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Research Article

FORMULATION AND EVALUATION OF TRANSFEROSOMAL MUPIROCIN GEL FOR ENHANCED SKIN PERMEATION

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ABSTRACT

Skin infections are among the most frequent bacterial infections and often require effective topical antibiotic therapy. Mupirocin is a widely used topical antibiotic, but conventional formulations may show limited skin penetration, short residence time and the need for frequent application, which can reduce therapeutic performance and patient compliance. This article aimed to formulate a Mupirocin loaded transferosomes using the ethanol injection method and added into a Carbopol based gel to improve topical delivery and antibacterial efficacy. The prepared transferosomal system was evaluated for particle size, polydispersity index, zeta potential, pH, drug content, spreadability, in vitro drug release, ex vivo permeation and antimicrobial activity. The optimized formulation showed nanosized vesicles, acceptable physicochemical properties, good stability and improved drug permeation compared with conventional gel. In vitro and ex vivo studies indicated sustained release and enhanced skin transport, while antimicrobial testing confirmed better antibacterial performance. The study concluded that the Mupirocin-loaded transferosomal gel is a promising topical drug delivery system for the effective treatment of bacterial skin infections due to its improved penetration, sustained release, and enhanced antibacterial efficacy.

Keywords: Franz diffusion cells, Sustained Release, Polydispersity Index, Dialysis Membrane.

INTRODUCTION

Skin and soft tissue infections are common clinical problems, and many of them are caused by pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Topical antibiotics are often preferred for localized skin infections because they can deliver the medication straight to the infected site while reducing systemic exposure. Mupirocin is an established topical antibiotic used for the treatment of bacterial skin infections, but conventional topical dosage forms may not provide optimal penetration into the skin barrier. Their limited residence time on the skin, frequent dosing requirements and reduced drug permeation can lower therapeutic effectiveness and affect patient adherence (Opatha *et al*., 2020). To get around these limitations, advanced vesicular drug delivery systems have gained increasing attention. Transferosomes are ultra-deformable phospholipid vesicles designed to enhance drug passage through the stratum corneum and improve transdermal and topical delivery. Their flexible nature allows them to squeeze through skin pores much smaller than their own size, thereby increasing penetration efficiency, prolonging drug retention, and supporting sustained release at the target site. When transferosomes are incorporated into a gel base, the formulation becomes more suitable for topical use because of improved stability, easier application and better patient acceptability (Stevens *et al*., 2014, Arpitha *et al*., 2019).

In this study, mupirocin was formulated into transferosomes and then converted into a gel dosage form to enhance its skin permeation and antibacterial activity. The formulation was characterized through physicochemical and performance studies, including vesicle size analysis, zeta potential, drug content, in vitro release, ex vivo permeation and antimicrobial evaluation.

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The overall aim was to develop a stable, effective and patient-friendly topical delivery system for the treatment of bacterial skin infections.

MATERIALS AND METHODS

Materials

The Mupirocin was received as gift sample from Endoc Biotech PVT.LTD and other excipients were obtained from Oxford Laboratories, Pune.

Formulation of Mupirocin Loaded Trasferosomes

The ethanol injection approach was used to create mupirocin-loaded transferosomes. To obtain a organic phase phospholipid, Span 60 and mupirocin are dissolved in ethanol. This solution is subjected to magnetic stirring for the appropriate amount of time, to obtained a clear solution. Aqueous phase is prepared by adding water soluble material in phosphate buffer. The temperature of both solutions is raised to 45-50 °C. The ethanolic phospholipid solution is then gradually added to the aqueous solution while being continuously stirred for the appropriate amount of time. The resulting dispersion is placed in a vacuum evaporator and sonicated to reduce particle size in order to remove the ethanol. (Chandrapalka *et al*., 2024).

Formulation of Transferosomal Mupirocin gel

1 % w/w Carbopol 934 was dispersed in distilled water under continuous stirring and allowed to hydrate completely. Methyl paraben (0.02% w/w) was dissolved in distilled water and mixed with propylene glycol (0.1% w/w). The optimized mupirocin-loaded transferosomal dispersion (0.4 g) was incorporated into this mixture and added slowly to the hydrated Carbopol under gentle stirring. Finally, pH is adjusted with triethanolamine. (Opatha *et al*., 2024). Similarly plain gel of mupirocin was formulated.

CHARACTERIZATION

Calibration & Linearity Curve

Calibration curve was plotted for determination of wavelength of maximum absorption using UV spectrophotometer for mupirocin. A linearity curve was developed in phosphate buffer having pH 6.8 covering a concentration range from 1 mg/ml.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was utilized to produce a spectrum of mupirocin and physical mixture of mupirocin with cholesterol, soya lecithin. The spectra were obtained using the KBr disc method under a hydraulic pressing the range of 400-4000 nm. FTIR analysis was performed to investigate drug-excipient compatibility and examine the structural features of the formed niosomes (Nair *et al*., 2020).

Particle Size & PDI

Mupirocin transferosomes' mean particle size and polydispersity index (PDI) were measured using using the Zetasizer Nano ZS-90 Instrument. Before analysis, mupirocin transferosomes suspensio were appropriately diluted with distilled water. The size distribution is indicated by the PDI; narrow transferosomal distributions are represented by modest PDI values. (Bhattacharjee *et al*., 2016).

Zeta Potential

The mupirocin transferosomes suspension was diluted with deionized water to an appropriate concentration. The diluted sample was placed in a electrophoretic cell and analyzed using the Nano-ZS Zetasizer at 25°C. To evaluate the transferosomes' stability and surface charge, the zeta potential was evaluated. (Jawale J *et al*., 2025)

pH Determination

A digital pH meter was used to measure the pH of Transferosomal gel. A magnetic stirrer was used to disperse 0.5 g of the formulation in 20 ml of distilled water for 20 minutes. The pH electrode was then submerged in the dispersion to determine the pH. Every measurement was made in triplicate (Devne *et al*.,2026)

Percentage Drug Content

To determine drug content 100 mg of the formulation is dissolved in 10 mL of ethanol with appropriate dilutions, the amount of drug present in the transfersomal gel was determined using UV-visible spectrophotometer. (Gupta *et al*., 2012).

Spreadability

A 50 g weight was added to the pan after an excess of sample was placed between two glass slides and compacted to a uniform thickness using a 100 mg weight for five minutes in order to determine spreadability. The spread ability was measured by timing how quickly the upper glass slide moved to the lower plate (Nooreen *et al*., 2023).

$$S = M.L / T$$

M- Weight tied to the upper slide

L - Length moved on the glass.

T - Time Taken

In-Vitro Drug Release Study

A Franz diffusion cell was used for the in vitro drug release determination of the prepared transferosomal gel. Prior to the experiment, a dialysis membrane (Molecular Weight Cut off 12,000-14,000) was soaked in phosphate buffer saline (PBS, pH 6.8) for an entire night. With the glossy side toward the donor compartment, the hydrated membrane was carefully positioned between the Franz

diffusion cell's donor and receptor compartments. PBS, pH 6.8 was added to the receptor compartment and to ensure even mixing and sink conditions, the medium was constantly agitated with a magnetic bead at a steady pace. Throughout the investigation, the receptor medium's temperature was kept at 37 ± 0.5°C to replicate physiological skin circumstances. The donor compartment was covered to stop evaporation after a predetermined amount of the produced transferosomal gel was equally applied to the membrane surface. Aliquots of the receptor medium were removed at prearranged intervals and promptly replaced with an equivalent volume of fresh PBS (pH 6.8) kept at the same temperature. The drug content of the samples was determined by analyzing them at 221 nm using a UV-visible spectrophotometer. For accuracy and repeatability, the study was conducted in duplicate and the cumulative % drug release was computed. (Azeez *et al*., 2024).

Ex Vivo Permeation

Fresh goat skin was collected from slaughter house and clean it thoroughly with a saline solution. Install the skin: With the epidermal side facing up, place the skin between the Franz diffusion cell's donor and receptor compartments. Fill the receptor compartment: Fill the receptor compartment with phosphate buffer (pH 6.8 or an appropriate medium) and stir continuously to keep the temperature at 37 ± 0.5°C. Use the formulation: Cover the skin's surface with the necessary amount of gel or formulation in the donor compartment. Sample collection: At prearranged intervals, remove a little portion of the sample and replace it with new buffer. Drug analysis: Use a UV spectrophotometer or an appropriate analytical technique to examine the samples that were gathered (Shahiwala *et al*., 2002).

Calculation of Skin Permeation Parameters

Plotting the cumulative amount of medication permeated per unit area versus time allowed for the calculation of skin permeation parameters, including a steady-state permeation rate (Jss) and lag time (LT, hours). The following formula was used to determine the permeability coefficient (Kp) from the slope and X-intercept of the linear section, respectively (Modi C et. Al 2023).

$$Kp = Jss/Co$$

Antimicrobial Activity

The antimicrobial activity of the optimized mupirocin-loaded transferosomal gel was evaluated by the agar well diffusion method using Staphylococcus aureus. Sterile nutrient agar plates were inoculated with a fresh bacterial suspension and wells were prepared using a sterile cork borer. Equal volumes (100 µL) of the optimized transferosomal gel and plain mupirocin gel having equivalent amount of mupirocin were introduced into separate wells. The plates were allowed to diffuse for 30 min and incubated at 37 ± 0.5°C for 24 h. The antibacterial activity was determined by measuring the zone of inhibition (Balouiri 2016).

RESULTS AND DISCUSSION

Mupirocin was quantitively analysed using UV-Visible spectrophotometry. It shows maximum absorbance at 221 nm as shown in figure 1a. Figure 1b. shows calibration curve and it was found to be linear within the studied range with Correlation Coefficient (R^2) 0.9918.

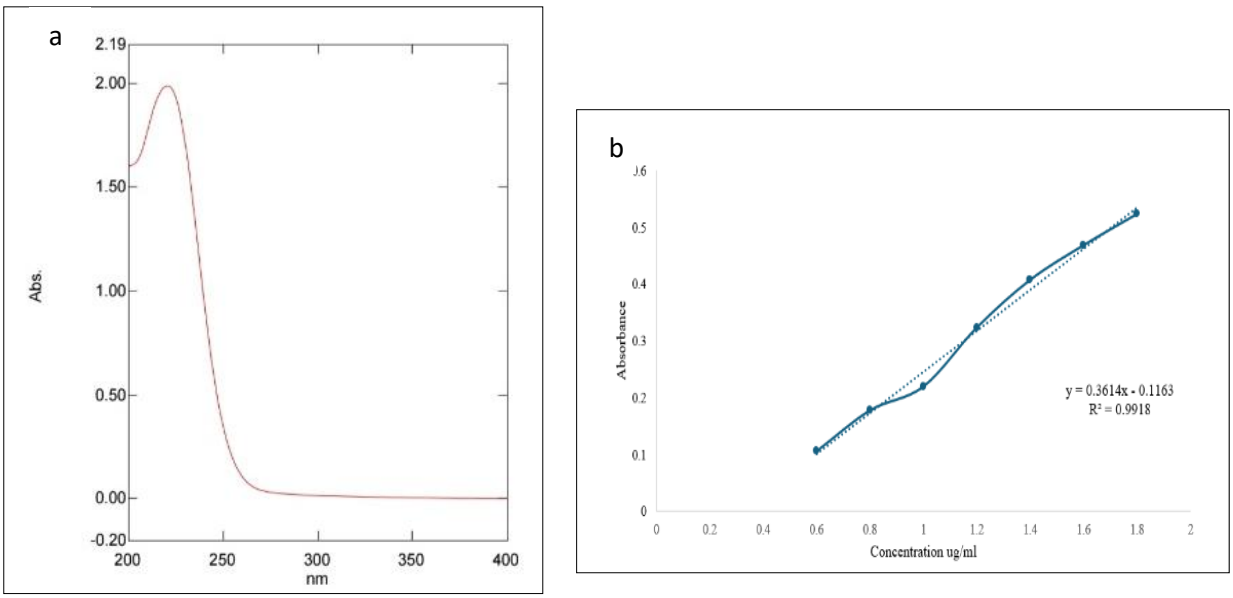

Figure 1 . a. UV Spectra of Mupirocin b. Calibration curve of Mupirocin

The FTIR spectral analysis confirms the identity of Mupirocin through its key molecular markers. The drug's signature structures are clearly verified by a broad O-H stretching peak at 3649.43 cm^{-1}, an alkane C-H stretch at 2933.88 cm^{-1} and a highly prominent ester C=O stretching peak at 1712.15 cm^{-1}. Additionally, the aliphatic ether chain networks characteristic of the monic acid core in mupirocin is validated by distinct C-O and C-O-C stretching peaks at 1220.30 cm^{-1}, 1161.51 cm^{-1} and 1022.41 cm^{-1}. The FTIR spectrum of physical mixture (Figure 3) confirms the presence of cholesterol via its characteristic peak at 3480.22 cm^{-1} for O-H stretching and steroid methyl bending at 1362.26 cm^{-1}. Concurrently, soya lecithin is accurately identified by its characteristic peak of fatty acid ester C=O carbonyl stretch at 1726.49/1712.15 cm^{-1}. In the FTIR spectra of physical mixture, all the characteristics peaks are reappeared which confirm the Mupirocin, Cholesterol & Soya lethicin are compatible for formulation. There is slight drifting of FTIR peaks in physical mixture it may be due to the mixing of the components. This concluded the absence of chemical interaction and confirms that the drug is compatible with the selected excipients.

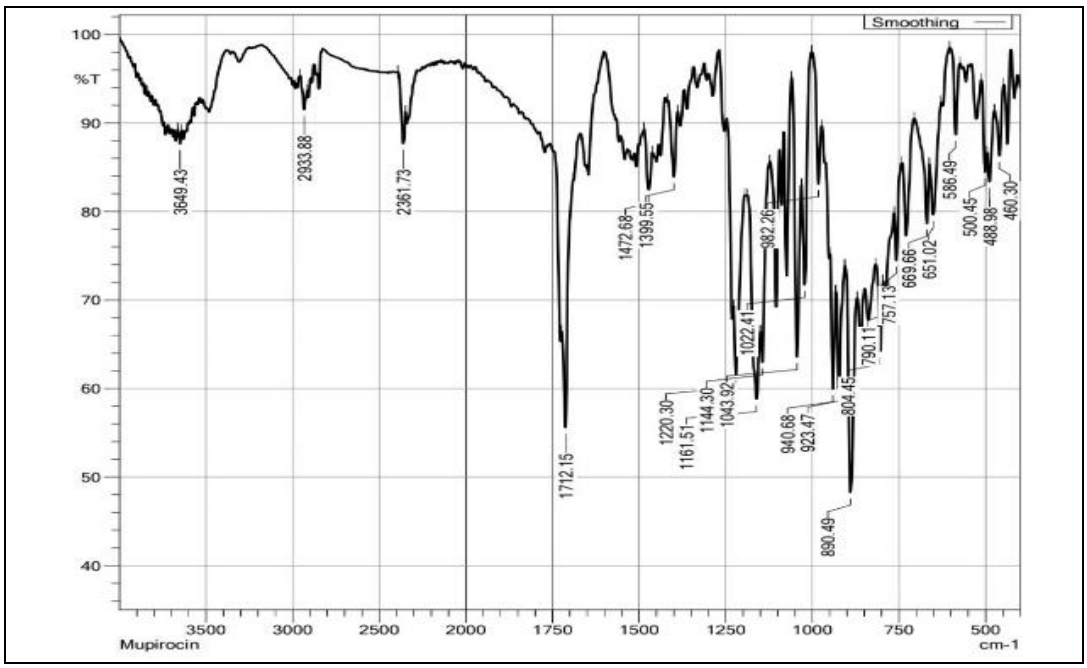

Figure 2. FTIR Spectrum of Mupirocin.

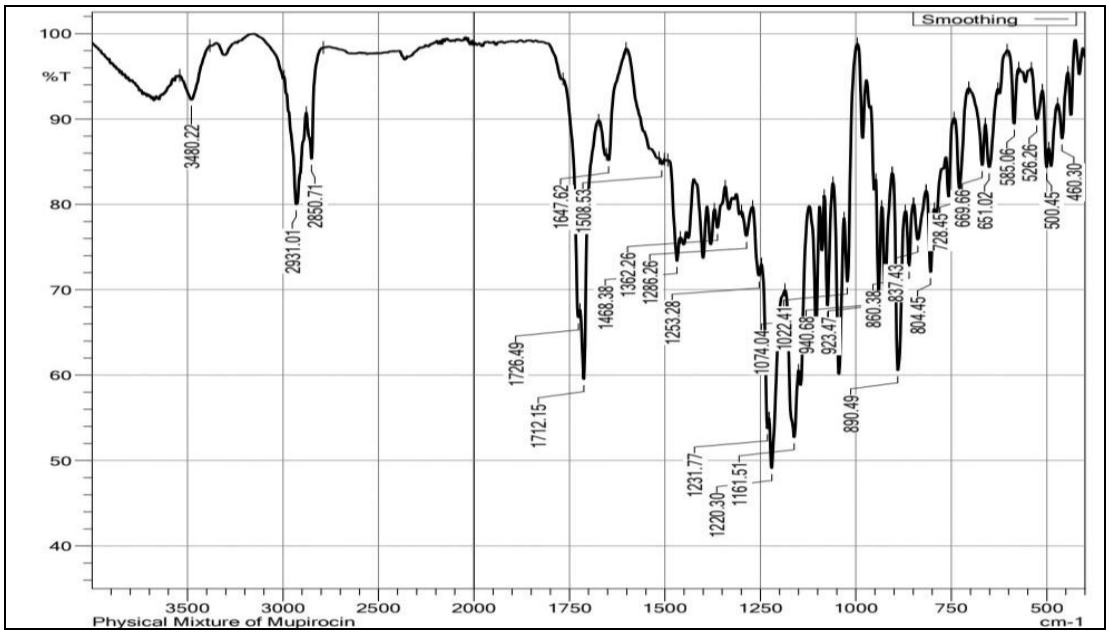

Figure 3. FTIR spectrum of Physical Mixture.

The formulation exhibited a average particle size of 212.0 nm with a PDI value of 0.411 (Figure 4). The obtained PDI value indicates a relatively narrow and uniform particle size distribution, suggesting good homogeneity and stability of the Transferosomes. Generally, a PDI value in the range of 0.3-0.7 is considered acceptable for nanoparticulate systems, indicating moderate to good uniformity in particle size distribution. The particle size in the nanometre range confirms the successful formation of transferosomal gel particles with suitable dispersion characteristics. The lower PDI value reflects improved uniformity and consistency of the formulation, which may enhance its stability and performance. Based on the particle size and PDI analysis, the formulation demonstrated promising nanoscale characteristics suitable for further evaluation.

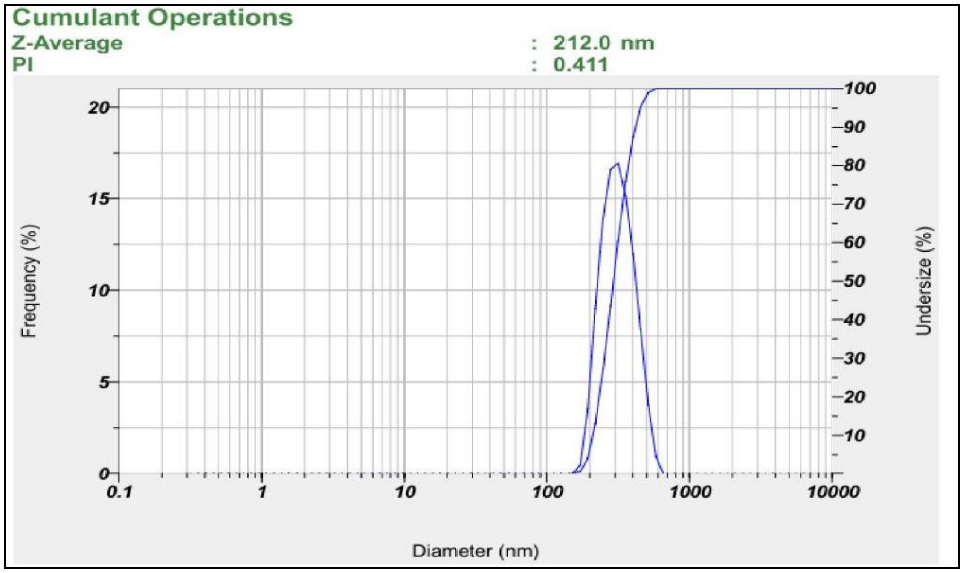

Figure 4. Particle size & PDI.

The formulation showed a mean zeta potential value of -64.7 mV (Figure 5). The highly negative zeta potential value indicates good electrostatic repulsion between the vesicles, suggesting excellent stability of the colloidal system and reduced chances of particle aggregation. The negative surface charge may be due to the presence of phospholipids and other functional groups in the formulation, which undergo ionization in the aqueous medium. The sharp and narrow peak observed in the graph further confirms the uniform distribution and stable nature of the transferosomal gel particles. Based on the zeta potential analysis, the formulation demonstrated promising stability characteristics.

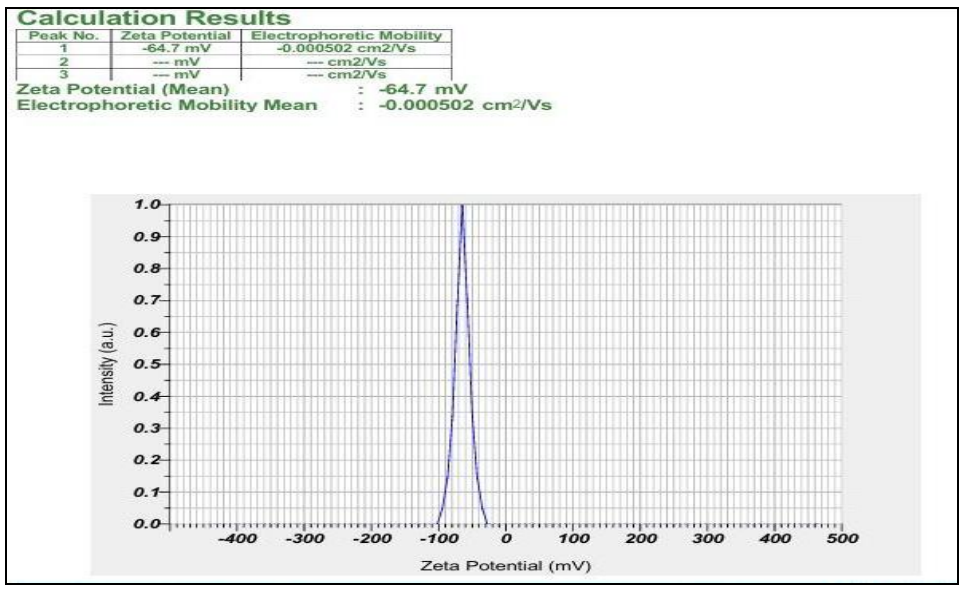

Figure 5. Zeta Potential.

The pH of formulation was found to be 6.8 ± 0.124. The obtained pH was within the acceptable skin pH range, indicating good compatibility and stability of the formulation.

The drug content of the formulated mupirocin transferosomal gel was determined to evaluate the uniform distribution of the drug within the formulation. The prepared formulation showed a drug content of 86.96 ± 0.868 %, indicating satisfactory incorporation of mupirocin into the transferosomal gel system. The spreadability of the formulated transferosomal Mupirocin gel prepared by the ethanol injection method was found to be 6.8 ±0.162 g.cm/sec, indicating good spreadability and ease of application on the skin. Transferosomal mupirocin gel released the drug faster and in larger amounts than the plain mupirocin gel. The transferosomal gel showed a quick release of about 30-50% of the drug within the first hour and reached around 60% release after 4 hours. In comparison, the plain mupirocin gel released the drug more slowly, with about 33% at 2 hours and 43% at 4 hours (Figure 6).

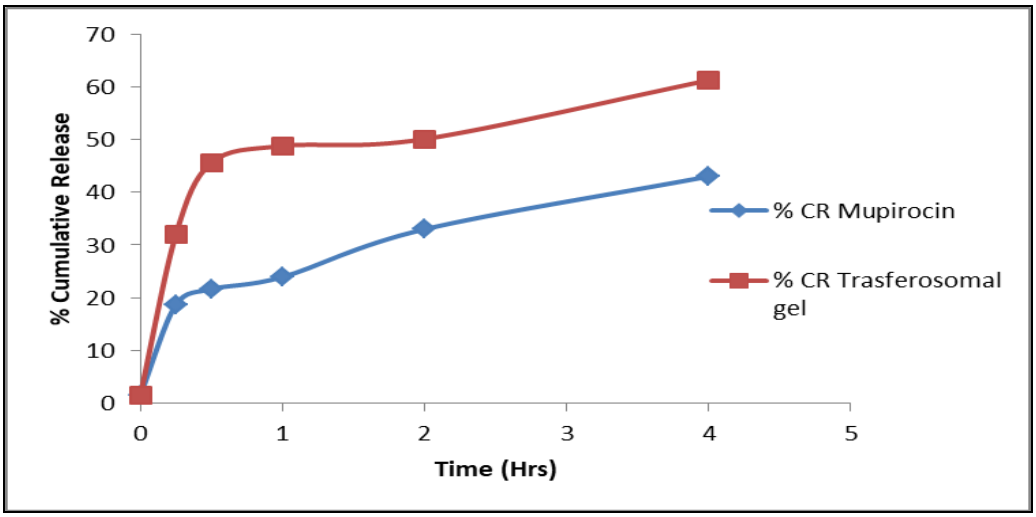

Figure 6. In-vitro drug release of Mupirocin

The transferosomal gel showed a two-step release pattern, with an initial fast release followed by a slower release as compare to pure drug. This suggests that transferosomes improve the availability of mupirocin at the site of application by helping the drug pass through the skin more effectively. Therefore, the transferosomal gel may provide a faster antibacterial effect and higher drug concentration in the skin. The ex vivo permeation study of the transferosomal gel of mupirocin demonstrated significantly enhanced drug permeation across the membrane compared to conventional mupirocin gel. The graph showed an initial rapid increase in the cumulative amount (Figure 7) of drug permeated (Q), followed by sustained and controlled release behaviour. The calculated transdermal flux value of 23.91 µg/cm²/h indicated efficient drug transport through the membrane. In comparison to plain mupirocin gel, the transferosomal formulation exhibited superior permeation due to the ultra-deformable and elastic nature of transferosomes, which facilitate deeper penetration through membrane pores.

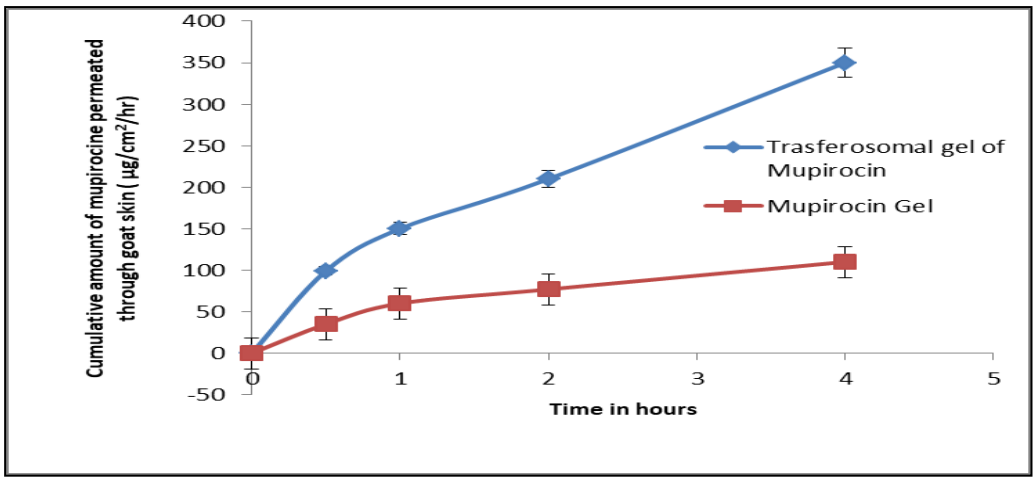

Figure 7. Cumulative amount of Mupirocin

Conventional mupirocin gel generally shows lower permeation and reduced flux because of limited penetration ability and poor passage through the stratum corneum barrier. The presence of edge activators Tween 80 in transferosomes enhances membrane fluidity and improves drug diffusion, resulting in higher cumulative drug permeation and better transdermal delivery. Therefore, the transferosomal mupirocin gel can be considered a more effective topical carrier system than conventional mupirocin gel for improved drug permeation and sustained release. Antibacterial activity of mupirocin loaded transferosomal gel was studied against Staphylococcus aureus and compared with a plain mupirocin gel containing the same drug concentration. The transferosomal gel exhibited a significantly larger zone of inhibition i.e 14.84 ± 0.62 mm than the plain mupirocin gel 8.76 ± 0.48 mm, indicating enhanced antibacterial efficacy. The improved antimicrobial activity of the transferosomal formulation can be due to the very small size, ultra-deformable vesicles, which facilitated better drug diffusion and sustained release, thereby increasing the availability of mupirocin at the site of bacterial growth. In contrast, the plain mupirocin gel exhibited a comparatively smaller inhibition zone due to its relatively limited drug diffusion. These findings demonstrate that incorporation of mupirocin into transferosomes significantly enhanced its antibacterial activity without altering the drug concentration, confirming the superiority of the transferosomal gel over the conventional plain mupirocin gel for topical treatment of bacterial skin infections.

CONCLUSION

An optimized topical transferosomal gel formulation of Mupirocin was successfully developed using the ethanol injection method, which produced vesicles with submicron particle size, spherical morphology, high entrapment efficiency and satisfactory stability characteristics. The rationale of the study was to enhance the skin permeation and therapeutic efficacy of Mupirocin through a novel vesicular carrier system. Transferosomes prepared by the ethanol injection method demonstrated enhanced deformability and permeability, enabling deeper penetration into skin layers compared to conventional topical formulations. This improved permeation resulted in sustained and higher Therefore, the developed Mupirocin transferosomal gel prepared using the ethanol injection method can be considered a promising topical delivery system for the effective treatment of skin infections such as impetigo, furuncles and other bacterial skin disorders.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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