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Eur. Pharm. J. 2025, 72(1), 47-53.
ISSN 1338-6786 (online) and ISSN 2453-6725 (print version),
DOI: 10.2478/afpuc-2025-0007

EUROPEAN PHARMACEUTICAL JOURNAL

Formulation of stimuli-responsive gels containing silver complex with nicotinamide

Research paper

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Received 9 June, 2025, accepted 8 July, 2025

Abstract The use of stimuli-responsive gels is increasingly widespread and includes almost all routes of administration. Their main property is the phase transition from sol to gel under the influence of physiological stimuli such as temperature, pH, ion presence, or enzymes. The aim of this formulation study was to prepare oral *in situ* gel containing silver complex with nicotinamide. We prepared formulations based on the thermosensitive polymer Pluronic® F-127 (15% w/w), methylcellulose (0.25% w/w), and various concentrations of the ion-sensitive polymer sodium alginate (0.2%–4% w/w). Various properties were evaluated, such as pH, injectability, critical sol–gel transition temperature of sols, gelation capacity, as well as dissolution profile and antimicrobial activity. We found that sodium alginate affects the critical sol–gel transition temperature of sols, and with higher concentrations, the sol–gel transition temperature decreases. At a 4% w/w sodium alginate concentration, the presence of calcium cations was necessary for gel formation. Sodium alginate also significantly influenced the viscosity of sols; higher concentrations led to more viscous sols. The most suitable formulation contained 4% sodium alginate and was used to incorporate silver complex with nicotinamide, a new potential antimicrobial agent. The drug release kinetics most closely correlated with the first-order kinetics, and the drug was released via Fickian diffusion. During antimicrobial activity testing, the formulation demonstrated higher efficacy against *Pseudomonas aeruginosa* compared to a commercial dental gel containing chlorhexidine gluconate, although this difference was not statistically significant. Overall, the chlorhexidine gel showed greater efficacy against most of the tested bacteria, while the antimicrobial *in situ* gel maintained a consistent moderate level of antimicrobial activity.

Keywords *in situ gels – silver nicotinamide – sodium alginate – methylcellulose – poloxamer*

INTRODUCTION

Stimuli-responsive gels (*in situ* gels) represent a modern trend in the development of dosage forms. The main characteristic of *in situ* gels is their phase change from sol to gel under certain conditions such as change in pH, temperature, influence of ions, or enzymes (Madan *et al.*, 2009). Polymers of natural or synthetic origin are used in their preparation, many of which also have mucoadhesive properties, thus providing longer drug contact with the mucosa, increasing bioavailability and reducing the frequency of drug administration. In recent years, there has been increasing interest in their use in various routes of administration including oral, ocular, rectal, or topical application (Mohanty *et al.*, 2018).

Poloxamers and methylcellulose (MC) are thermosensitive polymers. Upon changing the temperature, the dispersed particles change into a dense three-dimensional network structure. This phase separation occurs due to the formation of hydrophobic interactions between the polymer chains, which form a network structure in an aqueous environment. The sol–gel transition occurs at the so-called lower or upper critical solution temperature (Fan *et al.*, 2022).

Ion-sensitive polymers used for the preparation of *in situ* gel systems generally contain ionizable groups. The polymer anions crosslink with monovalent (Na^+, K^+) and/or divalent (Mg^{2+}, Ca^{2+}) cations that are present in various physiological fluids such as tears, saliva, etc. The type and concentration of

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the cation determine the viscosity of the crosslinked polymer and the rate of sol–gel transition. This group mainly includes polymers of natural origin and their derivatives such as pectin, gellan gum, methacrylated gellan gum, alginic acid, and sodium alginate (SA) (Kolawole and Cook, 2023). SA is a linear polymer composed of α(1→4)-l-guluronic acid and β(1→4)-d-mannuronic acid, derived from brown algae. It forms a water-insoluble gel in the presence of Ca^{2+} ions (Afrin Shefa *et al.*, 2022). It has good mucoadhesive properties and is biocompatible and nontoxic (Majeed and Khan, 2019).

Silver complex with nicotinamide (AgNam) was prepared by chemical reaction of nicotinamide with silver nitrate for 2 weeks. The synthesized compound $[Ag(Nam)_2]NO_3 \cdot H_2O$ is insoluble in water but soluble in other polar solvents and should be stored in the absence of light. AgNam was found to have higher antimicrobial potency than $AgNO_3$ against *Staphylococcus aureus* and *Escherichia coli*. Compared to the commercially used drug silver sulfadiazine, the antimicrobial activity of AgNam was slightly higher against *Sta. aureus* and *E. coli* and the antifungal activity against *Candida parapsilosis* was 30 times higher and approximately three times higher against *Rhizopus oryzae* (Rendošová *et al.*, 2017).

METHODS

Chemicals: Pluronic® F-127 (PF127) and SA were purchased from Sigma-Aldrich (St. Louis, USA), MC from Dr. Kulich Pharma (Hradec Králové, CZ), methylene blue (MB) and KH_2PO_4 from Centralchem (Bratislava, SR), NaCl from Mikrochem (Prešov, SR), $CaCl_2$ from Merck (Rahway, USA), and NaOH from Lachema (Brno, CZ). Silver complex with nicotinamide was synthesized at the Department of Inorganic Chemistry at the University of Pavol Jozef Šafárik (SR). Purified water ISO 2, artificial saliva medium (12 mmol/l KH_2PO_4, 40 mmol/l NaCl, 1.5 mmol/l $CaCl_2$, 0.2 mol/l NaOH, pH 6.8), and phosphate buffer (pH 6.8) were prepared at the University of Veterinary Medicine and Pharmacy in Košice (UVLF) (SR).

Material for antimicrobial activity: Mueller–Hinton agar, antibiotic disks containing 10 µg of gentamicin, and disks with 6-mm diameter were purchased from Oxoid (Hampshire, UK), blood agar from HiMedia (Maharashtra, IN), Corsodyl® from Purna Pharmaceuticals N.V. (Puurs-Sint-Amands, BE), and *aqua pro injectione* from B. Braun (Melsungen, DE)**.** Bacterial strains *Sta. aureus*, *Streptococcus pyogenes*, *E. coli*, *Pseudomonas aeruginosa* were clinical or environmental isolates from UVLF (SR). Human oral swabs were obtained from the collection of the Department of Microbiology and Immunology at UVLF (SR).

Preparation of formulations: We prepared 11 different formulations consisting of 15% w/w of thermosensitive PF127, 0.25% w/w of MC, and various concentrations of ion-sensitive SA (0.2%–0.8% and 4% w/w). Polymers were dispersed in cold water (4 °C–5 °C) using a shaft mixer (Witeg Labortechnik, Wertheim, DE) for 15 min at 1000 rpm. This was followed by 24-h storage of samples in refrigerator.

Table 1. Composition of formulations.

Formulation	PF127 (% w/w)	MC (% w/w)	SA (% w/w)	AgNam (% w/w)
PMSA 0.2	15.00	0.25	0.20	-
PMSA 0.3	15.00	0.25	0.30	-
PMSA 0.4	15.00	0.25	0.40	-
PMSA 0.5	15.00	0.25	0.50	-
PMSA 0.6	15.00	0.25	0.60	-
PMSA 0.7	15.00	0.25	0.70	-
PMSA 0.8	15.00	0.25	0.80	-
PSA	15.00	-	4.00	-
PMSA	15.00	0.25	4.00	-
MSA	-	0.25	4.00	-
SA	-	-	4.00	-
PMSA AgNam	15.00	0.25	4.00	1.00

Formulation containing AgNam was prepared similarly. AgNam was primarily suspended in cold water, followed by the addition of the polymer. Tab. 1. shows the composition of prepared formulations.

Appearance of sols and gels was visually evaluated vertically and horizontally against a black and white background. We looked for transparency, color, opalescence, and the presence of any aggregates or sediments. Samples were characterized by the following signs: (+) turbid – turbidity is present; (++) transparent – minimal turbidity or opalescence is present; (+++) glassy – without turbidity or opalescence (Bhandwalkar and Avachat, 2013).

pH of sols was evaluated using the pH meter Seven Compact S220 (Mettler Toledo, Columbus, USA). Samples were measured in triplicate at a temperature of 25 °C (Bhandwalkar and Avachat, 2013).

Injectability of sols was examined using a 5-ml syringe with injection needles of various diameters (0.5, 0.6, 0.7, 0.8 mm). We tried to squeeze out 1 ml of polymer solution smoothly. The polymeric solutions were evaluated as follows: injectable, the sol exits dropwise (+); injectable, the sol exits dropwise almost forming a stream (++); injectable, the sol exits as a stream (+++); noninjectable (–).

Gelation capacity: One gram of sample was colored by 1 drop of MB. Five hundred micrograms of colored sample was added to heated phosphate buffer (37 °C ± 0.5 °C) in testing tubes representing artificial saliva. The testing tubes were tempered in a heating bath (Vevor, Shanghai, CN) (37 °C) and gel stability was evaluated at several time intervals after 10, 20, 30, 60, 90, 120, and 150 min after applying vortex at 300 rpm (Usmate MB, VELP Scientifica, IT).

Critical sol–gel transition temperature (T_{sg}) was determined by increasing the temperature of sols placed into a water bath from 25 °C to 40 °C by 1 °C increments. The test tube with

sample was tilted after every temperature increase to assess if gelation occurred. T_{sg} was the temperature at which a gel was formed (Bhandwalkar and Avachat, 2013).

Pre-dissolution, dissolution: The pre-dissolution test was carried out to examine the release kinetics of MB from samples. Modified dissolution apparatus USP 2 Sr-8 Plus (Hanson Research, Chatsworth, USA) was used. For stirring, a shaft impeller with paddles was used and set at 50 rpm. Beakers with samples (1 g) colored by MB (one or two drops/g of sample) and dissolution medium (50 ml of artificial saliva with $CaCl_2$) were placed into the water bath of the dissolution apparatus (37 °C ± 0,5 °C). The sampling schedule was 0, 10, 20, 30, 40, 50, and 60 min and consisted of withdrawing 2 ml of dissolution medium and replacing it with 2 ml of fresh tempered medium. The last sample was taken after homogenization of the volume and represented completed dissolution. The absorbance of the sample was measured using Cary 60 UV–Vis spectrophotometer (Agilent, Santa Clara, USA) by scanning the sample at 665 nm. Dissolution profile of the samples containing AgNam was determined in the same manner; however, two more sampling times were added (0, 5, 10, 15, 20, 30, 40, 50, and 60 min). The absorbance of the sample was measured by scanning the sample at 260 nm.

Antimicrobial activity of *in situ* gels with AgNam was tested using the disk diffusion method. Activity was tested on two gram-positive bacterial strains, *Sta. aureus* and *Str. pyogenes*, two gram-negative bacterial strains, *E. coli* and *P. aeruginosa*, and two human oral swabs. Part of the colony of the bacterial culture cultivated on blood agar was retrieved and suspended in 3 ml of physiological solution in sterile testing tubes. Bacterial suspension was thoroughly mixed using vortex (VELP Scientifica, IT), and turbidity was measured with a densitometer (BioSan, Riga, LV). Bacteria were inoculated onto Mueller–Hinton agar in 90-mm-diameter Petri dishes. Five disks with 6-mm diameter were placed onto the agar. Clear disks were treated with 10 mg of samples: *in situ* gel without active ingredient (negative control), *in situ* gel with 1% of AgNam, and Corsodyl® with 1% of chlorhexidine gluconate. Antibiotic disk with 10 µg of gentamicin was used as a positive control. In addition, 10 µg of water suspension containing 1% of AgNam was used. Incubation took place during 24 h at 37 °C. The diameter of the inhibition zone (IZD) was measured in millimeters. The antibacterial activity was calculated according to the following formula, where RIZD expresses the percentage of the relative inhibition zone diameter (Rojas *et al.*, 2006):

$$\%\mathrm{RIZD} = \frac{\mathrm{IZD\ (sample)} - \mathrm{IZD\ (negative\ control)}}{\mathrm{IZD\ (pozitive\ control)}} \times 100$$

Since *in situ* gels were not sterile, a control of microbial contamination was used Blood agar with *in situ* gel containing AgNam was incubated for 24 h. MS Excel and Minitab were used for the statistical analysis by analysis of variance and Tukey test for the identification of statistically significant differences. The results were marked based on the results of the Tukey test: A, B, C represent statistically significant differences in the efficacy of tested samples. Values marked with the same letter are not significantly different from each other. Statistically significant differences were marked as follows: * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.

RESULTS ANS DISCUSSION

PF127-based hydrogels are liquid at room temperature and transition to a gel form at body temperature. Their main limitation is their poor mechanical strength and stability in physiological media (Giuliano *et al.*, 2018). The addition of MC to formulations with PF127 and SA should ensure controlled drug release and increase the viscosity and gel strength (Banerjee *et al.*, 2013; Pham *et al.*, 2021). The ideal oral gel should have high mucoadhesiveness since the oral mucosa is in constant contact with saliva, and food intake, chewing, and speech also have an effect (Bansal *et al.*, 2009). Since poloxamers mix relatively quickly with saliva, it is advisable to use additional polymers in the preparation. In a study by Chanaj-Kaczmarek et al. (2021), they prepared thermosensitive hydrogels containing PF127, SA, and MC, and evaluated their rheological and mucoadhesive properties. The results showed that SA increased the strength of the hydrogel, prolonged the gel residence time on the mucosa, and provided suitable rheological properties of the formulation. Together with MC, they improved the mucoadhesiveness of the hydrogel (Chanaj-Kaczmarek *et al.*, 2021).

Most of the sol samples were glassy. The formulations of PMSA 0.7, PMSA 0.8, MSA, and SA were opalescent. The appearance remained the same after gelation of the sols. The PMSA AgNam formulation was brown in color, with turbidity, and had the consistency of a thick sol, almost a gel even at room temperature. Under light, it darkened over time due to a photochemical reaction of the silver compounds (Long and Cai, 2014). Tab. 2 shows the appearance of samples.

The average pH (Tab. 3) ranged from 5.61 ± 0.02 to 6.78 ± 0.00. As the concentration of SA in the formulations increased, the pH decreased slightly from 6.78 ± 0.00 to 6.60 ± 0.01. For the formulations with the highest concentration of SA (4.0% w/w), the average pH values ranged from 5.61 ± 0.02 to 6.26 ± 0.00. The presence of MC and PF127 in the formulations caused a slight increase in pH. AgNam was effective in lowering the pH of the formulation from an average of 6.26 ± 0.00 for PMSA to 5.61 ± 0.02 for PMSA AgNam. The pH value fluctuated slightly for PMSA AgNam. Decrease in pH after the addition of AgNam to the cream and fluctuation of the measured pH values were also observed in the study by Sovova et al. (2023). Repeated pH measurements after 2 and 4 weeks showed a slight increase in the pH of the cream with AgNam (Sovová *et al.*, 2023).

On testing injectability of the sols, it was found that the combination of SA and MC with PF127 increases the viscosity

Table 2. Appearance and injectability of sols and gels.

Formulation	Appearance		Injectability (needle diameter)			
	Sol	Gel	0.5 mm	0.6 mm	0.7 mm	0.8 mm
PMSA 0.2	+++	–	+	++	++	+++
PMSA 0.3	+++	–	+	+	++	+++
PMSA 0.4	+++	+++	+	+	+	+++
PMSA 0.5	+++	+++	+	+	+	+++
PMSA 0.6	+++	+++	+	+	+	++
PMSA 0.7	++	++	+	+	+	++
PMSA 0.8	++	++	+	+	+	++
PSA	+++	–	–	–	+	+
PMSA	+++	–	–	–	+	+
MSA	++	–	–	+	+	+
SA	++	–	–	+	+	+
PMSA AgNam	+	–	–	–	+	+

Appearance: (+) turbid – turbidity or suspended particles are present; (++) transparent – minimal turbidity or opalescence is present; (+++) glassy – without turbidity or opalescence; (–) no gelation.
Injectability: injectable, the sol exits dropwise (+), injectable, the sol exits dropwise almost forming a stream (++), injectable, the sol exits as a stream (+++), noninjectable (–)

Table 3. pH and critical sol–gel transition temperature of the formulations.

Formulation	pH ± SD (n = 3)	T_{sg} (°C) ± SD (n=3)
PMSA 0.2	6.78 ± 0.00	-
PMSA 0.3	6.72 ± 0.00	-
PMSA 0.4	6.70 ± 0.00	34.10 ± 0.28
PMSA 0.5	6.68 ± 0.01	33.36 ± 0.13
PMSA 0.6	6.64 ± 0.00	32.66 ± 0.17
PMSA 0.7	6.61 ± 0.00	33.60 ± 0.08
PMSA 0.8	6.60 ± 0.01	31.50 ± 0.36
PSA	6.16 ± 0.02	-
PMSA	6.26 ± 0.00	-
MSA	5.84 ± 0.00	-
SA	5.75 ± 0.01	-
PMSA AgNAM	5.61± 0.02	-

of the formulations. The higher the concentration of SA in the formulation, the harder the salt passed through the needle. The addition of AgNam to the PMSA formulation did not affect the injectability test. Tab. 2 shows the injectability of formulations.

With increasing concentration of SA in the formulations, there was longer gel residence time. PMSA 0.8 formulation was stable even after 150 min. For formulations with 4% w/w SA content (PSA, PMSA, MSA, SA), there was immediate disintegration of all gels.

The combination of PF127 and SA causes a reduction in T_{sg} (Tab. 3). SA promotes PF127 micelle formation and decreases micelle–micelle interactions, weakening the elastic component of the gel, thus leading to a faster gelation process and a decrease in T_{sg} (Thouvenin *et al.*, 2022). For PMSA 0.2 and PMSA 0.3 formulations, gel formation did not occur even at 40 °C. Here, the concentration of SA and MC was probably low for T_{sg} reduction to occur. According to Altuntaş and Yener (2017), the sol–gel transition for PF127 at a concentration of 16.0% w/w occurred only at 50 °C. As the concentration of PF127 increased, T_{sg} decreased. Likewise, the addition of mucoadhesive polymers decreased T_{sg} (Altuntaş and Yener, 2017). For formulations from PMSA 0.4 (34.10 °C) to PMSA 0.8 (31.50 °C), we observed a decrease in T_{sg} with increasing SA concentration, except for the formulation PMSA 0.7 (33.60 °C), which unexpectedly showed a 1 °C increase in T_{sg} compared to the formulation PMSA 0.6. On the contrary, at high SA concentrations (4.0% w/w), no sol–gel phase transition occurred up to 40 °C, which can be explained by the fact that such a high SA concentration in the formulation needs the presence of Ca^{2+} for transition to the gel form.

Due to the limited amount of the synthesized silver complex, methylene blue (MB) was selected as a model substance for the dissolution test. Since both compounds are hydrophilic, we assumed a similar release profile (Rendošová et al., 2017; Bollinger et al., 2025). In the MB pre-dissolution test (Fig. 1), all PMSA 0.2 to PMSA 0.8 formulations immediately underwent gel disintegration. On the contrary, PSA, PMSA, MSA, and SA formulations were stable. In a study by Thouvenin et al. (2022), the kinetics of ketamine release from the PF127- and SA-based gel most closely fitted the Korsmeyer–Peppas model and the type of release according to the value of the exponential factor n corresponded to non-Fick diffusion (Thouvenin *et al.*, 2022). In our case, for MB pre-dissolution, the PSA (R^2_{adj} 0.996), PMSA (R^2_{adj} 0.991), and MSA (R^2_{adj} 0.998) formulations showed the best correlation with the Hixson–Crowell model and the SA formulation (R^2_{adj} 0.995) correlated with first-order kinetics. From the PSA (n = 0.473), MSA (n = 0.289), and SA (n = 0.193) formulations, MB was released by diffusion; from the PMSA (n = 0.534) formulation, it was released by non-Fick diffusion. MB was released most rapidly from the SA formulation. The addition of MC to the formulation slightly slowed the release of MB. Better results were obtained by PSA and PMSA formulations, which had very similar dissolution profiles; however, MC in the PMSA formulation caused slower release of MB. After 40 min, more than 90% of MB was released from all formulations. Since PMSA sample provided the slowest release profile, we selected this formulation to prepare an *in situ* gel with AgNam.

In the PMSA AgNam dissolution test (Fig. 2), we found that the drug release kinetics most closely correlated with the

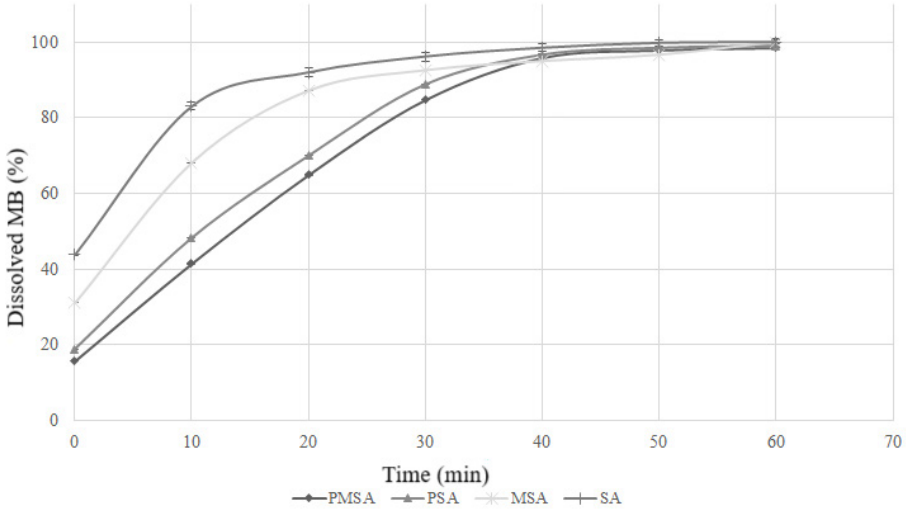


Figure 1. Dissolution profile of methylene blue.

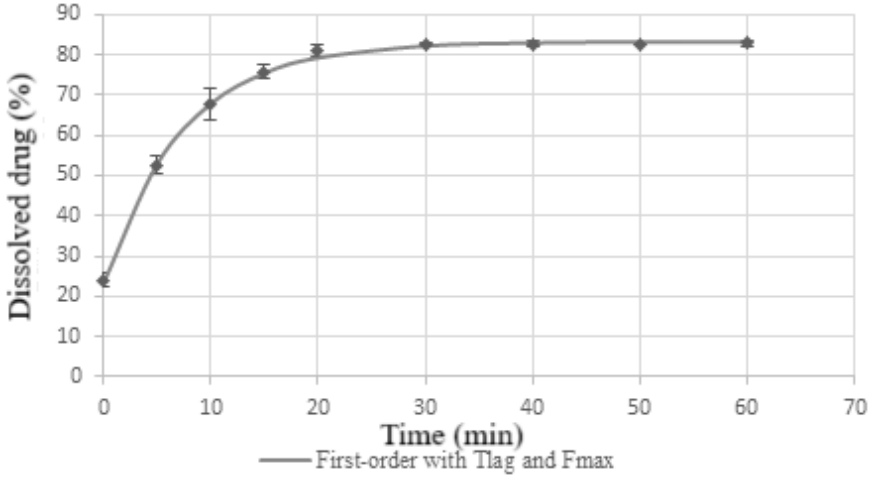


Figure 2. Dissolution profile of PMSA AgNam.

first-order kinetics (R^2_{adj} 0.997) and the drug was released by Fick diffusion (n = 0.213). AgNam was released from the formulation faster than MB, with AgNam being suspended in the formulation and MB being dissolved. After 10 min, 67.52% of AgNam was released from PMSA and after 20 min, it exceeded almost 81% (30 min: 82.6%). From this point onward, the release slowed down and even after 60 min, the amount of released AgNam did not exceed 83%. Sovová et al. (2023) dissolved AgNam in propylene glycol for preparing the cream (Sovová *et al.*, 2023). According to Choi et al. (1999), propylene glycol, ethanol, and hydrochloric acid increased T_{sg} and slightly decreased the gel strength and bioadhesiveness (Choi *et al.*, 1999).

The PMSA AgNam formulation had higher antibacterial activity compared to the 1% aqueous AgNam suspension for all bacterial strains and swabs, probably due to the evenly dispersed substance throughout the formulation volume (Figs 3 and 4). Despite thorough mixing of the aqueous AgNam suspension before each application to the disk, it is possible that insufficient amounts of AgNam were deposited on the disk or that AgNam did not diffuse efficiently from the disk into the agar. The PMSA AgNam formulation demonstrated the highest efficacy against *P. aeruginosa* (73.47%). In this case, the efficacy was also higher compared to the gel containing 1% chlorhexidine (67.87%), but this difference was not statistically significant (Fig. 3). In other cases, the chlorhexidine-containing gel had higher efficacy, but PMSA AgNam maintained a stable intermediate level of antimicrobial activity (Fig. 3). Sovova et al. (2023) reported higher antimicrobial activity of AgNam cream for *Sta. aureus* and *Bacillus subtilis* compared to commercially available silver sulfadiazine cream (Sovová *et al.*, 2023).

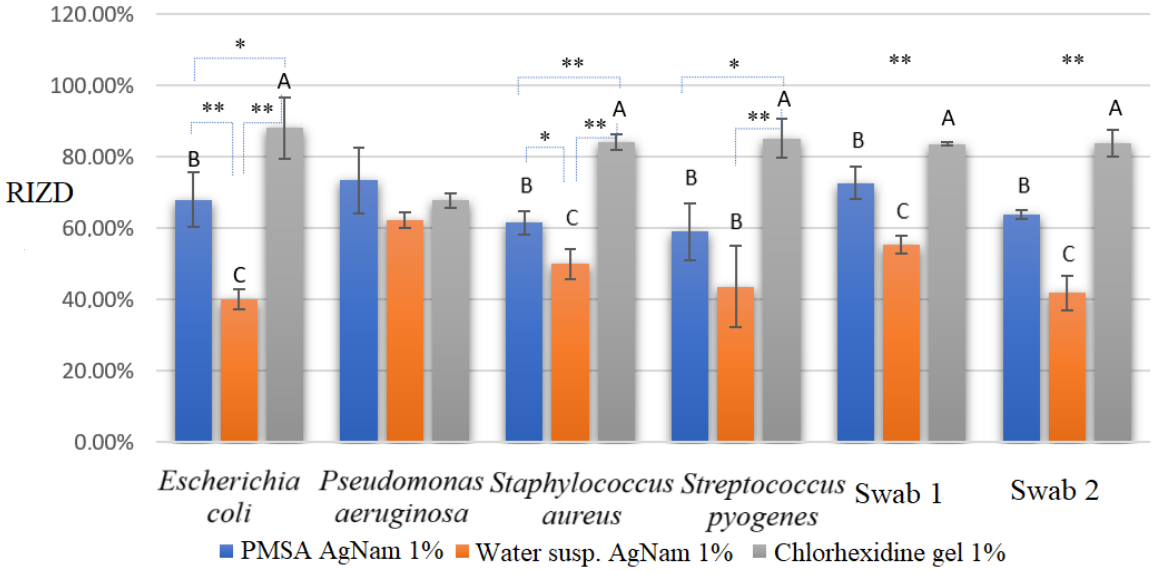


*Figure 3. Antibacterial activity of PMSA AgNam. n = 3 ± SD. A, B, C represent statistically significant differences in the efficacy of tested samples. Values marked with the same letter are not significantly different from each other. Statistically significant differences are marked as follows: * for p < 0.05; ** for p < 0.01; *** for p < 0.001. The same significance applies for swab 1 and 2 for all pairs.*

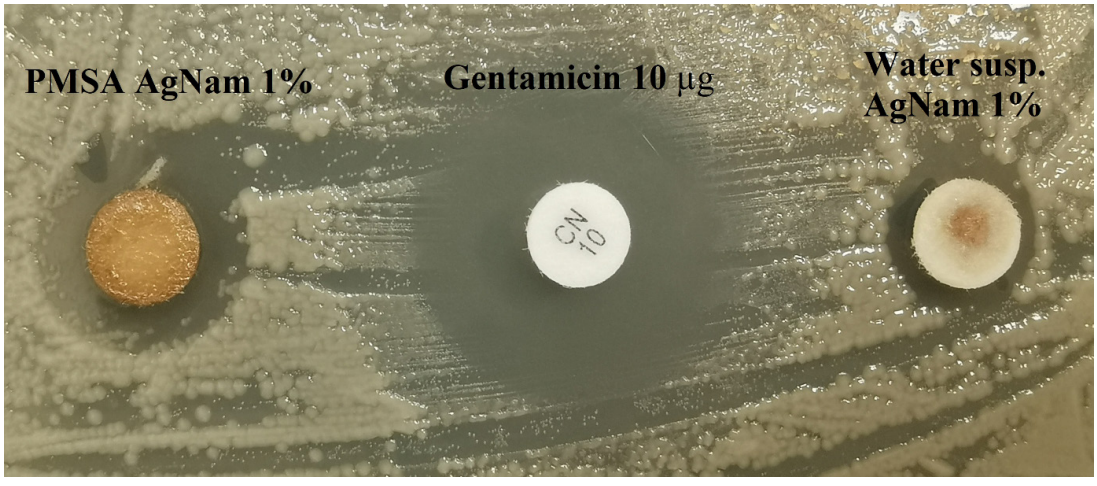


Figure 4. Inhibition zones for Streptococcus pyogenes.

After an overall evaluation of the results, we can assume that the *in situ* PMSA AgNam gel has potential for the treatment of oral infections. In the future, it would be advisable to investigate the difference in dissolution and antimicrobial activity if AgNam was dissolved in the formulation, or to adjust the ratios of the components of the *in situ* gel to ensure sufficient mucoadhesiveness and appropriate rheological properties.

CONCLUSION

The PMSA formulation exhibited the most favorable dissolution profile for oral administration, with a preference for slower drug release and longer residence time of the dosage form. The PMSA AgNam formulation achieved higher antimicrobial activity against all bacterial strains used and both human oral swabs compared to the aqueous AgNam suspension. Overall, the chlorhexidine gel had higher efficacy against most of the bacteria tested, while the *in situ* PMSA AgNam gel maintained a consistent intermediate level of antimicrobial activity. The prepared *in situ* gel with silver nicotinamide showed relatively good results, but it needs to be investigated what effect dissolving AgNam in the formulation would have on its properties and drug release kinetics and possibly on the antimicrobial activity.

ACKNOWLEDGMENT

This work was supported by KEGA 002UVLF-4/2024, 3D printing – Innovative preparation of medicines and APVV-23-0005, Microbiome modulators for maintaining dermal health in animals.

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