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Formulation and Evaluation of Ciprofloxacin Loaded Invasomes for Bacterial Infection

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ABSTRACT

Topical drug delivery enables targeted treatment through different methods, including ophthalmic, rectal, vaginal and skin applications. For skin conditions caused by bacteria, medications like Ciprofloxacin are used. This research aimed to create and assess Ciprofloxacin -loaded Invasomes to improve the drug's ability to penetrate the skin. The researchers made Invasomes using a mechanical dispersion method with soya lecithin, terpenes and ethanol. They evaluated formulations (F1-F5) based on appearance, pH level, particle size and zeta potential, drug content, entrapment efficiency, *in-vitro* drug diffusion and antibacterial effectiveness. The particle size measured 0.052µm, while the drug content ranged from 47.24% to 91.67%. The entrapment efficiency varied between 54.05% and 80.00%, with formulation F3 showing the highest drug diffusion rate of 0.039Conc within one hour. No interaction occurred between the drug and the formulation components. F3 demonstrated the strongest antibacterial activity among the Ciprofloxacin-loaded invasomes. Stability studies showed that F3 remained stable over time. This work focuses on creating and evaluating Ciprofloxacin-loaded Invasomes as a new way to enhance drug penetration for treating bacterial infections by encapsulating Ciprofloxacin within invasomes to improve its ability to pass through the skin barrier.

Keywords: Invasomes, Soya lecithin, Terpenes, Ethanol, Mechanical Dispersion.

INTRODUCTION

Invasomes are a kind of artificial vesicle nano carrier that help move substances through the skin, which is the body's outermost barrier. These tiny particles are a special type of liposome that are flexible and made up of phospholipids, ethanol, and either one terpene or a mix of them. Ethanol makes the lipids in the vesicles more fluid, giving them a softer and more flexible shape compared to regular liposomes, which helps them penetrate the skin better.[1] The use of penetrative boosters like terpenes and ethanol gives invasomes a strong ability to penetrate. By adding ethanol and terpenes to invasomes, the fluidity of the lipids within the vesicle structure increases, making them more adaptable and less stiff than standard liposomes. Ethanol works with the lipids in the outer layer of skin (the stratum corneum), causing changes in both keratinized and oily areas, lowering lipid transition temperatures and breaking apart tightly packed lipids. Studies show that terpenes enhance penetration by disrupting the compact structure of these skin lipids.[2]

COMPARISION OF INVASOME IN OTHER VESICLES

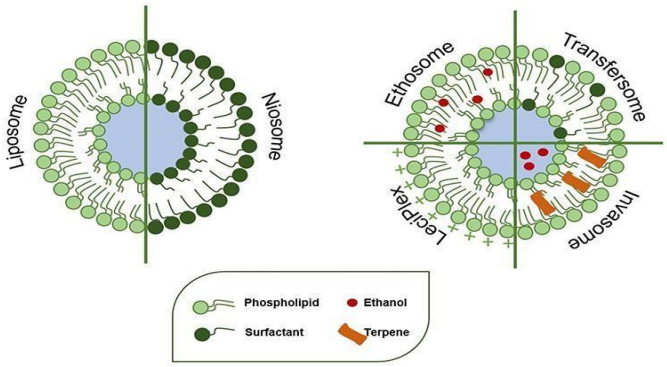

Figure1: Comparison of Invasomes

Advantages

- It is a method for delivering medicine that does not require any surgery.
- Better patient compliance.
- It is more stable compared to other ultra-deformable vesicles.
- The formula does not include any harmful ingredients.[3]
- It is possible to deliver both water-loving and fat-loving drugs directly to their target.
- It can easily penetrate through skin layers.
- This method for delivering drugs is easier than iontophoresis, phonophoresis, and other complicated techniques.
- Patients are more likely to follow their treatment because the medication comes in a semisolid form.[4,5]

Disadvantages

- Producing it costs a lot of money.
- Risk of leakage and merging of the enclosed active component.
- Invasomes that contain phospholipids can be oxidized or hydrolyzed, which may impact the stability of the vesicles[6].

Structure and Composition of Invasomes

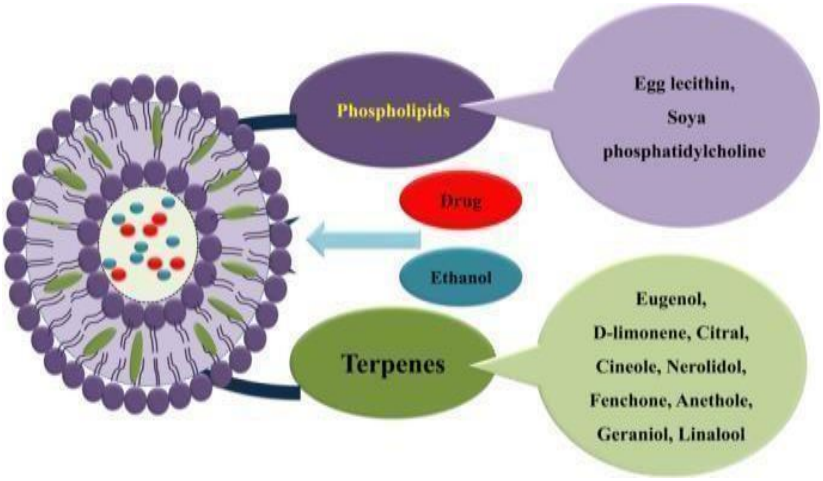

Figure 2: Composition of Invasomes

Composition: The soft liposomal vesicles known as invasomes, which may operate as carriers with improved skin penetration, contain small quantities of ethanol and different terpenes or terpene combinations. They include phospholipids and a tiny amount of alcohol, as well as terpenoids (such as citral, limonene, and cineole), water and a small quantity of ethanol (e.g., 3-3.3 percent by volume).[7].

Materials and Methods

Materials:

The drug Ciprofloxacin was purchased from Loba Chem Pvt.Ltd. Other materials like Soya Phosphatidylcholine, Ethanol, Menthol, Thymol and Camphor used in research work with analytical grades are used as supplied by manufacturer. Distilled water used throughout the study[8].

Methods:

Formulation of Ciprofloxacin Loaded Invasomes

Ciprofloxacin (100mg) was loaded into invasomes by Mechanical dispersion technique as per Table1. Following a five-minute vortex, 0.5 to 0.75% w/v of soy phosphatidylcholine was added to ethanol [9]. After adding the drug and terpenes (0.25% to 0.75%) and continuously vortexing the mixture, it was sonicated for five minutes. Fine stream of Phosphate buffer saline (upto 10% w/v) was added with syringe under constant vortexing.[10-13] for an extra five minutes, it was vortexed to obtain the invasomal preparation.

Table 1: Formulation Table of Ciprofloxacin Loaded Invasomes

S.NO	INGREDIENTS	F1	F2	F3	F4	F5
1	Ciprofloxacin (mg)	100	100	100	100	100
2	Soya lecithin (mg)	0.25	0.5	0.75	0.25	0.5
3	Camphor(mg)	0.75	-	-	-	0.75
4	Thymol (mg)	-	0.75	-	-	0.75
5	Menthol (mg)	-	-	0.75	-	0.75
6	Ethanol (ml)	10	10	10	10	10
7	Buffer	qs	qs	qs	qs	qs

Evaluation of Ciprofloxacin Loaded Invasomes

a. **Appearance**

The appearance of the drug-loaded Invasomal dispersion was checked by looking at it.

b. Microscopic Vesicle Size and Shape

One can determine the tiny makeup of invasomes.

c. pH

The pH values for the invasomes formulation batches F1 to F5 were measured using a digital pH meter with a glass electrode. The pH shows how active hydrogen ions are in a solution. A specific amount of the formulation was taken, diluted with calibrated distilled water and mixed thoroughly. To find the pH value, the electrode was placed in the prepared formulation.[14]

d. Drug content analysis

After examining the invasomes in 95% ethanol and shaking the vesicles well to make sure they fully broke apart, we calculated the amount of drug in the mixture. We then diluted it properly with PBS at a pH of 7.4 and measured how much light was absorbed at 256 nm by using UV-visible spectroscopy, with empty invasomes as our blank. We used a formula to find out the percentage of drug content based on the standard curve.[15]

% Drug content = (Sample absorbance /Standard absorbance) × 100

e. Entrapment Efficiency (EE)

The efficiency of invasome entrapment was measured using the Ultracentrifugation method. In this process, the invasome mixture was span at 15,000 rpm for 45 minutes. After centrifugation, the clear liquid on top was taken and mixed with a pH 7.4 phosphate buffer. Then, researchers analyzed it spectrophotometrically to check for Ciprofloxacin. Finally, they calculated the percentage of entrapment efficiency using a specific formula.[16]

% Entrapment Efficiency = [(Total drug - Diffused drug)/ Total drug] × 100

f. Particle Size Analysis

Samples were first diluted with deionized water 1:50 dilution and sonicated (ELMA, GERMANY) for 5 mins before being measured. Samples were measured using the Malvern zetasizer Nano-ZS, Malvern instruments (Malvern UK) at 25^0 C 173 ^{0} angle to access the particle size (PS), zeta potential. Measurement were done in triplicate and all the results were expressed as ± (SD).

g. *In-vitro* drug diffusion studies

The membrane diffusion method was used to examine how the invasomal formulation of the drug spreads in a lab setting. A diffusion cell was created using a cellophane membrane that had been soaked in warm water to activate it. This cell included a beaker, a magnetic stirrer with temperature control and an open-ended test tube. One end of the test tube was sealed with the treated cellophane membrane while the other end remained open for adding the invasomal formulation. The diffusion medium was freshly prepared phosphate-buffered saline (PBS) at a pH of 7.4, with a temperature kept at 37°C ±0.5 °C. A 5ml sample of the invasomal formulation was placed into the diffusion cell through the open end of the test tube on top of the cellophane membrane. To keep the volume steady, 5 ml portions of the medium were taken out at regular intervals and replaced with fresh PBS (pH 7.4). The samples were then analyzed for drug content using a UV-Visible spectrophotometer set to 256 nm.[17,18]

h. FT-IR Studies for the Optimized Formulation

The FT-IR analysis was done to identify the compounds qualitatively. To get the FT-IR spectra for the pure drug and its other ingredients, we placed the drug directly into the machine's cavity. The FT-IR spectrophotometer measured it in the wave number range of 4000-400 cm.[19]

i. Stability study

Researchers conducted a stability study on the enhanced formulation (F3) of Ciprofloxacin invasomes. They put the mixture into a sealed 20 ml glass vial and stored it for one month at 4 ± 2°C. Every week, they took samples from each batch to test how well the drug was released and how efficiently it was trapped.

j. Anti- Bacterial Activity for Optimized Formulation

The anti-bacterial activity of the Ciprofloxacin loaded invasomes was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth. After inoculation, plates were dried for 15 minutes and the wells were punched using sterile corn borers. Once wells were formed, they were filled with 100 µL invasomes and blank water. Commercially available product was used as a positive control in this study. Plates were incubated for24 h at 37 °C to allow invasomes to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different formulations against different bacteria was measured in millimeter for further analysis. An agar well (6 mm) showing zone of inhibition was considered as antibacterial activity. All experiments were done in triplicate and the average values were used for drawing bar diagram.[20]

Results and Discussion

Evaluation Parameters of Invasomes:

a. **Appearance:**

The Invasomal dispersion had a yellow-golden color. It remained stable and did not settle at the bottom.

b. pH Measurement:

The researchers used a digital pH meter to check the pH of all the formulations (F1–F5). They found that the pH ranged from 6.2 to 6.8.

c. Microscopic vesicle size and shape:

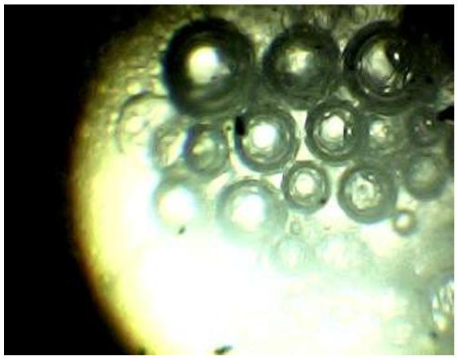

Figure: 3 Microscopic Image

d. Drug Content:

The amount of percentage of drug percent in formulation of Ciprofloxacin loaded invasomes were estimated by UV spectrophotometric method. The drug content of all the formulations of Ciprofloxacin loaded invasomes were found in the range of 47.24 % to 91.67 %. The formulation (F3) showed maximum drug content (91.67 %) than other formulations.

Table 2: The drug content of all the batches of invasomes

S.No	Formulation	Drug content
1	F1	47.24%
2	F2	65.76%
3	F3	91.67%
4	F4	76.31%
5	F5	88.04%

e. % Entrapment Efficiency (EE):

We measured how well the Ciprofloxacin loaded invasomes trapped the drug using a UV spectrophotometric method. The entrapment efficiency of those formulations ranged from 54.05% to 80.00%. Among all the formulations, F3 (80.00%) had the highest drug content.

Table 3: The entrapment efficacy of all 5 batches

S.No	Formulation code	Entrapment efficacy
1	F1	71.94%
2	F2	65.78%
3	F3	80.00%
4	F4	75.75%
5	F5	54.05%

f. Particle size analysis:

Median Diameter (D_{50}): 0.052 µm

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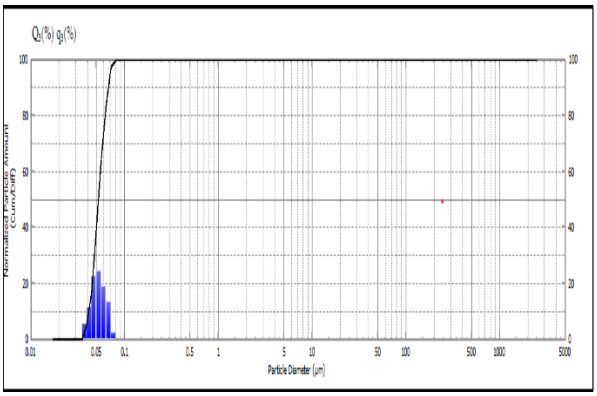
Modal Diameter: 0.050 µm

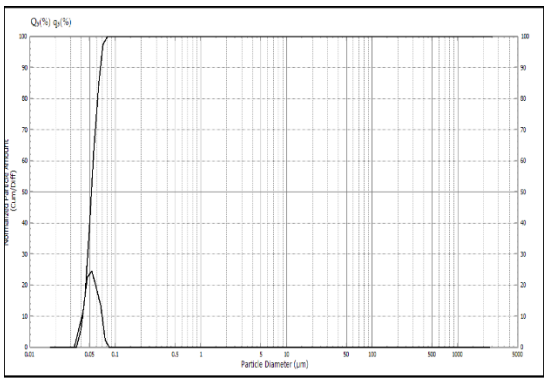
Cumulative Diameters (D75, D50, D25): 0.060 µm, 0.052 µm, and 0.046 µm respectively

Cumulative % of Particle Diameter: 100% for sizes of 1.000 µm, 10.000 µm, and 100.000 µm

Standard Deviation: 0.000 (showing consistency in the measurements)

Maximum and Minimum Values: Same as the average values, indicating no differences in the measurements.





	File Name	Median D (µm)	Modal D (µm)	Diameter of Cumulative %			Cumulative % of Particle Diameter		
				75.000%D	50.000%D	25.000%D	1.000	10.000	100.000
	Mean Value	0.052	0.050	0.060	0.052	0.046	100.000	100.000	100.000
	Std Dev	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Maximum	0.052	0.050	0.060	0.052	0.046	100.000	100.000	100.000
	Minimum	0.052	0.050	0.060	0.052	0.046	100.000	100.000	100.000

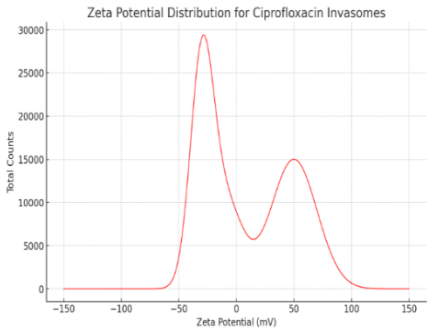


Figure 4: Particle Size Analysis of Ciprofloxacin loaded invasomes

g. *In-vitro* drug diffusion studies:

In vitro drug release of Ciprofloxacin Loaded Invasomes formulations were carried out as per the procedure. The concentration of drug release of different Formulations. The formulation F3 produce maximum drug release at 70 concentration.

Table 4: *In-vitro* drug diffusion

s.no	concentration	F1	F2	F3	F4	F5
1	10	0.007	0.009	0.008	0.007	0.007
2	20	0.009	0.011	0.01	0.009	0.01
3	30	0.023	0.015	0.015	0.015	0.014
4	40	0.027	0.019	0.019	0.019	0.017
5	50	0.029	0.023	0.022	0.024	0.024
6	60	0.03	0.027	0.026	0.029	0.029
7	70	0.032	0.032	0.039	0.032	0.034

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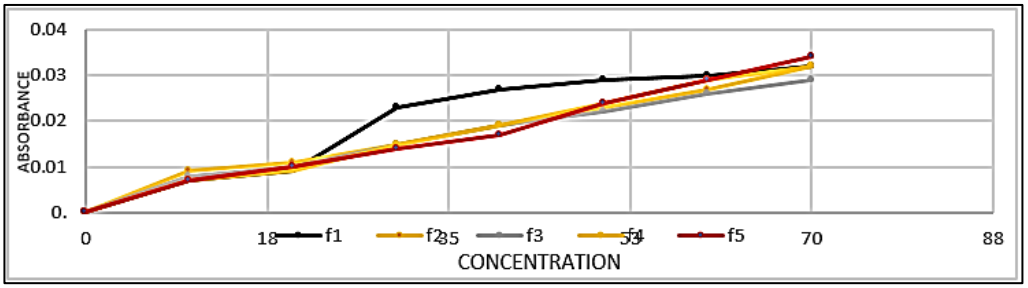

Figure 5: *In-vitro* drug diffusion study of All 5 Batches

h. **FT - IR Spectral Studies:**

The FT-IR spectrum of physical mixture showed all the characteristic peaks of Ciprofloxacin thus conforming that no interaction of drug occurred with the components of the formulation.

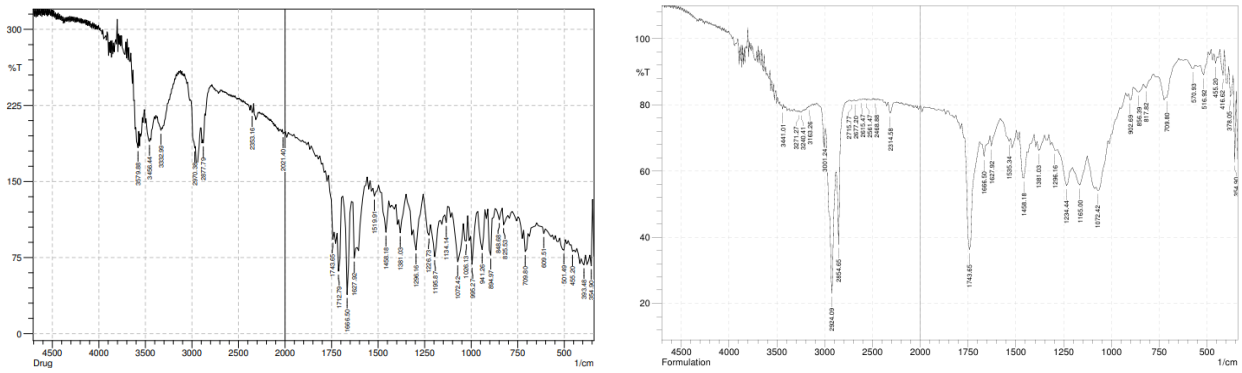

Figure 6: FT-IR Spectra of ciprofloxacin FT-IR Spectra of Ciprofloxacin Loaded Invasomes

h. Stability studies:

The stability study of the optimized Ciprofloxacin Invasomal formulation (F3) was carried out at 4±2°C. At the interval of 7, 15, 21 and one month the invasomes evaluated for Appearance, pH, %Drug content, %Entrapment efficiency and *in-vitro* drug diffusion. The stability study showed in Table that Invasomal formulations are more stable at 4°C (refrigerator) temperature.

Table 5: Stability studies of optimized formulation F3 at 4±2°C

Parameters	Initial	After 7 days	After 14 days	After 21 days	After one month
Appearance	Yellow-Golden	No Change	No Change	No Change	No Change
pH	6.7	No Change	No Change	No Change	No Change
%Entrapment efficiency	80.00%	80.00%	80.00%	80.00%	80.00%
% Drug content	91.67%	91.67%	91.67%	91.67%	91.67%
***In-vitro* drug diffusion**	0.039	0.039	0.039	0.039	0.039

i. Anti-Bacterial Activity of Ciprofloxacin Loaded Invasomes

In this study of gram positive and gram negative micro-organism were used in agar well diffusion method. Formulation (F3) containing Ciprofloxacin, phosphatidylcholine and terpenes and ethanol is showed maximum zone of inhibition against 2 microorganism. Hence formulation F3 containing Ciprofloxacin loaded invasomes is maximum Anti-bacterial activity than other formulation.

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Table 6: The anti-bacterial activity of formulation of ciprofloxacin loaded invasomes

S.NO	FORMULATION	MICRO ORGANISM	Standard zone of inhibition	Sample zone of inhibition	Andi biotic zone of inhibition	Control
1	F1	Escherichia coli	2	0.3	0.2	0
2	F2		2	0.4	0.1	0
3	F3	Staphylococcus Aureus	0.8	0.4	0.3	0
4	F4		0.8	0.4	0.5	0
5	F5		0.8	0.2	0.5	0

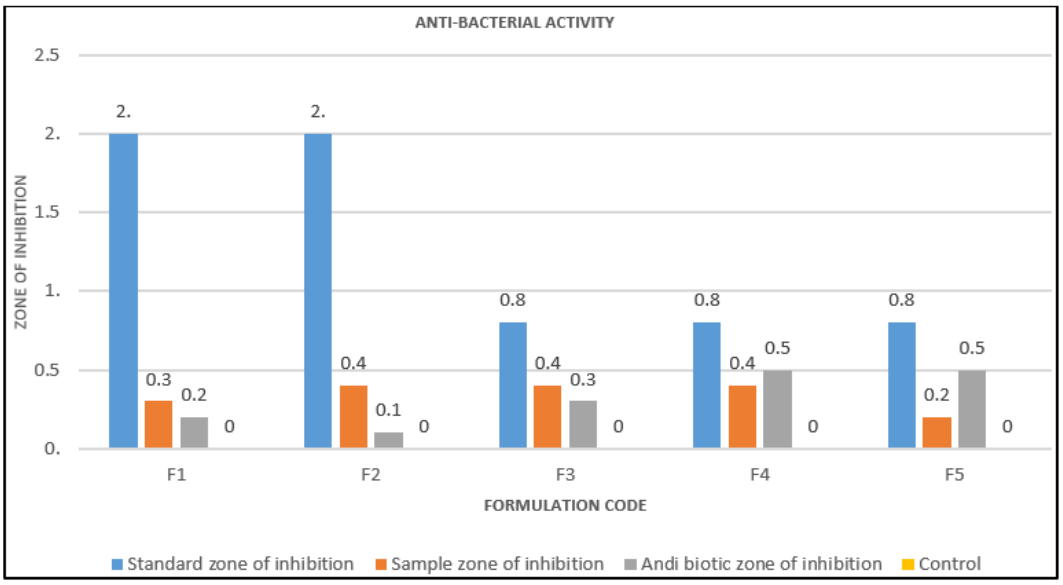

Figure 7: Anti-bacterial Activity of Ciprofloxacin Loaded Invasomes

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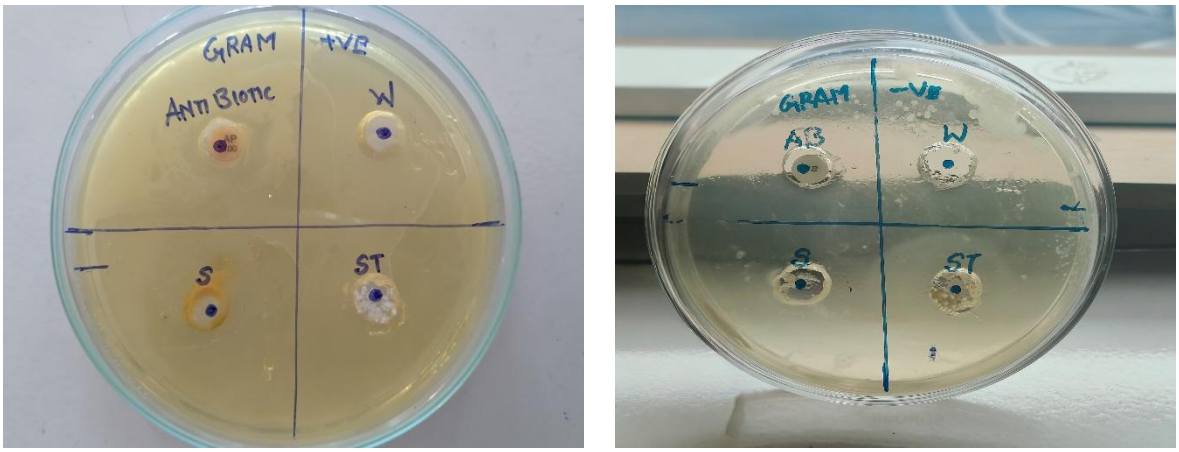

Figure 8: Zone of Inhibition and Anti-Bacterial Activity of Ciprofloxacin Loaded Invasomes.

Conclusion

The formulation and testing of invasomes that contain ciprofloxacin, along with an examination of their antibacterial properties, have been successfully completed. Researchers conducted a study on the interaction between the drug and excipients using FT-IR,

created ciprofloxacin invasomes and assessed various evaluation parameters. Among all the formulations tested, formulation F3 achieved the highest drug release at 91.67%, with a drug entrapment efficiency of 80% and a faster drug release rate (0.039 concentration) within one hour. This formulation showed promising antibacterial effects against both gram-positive and gram-negative bacteria. The results indicate that formulation F3, which includes 100mg of Ciprofloxacin combined with 0.75 mg of phospholipids and 0.75 mg of terpenes, is a promising option for treating certain skin infections caused by bacteria. By encapsulating ciprofloxacin in invasomes, researchers aim to enhance the drug's ability to penetrate through the skin barrier. These findings emphasize the potential of invasomal systems to improve drug delivery, increase patient adherence to treatment and reduce side effects, representing a significant step forward in pharmaceutical development.

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Conflict of Interest Statement: All authors have nothing else to disclose.