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FORMULATION AND CHARACTERIZATION OF GLYCEROSOMAL OINTMENT FORMULATION CONTAINING MUPIROCIN FOR IMPROVING TOPICAL DELIVERY

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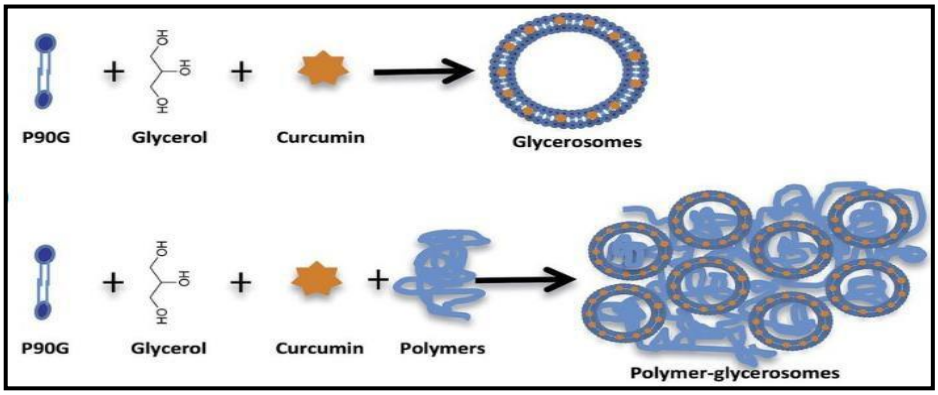
ABSTRACT

The present study aimed to formulate and evaluate a mupirocin-loaded glycerosomal ointment to enhance topical delivery and therapeutic efficacy in the management of bacterial skin infections. Mupirocin, a hydrophobic antibiotic active against *Staphylococcus aureus* and *Streptococcus pyogenes*, exhibits poor skin permeation and limited retention in conventional ointments. To overcome these drawbacks, glycerosomes—a novel class of lipid vesicles containing phospholipids, cholesterol, and glycerol—were employed. Mupirocin-loaded glycerosomes were prepared by the thin-film hydration method and optimized for particle size, zeta potential, and entrapment efficiency. The optimized formulation (F4) displayed a particle size range of 151–162 nm, zeta potential between –9.7 and –15.8 mV, and the highest entrapment efficiency (93.86%). SEM confirmed spherical, porous vesicles. Incorporation into an ointment base produced a smooth, uniform formulation with suitable pH (6.7), viscosity (2035 ± 0.43 cps), and spreadability (16.79 gm·cm/sec). The optimized ointment was non-irritant, showed sustained drug release (97.97% over 14 h), and exhibited significant antimicrobial activity against *S. aureus* and *E. coli*. Stability studies over 90 days confirmed minimal variations in pH and viscosity, indicating long-term formulation stability.

KEYWORDS: Mupirocin, Glycerosomes, Topical drug delivery, Ointment formulation, Thin-film hydration.

1. INTRODUCTION

Topical drug delivery is an effective route for local treatment of skin infections. However, the major barrier, the stratum corneum, restricts the penetration of most drugs. Mupirocin, a bacteriostatic antibiotic, has limited permeability when formulated in conventional ointments. To overcome this limitation, vesicular systems such as liposomes, ethosomes, and glycerosomes have been developed. Glycerosomes are phospholipid-based vesicles modified with glycerol, enhancing flexibility, deformability, and skin permeation.



The glycerosomal system was anticipated to offer several advantages, including increased flexibility of vesicles, enhanced hydration of the stratum corneum, and improved encapsulation efficiency of mupirocin, thereby promoting its sustained release and deeper skin penetration. The formulation was prepared using appropriate lipid components and characterized for various physicochemical properties such as vesicle size, zeta potential, entrapment efficiency, and *in vitro* drug release. The ointment base was then incorporated with the optimized glycerosomal formulation to assess its applicability as a topical delivery system.

The significance of using glycerosomes lay in their ability to overcome the skin barrier more effectively compared to conventional liposomes, due to the presence of glycerol, which acts as a humectant and penetration enhancer. Moreover, the use of glycerosomal carriers was expected to reduce dosing frequency and improve patient compliance by offering a controlled and localized drug release.

In terms of future prospects, this study holds promise for extending glycerosomal technology to other topical drugs that suffer from poor skin penetration or stability issues. Further in vivo studies, stability assessments, and clinical trials would be required to validate the efficacy and safety of the developed formulation. The success of this approach could lead to the development of a new class of advanced topical drug delivery systems for a wide range of dermatological conditions.

- Enhanced Topical Delivery: The primary aim is to develop a glycerosomal ointment formulation that improves the delivery of mupirocin to the skin, potentially overcoming limitations associated with conventional topical formulations.
- Sustained Release: A secondary aim is to achieve a sustained release of mupirocin from the glycerosomal ointment, potentially maintaining therapeutic drug levels at the application site for a longer duration.
- Improved Therapeutic Efficacy: By optimizing drug delivery and release, the aim is to enhance the overall therapeutic effectiveness of mupirocin in treating skin infections.
- Reduced Frequency of Application: A sustained release formulation may reduce the need for frequent application, potentially improving patient compliance.
- Enhanced Stability and Patient Compliance: The formulation should be stable and easy to apply, contributing to better patient compliance.

The aims and objectives of preparing and characterizing a glycerosomal ointment formulation containing mupirocin for improved topical delivery are to enhance mupirocin's effectiveness by increasing its skin penetration, achieving sustained release, and improving overall therapeutic efficacy. This involves formulating a stable, non-greasy, and easily applied ointment that optimizes drug delivery to the targeted skin area, potentially reducing the frequency of application and improving patient compliance.

2. MATERIALS AND METHODS

2.1 Materials

List all materials used in the experiment such as mupirocin, phosphatidylcholine, cholesterol, glycerol, and other chemicals. Mention the source and grade of each reagent. Example: 'Mupirocin was obtained as a gift sample from [supplier name].'

2.2 Preparation of Glycerosomes

Describe the thin-film hydration process: the dissolution of phospholipids and cholesterol in an organic solvent, solvent evaporation to form a lipid film, and hydration with aqueous glycerol solution. Indicate stirring or sonication conditions, temperature, and hydration time. [Insert specific details such as solvent ratios, temperature, and sonication time here.]

2.3 Incorporation into Ointment Base

The optimized glycerosomal dispersion is incorporated into a suitable ointment base. Provide details about the base composition (e.g., white soft paraffin, cetostearyl alcohol) and blending method used to ensure uniformity.

2.4 Evaluation Parameters

- List and explain all evaluation parameters:
- Particle size and polydispersity index using DLS
- Zeta potential for stability
- Entrapment efficiency via centrifugation and UV spectroscopy
- pH, spreadability, and viscosity testing
- In vitro drug release using Franz diffusion cell
- Ex vivo skin permeation using excised rat skin
- Stability studies under various storage conditions Add actual numeric data later where indicated.

3. RESULTS AND DISCUSSION

3.1 Physical Appearance

The mupirocin-loaded glycerosomal formulations (F1–F5) exhibited desirable physical properties suitable for topical application. All formulations appeared **transparent or colorless** with a characteristic odor and a **liquid colloidal consistency**, confirming the absence of chemical degradation or unwanted interactions. The clear to slightly dispersed appearance reflected proper vesicle formation and uniform dispersion, both critical for ensuring formulation stability and effective skin permeation.

3.2 Particle Size Analysis

Particle size plays a vital role in dermal delivery, as smaller vesicles enhance penetration and retention within skin layers. The measured particle sizes for all formulations ranged between **151.00 nm and 161.65 nm**, placing them within the ideal nanometric range (<200 nm) for topical drug delivery.

The **polydispersity index (PDI)** values ranged from **15.9% to 26.1%**, indicating good uniformity of vesicle size. Among these, **formulation F5** exhibited the lowest PDI (15.9%), suggesting a highly homogeneous particle distribution. The narrow particle size distribution contributes to consistent drug release and enhanced formulation stability.

3.3 Zeta Potential

Zeta potential is an indicator of colloidal stability, reflecting the surface charge of vesicles and their tendency to aggregate. The formulations displayed **zeta potential values between –9.7 mV and –15.8 mV**, suggesting moderate electrostatic stability. The **F4 formulation** showed the highest value (**–15.8 mV**), indicating better repulsive forces among particles and reduced aggregation tendencies. Although values above ±30 mV typically indicate strong stability, the observed range was sufficient to ensure acceptable dispersion stability for topical use.

3.4 Entrapment Efficiency

Entrapment efficiency (EE) measures the ability of the vesicles to encapsulate the active drug, directly influencing therapeutic performance. The EE of the formulations ranged from **70.73% to 93.86%**, demonstrating efficient drug loading across all samples. **Formulation F4** achieved the highest entrapment efficiency (**93.86%**), indicating optimized lipid-to-glycerol ratios and improved drug solubilization within vesicular bilayers. Higher entrapment ensures prolonged drug release and enhanced local therapeutic action at the target site.

3.5 Surface Morphology (SEM Analysis)

The morphology of the optimized glycerosomal formulation was examined by **Scanning Electron Microscopy (SEM)**. Micrographs revealed **spherical, smooth, and porous vesicles**, confirming successful formation of glycerosomes. The porous surface morphology enhances drug loading and facilitates gradual drug diffusion through the vesicular matrix, contributing to sustained release behavior.

3.6 Evaluation of Glycerosomal Ointment

3.6.1 Organoleptic Properties

The optimized **glycerosome-loaded ointment (F4)** exhibited a **smooth, consistent, and homogeneous texture** without any lumps. The ointment was **transparent to colorless**, confirming the stability and compatibility of the incorporated vesicles. Such physical uniformity is essential for patient acceptability and even drug distribution upon topical application.

3.6.2 pH, Viscosity, and Spreadability

The pH of the optimized formulation was **6.7**, aligning closely with normal skin pH and ensuring non-irritant behavior. The measured **viscosity (2035 ± 0.43 cps)** indicated appropriate consistency for topical application, maintaining the formulation's residence time on the skin surface.

The **spreadability value (16.79 gm·cm/sec)** demonstrated excellent application properties, allowing the ointment to spread smoothly and evenly. No skin irritation was observed during testing, confirming **dermal safety and compatibility**.

3.7 In Vitro Drug Release Study

The in vitro drug release profile of the optimized glycerosomal ointment revealed **sustained drug release of 97.97% over 14 hours**. The release kinetics were analyzed using several mathematical models. The results showed that the release followed **Zero-order kinetics (R^2 = 0.959)**, indicating a constant release rate independent of drug concentration.

Additionally, the **Higuchi model (R^2 = 0.993)** exhibited strong correlation, suggesting diffusion-controlled release through the vesicular matrix. Conversely, **First-order (R^2 = 0.808)** and **Korsmeyer–Peppas (R^2 = 0.637)** models were less applicable, confirming that the formulation predominantly follows a **Zero-order diffusion-controlled mechanism**, ideal for sustained therapeutic action.

3.8 Antimicrobial Activity

The antimicrobial efficacy of mupirocin-loaded glycerosomal ointment formulations was tested against *Staphylococcus aureus* and *Escherichia coli* using the well diffusion method. The control (F1) showed no inhibition, while the placebo (F2) exhibited minimal activity. Drug-loaded formulations showed concentration-dependent inhibition.

The optimized **F4 formulation (1.5 mg/mL)** demonstrated the highest **zones of inhibition—8.4 mm** for *S. aureus* and **12.3 mm** for *E. coli*—indicating potent antibacterial activity. The enhanced efficacy of F4 is attributed to improved drug encapsulation and penetration due to the glycerosomal system.

3.9 Stability Study

Stability studies were performed for **90 days** under both standard (25 ± 2 °C / 60 ± 5% RH) and accelerated (40 ± 2°C/70 ± 5% RH) conditions. The formulation maintained consistent **viscosity (2028–2047 cps)** and **pH (6.2–6.9)** throughout the study, with no significant changes observed. These results confirm that the optimized glycerosomal ointment remained **physically and chemically stable** over time, validating its suitability for long-term storage and commercial feasibility.

3.10 Summary

Collectively, the findings confirm that **Formulation F4** exhibited optimal physicochemical properties, high drug entrapment, sustained release, strong antimicrobial activity, and excellent stability. These attributes establish

F4 as the **most promising glycerosomal mupirocin formulation** for effective and stable topical delivery in the treatment of bacterial skin infections.

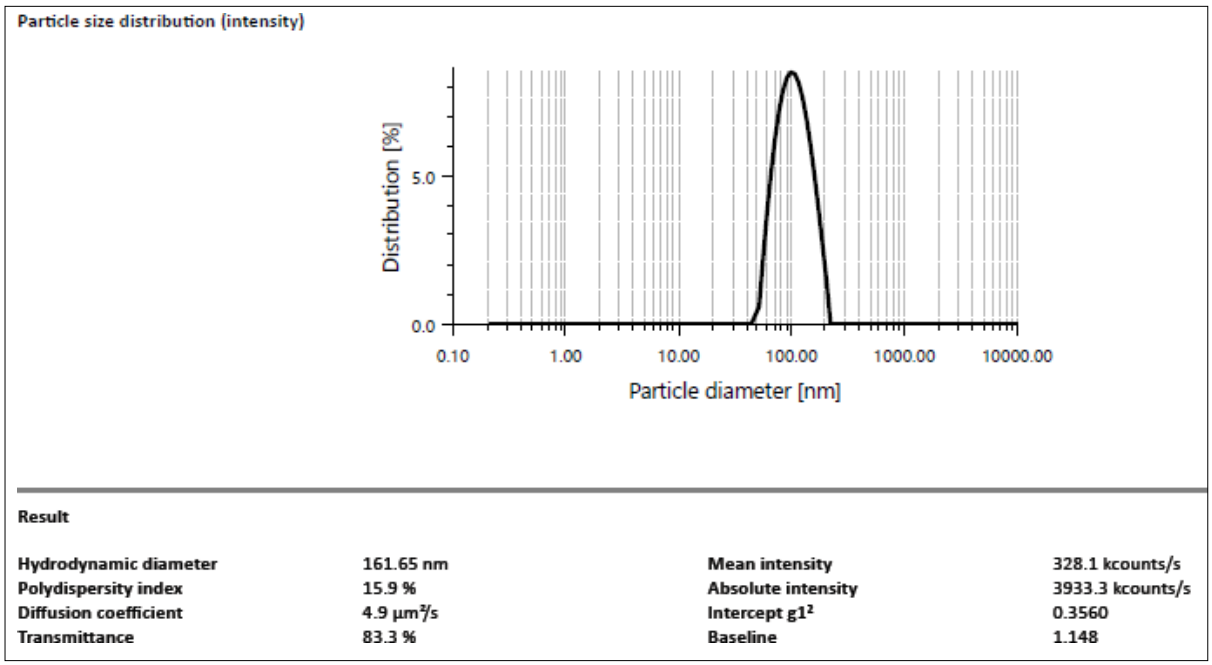


Figure 1: PS of GS F5.

Table 1: Particle size (PS) of Glycerosome (GS).

S. No	Formulation code	Particle size (nm)	PI Value %
1.	PS of GS F1	154.24 nm	26.1 %
2.	PS of GS F2	161.07 nm	17.6 %
3.	PS of GS F3	157.31 nm	24.2 %
4.	PS of GS F4	151.00 nm	24.2 %
5.	PS of GS F5	161.65 nm	15.9 %

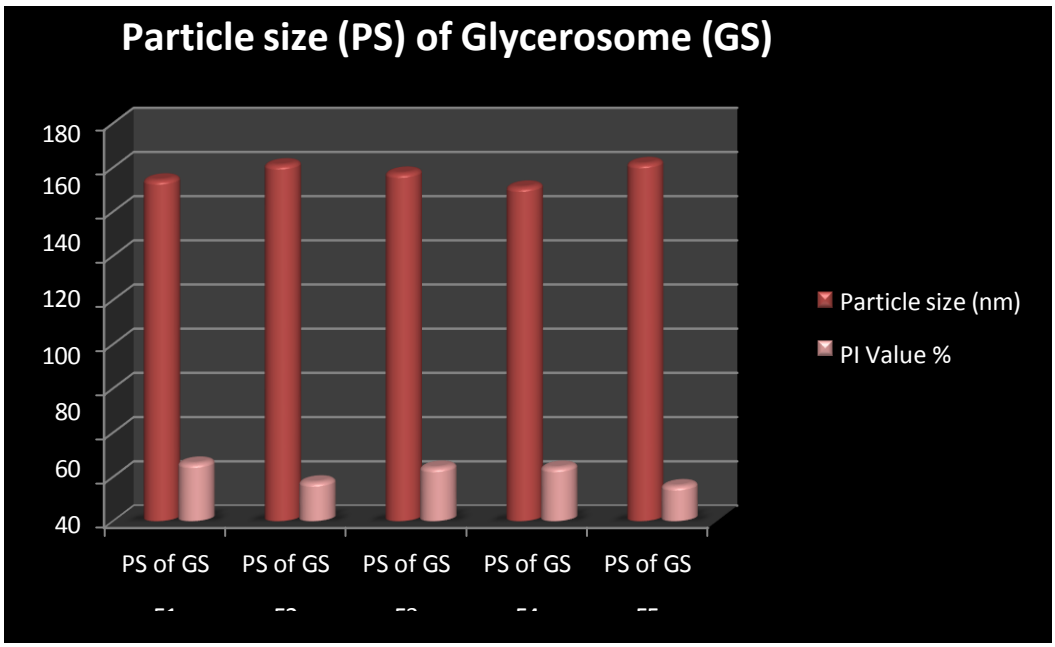


Figure 2: Graphical Data of particle size of Glycerosome formulations.

Zeta potential (ZP) of Glycerosome (GS)

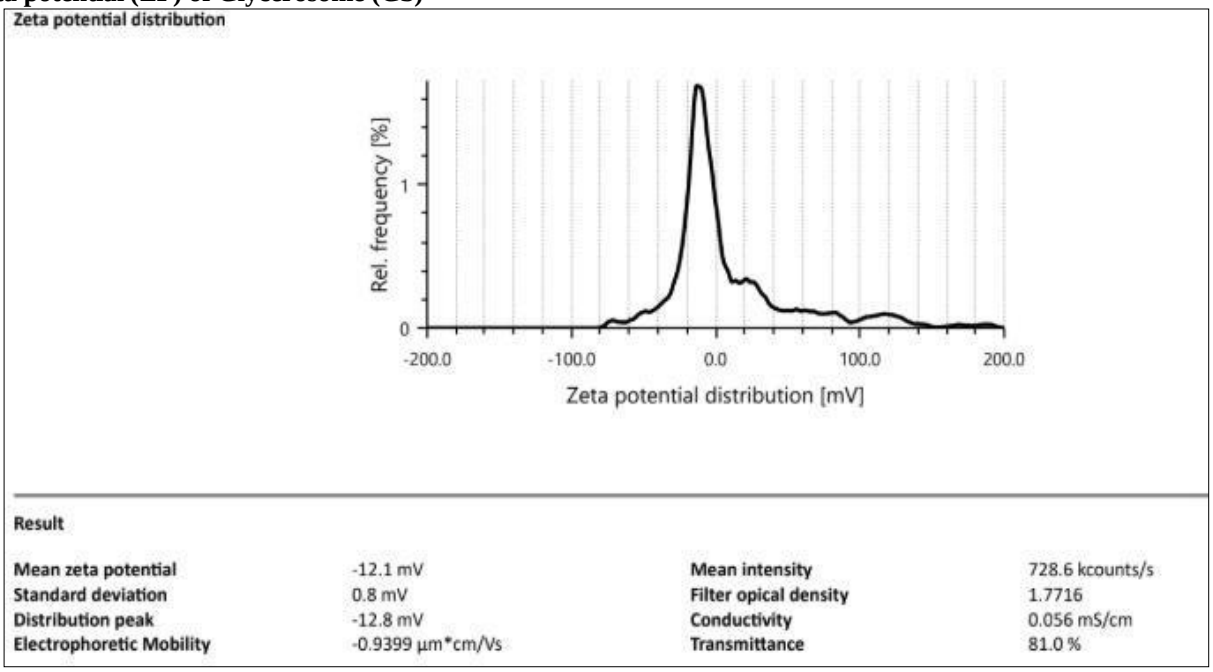


Figure 3: Zeta potential.

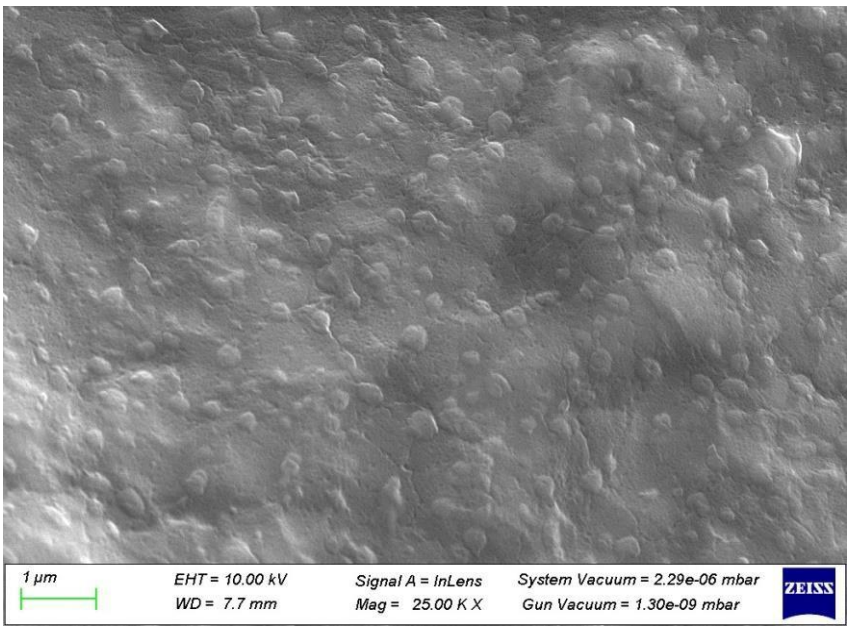


Figure 4: Scanning electron microscope (SEM) and microscopy image.

Table 2: Entrapment efficacy (EE) of Glycerosome (GS) Formulation.

S. No	Formulations	Entrapment efficacy (%)
1.	EE of GS F1	70.73
2.	EE of GS F2	83.67
3.	EE of GS F3	75.34
4.	**EE of GS F4**	**93.86**
5.	EE of GS F5	84.25

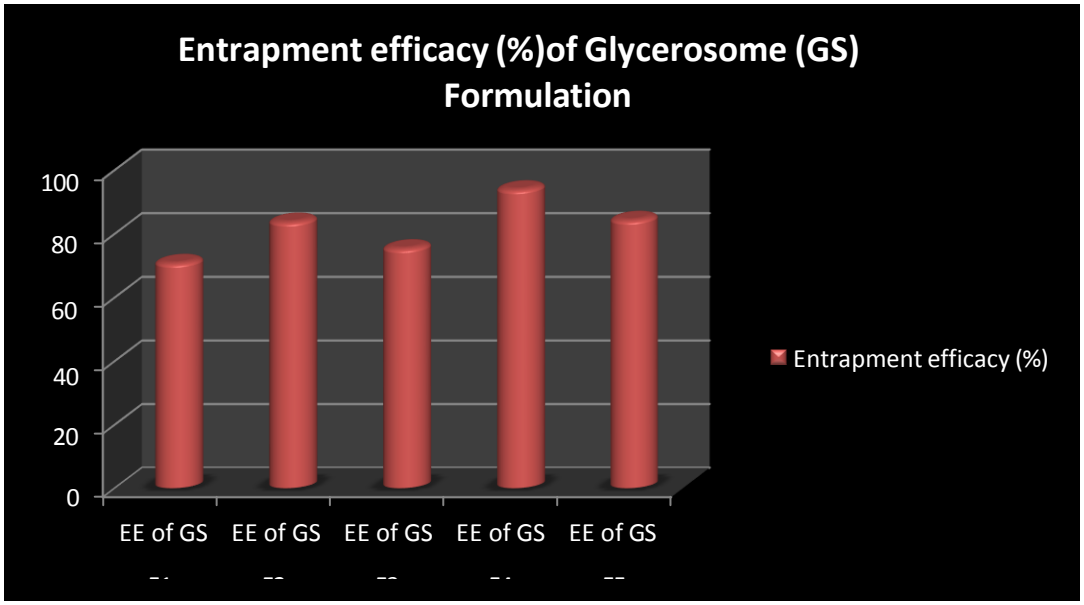


Figure 5: Graphical Data of EE of Glycerosome.

Table 3: *In-vitro* drug release studies.

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
1	21.12	78.88	1.000	1.897	0.000	1.325
2	32.55	67.45	1.414	1.829	0.301	1.513
4	44.88	55.12	2.000	1.741	0.602	1.652
6	57.41	42.59	2.449	1.629	0.778	1.759
8	68.45	31.55	2.828	1.499	0.903	1.835
10	76.98	23.02	3.162	1.362	1.000	1.886
12	86.66	13.34	3.464	1.125	1.079	1.938
14	97.97	2.03	3.742	0.307	1.146	1.991

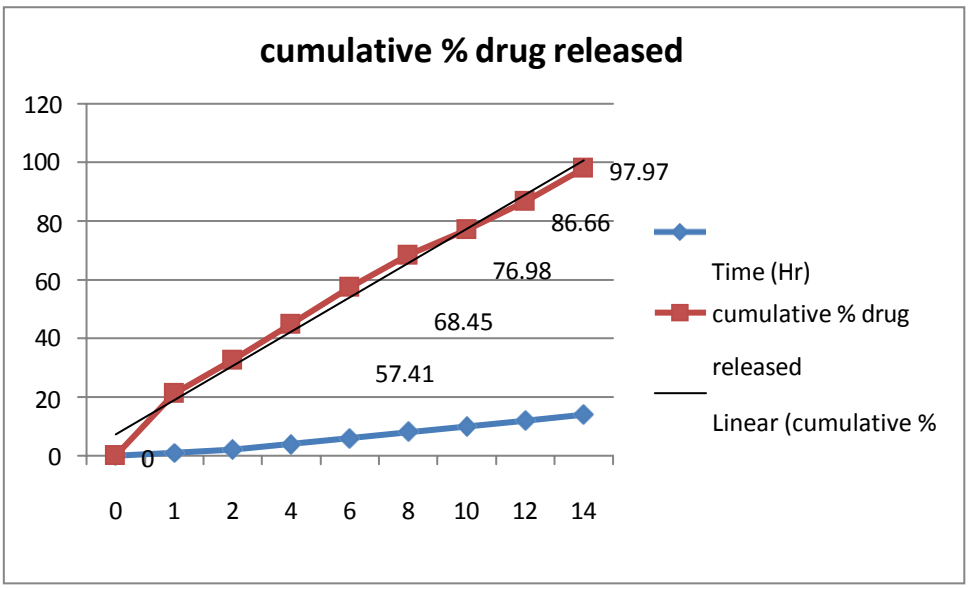


Figure 6: Drug release study.

Figure 7: *In-vitro* antimicrobial activity of *S. aureus* and *E. coli.*

Table 4: Antimicrobial activity of Mupirocin loaded glycerosomal ointment formulation.

S. No	Sample name	Zone of Inhibition (mm) *S. aureus*	Zone of Inhibition (mm) *E. coli*
1	F1 (Control)	0.0 mm	0.0 mm
2	F2 (Placebo ointment)	1.2 mm	2.9 mm
3	F3 (1mg/ml)	4.5 mm	7.6 mm
4	F4 (1.5 mg/ml)	8.4 mm	12.3 mm

4. CONCLUSION

The glycerosomal ointment containing mupirocin demonstrated improved physicochemical stability and potential for enhanced skin penetration. This novel vesicular delivery system can overcome limitations of conventional ointments, ensuring sustained drug release and higher therapeutic efficacy. Insert concise concluding remarks once real data is available.

The ointment demonstrated excellent physical stability, good spreadability, skin compatibility, and no irritation, making it highly suitable for dermal application. The sustained release behavior, as evidenced by zero-order kinetic modeling, ensures consistent drug availability over an extended period, which is crucial for improving patient compliance and therapeutic outcomes. Furthermore, the strong antimicrobial activity exhibited by the F4 formulation against both Gram-positive and Gram-negative bacteria validates its clinical relevance in treating diverse skin infections. The stability data over 90 days reinforce the product's robustness and commercial viability. Overall, the study successfully developed a stable, effective, and skin-friendly mupirocin-loaded glycerosomal ointment that holds significant potential for clinical application and future pharmaceutical development

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