Journal of Basic and Applied Pharmacology
Vol. 5 No.1 January - June 2025

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Original research article

Antimicrobial efficacy and cytotoxicity evaluation of silver nanoparticle microemulsions for potential intranasal therapy

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Received 14 March 2025; Received in revised form 22 April 2025
Accepted 24 April 2025; Available online XX June 2025

ABSTRACT

Background:
Acute Bacterial Rhinosinusitis (ABRS) is commonly treated with antibiotics, but increasing resistance underscores the need for alternative therapies. Silver nanoparticles (AgNPs), known for their broad-spectrum antimicrobial activity, may offer promise when delivered intranasally via microemulsions.

Objectives:
To synthesize and characterize silver nanoparticle microemulsions and evaluate their antimicrobial activity and cytotoxicity for potential intranasal use in ABRS.

Methods:
Two microemulsion formulations (A and B) were synthesized via chemical reduction. Characterization included UV–Vis spectroscopy, dynamic light scattering, and zeta potential analysis. Antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Candida albicans* were evaluated using disk diffusion and broth dilution methods. Cytotoxicity was assessed in RAW 264.7 macrophages using the MTT assay. VC_{50} values were calculated.

Results:
Formulations A and B had particle sizes of 256.7 ± 0.97 nm and 147.1 ± 2.7 nm, respectively, with zeta potentials near −2 mV. Both demonstrated limited antimicrobial effects (inhibition zones: 8–11 mm; MIC/MBC > 62.5 µg/mL). Cytotoxicity testing showed >70% cell viability

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at concentrations ≤4.0 µg/mL. VC_{50} values were 8.6 µg/mL (A) and 4.1 µg/mL (B), indicating greater cytotoxicity for formulation B.

Conclusion:

Both silver microemulsions exhibited acceptable in vitro cytocompatibility at concentrations ≤4.0 µg/mL but limited antimicrobial potency. Further formulation refinement is needed to enhance efficacy for potential intranasal applications in ABRS treatment.

Keywords: silver nanoparticles, microemulsion, antimicrobial efficacy, cytotoxicity, acute bacterial rhinosinusitis

1. Introduction

Acute bacterial rhinosinusitis (ABRS) represents one of the most common infections of the upper respiratory tract, typically necessitating antibiotic intervention. However, the rising prevalence of antibiotic-resistant pathogens has increasingly compromised the efficacy of conventional antimicrobial therapies, presenting a significant clinical challenge in the effective management of ABRS.[1] Compounding this issue, various pharmacological limitations inherent to intranasal antibiotic formulations further restrict therapeutic success. These limitations include suboptimal mucosal penetration, rapid mucociliary clearance which reduces the residence time of drugs within the nasal cavity and unintended disruption of the native nasal microbiota, which may predispose patients to secondary infections and impair local immune defense mechanisms. Moreover, the frequent or prolonged use of systemic or topical antibiotics can lead to undesirable systemic side effects and further accelerate the emergence of multidrug-resistant organisms, thereby narrowing future treatment options.

Considering these limitations, there is a compelling need to develop alternative therapeutic modalities that not only demonstrate potent antimicrobial activity but also possess an improved safety and pharmacological profile. Among the most promising candidates are silver nanoparticles (AgNPs), which have garnered significant attention due to their broad-spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria.[2,3] Their unique mechanisms of action include the induction of reactive oxygen species (ROS), leading to oxidative damage of bacterial cell membranes, intracellular proteins, and nucleic acids, ultimately resulting in cell death.[4-6] Additionally, the sustained release of silver ions disrupts key metabolic and structural processes within bacterial cells. Notably, AgNPs with diameters smaller than 10 nanometers exhibit superior surface area-to-volume ratios, allowing for enhanced cellular uptake and bactericidal activity, while simultaneously reducing the potential for resistance development.[7,8] These multifactorial mechanisms of action make AgNPs particularly attractive for treating infections that are refractory to standard antibiotics.

To further optimize the clinical utility of AgNPs, microemulsion-based delivery systems have been introduced as innovative platforms to enhance drug solubility, stability, and bioavailability. Microemulsions are thermodynamically stable, isotropic systems composed of oil, water, surfactants, and co-surfactants, with droplet sizes typically in the nanometer range. When used to deliver AgNPs, these systems offer critical advantages, including uniform nanoparticle dispersion, prevention of aggregation, and controlled, sustained release, which collectively enhance therapeutic efficacy while minimizing cytotoxicity.[9] The biphasic nature of microemulsions also facilitates improved permeation across biological membranes, enabling more efficient localization of AgNPs at the site of infection and prolonging their antimicrobial activity. Preclinical studies have demonstrated that silver-containing

microemulsions not only potentiate the bactericidal effects of AgNPs but also reduce their toxicity to surrounding tissues, making them especially suitable for intranasal applications in the treatment of ABRS.[10,11]

The potential for silver nanoparticles in nasal and respiratory applications has been well documented.[12-15] AgNPs have been incorporated into nasal sprays, inhalable aerosols, and mucosal formulations due to their strong antimicrobial properties and low mucosal irritancy at appropriate concentrations. For example, silver-based nasal sprays have demonstrated efficacy in reducing bacterial loads and biofilm formation in rhinosinusitis models, with minimal toxicity to nasal epithelium.[12,13] *In vivo* studies on inhaled AgNPs have shown their capacity to mitigate inflammation and bacterial colonization in the respiratory tract.[14] Furthermore, some formulations exhibit mucoadhesive properties, increasing residence time in the nasal cavity and enhancing localized therapeutic effects.[15] These findings provide a solid foundation for investigating AgNP-based microemulsions as a potential treatment for ABRS, particularly in the context of antibiotic resistance.

In this study, silver nanoparticles were synthesized using a controlled chemical reduction method within microemulsion systems to enhance their therapeutic profile. The primary objectives were to characterize the physicochemical properties of the formulations and to evaluate their antimicrobial activity and cytotoxicity. By assessing both efficacy and safety, this study aims to determine whether silver microemulsions could serve as a viable and sustainable alternative for the treatment of ABRS.

2. Materials and Methods

The study protocol was approved by the Biosafety Committee of Naresuan University, Phitsanulok, Thailand, under approval number NUIBC MI 67-11-65. To comprehensively evaluate the potential of silver microemulsion formulations for therapeutic use, the research was strategically structured into three key phases: synthesis and characterization, cytotoxicity assessment, and antimicrobial evaluation.

In the first phase, silver nanoparticles (AgNPs) were synthesized using a controlled chemical reduction method within microemulsion systems. This approach allowed stable colloidal formulations, which were subsequently characterized in terms of particle size, zeta potential, and optical stability to ensure consistency and functionality. The second phase focused on assessing the safety profile of the formulations. Cytotoxicity was evaluated in vitro using MTT assays on RAW 264.7 murine macrophage cells. This step was critical to determine the concentration thresholds that maintain cell viability, identifying non-cytotoxic doses suitable for biological applications, particularly in sensitive nasal tissues. In the final phase, antimicrobial efficacy was investigated against a panel of clinically relevant bacterial and fungal strains: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, and *Candida albicans*. Disk diffusion and broth dilution methods were employed to measure inhibition zones, minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs), offering a robust evaluation of the formulations' antimicrobial potential.

The selected microorganisms reflect a clinically meaningful range of pathogens commonly associated with ABRS and its complications. *S. aureus* and *P. aeruginosa* are frequently isolated in chronic or post-viral rhinosinusitis and are notorious for their antibiotic resistance and biofilm formation.[16,17] *S. mutans*, while predominantly an oral bacterium, has been detected in the upper respiratory tract and may act as a secondary pathogen in immunocompromised patients.[18] *C. albicans*, an opportunistic fungal species, is frequently encountered in chronic rhinosinusitis cases, particularly among individuals undergoing long-term antibiotic therapy or with compromised mucosal defenses.[19]

By incorporating this diverse microbial panel, the study not only addresses primary ABRS pathogens but also anticipates secondary infections, thereby enhancing the clinical relevance of silver microemulsions as a potential intranasal therapeutic strategy.

2.1 Preparation and characterization of synthesized AgNPs

The preparation method and composition of the silver nanoparticle microemulsion system were thoroughly designed to meet the established criteria for microemulsion formulations. The silver nanoparticles, commercially obtained from Chanjao Longevity Co., Ltd. (Bangkok, Thailand) or TTK Science Co., Ltd. (Bangkok, Thailand), were incorporated into microemulsion systems for this study. These nanoparticles were characterized based on their physical and chemical properties, including appearance, concentration, and pH. Two types of silver nanoparticle suspensions were tested: Type A, with a concentration of 2000 ppm, nearly colorless to colorless in appearance, pH ranging from 4 to 6, and Type B, with a concentration of 2000 ppm, yellow to brown in appearance, pH ranging from 3.5 to 4.5, and particle size (D50) between 10 and 25 nm.

The formulation process involved a five-step preparation method (Fig. 1) to ensure precision, consistency, and homogeneity. First, approximately 22 mL of ultrapure water (18.2 MΩ·cm at 25°C) was measured, and 0.05 g of polyethylene glycol (PEG) was added as a dispersing agent. Subsequently, 0.025 g of citrate- or polyvinyl alcohol (PVA)-coated silver nanoparticles were gradually introduced into the base solution under continuous stirring to achieve uniform dispersion. Following this, 0.125 g of PVA stabilizer and 0.025 g of Tween 80 surfactant were added, with continuous stirring until complete homogenization was achieved. The final volume was adjusted to exactly 25 mL using ultrapure water. To ensure sterility and purity, the solution was subjected to appropriate sterilization methods, such as filtration or autoclaving. This carefully controlled preparation process resulted in a stable, homogenous silver nanoparticle microemulsion system suitable for further pharmacological evaluation.

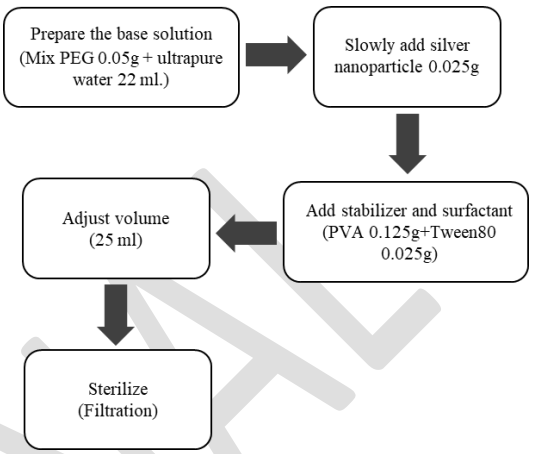

Fig. 1. Preparation of silver nanoparticle microemulsion involving a five-step procedure.

The characterization of silver nanoparticles within microemulsion systems involved a comprehensive analysis to ensure their stability and efficacy. UV-visible spectral analysis was conducted within the wavelength range of 250 to 650 nm to assess the optical properties and confirm the presence and stability of the nanoparticles. Zeta potential analysis was employed to evaluate the particle size distribution and surface charge, which are critical for determining the stability and dispersibility of the nanoparticles within the microemulsion systems, based on their refractive index.

2.2 Cytotoxicity testing

2.2.1 Preparation of cell line

Murine macrophage RAW 264.7 (ATCC, Manassas, VA, USA) cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were maintained at 37°C in an environment with 95% humidity and 5% CO_2, with media changes performed every 2 days.

Upon reaching 80% confluence, the cell culture medium was discarded, and the cells were rinsed twice with 2 mL of sterile PBS (pH 7.2-7.4). The cells were then subjected to trypsinization with 0.25% trypsin-EDTA solution for 5-10 minutes, and the trypsin activity was halted by adding an equal volume of complete medium. The detached cells were transferred to a centrifuge tube and centrifuged at 3000 rpm for 5 minutes. Finally, the cells were counted or seeded for the experiment and continued to be incubated at 37°C with 5% CO_2. The cell culture was monitored daily using an inverted microscope.

2.2.2 Sample preparation

Silver microemulsion (SMe) concentrations were prepared by 2-fold serial dilution, ranging from 0.25 to 8 µg/mL for silver microemulsions A and B.

2.2.3 Cytotoxicity assays

The cytotoxicity of silver nanoparticles in microemulsion systems was evaluated using the MTT assay, which measures cell viability by assessing mitochondrial activity. Viable cells reduce MTT to purple formazan crystals, detectable by spectrophotometry at 570 nm, with a darker colour indicating higher cell viability.

2.2.4 MTT assay

RAW 264.7 cells were seeded at a density of 1×10^5 cells per well in a 96-well plate. After 24 hours of incubation, cells were treated with 100 µL of various concentrations of silver microemulsion samples. Untreated cells were used as control. Following another 24 hours of incubation, the supernatants were removed, and 100 µL of MTT solution (5 mg/mL) dissolved in DMEM without phenol red was added to each well. After 3 hours of incubation at 37°C, the MTT solution was removed, and the formazan crystals were solubilized by adding 100 µL of pure DMSO to each well. The absorbance was measured at 570 nm using a Microplate Absorbance Spectrophotometer, with pure DMSO serving as the blank.[10]

2.2.5 Cytotoxicity interpretation

Absorbance was measured at 570 nm using a microplate absorbance spectrophotometer. The percentage of cell viability was calculated using the following equation:

$$\% \text{ Cell viability} = \frac{OD_{570} \text{ of treated cells}}{OD_{570} \text{ of untreated cells}} \times 100$$

This testing aims to determine the dose ranges that are safe for mammalian cell lines. These identified safe dose ranges are then utilized in subsequent efficacy investigations. The cytotoxicity MTT assay was employed for this purpose due to its empirically confirmed effectiveness in assessing the safety of nanoparticles in cell lines and its ease of execution, making it suitable for obtaining preliminary safety data.[10]

2.3 Evaluation of antimicrobial activity

2.3.1 Antimicrobial activity testing

The antimicrobial activity of silver nanoparticles formulated within microemulsion systems was evaluated using two standardized methods: the disk diffusion method and the broth dilution test.[11-13]

2.3.2 Disk diffusion method

The microbial strains used in this study were obtained from the American Type Culture Collection (ATCC), including *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *S. mutans* A32-2, and *C. albicans* ATCC 10231. To ensure viability, all cultures were regularly subcultured and maintained in appropriate growth media. *S. aureus*, *P. aeruginosa*, and *S. mutans* were cultured on Brain Heart Infusion (BHI) agar, while *C. albicans* were grown on Sabouraud Dextrose Agar (SDA). Bacterial strains were incubated at 37°C for 24 hours under aerobic conditions, except for *S. mutans*, which were incubated anaerobically. *C. albicans* was cultured at either 25°C for 48 hours or 37°C for 24 hours.[12]

Inocula were prepared using the direct colony suspension method. Colonies were suspended in 0.85% normal saline solution (NSS) to achieve a turbidity

equivalent to the 0.5 McFarland standard (optical density of 0.08–0.10 at 625 nm), corresponding to approximately 1×10^8 CFU/mL for bacteria. For *C. albicans*, the suspension was standardized to an optical density measured at 530 nm, yielding a final inoculum of approximately 2×10^8 PFU/mL. Silver microemulsion formulations A and B were prepared in two-fold serial dilution series, ranging from 500 µg/mL to 7.81 µg/mL. Each microbial suspension was evenly spread onto Mueller-Hinton Agar (MHA) plates using sterile cotton swabs. Sterile 6 mm paper disks were loaded with 20 µL of the test samples and allowed to dry before being placed on the inoculated agar surface. Plates were left at room temperature for 5 min to allow diffusion, then incubated under appropriate conditions for 24 hours.

Distilled water was used as the negative control, and 0.2% chlorhexidine (CHX) served as the positive control for both bacterial and fungal strains. CHX was selected due to its well-documented broad-spectrum antimicrobial activity and its widespread clinical use in nasal and oral formulations. As a reliable benchmark, it enabled direct comparison of the efficacy of silver microemulsions against a standardized reference.[20]

After incubation, the diameters of the inhibition zones were measured in millimeters using Vernier calipers, following the Clinical and Laboratory Standards Institute (CLSI) guidelines.[21,22]

2.3.3 Broth dilution test

The broth dilution method, specifically the micro broth dilution technique, was employed to determine the efficacy of the silver nanoparticle emulsions against microorganisms.[14] The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that inhibits the growth of microorganisms, while the Minimum Bactericidal Concentration (MBC) is the lowest concentration required to kill the microorganisms. The prepared samples underwent 2-fold serial dilutions across six concentrations and were added to a 96-well plate at 100 µL/well. Each well was inoculated with 100 µL of the microorganism suspension, following CLSI guidelines to achieve a cell density of 1×10^8 CFU/mL, resulting in a final volume of 10 µL/well. Broth alone was used as the negative control, and broth with microorganisms as the positive control.[13]

The plates were then incubated under the respective conditions for each microorganism. The MIC was determined by identifying the concentrations that showed no change in optical density and turbidity, indicating minimal microbial growth. To determine the MBC, the concentrations that showed the least microbial growth were transferred to suitable agar plates using the simple drop plate technique. These plates were incubated under the appropriate conditions for 24-48 hours. The lowest concentration at which no microorganism growth was observed on the drop plates was identified as the MBC.[13]

2.4 Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using computer software. For cytotoxicity data (MTT assay), a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare cell viability across different concentrations of silver microemulsions. For antimicrobial testing (disk diffusion), the diameters of inhibition zones were compared descriptively, while MIC and MBC results from broth dilution assays were interpreted qualitatively due to their categorical nature. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Preparation and characterizations of synthesized AgNPs

In the preparation of silver nano-particles, the potential value of Zeta is important as it can indicate stability and predict

the stability of particle dispersion. The results are shown in Table 1.

Table 1. Particle size and zeta potential of silver microemulsion samples.

Test sample	Particle size (nm)	Zeta potential (mV)
Silver microemulsion A	256.67 ± 0.97	-2.91 ± 0.42
Silver microemulsion B	147.13 ± 2.67	-1.97 ± 2.10
Microemulsion C (No added silver nano)	222.37 ± 6.02	-4.20 ± 2.97

Nanoparticles are defined as particulate matter with a diameter of less than 100 nm in at least one dimension. Microemulsions are thermodynamically stable emulsions of oil-in-water (o/w) type, characterized by an average droplet size ranging from approximately 100 to 400 nm. The results of the stability assessment of the silver nanoparticles within the microemulsion systems, after 3 months of storage, are detailed in Tables 2 and 3. These analyses are crucial for confirming the quality and reliability of the synthesized nanoparticles for pharmacological applications. The ultraviolet-visible (UV-vis) spectroscopy analysis revealed a sharp narrow peak with a maximum absorbance at 435 nm for silver microemulsion B (Table 3), which is in the range specified for silver nanoparticles.

According to Table 2, the stability of silver microemulsion A at room temperature was evaluated over 12 weeks, focusing on pH, λ_{max}, OD, and colour characteristics. The pH slightly decreased from 5.20 ± 0.00 at week 0 to 5.12 ± 0.01 by week 12, suggesting minor acidification. The λ_{max} remained stable at 250 nm, indicating consistent optical properties. However, OD values fluctuated, decreasing initially from 0.089 to 0.031 by week 1, then rising to 0.073 by week 12, suggesting potential nanoparticle aggregation. Colour changes correlated with these findings, as the initially clear solution developed a faint yellow tint by week 4, which intensified through week 12. This progressive discoloration, alongside OD fluctuations, indicates partial instability, highlighting the need for formulation optimization to maintain nanoparticle integrity and ensure consistent pharmacological performance.

The stability of silver microemulsion B was evaluated at room temperature over a 12-week period (Table 3), focusing on pH, λ_{max}, OD, and colour characteristics. The pH exhibited a slight decrease from 4.91 ± 0.00 at week 0 to 4.79 ± 0.01 by week 12, indicating minor acidification over time. The λ_{max} remained consistently at 435 nm throughout the study, suggesting stable optical properties of the silver nanoparticles. OD values demonstrated minimal fluctuations, decreasing from 1.188 at baseline to 1.169 by the end of the study, reflecting slight variations in nanoparticle dispersion. Visually, the microemulsion retained its yellow hue from week 0, with only subtle intensification observed over time. The stable λ_{max}, limited OD changes, and consistent coloration indicate that silver microemulsion B exhibited relatively high stability, with minimal signs of nanoparticle aggregation or degradation. These findings suggest that the formulation maintains its physicochemical integrity under ambient conditions, although further optimization could enhance long-term stability and pharmacological efficacy.

3.2 Cytotoxicity testing

The cytotoxicity of the silver microemulsions was assessed using the MTT assay on RAW 264.7 murine macrophage cells. The silver microemulsions were non-cytotoxic at concentrations up to 4.0 µg/mL, with cell viability exceeding 70%. However, at 8.0 µg/mL, cell viability decreased, and changes in cell morphology were observed.

Effects of AgNPs on RAW 264.7 cell viability

To evaluate the cytotoxicity effect of silver microemulsions A and B on RAW 264.7 macrophage cells viability, cells were exposed to different concentrations of silver microemulsions A and B ranging from 0.25 to 8.0 µg/mL, for 24 hours. The cytotoxicity

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of the RAW 264.7 macrophage cells was detected using an MTT assay (Fig. 2).

According to the ISO 10993-5:2009 standard for in vitro cytotoxicity testing, a

Table 2. Stability of silver microemulsion A at room temperature.

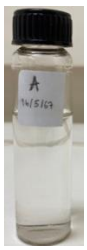
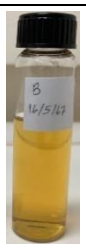
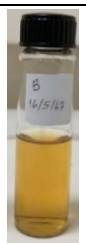
Parameter	Time (weeks)					
	0	1	2	4	8	12
pH	5.20±0.00	5.15±0.00	5.19±0.01	5.14±0.00	5.06±0.01	5.12±0.01
λ_{max} (nm)	250	250	250	250	250	250
OD	0.089	0.031	0.061	0.056	0.066	0.073
Colour Clear						

Table 3. Stability of silver microemulsion B at room temperature.

Parameter	Time (weeks)					
	0	1	2	4	8	12
pH	4.91±0.00	4.86±0.01	4.91±0.00	4.93±0.00	4.80±0.01	4.79±0.01
λ max (nm)	435	435	435	435	435	435
OD	1.188	1.183	1.186	1.175	1.180	1.169
Colour Yellow						

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According to the ISO 10993-5:2009 standard for in vitro cytotoxicity testing, a material is considered non-cytotoxic if ≥70% cell viability is maintained compared to untreated controls. [21] In this study, both silver microemulsions A and B exhibited cell viability above this threshold at concentrations ranging from 0.25 to 4.0 µg/mL, indicating acceptable in vitro biocompatibility in RAW 264.7 macrophage cells. A notable decline in cell viability was observed at 8.0 µg/mL, accompanied by visible alterations in cell morphology, suggesting cytotoxic effects at this higher dose.

Importantly, the calculated VC_{50} values, defined as the concentration at which 50% of cell viability is observed, were approximately 8.6 µg/mL for silver microemulsion A and 4.1 µg/mL for silver microemulsion B. These values quantitatively reinforce the greater cytotoxic potential of microemulsion B compared to microemulsion A. Based on these findings, 4.0 µg/mL was established as the maximum non-cytotoxic concentration for both formulations and was thus selected for subsequent biological evaluations.

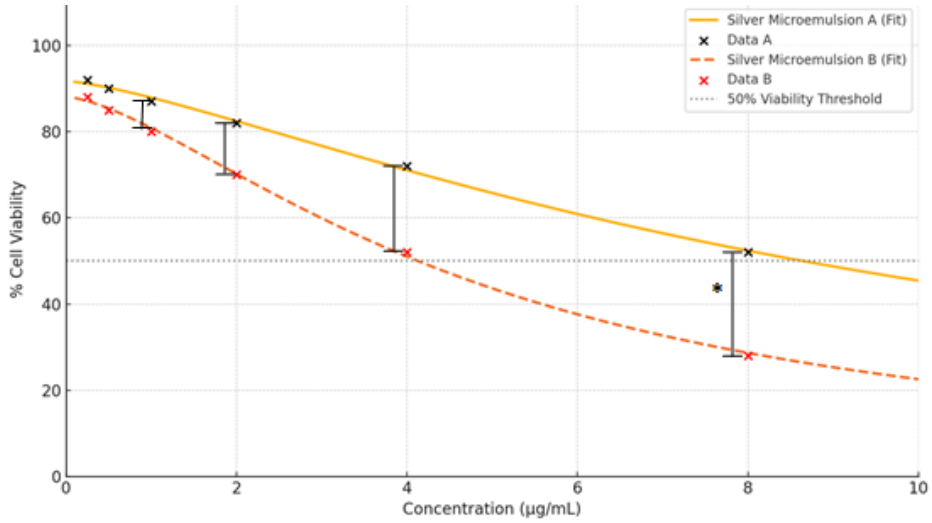

Fig. 2. Percentage of cell viability of RAW 264.7 macrophages following 24-hour exposure to various concentrations of silver microemulsion A and silver microemulsion B, as measured by MTT assay. Data are presented as mean ± SD (n=3). A significant reduction in cell viability was observed at 8.0 µg/mL for both formulations compared to control ($p < 0.05$). * indicates statistically significant difference from control.

Note: VC_{50} (cell viability concentration at 50%) for Silver Microemulsion A ≈ 8.6 µg/mL and VC_{50} for Silver Microemulsion B ≈ 4.1 µg/mL

While additional validation using nasal epithelial models is warranted to confirm safety *in vivo*, these in vitro results provide preliminary support for the biocompatibility of silver microemulsions, underscoring their potential as safe candidates for intranasal antimicrobial applications.

3.3 Antimicrobial activity evaluation

The disk diffusion method was used to evaluate the antimicrobial susceptibility by measuring the inhibition zone against the test microorganisms. The results of the antimicrobial activity of silver microemulsions A and B tested against microorganisms by disk diffusion method are shown in Table 4. The silver microemulsions showed limited

antimicrobial activity. The inhibition zones for *S. aureus, P. aeruginosa, and S. mutans* ranged from 8 to 11 mm, indicating resistance. No antifungal activity was observed against *C. albicans*.

Table 4. Diameters of the growth inhibition zones of silver microemulsions A and B.

Organism	Treatment	Diameter of growth inhibition zone (mm)		
		results	positive	negative
S. aureus	Silver microemulsion A	8	14	NA
	Silver microemulsion B	8	14	NA
	Control (no silver) C	NA	NA	NA
P. aeruginosa	Silver microemulsion A	9	13	NA
	Silver microemulsion B	10	13	NA
	Control (no silver) C	NA	NA	NA
S. mutans	Silver microemulsion A	10	20	NA
	Silver microemulsion B	11	20	NA
	Control (no silver) C	NA	NA	NA
C. albicans	Silver microemulsion A	Colonies within zone	14	NA
	Silver microemulsion B	Colonies within zone	14	NA
	Control (no silver) C	NA	NA	NA

Note: "Colonies within zone" indicates incomplete inhibition of *C. albicans*, with visible fungal growth within the inhibition area, suggesting limited antifungal activity of the silver microemulsions. Positive Control: Antimicrobial: 0.2% Chlorhexidine (CHX). Cytotoxicity: Doxorubicin (10.0 µg/mL). Negative Control: Antimicrobial: Sterile distilled water. Cytotoxicity: Untreated cells in culture medium. NA: No activity.

The antimicrobial activity of silver microemulsions A and B was evaluated using the disk diffusion method against *S. aureus*, *P. aeruginosa*, *S. mutans*, and *C. albicans* (Table 4, Fig. 3 and 4). Both microemulsions demonstrated limited inhibitory effects compared to the positive control (0.2% CHX). For *S. aureus*, the inhibition zones were 8 mm for both microemulsions, while CHX produced a 14 mm zone. Against *P. aeruginosa*, microemulsion A exhibited a 9 mm zone and microemulsion B a 10 mm zone, compared to the 13 mm zone observed with CHX. For *S. mutans*, microemulsion A produced a 10 mm zone, while microemulsion B showed an 11 mm zone, whereas the positive control resulted in a 20 mm inhibition zone. Both microemulsions displayed incomplete inhibition against *C. albicans*, as evidenced by colonies within the zone, while CHX produced a clear 14 mm zone. The negative control (distilled water) showed no antimicrobial activity. These findings highlight the limited antimicrobial efficacy of silver microemulsions A and B relative to the positive control.

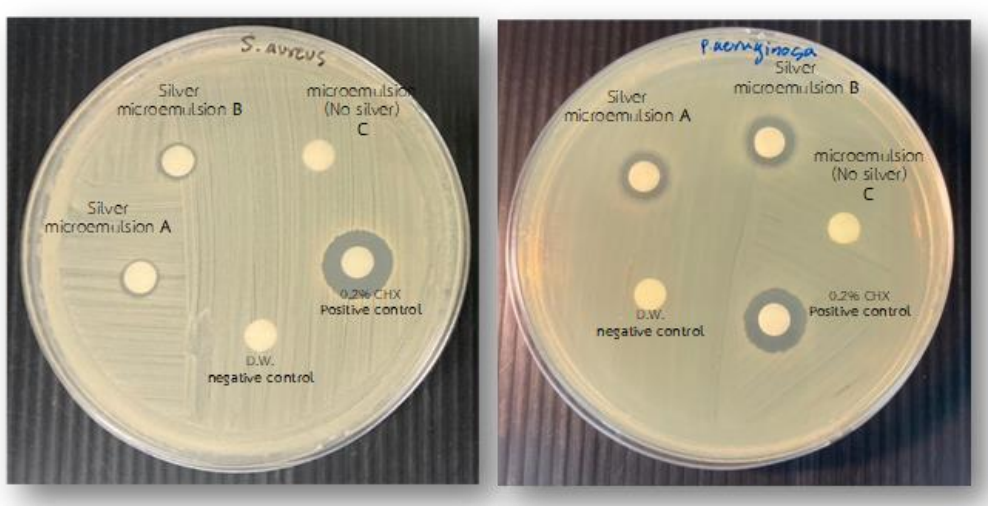

Fig. 3. Inhibition zones of silver microemulsion A, silver microemulsion B, silver microemulsion (no silver) C, 0.12% CHX (positive control) and distilled water (negative control) against *S. aureus* ATCC25923 and *P. aeruginosa* ATCC27853.

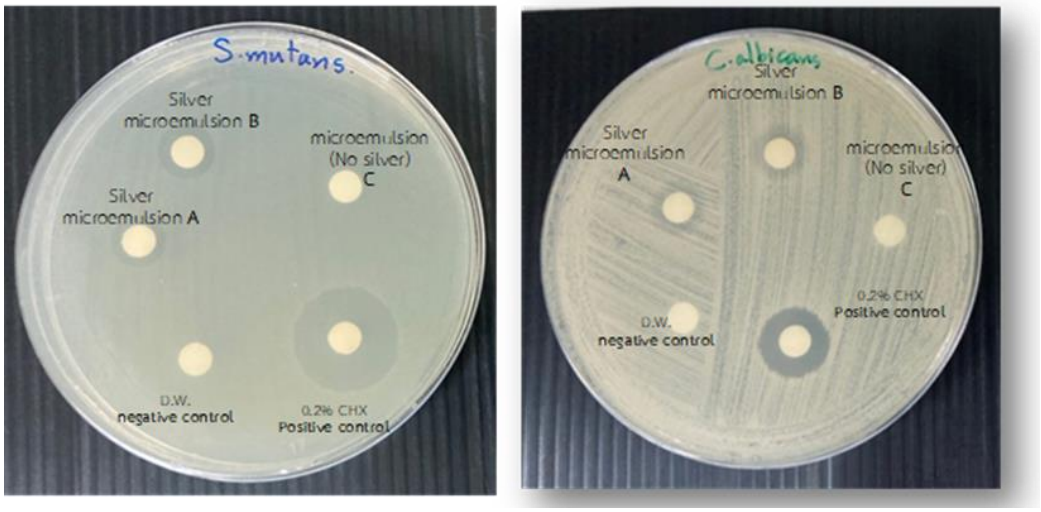

Fig. 4. Inhibition zones of silver microemulsion A, silver microemulsion B, microemulsion (no silver) C, 0.2% CHX (positive control) and distilled water (negative control) against *S. mutans* A32-2 and *C. albicans* ATCC10231.

3.4 Antimicrobial susceptibility by broth dilution test

The disk diffusion method yielded inconclusive results regarding the antimicrobial effectiveness of the samples. Consequently, the microbroth dilution method was employed to determine the MIC and MBC of silver microemulsions A and B against *S. aureus*, *P. aeruginosa*, *S. mutans*, and *C. albicans*. The MIC and MBC values were assessed using the microbroth dilution technique, with the highest tested concentration set at 62.5 µg/mL. Both silver microemulsions exhibited MIC and MBC values exceeding the tested concentrations, indicating limited efficacy in inhibiting or eliminating the target microorganisms. The detailed results are presented in Table 5.

Table 5. The diameter of the growth inhibition zone of silver microemulsions A and B.

Organism	Treatment	MIC (µg/mL)	MBC/ MFC (µg/mL)
S. aureus	Silver microemulsion A	> 31.3	> 31.3
	Silver microemulsion B	> 31.3	> 31.3
	Control (no silver) C	NA	NA
P. aeruginosa	Silver microemulsion A	> 15.6	> 31.3
	Silver microemulsion B	> 15.6	> 31.3
	Control (no silver) C	NA	NA
S. mutans	Silver microemulsion A	> 7.8	> 15.6
	Silver microemulsion B	> 7.8	> 15.6
	Control (no silver) C	NA	NA
C. albicans	Silver microemulsion A	> 15.6	> 62.5
	Silver microemulsion B	> 15.6	> 62.5
	Control (no silver) C	NA	NA

Note: MIC indicates the lowest concentration of a substance (µg/mL) required to inhibit visible microbial growth in vitro. MBC/MFC (Minimum Bactericidal/Fungicidal Concentration) refers to the lowest concentration of a substance (µg/mL) needed to achieve complete eradication of bacteria or yeast. NA: No activity.

4. Discussion

This study introduces an innovative approach to managing microbial infections by utilizing silver microemulsions that target microorganisms at the molecular level. The findings suggest that these formulations have potential as multifunctional antimicrobial agents, exhibiting acceptable cytotoxicity profiles in human cell lines. While the results show promise for applications such as treating sinusitis, particularly ABRS, further optimization is necessary to enhance both their antimicrobial efficacy and long-term stability.

4.1 Formulation characterization and stability

Among the tested formulations, microemulsion B exhibited a particle size of 147 ± 2.7 nm, which falls within the optimal range for improved cellular uptake and mucosal bioavailability. However, both formulations displayed suboptimal zeta potential values (−2.0 to −2.9 mV), which are inadequate for colloidal stability. A zeta potential greater than ±30 mV is generally required to prevent nanoparticle aggregation and ensure long-term dispersion.[22]

Twelve-week stability testing at room temperature showed that both microemulsions A and B maintained acceptable physical characteristics, with only minor fluctuations in pH and OD. Microemulsion A demonstrated a slight decline in pH over time, possibly indicating chemical degradation, while changes in OD suggested some particle aggregation. Conversely, Microemulsion B exhibited more stable pH and OD values, indicating superior structural integrity. Although these short-term data are promising, long-term studies under various environmental conditions, following ICH guidelines, are necessary to determine true shelf-life and optimize storage parameters.[23]

4.2 Antimicrobial efficacy

The antimicrobial properties of the silver microemulsions were evaluated using disk diffusion and broth dilution methods. Both formulations showed limited antibacterial activity, with inhibition zones ranging from 8 to 11 mm against *S. aureus*, *P. aeruginosa*, and *S. mutans*. No inhibitory effect was observed against *C. albicans*. Broth dilution assays confirmed these findings, with MIC and MBC values exceeding the highest tested concentration (62.5 µg/mL). Specifically, MICs were greater than 31.3 µg/mL for *S. aureus* and *P. aeruginosa*, 7.8 µg/mL for *S. mutans*, and 15.6 µg/mL for *C. albicans*. These results indicate insufficient antimicrobial potency at the tested concentrations and highlight the need for formulation refinement.

4.3 Cytotoxicity and biocompatibility

The cytotoxicity of the silver microemulsions was assessed using the MTT assay on RAW 264.7 murine macrophage cells. Both formulations were found to be non-cytotoxic at concentrations up to 4.0 µg/mL, maintaining cell viability above the 70% threshold defined by ISO 10993-5:2009 standards.[21] However, a concentration of 8.0 µg/mL significantly reduced cell viability and induced morphological alterations, suggesting cytotoxic effects at higher doses. These findings define 4.0 µg/mL as the maximum safe concentration for further biological evaluation and underscore the importance of strict dose control to ensure cellular safety.

While the RAW 264.7 cell line provides initial safety insights, it does not fully replicate the nasal epithelial environment. Therefore, future studies should employ more physiologically relevant models—such as primary human nasal epithelial cells or air–liquid interface (ALI) cultures to evaluate mucosal compatibility, barrier integrity, and immune responses.[24]

4.4 Pathogen relevance

The microbial strains selected for this study, *S. aureus*, *P. aeruginosa*, *S. mutans*, and *C. albicans*, are not primary pathogens of ABRS but are frequently implicated in secondary infections, particularly in chronic or recurrent cases. *S. aureus* and *P. aeruginosa* are common opportunistic pathogens in the nasal cavity and sinuses, often forming biofilms and displaying antibiotic resistance. *S. mutans*, typically associated with oral biofilms, have been isolated from the upper respiratory tract, especially in immunocompromised individuals. *C. albicans*, a frequent isolation in patients undergoing prolonged antibiotic therapy, is a key fungal pathogen in chronic rhinosinusitis. Assessing the efficacy of silver microemulsions against these organisms provides insight into their potential as adjunctive therapies that can address both primary infection and secondary complications.

4.5 Strategies for enhancing efficacy and safety

Improving the antimicrobial performance of silver microemulsions requires a multifaceted approach. Reducing nanoparticle size can increase the surface area-to-volume ratio, promoting better interaction with microbial membranes and improving bactericidal activity at lower concentrations. Controlled synthesis using specific surfactants or stabilizers can help achieve uniform particle size and enhance potency.[25,26] Surface modification with biocompatible agents such as polyethylene glycol (PEG) or chitosan may further improve stability, reduce cytotoxicity, and enhance microbial targeting.[27]

Combining silver microemulsions with synergistic agents such as antibiotics or plant-derived compounds could also produce additive or synergistic effects, enhancing efficacy while minimizing the cytotoxic burden.[28] Moreover, optimizing the surfactant-to-co-surfactant ratio and increasing the zeta potential could improve both dispersion and long-term stability, making the formulation more viable for clinical use.[29]

4.6 Future directions

Silver microemulsions exhibit several favorable characteristics, including appropriate particle size for mucosal delivery, short-term physicochemical stability, and non-cytotoxicity

at therapeutic concentrations. Microemulsion B demonstrated promising structural integrity and biological safety. However, limitations in antimicrobial efficacy, insufficient zeta potential, and cytotoxic effects at higher doses indicate the need for further formulation refinement.

Future work should focus on improving nanoparticle properties, optimizing formulation stability, and evaluating long-term safety in nasal tissue models. Exploring synergistic combinations with established antibiotics may also improve therapeutic outcomes. Achieving a balanced formulation that maximizes efficacy while ensuring safety and stability will be key to developing silver microemulsions into a viable alternative treatment for ABRS and other infections amid rising antibiotic resistance.

5. Conclusion

Silver nanoparticle microemulsions A and B demonstrated acceptable cytocompatibility, with cell viability maintained above 70% at concentrations up to 4.0 µg/mL. The calculated VC_{50} values of 8.6 µg/mL for Microemulsion A and 4.1 µg/mL for Microemulsion B support this threshold as the maximum non-cytotoxic dose. However, both formulations exhibited limited antimicrobial efficacy, with inhibition zones of 8–11 mm and MIC/MBC values exceeding 62.5 µg/mL. While Microemulsion B showed better physicochemical stability, suboptimal zeta potentials in both formulations suggest a need for improved colloidal stability. Overall, these results support the preliminary safety of silver microemulsions for intranasal use but highlight the need for formulation optimization to enhance antimicrobial performance.

Acknowledgements

The present study was supported by the revenue budget of the Faculty of Pharmaceutical Sciences, Naresuan University (grant number R2567-A02), for the fiscal year 2024.

Conflicts of interest

The authors declare no conflict of interest.

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