**Pharmacophore,** 15(2) 2024, Pages: 119-123

**Pharmacophore**

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

# DEVELOPMENT OF VAGINAL SUPPOSITORIES MODIFIED WITH SILVER NANOPARTICLES

**Diana Abdulovna Yakubova[1*], Hava Said-Selimovna Arsanukayeva[1], Iman Anvarovna Zakaeva[2], Zaira Ikramovna Nukhova[1], Kamila Teimurovna Sargulanovna[1], Elina Beslanovna Dadaeva[1], Madina Vakhaevna Mutsaeva[2], Tahmina Teyub Kyzy Hasanova[1]**

1. ***Department of Therapy, Faculty of Medicine, Saratov State Medical University named after V.I.Razumovsky, Saratov, Russia.***
2. ***Department of Therapy, Faculty of Medicine, Astrakhan State Medical University, Astrakhan, Russia.***

ARTICLE INFO

**Received:**
26 December 2023
**Received** in revised form:
06 April 2024
**Accepted:**
14 April 2024
**Available online:**
28 April 2024

***Keywords:*** Suppositories, Silver nanoparticles, Poviargol, Antimicrobial activity

ABSTRACT

The article presents the results of a study on the development of vaginal suppositories with silver nanoparticles. The composition of excipients was selected, the main parameters of the technological process were determined: the temperature of preparation of the suppository mass, the time of dispersion of the substance, the conditions and cooling time. It was found that the use of Macrogol 4000 in suppositories with poviargol is difficult, since the temperature of their preparation is lower than the solidification temperature of the auxiliary substance. The optimal composition and the temperature regime of preparation of suppositories (≤40-42 °C) were selected. It is shown that the experimental and semi-industrial series of suppositories fully comply with the developed specifications. They have deviations from the nominal weight value of no more than 0.63%, deviations from the nominal content of poviargol no more than 3.34%, acceptable decay time and microbiological purity. A wide antimicrobial spectrum of action of suppositories in relation to gram-negative, gram-positive microorganisms and fungi was identified.



**To Cite This Article:** Yakubova DA, Arsanukayeva HSS, Zakaeva IA, Nukhova ZI, Sargulanovna KT, Dadaeva EB, et al. Development of Vaginal Suppositories Modified with Silver Nanoparticles. Pharmacophore. 2024;15(2):119-23. https://doi.org/10.51847/uZV70TmXhl

## Introduction

Metal nanoparticles (Me NPs) exhibit pronounced biological activity [1]. It is known that they act on organisms at the level of enzymes, regulatory biosystems, cells, organs and the body as a whole [2-4]. In recent years, a number of studies have been devoted to Ag NPs [5, 6]. Among all metals, Ag NPs have the most pronounced bactericidal [7], antiviral [8], fungicidal [9] and immunomodulatory effects [10], while remaining low-toxic [11].

Ag NPs are of interest because its action is specific not by the type of microorganism, as in antibiotics, but by the cellular structure [12]. It affects bacterial cells, viruses and other organisms without a cell wall. Mammalian cells have a different, denser type of membrane, and Ag NPs are not active against them [13]. Particular interest in the antimicrobial action of Ag NPs is associated with the spread of strains of microorganisms resistant to antibiotics. Antibiotic resistance is explained by various reasons: a decrease in the permeability of the bacterial cell wall, changes in the structure and physiology of microorganisms, and the production of specific enzymes by the target cells that destroy drug molecules [14, 15]. Unlike antibiotics, Ag NPs are largely devoid of disadvantages associated with the problem of resistance of pathogenic microorganisms to it [16, 17].

One of the possible applications of active substances with such properties is the treatment of cervical erosion. This disease occurs in 40% of women and is a defect in the mucous membrane of the outer part of the cervix [18, 19]. The trigger mechanism in the development of the disease is most often an infection [20]. Considering that the task of creating ready-made medicines with prolonged antimicrobial action is urgent [21], the purpose of this study was to develop the composition and technology of a new drug with Ag NPs.

**Corresponding Author:** Diana Abdulovna Yakubova; Department of Therapy, Faculty of Medicine, Saratov State Medical University named after V.I.Razumovsky, Saratov, Russia. E-mail: bucky99@yandex.ru.

## Materials and Methods

Suppositories were used as the object of the study, which included poviargol containing Ag NPs (Biopharm, Minsk, Belarus) as a pharmaceutical substance. Experimental and semi-industrial series of suppositories were prepared using the following technology: components of the suppository mass were melted in a water bath at a temperature of 60-65 ºC, then samples 1,2,6 were cooled to a temperature of 40-42 ºC, and samples 3,4,5 – to a temperature of 44-45 ºC. A stabilizer and poviargol were added to the cooled suppository mass and the mixture was stirred for 15 minutes using an Ultra-turrax T50 dispersant at 6000 rpm. The finished suppository mass was poured into disposable cells and sealed with a DottBonapace 8C device. The finished suppositories were cooled at 15 °C for 15 minutes. In total, 6 experimental samples of suppositories were prepared **(Table 1)**. Experimental and pilot-industrial series of suppositories were tested according to the following indicators: potentiometric determination of pH, disintegration of suppositories and pessaries, uniformity of mass for a unit of a dosed drug, uniformity of the active substance content in a unit of a dosed drug, microbiological purity. The authenticity of poviargol was determined by qualitative reactions:

1. with the addition of hydrochloric acid, a white curd precipitate was observed, dissolving with the addition of 3 ml of a dilute ammonia solution.
2. a shiny coating of metallic silver forms on the walls of the test tube when reacting with a solution of ammonia and formaldehyde solution.

**Table 1.** The composition of the experimental series of suppositories with poviargol

| Sample | Composition of excipients of experimental series of suppositories | | | | | |
|---|---|---|---|---|---|---|
| | Macrogol 400 | Macrogol 1000 | Macrogol 1500 | Macrogol 4000 | Polysorbate 80 | Stabillizer |
| 1 | | + | | | + | + |
| 2 | | + | | | | + |
| 3 | + | | + | + | | + |
| 4 | + | | + | | | + |
| 5 | + | | + | | | + |
| 6 | + | + | | | | + |

Quantitative determination of poviargol was carried out by the method of rhodanometric titration [22]. The study of antimicrobial activity was carried out by diffusion into agar using stainless steel cylinders [23]. The test samples of suppositories were melted in water at a temperature of 40 ºC, the primary packaging was opened and the contents were transferred to cylinders. It was left at room temperature until solidification. 100 µl of a suspension of microorganisms with a microbial load of 100 $\times 10^6$ microbial cells in 1 ml were applied to the surface of the agar. Cylinders with samples of suppositories were placed on the seeded surface. Antimicrobial activity was determined by the diameter of the growth retardation zone [24, 25]. Gram-positive (*Staphylococcus aureus* ATCC 6538), gram-negative (*Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 8739) and spore-forming (*Bacillus subtilis* ATCC 6633) microorganisms and fungi (*Candida albicans* ATCC 10231) were used as test cultures.
Statistical data processing was carried out using the Microsoft Excel 2010.

## Results and Discussion

Suppositories of all samples were torpedo-shaped with smooth surface and a funnel-shaped recess. It was found that the solutions obtained during the sample preparation of suppositories of samples 1 and 2 had pH of 4.15-4.28, corresponding to the physiological pH of the vagina (4-4.5). The pH value of the remaining series of suppositories was at least 7.08 **(Table 2)**. The lowest decay time was observed in sample 6, while in all series it met the state requirements. It was shown that the emulsifier polysorbate 80 did not statistically significantly affect ($p=0.08$) the disintegration of suppositories with poviargol (samples 1 and 2) **(Table 2)**.

**Table 2.** Quality indicators of experimental series of suppositories with poviargol

| Sample | pH | Disintegration, min | Authenticity | Color |
|---|---|---|---|---|
| 1 | 4.28 | 22.3±0.03 | satisfy | Light brown |
| 2 | 4.15 | 22.0±0.3 | satisfy | Light brown |
| 3 | 7.98 | 35.8±0.7 | satisfy | Dark brown |
| 4 | 7.83 | 26.7±0.2 | satisfy | Dark brown |
| 5 | 7.08 | 27.5±0.4 | satisfy | Dark brown |
| 6 | 7.21 | 15.3±0.4 | satisfy | Brown |

It was found that with an increase in the molecular weight of the macrogols that make up the suppositories, their decay time increases **(Figure 1)**. When studying the uniformity of the mass of the experimental samples of suppositories, it was found that samples 1,2,4-6 fully comply with the state requirements in this indicator. Samples 1 and 2 had the best indicators (mass deviation of 0.07% and 0.08%). It was determined that the difference in the weight of individual suppositories of sample 3 reached 15.7%. It was found that at the bottling stage at a temperature of 44-45 ºC, macrogol 4000, which is part of the suppositories, solidified, while the rest of the suppository mass remained liquid. At the same time, it remained on the walls of the bunker and partially blocked the outlet. As the filling temperature increased, the suppository mass began to darken **(Table 3)**.

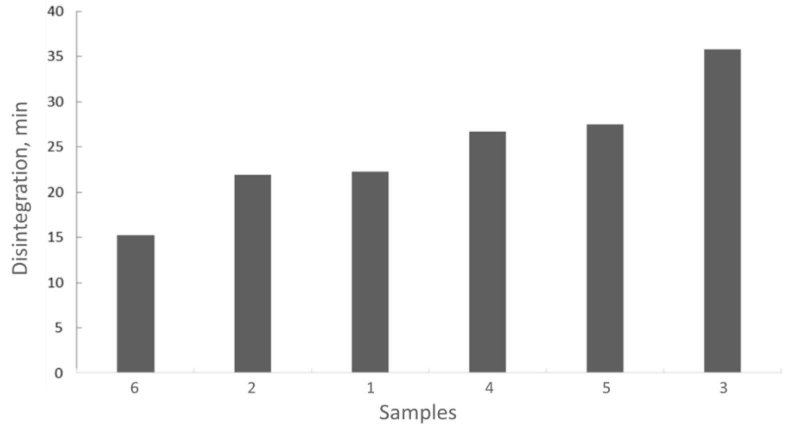


**Figure 1.** The effect of the molecular weight of macrogol on the disintegration of suppositories

**Table 3.** Uniformity of mass of experimental series of suppositories with poviargol (n=20)

| Index | Sample | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Average weight, mg | 3003±1 | 3004±1 | 2722±225 | 2994±7 | 2999±6 | 2997±5 |
| The weight range of suppositories, mg | 3001-3005 | 3002-3006 | 2488-2950 | 2981-3003 | 2989-3010 | 2990-3007 |
| Acceptance criteria, mg | 2850 – 3150 | | | | | |
| Deviation in mass, % | 0.07 | 0.08 | 8.61 | 0.43 | 0.38 | 0.33 |
| Acceptance criteria, % | 5 | | | | | |

According to the results of microbiological studies, it was found that suppositories with poviargol have a wide antimicrobial effect. It was found that sample 2 has statistically significantly higher antimicrobial activity against *Escherichia coli* ($p<0.05$), samples 1 and 2 have higher activity than samples 5 and 6 with respect to *Pseudomonas aeruginosa* ($p<0.05$). Activity against *Candida albicans* and gram-positive microorganisms did not significantly differ from different series ($p>0.05$) **(Table 4)**. According to the totality of all data, the greatest antimicrobial effect was observed in suppositories prepared on the basis of sample 2. Based on the conducted research, the composition and technology of production of suppositories with poviargol (sample 2) have been developed. As result, 3 semi-industrial series have been manufactured.

**Table 4.** Antimicrobial activity of experimental series of suppositories with poviargol

| Sample | Diameter of the inhibition zone of test strains, mm, n=3 | | | | |
|---|---|---|---|---|---|
| | *E. coli* | *Ps. aeruginosa* | *B. subtilis* | *St. aureus* | *C. albicans* |
| 1 | 21±1 | 23±2 | 23±1 | 20±2 | 24±1 |
| 2 | 24±1 | 25±2 | 25±2 | 23±2 | 25±1 |
| 3 | 17±2 | 21±1 | 21±1 | 20±2 | 23±1 |
| 4 | 21±1 | 21±1 | 21±2 | 22±1 | 23±1 |
| 5 | 19±1 | 18±1 | 18±1 | 22±1 | 22±3 |
| 6 | 22±1 | 20±1 | 20±1 | 20±2 | 24±1 |

It was found that semi-industrial series of suppositories with poviargol correspond to the draft pharmacopoeia article in all indicators. They have a deviation from the nominal mass value of no more than 0.63% (an acceptable value of 5%) and the value of the interval between the mass of suppositories of one series from 0.07% (sample 2) to 0.20% (sample 3). It was shown that suppositories have a uniformity of poviargol content from 2.9% (sample 3) to 3.34% (sample 2) with an acceptance criterion of 15% **(Table 5)**.

**Table 5.** Quality indicators of semi-industrial series of suppositories with poviargol

| **Index** | **Semi-industrial series of suppositories with poviargol** | | | **Acceptance criteria** |
|---|---|---|---|---|
| | **1** | **2** | **3** | |
| Description | + | + | + | Torpedo-shaped suppositories with light brown color. A funnel-shaped recess is allowed. An air rod is allowed on the longitudinal section |
| Uniformity of mass, mg | 3009-3012 | 3000-3002 | 3012-3019 | 2850 – 3150 |
| Authenticity of the poviargol | + | + | + | Reaction with hydrochloric acid |
| | + | + | + | Reaction with ammonia solution |
| Disintegration, min | + | + | + | ≤ 60 |
| Uniformity of metered units, % | 100-103 | 100-103 | 101-103 | 85-115 |
| The quantitative content of poviargol, mg | 90.0-91.5 | 90.0-93.1 | 91.5-93.1 | 76.5-103.5 |
| Microbiological purity | + | + | + | According to state requirements |

**Conclusion**

It was determined that the physiological pH of the vagina was observed in solutions obtained during the sample preparation of suppositories with Ag NPs of samples 1 and 2 prepared on the basis of Macrogol 1000. It was found that the addition of macrogols with a high molecular weight to the composition of suppositories increases the decay time. It has been shown that the use of Macrogol 4000 in suppositories with poviargol is difficult, since the temperature of their preparation is lower than the solidification temperature of the auxiliary substance. The optimal composition (sample 2) and the temperature regime of preparation of suppositories (≤40-42 ºC) were selected. It has been established that suppositories exhibit a wide antimicrobial spectrum of action in relation to gram-negative (*Pseudomonas aeruginosa, Escherichia coli*), gram-positive (*Staphylococcus aureus*), gram-positive and spore-forming (*Bacillus subtilis*) microorganisms and fungi (*Candida albicans*). It is shown that the pilot series of suppositories with Ag NPs meet the specifications of the draft pharmacopoeia article in all respects.

**Acknowledgments:** None

**Conflict of interest:** None

**Financial support:** None

**Ethics statement:** None

**References**

1. Gabrielyan L, Trchounian A. Antibacterial activities of transient metals nanoparticles and membranous mechanisms of action. World J Microbiol Biotechnol. 2019;35(10):162. doi:10.1007/s11274-019-2742-6
2. Rohner E, Yang R, Foo KS, Goedel A, Chien KR. Unlocking the promise of mRNA therapeutics. Nat Biotechnol. 2022;40(11):1586-600. doi:10.1038/s41587-022-01491-z
3. Patil S, Chandrasekaran R. Biogenic nanoparticles: A comprehensive perspective in synthesis, characterization, application and its challenges. J Genet Eng Biotechnol. 2020;18(1):67. doi:10.1186/s43141-020-00081-3
4. Joudeh N, Linke D. Nanoparticle classification, physicochemical properties, characterization, and applications: A comprehensive review for biologists. J Nanobiotechnology. 2022;20(1):262. doi:10.1186/s12951-022-01477-8
5. Shah DD, Chorawala MR, Mansuri MKA, Parekh PS, Singh S, Prajapati BG. Biogenic metallic nanoparticles: From green synthesis to clinical translation. Naunyn Schmiedebergs Arch Pharmacol. 2024. doi:10.1007/s00210-024-03236-y
6. Arif M, Rauf A, Akhter T. A review on Ag nanoparticles fabricated in microgels. RSC Adv. 2024;14(27):19381-99. doi:10.1039/d4ra02467b
7. Chapa González C, González García LI, Burciaga Jurado LG, Carrillo Castillo A. Bactericidal activity of silver nanoparticles in drug-resistant bacteria. Braz J Microbiol. 2023;54(2):691-701. doi:10.1007/s42770-023-00991-7
8. Rios-Ibarra CP, Salinas-Santander M, Orozco-Nunnelly DA, Bravo-Madrigal J. Nanoparticle based antiviral strategies to combat the influenza virus (Review). Biomed Rep. 2024;20(4):65. doi:10.3892/br.2024
9. Madkhali OA. A comprehensive review on potential applications of metallic nanoparticles as antifungal therapies to combat human fungal diseases. Saudi Pharm J. 2023;31(9):101733. doi:10.1016/j.jsps.2023.101733

10. Zheng Y, Song J, Qian Q, Wang H. Silver nanoparticles induce liver inflammation through ferroptosis in zebrafish. Chemosphere. 2024:142673. doi:10.1016/j.chemosphere.2024.142673
11. Merino JJ, Cabaña-Muñoz ME. Nanoparticles and mesenchymal stem cell (MSC) therapy for cancer treatment: Focus on nanocarriers and a si-RNA CXCR4 chemokine blocker as strategies for tumor eradication in vitro and in vivo. Micromachines (Basel). 2023;14(11):2068. doi:10.3390/mi14112068
12. Zharkova MS, Golubeva OY, Orlov DS, Vladimirova EV, Dmitriev AV, Tossi A, et al. Silver nanoparticles functionalized with antimicrobial polypeptides: Benefits and possible pitfalls of a novel anti-infective tool. Front Microbiol. 2021;12:750556. doi:10.3389/fmicb.2021.750556
13. Slavin YN, Asnis J, Häfeli UO, Bach H. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. J Nanobiotechnology. 2017;15(1):65. doi:10.1186/s12951-017-0308-z
14. Sultan I, Rahman S, Jan AT, Siddiqui MT, Mondal AH, Haq QMR. Antibiotics, resistome and resistance mechanisms: A bacterial perspective. Front Microbiol. 2018;9:2066. doi:10.3389/fmicb.2018.02066
15. Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, et al. Molecular mechanisms of antibiotic resistance revisited. Nat Rev Microbiol. 2023;21(5):280-95. doi:10.1038/s41579-022-00820-y
16. Stabryla LM, Johnston KA, Diemler NA, Cooper VS, Millstone JE, Haig SJ, et al. Role of bacterial motility in differential resistance mechanisms of silver nanoparticles and silver ions. Nat Nanotechnol. 2021;16(9):996-1003. doi:10.1038/s41565-021-00929-w
17. Abootaleb M, Mohammadi Bandari N, Karimli M, Mobayen M, Feizkhah A, Masoumi S, et al. Assessing the safety of probiotic spray as an antibiotic alternative: A clinical trial at velayat burn injuries hospital. J Med Pharm Chem Res. 2024;6(4):466-73. doi:10.48309/jmpcr.2024.427850.1045
18. Alani S, Wang J, Suarthana E, Tulandi T. Complications associated with cervical cerclage: A systematic review. Gynecol Minim Invasive Ther. 2023;12(1):4-9. doi:10.4103/gmit.gmit_61_22
19. Dioguardi M, Polverari D, Spirito F, Iacovelli G, Sovereto D, Laneve E, et al. Introspection of the etiopathological mechanisms underlying noncarious cervical lesions: Analysis of the different theories and their impact on the mineralized structures of the tooth. Int J Dent. 2023;2023:8838314. doi:10.1155/2023/8838314
20. Roberts WE, Mangum JE, Schneider PM. Pathophysiology of demineralization, part I: Attrition, erosion, abfraction, and noncarious cervical lesions. Curr Osteoporos Rep. 2022;20(1):90-105. doi:10.1007/s11914-022-00722-1
21. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 2021;9(10):2041. doi:10.3390/microorganisms9102041
22. Manukyan H, Lal M, Zhu C, Singh O, Lin TL, Tritama E, et al. Application of MPBT assay for multiplex determination of infectious titers and for selection of the optimal formulation for the trivalent novel oral poliovirus vaccine. Viruses. 2024;16(6):961. doi:10.3390/v16060961
23. Blinova A, Blinov A, Kravtsov A, Nagdalian A, Rekhman Z, Gvozdenko A, et al. Synthesis, characterization and potential antimicrobial activity of selenium nanoparticles stabilized with cetyltrimethylammonium chloride. Nanomaterials (Basel). 2023;13(24):3128. doi:10.3390/nano13243128
24. Blinov AV, Nagdalian AA, Povetkin SN, Gvozdenko AA, Verevkina MN, Rzhepakovsky IV, et al. Surface-oxidized polymer-stabilized silver nanoparticles as a covering component of suture materials. Micromachines (Basel). 2022;13(7):1105. doi:10.3390/mi13071105
25. Filipović N, Ušjak D, Milenković MT, Zheng K, Liverani L, Boccaccini AR, et al. Comparative study of the antimicrobial activity of selenium nanoparticles with different surface chemistry and structure. Front Bioeng Biotechnol. 2021;8:624621. doi:10.3389/fbioe.2020.624621