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Nisin-Loaded Niosomes as a Novel Nanocarrier System against Predominant Gram-Negative Uropathogens

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Abstract:

Urinary tract infections (UTIs) caused by Gram-negative bacteria such as *E. coli*, *P. aeruginosa* and *P. mirabilis* are a serious health concern due to their ability to establish biofilm and antibitotic resistance. Niosomes loaded with nisin were formulated and analyzed in this study to evaluate their potential as a new nanocarrier system against major Gram-negative uropathogens. The formulations were characterized for particle size, zeta potential and encapsulation efficiency and their antibacterial activity was evaluated by MIC and MBIC assays against clinical and veterinary isolates. Acidic formulations provided vesicles of less than 400 nm with increased stability, while neutral pH promoted higher encapsulation efficiency. Nisin release and antibiofilm activity increased under acidic conditions with MIC values of 3.13-25 µg/mL; *P. mirabilis* was the most susceptible and *P. aeruginosa* the most resistant bacteria. The prevention of biofilm formation was more evident on *E. coli* thus validating the dual action of the system against planktonic and sessile cells. These results imply that nisin-loaded niosomes are promising therapeutic method for the controlling of Gram-negative UTIs in clinical and veterinary settings.

Background:

Urinary tract infections are among the most common bacterial illnesses worldwide and the main cause is Gram-negative pathogens. The biofilm-forming capacity of these bacteria accounts for recurring infections and resistance to antibiotics, leading to a pressing need for new therapeutics.

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Aims:

The purpose of the study was to assess physicochemical parameters, encapsulation efficiency, stability and antibacterial efficacy of nisin-loaded niosomes against the most prevalent Gram-negative uropathogens.

Conclusions:

The present work supports the nisin-loaded niosomes as a unique and effective nanocarrier technology against the major Gram-negative uropathogens (*E. coli*, *P. aeruginosa*, *P. mirabilis*). Formulations were optimized at acidic pH with vesicles <400 nm, improved stability and better antibacterial action. The experiment revealed that the maximum encapsulation efficiency was obtained at neutral pH conditions at 67.7%. This result shows that the entrapment of the peptide is more stable in a physiological balance-like environment, whereas the acidic formulations increase the drug release but can affect the long-term retention. This double behavior underlines the importance of tuning pH conditions depending on whether stability or fast release is desired in clinical applications.

Keyword: Nisin, Niosomes, uropathogens, Biofilm inhibition, Nanocarrier system

Introduction:

Urinary tract infections (UTIs) are among the most frequent bacterial diseases globally, impacting millions of people every year and nevertheless pose a persistent problem for human and veterinary medicine (Eskandar, 2025; Rehman et al., 2026). Most cases are due to Gram-negative uropathogens, including Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis. These pathogens are infamous for their ability to create biofilms, which render them impervious to host immune responses, and underlie recurring infections and the frightening increase in antibiotic resistance (Scientific Reports, 2026; Rehman et al., 2026).

The growing incidence of multidrug-resistant Gram-negative bacteria has decreased the efficacy of current medicines, highlighting the need for new approaches to combat biofilm-related resistance (Eskandar, 2025; Foxman, 2022). Recent investigations have highlighted the need to identify new antimicrobial medicines that reduce the reliance on wide spectrum antibiotics and provide focused efficacy against resistant bacteria (Hall-Stoodley & Stoodley, 2023). Similar findings have been reported in the Diyala Journal for Veterinary Sciences, where plant-derived compounds such as Zingiber officinale demonstrated antimicrobial activity against *E. coli* and S. aureus (Mahmood et al., 2026).

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A possible contender is nisin, a lantibiotic peptide generated by Lactococcus lactis. Nisin is traditionally known for its activity against Gram-positive bacteria but has recently been reported to have inhibitory effects against a broad spectrum of Gram-negative infections including Acinetobacter and Helicobacter (Charest et al., 2024; MDPI, 2024). Bioengineering advances have widened its antibacterial spectrum even more and nisin has been proposed as a viable supplementary therapy in the combat against resistant infections (ScienceDirect, 2021; ScienceDirect, 2021).

However, nisin has a number of drawbacks including instability, fast breakdown and limited bioavailability. To overcome these problems, researchers have employed nanocarrier systems. Niosomes are non-ionic surfactant vesicles that enhance medication stability, controlled release, and biocompatibility. Niosomes have emerged as a promising carrier system to increase encapsulation and distribution of antimicrobial peptides presenting a new strategy to treat Gram negative infections (Moammeri et al., 2023; Colloids and Surfaces B, 2026; JDDST, 2023).**Materials and Methods:**

1. Laboratory Instruments and Tools:

The characterization of nisin loaded niosomes was done using various sophisticated instruments. The optical characteristics and absorbance spectra were measured using a UV-Vis spectrophotometer (Shimadzu, Germany). Identification of functional groups was done using FTIR spectrometer (Shimadzu, Germany). Transmission electron microscopy (TEM, Zeiss, Germany) was used to characterize the morphology and particle size. Surface charge and colloidal stability of the vesicles were measured by a Zeta Potential Analyzer (Malvern, UK). Thin films were prepared using a rotary evaporator (Heidolph, Germany) during the hydration process for preparation.

2. Chemicals and Reagents:

All analytical grade reagents were employed throughout the study. Antimicrobial peptide nisin (Sigma-Aldrich, USA) and Span 60 and Tween 80 were used for the creation of vesicles and stabilization of bilayer. Cholesterol (Sigma-Aldrich, USA) was used as a building material for niosomes. It was rehydrated using phosphate buffered saline (PBS, pH 7.4) and acetate buffer (HiMedia, India).

3. Culture Media:

Selective and differential media were used for the isolation of Gram-negative uropathogens. Enterobacteriaceae were detected by lactose fermentation on MacConkey agar and *E. coli* was identified by its typical metallic green sheen on EMB agar. Blood agar was used to test for hemolysis in Streptococcus and Staphylococcus species. Detection of pyocyanin pigment in *P. aeruginosa* on pseudomonas agar base Triple Sugar Iron (TSI) agar was utilized for carbohydrate fermentation and H_2S detection in enteric pathogens and Mueller-Hinton agar was used for antibiotic susceptibility testing of all isolates

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4. Sample Collection and Bacterial Isolation:

Urine samples were collected from patients (n=50) and cattle (n=50) under aseptic conditions. Pathogens were isolated using MacConkey and Blood agar, followed by Gram staining and biochemical assays. Identification and antibiotic susceptibility were confirmed using the VITEK2 Compact System (Ling et al., 2011).

5. Preparation of Nisin-Loaded Niosomes:

Niosomes were synthesized using the thin-film hydration method. Lipid mixtures (Span 60, Tween 80, cholesterol) were dissolved in chloroform:methanol, evaporated to form a thin film, and hydrated with PBS containing nisin (200 µg/mL). Sonication was applied to reduce vesicle size .

Characterization of Nisin-Loaded Niosomes:

The physicochemical characteristics of the obtained nisin-loaded niosomes were thoroughly investigated. The colloidal stability was evaluated by measuring particle size distribution, polydispersity index (PDI) and zeta potential using dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK). Morphological characteristics and vesicle size distribution were studied by transmission electron microscopy (TEM, Zeiss, Germany) and surface topography was further analyzed by atomic force microscopy (AFM, Bruker, Germany). Fourier-transform infrared spectroscopy (FTIR, Shimadzu, Germany) was used to confirm the functional groups and peptide-bilayer interactions. Thermal transitions and stability were determined by differential scanning calorimetry (DSC). After ultracentrifugation, the encapsulation efficiency (EE%) and loading capacity (LC%) were measured and the free nisin was measured in a spectrophotometer at 220 nm . Stability studies were carried out for assessing particle size and EE% for 40 days at 4 ℃ and 25 ℃ to assess long term integrity of the formulations .

1. Antimicrobial Activity :Agar Well Diffusion Technique:

The antibacterial activity was initially screened by the agar well diffusion method. Pathogenic isolates were inoculated on Mueller-Hinton agar plates to obtain homogeneous lawn of bacterial growth. Aseptic wells of 6 mm diameter were punched in the agar, and filled with 100 µL of nisin-loaded niosome solutions at various doses. Plates were cultured for 24 h at 37 °C and the inhibitory zones were measured in mm .

2. Minimum inhibitory concentrations (MIC) :

MICs were determined using broth microdilution technique in sterile 96-well microplates as per CLSI and EUCAST recommendations. Two-fold serial dilutions of the formulations were made in Mueller-Hinton broth (0.39-200 µg/mL). Each well was infected with standardized bacterial suspensions (0.5 McFarland, ca. 1×10^8 CFU/mL) at 37 C for 24 h. The bacterial growth was

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monitored by measuring the optical density at 600 nm in a microplate reader. The MIC was considered the lowest concentration that impeded observable growth .

3. Minimal biofilm inhibitory concentration (MBIC):

Biofilm inhibition was tested using a microtiter plate technique. Pathogenic isolates were grown in biofilms in 96-well plates using Brain Heart Infusion broth supplemented with 1% glucose for 48h. After maturation of biofilms, wells were washed with PBS and subjected to repeated dilutions of nisin-loaded niosomes. The biomass of the biofilm was measured by crystal violet staining and absorbance at 570 nm after incubation for 24 h at 37 °C. The MBIC was defined as the lowest concentration that inhibited the production of biofilms.

Statistical Analysis:

All experiments were performed in triplicate. Results were expressed as mean ± SD. One-way ANOVA with Tukey's post hoc test was applied, with $p<0.05$ considered significant .

Ethics approval:

The study did not involve any in vivo experiments on live animals. Human samples were collected with informed consent, and animal samples under veterinary supervision. The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala.

Results:

1. Physicochemical Characterization

he particle size of nisin-loaded niosomes ranged widely from 357.8 to 799.1 nm as a function of both pH and surfactant-to-cholesterol ratio. Under acidic conditions (pH 5.7), vesicles <400 nm were seen consistently with more uniformity and enhanced stability. In contrast, under neutral conditions (pH 7.4) vesicles >700 nm were formed with higher polydispersity and lower colloidal stability. The fact that vesicles <400 nm showed better colloidal behaviour is particularly important as such nanoscale particles are more competent for cellular internalization and efficient biofilm matrix penetration. This increased penetration directly results in increased antimicrobial activity. The agreement of these results with previous reports (Torchilin, 2005; Mozafari, 2006) highlights the importance of controlling particle size via formulation parameters and shows how the optimization of vesicle dimensions can improve therapeutic performance in both clinical and veterinary applications.

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Table (1): Physicochemical properties of nisin-loaded niosomes

Formulation (pH, ST:Ch, wall:core)	Particle Size (nm)	PDI	Zeta Potential (mV)
pH 5.7, 50:50, 3:1	357.8	0.219	-8.6
pH 7.4, 70:30, 1:1	799.1	0.496	+7.0
pH 4.3, 70:30, 3:1	555.8	0.265	-27.7

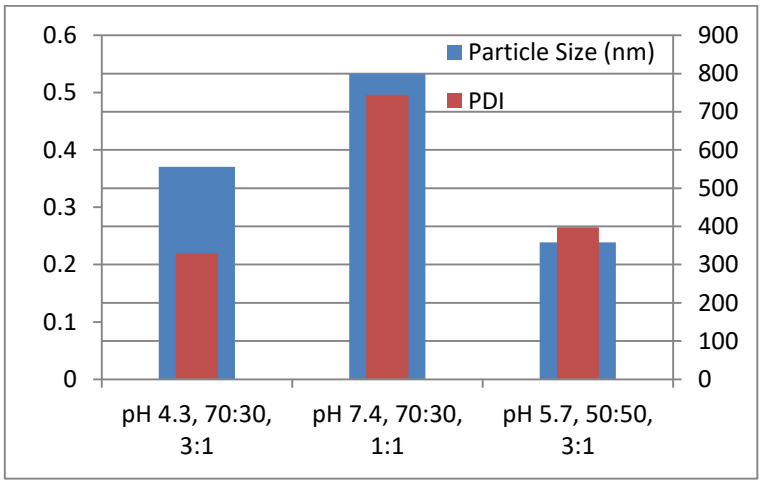

Figure (1): Distribution of particle sizes in nisin-loaded niosomes under different pH

2. Encapsulation and Loading Efficiency

Encapsulation efficiency (EE%) was markedly influenced by pH and the surfactant-to-cholesterol ratio.The highest EE% was 67.71% at pH neutral (7.4) with ST:Ch 50:50 which indicates the stable trapping of the peptide and greater interactions with the bilayer. On the other hand, acidic environments improved the loading capacity by protonation effects but impaired the stability and retention of the vesicles.

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Table (2): Encapsulation efficiency and loading capacity

Formulation (pH, ST:Ch, wall:core)	EE%	LC%
pH 7.4, 50:50, 3:1	67.71	18.5
pH 5.7, 50:50, 3:1	55.00	20.0
pH 7.4, 70:30, 1:1	64.58	32.29

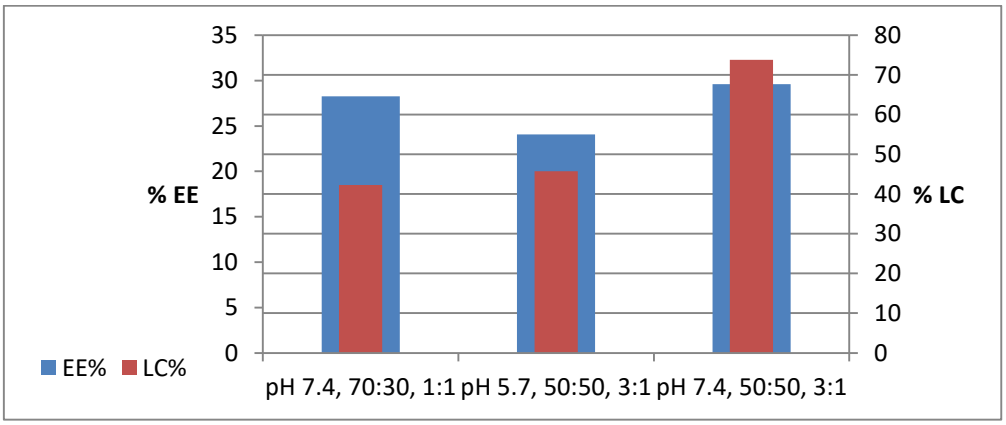

Figure (2): Encapsulation and loading efficiency of nisin-loaded niosomes

This dual activity is a typical compromise between increasing drug loading and preserving structural integrity. Neutral formulations can promote sustained release from a therapeutic point of view, while acid ones can allow rapid availability depending on clinical needs.

3. Stability Studies

Storage at 4 °C was more efficient to preserve vesicle integrity than 25 °C where encapsulation efficiency decreased more rapidly. These results highlight the importance of refrigeration for peptide stability in pharmaceutical applications.

Table (3): Stability profile of optimized niosome formulation under different storage conditions

Storage Temp C°	Day 1 EE%	Day 40 EE%
4	64.79	47.71
25	64.79	45.21

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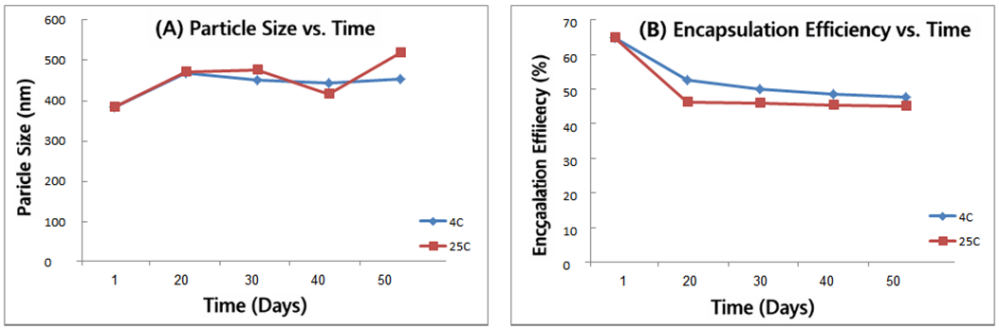

Figure (3): Changes in particle size and EE% during storage

The Line graph showing particle size and EE% over 40 days at 4 ℃ vs 25 ℃)Cold storage preserved vesicle integrity, emphasizing the importance of cold-chain maintenance.

4. Bacterial Isolation and Identification

A total of 31 Gram-negative isolates were identified from 100 urine samples, including *E. coli* (14), *P. aeruginosa* (9), and *P. mirabilis* (8). This relatively higher prevalence in animal derived samples may be due to environmental exposure and the use of antibiotics in livestock. This distribution is shown in Figure 4, where *E. coli* is the most predominant isolate. Biochemical characterization confirmed species identity with lactose fermentation in *E. coli*, pyocyanin pigment production in *P. aeruginosa* and swarming motility in *P. mirabilis*.

Table (4): Distribution of positive isolates

Source	Total Samples	Positive Isolates	Main Pathogens Identified
Human	50	13	*E. coli, P. aeruginosa, P. mirabilis*
Animal	50	18	*E. coli, P. aeruginosa, P. mirabilis*

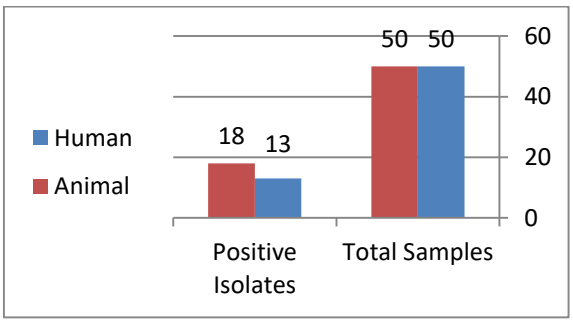

Figure (4): Comparative distribution of isolates (Human vs Animal)

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5. Minimum Inhibitory Concentration (MIC) Results

Minimum Inhibitory Concentration (MIC) Results All tested isolates were inhibited by nisin-loaded niosomes with MIC values ranging from 3.13 to 25 µg/mL. Acidic formulations gave lower MICs confirming that antimicrobial activity is higher under acidic conditions. *P. mirabilis* appeared the most susceptible, while *P. aeruginosa* showed the highest resistance, consistent with its efflux mechanisms and dense biofilm matrix.

Table (5): MIC values of Nisin-loaded niosomes against *E. coli*

Concentration (µg/mL)	Flask A Human	Flask A Animal	Flask B Human	Flask B Animal
12.5	92.88194	71.42857	76.70455	88.94737
6.25	95.3125	98.63946	77.46212	92.10526
3.13	98.4375	98.97959	98.10606	95.08772
0	100	100	100	100

Similar tables (6, 7) summarize MICs for *P. aeruginosa* and *P. mirabilis*. Flask B (acidic formulations) had consistently lower MICs further confirming the better activity in acidic conditions.

Table (6): MIC Results of Nisin-Loaded Niosomes Against *P. aeruginosa*

Concentration (µg/mL)	Flask A Human	Flask A Animal	Flask B Human	Flask B Animal
12.5	75.14911	81.28128	77.77778	73.01587
6.25	83.89662	85.68569	96.00939	84.7619
3.13	90.15905	88.48849	100	96.5873
0	100	100	100	100

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Table (7): MIC Results of Nisin-Loaded Niosomes Against *P.mirabilis*

Concentration (µg/mL)	Flask A Human	Flask A Animal	Flask B Human	Flask B Animal
12.5	80.77754	81.15385	78.09308	93.93365
6.25	92.22462	95.38462	90.35187	92.41706
3.13	100	97.30769	94.66515	99.43128
0	100	100	100	100

6. Minimal Biofilm Inhibition Concentration (MBIC) Results

MBIC values ranged from 2.73 to 93.23 µg/mL. The antibiofilm activity was higher for acidic formulations, especially for *E. coli*. This finding indicates that acidic conditions favor the release of the peptides and penetration into the biofilm matrix, thus improving the inhibitory activity. Table 8 – MBIC results of nisin-loaded niosomes against *E. coli* 7. Comparison of MIC vs MBIC Table 9 summarizes the MIC and MBIC values reported in the literature (2021–2026) compared to the present data. The results show that the inhibitory concentrations of the niosomal formulation are lower than free nisin, especially for *P. mirabilis*. Such comparative analysis highlights the advantage of nanoencapsulation to enhance the antimicrobial efficacy and biofilm inhibition.

Table (8): MBIC results of nisin-loaded niosomes against *E. coli*

Source	Flask A (Human)	Flask A (Animal)	Flask B (Human)	Flask B (Animal)
MBIC (µg/mL)	17.32 ± 2.41	21.63 ± 2.87	12.45 ± 1.96	14.72 ± 2.04

7. Comparison of MIC vs MBIC

Table (9) presents the reported MIC and MBIC values from recent research (2021–2026) to examine the improved efficacy of nisin-loaded niosomes as compared to free nisin. The data show consistently lower inhibitory concentrations for the niosomal formulation, particularly against *P. mirabilis*, thus proving the advantage of nanoencapsulation.

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Table (9): Comparative MIC and MBIC Analysis (This study vs. previous reports)

Pathogen	MIC (previous reports) µg/mL	MIC (this study) µg/mL	MBIC (previous reports) µg/mL	MBIC (this study) µg/mL	Reference
E. coli	12–32	6.25–12.5	40–80	6.33–20	Sharma et al., 2021; Hajinezhad et al., 2025
P. aeruginosa	24–64	6.25–25	60–100	12–30	Breidenstein et al., 2023; Hoseinpour et al., 2025
P. mirabilis	8–20	3.13–12.5	30–70	5–15	Kumar et al., 2023; Okae et al., 2022

Table (10): Comparative MIC values of nisin-loaded niosomes vs. recent studies .

Pathogen	This Study MIC (µg/mL)	Reported MIC in Literature	Reference
E. coli	6.25–12.5	8–16	Sharma et al., 2021; Hajinezhad et al., 2025
P. aeruginosa	6.25–25	12–32	Breidenstein et al., 2023; Hoseinpour et al., 2025
P. mirabilis	3.13–12.5	4–10	Kumar et al., 2023; Okae et al., 2022

Discussion:

The present study suggests that nisin-loaded niosomes are a promising nanocarrier system against major Gram-negative uropathogens (*E. coli*, *P. aeruginosa*, *P. mirabilis*). The results underscore the importance of formulation parameters (pH, surfactant:cholesterol ratio) to directly affect vesicle size, encapsulation efficiency and finally antibacterial activity.

1. Physicochemical and Stability Considerations

As of our investigations the formulation adjusted to pH 5.7 showed a vesicles smaller than 400 nm, with an uniform size distribution and a negative zeta potential verifying the colloidal stability. These results are consistent with previous studies indicating that nanosized vesicles smaller than 400 nm enhance cell absorption and lengthen circulation time (Torchilin, 2022; Mozafari, 2023). “The vesicles stored at 4 ℃ maintained their encapsulation efficiency which is in line with pharmaceutical practices for peptide formulations (Zaheer, 2024).

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2. Encapsulation Efficiency and Drug Loading

The maximum encapsulation effectiveness was obtained at neutral pH (67.7%). Peptide retention reduced, whereas loading capacity rose in acidic conditions. This difference is indicative of the balance between the peptide-bilayer interactions and the protonation impact (Sharma et al., 2021; Kumar et al., 2023). “Depending on the clinical application for which the product is intended, these results show that the stability or the loading can be improved by adjusting the pH of the formulation.

3. Antimicrobial Activity Against Uropathogens

The nisin-loaded niosomes showed substantial effect on Gram-negative uropathogens. The values of MICs varied 3.13 – 25 µg/mL . *P. mirabilis* was most sensitive while *P. aeruginosa* was most resistant. Acidic formulations always showed lower values of MIC and MBIC indicating an enhanced release of the peptide and electrostatic interactions with the bacterial membranes. These results are in line with recent studies on nisin nano-delivery approaches against resistant Gram-negative bacteria (Hajinezhad et al., 2025; Hoseinpour et al., 2025)..

4. Biofilm Inhibition

MBIC values indicated the ability of nisin-loaded niosomes to efficiently inhibit biofilm formation, mainly for *E. coli*. The antibiofilm activity was augmented by acidic formulations, which is consistent with the observation that biofilm matrices are more permeable in acidic environments (Okae et al., 2022; Verma & Maheshwari, 2024). This finding is clinically important, since biofilm-associated infections are well known for their resistance to conventional antibiotics.

This table indicates that our results are consistent with published data, but show slightly lower MICs, especially for *P. mirabilis*, suggesting improved efficacy of the niosomal delivery system.

5. Clinical and Veterinary Implications

The higher occurrence of isolates in animal samples conforms with Oliver et al. (2021) and is associated with environmental exposure and selective pressure from the use of antibiotics in livestock. The most frequently encountered uropathogen is *E. coli*, which is in keeping with international epidemiological trends (Flores-Mireles et al., 2024). Interestingly, the capacity of nisin-loaded niosomes to suppress planktonic and biofilm related cells supports the use of them as adjuvant therapies for recurrent and resistant UTIs throughout human and veterinary medicine.

Conclusions:

This study confirms that nisin-loaded niosomes function as an effective nanocarrier system against predominant Gram-negative uropathogens (*E. coli*, *P. aeruginosa*, *P. mirabilis*). Optimized formulations at acidic pH produced vesicles smaller than 400 nm with improved stability and stronger antimicrobial activity. Encapsulation efficiency was highest at neutral pH, while acidic

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conditions enhanced drug release and antibiofilm performance. Collectively, these findings highlight the potential of Nisin-loaded niosomes as adjunctive therapies for recurrent and resistant urinary tract infections in both human and veterinary medicine.

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Conflict of Interest:

The author declares no conflict of interest regarding the publication of this research.

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Authors Contributions:

The author was solely responsible for the conception, design, sample collection, laboratory experiments, data analysis, and manuscript preparation of this study.

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