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DEVELOPMENT AND EVALUATION OF MUPIROCIN INVASOMES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Mupirocin invasomes, incorporating soy lecithin and various terpenes (eugenol, citral, and D-limonene), were prepared using a mechanical dispersion method. Terpene concentrations influenced particle size, with citral containing invasomes (F5) exhibiting the smallest size (92nm to 1250nm).The polydispersibility index indicated uniform particle size distribution (0.136-0.624), confirming homogeneous preparation. Increasing terpene concentration correlated with larger particle sizes. Zeta potential values ranged from -62.2mV to +8.4mV, favoring drug penetration and formulation stability. Formulation F5 demonstrated the highest entrapment efficiency (83%) and drug content (90%), suggesting effective Mupirocin encapsulation. *In-vitro* release kinetics showed percentages between 62% and 86% at 30 hours, following first-order kinetics. Anti-bacterial activity against *Staphylococcus aureus* varied across formulations (10mm to 20mm zones of inhibition), with F2 exhibiting the highest and F10 the lowest inhibition zones. Stability studies indicated consistent performance of F5 over three months. Overall, Mupirocin invasomes, especially F5, present a promising strategy for the topical treatment of bacterial skin infections, offering sustained release, enhanced penetration, and antibacterial efficacy.

Keywords: Mupirocin, invasomes, terpenes, topical delivery, antibacterial.

INTRODUCTION

The transdermal route is essential for localized or systemic effects. The stratum corneum, or top layer of skin, acts as a vital barrier to prevent skin penetration for many medications. Many approaches have been developed to overcome

this challenge, such as the use of methods like iontophoresis, electroporation, and ultrasound that change the continuity of the stratum corneum (SC) and the use of vehicles and nanocarriers to improve drug penetration. Many kinds of nanocarriers have recently been created to improve the cutaneous and transdermal distribution of drugs. Since the physiochemical properties, such as deformability, size, and charge can be adjusted by changing the lipid content and production methods, vesicular system appear to be suitable carriers[(1)].Topical antibacterials are frequently used to treat or prevent infections after minor burns, abrasions, scrapes, and surgical wounds. They are also commonly used to treat superficial pyodermas like impetigo. Topical antibiotics are commonly employed in the management of folliculitides and furuncles; however, their efficacy may be restricted to prevent the infection from propagating from the original lesion to the neighboring follicles[(2)].When treating burns, prophylactic topical therapy is frequently utilized to avoid the potentially fatal secondary infection. Furthermore, preoperative surgical cleaning and hand hygiene are always performed. Macrolides are a class of antibiotics that are primarily used to treat bacterial infections. They are characterized by a macrocyclic lactone ring and are effective against a wide range of bacteria. Mupirocin is one of several antibiotics derived from the fermentation of *P. fluorescens* and is also known as pseudomonic acid Although the derived from cultures activity of *P. fluorescens* was first reported over a century and a half ago, this agent could not be used as an antimicrobial until had executed a more complete isolation and purification of pseudomonic acid. Mupirocin has a unique chemical structure composed of a short fatty acid chain linked to monic acid; it inhibits bacterial RNA and protein synthesis by binding to isoleucyl-tRNA synthetase to prevent incorporation of isoleucine into bacterial proteins. infection rate due to resistance development or reinfection from other body areas[(3)].

Invasomes are novel elastic phospholipid vesicles composed of phosphatidylcholine, ethanol and one or mixture of terpenes. Many researchers have confirmed the capability of terpenes in enhancing percutaneous penetration. Their penetration enhancing activity is through the disruption of the stratum corneum lipids, interaction with intracellular proteins, and improvement of partitioning of the drug into the stratum corneum. Ethanol improves the vesicular ability to penetrate the stratum corneum. In addition, ethanol provides net negative surface charge and prevents vesicle aggregation due to electrostatic repulsion· A synergistic effect between terpenes and ethanol on the percutaneous absorption has been significantly observed. Terpenes, the naturally occurring volatile oils are included in the list of generally recognised as safe substances with low irritancy at lower concentrations (1-5%), with reversible effect on the

lipids of stratum corneum are considered as clinically acceptable penetration enhancers. Invasomes are characterised for size, surface morphology, zeta potential, stability[4].

Penetration Mechanism of Invasomes :

Terpenes and ethanol in the invasomes cause deformability of the vesicles, disrupt the stratum corneum bilayer skeleton, and act as penetration enhancers, improving the permeability of the invasomes[5] during penetration of the invasome, one part of the vesicle disintegrates and releases its components, such as terpenes, phospholipid segments, and single phospholipid molecules, which enhance the penetration and fluidize the stratum corneum lipids. Smaller invasome vesicles, which do not disintegrate, penetrate through the stratum corneum intact,upon penetration, intact invasomes may reach the inner parts of the stratum corneum by the follicular transport pathway or via the narrow hydrophilic channels existing in the intercellular region of the stratum corneum revealed that smaller intact invasomes can penetrate the deeper part of the stratum corneum through the channel-like areas[6]. This was reduced by the flexible vesicles of various sizes that were discovered at the channel-like areas in the deeper layer of the stratum corneum and skin surface vesicles. In general, a number of invasomes disintegrate when penetrating the stratum corneum whereas smaller vesicles and flexible invasomes penetrate the deeper layers intact[7].

Effect of Composition on the Physicochemical Characteristics of Invasomes

Effect of Ethanol

The addition of ethanol in the formulation of lipid nanovesicles is an effective strategy to increase the fluidity of the lipid bilayer of the skin. The interaction of ethanol with the lipid elements in the polar group area of the SC leads to alterations in the structure of the keratinized or lipophilic domains, decreased transition temperature of lipids, and consequently fluidization and disruption of the tightly packed SC lipids Ethanol-based nanocarriers can fluidize and disturb the SC lipids[8].The presence of ethanol increases the flexibility of the intercellular lipid matrix due to the rotating freedom of the lipid acyl chains. Thus, ethanol increases the fluidity of lipids in the vesicle structure, resulting in a structure that has softer and less rigid properties than conventional liposomes. In addition to enhanced penetration ability, ethanol creates a net negative surface charge and limited vesicle aggregation due to electrostatic repulsion, leading to increased stability of invasomes under storage conditions[9].

Effect of Terpenes

Effect of Terpene on Penetration

X-ray diffraction and differential scanning calorimetry (DSC) results showed that terpenes lead to increased drug penetration by disrupting the tight bilayers and lipid packing in the SC. Furthermore, breaking the hydrogen bonds and extracting SC lipids enhancing the partition into the SC by improving lipid fluidity and increasing diffusion via the intercellular lipids are another mechanisms that have been reported to increase drug permeability by terpenes[10].

Effect of terpenes on the size of the invasomes

Particle size analysis revealed a direct relationship between the size of the invasomes and the number of terpenes; as terpene levels rise, so does the size of the invasomes. Skin's lipid bilayer more fluid .Ethanol interaction with the lipid elements in the polar groups area of the stratum corneum changes in the keratinized or lipophilic[11].

MATERIALS AND METHODS

These are the materials used in these formulations (Drug: Mupirocin), (Source: Micro Labs PVT LTD), (Excipients: Soy lecithin), (Source: Kanon laboratories), (Terpene: Citral, Eugenol, D-limonene), (Source: Sri Mahalakshmi Scientific Company).

Mechanical dispersion method

Drug and terpene are dissolved in ethanolic phospholipid solution. The mixture was vortexed for 5 min and then sonicated for 5 min to obtain a clear solution. Phosphate buffer saline (PBS) (pH: 6.4) was added to the solution by a syringe under constant vortexing. The vortexing is continued for an additional 5 min to obtain final invasomal preparation[12]

MECHANICAL DISPERSION METHOD:

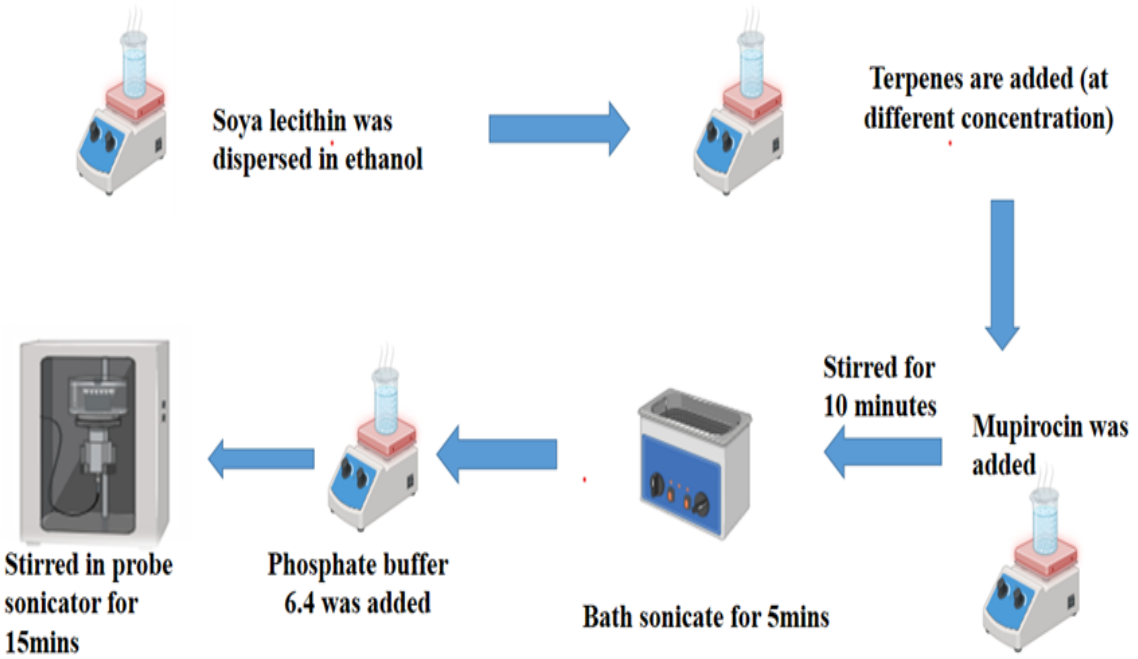

Figure 1 : Preparation of invasomes formulation

Table 1: Composition of invasomes formulation

INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Mupirocin (mg/ml)	100	100	100	100	100	100	100	100	100	100	100	100
Soy lecithin (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Eugenol (%)	0.5	1	2	4	-	-	-	-	-	-	-	-
Citral (%)	-	-	-	-	0.5	1	2	4	-	-	-	-
D-limonene (%)	-	-	-	-	-	-	-	-	0.5	1	2	4
Ethanol(ml)	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Phosphate buffer (ml)	100	100	100	100	100	100	100	100	100	100	100	100

Evaluation of invasomes

Particle size

The size of the formulation was analyzed using a Horiba Zetasizer .The formulation was placed in the sample holder and the particles size was measured[13].

Zeta potential analysis

Zeta potential analysis was used to measure the stability of invasome by studying its colloidal property. Aggregation is attributed to the shielding of the vesicle surface charge by ions in solution and thereby reducing the electrostatic repulsion. Vesicle surface charge can be estimatedby measurement of particle electrophoretic mobility and is expressed as the Zeta potential. The study was conducted using Horiba Zeta Analyzer[13,4].

Drug content

1 ml of Invasome preparation was taken in a 100ml volumetric flask. 2ml of methanol was added and volume was made up with phosphate buffer pH 6.4. Samples were filtered through Whatman filter paper number 40 and diluted with phosphate buffer pH 6.4.Drug content was determined spectrophotometrically at 220 nm[14].

Drug entrapment

5ml of the sample was centrifuged at 5000rpm at 40˚C for 60 minutes. The supernatant liquid was separated without disturbing the sediment layer using a micropipette. The sample withdrawn was diluted using phosphate pH 6.4 and analyzed by UV Spectrophotometer at 220nm[15].

Entrapment Efficiency % = total drug – free drug / total drug × 100

In vitro drug release

In vitro drug release was determined using Franz's diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^2 respectively. The donor cell containing the invasomal formulation was placed over the receptor cell in which phosphate buffer saline (pH 6.4) was filled. The experiment was conducted for 24 h at a temperature of 37 ± 1 ˚C with constant magnetic stirring at 600 rpm. Samples were withdrawn at 1,2,3,4,5,6,8,12,24h the absorbance was recorded at 220nm using UV-visible specterometer. The percentage drug release was calculated from the absorbance measured for the sample[15].

Drug release kinetic studies

The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows.

1. Log cumulative percent drug remaining versus time (first order kinetic model).
2. Cumulative percent drug release versus square root of time (Higuchi's model)
3. Cumulative percent drug release versus time (zero order kinetic model)
4. Log cumulative Percent Drug release versus log time (Korsemeyer-peppas model)[16].

Anti-bacterial activity

The antibacterial activity of nanocarriers was determined in vitro by measuring the zone of inhibition against suitable microorganisms such as *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus,* etc[3]. The microorganisms of interest were grown on a compatible sterilized nutrient agar medium. The test composites were transferred onto square plastic grids or disks on the solidified agar medium and incubated for 24 hours at 37°C. After the incubation period, the zones of inhibition were observed in four directions for each tested sample[17].

Stability studies

The stability of invasomal dispersion, gel, and suspension was estimated by measuring the change in invasome vesicle size and polydispersity index (PDI) using Dynamic Light Scattering (DLS)[18]. As part of the stability study, the shape of the invasomal formulation was ensured by employing scanning electron microscopy (SEM), transmission

electron microscopy (TEM), or other appropriate techniques[19]. The particle-containing charge changes were worked out by evaluating the zeta potential of invasomal vesicles. The invasomes were placed in the stability chamber at 4°C and 25°C temperatures for 120 days. After 120 days, the zeta potential and entrapment efficiency of vesicles stored at 4°C were observed[20].

RESULTS AND DISCUSSION

Determination of solubility

Soluble in ethanol, acetone and slightly soluble in water

Determination of melting point

The melting point of Mupirocin was determined by the capillary tube method, which was found to be 77°C. This value matched with the literature reference standard drug value of Mupirocin.

Table 2 :Determination of melting point

Compound name	Melting point	
Mupirocin	Observed	Reported
	77°C	77°C-780°C

Absorbance

Standard Calibration curve for Mupirocin

Mupirocin was dissolved in phosphate buffer pH 6.4 and suitable dilutions were made to prepare 2, 4, 6, 8 and 10 µg/ml. The absorbance of each solution was measured at 220nm against phosphate buffer pH 6.4 as a blank. A graph of the concentration of the drug versus absorbance was plotted. The absorbance obtained was found to obey Beer's Lambert's law with a regression coefficient R^2 value of 0.997 in phosphate buffer pH 6.4.

Table 3 : Calibration graph curve of Mupirocin

S.No	Concentration (µg/ml)	Absorbance at 220 nm
1	0.2	0.135
2	0.4	0.228
3	0.6	0.339
4	0.8	0.416
5	1	0.569

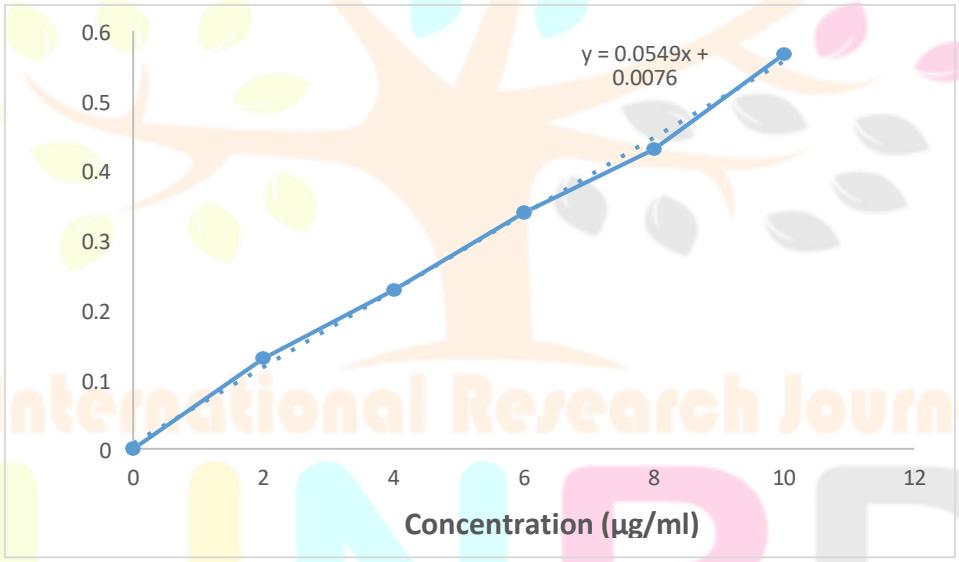

Figure 2 : Calibration curve of Mupirocin

Compatibility studies by FTIR spectroscopy

The IR spectra of the drug and its other excipients were shown. The FT-IR study showed no major change in the position of the peak obtained in the drug and the mixture of the drug with excipients, thus indicating no interaction between the drug and excipients.

Table 4 : FT-IR peaks of drug, excipients, physical mixture of drug with excipients andFormulations

Functional Group	Wavelength (Cm-1)							
	Mupirocin	Mupirocin and soylecithin	eugenol	Mupirocin and Eugenol	citral	Mupirocin and citral	D-limonene	Mupirocin & D-Limonene
C-X str	652	-	643	648	-	-	-	-
C=C ben	886,1653	718	797,1610	-	981,1630	666,836,985	889,1646	1643
C-H ben	783	-	909	748,794	748	2854,2966	2920	879,1446
C-O str	1715	1218	1738	1267,1149	1116,1670	1120,1209, 1672,1735	1218	1153,1251
S=O str	1070,1400	-	-	1030	-	1370	1367	-
O-H str	2848,2913, 3723	-	2841,2969	2930,3522	2851,2911	3453	1435,3456	2913,2963
N-O str	-	-	-	1508	-	-	-	-
C-N str	-	1080	-	-	-	-	-	-
O-H ben	-	-	-	-	-	-	-	1376

Characterization Mupirocin invasome

Particle size

The particle size of all the formulations was analyzed by using the Horiba Nanoparticle analyzer. The formulations were placed in the sample holder and particle size was measured. The results are given in the (Table 1) .The mean particle size of the formulation F1 to F12 is found to be in the size range of 92nm – 1250nm.The polydispersibility index of the formulation F1 to F12 is found to be in the range of 0.136 – 0.624 that concluded the homogenous preparation of vesicles. A lower polydispersibility index indicates a more homogenous sample with particles that are more uniform in size.

Table 5 : Particle size of invasomes formulations

S.No	Formulation Code	Particle Size
1	F1	875nm
2	F2	198nm
3	F3	487nm
4	F4	838nm
5	F5	92nm
6	F6	98nm
7	F7	320nm
8	F8	1250nm
9	F9	173nm
10	F10	288nm
11	F11	296nm
12	F12	849nm

Zeta Potential

The zeta potential of the invasome was measured using a Horiba Nanoparticle analyzer SZ -100. The zeta analysis, software produces a frequency spectrum from which the electrophoretic mobility hence the zeta potential, is calculated. The negative zeta potential values usually indicate repulsive forces between particles, leading to dispersion stability, while positive values suggest attractive forces, which may lead to aggregation.Zeta potential of invasomes F1

to F12 ranges from -62.2mV to +8.4mV.The negative potential of the invasomes can enhance the penetration rate of the drug as well as the stability of the formulation.

Table 6 : Zeta potential of invasomes formulation

S.No	Formulation Code	Zeta Potential
1	F1	-3.2mV
2	F2	-12.1mV
3	F3	-5.9mV
4	F4	8.4mV
5	F5	-3.9mV
6	F6	-2.1mV
7	F7	-22.1mV
8	F8	-62.2mV
9	F9	-8.0mV
10	F10	-4.1mV
11	F11	-25.5 mV
12	F12	8.4mV

Drug Entrapment

The % entrapment efficiency of the formulations was calculated from the absorbance obtained from the supernatant after centrifugation. All the formulations showed a percentage entrapment efficiency in the range of 43 - 83%. It was observed that the F5 Formulation showed the maximum entrapment when compared to other formulations.

Table 7 : Percentage Entrapment efficiency of prepared invasomes

Formulation code	% Entrapment efficiency
F1	43%
F2	78%
F3	65%
F4	57%
F5	83%
F6	80%

F7	66%
F8	45%
F9	78%
F10	72%
F11	70%
F12	53%

Drug content

The % drug content of invasomes preparation ranges from 50% -90%. F5 formulation showed the maximum drug content of 90% Mupirocin in the invasomes.

Table 8 : Percentage Drug Content of prepared invasomes

Formulation code	% Drug content
F1	52%
F2	81%
F3	70%
F4	63%
F5	90%
F6	89%
F7	72%
F8	50%
F9	84%
F10	81%
F11	77%
F12	62%

***Invitro* drug release**

In-vitro drug release of Mupirocin invasomes was found to be 86%, 82%,78%, 75%, 74%, 70%, 69%, 68.39%, 64.74%, 66.26%, 62% for the formulation F1 to F12 respectively at the end of 30 hrs.

Table 9: % Cumulative drug release of invasomes formulations (F1 to F12)

Time	% Cumulative drug release											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0hr	0	0	0	0	0	0	0	0	0	0	0	0
1hr	17.36	25.84	20.36	18.14	22.43	21.32	21.01	16.17	25.32	24.93	20.36	18.83
2hr	23.61	29.69	27.61	21.15	36.85	34.15	28.62	21.41	30.43	30.811	27.61	22.02
3hr	27.84	34.45	32.54	26.32	49.52	44.61	30.96	27.12	36.64	39.43	30.35	28.55
4hr	33.26	46.58	40.23	30.11	61.43	59.32	42.78	38.56	40.13	45.23	45.26	33.14
5hr	41.13	53.01	48.72	43.43	70.68	69.72	50.25	44.75	47.87	50.65	58.01	40.75
6hr	44.62	60.54	55.65	50.09	79.63	76.42	59.14	51.43	56.13	59.67	63.45	45.98
24hr	55.43	72.21	62	57.87	83.72	80	65.48	60.54	68.43	65.78	68.04	56.87
30hr	68.39	78	69	66.26	86	82	72	64.74	75	70	74	62

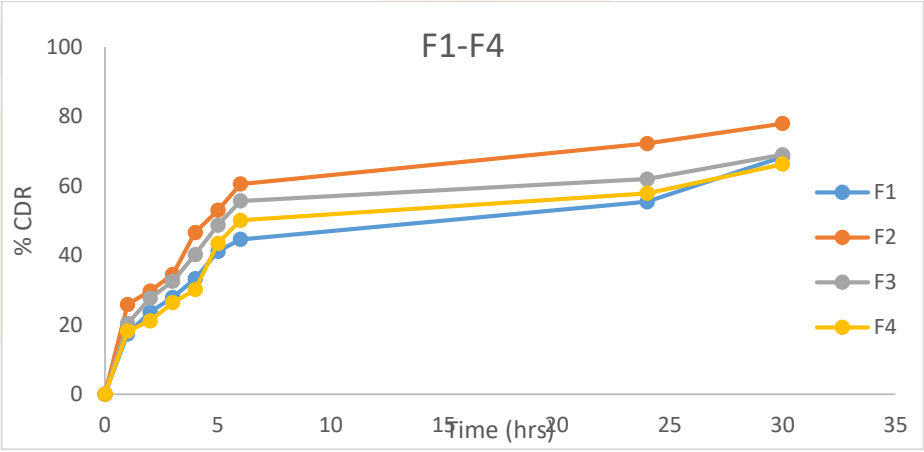

Figure 3: FORMULATION F1TO F4

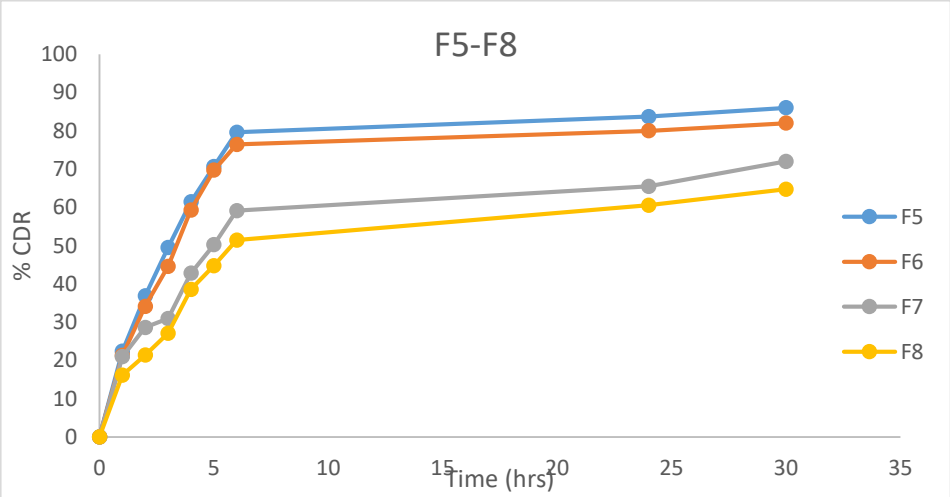

Figure 4 : FORMULATION F5TO F8

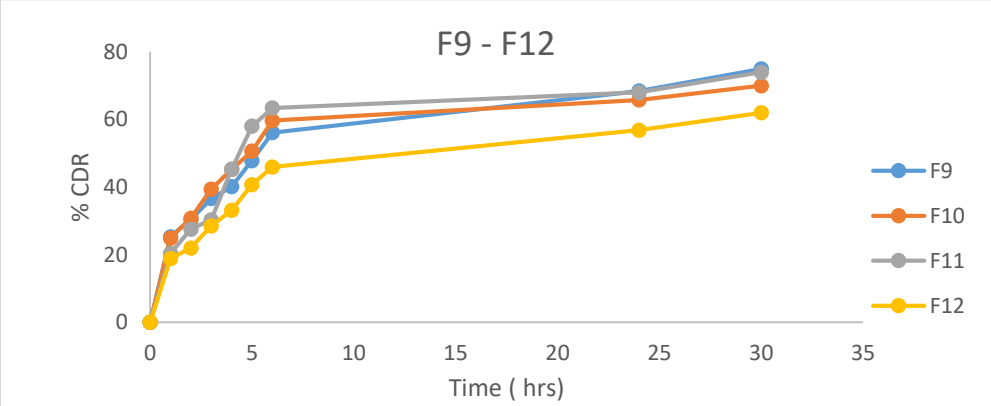

Figure 5: FORMULATION F9 TO F12

Drug release kinetic study

In order to investigate the drug release pattern and mechanism of Mupirocin invasomes formulations in the percentage cumulative drug release data was analysed with the mathematical models zero order, first order, Higuchi and Korsmeyer- Peppas plot. The regression coefficient (r) values for F5 the formulations were shown in Table. The high correlation coefficient was obtained for the First order for F5 formulation. The results obtained from the release kinetics study F5 formulation follows First order kinetics. First order equation shows r^2 0.872, indicating the drug release is directly proportional to the remaining drug concentration in the formulation. A higher coefficient of determination compared to Zero order indicates that the first-order kinetics model might better fit the data, implying that drug release is likely dependent on the concentration of Mupirocin within the invasomes.

Zero order

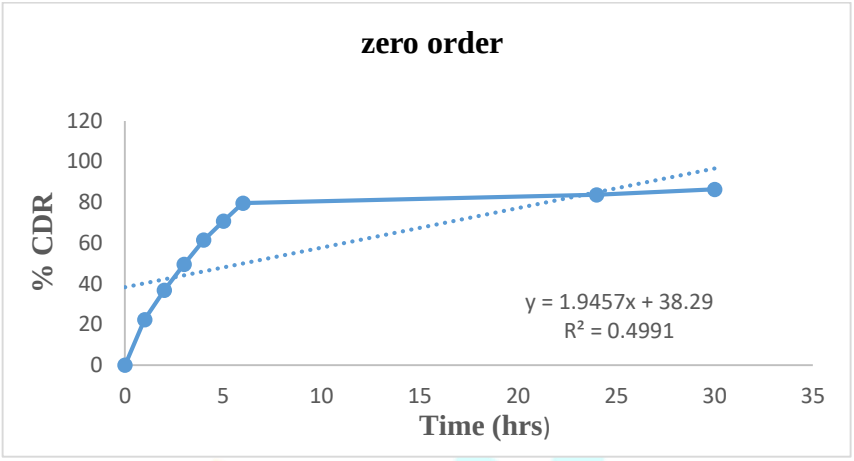

Figure 6: Zero order of F5 invasomes formulations – Kinetic model

First order kinetics

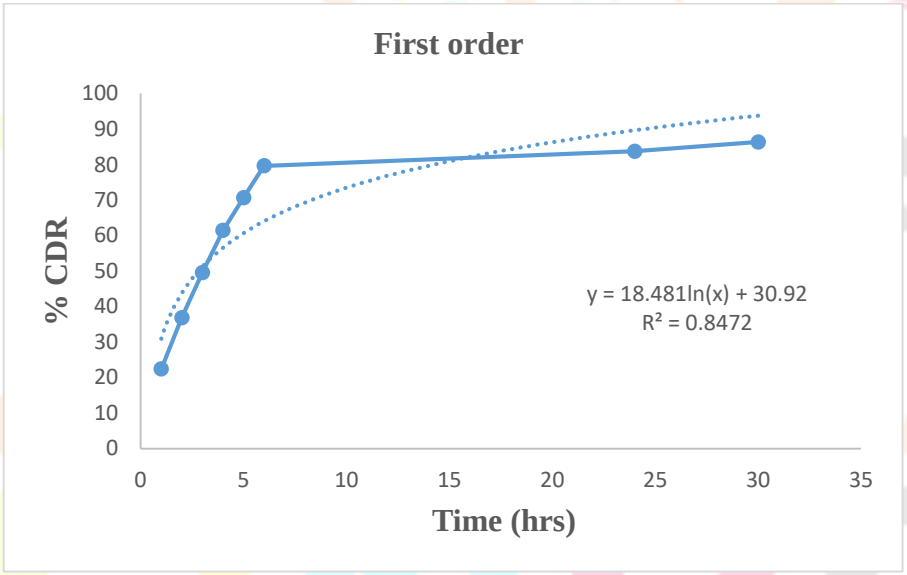

Figure 7: First order of F5 invasomes formulations – Kinetic model

Higuchi plot

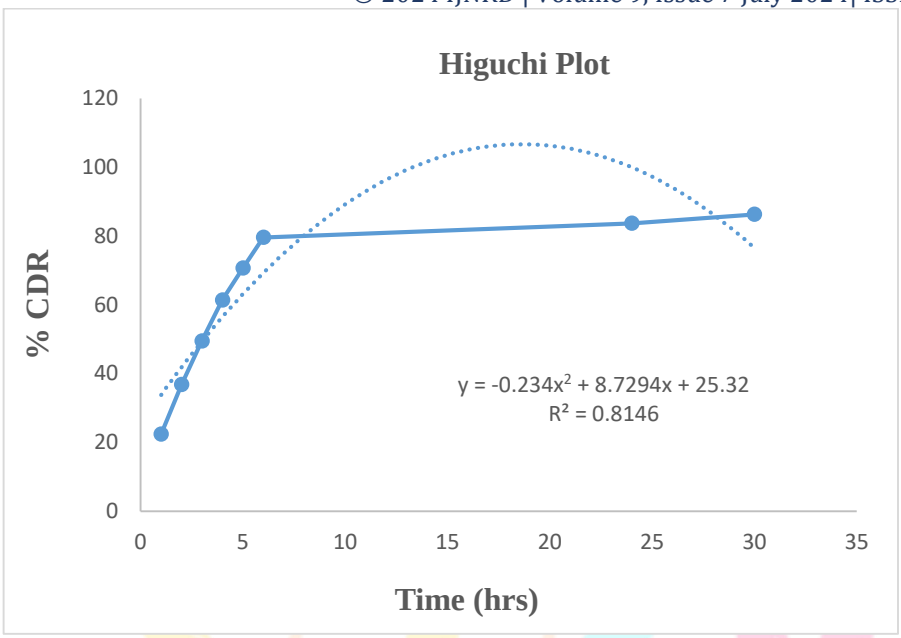

Figure 8: Higuchi of F5 invasomes formulations – Kinetic model

Korsmeyer-Peppas

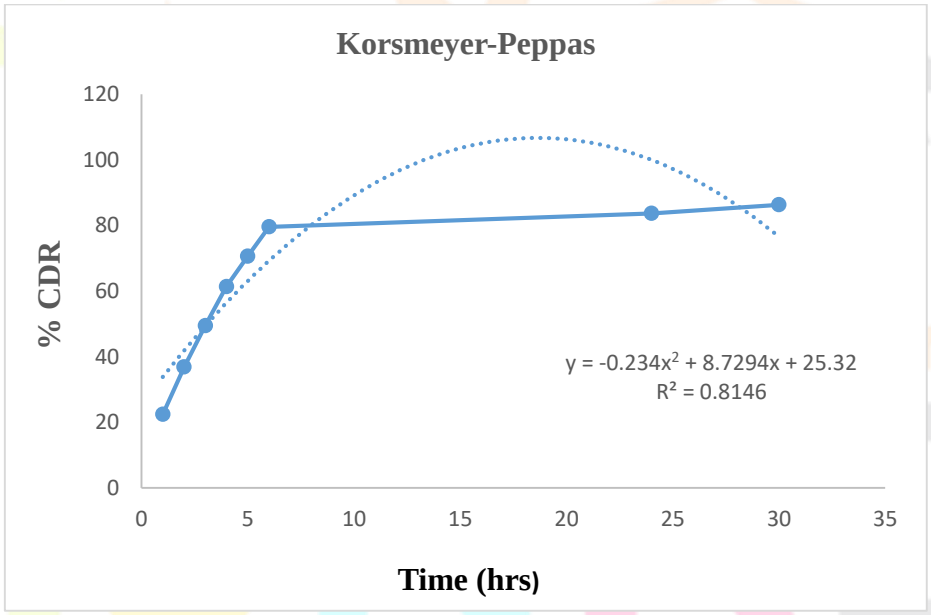

Figure 9: Korsmeyer Peppas of F5 invasomes formulations – Kinetic model

Table 10: *In-vitro* drug release kinetics of F5

Release Kinetics	Zero order r^2	First order r^2	Higuchi r^2	Korsmeyer-Peppas r^2
F5	0.4991	0.8472	0.8146	0.8146

Anti bacterial activity

The invasomes formulation shows antibacterial activity with a zone of inhibition of 16,20,19,16,15,17,13,10,18,12,19,14 mm for F1 to F12 respectively against *Staphylococcus aureus* by the Disc diffusion method .

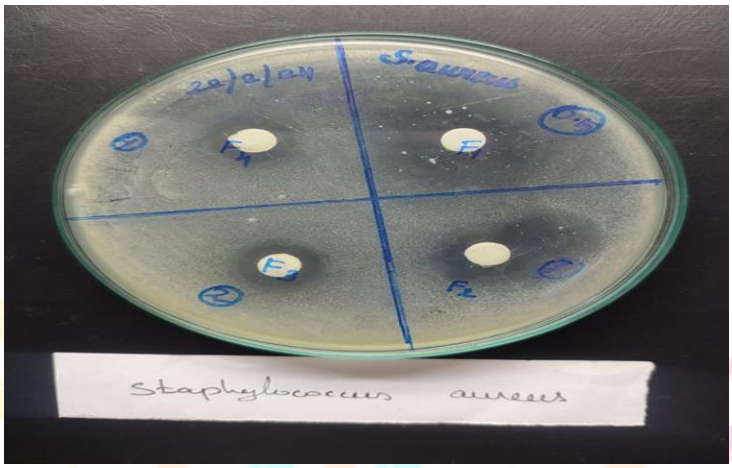

Figure 10: Mupirocin invasomes shows anti bacterial activity against *Staphylococcus aureus* (F1 to F4)

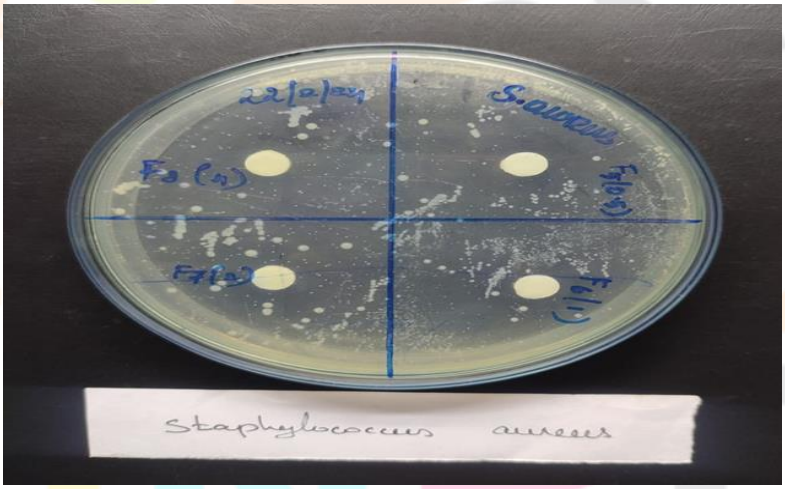

Figure 11: Mupirocin invasomes shows antibacterial activity against *Staphylococcus aureus* (F5 to F8)

Figure 12 : Mupirocin invasomes shows antibacterial activity against *Staphylococcus aureus* (F9 to F12)

Stability study

The stability study for invasome F5 was p e r f o r m e d at two temperature condition, zone. It 4-8˚C and 27-30˚C and room temperature Relative Humidity (42%) for 3 months. The physical appearance, drug content and pH of F5 were evaluated after 1 day, 1st month 2th month 3th month of storage. There was no significant change in the physical appearance of the F5 over 3 month at any temperature condition.

Table 11: Physical appearence of after 3 month (F5)

S.NO	Day of sample withdraw	Physical characteristic
1	Day 1	No change
2	30th day	No change
3	60th day	No change
4	90th day	No change

Table 12 : Drug content of after 3 month (F5)

S.NO	Day of sample withdrawing	Drug content%
		AT 4±8˚C
1	Day 1	99.67
2	30th day	99.54
3	60th day	99.43
4	90th day	99.21

CONCLUSION

In this study, Mupirocin invasomes was prepared by Mechanical dispersion method using Soy lecithin, eugenol, citral and D-limonene. It indicates that an increase in the terpene concentration increased the particle size of the invasomes. Zeta potential values ranged from F1 to F12 -62.2mV to +8.4mV, with negative potentials enhancing drug penetration and formulation stability. The entrapment efficiency ranged from 43% to 83%, with formulation F5 exhibiting the highest entrapment efficiency at 83%. Similarly, the drug content ranges between 50% and 90% for F1 to F 12 with formulation F5 demonstrating the maximum drug content at 90%. These results suggest that formulation F5 is highly efficient in encapsulating Mupirocin within the invasome structure, with a significant proportion of the drug being retained. This indicates the potential of formulation F5 to deliver a substantial amount of Mupirocin to the target site, enhancing its therapeutic efficacy in topical antibacterial applications. *In-vitro* drug release studies showed release percentages between 62% and 86% at 30 hours, following first-order kinetics. From the above results, it can be calculated that the formulation F5 containing citral 0.5% was optimized. The antibacterial activity of the formulated Mupirocin invasomes was assessed through zone of inhibition assays against *Staphylococcus aureus.* Across formulations F1 to F12, the observed zones of inhibition ranged from 10mm to 20mm.These results indicate that all formulations exhibited antibacterial activity against *Staphylococcus aureus*, with varying degrees of effectiveness. Formulation F2 demonstrated the highest zone of inhibition at 20mm, while formulation F10 exhibited the lowest at 10mm.Stability studies revealed that the physical appearance, drug content, and pH of F5 remained consistent over three months under different temperature conditions. Overall, Mupirocin invasomes, especially formulation F5, present a promising approach for the topical treatment of bacterial skin infections, including impetigo and other superficial

pyodermas. The study concludes that Mupirocin invasomes hold potential as effective carriers for delivering Mupirocin, offering advantages such as sustained release, enhanced drug penetration, and antibacterial efficacy.

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REFERENCES

1. Soraya Babaie, Azizeh Rahmani Del Bakhshayesh, Ji Won Ha, Hamed Hamishehkar, KiHyun Kim. Invasome: A Novel Nanocarrier for Transdermal Drug Delivery nanomaterials 2020.10(2):341
2. Hirschmann, MD. Topical Antibiotics in Dermatology: Arch Dermatol 1988; 124:1691-1700.
3. Van Rijen , Bonten , Wenzel , Kluytmans . Mupirocin ointment for preventing Staphylococcus aureus infections in nasal carriers. Cochrane Database Syst Rev. 2008 : 4 : 214-6
4. Uzma Afreen, Krishna Shailaja, Overall Review on Invasomes, Research Journal of Nanoscience and Engineering,2019 ,(3)4 ,5-9.
5. Jain Tripathi, Tripathi. Invasomes: Potential vesicular systems for transdermal delivery of drug molecules. Journal of Drug Delivery Science and Technology. 2021;61
6. Parikh, Ghosh, Feasibility of transdermal delivery of fluoxetine. AAPS Pharm SciTech 2005, 6(2), 144-149
7. Dragicevic-Curic, Gräfe,; Albrecht,Fahr, A Topical application of temoporfin-loaded invasomes for photodynamic therapy of subcutaneously implanted tumours in mice: A pilot study. J. Photochem. Photobiol. B Biol. 2008, 91, 41–50.
8. Nina Dragicevic, Daya Dass Verma & Alfred Fahr. Invasomes:Vesicles for Enhanced Skin Delivery of Drugs: Springerlink 2016 :77 – 92.
9. Honeywell-Nguyen, de Graaff, ; Groenink, Bouwstra,. The in vivo and in vitro interactions of elastic and rigid vesicles with human skin. Biochim. Et Biophys. Acta (Bba)-Gen. Subj. 2002, 1573, 130–140
10. Lakshmi , Kalpana Prasanthi Invasomes-novel Vesicular Carriers for Enhanced Skin Permeation. Systematic Reviews in Pharmacy. 2013;4(1):26-30.
11. Karimi, Ghanbarzadeh, Hamishehkar,; Keivani, Pezeshki, Gholian, Phytosome and liposome: The beneficial encapsulation systems in drug delivery and food application. Appl. Food Biotechnol. 2015, 2, 17–27.
12. Sopan Nangare Smart invasome synthesis, characterizations, pharmaceutical applications, and pharmacokinetic perspective: a review Future Journal of Pharmaceutical Sciences (2020); 6:123 ,1-21.
13. Amnuaikit , Ikeuchi, Ogawara , Higaki , Kimura Skin permeation of propranolol from polymeric flim containing terpene enhancers for transdermal use. Int J Pharm 2005,289.

14. A Krishna sailaja, Applications of Invasomal Drug Delivery System, Archives of pharmacy & pharmacology research 2021,3(1);1-8.

15. Thanaporn Amnuaikit .Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes 2018 ;13(5):472-484

16. Gauhar R. Qadri, Invasomes of isradipine for enhanced transdermal delivery against hypertension: formulation characterization, and *in vivo* pharmacodynamics study.An International Journal Artificial Cells, Nanomedicine, and Biotechnology 2017,45(1);139-145.

17. Iman Akbarzadeh. Optimaization,physiochemical characterization and antimicrobial activity of a novel simvastatin nano-niosomal gel against *E.coli* and *S.aureus*.Chemistry and physics of lipids.2021.

18. Dr.John CIMS India drug reference.2010,2031-2034 (http://medical.fundazone.com)

19. Ohradanova-Repic, Nogueira, Hartl, Gomes, Preto, Steinhuber, Mühlgrabner,Repic, Kuttke, Zwirzitz, Fab antibody fragment-functionalized liposomes for specific targeting of antigen-positive cells. Nanomed. Nanotechnol. Biol. Med. 2018, 14, 123–130.

20. Dragicevic-Curic,;Scheglmann,;Albrecht, Fahr, Temoporfin-loaded invasomes: Development, characterization and *in vitro* skin penetration studies. J. Control. Release 2008,127, 59–69.