

# Formulation Strategies for Linezolid- Encapsulated Transferosomes in Topical Antibacterial Therapy

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## Abstract

**Aim:** To develop and characterize linezolid-loaded transferosomes and evaluate their antibacterial activity for enhanced topical drug delivery.

**Materials and Methods:** Linezolid transferosomes were prepared using a modified reverse-phase evaporation method with soya lecithin, cholesterol, Tween 80, and sodium deoxycholate. Preformulation studies including organoleptic evaluation, solubility, melting point, and pH determination were performed. UV spectrophotometry was used for  $\lambda_{max}$  determination and calibration curve preparation, while FTIR analysis confirmed drug-excipient compatibility. The formulations were characterized for particle size, zeta potential, surface morphology (SEM), entrapment efficiency, and in-vitro drug release. Antibacterial activity was assessed against *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. **Results:** Linezolid exhibited a  $\lambda_{max}$  at 250 nm with good linearity. FTIR analysis confirmed the presence of characteristic functional groups, indicating drug stability. Transferosomal formulations showed particle sizes ranging from 188.3 to 396.4 nm, with TFF5 exhibiting the smallest size, highest entrapment efficiency (96.69%), and favorable zeta potential (25.7 mV). In-vitro drug release demonstrated sustained release up to 14 hours, following Higuchi kinetics ( $R^2 = 0.991$ ). Antibacterial studies revealed enhanced activity of the optimized formulation (TFF5), with zones of inhibition of 7 mm against *S. aureus* and 12 mm against *E. coli*. **Conclusion:** The study confirms that transferosomal encapsulation of linezolid significantly improves its stability, controlled release, and antibacterial efficacy. The optimized formulation (TFF5) shows promising potential as an

effective topical drug delivery system for the treatment of bacterial infections.

**Keywords:** Linezolid, Transferosomes, Antibacterial activity, Nanocarrier drug delivery, Entrapment efficiency,

## I. Introduction

Transferosome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer (Pandey et al., 2009). Interdependency of local composition and shape of the bilayer makes the vesicle both self-regulating and self-optimizing. This enables the Transferosome to cross various transport barriers efficiently, and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents (Jyolsna, 2020). Linezolid is the first member of a new class of synthetic antibacterial agents known as oxazolidinones (Hashemian et al., 2018). This antimicrobial has a potent spectrum of activity against Gram-positive micro-organisms, including multiresistant pathogens. Its mechanism of action is unique (early inhibition of the protein synthesis), so no cross-resistance with other anti-Gram-positive drugs is expected. It can be administered by oral or parenteral route and is well tolerated (Jain et al., 2006). Our work is directed to investigate antimicrobial activity of Linezolid transferosomal formulation. Since there is no scientific study to substantiate the traditional claim on antimicrobial activity of the plant, the present study is being taken up.

## II. Materials and methods

### 2.1 Chemical used

Linezolid served as the active pharmaceutical ingredient in this study, with soya

lecithin, cholesterol, and sodium deoxycholate as key components for transferosomal vesicle preparation. Tween 80 acted as a surfactant, while dimethyl sulfoxide enhanced permeability. Organic solvents like methanol, chloroform, diethyl ether, and petroleum ether were utilized for extraction and formulation. All chemicals were of analytical grade, and distilled water was used throughout the experiments.

## 2.2 Pre-formulation studies

Pre-formulation studies are crucial initial investigations that assess the mechanical, chemical, and physical characteristics of the active pharmaceutical ingredients (APIs) of a drug such as linezolid. These investigations guarantee that medications such as linezolid continue to be efficacious when combined and made into creams or ointments (Arya et al., 2025).

### 2.2.1 Organoleptic evaluation

Visual observation was used to determine the organoleptic qualities of linezolid. Results of the organoleptic investigations of Linezolid's general appearance, including color, odor, condition, etc are mentioned below (Maji et al., 2021).

### 2.2.2 Solubility Study

In a solubility study, a drug's capacity to dissolve in a particular solvent under predetermined parameters such as temperature, pH, and agitation is evaluated. Since it directly affects a drug's bioavailability, absorption, and therapeutic efficacy, solubility is a crucial factor in drug development. USP NF, 2007 was used to test the drug's qualitative solubility in several solvents (Sharma et al., 2022).

### 2.2.3 Determination of the Melting point

Using a melting point apparatus, coarsely powder the solid sample and pack a tiny amount (2–3 mm height) into a capillary tube with a sealed end to find the melting point of linezolid. Note the temperature at which the sample starts to melt (onset temperature) and the temperature at which it completely liquefies (end-point temperature). A range between these two temperatures is used to describe the melting point.

### 2.2.4 Determination of the pH

Determine the pH of Linezolid drug using a digital pH meter. 10 mg of drug was dissolved in 10 ml of distilled water and the pH was determined by digital pH meter and was recorded (Kharwade et al., 2023).

## 2.3 Determination of Maximum Wavelength ( $\lambda_{max}$ )

### 2.3.1 Preparation of Linezolid standard stock solution in methanol

Standard solution of Linezolid was prepared by dissolving accurately weighed 10 mg of Linezolid with 5 ml of methanol solvent, in a 10 ml volumetric flask. The volume was made up to 10 ml with methanol to obtain a stock solution of 100  $\mu\text{g/ml}$ . 1 ml of this stock solution was taken and then diluted up to 10 ml using respective solvent (methanol) to obtain a solution that has a concentration 100  $\mu\text{g/ml}$  which is standard stock solution (Golhar et al., 2020).

### 2.3.2 Lambda max

Using distilled water as a blank, the drug's working standard solution was scanned in the UV range of 200 to 400 nm in normal mode. After that, the absorption maxima were identified and the obtained peaks were documented (Sahu et al., 2019).

### 2.3.3 Linearity and Calibration Curve

At Linezolid 250.0 nm, the absorbance of the resultant solutions was measured in comparison to a blank of methanol. A calibration curve was created by graphing the drug's absorbance against concentration. A seven-point calibration curve was produced for Linezolid concentrations ranging from 5 to 25  $\mu\text{g/ml}$  (Bnyan et al., 2019).

### 2.3.4 Functional group identified by FTIR

To ascertain the structure of specific molecules and the makeup of molecular mixtures, academic laboratories and the pharmaceutical sector use Fourier Transform Infrared (FTIR) spectroscopy. To examine a sample, FTIR spectroscopy used modulated mid-infrared energy (Abdallah et al., 2021).

## 2.4 Formulation of linezolid-loaded Transferosome

Transferosomes were developed using a modified reverse phase evaporation method. Initially, soya lecithin and cholesterol were combined in a beaker with Tween 80 surfactant, dissolved in a diethyl ether-chloroform solvent mixture (1:1). The mixture was left at room temperature for 24 hours to form a thin film. A 20 mg drug solution in water was added to the film and sonicated for 2 minutes. The film was then hydrated with sodium deoxycholate in phosphate buffer saline (pH 7.4) and sonicated again for 2 minutes to create transferosomal suspensions. Dimethyl sulfoxide (DMSO) was added as a chemical permeation enhancer, and the suspensions were filtered through Whatman® filter paper (No.

40) and stored in a cool, dark place(Sana et al., 2021).

## 2.5 Characterization of extract loaded phytosomes

### 2.5.1 Physical properties

The physical properties of the Linezolid Transferosomal formulation can be initially evaluated by visual inspection to assess clarity, color, and homogeneity(Rattanapak et al., 2012).

### 2.6.2 Particle size

Particle size was determined using a Malvern Zetasizer (Malvern Instruments, UK) based on the dynamic light scattering (DLS), the particle size of Transferosomal is determined. To eliminate numerous scattering effects, prepare a diluted version of Transferosomal in an appropriate liquid media before using a Malvern Zetasizer to measure the particle size(Imam, 2023).

### 2.6.3 Zeta potential

The zeta potential was measured in order to determine the particle charge and the speed at which the particles traveled in an electric field. For the current investigation, the Transferosomal was diluted ten times with distilled water and analyzed using Zetasizer Malvern equipment. All

### 2.6.4 Scanning Electron Microscopic (SEM)

A scanning electron microscope employed an electron beam to optimize the morphological features of Transferosomes, coated with a 2–20 nm metal layer in a vacuum. This interaction produced secondary electrons, allowing for surface topography images to be captured by processing electrons scattered at a 90° angle, utilizing Rutherford and Kramer's Law(Ghasemiyeh& Mohammadi-Samani, 2020).

### 2.6.5 %Entrapment Efficiency

Entrapment efficiency was assessed using an indirect method. The Linezolid Transferosomal formulation was subjected to centrifugation at 1500 rpm for 30 minutes using a REMI Ultra Centrifuge. The supernatant, containing the unencapsulated (free) drug, was carefully collected and analyzed using a UV spectrophotometer(Rai et al., 2017).

### 2.6.6 In Vitro Drug Release Study of Linezolid Transferosomal

The release data were fitted to various kinetic models to elucidate the drug release mechanism:

**Zero-order model:** Drug release occurs at a constant rate, independent of concentration.

**First-order model:** Release rate depends on the remaining drug concentration.

**Higuchi model:** Drug release follows a diffusion mechanism proportional to the square root of time.

**Korsmeyer-Peppas model:** A log-log plot used to analyse the drug release mechanism from the Transferosomal system(Kumar et al., 2018).

### 2.7 Anti-bacterial activity of extract-loaded phytosomes by Well diffusion assay

The preparation of Nutrient Agar Media involved dissolving 2.8 grams of nutrient media in 100 milliliters of purified water, measuring the pH, and sterilizing it by autoclaving for 15 minutes at 121°C and 15 pounds of pressure. After pouring the media into plates, they were placed in a laminar air flow to solidify. For the Well Diffusion Assay, bacterial strains (*S. aureus* and *E. coli*) were spread on the agar. Wells were created for inoculation with 100 µl of standardized bacterial suspension (10<sup>8</sup> CFU/ml). The plates were incubated at 37°C for 48-72 hours. After incubation, the clear zones of inhibition (ZOI) around the wells were measured to assess antimicrobial activity, using a ruler against a dark background for accuracy(Modi&Bharadia, 2017).

## III. Results

### 3.1 Preformulation Study

#### 3.1.1 Organoleptic properties of Linezolid

Table 1: Organoleptic properties of Linezolid

Plant name	Yield (gm)	Yield (gm)
Linezolid	Color	White to off-white
	Odor	Odorless
	Appearance	Crystalline powder
	State	Solid

### 3.1.2 Solubility study

**Table 2: Solubility study of Linezolid**

Extract	Solvents	Observation/Inference
<i>Tephrosia purpurea</i>	Water	Slightly soluble
	Ethanol	Sparingly soluble
	Methanol	Freely Soluble
	Chloroform	Practically insoluble
	DMSO	Freely Soluble

### 3.1.3 Melting point

**Table 3: M.P of Linezolid**

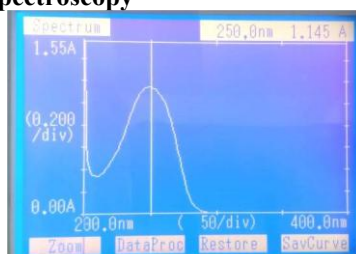
Sr. No.	Drugs	Observed	Reference
1	Linezolid	180 °C	176°C to 183°C

### 3.1.4 pH determination

**Table 4: pH of Linezolid**

No	Drugs	Observed	Reference
1.	Linezolid	5.2 pH	4.3 to 5.3.pH

### 3.2 Determination of $\lambda$ max by UV spectroscopy



**Figure 1: Lambda max of linezolid**

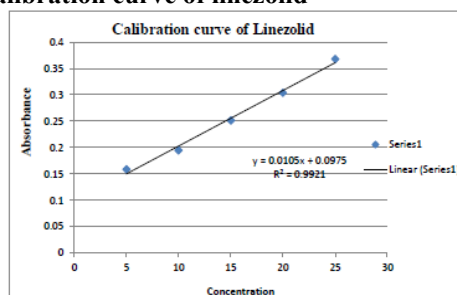
**Table 5: Lambda max of Linezolid**

S. No	Drug	UV absorption maxima (Lambda max)
1.	Linezolid	250.0nm

### 3.2.1 Calibration curve of Linezolid

**Table 6: Calibration curve of linezolid**

Concentration ( $\mu\text{g/ml}$ )	Absorbance
5	0.159
10	0.195
15	0.252
20	0.304
25	0.368
<b>Mean</b>	<b>0.2556</b>
<b>SD</b>	<b>0.083656</b>
<b>%RSD</b>	<b>36.88</b>



### 3.2.2 Functional group identified by Fourier transform infrared (FTIR) study

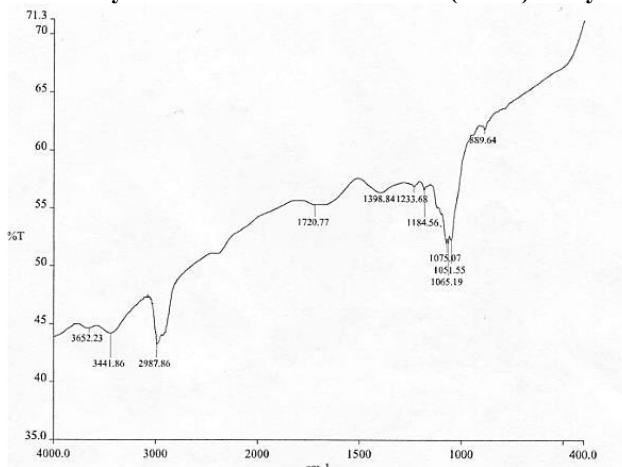


Figure 2: FTIR of Linezolid

Table 7: Interpretation of IR spectrum of Linezolid

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3652.23	3700-3584	O-H stretching	Alcohol
2	3441.86	3500- 3400	N-H stretching	primary amine
3	2987.86	3000-2840	C-H stretching	alkane
4	1729.77	1740-1720	C=O stretching	Aldehyde
5	1233.68	1275-1200	C-O stretching	alkyl aryl ether
6	1051.55	1070-1030	S=O stretching	sulfoxide

### 3.3 Characterization of Linezolid Transferosomes formulation

#### 3.3.1 Physical Appearance of Transferosomes

Table 8: Physical Appearance of Transferosomes

Formulation	Colour	White
Transferosomes	Odour	Characteristic
	Appearance	Liquid dispersion

#### 3.3.2 Particle size

Table 9: Particle size of Transferosomes formulation

S. No	Formulation code	Particle size (nm)	PI Value %
1.	TFF 1	254.1	2.65
2.	TFF 2	194.0	1.12
3.	TFF 3	330.9	5.10
4.	TFF 4	396.4	1.59
5.	TFF 5	188.3	1.41

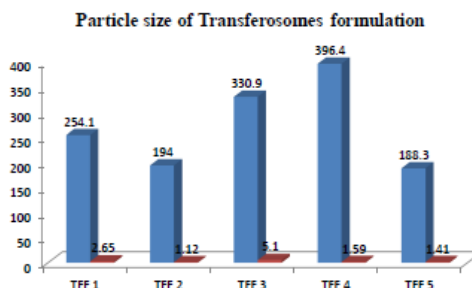


Figure 3: Particle size of Transferosomes formulation

### 3.3.3 Zeta potential

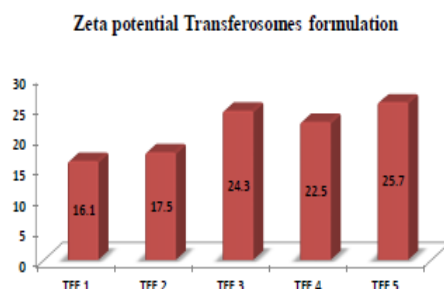


Figure 4: Graphical representation of Zeta potential of Transfersomes

Table 10: Zeta potential of Transfersomes

S. No	Formulation Code	Zeta potential mV
1.	TFF 1	16.1
2.	TFF 2	17.5
3.	TFF 3	24.3
4.	TFF 4	22.5
5.	TFF 5	25.7

### 3.6.4 SEM analysis of Optimized formulation

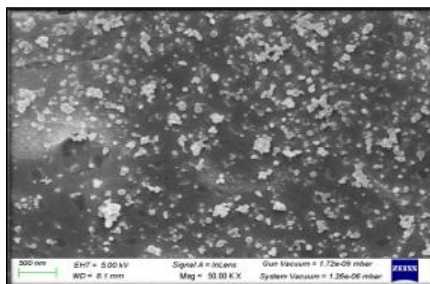


Figure 5: Scanning electron microscope (SEM)

### 3.3.5 Entrapment efficacy

Table 11: Entrapment efficacy

S. No	Formulations (TFF1-F5)	Entrapment efficacy (%)
1.	TFF 1	59.32
2.	TFF 2	67.57
3.	TFF 3	78.21
4.	TFF 4	89.41
5.	TFF 5	96.69

### 3.3.6 In-vitro drug release

Table 12: Release kinetics study of Transfersomes

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	Log Cumulative % drug remaining	Log time	Log Cumulative % drug released
0	0	100	0.000	2.000	0.000	0.000
1	22.12	77.88	1.000	1.891	0.000	1.345
2	32.42	67.58	1.414	1.830	0.301	1.511
4	44.88	55.12	2.000	1.741	0.602	1.652
6	56.11	43.89	2.449	1.642	0.778	1.749

8	67.91	32.09	2.828	1.506	0.903	1.832
10	78.77	21.23	3.162	1.327	1.000	1.896
12	86.66	13.34	3.464	1.125	1.079	1.938
14	98.97	1.03	3.742	0.013	1.146	1.996

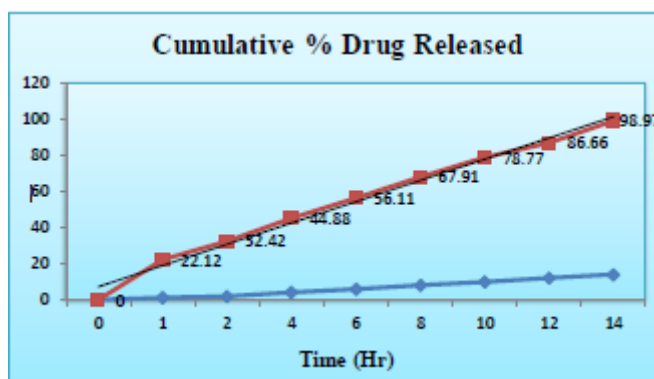


Figure 6: Drug release study

Table 13: Correlation value (R2 value)

Formulation	Model	Kinetic parameter values
Transfersome formulation	Zero Order	R <sup>2</sup> = 0.962
	First Order	R <sup>2</sup> = 0.787
	Higuchi	R <sup>2</sup> = 0.991
	Korsmeyerpeppas	R <sup>2</sup> = 0.631

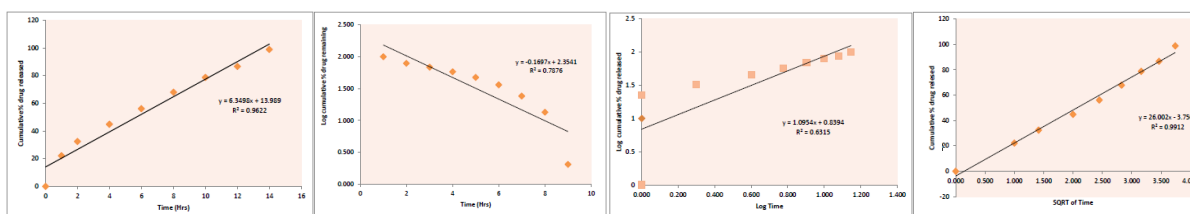


Figure 7: Zero order, First order, Korsmeyer peppas, Higuchi

### 3.7 Results of antimicrobial activity of phytosomes formulation

#### 3.7.1 Antimicrobial activity of phytosomes

Table 11: Antimicrobial activity of formulation against E.coli and S. aureus

S. No.	Sample Name (mg/ml)	Zone of Inhibition (mm) of S. aureus	Zone of Inhibition (mm) of E. coli
1.	C (control)	0mm	0mm
2.	Formulation C1 (0.5mg/ml)	3 mm	6 mm
3.	Formulation 5 C2 (0.5mg/ml)	7 mm	12mm

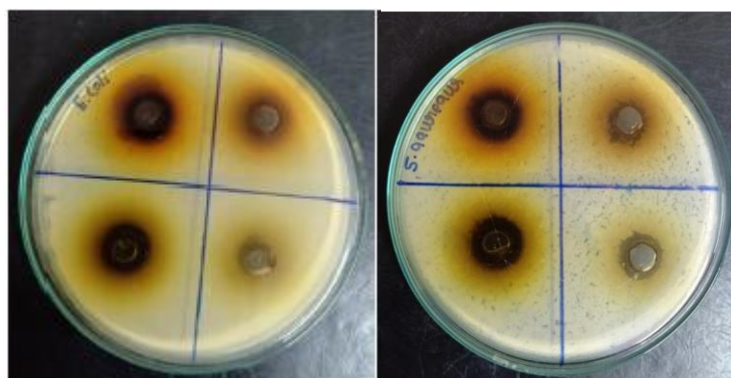


Figure 8: Antimicrobial activity against *E. coli* and *S. aureus*

#### IV. Discussion

The study developed and characterized Linezolid-encapsulated transferosomes for topical antibacterial therapy. Pre-formulation studies confirmed Linezolid's crystalline nature, slight water solubility, free solubility in methanol and DMSO, a melting point of 180°C, and a slightly acidic pH of 5.2, indicating its purity and stability. UV spectrophotometric analysis showed a  $\lambda$  max of 250 nm with a linear calibration curve in the 5–15  $\mu$ g/mL range for accurate quantification. FTIR studies identified essential functional groups such as O–H, N–H, C–H, C=O, C–O, and S=O, confirming the structural integrity of Linezolid. The transferosomal formulations (TFF1–TFF5) were assessed for various physical properties and drug release characteristics. All formulations were white, solid, and homogenous, indicating successful preparation. Particle sizes ranged from 188.3 to 396.4 nm, with TFF5 being the smallest. Zeta potential measurements showed moderate to good stability, with TFF5 at 25.7 mV, indicating minimal aggregation. SEM imaging revealed rough, aggregated vesicular surfaces. Entrapment efficiency varied, with TFF5 achieving 96.69%, indicating strong drug loading. In-vitro studies indicated a controlled release over 14 hours, with 98.97% cumulative release, primarily following Higuchi diffusion kinetics. Antimicrobial testing showed TFF5 enhanced the antibacterial activity of Linezolid against *S. aureus* (7 mm inhibition) and *E. coli* (12 mm inhibition), particularly highlighting its efficacy against gram-negative bacteria, attributed to its nanoscale size and vesicular structure that improve delivery at infection sites.

#### V. Conclusion

Linezolid-loaded transferosomes were effectively developed by the study as a potential topical antibiotic delivery method. Because of its

smallest particle size, highest entrapment effectiveness, good zeta potential, and sustained drug release profile, TFF5 was found to be the best formulation among those studied. When compared to the traditional medication solution, the nanosized, stable, and deformable vesicles greatly increased the antibacterial efficacy of linezolid against both gram-positive and gram-negative bacteria. These results show that, especially in topical applications, transferosomal encapsulation can improve drug delivery, stability, and therapeutic efficacy. With its potential for enhanced patient compliance, extended drug action, and targeted antibacterial therapy, the refined Linezolid transferosomal formulation presents a viable approach for cutting-edge topical treatment of bacterial infections.

#### References

- [1]. Pandey, S., Goyani, M., Devmurari, V., & Fakir, J. (2009). Transferosomes: A novel approach for transdermal drug delivery. *Der Pharmacia Lettre*, 1(2), 143-150.
- [2]. Abdallah, M. A. R. W. A., Neseem, D., Elgazayerly, O., & Abdelbary, A. L. Y. A. (2021). Topical delivery of quercetin loaded transferosomes for wound treatment: in vitro and in vivo evaluation. *Int J App Pharm*, 13(5), 189-97.
- [3]. Hashemian, S. M. R., Farhadi, T., & Ganjparvar, M. (2018). Linezolid: a review of its properties, function, and use in critical care. *Drug design, development and therapy*, 1759-1767.
- [4]. Arya, H., Dhamija, K., Singh, P., & Kumar, G. (2025). Preparation, Characterization and Ex vivo Evaluation of *Trigonella foenum-gracium* seeds extract-loaded Transferosomal gel for the Treatment of Rheumatoid Arthritis. *Research Journal of*

- Pharmacy and Technology, 18(3), 1118-1127.
- [5]. Bnyan, R., Khan, I., Ehtezazi, T., Saleem, I., Gordon, S., O'Neill, F., & Roberts, M. (2019). Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic. *Journal of Pharmacy and Pharmacology*, 71(10), 1508-1519.
- [6]. Ghasemiyeh, P., & Mohammadi-Samani, S. (2020). Potential of nanoparticles as permeation enhancers and targeted delivery options for skin: Advantages and disadvantages. *Drug design, development and therapy*, 3271-3289.
- [7]. Golhar, A. R., Ghume, V. K., Merekar, D. A., Dokhe, M. D., & Patil, D. B. (2020). Formulation and evaluation of liposomal topical gel of linezolid for the treatment of skin infection. *Journal of Emerging Technologies and Innovative Research*, 7(4).
- [8]. Imam, S. S. (2023). Topical formulation constituted with transferosomes for the treatment of non-melanoma skin cancer. *Asian Journal of Pharmaceutical and Clinical Research*.
- [9]. Jain, S., Mishra, D., Kuksal, A., Tiwary, A. K., & Jain, N. K. (2006). Vesicular approach for drug delivery into or across the skin: current status and future prospects. *Pharm Online*, 1, 1-32.
- [10]. Jyolsna, P. (2020). Overview on different organic nanomaterials in medical field. *Journal of Pharmaceutical Sciences and Research*, 12(7), 973-977.
- [11]. Kharwade, R., Ali, N., Gangane, P., Pawar, K., More, S., Iqbal, M., ... & Kaleem, M. (2023). DOE-assisted formulation, optimization, and characterization of tioconazole-loaded transferosomal hydrogel for the effective treatment of atopic dermatitis: in vitro and in vivo evaluation. *Gels*, 9(4), 303.
- [12]. Kumar, A., Nayak, A., Ghatuay, S. K., Dasgupta, S., & Jain, A. P. (2018). Transferosome: A recent approach for transdermal drug delivery. *Journal of Drug Delivery & Therapeutics*, 8.
- [13]. Maji, R., Omolo, C. A., Jaglal, Y., Singh, S., Devnarain, N., Mocktar, C., & Govender, T. (2021). A transferosome-loaded bigel for enhanced transdermal delivery and antibacterial activity of vancomycin hydrochloride. *International journal of pharmaceuticals*, 607, 120990.
- [14]. Modi, C. D., & Bharadia, P. D. (2012). Transfersomes: new dominants for transdermal drug delivery. *Am J Pharm Tech Res*, 2(3), 71-91.
- [15]. Rai, S., Pandey, V., & Rai, G. (2017). Transfersomes as versatile and flexible nano-vesicular carriers in skin cancer therapy: The state of the art. *Nano reviews & experiments*, 8(1), 1325708.
- [16]. Rattanapak, T., Young, K., Rades, T., & Hook, S. (2012). Comparative study of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous immunisation: characterisation and in vitro skin penetration. *Journal of Pharmacy and Pharmacology*, 64(11), 1560-1569.
- [17]. Sahu, J. P., Khan, A. I., Maurya, R., & Shukla, A. K. (2019). Formulation development and evaluation of Transferosomal drug delivery for effective treatment of acne. *Advance Pharmaceutical Journal*, 4(1), 26-34.
- [18]. Sana E, Zeeshan M, Ain QU, Khan AU, Hussain I, Khan S, Lepeltier E, Ali H. Topical delivery of curcumin-loaded transferosomes gel ameliorated rheumatoid arthritis by inhibiting NF- $\kappa$ B pathway. *Nanomedicine*. 2021 Apr 1;16(10):819-
- [19]. Sharma, M., Malik, G., Gulati, D., Kaushik, P., & Arora, S. (2022). Formulation and evaluation of fusidic acid based transferosome for burn wound infection. *Materials Today: Proceedings*, 68, 836-841.