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"Formulation and evaluation of mupirocin loaded invasomes for effective treatment of skin infections"

MAHENDRA THAKUR[1], ANIL NAGAR[2], ABHISHEK KUMAR JAIN[3], U. K. JAIN[4*]

[1, 2, 3, 4]Bhopal Institute of Technology and Science-Pharmacy, Bhopal, (Madhya Pradesh)
*Corresponding author Email: ukjain65@gmail.com

ABSTRACT

Invasomes are novel vesicular systems that exhibit improved transdermal penetration compared to conventional liposomes. These vesicles contain phospholipids, ethanol, and terpene in their structures; these components confer suitable transdermal penetration properties to the soft vesicles. The main advantages of these nanovesicles lie in their ability to increase the permeability of the drug into the skin and decrease absorption into the systemic circulation, thus, limiting the activity of various drugs within the skin layer. The purpose of this work was to prepare and characterize invasomes carrier for Mupirocin, and to evaluate the optimized formulation obtained for pharmacodynamic study. Mupirocin -loaded invasomes were prepared by mechanical dispersion technique using Soya phosphatidylcholine, terpene and ethanol. Prepared formulations were characterized in terms of Entrapment efficiency, Particle Size, Drug Content, Vesicular Size and Shape and In vitro drug release. It was observed that prepared Mupirocin-loaded invasomes delivers reasonable entrapment efficiency, and more effectiveness for transdermal delivery. Among all 6 formulation the optimized formulation F3 exhibits a mean particle size of 359.58 nm, entrapment efficiency of 85.29%, and drug content of 98.28%. These values collectively suggest that F3 is a promising formulation with desirable characteristics for drug delivery. The *in-vitro* drug release data of Mupirocin from F3 follows Higuchi kinetics. This implies controlled release through diffusion as the dominant mechanism.

Keywords: Invasomes, Mupirocin, Mechanical Dispersion, Soya phosphatidylcholine

INTRODUCTION

Invasome a potential approach for transdermal drug delivery. Invasomes are innovative vesicular systems, play an important role to improve transdermal penetration of active drug molecules as compare to other conventional vesicles. These vesicles are composed of phospholipids, ethanol and terpene or mixture of terpenes in their structures. These components

worked as suitable transdermal penetrator with good penetration properties (**Lakshmi *et al*., 2013**). Invasomes are the soft liposomal vesicles embodying trivial quantities of ethanol and terpene or terpene assortments, which deed as potential transporters with amplified skin penetratio. These unique lipid vesicles are poised of phospholipids (i.e. phosphatidylcholine, phosphatidylserine, soya phospholipid, egg lecithin, phosphatidylinositol, phosphatidic acid and phosphatidylglycerol), low concentration of ethanol (3% to 3.3% v/v), terpenes or a mixture of terpenes (i.e. citral, cineole, limonene, eugenol; 1-5 % v/v) and water. Terpenes have general formula (CsH8) n, increases the percutaneous absorption of hydrophilic and hydrophobic drugs. The terpenes, which are constituents of essential oils obtained from natural sources, and used broadly as penetration enhancers. However, terpenes have additional advantages of low skin irritancy at low concentrations. Furthermore, FDA classifies terpenes as generally safe (**Jain *et al.*, 2021**). Transmission electron microscopy (TEM) and scanning electron microscopy are excellent tools for observing invasomes. Vesicles of the Temoporfin peptide have been shown to be spherical, oval, unilamellar, bilamellar, and even oligolamellar in shape. A spherical, unilamellar form was described for finasteride invasomes. Carboxyfluorescein and temoporfin invasomes were found to be nearly unilamellar and bilamellar, respectively, by cryo-TEM. This is why they can be identified as spherical or distorted vesicles with one, two, or more lamellae (**Chen *et al.*, 2011**). Liposomes are phospholipid-based vesicular structures composed of anionic, cationic, and neutral lipids and cholesterol that improve the encapsulation of lipophilic, hydrophilic, and amphiphilic drugs. Lipophilic drugs are placed in the inner part of the lipid bilayer, hydrophilic drugs in the aqueous core, and amphiphilic types in the interlayer of the vesicles. Contrary to this, invasomes are flexible liposomes consisting of phospholipids, ethanol, and one terpene molecule or a mixture of terpenes. Ethanol increases the fluidity of lipids in the vesicle structure, creating a soft structure less rigid than conventional liposomes and, therefore, enhancing its permeability into the skin. Similarly, terpenes have also been shown to improve penetration by disrupting the tight structure of the SC lipids. SEM photographs indicated that all vesicles displayed smooth surface and spherical structure. TEM photographs showed the surface morphology of liposomes and invasomes were unilamellar, while unilamellar to multilamellar was revealed in the case of transfersomes (**Ohradanova-Repic *et al*., 2018, Karimi *et al*., 2015**).

MATERIAL AND METHODS

Pre formulation Studies

- **Organoleptic Properties of Mupirocin**

The physical examination of Mupirocin was performed in day-light and drug was observed for its appearance, colour, and odour.

- **Solubility Determination**

A qualitative determination of the solubility was made by adding solvent in small incremental amount to a test tube containing fixed quantity of solute or vice versa. After each addition, the system was vigorously shaken and observed visually (**Indian Pharmacopoeia, 1996**).

- **Melting Point of Mupirocin**

Melting point measurement of Mupirocin was carried out by melting point apparatus (digital melting point apparatus).

- **Partition coefficient of Mupirocin**

At room temperature (30°C), the partition coefficient of n-octanol/water was determined. 10 mL of n-octanol and 10 mL of distilled water were placed in a glass stoppers graduated tube, and 5 mg of accurately weighted drug was added. For 24 hours, the mixture was shaken at room temperature using a mechanical shaker, before being transferred to a separating funnel and allowed to equilibrate for 6 hours. The drug content in the aqueous phase was determined using a UV-Visible spectrophotometer after the aqueous and octanol phases were separated and filtered through membrane.

The partition coefficient was determined using the formula - Ct-Ca/Ca.

Where Ct is the total drug concentration (5 mg), and Ca is the drug concentration in aqueous phase. Log P was computed.

- **Determination of λ_{max} of Mupirocin:**

Ultraviolet Visible spectrophotometry (Shimadzu 1700, Japan) has been used to obtain specific information on the chromophoric part of the molecules (**Singh *et al.*, 2023**).

- **Preparation of Standard Curve of Mupirocin in phosphate buffer (pH 7.4)**

Accurately weighed 10 mg of Mupirocin and was dissolved in 10 ml of phosphate buffer (pH 7.4, from this stock solution 1 ml was withdrawn and the volume was made up to 10 ml which was named as a stock solution. Form this standard stock solution, a series of dilution (5, 10, 15, 20, 25 µg/ml) were prepared using methanol. The absorbance of these solutions was measured spectrophotometrically against blank of methanol at 222 nm for Mupirocin.

- **Compatibility study by FT-IR**

Compatibility was confirmed by carried out IR studies. The pure drug and its formulation along with excipients were subjected to IR studies. In present studies potassium bromide disc (pellets) method were used.

Preparation of Mupirocin Invasomes

Invasomes of Mupirocin were prepared by mechanical dispersion technique. Soya phosphatidylcholine was added to ethanol and the mixture was vortexed for 5 minutes. Mupirocin and terpenes were added while the mixture was constantly vortexed and sonicated for 5 minutes. Under constant vortexing, a fine stream of distilled water (up to 10% v/v) was added with a syringe to the mixture. To obtain the final invasomal preparation, the formulation was vortexed for an additional 5 minutes (**Dragicevic-Curic *et al.*, 2010**).

Table 1: Composition of different invasomal formulation

Formulation	Drug (% w/v)	Terpene (5% v/v)		Ethanol (% v/v)	Polymer (% w/v)
		Nerolidol	Citral		
F1	1	0.5	-	10	1
F2	1	1	-	10	1
F3	1	1.5	-	10	1
F4	1	-	0.5	10	1
F5	1	-	1	10	1
F6	1	-	1.5	10	1

Characterization and optimization of Mupirocin-loaded invasomes

- **Entrapment efficiency**

Ultracentrifugation method was used for determining the percentage drug entrapment of the invasomal formulation (**Aggarwal and Goindi, 2013**). Percentage drug entrapment was calculated using the equation:

- **Particle Size**

The size of the vesicles was determined using Zeta Sizer (Nano- ZS, Malvern, U.K.) (**Amnuaikit *et al.*, 2018; Kumar *et al.*, 2022**).

- **Surface morphology**

It was studied by placing a drop of preparation on clear glass slide, air dried, coated with gold using sputter coater (Polaron E5100, Watford, UK) and visualized under scanning electron microscopy.

- **Drug Content**

Drug content of the invasomes can be determined by using ultraviolet spectrophotometer.

- **Vesicular Size and Shape**

Invasomes can be visualised by using Transmission Electron Microscopy (TEM) and by Scanning Electron Microscopy (SEM).

- ***In vitro* drug release**

In vitro drug release study was conducted using Franz's diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^3 respectively (**Kumar *et al.*, 2021).**

- ***In vitro* antimicrobial activity of optimized formulation F3**

The well diffusion method was used to determine the antimicrobial activity of the invasomes optimized formulation (F3) prepared from mupirocin using standard procedure.

RESULTS

Results of Organoleptic properties

Table 2: Organoleptic characteristics of Mupirocin

S. No.	Properties studied	Results
1	Colour	White to off-white
2	Odour	Unpleasant odour
3	Taste	Bitter
4	Appearance/Morphology	Crystalline powder

Results of Solubility analysis

Table 3: Solubility determination of Mupirocin in various solvent

Solvents	Results of Solubility
Methanol	Soluble
Ethanol	Soluble
Chloroform	Sparingly soluble
Distilled water	Sparingly soluble
7.2 pH phosphate buffer	Soluble
0.1 N HCI	Sparingly soluble
0.1 N NaOH	Soluble

It has been observed that Mupirocin was soluble in ethanol, methanol, 7.2 pH phosphate buffer and 0.1 N NaOH, sparingly soluble in chloroform, 0.1 N HCI and distilled water.

Results of Melting point and partition coefficient of Mupirocin

Table 4: Results of Melting point and partition coefficient

S. No.	Melting point of Mupirocin	Partition coefficient of Mupirocin
1	77-78°C	2.23

Determination of λ_{max} and Calibration curve of Mupirocin

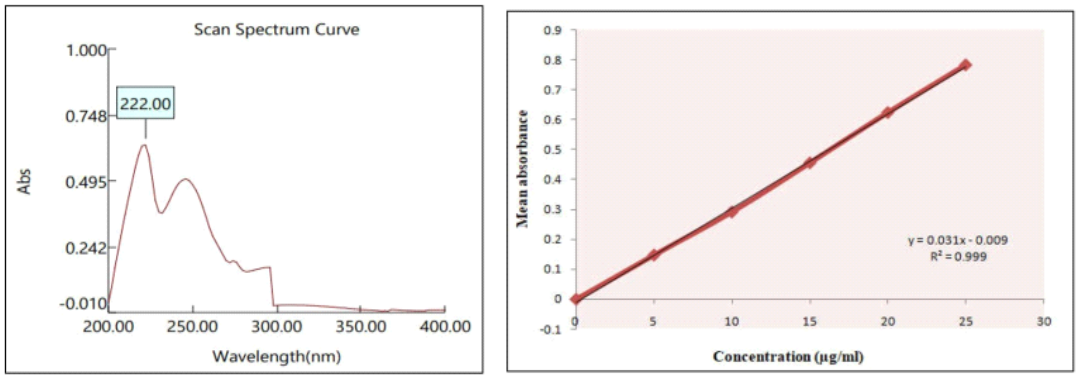

Figure 1: λ_{max} and Calibration curve of Mupirocin

Table 5: Calibration curve of Mupirocin

S. No.	Concentration (µg/ml)	Mean absorbance
•	5	0.148±0.002
•	10	0.292+0.002
•	15	0.456±0.005
•	20	0.624+0.003
•	25	0.783+0.005

All values are expressed in S.D (n=3)

FTIR spectroscopy of Mupirocin

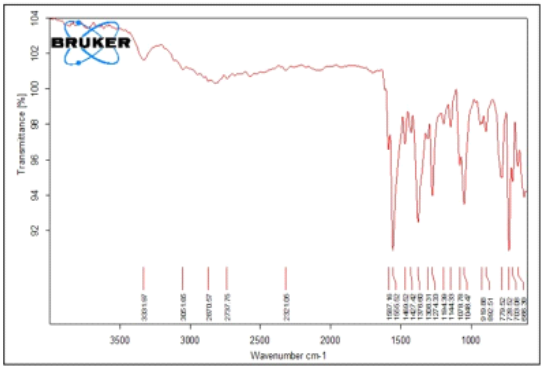

Figure 2: FTIR spectra of Mupirocin

Characterization of Invasomes

Table 6: Vesicle Size (nm), Entrapment Efficiency and Drug Content analysis of Mupirocin loaded invasomes

S. No.	Formulation Code	Vesicle size (nm)	Entrapment Efficiency (%)	Drug Content (%)

•	FI	308.45±0.10	82.53+0.08	92.06-0.13
•	F2	348.38±0.11	84.94±0.16	93.51±0.11.
•	**F3**	**359.58+0.03**	**85.29+0.13**	**98.28±0.06**
•	F4	259.36±0.06	81.86±0.06	93.53+0.12
•	F5	276.57+0.13	83.85+0.11	94.27±0.10
•	F6	283.28±0.08	84.04+0.09	95.19±0.13

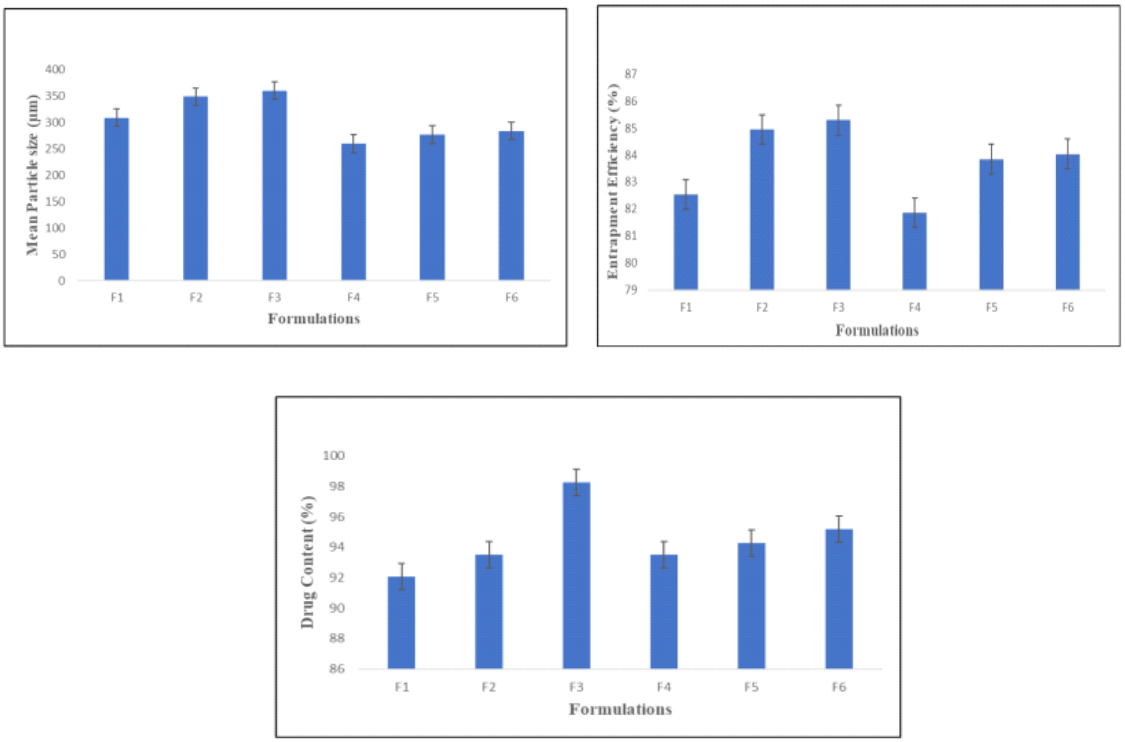

Figure 3: Vesicle Size (nm), Entrapment Efficiency and Drug Content of Mupirocin loaded invasomes

Discussion

The evaluation of percentage yield, entrapment efficiency, and drug content is crucial in determining the overall success of a formulation. The results obtained for the optimized formulation, F3, The mean particle size of 359.58±0.03 µm for F3 indicates a stable and reproducible particle size distribution. Consistency in particle size is essential for ensuring uniformity in the formulation, which, in turn, influences factors such as stability, bioavailability, and patient compliance. The entrapment efficiency of 85.29 0.13% in formulation F3 is indicative of the formulation's ability to encapsulate and retain a significant proportion of the drug within the invasomes. This high entrapment efficiency is advantageous as it ensures that a substantial amount of the active pharmaceutical ingredient (API), in this case, Mupirocin, is effectively utilized, minimizing wastage. The drug content of 98.28 ± 0.06% in F3 is exceptionally high and signifies that almost the entire mass of the formulation

consists of the intended drug. Achieving such high drug content is beneficial for therapeutic applications as it maximizes the dosage delivered with each administration.

The evaluation of percentage yield, entrapment efficiency, and drug content for the optimized formulation, F3, reveals a formulation that is not only consistent in particle size but also highly efficient in encapsulating and delivering the active drug. These characteristics position F3 as a strong candidate for further development and potential clinical applications in the realm of Mupirocin-loaded invasomes.

- **Scanning electron microscopy**

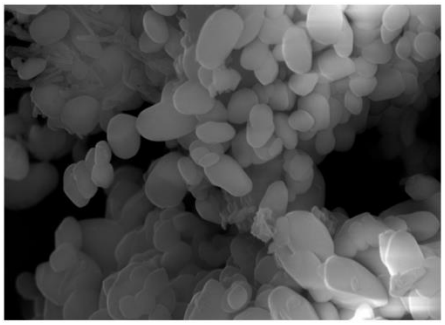

Figure 4: SEM images of Mupirocin loaded invasomes (F3)

DISCUSSION

SEM analyses of the formulated Mupirocin loaded invasomes were performed to evaluate the surface morphology of invasomes. The SEM images of formulation F3 showed the invasome was porous with a smooth surface morphology and spherical in shape. The spongy and porous nature of the invasomes can be seen in the figures.

- ***In vitro* drug release studies**

Table 7: In-vitro drug release data for optimized formulation F3

Time (h)	Square Root of Time(h)	Log Time	Cumulative % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	14.65	1.166	85.35	1.931
1	1	0	23.85	1.377	76.15	1.882
1.5	1.225	0.176	38.85	1.589	61.15	1.786
2	1.414	0.301	49.98	1.699	50.02	1.699
3	1.732	0.477	58.85	1.770	41.15	1.614
4	2	0.602	65.74	1.818	34.26	1.535

6	2.449	0.778	76.65	1.885	23.35	1.368
8	2.2828	0.90.	89.98	1.954	10.02	1.001
12	3.464	1.079	93.32	1.970	6.68	0.825

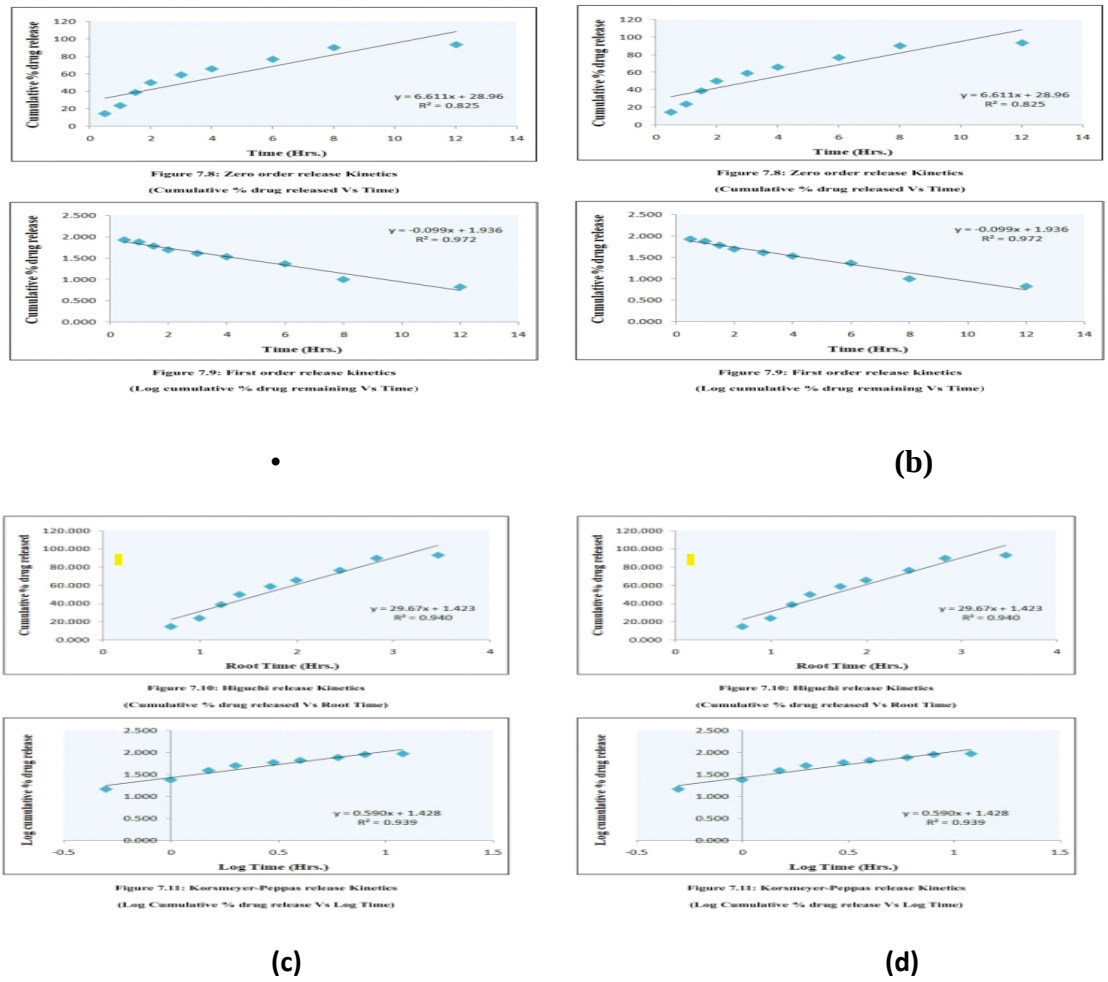

Figure 5: (a) Zero order release Kinetics (b) First order release kinetics (c) Higuchi release Kinetics (d) Korsmeyer-Peppas release Kinetics

Table 8: *In-vitro* drug release kinetics data for optimized formulation F3

Batch	Zero Order	First Order	Higuchi	Korsmeyer-Peppas
	r^2	r^2	r^2	r^2
F3	**0.825**	**0.972**	**0.940**	**0.939**

This indicates that the primary mechanism governing drug release is likely diffusion through the invasome structure. The *in-vitro* drug release kinetics data for formulation F3 supports the notion that the release mechanism follows Higuchi kinetics. This finding provides valuable

information for further development and optimization of the Mupirocin-loaded invasomal formulation, with potential benefits for controlled and sustained drug release.

In vitro antimicrobial activity of optimized formulation F3

Table 9: Antimicrobial activity of Mupirocin-loaded invasomal formulation (F3) against selected microbes

S.NO	Microbes	Zone of Inhibition		
1.		25 µg/ml	50 µg/ml	100 µg/ml
	Escherichia coli	8+0.5	10±0.5	12+0.47
2.	Staphylococcus aureus	7+0.47	12+0.94	15+0.86

For *Escherichia coli*, the zone of inhibition was 8+0.5 mm, 10+0.5 mm, and 12+0.47 mm at concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml respectively. Similarly, for Staphylococcus aureus, the zone of inhibition was 7±0.47 mm, 12±0.94 mm, and 15+0.86 mm at the corresponding concentrations. These findings indicate that the Mupirocin-loaded invasomal formulation (F3) possesses significant antimicrobial activity against both Gram-negative (*Escherichia coli*) and Gram- positive (*Staphylococcus aureus*) bacteria. The increase in the zone of inhibition with increasing concentrations suggests a dose-dependent response, with higher concentrations exhibiting greater inhibitory effects against the tested microbes.

SUMMARY AND CONCLUSION

In order to gain further insights into the surface morphology and structural characteristics of the optimized Mupirocin-loaded invasome formulation (F3), scanning electron microscopy (SEM) was conducted. SEM is a valuable technique for visualizing the surface topography of particles and vesicles. Unfortunately, the provided data lacks SEM results. Nevertheless, SEM images could reveal the shape, size, and surface characteristics of the invasomes, providing a comprehensive understanding of their structural attributes.

The *in-vitro* drug release data for the optimized Mupirocin-loaded invasome formulation (F3) elucidates the release kinetics and behavior of the entrapped drug. The evaluation involves various kinetic models to understand the mechanism governing drug release from the invasomal vesicles. The vesicle size analysis indicates that the formulations (F1-F6) exhibit nanoscale vesicles, with F3 having a mean vesicle size of 359.58 nm. This suggests the successful formation of invasomes in the nanosize range.

The entrapment efficiency values (82.53%-85.29%) suggest that a significant proportion of Mupirocin is effectively entrapped within the invasomes. Higher entrapment efficiency is desirable for maximizing drug payload. The drug content results (92.06%-98.28%) indicate

that the invasomes are loaded with a high percentage of Mupirocin. This is essential for ensuring that each vesicle carries a substantial amount of the therapeutic agent.

The optimized formulation (F3) exhibits a mean particle size of 359.58 nm, entrapment efficiency of 85.29%, and drug content of 98.28%. These values collectively suggest that F3 is a promising formulation with desirable characteristics for drug delivery. The in-vitro drug release data, analyzed using various kinetic models, suggests that the release mechanism of Mupirocin from F3 follows Higuchi kinetics. This implies controlled release through diffusion as the dominant mechanism.

The formulation and characterization results collectively indicate that the Mupirocin-loaded invasome formulation, particularly F3, possesses favorable attributes such as nanoscale size, high entrapment efficiency, and controlled drug release behavior. These findings provide a solid foundation for further preclinical and, eventually, clinical development of the optimized invasomal formulation for effective and controlled Mupirocin delivery. The absence of SEM results underscores the importance of complementary techniques for a comprehensive understanding of the formulation's characteristics.

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