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PREPARATION AND CHARACTERIZATION OF GLYCEROSOMES FORMULATION CONTAINING TOFACITINIB FOR ENHANCED TRANSDERMAL DELIVERY

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ABSTRACT

The present study was aimed at the preparation and characterization of Tofacitinib-loaded glycerosomes for enhanced transdermal drug delivery. Tofacitinib, a poorly water-soluble drug, exhibits limited bioavailability, thereby necessitating the development of a suitable vesicular carrier system to improve its solubility, stability, and controlled release profile. Pre-formulation studies confirmed that Tofacitinib is a white to off-white crystalline solid with a melting point of 199°C, λ max at 286 nm, and pH of 5.3, indicating good purity and physicochemical suitability for formulation development. The drug showed poor aqueous solubility but was freely soluble in methanol and DMSO, supporting the need for a lipid-based delivery system. Glycerosomes were prepared using appropriate lipid and glycerol concentrations and evaluated for physicochemical properties, including particle size, zeta potential, entrapment efficiency, morphology, in vitro drug release, and stability. Among the formulated batches (GSF1–GSF5), GSF3 was identified as the optimized formulation, showing a particle size of 141.8 nm, zeta potential of −43.5 mV, and highest entrapment efficiency of 93.65%, indicating superior vesicular stability and drug loading capacity. Scanning electron microscopy revealed submicron-sized, porous vesicles with irregular morphology, favorable for enhanced drug permeation. The in vitro release study demonstrated a sustained release of 95.97% over 14 hours, following zero-order kinetics (R^2 = 0.9826), suggesting controlled and predictable drug release behavior. Stability studies performed as per ICH guidelines confirmed that the optimized formulation remained stable over 90 days under both normal and accelerated conditions, with negligible changes in particle size (approximately 166–168 nm) and entrapment efficiency (> 92%). Overall, the study concludes that glycerosomes are an effective vesicular delivery system for Tofacitinib, significantly enhancing its stability, controlled release, and transdermal delivery potential. The optimized formulation (GSF3) demonstrated promising characteristics for improving therapeutic efficacy and may serve as a potential carrier system for transdermal drug delivery applications.

KEYWORDS: Tofacitinib; Glycerosomes; Vesicular drug delivery system; Transdermal delivery; Entrapment efficiency; Controlled drug release; Zeta potential; Stability studies.

1. INTRODUCTION

Transdermal drug delivery systems (TDDS) have emerged as an attractive alternative to conventional oral and parenteral routes due to their ability to provide controlled drug release, avoid first-pass hepatic metabolism, improve patient compliance, and reduce systemic adverse effects **(Alkilani *et al.*, 2022).** However, the effectiveness of transdermal delivery is significantly limited by the stratum corneum, the outermost layer of the skin, which acts as a highly efficient barrier against the permeation of most therapeutic agents. Consequently, considerable research has focused on developing novel vesicular carrier systems capable of overcoming this barrier and enhancing drug penetration into deeper skin layers **(Yu *et al.*, 2021).**

Glycerosomes are bilayer vesicles used for dermal and transdermal drug delivery. These vesicles differ from conventional liposomes in bilayer fluidity, formed by the addition of phospholipids and varying concentrations of glycerol (10-30 % v/v). These are so named, as they contain high amount of glycerol. These vesicles deliver the active ingredients to skin with high efficiency **(Ashtiani *et al.*, 2016).** Glycerosomes are found to be more stable and possess greater fluidity then liposomes and hence are predominantly used as topical drug delivery systems. Glycerol ameliorates the deformability index of liposomal bilayers, thus enhancing skin penetration **(Abdallah *et al.*, 2025).** Glycerosomes are new vesicular systems composed of phospholipids and cholesterol just like conventional liposomes. When dispersed in water, phospholipids quickly assemble themselves as bilayer vesicles **(Lombardo *et al.*, 2016).**

Tofacitinib, an oral Janus kinase (JAK) inhibitor, demonstrates significant clinical efficacy in autoimmune therapies, yet its therapeutic potential is hindered by a short elimination half-life and poor systemic bioavailability. It acts by inhibiting Janus kinases, where it is considered a selective Janus kinase inhibitor (JAK) with preferential repression/inhibition of JAK3 and JAK1 and to some extent, JAK2 **(Lundquist *et al.*, 2014).** According to biopharmaceutics classification system (BCS), it belongs to Class III, i.e., highly soluble with low permeability **(Kushner *et al.*, 2020).** Tofacitinib is commercially available in dosage forms such as immediate release (IR) tablets, extended release (XR) tablets, and as oral solution preparations. Adverse effects of tofacitinib include increased levels of alanine transaminase (ALT) and aspartate aminotransferase (AST), nasopharyngitis, dyspnea, anemia, hyperlipidemia, and increased risk of cardiovascular diseases such as leukopenia and thromboembolism **(Morrissey *et al.*, 2019).**

Recent studies have demonstrated that glycerosomal formulations significantly improve the permeation, retention, and bioavailability of various anti-inflammatory, antifungal, antioxidant, and anticancer agents. Their ability to encapsulate both hydrophilic and lipophilic drugs, combined with sustained drug release and excellent stability, makes them particularly suitable for delivering immunomodulatory agents such as tofacitinib. Furthermore, glycerosomes can be incorporated into hydrogels or other semisolid dosage forms to improve viscosity, spreadability, residence time, and patient acceptability for topical application **(Banode *et al.*, 2025).**

This review article aims to provide a comprehensive overview of the preparation, characterization, and therapeutic potential of glycerosomal formulations containing tofacitinib for enhanced transdermal delivery.

2. MATERIALS AND METHODS

2.1 Chemicals

Tofacitinib, were obtained from ZES Bioscience. Renkem provided the DMSO, Methanol and Methanol. Shree Renuka Sugars provided the Ethanol while Sisco Research Laboratories Pvt. Ltd supplied Glycerol. Methyl paraben were procured from Shreeji Pharma International. Phospholipid was supplied by Jigs Chemical Limited. Triethanolamine were obtained from Alpha Chemicals Pvt. Ltd. (Panvel) while Chloroform supplied Tirkuta Chemicals Private Limited.

2.2 Pre-formulation studies

Pre-formulation studies are preliminary researches that investigate the physicochemical and mechanical properties of the active pharmaceutical ingredient (API) Tofacitinib. Pre-formulation studies provide valuable information for selecting acceptable formulation components and designing an effective medication delivery system. Pre-formulation experiments give critical data required for establishing a stable, safe, and efficient Tofacitinib dosage form. **(Srinath K.R. *et al.*, 2011).**

2.2.1 Organoleptic Properties

Organoleptic properties characterize a drug characteristic, such as color, odor, and general look. Tofacitinib organoleptic qualities were assessed by visually inspecting the medicine for color and physical appearance, as well as detecting any characteristic odor under controlled laboratory circumstances **(Sharma D *et al.*, 2025).**

2.2.2 Solubility study Determination

Tofacitinib solubility was tested by adding approximately 1 mg of the drug to separate test tubes containing various polar and non-polar solvents such as distilled water, ethanol, methanol, chloroform, and dimethyl sulfoxide (DMSO). To help the medicine dissolve, each mixture was thoroughly agitated and allowed to stand for a brief length of time. A clear solution indicated that the drug was well dissolved in the solvent, whereas turbidity or the presence of sediment indicated low solubility. The results of this investigation helped in understanding the solubility behavior of Halcinonide in different solvents, as well as selecting acceptable solvents for subsequent formulation and analytical experiments. **(Pignatello R *et al.*, 2022).**

2.2.3 Melting Point Determination

Tofacitinib melting point was measured to verify its identification and purity. Approximately 1 mg of the finely powdered drug was precisely weighed and transferred to a clean, dry capillary tube. The capillary tube with the sample was then placed in a melting point instrument **(Xu J *et al.*, 2022).**

2.2.4 pH determination

The pH of the Tofacitinib solution was determined using a calibrated digital pH meter. A small quantity of the

drug was dissolved in an appropriate solvent to prepare the sample solution. Before measurement, the pH meter was calibrated using standard methanol solutions of known pH values. The measurement was carried out at room temperature, and the determination was performed in triplicate to ensure accuracy and reliability of the results **(Noman MA *et al.*, 2024).**

2.2.5 Lambda max determination

- **Preparation of Standard Stock Solution**

Dissolve the drug in a suitable solvent (commonly distilled water, ethanol, or methanol) to make a 1 mg/mL stock solution. Ensure complete dissolution by gentle stirring or sonication if required. Prepare serial dilutions of the stock solution to obtain concentrations suitable for scanning.

- **Lambda max Determination Procedure**

Using the UV–Visible spectrophotometer, scan the prepared solution over a wavelength range of 200–400 nm. Record the absorbance spectrum of the drug. Identify the wavelength at which the highest absorbance occurs. This wavelength is noted as λ max, which will be used for further quantitative estimations, such as calibration curves or drug content analysis. This method ensures accurate identification of the drug's absorption characteristics, which is critical for stability studies, formulation analysis, and release profiling **(Bitencourt *et al.*, 2015).**

2.2.5.1 Preparation of Tofacitinib standard stock solution in methanol

Tofacitinib (1mg) was carefully weighed and placed to a clean, dry volumetric flask. A tiny amount of methanol (1mL) was added to thoroughly dissolve the medication, producing a standard stock solution at a concentration of 1mg/mL. The solution was carefully combined by moderate shaking or vortexing until the medication was fully dissolved. This prepared stock solution was utilized for making adequate serial dilutions to obtain working standard solutions required for determining λ max, preparing the calibration curve, and quantitative estimate of Tofacitinib in different formulations **(Joseph JA *et al.*, 2022).**

2.2.5.2 Linearity and Calibration Curve

To determine the linearity and construct a calibration curve of Tofacitinib, a series of standard solutions were prepared from the 1mg/mL methanolic stock solution by serial dilution. Typically, five different concentrations (5-25µg/mL) were prepared in methanol. Each solution absorbance was measured at the λ max of Tofacitinib using a UV-Visible spectrophotometer. The absorbance values were then plotted against the corresponding concentrations to generate the calibration curve. The resulting graph showed a linear relationship, typically following Beer's Law, indicating that the absorbance is directly proportional to the concentration within the tested range. **(Gegenschatz SA *et al.*, 2022).**

2.2.6 Fourier Transform Infrared (FT-IR) Spectroscopy of Tofacitinib

The FT-IR spectroscopy of Tofacitinib was performed to identify its characteristic functional groups and confirm its structural integrity. The drug was mixed with dry potassium bromide (KBr), compressed into a transparent pellet, and analyzed using an FT-IR spectrophotometer over the 4000-400 cm^{-1} spectral range. The background spectrum was recorded prior to analysis and automatically subtracted. The obtained spectrum was evaluated for characteristic absorption peaks corresponding to the functional groups of Tofacitinib, thereby confirming the identity and purity of the drug before formulation development **(Drăgan LR *et al.*, 2023).**

2.3 Preparation of Glycerosomes formulation

Tofacitinib-loaded glycerosomes were prepared by the thinfilm hydration method. Phospholipid (10–50 mg), cholesterol (40 mg), and Tofacitinib (2% w/w) were dissolved in a methanol:chloroform (1:1, 20 mL) mixture and stirred at 40°C until a clear solution was obtained. The organic solvents were evaporated under reduced pressure using a rotary evaporator to form a thin lipid film, which was further dried under vacuum overnight. The film was hydrated with 15ml phosphate buffered saline (PBS, Ph 6.8) containing 10% v/v glycol at 40°C with continuous stirring for 1 hour to obtain glycerosomes. The vesicular dispersion was probe-sonicated to reduce particle size and centrifuged at 1500 rpm for 10 min at 4°C to remove unentrapped drug. The purified formulations were lyophilized and stored at 4°C until further evaluation. Five formulations (GF1-GF5) were prepared by varying the phospholipid concentration (10–50 mg) while keeping all other formulation components constant **(Sharma D *et al.*, 2023).**

Table 1: Composition of Glycerosomes formulation.

Formulation code	Tofacitinib Drug (%)	Phospholipid (mg)	Glycerol (%)	Cholesterol (mg)	Methanol (ml)	Chloroform (ml)
GSF 1	2.0	10.0	10.0	40.0	10.0	10.0
GSF 2	2.0	20.0	10.0	40.0	10.0	10.0
GSF 3	2.0	30.0	10.0	40.0	10.0	10.0
GSF 4	2.0	40.0	10.0	40.0	10.0	10.0
GSF 5	2.0	50.0	10.0	40.0	10.0	10.0

2.4 Evaluation parameter of drug loaded Glycerosomes formulation

2.4.1 Physical properties

The glycerosomes formulation was carefully evaluated for its physical attributes, including color, odor, texture, and homogeneity. These characteristics were assessed to ensure the formulation consistency, quality, and suitability for intended application, confirming it meets the required standards for stability and patient acceptability.

2.4.2 Determination of Particle size

The particle size of the drug-loaded glycerosomes was determined using a Malvern Zetasizer, which employs dynamic light scattering (DLS) technology. To limit the effects of multiple scattering, the glycerosomes suspension was diluted in an appropriate medium prior to analysis **(Danaei MR *et al*., 2018).**

2.4.3 Determination of Zeta potential

Zeta potential analyses were used to assess the surface charge and electrophoretic mobility of the glycerosomes particles, which are important markers of formulation stability. The zeta potential was then measured with a Malvern Zetasizer **(Kaur *et al*., 2020).**

2.4.4 SEM analysis

The surface morphology of the generated glycerosomes was investigated using scanning electron microscopy (SEM). To secure the samples, double-sided adhesive tape was gently applied to the metal stubs. The prepared stubs were then inserted into the SEM chamber for imaging. High-resolution photos were obtained with an accelerating voltage of 15 kV to reveal detailed properties of the glycerosomes surface, such as shape, size, and texture. SEM micrographs offered vital insights into the physical properties and homogeneity of the glycerosomes formulation. **(Kendre *et al*., 2022).**

2.4.5 Entrapment Efficiency (EE)

The drug-loaded glycerosomes' entrapment effectiveness was assessed by centrifugation of the formulation for 30 minutes in a REMI Ultra Centrifuge. Following centrifugation, the supernatant with the encapsulated drug was carefully separated and filtered. The concentration of free drug in the supernatant was determined using UV spectrophotometry at 247 nm. The percentage of drug entrapped within the glycerosomes (% EE) was measured by comparing the initial drug amount to the unentrapped drug present in the supernatant, using the conventional entrapment efficiency formula **(Kapoor, *et al*., 2021).**

$$\% EE = \frac{(\text{Initial amount of drug added} - \text{Drug amount in supernatant}) \times 100}{\text{Initial amount of drug added}}$$

2.5 *In-vitro* drug release Study of drug loaded glycerosomes formulation

The *in vitro* drug release profile of the glycerosomes formulation was assessed using the dialysis bag diffusion method. A measured amount of the drug-loaded glycerosomes was placed inside a dialysis membrane, which was then submerged in 100 mL of phosphate buffer solution (pH 7.4). The system was maintained at 37 ± 2°C to simulate physiological conditions and stirred continuously at 100 rpm with a magnetic stirrer to ensure homogenous mixing of the release medium. At specified time intervals, 2 mL aliquots were withdrawn from the release medium and replaced immediately with fresh buffer to maintain sink conditions. The withdrawn samples were appropriately diluted and analyzed via UV-visible spectrophotometry at 301 nm to quantify the amount of drug released.

The drug release data were fitted to various kinetic models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations to elucidate the release mechanism and rate. Zero-order kinetics represent a system where the drug release rate is constant and independent of concentration, whereas first-order kinetics indicate a release rate proportional to drug concentration. The Higuchi model describes drug release as a diffusion-controlled process dependent on the square root of time, based on Fickian diffusion principles. By plotting the release profiles according to these models, insights into whether the drug release is governed by diffusion, erosion, or a combination were obtained, helping to better understand and optimize the formulation's performance. The following kinetic models are commonly used to analyze drug release data:

Zero order kinetics

$$A_t = A_0 - K_0 t \quad \text{----------Eq. (1)}$$

Zero order release would be predicted by the following equation:

Where

At=Drug release at time 't'

A0=Initial drug concentration.

K0=Zero-order rate constant (hr^{-1})

The data will obey zero-order kinetics if the plot, which shows the cumulative percent Time versus drug release, is linear and has a slope equal to the zero-order release constant, K0.

First order kinetics

$$\text{LogC} = \text{logCo} - Kt/2.303 \quad \text{---------- Eq. (2)}$$

First-order release could be anticipated using the subsequent formula:

Where,

C=Amount of drug remained at time 't'

C0=Initial amount of drug.

K=First-order rate constant (hr^{-1}).

First order kinetics is followed by the release, as seen by the straight line that results from plotting the information as log cumulative percent medication remaining vs time. It is feasible to obtain the constant "K1." The release follows first order kinetics, as seen by the straight line that results from plotting the data as log cumulative

percent medication remaining vs time. The slope value multiplied by 2.303 yields the constant "K1."

Higuchi's Model

Higuchi's classical diffusion equation has been employed to characterize the release of drugs from matrix devices by diffusion:

$$Q = [D\epsilon / \tau (2A - \epsilon Cs) Cst]^{1/2} \quad \text{----------Eq. (3)}$$

Where,

Q= Amount of drug release at time 't'

D= Diffusion coefficient of the drug in the matrix.

A= Total amount of drug in unit volume of matrix.

Cs= Solubility of drug in the matrix

Є= Porosity of the matrix.

τ= Tortuosity.

t= Time hrs at which q amount of drug is released.

Above equation can be simplified as if we assume, that 'D', 'Cs' and 'A' are constant. Then equation: becomes**.**

Korsmeyer-Peppas model

Using equation (4) for a polymeric system, a straightforward connection that explained drug release was obtained. To ascertain the release of the medication mechanism, we used this formula:

$$Mt / M\infty = K t^{n} \cdots \quad \text{----------Eq. (4)}$$

Where n is the release exponent, k is the release rate constant, and Mt / M∞ is the fraction of medication released at time t. The n value is used to characterize different release for cylindrical shaped matrices **(Anwer *et al*., 2025).**

2.6 Stability Profiling of the Glycerosomes

To firmly establish the shelf-life resilience and environmental durability of the drug loaded Glycerosomes, a rigorous three-month stability protocol was initiated in strict compliance with current ICH mandates. The finalized batches were partitioned and securely housed under two distinct thermal environments: standard ambient room conditions (maintained at 25°C ± 2°C with a relative humidity of 60 ± 5%) and severe accelerated stress parameters (locked at 40°C ± 2°C against an aggressive humidity of 70 ± 5%). At highly specific intervals specifically day 0, 30, 60, and 90 samples were extracted and subjected to a full spectrum of physical evaluations. These checks monitored any degradation in appearance, Particle Size and Entrapment Efficiency. By directly contrasting these chronological data points against the day-zero baseline metrics, formulators can definitively predict the system long-term commercial viability and structural reliability for advanced clinical applications **(Abd-Allah FI *et al* ., 2010).**

3. RESULTS AND DISCUSSION

3.1 Pre-formulation study of Tofacitinib

3.1.1 Organoleptic properties

Table 2: Organoleptic properties of Tofacitinib.

Parameter	Result
Color	White to off-white
Odor	Odorless
Appearance	Crystalline powder
State	Solid

3.1.2 pH and Melting Point determination

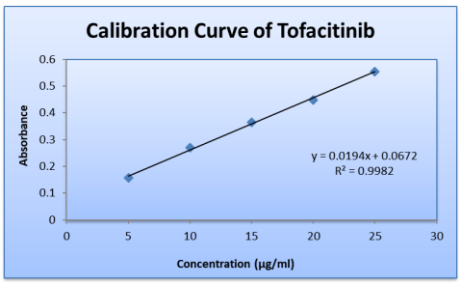
Table 3: pH, Melting Point of Tofacitinib.

Drugs	Observed (pH)	Reference (pH)	Observed (Melting Point)	Reference (Melting Point)
Tofacitinib	5.3	4.5–5.5	199°C	198°C - 202°C

3.1.3 Calibration curve of Tofacitinib

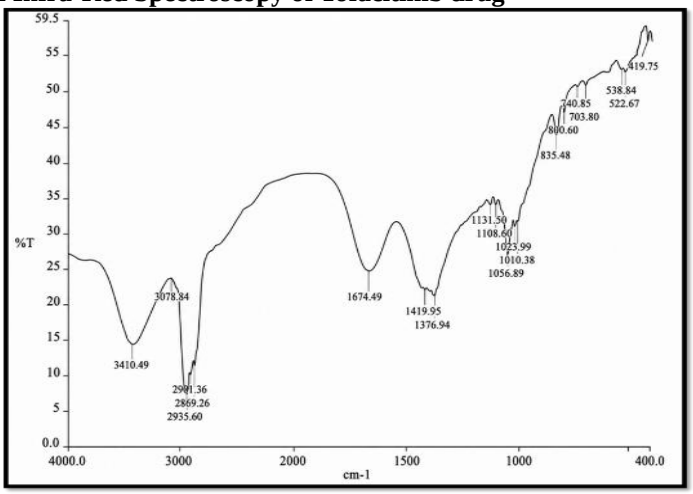
Table 4: Calibration curve of Tofacitinib.

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.159
2	10	0.269
3	15	0.364
4	20	0.449
5	25	0.554
Mean		**0.3588**
SD		**0.153827**
R.S.D.%		**42.737**



Graph 1: Calibration Curve of Tofacitinib.

3.1.4 Fourier transmission Infra-Red Spectroscopy of Tofacitinib drug



Graph 2: FTIR study of Tofacitinib.

Table 5: Interpretation of IR spectrum of Tofacitinib.

Peak obtained	Reference peak cm-1	Functional group	Name of functional group
3410.19	3500- 3400	N-H stretching	Primary amine
3078.84	3300-2500	O-H stretching	Carboxylic acid
2901.36	3000-2800	N-H stretching	Amine salt
2869.26	3000-2840	C-H stretching	Alkane
1674.49	1690-1640	C=N stretching	Imine / Oxime
1419.95	1440-1395	O-H bending	Carboxylic acid

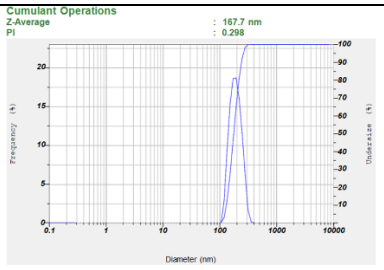
3.2 Characterization Parameters of drug loaded glycerosomes formulation

3.2.1 Physical appearance

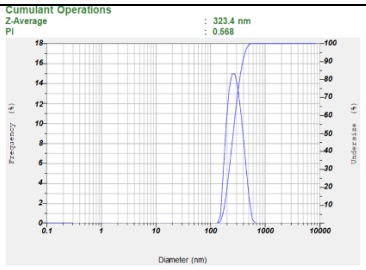
Table 6: Physical appearance of drug loaded glycerosomes formulation.

Parameter	Observation
Color	Milky white dispersion
Odor	characteristic odor
Texture	Smooth, soft, and moderately viscous consistency
Homogeneity	Uniform system with no visible phase separation, precipitation, or aggregation
Physical Appearance	Stable, homogeneous vesicular dispersion indicating proper glycerosomes formation

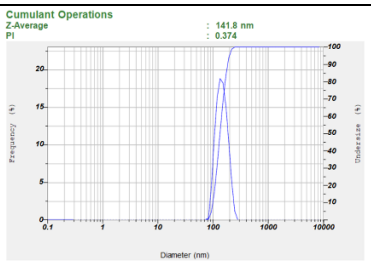
3.2.2 Particle size



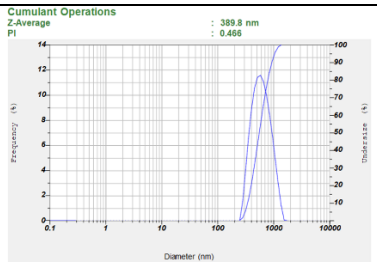
Graph 3: Particle Size (GSF1)



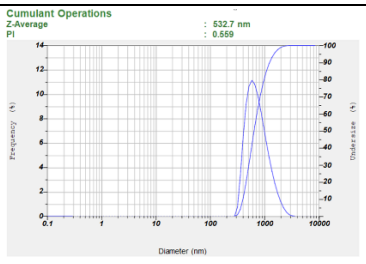
Graph 4: Particle Size (GSF2)



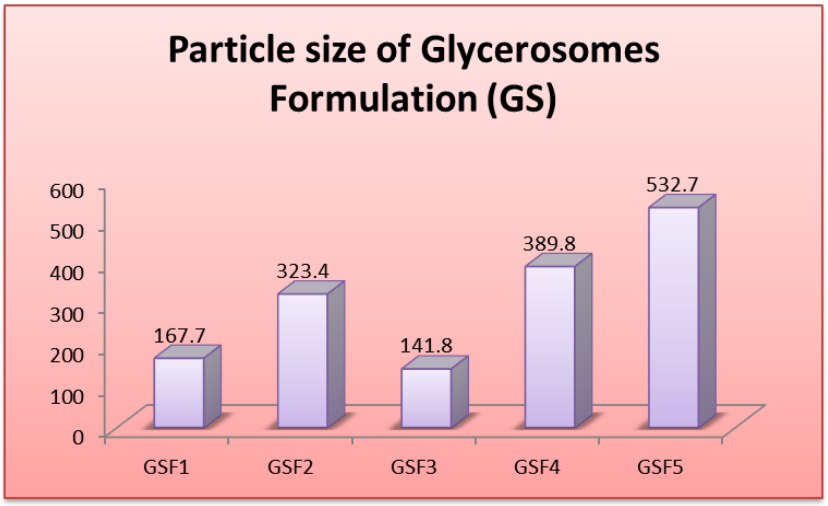
Graph 5: Particle Size (GSF3)



Graph 6: Particle Size (GSF4)

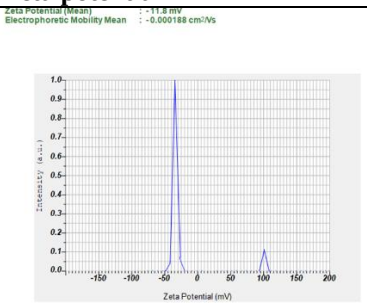


Graph 7: Particle Size (GSF5)

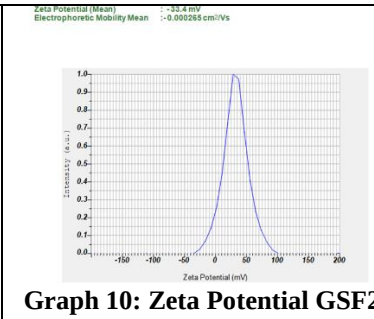


Graph 8: Particle size of glycerosomes formulation

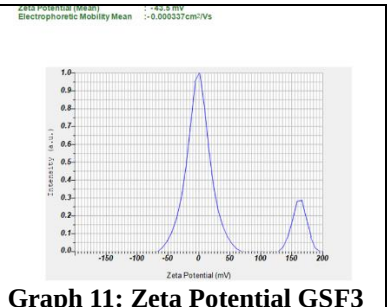
3.2.3 Zeta potential



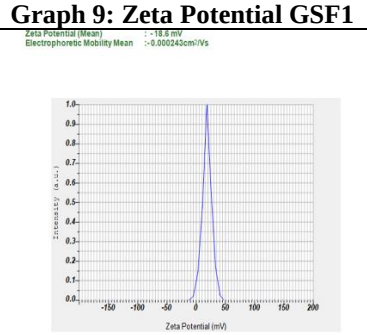
Graph 9: Zeta Potential GSF1



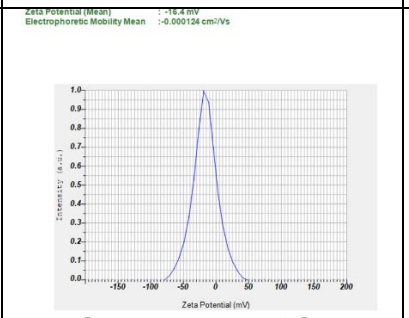
Graph 10: Zeta Potential GSF2



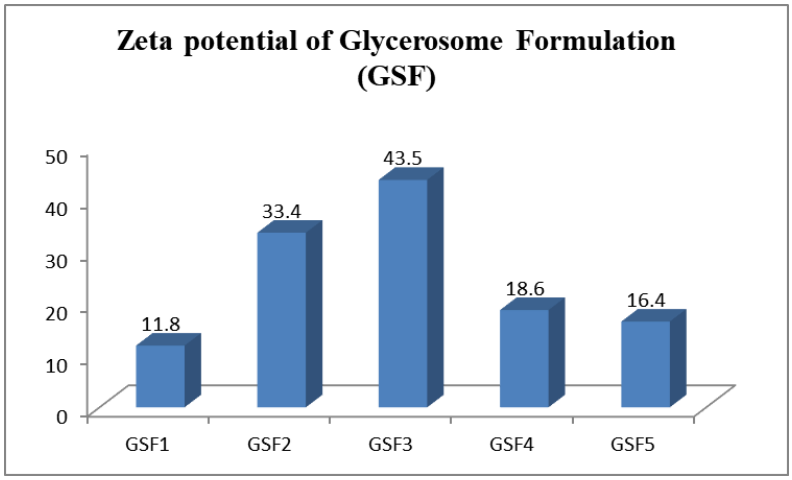
Graph 11: Zeta Potential GSF3



Graph 12: Zeta Potential GSF4



Graph 13: Zeta Potential GSF5



Graph 14: Zeta potential of Glycerosomes Formulation.

3.2.4 Scanning electron microscope (SEM)

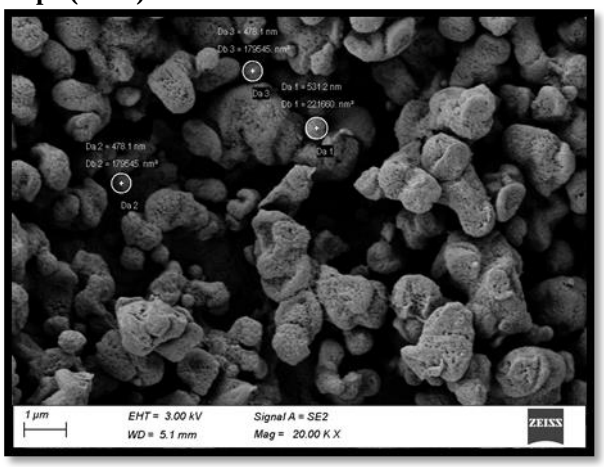


Figure 1: Scanning electron microscope (SEM)

3.2.5 Entrapment efficacy determination

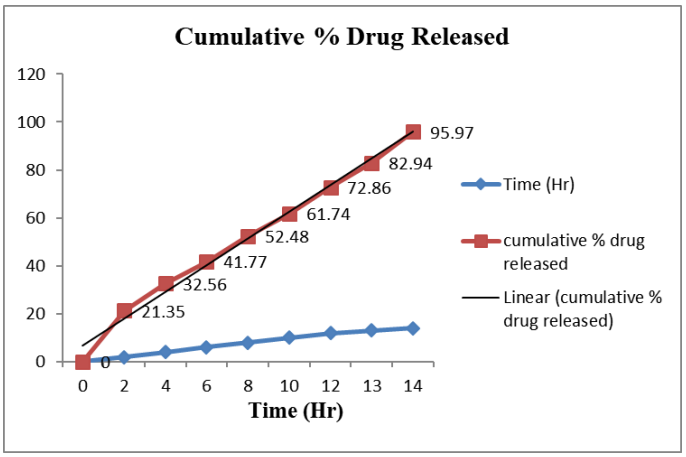
Table 7: Entrapment efficacy.

Formulations (GSF1-GSF5)	Entrapment efficacy (%)
GSF1	84.43
GSF2	86.54
GSF3	**93.65**
GSF4	87.74
GSF5	79.56

3.3 *In Vitro* drug release study

Table 8: *In Vitro* Drug Release Study.

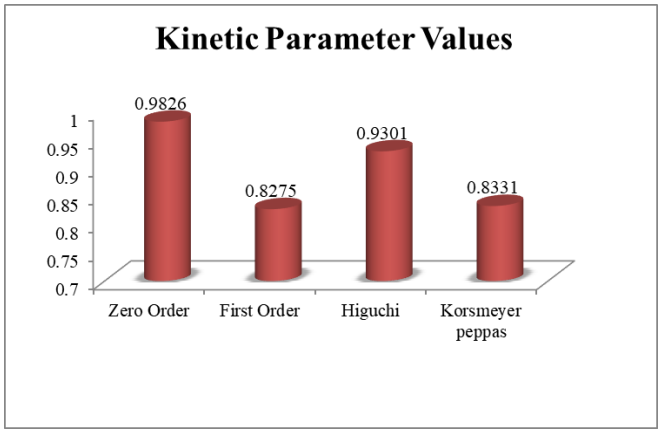
Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
2	21.35	78.65	1.414	1.896	0.301	1.329
4	32.56	67.44	2.000	1.829	0.602	1.513
6	41.77	58.23	2.449	1.765	0.778	1.621
8	52.48	47.52	2.828	1.677	0.903	1.720
10	61.74	38.26	3.162	1.583	1.000	1.791
12	72.86	27.14	3.464	1.434	1.079	1.862
13	82.94	17.06	3.606	1.232	1.114	1.919
14	95.97	4.03	3.742	0.605	1.146	1.982



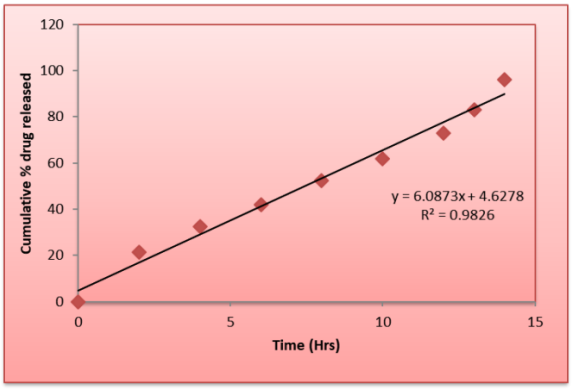
Graph 15: Drug Release Study.

Table 9: Correlation value (R2 value).

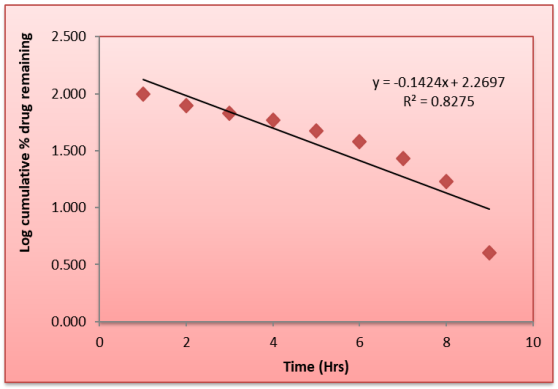
Formulation	Model	Kinetic parameter values
Glycerosomes Formulation	Zero Order	$R^2 = 0.9826$
	First Order	$R^2 = 0.8275$
	Higuchi	$R^2 = 0.9301$
	Korsmeyerpeppas	$R^2 = 0.8331$



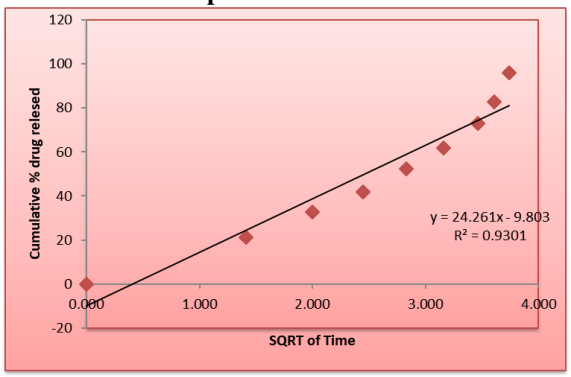
Graph 16: Kinetic Parameter.



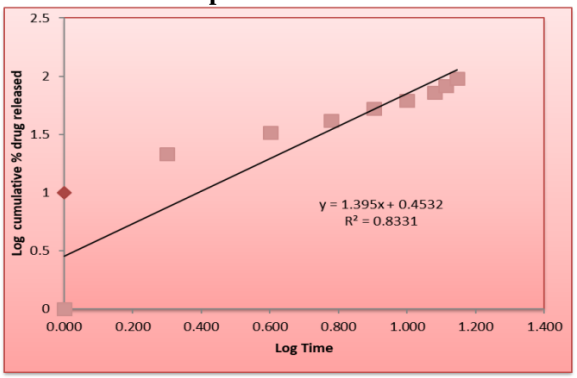
Graph 17: Zero order



Graph 18: First order



Graph 19: Higuchi



Graph 20: Korsmeyer peppas

3.4 Stability Profiling

Time (Days)	25°C ± 2°C / 60 ± 5% RH		40°C ± 2°C / 75 ± 5% RH	
	Particle Size (nm)	Entrapment Efficiency (%)	Particle Size (nm)	Entrapment Efficiency
0	167.7	93.65	166.2	94.89
30	167.8	94.21	167.56	94.11
45	166.9	93.88	167.68	93.72
60	167.2	93.15	165.89	92.87
90	167.6	93.45	166.98	93.65

DISCUSSION

The pre-formulation studies confirmed that Tofacitinib possesses suitable physicochemical properties for glycerosome formulation. The drug was observed as a white to off-white, odorless, crystalline solid with a melting point of 199°C, pH of 5.3, and λmax at 286 nm, confirming its identity, purity, and stability. Solubility studies showed high solubility in DMSO and methanol, moderate solubility in ethanol, and poor solubility in water, supporting the need for a lipid-based carrier system to improve drug delivery. The calibration curve exhibited excellent linearity ($R^2 = 0.9982$), validating the UV analytical method. FTIR analysis confirmed the presence of characteristic functional groups without significant shifts, indicating compatibility between Tofacitinib and the formulation excipients.

Among the five glycerosome formulations (GSF1–GSF5), GSF3 was identified as the optimized formulation, exhibiting the smallest particle size (141.8 nm), highest negative zeta potential (−43.5 mV), and maximum entrapment efficiency (93.65%), indicating excellent stability and drug-loading capacity. SEM analysis revealed irregular, porous submicron particles favorable for enhanced drug release and skin permeation. Stability studies performed for **90 days** under both long-term and accelerated conditions showed only minimal changes in particle size and entrapment efficiency, confirming the excellent physical and chemical stability of the optimized formulation. Overall, the results demonstrate that Tofacitinib-loaded glycerosomes are a promising nanocarrier system for improving drug stability, encapsulation, and controlled topical/transdermal delivery.

4. CONCLUSION

The present study successfully developed and characterized Tofacitinib-loaded glycerosomes for enhanced topical and transdermal drug delivery. Pre-formulation studies confirmed the suitability of Tofacitinib for formulation development. Among the five formulations (GSF1–GSF5), GSF3 was identified as the optimized formulation, exhibiting the smallest particle size (141.8 nm), highest zeta potential (-43.5 mV), and maximum entrapment efficiency (93.65%), indicating excellent stability and drug-loading capacity. The optimized glycerosomes showed sustained drug release (95.97% in 14 hours) following zero-order kinetics ($R^2 = 0.9826$) and remained stable for 90 days under both long-term and accelerated storage conditions with only minor changes in physicochemical properties. Overall, the findings demonstrate that glycerosomes are a promising carrier system for improving the stability, entrapment, and controlled release of Tofacitinib, making them suitable for topical and transdermal applications. Further ex vivo, in vivo, and clinical studies are recommended to confirm their therapeutic efficacy and safety.

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