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An effective method for preparation of high purity oligohexamethylene guanidine salts

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Objectives. *Given that microorganisms can become resistant to certain groups of drugs and considering also their ability to form biofilms, the development of new drugs that are active against adapted microflora is required. This study focused on the development of a new method for the synthesis of a promising compound, the branched hydrosuccinate oligohexamethylene guanidine (OHMGsucc), with high purity that meets the standards of the 14th edition State Pharmacopeia of the Russian Federation (SPRF). Previously proposed methods have managed to isolate this product, which, however, complies with the requirements of the outdated SPRF. Therefore, the main aim of this study was to update the regulatory framework for the indicated OHMG salt for its further use in the pharmaceutical industry according to modern standards.*

Methods. *To control the residual impurities of hexamethylenediamine (HMDA) and guanidine hydrochloride (GHC), high-performance liquid chromatography (HPLC) was applied using a Thermo Scientific Dionex UltiMate 3000 chromatograph, and the chromatographic signals of the test solution with those of a standard sample solution obtained by a previously published conventional method were compared.*

Results. *The HPLC experimental data indicated a significant difference in the quantitative content of HMDA and GHC observed for the new and older preparation method of the branched OHMGsucc, suggesting that the method disclosed in this article can be used to obtain highly pure OHMGsucc.*

Conclusions. *The specified compound was standardized with the parameter "related impurities" according to the current (14th) edition of the SPRF. The effectiveness and reproducibility of the proposed method was experimentally confirmed. In addition, a process diagram for the preparation of the indicated OHMG salt was prepared.*

Keywords: *biocide, antibacterial agent, purification, related impurities, oligohexamethylene guanidine salt, disinfectant.*

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Способ получения соли олигогексаметиленгуанидина высокой степени чистоты

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Цели. *На фоне приобретения микроорганизмами резистентности к определенным группам лекарственных средств, а также способностей образовывать биопленки, требуются новые препараты, активные против адаптированной микрофлоры. Статья посвящена изысканию способа получения перспективного соединения – разветвленного гидросукцината олигогексаметиленгуанидина с высокой степенью чистоты, соответствующей нормам Государственной Фармакопеи 14 издания. Так как предложенные ранее методы позволяли получить продукт, удовлетворяющий требованиям устаревшей Государственной Фармакопеи, то основной целью являлась актуализация нормативной базы в отношении указанной соли олигогексаметиленгуанидина для ее дальнейшего применения в фармацевтической отрасли согласно современным стандартам.*
Методы. *Для контроля примесных соединений – гексаметилендиамина и гуанидина гидрохлорида применяли высокоэффективную жидкостную хроматографию, которую проводили на хроматографе Thermo Scientific Dionex UltiMate 3000 методом внешнего стандарта.*
Результаты. *На основании экспериментальных данных, полученных с помощью высокоэффективной жидкостной хроматографии, отображена разница в количественном содержании остаточных примесей в составе субстанции разветвленного гидросукцината олигогексаметиленгуанидина, полученной двумя разными способами. Отмечено, что способ, предложенный авторами настоящей статьи, позволяет снизить их содержание по сравнению с конвенциональным методом.*
Выводы. *Согласно представленным данным проведена стандартизация указанного соединения по параметру «Родственные примеси» в соответствии с актуальным на данный момент изданием Государственной Фармакопеи. Вследствие того, что эффективность предложенного метода экспериментально подтвердилась, на заключительном этапе работы была составлена технологическая схема получения указанной соли олигогексаметиленгуанидина.*

Ключевые слова: *биоцид, антибактериальный агент, очистка, родственные примеси, соль олигогексаметиленгуанидина, дезинфектант*

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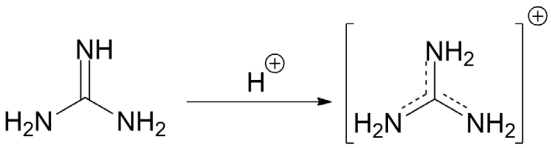
INTRODUCTION

Infectious diseases are one of the most common human pathologies in the modern world. At the beginning of the last century, the fight against this group of diseases was remarkably successful. However, an outbreak of various epidemics is currently being recorded worldwide [1], which is mainly associated with the tendency of microorganisms to form biofilms and their increasing resistance to various drugs [2]. In this regard, there is a need to develop new pharmaceutical agents with a wide spectrum of antimicrobial activity that could serve as a basis for the development of effective drugs against the highly resistant pathogenic microflora. Oligohexamethylene guanidines (OHMG), which are synthetic derivatives of the nitrogenous base guanidine, are an example of such pharmaceutical substances. Oligoguanidines are a class of polymeric biocides that consist of repeating units of the macromolecular chain of a positively charged guanidine moiety and exhibit antimicrobial, antiviral, sporicidal, fungicidal, insecticidal, pesticidal, and algicidal activity. In

addition, they can be used against aerobic and anaerobic microflora [3], while they can also destroy the biofilms formed by pathogenic microflora [4]. It should be noted that the biocidal properties of the OHMG derivatives are significantly determined by their positive charge.

The mechanism of action of the OHMG derivatives is activated after protonation of a guanidine molecule, as shown in Scheme 1, which leads to the uniform distribution of the positive charge throughout the molecule. These polycations are then adsorbed on the cell membrane of the pathogen, which is negatively charged due to the phosphate groups. This, in turn, hinders the respiration and metabolite transfer processes through the bacterial cell wall, while the oligoguanidine macromolecules penetrate into the cell, causing irreversible damage to the cytoplasmic membrane and nucleotide structure, which ultimately leads to cell death (Fig. 1).

Furthermore, the positive charge of the OHMG derivatives enables them to act as organic bases or form salts with various inorganic or organic acids such as hydrochlorides, hydrocyrates, hydrosalicylates, hydrosuccinates, etc. Especially the properties of the branched oligohexamethylene guanidine hydrochloride salt (OHMG-HC) have been earlier established [5, 6], indicating its low toxicity and antibacterial activity against various bacteria and fungi [7]. Therefore, it has been used as an active base for dental gels [8] or for sprays to treat diseases of the oral cavity [9]. Another promising OHMG salt is the branched oligohexamethylene guanidine hydrosuccinate (OHMGsucc), which exhibits modified activity. A study conducted with a compound related to OHMG showed that the succinic acid salt is similar to the hydrochloric acid salt in the spectrum of antimicrobial activities; however, succinate significantly exceeds the hydrochloride in the disintegrating effect on the formed biofilms of the microorganism *P. aeruginosa* [10]. This is the modified activity of this compound. The branched OHMGsucc can be used for the development of various drugs, thus creating new possibilities in the medical field to combat bacterial diseases. This salt has already been synthesized with purity [11] that complied with the earlier (12th) edition of the State Pharmacopeia of the Russian Federation (SPRF). In this study, we aimed to develop a new preparation method of this compound with high purity and adapt its characteristics to the requirements of the current 14th edition of the SPRF.



Scheme 1. Protonation of a guanidine molecule.

MATERIALS AND METHODS

Oligo(imnocarbonimidoylimino-1,6-hexanediyl) hydrochloride was obtained from the *Institute of Pharmaceutical Technologies* (Russia). Chloroform (GOST 20015-88), potassium hydroxide (GOST 24363-80), and succinic acid (GOST 6341-75) were obtained from *CHIMMED* (Russia). Ethyl alcohol (95%) was purchased

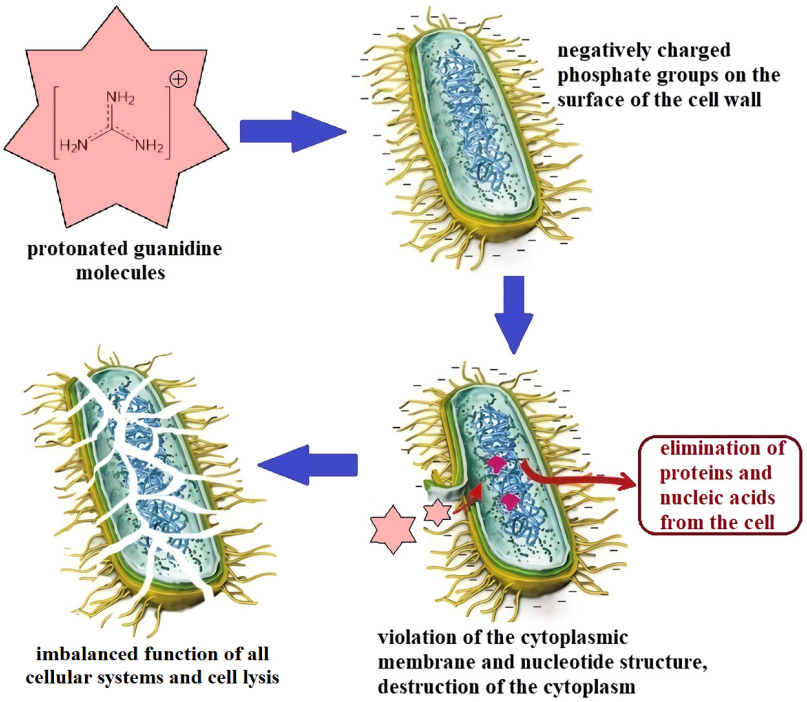


Fig. 1. Effect of OHMG on a bacterial cell.

from *Flora Kavkaza* (Russia), hexamethylenediamine (1,6-diaminohexane) from *Acros Organics* (Belgium), and guanidine hydrochloride from *Sigma-Aldrich* (USA).

The OHMGsucc samples were obtained using a conventional (Method 1) and a novel (Method 2) method, as described below.

Method 1. The conventional synthetic methodology has been in detail described in ref. [11], where carbon dioxide was used to fully saturate the solution for the preparation of the OHMG base.

Method 2. For the preparation of the base, a solution of potassium hydroxide (60 ml of 5-M KOH solution) was prepared in ethyl alcohol, followed by the addition of technical OHMG-HC (1 eq. per 1–4 eq. of solution). The resulting mixture was then added upon stirring to 1 volume part of an aqueous solution (40–60%) of OHMG-HC and the mixture was allowed to stand for about 16 h, until the phases separated. Afterward, 1–3 eq. of potassium hydroxide per 1 eq. of OHMG-HC were added to the phase with the highest OHMG base content. The resulting mixture was stirred for 3–4 h and then was allowed to stand for 16 h until phase separation. A portion of succinic acid was subsequently added to the phase with the highest OHMG base content until the precipitation stopped. The precipitate was separated by filtration, the filtrate was evaporated, and the dry residue of OHMGsucc was dissolved in 1 mass part of water. Ethyl alcohol and chloroform in a 2 : 1 ratio were then added to the aqueous solution, followed by stirring for 1 h. After phase separation, the lower phase, which contained the purified OHMGsucc, was collected and evaporated to afford the desired analogue as a solid salt. Its content in impurities, i.e., hexamethylenediamine (HMDA) and guanidine hydrochloride (GHC), was determined by high-performance liquid chromatography (HPLC) using a Thermo Scientific Dionex UltiMate 3000 chromatograph (USA), and the chromatographic peaks of the test solution were compared with those of the standard sample solution obtained by the conventional Method 1.

RESULTS AND DISCUSSION

In this study, it was expected that the application of Method 2 will provide better results and overcome the significant disadvantages of the previously developed Method 1 [11]. In order to confirm the effectiveness of the proposed method, the OHMGsucc salt was synthesized both by the conventional method and the currently described method, which has not been previously used in practice. Thus, a comparative analysis of the amount of residual impurities contained in the OHMGsucc salt was performed. More specifically, the chromatograms of HMDA (Fig. 2) and GHC (Fig. 3) were recorded to identify the effectiveness of the proposed method (Method 2) compared to the conventional process (Method 1). As observed in Figs. 2 and 3, irrespective of the applied method, the peaks of HMDA and GHC could be detected at retention time ranges of 14.0–15.0 and 4.0–4.5 min, respectively. However, it is clear that

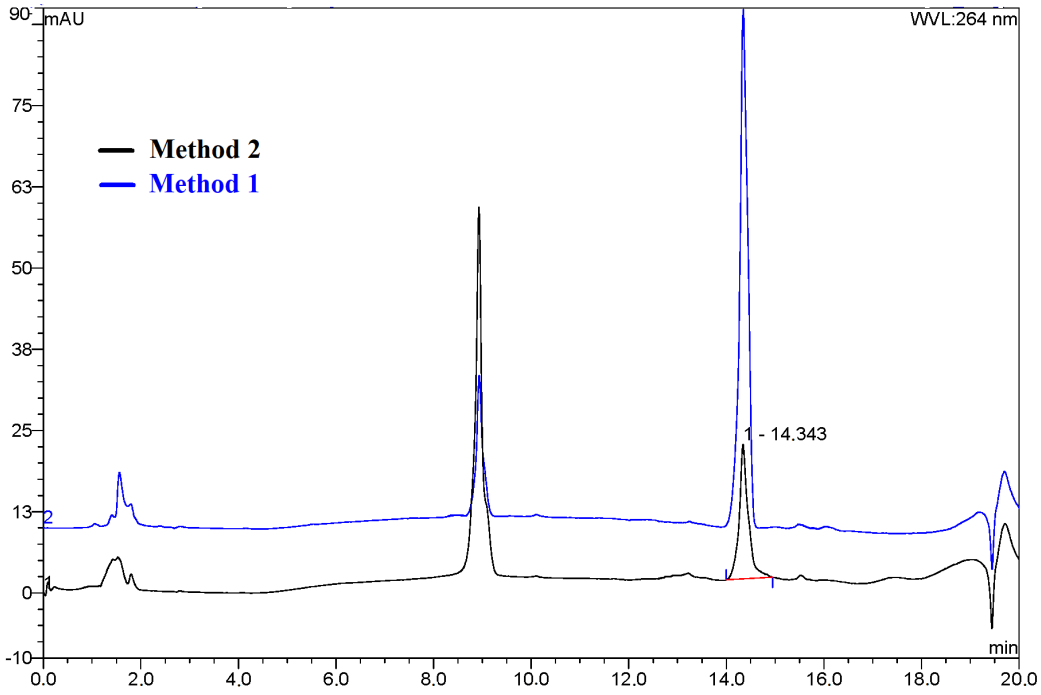


Fig. 2. Chromatogram of the quantitative content of HMDA in OHMGsucc samples purified by Methods 1 and 2.

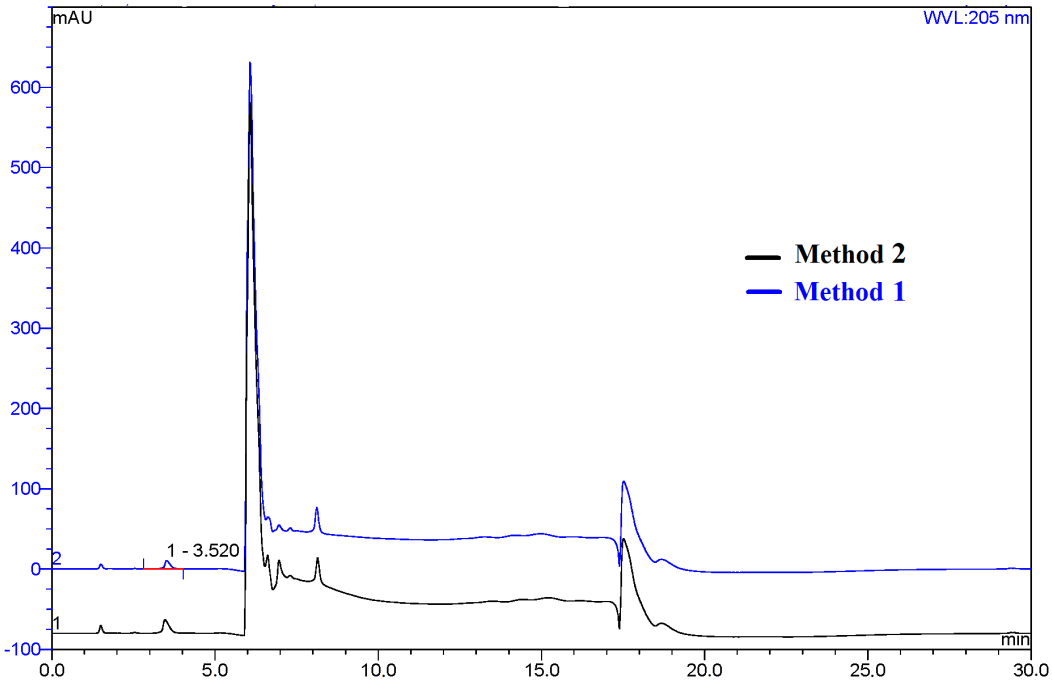


Fig. 3. Chromatogram of the quantitative GHC content in OHMGsucc samples purified by Methods 1 and 2.

Method 2 was more efficient than Method 1, as the respective HPLC peaks of the residual impurities were lower in both cases.

Furthermore, to confirm the reproducibility of the results, each purification process was performed in triplicate, resulting in six batches. It is clear from the data in table that the results of both methods were reproducible, while the higher efficiency of Method 2 in purifying OHMGsucc compared to that of Method 1 was again indicated by the lower mol % values of the residual impurities.

A process diagram of the proposed method was also created, which shows in detail all the necessary steps required for the implementation of Method 2 and the acquisition of high purity OHMGsucc (Fig. 4). This scheme clearly demonstrates the simplicity of the proposed technology for obtaining compounds of high purity.

Content of HMDA and GHC impurities obtained after purification of OHMGsucc with Methods 1 and 2.

Batch	Method	HMDA, mol %	GHC, mol %
1.1	Method 1 according to ref. [10]	0.076	0.18
1.2		0.071	0.17
1.3		0.077	0.16
2.1	Method 2	0.048	0.14
2.2		0.049	0.14
2.3		0.046	0.15

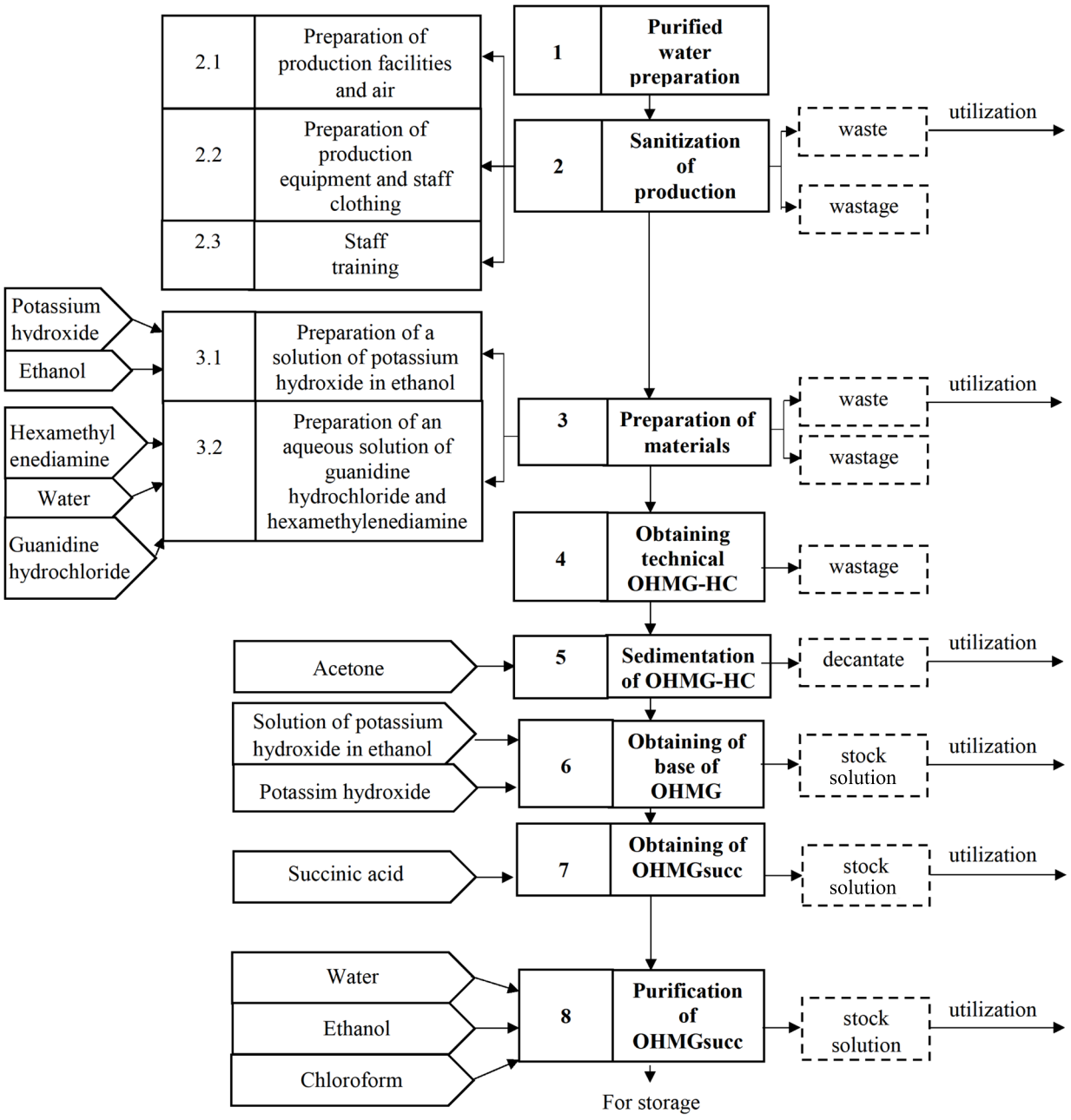


Fig. 4. Process diagram of Method 2 for the preparation and isolation of high purity OHMGsucc.

CONCLUSIONS

A new synthesis and purification method was developed to obtain the branched OHMGsucc salt with high purity by limiting the content of HMDA and GHC residues. The described method also proved to be more efficient than an earlier reported conventional method, as it can reduce the number of toxic impurities in the composition of the target compound, while complying with the requirements of the most recent 14th edition of the SPRF. Therefore, the suggested preparation process would be useful for the effective production of highly pure OHMGsucc, so that it can be further used in the pharmaceutical industry to develop new drugs that can fight the resistant harmful microorganisms.

The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

REFERENCES

1. Dieleman J.L., Schneider M.T., Singh L., Sadat N., Birger M., Reynolds A., Templin T., Hamavid H., Chapin A., Murray C.J.L., Haakenstad A. Development assistance for health: past trends, associations, and the future of international financial flows for health. *The Lancet.* 2016;387(10037):2536-2544. https://doi.org/10.1016/S0140-6736(16)30168-4

2. Sadekuzzaman M., Yang S., Mizan M.F.R., Ha S.D. Current and recent advanced strategies for combating biofilms. *Compr. Rev. Food Sci. F.* 2015;14(4):491-509. https://doi.org/10.1111/1541-4337.12144

3. Vointseva I.I., Gembitsky P.A. *Poliguanidiny - dezinfektsionnye sredstva i polifunktsional'nye dobavki v kompozitsionnye materialy* (Polyguanidines - disinfectants and multifunctional additives in composite materials). Moscow: LKM-Press Publishing House, 2009; 304 p. (in Russ). ISBN 978-5-9901286-2-0

4. Zhou Z.X., Wei D.F., Guan Y., Zheng A.N., Zhong J.J. Damage of Escherichia coli membrane by bactericidal agent polyhexamethylene guanidine hydrochloride: Micrographic evidences. *J. Appl. Microbiol.* 2010;108(3):898-907. http://dx.doi.org/10.1111/j.1365-2672.2009.04482.x

5. Kedik S.A., Sedishev I.P., Panov A.V., Zhavoronok E.S., Kha K.A. Branched oligomers based on a guanidine derivative and a disinfectant containing them: RF Pat. 2443684. Publ. 27.02.2012 (in Russ.).

6. Rodlovskaya E.N., Izmailov B.A., Vasnev V.A., Mishina E.S. Protection of Textile Materials against Biodamage. Immobilization of Oligohexamethylene Guanidine Hydrochloride on the Surface of Fiber. *International Polymer Science and Technology.* 2013;40(6):39-42. https://doi.org/10.1177/0307174X1304000608

7. Garcia I.M., Rodrigues S.B., Leitune C.B., Collares F.M. Antibacterial, chemical and physical properties of sealants with polyhexamethylene guanidinehydrochloride. *Braz. Oral Res.* 2019;33(0). https://doi.org/10.1590/1807-3107bor-2019.vol33.0019

8. Shatalov D.O., Kedik S.A., Aidakova A.V., Krupenchenkova N.V., Kovalenko A.V., Ivanov I.S. Current approaches to the development of dosage forms for the treatment of diseases of the oral cavity (review). *Biofarmatsevticheskii Zhurnal = Russian Journal of Biopharmaceuticals.* 2019;11(4):15-28 (in Russ.).

9. Shatalov D.O., Kedik S.A., Panov A.V., Aidakova A.V., Ivanov I.S., Belyakov S.V. The combined drug in the form of a solution for obtaining a spray for the treatment of diseases of the oral cavity: RF Pat. 2687745. Publ. 16.05.2019 (in Russ.).

10. Kha K.A., Grammatikova N.E., Vasilenko I.A., Kedik S.A. Comparative *in vitro* Antibacterial Activity of Polyhexamethylene Guanidine Hydrochloride and Polyhexamethylene Guanidine Succinate. *Antibiotics and Chemotherapy*. 2013;(58):3-7. PMID: 24640138.

11. Kha K.A. Development of a technology for producing a substance of oligoghexamethylene guanidine hydrosuccinate and eye drops based on it: Dis. Cand. Sci. Farm. Moscow, 2013. 131 p. (in Russ.).

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