in wound treatment can be justified by the necessity of soft action on the wound, required cooling effect, good release of AFIs from the matrix, and prevention of microbial growth.

Keywords:chitosan – miramistin complex (CMC), chitosan – chymopsin complex (CCC),infected wounds, wound treatments,lysozyme, chlorhexidine, miramistin,hydroxypropyl methylcellulose, polyacrylamide.

INTRODUCTION

As the result of experimental studies, chitosan was chosen as an optimal carrier for obtaining complexes with chymopsin and miramistin[1].Chitosan is an amino polysaccharide (2-amino-2-deoxy-beta-D-glucan), which is derived by the deacetylation of chitin(Figure 1)[2].

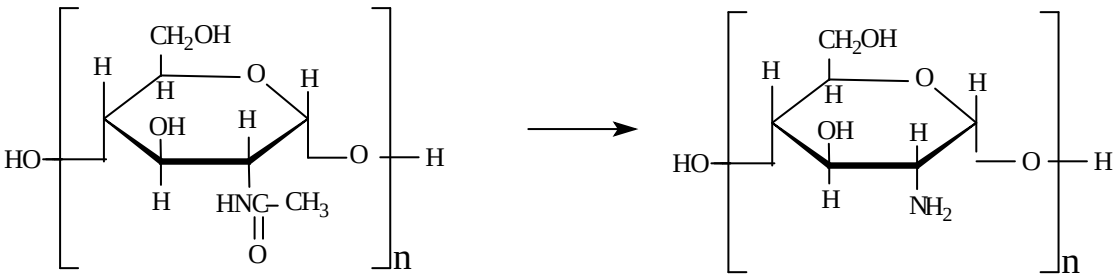

Fig. 1: Chitosan Derivation Scheme

Deacetylation is the process of removal of acetyl functional groups from a chemical compound. When chitin deacetylation degree reaches about 50% it becomes acid-soluble chitosan. Dissolution in acidic media is possible due to protonation of aminogroup adjacent to 2nd carbon atom in D-glucosamine repeating unit which provides polysaccharide with properties of polyelectrolyte. Properties of chitosan solutions depend not only on deacetylation degree, but also on distribution of acetyl groups in polymer chain[3].Chitosan dissolves in dilute solutions of organis acids. Usually, aqueous solutions of acetic acids are used; viscosity of obtained gel is proportional to the chitosan concentration. Free

amino group predetermines polyelectrolytic properties of chitosan[3-5].Unique properties of chitosan – biocompatibility, biodegradability, high biological activity and sorption capacity, and absence of toxic effects – makes this amino polysaccharide one of the few commercially available, ecologically safe polymers, which can, in perspective, serve as novel biomaterials for development and manufacturing of novel active pharmaceutical ingredients (AFIs) for treatment of open wound and burns[6, 7].In order to select proteolytic AFI, interaction of chitosan solution with different enzymatic products (trypsine, proteolytic complex from crab hepatopancreas, and chymopsin) was assessed; chymopsin was chosen as the result of experimental studies[1]. The components of chymopsin cleave different peptide bonds in protein molecule: trypsine hydrolyzes peptide binds between lysine and arginine residues, chymotrypsin breaks bonds between tyrosine and tryptophan residues. Chymopsinrestores microcirculation in wound walls, enhances metabolic processes resulting in local inflammation reduction[8-12].Miramistin was chosen as an antimicrobial AFI due to its pronounced bactericidal activity against both aerobic and anaerobic bacteria, Gram-positive and Gram-negative microorganisms(Figure 2). It is effective against monocultures, microbial associates, and poly resistant hospital strains[10, 11]. Miramistin molecules affect outer membrane of microbialcell, which leads to its destruction, and death of microorganism[13, 14].

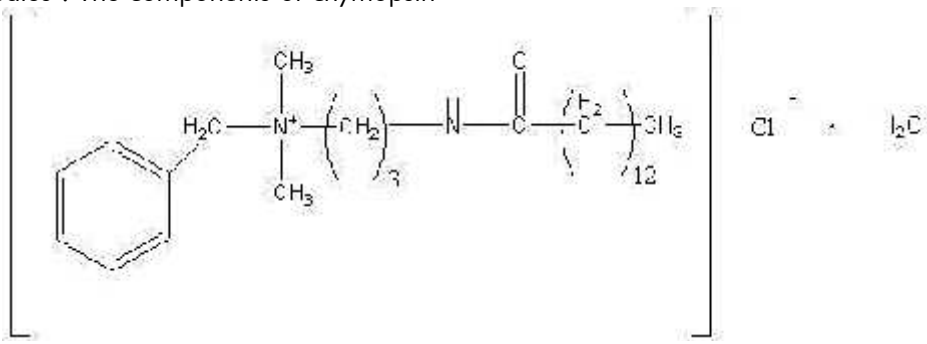

Fig. 2: Structure Formula Of Miramistine

(Benzyl-Dimethyl-[3-(Tetradecanoylamino)Propyl]Azanium Chloride Monohydrate**, $C_{26}h_{47}cln_2o\cdot H_2o$, Mw = 457.14 G/Mol)**

Polysaccharide complex of chitosane and miramistine possesses prolonged antimicrobial and fungicidal activity, enhances functional activity of immune cells, and stimulates local, non-specific immunity. Lidocaine hydrochloride was used as a local anesthetic(Figure 3). It is widely utilized for block, infiltration, and terminal anesthesia. Anesthetic effect mechanism is explained by suppression of nerve conductivity due to sodium channels blockage.

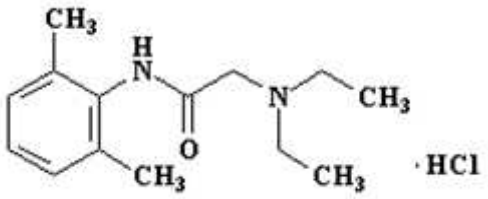

Figure 3.Structure formula of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl \cdot H_2O$, MW = 288.81 g/mol)

Materials And Methods

The following materials and reagents were used in the study: chitosan (NPO "Bioprogress", Russia) (10% moisture content, Product Specification 9289-067-00472124-03, degreeofdeacetylation – 80.0%; kinematic viscosity – not less than 383.7 cSt; molecular weight - about 500 kDa), proteolytic complex from crab hepatopancreas (NPO "Bioprogress", Russia) (Product Specification 9154-032-11734126-10; proteolytic activity (casein substrate) – 0.9 U/mg; proteolytic activity (BApNa substrate) -43 nMol/mg*min;proteolytic activity (azocoll substrate) – 28 U/mg); trypsin from bovine pancreas ("SamsonMed", Russia) (Manufacturer's monograph 42-0179-5943-04, proteolytic activity (casein substrate) – 8.2 U/mg); chymopsin("SamsonMed", Russia) (Manufacturer's monograph 42-0179-5944-04, proteolytic activity (casein substrate) – 9.0 U/mg); miramistine ("Infamed " Ltd., Russia) (Manufacturer's monograph 42-0414-2768-02). All other reagents, unless otherwise stated, were manufactured in the Russian Federation and were of" chemically pure" grade.

Results

Aqueous gel is the preferable dosage form for wound treatment, especially in the case of complicated, open wounds, due to several advantages, namely gentle action with preservation of moist environment, and anesthetic effect. General

rules for gel development require not only careful AFIs selection, but also proper choice of the vehicle (excipients) for the AFIs. During course of the experiment, the choice of excipients was based on the bio pharmacy principles, which govern the presence of AFIs in the dosage form in the soluble state. Selection of matrix gel formers, which also serve as the viscosity modifiers, was optimized in terms of biopharmaceutical characteristics of the dosage form. The gel is usually present on the affected surface for about 24 hours and depends on the following parameters: body and wound temperature; pH of the wound; and its condition. Averagely, wound pH is about 6.4 (slightly acidic), but the progressing acidosis can lower the value to 5.4, whereas pH of undamaged skin is between 3.8 and 5.0.The viscosity of the wound exudate is 0.6-0.8 Pa•s. Our experimental studies have confirmed the literature data about aqueous solutions of cellulose derivatives being the optimal gel forming excipients[1, 4]. Therefore, they were used for increasing aggregate stability and rheological characteristics optimization.

The solutions of the following cellulose derivatives were assessed during the study:

- sodium carboxymethyl cellulose (NaCMC);
- methyl hydroxyethyl cellulose;
- hydroxypropyl methylcellulose (HPMC).

The viscosity of different concentrations of the aqueous solutions of the mentioned excipients (NaCMC – 1-2%, methyl hydroxyethyl cellulose – 1-2%, and HPMC – 0.5-6.0%) was tested on the RHEOTEST-2 viscosimeter (according to GOST 10028 requirements). The assessment of relationship between concentration of tested solutions and their viscosity was done based on the data presented in Table 1.

Table 1: Viscosity Of Tested Cellulose Derivatives Solutions

NaCMC solutions					
Concentration, %	1.0 ± 0.1	2.0 ± 0.1	-	-	-
Viscosity, Pa•s	0.4	0.68	-	-	-
Methyl hydroxyethyl cellulose solutions					
Concentration, %	1.0 ± 0.1	2.0 ± 0.1	-	-	-
Viscosity, Pa•s	0.3	0.45	-	-	-
HPCM solutions					
Concentration, %	0.5 ± 0.1	1.0 ± 0.1	2.0 ± 0.1	2.5 ± 0.1	3.0 ± 0.1
Viscosity, Pa•s	0.1	0.36	1.0 ± 0.1	1.1± 0.1	1.1 ± 0.2
Concentration, %	3.5 ± 0.1	4.0 ± 0.1	4.5 ± 0.1	5.0 ± 0.1	6.0 ± 0.1
Viscosity, Pa•s	1.4 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	2.7 ± 0.1	3.2 ± 0.1

Based on the results of the experimental assessment, the HPMC solutions were selected as excipients, their concentration range corresponding to the viscosity of wound exudate, i.e. between 1.0 ± 0.1 and 1.1 ± 0.1 Pa•s. HPMC solutions of other concentrations were also used in the latter preclinical studies, namely in the osmotic activity assessment. The obtained data served as the basis for preparing the vehicle for wound-healing gel. The AFIs for the gel (chitosan-chymopsin complex (CCC) and chitosan-miramistin complex (CMC)) were prepared in advance. In order to do so, acetic acid was added to the 1% chitosan solution, and the solution was left for 24 hours for swelling and structuring. After that, calculated amount of chymopsin (2.0 g per 100 g of the product) was added to the gel-like solution, and it was left at room temperature for two hours, in order for the proteolytic enzyme to become incorporated in the gel structure. The CMC AFI was prepared in the similar manner: miramistin (0.05 g per 100 g of the product) was added to the chitosan solution, and was left for two hours at room temperature. Obtained gel-like solutions were freeze-dried, i.e., the water contained in the chitosan-based solution of enzymes and antiseptic agent was removed by ice sublimation, bypassing the liquid state. Water vapor is pumped out of the working chamber by vacuum pump and is condensed on the coils of the low-temperature condenser. The freeze-dried material preserves its structure integrity and biological activity to a greater extent, than the air-dried films. After wetting, the original properties of the material are restored. An additional benefit of this procedure is that the excess of the acetic acid used for chitosan dissolution is removed during the process. Using analytical balances, 16.0 g of CCC lyophilized powder, 14.0 g of CMC lyophilized powder, 1.0 g of lidocaine, 20.0 g of HPMC, and 1.0 g of polyacrylamide (PAA) were weighed. Using graduated cylinder, 50.0 ml (62.5 g) of glycerol (relative density at 20ºC – 1.25 g/cm^3) were measured. The vehicle preparation procedure included dissolution and swelling stages. The calculated amount of purified water, preheated to

60-65℃, was measured into the process vessel. Weighed amount of HPMC powder was layered on the water surface and was left for 2-3 hours for swelling (until blobs of the polymer disappear). After that, the solution was mixed using the propeller mixer until a homogenous gel was obtained. Under constant mixing, calculated amount of PAA with predissolved lidocaine were added to the mixture. Addition of second gelling agent leads to the thickening of the gel and increased viscosity.The obtained gel was left for 24 hours to complete structuration and for degasing.

Discussion

On the basis of developed substances, the composition of the combined drug product, containing chymopsin, miramistin, and lidocaine, and manufactured in form of a topical gel, was developed and optimized on the data presented in Table 2.

Table 2: Optimal Composition Of The Product

Gel for topical application	Mass (g) per 100 gram
Chymopsin	0.20
Miramistin	0.05
Chitosan	2.00
Lidocaine	0.10
Polyacrylamide	0.10
HPMC	2.00
Glycerol	5.00
Purified water	up to 100.0

Generalizing the literature data about the products used in the infected wounds treatment, the following AFIs were chosen for the development of the topical gel: complex of proteolytic agent chymopsin and chitosan, chitosan-miramistin complex, and lidocaine anesthetic. Hydroxypropyl methylcellulose, polyacrylamide, and glycerol were utilized as excipients. Proper development of vehicles for gels used in wound treatment can be justified by the necessity of soft action on the wound, required cooling effect, good release of AFIs from the matrix, and prevention of microbial growth. The therapeutic effect of the developed gel is due to the combination of AFIs with different biological activity. The choice of dosage form, especially the excipients used in ints preparation, are crucially important in case of topical preparations, because the ultimate aim of the dosage form development is the maximal bioavailability of the AFI at the wound. Products for local wound healing should have complex action that is provided both by the AFIs and the excipients. Taking into account the place of action, i.e. the wound, the following requirements are specified for the complex medicinal product: biocompatibility with human tissues, and absence of allergic, pyrogenic, and toxic action on healthy tissues. Moist aerated environment is necessary for the proper reparative regeneration of the cover tissues, therefore, introduction of the described AFIs and excipients provide such environment, thus making regeneration faster.

Conclusion

Taking into account the specific characteristics of the wound healing process, the composition of typical drug products utilized at the Phase I of this process (inflammation stage), it was confirmed that only the complex gels with wide range of therapeutic activities would improve wound debridement and healing process.The pharmaceutical composition is based on two AFIs and provides four different types of pharmacological action: necrolythic, antimicrobial, wound-healing, and anesthetic. Gel dosage form also provides indirect anesthetic effect after application to the wound surface, due to cooling sensation.

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