https://doi.org/10.33380/2305-2066-2021-10-4-129-137
UDC 577.182.26

Research article / Оригинальная статья

Investigation of Colloidal Structure and Biopharmaceutical Properties of New Antibacterial Composition of Gramicidin S

Aleksandr A. Drannikov[1,2]*, Ivan S. Vatlin[1,2], Marina E. Trusova[1], Antonio Di Martino[1], Sergei V. Krivoshchekov[3], Artem M. Guriev[3], Mikhail V. Belousov[1,3]

[1] The National Research Tomsk Polytechnic University, 30, Lenina ave., Tomsk, 634034, Russia
[2] JSC "Industrial pharmaceutical company Obnovlenie", 80, Stantsionnaya str., Novosibirsk, 630096, Russia
[3] Siberian State Medical University, SSMU, 2, Moskovsky tract, Tomsk, Tomsk region, 634050, Russia

***Corresponding author:** Aleksandr A. Drannikov. **E-mail:** alexdr2037@gmail.com

ORCID: Aleksandr A. Drannikov – https://orcid.org/0000-0002-4839-1667; Ivan S. Vatlin – https://orcid.org/0000-0002-7663-7863; Marina E. Trusova – https://orcid.org/0000-0002-1761-264X; Antonio Di Martino – https://orcid.org/0000-0002-2664-4483; Sergei V. Krivoshchekov – https://orcid.org/0000-0001-5505-7141; Artem M. Guriev– https://orcid.org/0000-0002-1120-4979; Mikhail V. Belousov – https://orcid.org/0000-0002-2153-7945.

Received: 07.10.2021 **Revised:** 19.11.2021 **Published:** 25.11.2021

Abstract

Introduction. Gramicidin S has been conventionally manufactured as buccal tablets. However, in the past decade, the interest in the development of spray formulations has been growing. Those formulations contain excipients that enhance the solubility of the antibiotic in water solutions. However, the real structure of gramicidin S containing sprays remains unrevealed.

Aim. Investigation of colloidal structure and biopharmaceutical properties of new gramicidin S antibacterial composition.

Materials and methods. The composition sample was obtained using gramicidin S dihydrochloride, propylene glycol, polysorbate-80, ethanol and purified water. Raman spectroscopy has been performed to determine the composition of the phases. Dynamic light scattering analysis was performed to characterize the composition particles. Release of gramicidin S was performed by dialysis method and the concentration was determined by HPLC. The antimicrobial properties were investigated in accordance with the requirements of the XIV edition of the Russian pharmacopoeia.

Results and discussion. Dynamic light scattering analysis results show gramicidin S formulation particles having an average size in solution 5–50 nm and ζ-potential (–1.1: +7.9 mV). Based on the obtained data on the composition properties and formulation parameters it was classified as colloidal solution. The kinetic stability evaluation was performed. We compared the solubility in water and release parameters of the active pharmaceutical ingredient in the native state and in the micelles. The enhancement of the antimicrobial activity of the peptide in the colloidal solution was confirmed and ascribed to the synergic effect gramicidin S – surfactant.

Conclusion. We reported the colloidal type of the composition, that aggregate gramicidin S at a concentration of 8 mg/mL. We found that gramicidin S inclusion into the colloidal solution led to significant efficiency increase, which reveals the potential to reduce the drug dose and side effects level.

Keywords: gramicidin S, antibiotic, colloidal solution, micelles

Conflict of interest. Aleksandr A. Drannikov is an author of patents RU2659418 and EA036926 which reveal the content of the composition. Ivan S. Vatlin, Aleksandr A. Drannikov claim the employment at JSC "PFK Obnovlenie". The rest of the authors claim the absence of obvious and potential conflicts of interest.

Contribution of the authors. Aleksandr A. Drannikov, Ivan S. Vatlin developed the experimental design, held the experiment. Aleksandr A. Drannikov, Sergei V. Krivoshchekov, Artem M. Guriev, analyzed the obtained results. Mikhail V. Belousov, Marina E. Trusova were supervisors of this work. Aleksandr A. Drannikov, Ivan S. Vatlin, Sergei V. Krivoshchekov, Antonio Di Martino prepared the manuscript. All authors took part in the discussion.

For citation: Drannikov A. A., Vatlin I. S., Trusova M. E., Di Martino A., Krivoshchekov S. V., Guriev A. M., Belousov M. V. Investigation of colloidal structure and biopharmaceutical properties of new antibacterial composition of gramicidin S. *Razrabotka i registratsiya lekarstvennykh sredstv = Drug development & registration*. 2021;10(4):129–137. https://doi.org/10.33380/2305-2066-2021-10-4-129-137

Исследование коллоидной структуры и биофармацевтических свойств новой антибактериальной композиции грамицидина С

А. А. Дранников[1,2]*, И. С. Ватлин[1,2], М. Е. Трусова[1], А. Ди Мартино[1], С. В. Кривощеков[3], А. М. Гурьев[3], М. В. Белоусов[1,3]

[1] ФГАОУ ВО « Национальный исследовательский Томский политехнический университет» (ТПУ), 634034, Россия, г. Томск, пр. Ленина, д. 30
[2] АО «Производственная фармацевтическая компания Обновление», 630096, Россия, г. Новосибирск, ул. Станционная, д. 80
[3] ФГБОУ ВО «Сибирский государственный медицинский университет» Министерства здравоохранения Российской Федерации (ФГБОУ ВО СибГМУ Минздрава России), 634050, Россия, Томская обл., г. Томск, Московский тракт, д. 2

***Контактное лицо:** Дранников Александр Алексеевич. **E-mail:** alexdr2037@gmail.com

ORCID: А. А. Дранников – https://orcid.org/0000-0002-4839-1667; И. С. Ватлин – https://orcid.org/0000-0002-7663-7863; М. Е. Трусова – https://orcid.org/0000-0002-1761-264X; А. Ди Мартино – https://orcid.org/0000-0002-2664-4483; С. В. Кривощеков – https://orcid.org/0000-0001-5505-7141; А. М. Гурьев – https://orcid.org/0000-0002-1120-4979; М. В. Белоусов – https://orcid.org/0000-0002-2153-7945.

Статья поступила: 07.10.2021 **Статья принята в печать:** 19.11.2021 **Статья опубликована:** 25.11.2021

Резюме

Введение. Грамицидин С традиционно выпускается в виде таблеток буккальных. В последнее время активно ведутся разработки лекарственной формы грамицидина С в виде спрея. Разработанные составы содержат вспомогательные вещества, которые способствуют солюбилизации малорастворимого антибиотика, однако истинная структура жидких лекарственных форм грамицидина С остается неопределенной.

Цель. Определить коллоидную структуру и биофармацевтические свойств новой антибактериальной композиции грамицидина С.

Материалы и методы. Образец композиции получали в результате самоорганизации с использованием субстанции грамицидина С дигидрохлорида и вспомогательных веществ: пропиленгликоль, полисорбат-80, этиловый спирт, вода очищенная. Для определения качественного состава фаз использовалась рамановская спектроскопия. Параметры частиц в композиции устанавливали с использованием метода динамического рассеяния света. Для сравнительной оценки параметров высвобождения использовали диализ, детектируя концентрацию грамицидина С методом ВЭЖХ. Исследование антимикробного действия проводили в соответствии с требованиями Государственной фармакопеи РФ XIV издания.

Результаты и обсуждение. Проведены структурные исследования композиции грамицидина С: определены размер (5–50 нм), ζ-потенциал частиц (–1,1: +7,9 мВ). Проведена классификация композиции на основании данных о параметрах и особенностях формирования системы, что позволило отнести ее к классу коллоидных растворов. Показано, что исследуемая композиция обладает кинетической устойчивостью. Продемонстрирован характер высвобождения действующего вещества в сравнении с исходной субстанцией и повышенная растворимость антибиотика в воде. Подтверждено усиление антимикробных свойств пептида в составе коллоидного раствора, что связано с синергетическим действием поверхностно-активных веществ и грамицидина С.

Заключение. Установлена коллоидная структура композиции грамицидина С с содержанием 8 мг/мл. Установлено, что включение грамицидина С в состав коллоидного раствора приводит к значительному повышению эффективности антибиотика, что открывает перспективы к уменьшению дозировки действующего вещества и снижению уровня побочных эффектов при разработке лекарственного препарата.

Ключевые слова: грамицидин С, антибиотик, коллоидный раствор, мицеллы

Конфликт интересов. А. А. Дранников является автором патентов RU2659418 и EA036926 на состав исследуемой композиции. И. С. Ватлин, А. А. Дранников заявляют о трудоустройстве в АО «ПФК Обновление». Все остальные авторы декларируют отсутствие явных и потенциальных конфликтов интересов.

Вклад авторов. А. А. Дранников, И. С. Ватлин разработали дизайн исследования, провели исследование. А. А. Дранников, С. В. Кривощеков, А. М. Гурьев провели анализ полученных данных. М. В. Белоусов, М. Е. Трусова осуществляли научное руководство исследованием. А. А. Дранников, И. С. Ватлин, С. В. Кривощеков, А. Ди Мартино подготовили текст статьи. Все авторы участвовали в обсуждении результатов.

Для цитирования: Дранников А. А., Ватлин И. С., Трусова М. Е., Ди Мартино А., Кривощеков С. В., Гурьев А. М., Белоусов М. В. Исследование коллоидной структуры и биофармацевтических свойств новой антибактериальной композиции грамицидина С. *Разработка и регистрация лекарственных средств*. 2021;10(4):129–137. https://doi.org/10.33380/2305-2066-2021-10-4-129-137

INTRODUCTION

Gramicidin S is active against gram-positive or gram-negative bacteria as well as some fungi [1], which makes allows its application in the therapy of bacterial infections for more than 70 years [2].

However, due to the low water solubility with a consequent lack of systemic absorption, gramicidin S is administered only locally or topically as buccal tablets or solutions for local application [3].

The latest works aimed to develop new dosage forms of gramicidin S which is a spray, where the drug is combined with cetylpyridinium chloride and oxybuprocaine for the treatment of throat and oral cavity diseases [4-7]. In addition, a new antibacterial gramicidin S composition was developed based on the system of co-solvents and surfactants and with drug content in a range from 3.2 up to 8.0 mg/mL [8,9]. A common property of the developed compositions consists in the use of surfactants to increase the solubility of gramicidin S (Table 1).

The use of surfactants is the most promising approach to increase the water solubility of a component due to the possible formation of the self-aggregated systems [10]. Additional stability of those systems is proved by using co-solvents (ethanol, propylene glycol or glycerol), which have lower hydrophilic-lipophilic balance (HLB) value due to the formation of a film-like complex at the phase boundary (Figure 1) [11].

Table 1. **Comparative table of gramicidin S compositions containing surfactants**

Document №	Patent RU 2627423	Patent RU 2604575	Patent RU 2604576	Patent RU 2749902	Patent RU 2659418
Content in wt. %					
Gramicidin S	0,027–0,2	0,027–0,033	0,027–0,033	0,002–0,1	0,32–0,80
Oxybuprocaine hydrochloride	0,067–0,2		0,067–0,083		
Cetylpyridinium chloride	0,045–0,2	0,045–0,055	0,045–0,055	0,05	
Methylparaben	0,0736–0,1104	0,0736–0,1104	0,0736–0,1104	0,092	
Propylparaben	0,0080–0,0120	0,0080–0,0120	0,0080–0,0120	0,01	
Ethanol	7,3–10,9	7,3–10,9	7,3–10,9	9,1–9,5	16,00–40,00
Sucralose	0,09–0,11	0,09–0,11	0,09–0,11	0,1	
Glycerol	14,9–18,2	14,9–18,2	14,9–18,2	14,3–16,95	
Propylene glycol					10,00–12,00
Mint aromatizer	0,37–0,44	0,37–0,44	0,37–0,44	0,41	
Citric acid monohydrate	0,026–0,032	0,026–0,032	0,026–0,032	0,029	
Sodium citrate	0,0099–0,012	0,0099–0,012	0,0099–0,012	0,011	
Polysorbate-80	0,18–0,22	0,18–0,22	0,18–0,22	0,2	0,90–1,10
Purified water	rest	rest	rest	rest	rest

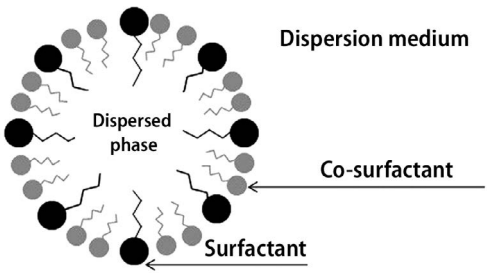

Figure 1. **Interaction between surfactants and co-solvents in micells**

With these assumptions, we suggest that the compositions reported in the literature are colloidal solutions [12].

This work presents a study of the colloidal structure and biopharmaceutical properties of the composition presented in patent RU 2659418 [8], which contains exclusively gramicidin S as the active pharmaceutical ingredient, surfactant and drug co-solvent system, which excludes the possible influence of other excipients on the experimental results.

MATERIALS AND METHODS

Gramicidin S dihydrochloride (TS 9348-023-47509455-2012, JSC «PFK Obnovlenie», Russia) was used as an object of this research, excipients – propylene glycol, EP/USP (Ineos Group Ltd, UK), polysorbate-80, EP/NF (Croda International plc., UK), ethanol 95 % (Russian Pharmacopeia XIV ed, FS.2.1.0036.15) and purified water (Russian Pharmacopeia XIV ed, FS.2.2.0020.18). Reagents – perchloric acid, analytical grade, (LLC "Kamkhimkom", Russia), acetonitrile for chromatography, high pure (JSC “LenReaktiv”, Russia).

The composition was prepared using the technology described in [8,9]. 75.0 g of purified water was put for stirring, afterwards subsequently 16.0 g of ethanol, 10.0 g of propylene glycol and 1.0 g of polysorbate-80 were added, stirred for 10 minutes followed by adding 0.8 g of gramicidin S. The mixture was stirred until the uniform solution formation which then was filtered through 0.45 μm syringe filter (LLC “Gluvex”, Russia)

Micelles formation was confirmed via Raman spectroscopy using the portable spectrophotometer Bruker BRAVO Raman spectrometer (Bruker Corporation, USA) in spectral range 3200-400 cm^{-1} and 10 cm^{-1} resolution. Each spectrum was recorded in triplicate to ensure the repeatability of the result and signal location and intensity change absence.

We determined the colloidal solution particle size and surface charge by applying the dynamic light scattering method. Zetasizer Nano ZS (Malvern Panalytical Ltd, UK) was used. The samples were analyzed directly upon preparation.

The kinetic stability of the colloidal solution was determined using a centrifuge Frontier 5000 FC5706 (Ohaus Corporation, Germany). We centrifuged samples at 5000 rpm for 15 min indicating a phase separation.

Antibacterial activity of gramicidin S composition was determined according to Russian Pharmacopeia OFS 1.2.4.0002.18. We indicated pH using the pH-meter MettlerToledo™ FiveEasyPlus™ FEP20 (Thermo Fisher Scientific Inc, USA).

We studied gramicidin S release from the composition following the next sequence: 18.75 ml of colloidal solution sample was placed in semi-permeable membrane Spectra/Por®7 (Thermo Fisher Scientific Inc, USA) with pore size 3.5 kDa, preventing the leakage, the sample was placed into the flask containing 100 ml of phosphate buffer saline with pH = 7.4 (Russian Pharmacopeia XIV ed., OFS.1.3.0003.15). Gramicidin S dihydrochloride in an amount of 150 mg was used as the reference. Both samples were shaken using the orbital shaker ("Bio-Rus" Ltd., Russia), periodically collecting the specimen followed by adding the buffer solution to save the constant volume.

To determine gramicidin S assay we applied the HPLC method, using the following solutions as mobile phase:

- *Mobile phase A*. In 1000 ml of water add 6.0 ml of perchloric acid 70 % and 10.0 ml of phosphoric acid solution 25 %, mix; maintain the pH of the obtained solution up to 2.50 ± 0.05 with sodium hydroxide solution 50 %.
- *Mobile phase B*. Acetonitrile for chromatography.

As a test solution, we used a colloidal solution of gramicidin S filtered through a nylon membrane filter with 0.45 µm pore size, discarding the first filtrate portions. The test volume was 20 µl.

As standard, we used the solution prepared by dissolution of 25 mg of gramicidin S dihydrochloride (gramicidin S dihydrochloride, substance-powder, gramicidin S dihydrochloride content 99.9 %, JSC "Proizvodstvennaya farmatsevticheskaya kompaniya Obnovlenie", Russia, batch 10719, expiring date 31.07.2024) in 25 ml of ethanol 95 %.

Chromatographic conditions: column Luna C18, 250 × 4,6 mm, 5 µm, Phenomenex, flow rate 1.0 ml/min, column temperature 25 °C, UV-detector, 210 nm. The gradient program is illustrated in the table 2.

Table 2. Gradient program

Time, min	Mobile phase A, %	Mobile phase B, %
0–7	85	15
7–8	85–50	15–50
8–25	50	50
25–26	50–40	50–60
26–35	40	60
35–65	40–20	60–80
65–70	20	80
70–71	20–85	80–15
71–80	85	15

Gramicidin S dihydrochloride $C_{60}H_{92}N_{12}O_{10} \cdot 2HCl$ content in 1 mL of colloidal solution in mg (X) was calculated as follow:

$$X = \frac{S \cdot a_0 \cdot P}{S_0 \cdot 25 \cdot 100}, \quad (1)$$

where S – gramicidin S dihydrochloride peak area on the chromatogram of the test solution; S_0 – gramicidin S dihydrochloride peak area on the chromatogram of the standard sample solution; a_0 – weight of the standard sample of gramicidin S dihydrochloride, in mg; P – assay in the standard sample of gramicidin S dihydrochloride, in %.

The developed method was validated according to [13].

RESULTS AND DISCUSSION

To confirm the structure of the composition containing gramicidin S, surfactant and co-solvents, the following sample was prepared (Table 3):

The obtained composition was analyzed by Raman spectroscopy. The result is presented at figure 2.

Table 3. Composition of colloidal solution containing gramicidin S hydrochloride, surfactant and co-solubilizers

Component name	Content, g
Gramicidin S dihydrochloride	0,8
Ethanol, 95 %	16,0
Propylene glycol	10,0
Polysorbate-80	1,0
Purified water	up to 100

In the Raman spectra analysis (Figure 2 b) the presence of ethanol and propylene glycol characteristic peaks in the range of 1500–500 cm^{-1} [14] is observable, which confirms their presence in the dispersion medium. At the same time the characteristic peaks Amide I of gramicidin S located in the range of 1700–1500 cm^{-1} [15] and 1000 cm^{-1} determined by aromatic structure of D-phenylalanine [16] (Figure 2 a), are absent, however, the presence of these signals in the spectrum of the dried dispersed phase (Figure 2 c) confirms that the active pharmaceutical ingredient is present in the composition. In this case, the spectrum of the dispersed phase shows signals at 850 and 1100 cm^{-1}, which are characteristic of polysorbate-80 [17].

Thus, the composition of the dispersion medium of the colloidal solution obtained contains water, ethanol and propylene glycol, while the dispersed phase contains gramicidin S and polysorbate-80.

The obtained gramicidin S colloidal solution was analyzed by dynamic light scattering to prove the presence of the particles and to determine their size and surface charge (Figure 3).

According to the data (Figure 3), the particle size of the colloidal solution falls in the range of 5 to 50 nm. The

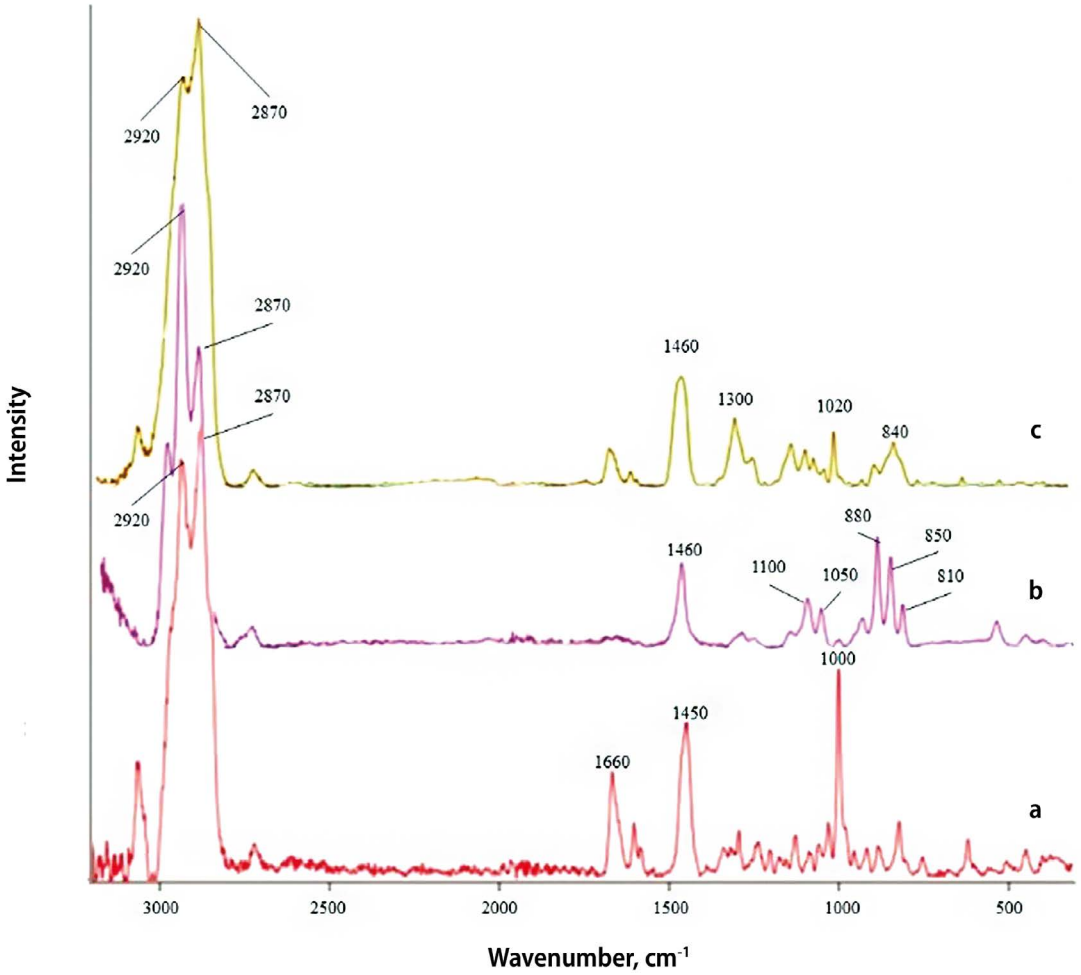

Figure 2. Raman spectra:
a – gramicidin S dihydrochloride; b – gramicidin S dihydrochloride colloidal solution; c – dispersed phase of gramicidin S colloidal solution

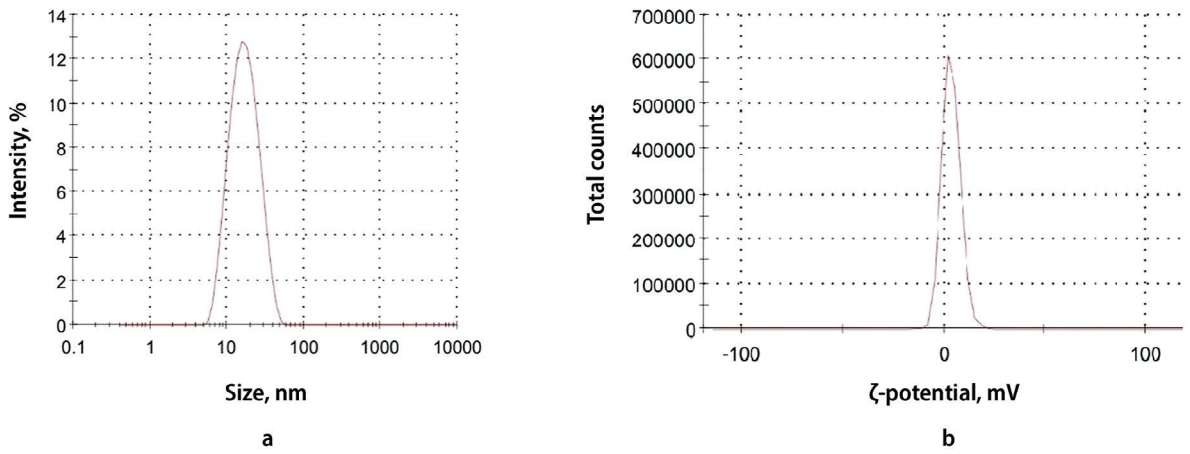

Figure 3. Gramicidin S colloidal solution analysis by dynamic light scattering: a – particle size; b – surface charge

surface charge (ζ-potential) of the particles in colloidal solution is in the range (–1.1; +7.9) mV.

The low ζ-potential value in the developed composition can be explained by the nonionic nature of the surfactant polysorbate-80 since this excipient stabilizes the micelles by steric repulsion of the particles, conserving the structure [18]. The particle charge deviates from zero, which is characteristic for the nonionic surfactant based micelles and indicates the presence of charged particles in the colloidal solution, which could be the impurities in the ionic polysorbate-80 in the form of free fatty acids [19].

At the same time, it is known that the micelles based on polysorbate-80 usually carry a negative charge [20]. Taking into account the chemical nature of propylene glycol, its effect on the shift in the charge of the particles to positive is highly limited, it can be concluded that the major contribution to the charge is coming by charge is by gramicidin S, which demonstrates cationic properties.

Thus, the obtained value of ζ-potential does not counter the condition of nonionic surfactant based colloidal solution stability.

In order to evaluate the stability of the colloidal solution, we performed the kinetic stability study. The centrifugation did not cause precipitation of the dispersed phase, which indicates the stability of the composition.

The HPLC was used for the quantitative analysis of gramicidin S. The validation criteria and the results are resumed in the Table 4.

The release patterns of gramicidin S from the composition and the native form are reported in the Figure 4.

The trends (Figure 4) show that gramicidin S is insoluble in simulated oral cavity media, however, upon the composition formation, the antibiotic is released at a high rate (more than 75 % within 1 hour), which can provide higher antimicrobial activity.

Table 4. Results of validation tests for Gramicidin S quantification method

Criteria	Result
Specificity	A peak with the retention time of gramicidin S (46.38 min) is absent on the chromatogram of «placebo»
Linearity	R = 0.9997 Linear dependence equation $y = 28495.6x - 17781.2$
Repeatability	RSD = 0,85 %
Intra-labprecision	$F_{fact.} = 2.893$ $F_{calc.} = 10.967$
Accuracy	RSD ≤ 2.0 % (0.85 %) Response 97.5–102.5 % (99.8 %) The confidence interval includes meaning 100 % (99.8 ± 0.9)
Analytical area	1–140 %

The antimicrobial effect of the composition was compared to the aqueous suspension of gramicidin S. The results of this study are resumed in the Figure 5.

From the results presented at Figure 5 it can be seen that gramicidin S exhibits 2–5 times higher antimicrobial activity towards to the test strains in colloidal solution. Additionally, a contribution can come from the presence of the surfactant. In fact,

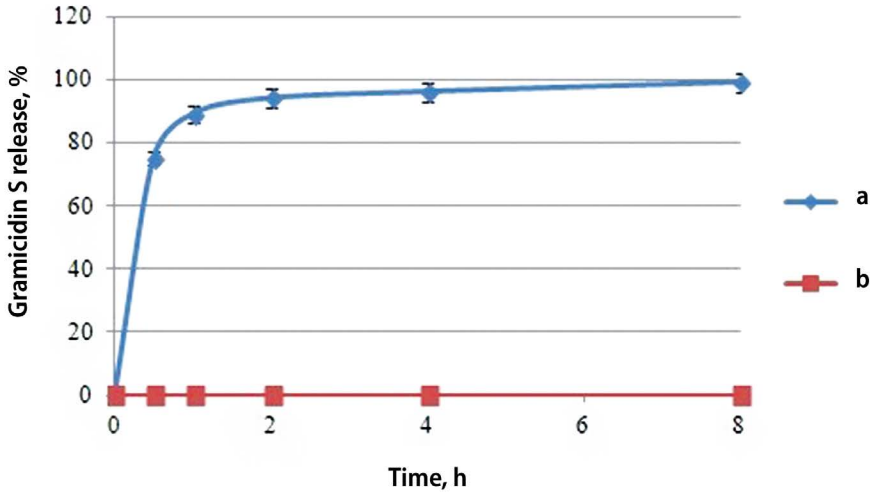

Figure 4. **Release parameters of gramicidin S from the composition (a) in comparison with the dissolution of gramicidin S dihydrochloride (b)**

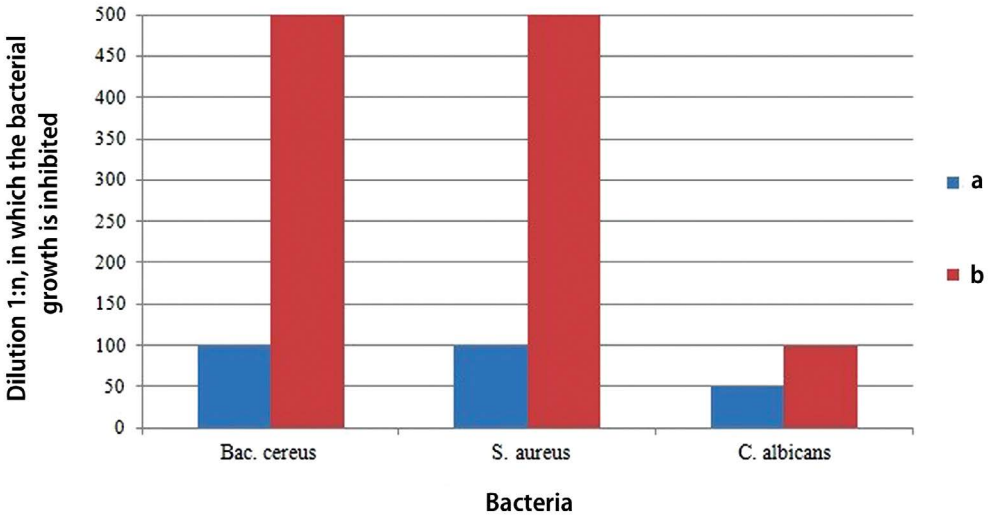

Figure 5. **Antimicrobial action of gramicidin S hydrochloride in suspension (a) and in colloidal solution (b)**

it is known that surfactants are able to increase antimicrobial activity by affecting the bacterial cell wall [21]. Taking into account that gramicidin S has a similar to surfactant mechanism of activity on the microorganism membrane, a synergistic effect of the peptide, surfactant and co-solubilizer system can occur.

CONCLUSION

As result of the present study, we determined the structure of a liquid composition containing gramicidin S in a co-solvent system, which made it possible to define it as a colloidal solution.

It has been observed that gramicidin S inclusion into the micelles of colloidal solution provides a sufficient increase in the antibiotic effectiveness, which reveals the prospective to decrease the dosage of the active pharmaceutical ingredient and consequently the side effects level upon the administration of the drug.

REFERENCES

1. Gause G. F., Brazhnikova M. G. Gramicidin S and its use in the treatment of infected wounds. *Nature*. 1944;154(3918):703–703. DOI: 10.1038/154703a0.
2. Wenzel M., Rautenbach M., Vosloo J. A., Siersma T., Aisenbrey C. H., Zaitseva E., Laubscher W. E., van Rensburg W., Behrends J. C.,

Bechinger B., Hamoen L. W. The multifaceted antibacterial mechanisms of the pioneering peptide antibiotics tyrocidine and gramicidin S. *mBio*. 2018;9(5). DOI: 10.1128/mBio.00802-18.

3. *Gosudarstvennyy reestr lekarstvennykh sredstv. Gramitsidin S.* [State register of medicines. Gramicidin S]. Available at: https://grls.rosminzdrav.ru/grls.aspx?s=%D0%B3%D1%80%D0%B0%D0%BC%D0%B8%D1%86%D0%B8%D0%B4%D0%B8%D0%BD&m=mnn. Accessed: 12.11.2021. (In Russ.)
4. Syrov K. K., Nesteruk V. V. *Farmatsevticheskaya kompozitsiya dlya lecheniya infektsionno-vospalitel'nykh zabolevaniy mestnogo primeneniya i sposob ee polucheniya i primeneniya* [Pharmaceutical composition for the treatment of infectious and inflammatory diseases of local use and method for its preparation and use]. Patent RUS № 22627423 C1. 08.08.2017. Available at: https://www1.fips.ru/ofpstorage/Doc/IZPM/RUNWC1/000/000/002/627/423/%D0%98%D0%97-02627423-00001/document.pdf Accessed: 12.11.2021. (In Russ.)
5. Syrov K. K., Nesteruk V. V. *Farmatsevticheskaya kompozitsiya dlya lecheniya infektsionno-vospalitel'nykh zabolevaniy mestnogo primeneniya i sposob ee polucheniya i primeneniya* [Pharmaceutical composition for the treatment of infectious and inflammatory diseases of local use and method for its preparation and use]. Patent RUS № 2604575 C1. 10.12.2016. Available at: https://new.fips.ru/Archive4/PAT/2016FULL/2016.12.10/DOC/RUNWC1/000/000/002/604/575/DOCUMENT.PDF. Accessed: 12.11.2021. (In Russ.)
6. Syrov K. K., Nesteruk V. V. *Farmatsevticheskaya kompozitsiya dlya lecheniya infektsionno-vospalitel'nykh zabolevaniy mestnogo primeneniya i sposob ee polucheniya i primeneniya* [Pharmaceutical composition for the treatment of infectious and inflammatory diseases of local use and method for its preparation and use]. Patent RUS № 2604576 C1. 10.12.2016. Available at: https://new.fips.ru/Archive4/PAT/2016FULL/2016.12.10/DOC/RUNWC1/000/000/002/604/576/DOCUMENT.PDF. Accessed: 12.11.2021. (In Russ.)
7. Pereverzev A. P. *Farmatsevticheskaya kompozitsiya dlya lecheniya infektsionno-vospalitel'nykh zabolevaniy* [Pharmaceutical composition for the treatment of infectious and inflammatory diseases]. Patent RUS № 2749902 C2. 18.06.2021. Available at: https://www1.fips.ru/ofpstorage/Doc/IZPM/RUNWC1/000/000/002/749/902/%D0%98%D0%97-02749902-00001/document.pdf. Accessed: 12.11.2021. (In Russ.)
8. Kalustov V. B., Biryukova E. A., Izmest'eva E. V., Drannikov A. A. *Antibakterial'naya kompozitsiya dlya dostavki Gramitsidina S k ochagu mestnogo vospaleniya, sposob prigotovleniya antibakterial'noy kompozitsii dlya dostavki Gramitsidina S k ochagu mestnogo vospaleniya, sposob dostavki Gramitsidina S k ochagu mestnogo vospaleniya* [Antibacterial composition to deliver gramicidin C to a site of local inflammation, method of producing an antibacterial composition to deliver gramicidin C to a site of local inflammation, and method of delivering gramicidin C to a site of local inflammation]. Patent RUS № 2659418 C1. 02.07.2018. Available at: https://www1.fips.ru/ofpstorage/Doc/IZPM/RUNWC1/000/000/002/659/418/%D0%98%D0%97-02659418-00001/document.pdf. Accessed: 12.11.2021. (In Russ.)
9. Kalustov V. B., Biryukova E. A., Izmest'eva E. V., Drannikov A. A. *Antibakterial'naya kompozitsiya dlya dostavki Gramitsidina S k ochagu mestnogo vospaleniya, sposob prigotovleniya antibakterial'noy kompozitsii dlya dostavki Gramitsidina S k ochagu mestnogo vospaleniya, sposob dostavki Gramitsidina S k ochagu mestnogo vospaleniya* [Antibacterial composition to deliver gramicidin C to a site of local inflammation, method of producing an antibacterial composition to deliver gramicidin C to a site of local inflammation, and method of delivering gramicidin C to a site of local inflammation]. Patent EA № 036926 B1. 15.01.2021. Available at: http://www.eapatis.com/Data/EATXT/eapo2021/PDF/036926.pdf. Accessed: 12.11.2021. (In Russ.)
10. Solomonov A. V., Marfin Y. S., Rumyantsev E. V., Ragozin E., Zahavi T. S., Gellerman G., Tesler A. B., Muench F., Kumagai A., Miyawaki A. Self-assembled micellar clusters based on Triton-X-family surfactants for enhanced solubilization, encapsulation, proteins permeability control, and anticancer drug delivery. *Materials Science and Engineering*. 2019;99:794–804. DOI: 10.1016/j.msec.2019.02.016.
11. Kassem A. A., Mohsen A. M., Ahmed R. S., Essam T. M. Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design, optimization, in vitro and in vivo evaluation. *Journal of Molecular Liquids*. 2016;218:219–232. DOI: 10.1016/j.molliq.2016.02.081.
12. Glatter O., Salentinig S. Inverting structures: From micelles via emulsions to internally self-assembled water and oil continuous nanocarriers. *Current Opinion in Colloid & Interface Science*. 2020;49:82–93. DOI: 10.1016/j.cocis.2020.05.003.
13. *Gosudarstvennaya farmakopeya Rossiyskoy Federatsii. 14-e izd. OFS.1.1.0012.15. Validatsiya analiticheskikh metodik* [State Pharmacopoeia of Russian Federation. 14th ed. OFS.1.1.0012.15. Validation of analytical procedures]. Available at: https://docs.rucml.ru/feml/pharma/v14/vol1/276/#zoom=z. Accessed: 12.11.2021. (In Russ.)
14. Le M. L. P., Tran N. A., Ngo H. P. K., Nguyen T. G. Liquid electrolytes based on ionic liquids for lithium-ion batteries. *Journal of Solution Chemistry*, 2015;44(12):2332–2343. DOI: 10.1007/s10953-015-0408-z.
15. Ogawa K., Oshima Y., Etoh T., Kaisyakuji Y., Tojigamori M., Ohno Y., Shiraishi N., Inomata M. Label-free detection of human enteric nerve system using Raman spectroscopy: A pilot study for diagnosis of Hirschsprung disease. *Journal of Pediatric Surgery*. 2021;56(7):1150–1156. DOI: 10.1016/j.jpedsurg.2021.03.040.
16. Zhang L., Yuan W.-L., Zhang Z., Zhang G.-H., Chen H., Zhao N., He L., Tao G.-H. Self-assembly ionic nanofibers derived from amino acid for high-performance particulate matter removal. *Journal of Materials Chemistry A*. 2019;7(9):4619–4625. DOI: 10.1039/c8ta11382c.
17. Mitsutake H., Castro S. R., de Paula E., Poppi R. J., Rutledge D. N., Breitkreitz M. C. Comparison of different chemometric methods to extract chemical and physical information from Raman images of homogeneous and heterogeneous semi-solid pharmaceutical formulations. *International Journal of Pharmaceutics*. 2018;552(1–2):119–129. DOI: 10.1016/j.ijpharm.2018.09.058.

18. Friedrich R. B., Kann B., Coradini K., Offerhaus H. L., Beck R. C., Windbergs M. Skin penetration behavior of lipid-core nanocapsules for simultaneous delivery of resveratrol and curcumin. *European Journal of Pharmaceutical Sciences*. 2015;78:204–213. DOI: 10.1016/j.ejps.2015.07.018.
19. Espinosa-Olivares M. A., Delgado-Buenrostro N. L., Chirino Y. I., Trejo-Márquez M. A., Pascual-Bustamante S., Ganem-Rondero A. Nanostructured lipid carriers loaded with curcuminoids: physicochemical characterization, in vitro release, ex vivo skin penetration, stability and antioxidant activity. *European Journal of Pharmaceutical Sciences*. 2020;155:105533. DOI: 10.1016/j.ejps.2020.105533.
20. Prabhakar K., Afzal S. M., Surender G., Kishan V. Tween 80 containing lipid nanoemulsions for delivery of indinavir to brain. *Acta Pharmaceutica Sinica B*. 2013;3(5):345–353. DOI: 10.1016/j.apsb.2013.08.001.
21. Guo L., Fang Y., Liang X., Xu Y., Chen J., Li Y., Fang S., Meng Y. Influence of polysorbates (Tweens) on structural and antimicrobial properties for microemulsions. *International Journal of Pharmaceutics*. 2020;590:119939. DOI:10.1016/j.ijpharm.2020.119939.