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PREPARATION AND DEVELOPMENT OF ECONAZOLE USING TRANSFERSOMALS GEL ON ANTIFUNGAL ACTIVITY

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ABSTRACT

This study aimed to enhance the penetration of Econazole into the skin through topical application by formulating a transferosomal gel. The research was conducted in three phases: preformulation studies and carrier optimization, preparation and characterization of transferosomal gel, and in vitro evaluation of the delivery system. In the preformulation phase, Econazole exhibited favorable physical and chemical properties, making it suitable for formulation. Transferosomal formulations were developed with nanoscale size, high encapsulation efficiency, and controlled release profiles. Formula F4, demonstrating optimal characteristics, was chosen for incorporation into the gel. Gel formulations displayed desirable viscosity, skin-compatible pH, and uniform drug content. Transferosomal gel formulations significantly enhanced the permeation of Econazole compared to plain gel, demonstrating their potential for improved drug delivery. Release kinetics analysis revealed a delayed drug release pattern consistent with diffusion and erosion mechanisms, indicating controlled release behavior. Stability studies indicated that refrigeration preserved the physical stability of transferosomal vesicles, while higher temperatures led to decreased encapsulation efficiency due to lipid bilayer fluidity. In conclusion, transferosomal gel formulations offer a promising strategy for enhancing the delivery of Econazole through the skin. These formulations have the potential to improve therapeutic outcomes in topical applications, providing a valuable option for the treatment of fungal infections.

KEYWORDS: Econazole, Transferosomes, Gel formulation, Topical delivery.

INTRODUCTION

Fungal infections represent a significant burden on global healthcare systems, necessitating effective and patient-friendly treatments. Among the arsenal of antifungal agents, econazole stands out for its broad-spectrum activity against various fungal pathogens.[1] However, traditional formulations of econazole often fall short in terms of efficacy and patient compliance.[2] To address these challenges, researchers have turned to innovative drug delivery systems, such as transfersomes, to enhance the therapeutic performance of econazole.[3]

Transfersomes, lipid-based vesicular carriers capable of traversing biological barriers, offer promising prospects for improving drug delivery efficiency.[4] By encapsulating econazole within transfersomes and incorporating them into a gel matrix, researchers aim to harness the synergistic advantages of both formulations.[5] This approach not only enhances the bioavailability of econazole but also facilitates targeted delivery to the site of infection, potentially enhancing its antifungal activity.[6]

According to Cevc and Blume (2001) introduced the concept of transfersomes as highly efficient carriers for topical and transdermal drug administration. Transfersomes, lipid-based vesicular structures, possess deformable properties that enable them to penetrate biological barriers effectively. This characteristic has sparked interest in their application in drug delivery, including antifungal agents like econazole.[7] El-Samaligy et al. (2006) explored the potential of transfersomal carriers for the topical delivery of aceclofenac, demonstrating improved drug permeation and bioavailability. Their study underscored the versatility and effectiveness of transfersomes in enhancing the delivery of pharmaceutical agents across biological membranes. Building upon this foundation, researchers have investigated the formulation and evaluation of econazole-loaded transfersomal gel formulations for topical antifungal therapy.[8] Sivasankar et al. (2014) conducted a study to develop and assess the performance of econazole nitrate transfersomes incorporated into a gel matrix. Their findings indicated enhanced drug permeation and sustained release characteristics compared to conventional gel formulations. Moreover, the econazole transfersomal gel exhibited superior antifungal activity against various fungal strains in vitro, highlighting its potential as a promising therapeutic option for fungal infections.[9] Furthermore, Fang and Tsai (2006) provided insights into the application of transfersomes for transdermal drug delivery,

emphasizing their role in improving drug bioavailability and minimizing systemic side effects. Their review highlighted the versatility of transfersomal carriers in delivering a wide range of therapeutic agents, including antifungals, across the skin barrier.[10]

These studies collectively underscore the potential of transfersomal gel formulations in enhancing the therapeutic outcomes of econazole in the treatment of fungal infections. By leveraging the unique properties of transfersomes, such as deformability and enhanced permeation, econazole transfersomal gels offer a promising approach to overcome the limitations of conventional formulations, including poor drug penetration and low bioavailability. This research paper aims to explore the preparation and development of econazole-loaded transfersomal gel formulations and their impact on antifungal efficacy. By investigating the physicochemical properties, drug release kinetics, and in vitro/in vivo antifungal activity of these formulations, we seek to elucidate their potential as advanced therapeutic options for fungal infections.

MATERIAL

During the course of the study, various materials and equipment were utilized to formulate and evaluate the transfersomal gel containing econazole nitrate. The materials included econazole nitrate obtained from GSK Pharmaceuticals Ltd., cholesterol, soybean lecithin, sodium cholate, sodium chloride, potassium dihydrogen orthophosphate, and disodium hydrogen orthophosphate sourced from S.D. Fine Chem. Ltd., India, while Span 80 and Brij-35 were procured from Loba Chemie, India. Additionally, chloroform, methanol, and n-propanol were acquired from Rankem and Nice Chemicals, India, respectively. The dialysis membrane used in the study was obtained from HiMedia, India.

Furthermore, various equipment were employed for formulation and characterization purposes, including an analytical balance, dissolution apparatus, optical microscope (Olympus), UV spectrophotometer (Systronic, India), FT-IR spectrometer (Bruker, India), scanning electron microscope (SEM, JSM – 840A, JEOL, Japan), digital pH meter (Digisun Electronics, Hyderabad), melting point apparatus, hot air oven (Techno Scientific, Bangalore), refrigerated centrifuge (Hitachi Ltd Japan), and laminar flow hood, all sourced from different manufacturers.

METHODOLOGY

Preformulation study

Preformulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms.

Organoleptic properties

Appearance

Transferred approximately 1gm of the sample on a white paper spreaded uniformly and examined visually.

Colour

A small quantity of pure drug powder was taken in a butter paper and viewed in well illuminated place.

Solubility

Aqueous solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy. Solubility of Econazole Nitrate was determined in water and methanol, ethanol, chloroform and ethyl acetate and other common solvents.

Table 5.3: Solubility specifications

Descriptive terms	Approximate volume of solvent in millilitres per gram of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	More than 10,000

Melting point determination

Melting point of Econazole Nitrate was determined by Open capillary method.

Determination of partition coefficient

25 mg of Econazole Nitrate with aqueous phase and n-octanol was taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for

equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phases was calculated.

Determination of λmax

A solution of Econazole Nitrate containing the concentration 1000µg/ ml was prepared in PBS pH 6.8 and UV spectrum was taken using double beam spectrophotometer(Systronic, 2200). The solution was scanned in the range of 200 – 400 nm.

Preparation of standard calibration curve of Econazole Nitrate in PBS 7.4 pH Buffer

From the above Econazole Nitrate standard stock solution (1000µg/ml), 1ml solution was diluted to 10 ml using PBS pH 6.8solution to get concentrations of 100 µg/ml. from this solution, aliquots of, 0.5 ml, 1 ml, 1.5 ml, and so on from standard drug solution were diluted to 10 ml to prepare 10-50 µg/ml dilutions. The absorbance of these solutions was measured against PBS pH 7.4 as a blank.

Drug – Excipient Interaction Studies by FTIR

Infra-red spectra matching approach was used for the detection of any possible chemical reaction between the drug and the excipients. A physical mixture (1:1) of drug and excipients was prepared and mixed with suitable quantity of potassium bromide. About 100 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 10 tones pressure. It was scanned from 4000 to 150 cm-1 in a Bruker FTIR spectrophotometer. The FTIR spectrum of the physical mixture was compared with those of pure drug and excipients and matching was done to detect any appearance or disappearance of peaks.

Preparation of econazole nitrate loaded transferosomal formulations[11]

Transferosomes were prepared using the thin film hydration method. Soybean phosphatidylcholine, cholesterol, sodium cholate, Span 80, and Brij 35 were dissolved in a mixture of methanol, chloroform, and ethanol (2:2:1 v/v/v) to form a thin lipid film on the internal surface of a round-bottomed flask. Econazole Nitrate (100 mg) was dissolved in isotonic phosphate buffer (pH 5.8) and used to hydrate the lipid film by rotation at 100 rpm for 2 hours. Large multilamellar vesicles were formed by allowing the suspensions to stand for 24 hours at 25°C, while smaller vesicles were obtained by sonication for 30 minutes.

The Econazole Nitrate transferosomes were separated from the entrapped drug by high-speed centrifugation at 20,000 rpm for 3 hours at −5°C using a cooling ultracentrifuge. The untrapped drug was separated by carefully removing the clear supernatant after centrifugation, leaving behind the precipitate containing the entrapped Econazole Nitrate. The precipitate was resuspended in isotonic phosphate buffer (pH 5.8) for evaluation, and the transferosomal dispersions were stored at 4°C in glass vials.

Table 1: Composition of transferosomal formulations.

Formulation code	Econazole Nitrate	Cholesterol	Lecithin	Sodium Cholate	Span 80	Brij 35
TF-1	100	2	1	4	-	-
TF-2	100	2	1	3	-	-
TF-3	100	2	1	2	-	-
TF-4	100	2	1	-	4	-
TF-5	100	2	1	-	3	-
TF-6	100	2	1	-	2	-
TF-7	100	2	1	-	-	4
TF-8	100	2	1	-	-	3
TF-9	100	2	1	-	-	2

Evaluation of transferosomal formulations

Morphological study

Vesicle formation was confirmed using optical microscopy at a resolution of 45×. The transferosomal suspension was placed onto a glass slide and fixed by drying at room temperature. The resulting dry thin film of the transferosomal suspension was observed for the formation of vesicles. Microphotographs of the transferosomes were obtained from the microscope using a digital camera.[12]

Particle size analysis

The vesicle sizes of transferosomes were determined by light scattering based on laser diffraction using a Malvern Mastersizer (Malvern Instruments, Malvern, UK). The apparatus consisted of a HeNe laser (5 mW) and a small-volume sample-holding cell. The sample was stirred using a magnetic stirrer bead to keep and maintain the sample in suspension.

Zeta potential

The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly

charged particles in dispersion. The zeta potential for the Transferosomal dispersion was determined using Malvern instruments.[13]

Entrapment efficiency

The percentage of Econazole Nitrate loading in transferosome was determined by using 4.0 mL of dispersion. Free Econazole Nitrate was separated from the transferosomal dispersions by subjecting the transferosomes to a high-speed centrifugation at 21,000 rpm at 10°C model T-70BL (Laby Instrument Industry, Haryana, India) for 3 hours. The supernatant was siphoned-off and analyzed using a UV spectrophotometer. The precipitate separated from supernatant was redispersed in 4 mL of isotonic phosphate buffer (pH 7). To perform the lysis of transferosomes for liberating the encapsulated Econazole Nitrate molecules, a 500 µL was diluted ten times with methanol (HPLC grade, ≥99.9%). The concentration of drug was determined spectrophotometrically.[14]

% Entrapment efficiency = [(TD-FD)/TD] × 100

where TD is the total drug amount, and FD is the amount of free drug.

***In-Vitro* drug release study**

The in vitro release study was performed via a dialysis membrane according to Hao's method. Briefly, an equivalent amount of 10 mg Econazole Nitrate -loaded transferosomal dispersion was introduced into dialysis bags with a molecular weight cutoff 12,000 kDa. The dialysis bags were suspended in an isotonic buffer solution (250 mL, pH 6.8, 37°C±2°C) at speed of rotation 1,500 rpm and placed within the dissolution flask of the USP dissolution apparatus. The samples (5 mL) were withdrawn and analyzed spectrophotometrically every 45 minutes for 12 hours. The withdrawn samples were replaced with the same volume of fresh an isotonic buffer solution (pH 6.8). The concentration percentage of Econazole Nitrate at time (t) was estimated.[14]

Formulation of transferosomes entrapped econazole nitrate gel

The gel was prepared by the same procedures described by Schmolka (1972). In brief, in 10 mL distilled water, a required quantities of poloxamer 407 and HPMC k15 were added slowly and stirred with the help of magnetic stirrer at 50 rpm for 1 hour. To ensure the maximum dissolution of polymers, the prepared solution was left in the quiescent state for 12 hours in a refrigerator. Then, the solution (poloxamer with HPMC k15) was stirred slowly at 5°C for 5 hours until a gel was formed. Various formulations were prepared as shown in Table 2.[15]

Table 2: Composition of transferosomal gel formulations.

Formulation code	Poloxamer 407	HPMC k15	Propylene glycol	DMSO
TFG-1	0	15	-	-
TFG-2	10	20	-	-
TFG-3	10	25	-	-
TFG-4	10	20	0.5	-
TFG-5	10	20	-	0.5

Evaluation of transferosomal gel

Physical appearance

The prepared gel was examined for clarity, colour, homogeneity and the presence of foreign particles.

pH

The pH of the dispersion was measured by using a digital pH meter.

Rheological study

Viscosity measurement: Viscosity was determined by Brookfield programmable DV III ultra viscometer. In the present study, spindle no. CP 52 with an optimum speed of 0.01 rpm was used to measure the viscosity of the preparation.

Content uniformity

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 10 mg of the drug and triton X-100 (1%) in small amount of water shaken it vigorously and taken in 100 ml volumetric flask and volume was made up to 100 ml with methanol. The content was filtered through Whatman filter paper No. 41. 5 ml of above solution was taken into a 25 ml volumetric flask and volume was made up to mark with methanol. The content of Econazole Nitrate was determined against blank by using the Shimadzu UV/visible spectrophotometer. The drug content was determined from the calibration curve of drug.

***In Vitro* drug release study**

The apparatus consists of a glass cylinder open at both ends. A dialysis membrane soaked in distilled water (24 h before use) is fixed to the one end of the cylinder with the aid of an adhesive. Gels equivalent to 10 mg of drug is taken inside the cell (donor compartment) and the cell is immersed in a beaker containing 100 ml of PBS pH 7.4 containing 10% v/v methanol (to maintain sink condition), act as receptor compartment. The whole assembly is

fixed in such a way that the lower end of the cell containing gel is just above the surface of the diffusion medium (1-2 mm deep) and the medium was agitated using a magnetic stirrer at the temperature 37 ± 0.5°C. Aliquots (5 ml) are withdrawn from the receptor compartment periodically and replaced with same volume with fresh buffer. The samples were analyzed by using UV-visible spectrophotometer. The tests were carried out in triplicate.

Kinetic modelling of *in -vitro* release rates of formulations

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

Zero-order kinetic model- Cumulative percentage drug release versus time.

First- order kinetic model- Log cumulative percentage drug release remaining versus time.

Higuchi's model- Cumulative percentage drug released versus square root of time.

Korsmeyer's equation/peppa's model- Log cumulative percentage drug released versus log time.

Zero-order kinetics

Zero order release would be predicted by the following equation

$$\mathbf{A_t = A_0 - K_0\, t}$$

Where,

A = Drug release at time 't'

A_0 = Initial drug concentration

K_0 = Zero order rate constant (hr-1)

When the data is plotted as cumulative percent drug release versus time, if the plot is linear then the data obeys zero-order release kinetics, with a slope equal to K0.

First- order kinetics

First-order release would be predicted by the following equation:-

$$\mathbf{Log\ C = log\ C0 - Kt\ /2.303}$$

Where,

C=Amount of drug remained at time't' C0=Initial amount of drug

K=First-order rate constant (hr-1)

When the data is plotted as log cumulative percent drug remaining versus time yields a straight line, indicating that the release follow first order kinetics. The constant K can be obtained by multiplying 2.303 with slope values.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation.

$Q = [D\varepsilon / \tau (2A - \varepsilon Cs) Cst]$ 1/2

Where,

Q=Amount of drug released at time‘t’ D=Diffusion coefficient of drug in the matrix A=Total amount of drug in unit volume of matrix Cs= The solubility of drug in the matrix

ε= Porosity of the matrix

τ= Tortuosity

t= Time (hrs) at which Q amount of drug is released

Above equation may be simplified if one assumes that D, Cs, and A, are constant.

Then equation becomes:

$Q = K t ½$

When the data is plotted according to equation i.e., cumulative drug release versus square root of time yields a straight line, indicating that the drug was released by diffusion mechanism. The slope is equal to 'K'.

Korsmeyer’s equation/ peppa's model

To study the mechanism of drug release from the solid dispersions, the release data were also fitted to the well-known exponential equation (Korsmeyer’s equation/peppa's law equation), which is often used to describe the drug release behaviour from polymeric systems.

$Mt/Ma = Ktn$

Where,

Mt/Ma = The fraction of drug released at time‘t’

K= Constant incorporating the structural and geometrical characteristics of the drug/polymeric

N= Diffusion exponent related to the mechanism of release

Above equation can be simplified by applying log on both sides, and we get:

$\text{Log } Mt/Ma = \text{Log } K + n \text{ Log } t$

When the data is plotted as log of drug released versus log time, yields a straight line with a slope equal to 'n' and the 'K' can be obtained from y-intercept. For Fickian release 'n' =0.5 while for anomalous (non-Fickian) transport 'n' ranges from 0.5 to 1.0 as shown below.

S. No	n Value	Drug release
1.	n < 0.5	Fickian release
2.	0.5<n<1	Non- Fickian release
3.	n>1	Case II transport

Stability studies

As soon as the product is developed, it is subjected to ageing; as a result, its physical properties, chemical composition and even its biological availability may be changed. To assess long-term stability, gel-based formulations were stored in gel tube at (4°C±1°C, 25°C±1°C, and 40°C±1°C), 75% relative humidity (RH) ±5% for a period of up to 3 months. They were evaluated periodically for the following parameters[16]

- Appearance
- Viscosity
- pH
- Drug Content analysis
- % Drug Release

RESULTS

Physical appearance

The drug was obtained as a kind gift from GSK Pharmaceuticals Ltd. The supplied powder of Econazole Nitrate was white, odourless White to yellowish white powder.

Melting point

Melting point of Econazole Nitrate was determined by melting point apparatus (Tempo) and found to be 174.5±2°C.

Table 3: Solubility of econazole nitrate in different solvents.

S. No.	Solvent	Solubility
1.	Water	Slightly Soluble
2.	Methanol	Sparingly Soluble
3.	Ethanol	Sparingly Soluble
4.	DMSO	Soluble
5.	Phosphate buffer	Soluble

++++ = Freely soluble 1-10 parts, +++ = Sparingly soluble 30-100 parts, ++ = Soluble 30-100 parts, + = Slightly soluble 100-1000 parts, – = Practically insoluble >10000 parts

Table 4: Partition coefficient value of econazole nitrate.

S. No.	Solvent system	Partition Coefficient
1.	n-Octanol/PBS (pH 6.8)	5.45

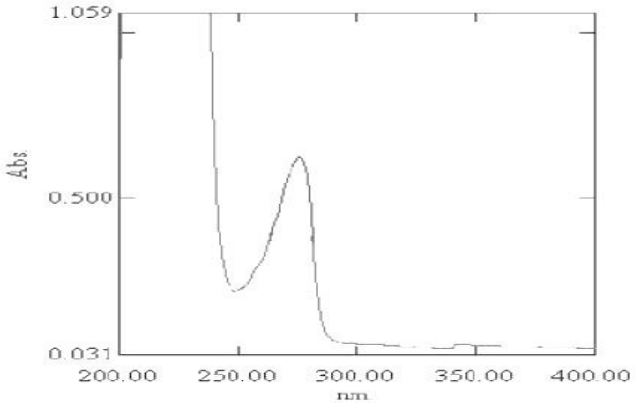

Figure 1: UV spectra of Econazole Nitrate in PBS Buffer.

Infrared spectroscopy

It was done by making pellets of the drug in KBr. FTIR spectra was taken at Thermo Instrument. The observed peaks were compared with those (Florey, 1973) reported for functional groups.

Table 5: Important band frequencies in FTIR spectrum of Econazole Nitrate.

S. No.	Named Group	Reported Band frequency	Band frequency obtained
1.	Imidazole C-N stretching	3140-1475	1409
2.	Aromatic C-H stretching	3000-3100	3107
3.	Aliphatic C-H stretching	2850-3000	2962
4.	C=C aromatic	1450-1590	1587
5.	C-Cl halogen attached at benzene ring	650-800	754
6.	Ether C-O-C stretch ether	1050-1250	1089

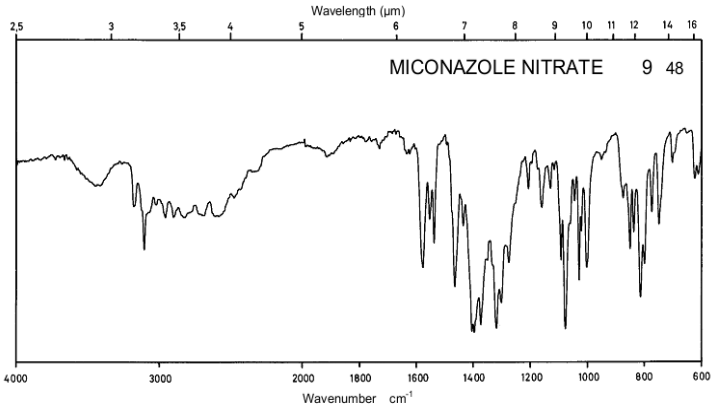

Figure 2: Reference FTIR spectra of Econazole Nitrate.

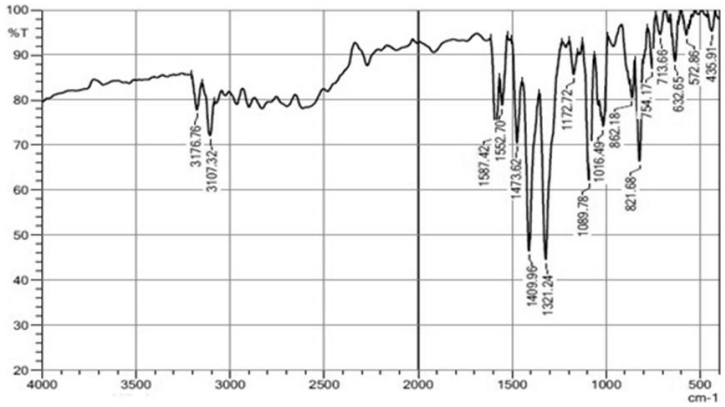

Figure 3: FTIR spectra of econazole nitrate.

Standard Curve of Econazole Nitrate in Phosphate Buffer Solution (pH 6.8)

All dilutions and measurements were made as above in phosphate buffer solution of pH 6.8 made as per formula (I.P.). The absorbance was taken at λ_{max} 265.6 nm against a reagent blank. The standard curve was plotted between absorbance and concentration.

Table 6: Standard Curve of Econazole Nitrate in Phosphate Buffer Solution (pH 6.8).

S. No.	Drug Conc. (µg/ml)	Absorbance at 272.2 nm
1.	10	0.141
2.	20	0.285
3.	30	0.429
4.	40	0.534
5.	50	0.653

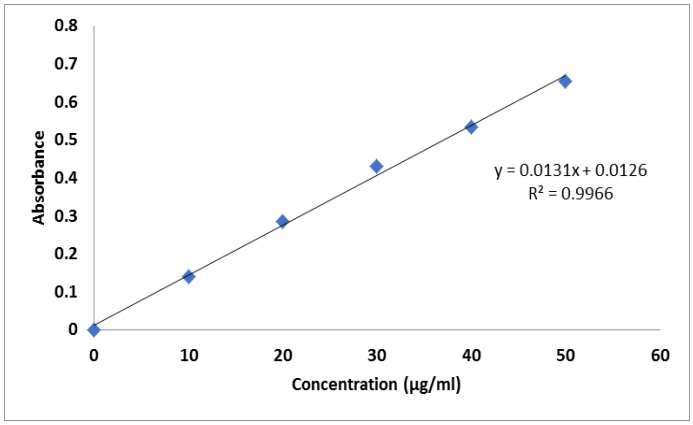

Figure 4: Standard curve of Econazole Nitrate in phosphate buffer solution (pH 6.8) at 272.2 nm

Table 7: Evaluation of econazole nitrate loaded transferosomal formulation.

Formulation Code	Mean particle size (µm)	Zeta potential (mv)	Encapsulation efficacy (%)
TS-1	171.57±2.10	-53.34±2.27	70.31±4.63
TS-2	178.61±2.35	-38.22±1.35	69.28±6.47

TS-3	184.38±4.13	-25.62±3.65	67.08±3.84
TS-4	188.48±2.61	-45.68±1.45	83.86±5.27
TS-5	192.89±3.16	-40.53±4.61	79.47±7.54
TS-6	197.93±2.27	-35.91±2.72	78.27±6.19
TS-7	162.54±1.20	-33.29±1.16	74.43±5.44
TS-8	171.68±3.32	-30.98±3.57	69.18±5.95
TS-9	193.83±3.50	-28.56±1.42	64.93±4.65

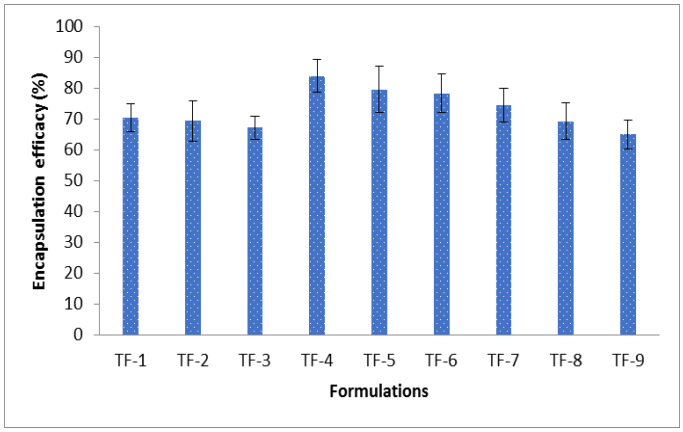

Figure 5: Entrapment efficiency of econazole nitrate loaded transferosomal formulation.

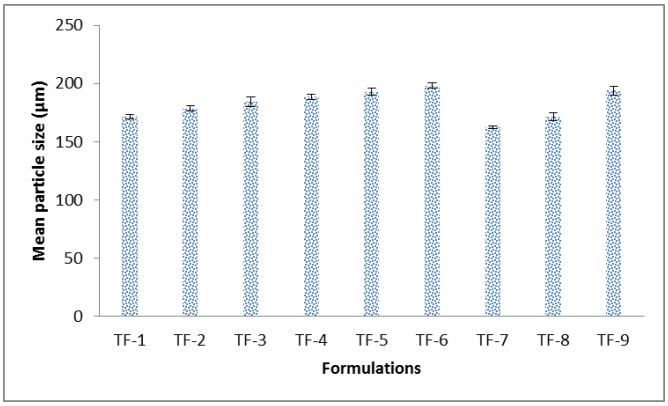

Figure 6: Mean particle size (µm)of Econazole Nitrate Loaded Transferosomal Formulation.

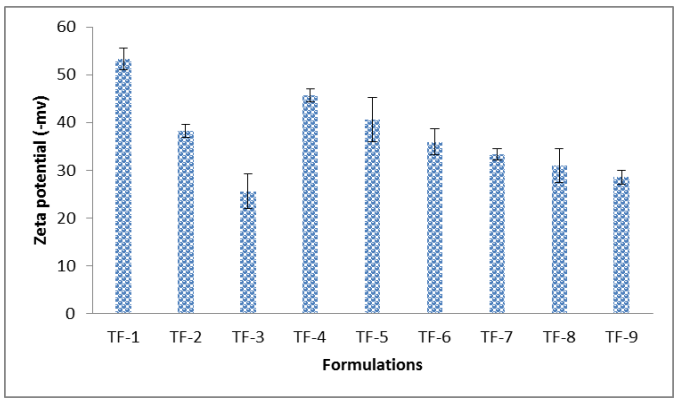

Figure 7: Zeta potential (-mv) of econazole nitrate loaded transferosomal formulation.

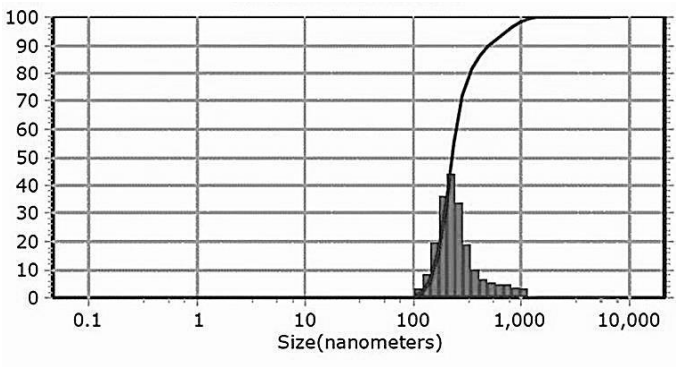

Figure 8: Particle size distribution of econazole nitrate loaded transferosomal formulation (TF3)

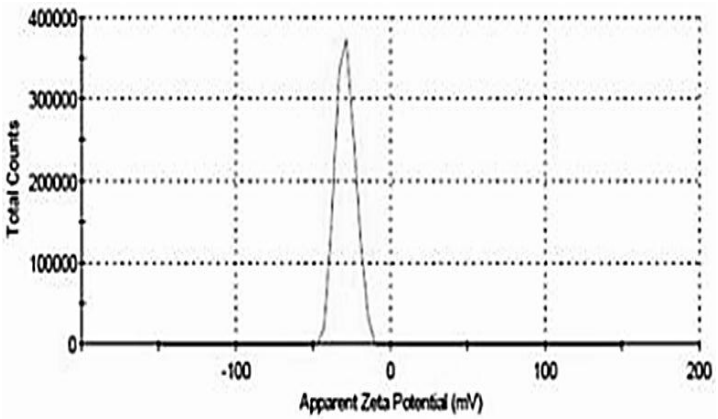

Figure 9: Zeta potential of econazole nitrate loaded transferosomal formulation (TF3).

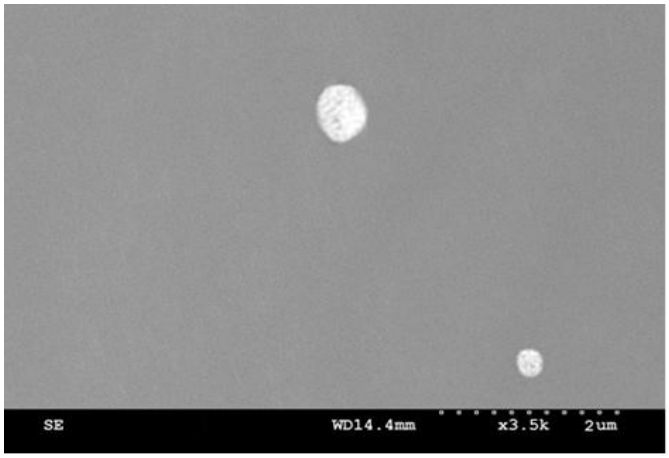

Figure 10: SEM photograph of econazole nitrate loaded transferosomal formulation (TF3)

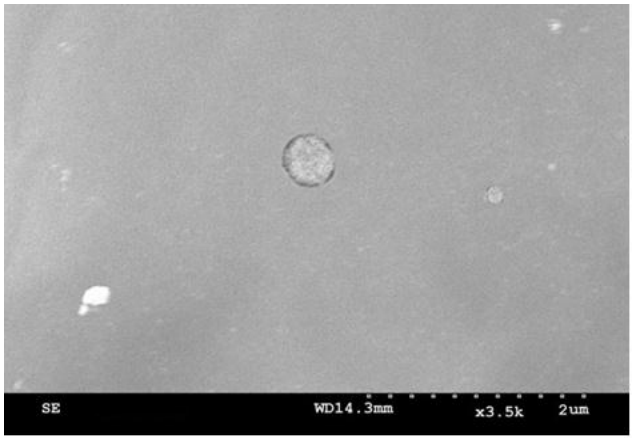

Figure 11: SEM photograph of econazole nitrate loaded transferosomal formulation (TF4)

Table 8a: Cumulative % of drug release of econazole nitrate loaded transferosomal formulation.

F. Code / Time	Cumulative % of drug release (in 10 hr.)				
	TF-1	TF-2	TF-3	TF-4	TF-5
0	0	0	0	0	0
0.25	11.85±1.56	9.94±5.33	18.08±1.18	19.32±2.23	17.35±4.66
0.5	22.29±1.32	14.85±1.67	26.61±2.09	29.67±3.86	15.96±4.53
1	37.82±1.98	20.98±3.54	27.32±3.08	35.09±2.06	25.97±3.79
2	45.97±2.15	31.54±5.17	47.98±2.62	47.86±3.56	33.53±3.43
3	55.76±2.28	47.47±6.15	55.47±1.32	62.33±2.98	44.79±3.08
4	63.11±8.06	55.44±5.18	63.8±2.67	67.95±3.54	51.44±1.69
5	72.17±1.33	67.82±2.15	70.43±3.09	79.43±3.08	60.72±3.23
6	78.42±2.18	74.22±3.24	74.62±4.86	88.11±2.15	74.27±2.66
8	80.92±3.23	78.96±3.24	78.11±1.16	96.56±2.86	89.64±2.23
10	82.55±3.75	84.67±2.47	89.95±2.28	99.16±1.62	96.28±4.35

Table 8b: Cumulative % of drug release of econazole nitrate loaded transferosomal formulation.

F. Code / Time	Cumulative % of drug release (in 10 hr.)			
	TF-6	TF-7	TF-8	TF-9
0	0	0	0	0
0.25	22.97±4.35	19.33±2.15	18.91±1.35	12.86±4.66
0.5	36.54±3.66	19.89±4.35	15.59±1.39	16.33±3.23
1	41±5.39	31.69±3.29	27.67±2.38	20.18±2.25
2	48±7.21	37.58±3.09	35.54±2.98	27.23±1.54
3	58.25±3.63	46.19±2.56	41.78±2.32	36.72±2.09
4	63.86±9.52	54.18±2.33	52.99±2.67	47.82±2.06
5	76.51±8.35	65.84±3.67	68.95±1.08	52.88±2.15
6	82.35±2.45	73.94±2.65	79.55±2.18	66.85±3.24
8	94.45±1.74	80.89±1.09	81.95±2.78	79.86±1.25
10	95.83±2.17	89.76±4.86	90.11±1.96	84.35±2.18

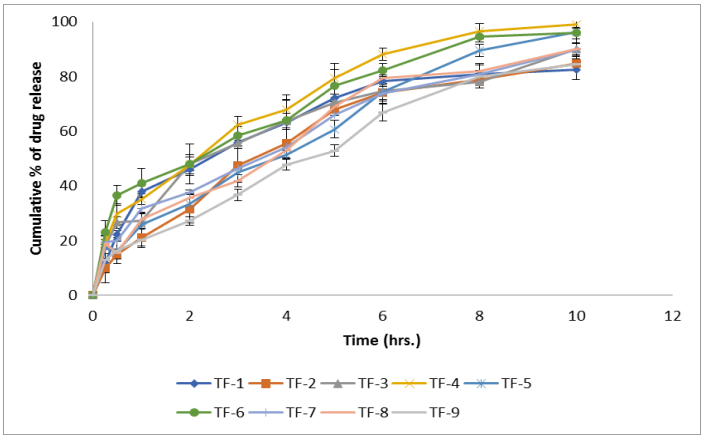

Figure 12: Cumulative % of drug release of econazole nitrate loaded transferosomal formulation.

Table 9: Evaluation of econazole nitrate loaded transferosomal gel formulation.

Formulation code	TFG-1	TFG-2	TFG-3	TFG-4	TFG-5
Appearance	Off-white	Off-white	Off-white	Off-white	Off-white
Homogeneity	Good	Good	Good	Good	Good
pH	5.88	6.55	7.38	6.76	5.26
Viscosity (Pascal Second)	10.56	16.95	24.12	20.68	17.85
Drug Content (%)	96.66	98.85	97.28	98.56	98.47

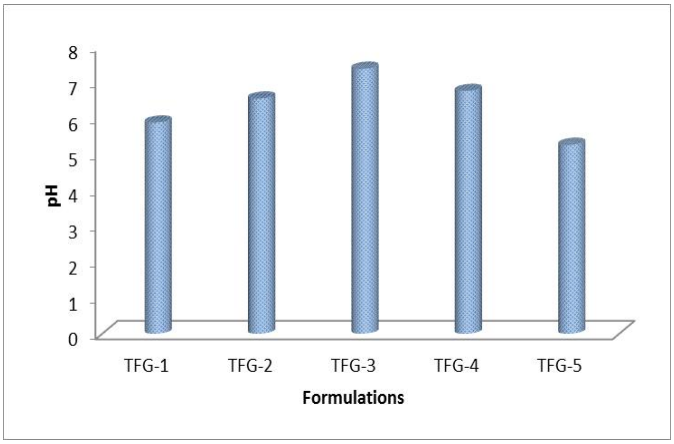

Figure 13: Evaluation of pH of econazole nitrate loaded transferosomal gel formulations.

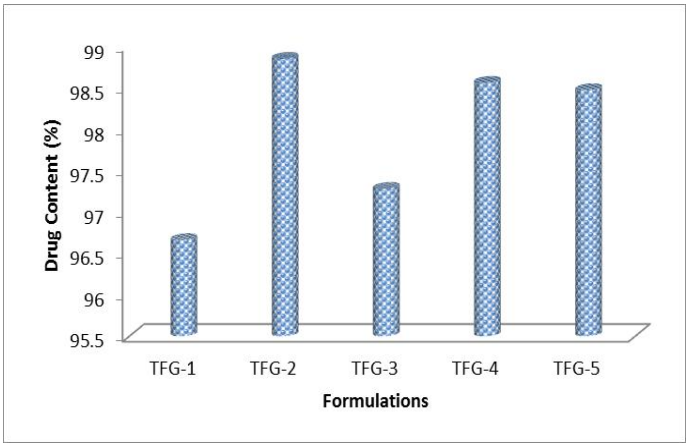

Figure 14: Evaluation of drug content (%) of econazole nitrate loaded transferosomal gel formulations.

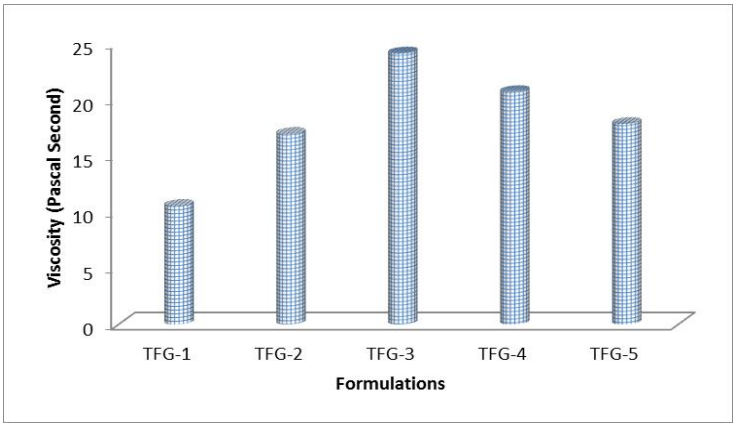

Figure 15: Evaluation of viscosity (pascal second) of econazole nitrate loaded transferosomal gel formulations.

Table 10: Comparative cumulative % in vitro drug permeation study of econazole nitrate loaded transferosomal gel formulation.

Time in(hrs)	Econazole Nitrate Containing Plain Gel	TFG-2	TFG-4	TFG-5
0.25	0	0	0	0
0.5	0	0	0	0
0.75	0	0	0	0
1	0.85±0.12	0.67±1.15	0.93±0.11	2.14±0.55
1.5	1.701±0.68	1.56±0.89	1.861±0.34	4.56±0.67
2	2.552±0.55	2.56±1.34	3.72±0.56	7.85±0.89
2.5	7.658±0.98	5.67±1.7	11.96±0.98	12.87±1.26
3	12.76±1.05	10.11±1.21	16.54±1.15	19.45±1.75
4	18.45±1.23	13.56±1.15	22.85±1.18	25.78±1.89
5	22.97±1.56	22.87±1.24	38.09±1.25	40.45±1.94
6	29.78±1.78	31.46±1.31	43.74±1.14	45.8±1.48
7	30.63±1.34	39.89±1.52	54.6±1.31	55.23±2.13

8	31.88±1.54	45.15±1.48	60.47±1.45	65.78±1.82
9	32.29±1.67	49.55±1.36	70.26±1.98	78.67±1.95
24	36.48±1.53	52.67±1.29	80.77±1.85	84.67±2.35

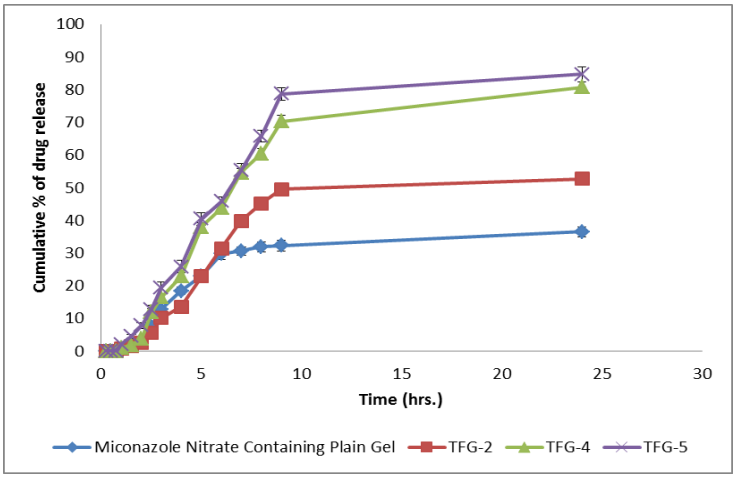

Figure 16: Comparative cumulative % *in vitro* drug permeation study of econazole nitrate loaded transferosomal gel formulation.

Table 11: Kinetic modelling fitting econazole nitrate loaded transferosome gel formulations (TFG-2&TFG-5).

Formulation Code	Model	Kinetic Parameter Value	
	Zero order	y = 7.8672x - 6.5327	R^2 = 0.9302
	First order	y = 20.475x - 17.557	R^2 = 0.8214
TFG-4	Higuchi	y = -0.0413x + 2.0364	R^2 = 0.9036
	Korsemeyer-peppas	y = 0.7577x + 1.0869	R^2 = 0.9974
	Zero order	y = 8.4438x - 5.9999	R^2 = 0.9643
TFG-5	First order	y = -0.0452x + 2.0353	R^2 = 0.9369
	Higuchi	y = 22.27x - 18.223	R^2 = 0.8744
	Korsemeyer-peppas	y = 0.7618x + 1.0746	R^2 = 0.9915

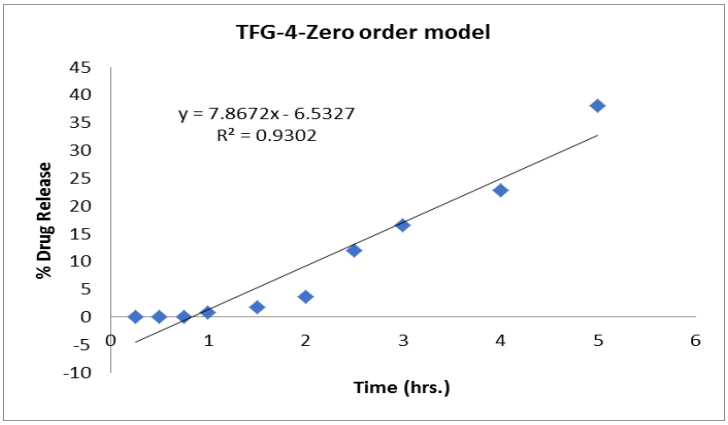

Figure 17: Kinetic Model fitting of econazole nitrate loaded transferosome gel formulations (TFG-4) zero order.

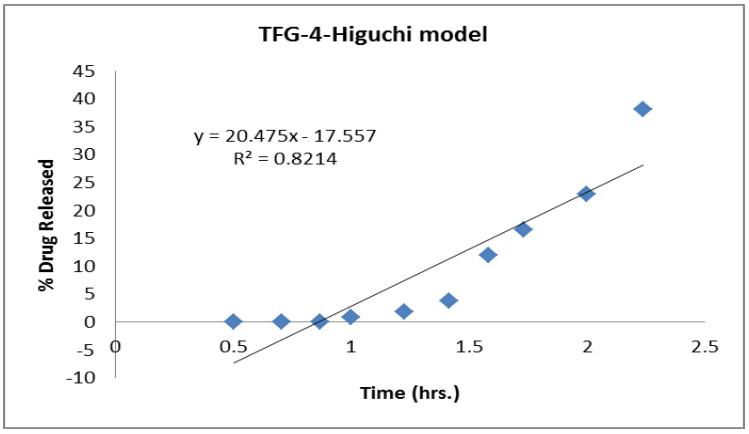

Figure 18: Kinetic Model fitting of econazole nitrate loaded transferosome gel formulations (TFG-4) -first order.

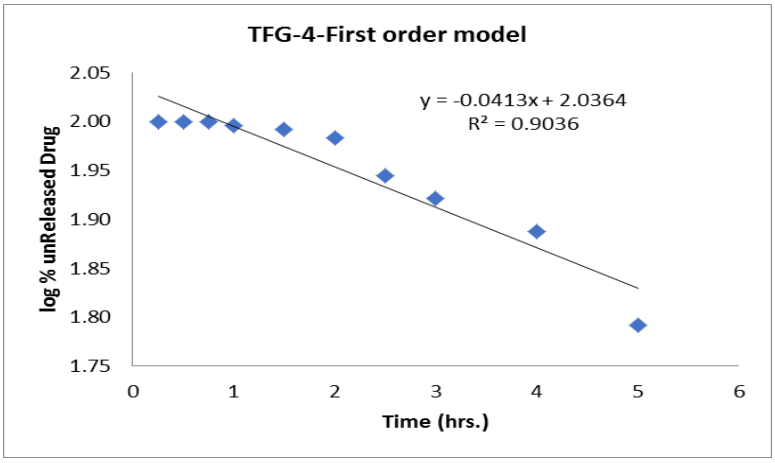

Figure 19: Kinetic model fitting of econazole nitrate loaded transferosome gel formulations (TFG-4) –Higuchi.

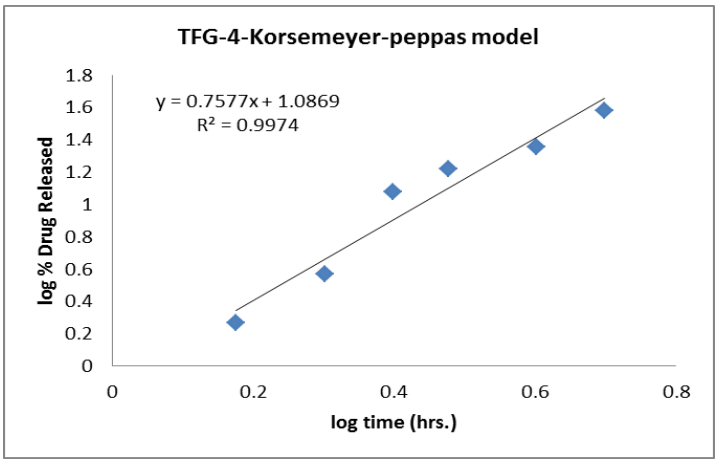

Figure 20: Econazole nitrate loaded transfersome gel formulations (TFG-4) - Korsemeyer-Peppas.

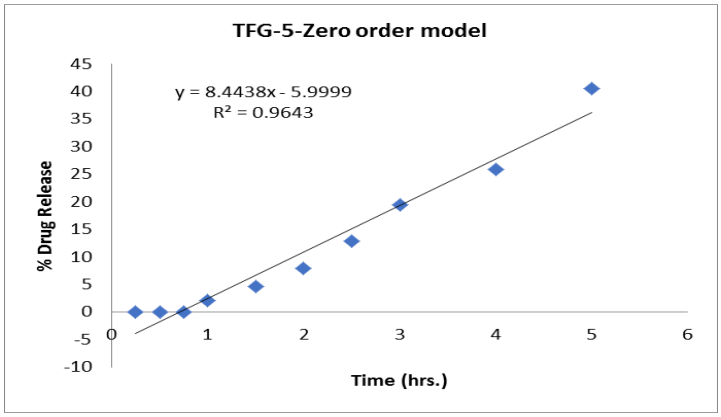

Figure 21: Kinetic model fitting of econazole nitrate loaded transferosome gel formulations (TFG-5) Zero Order.

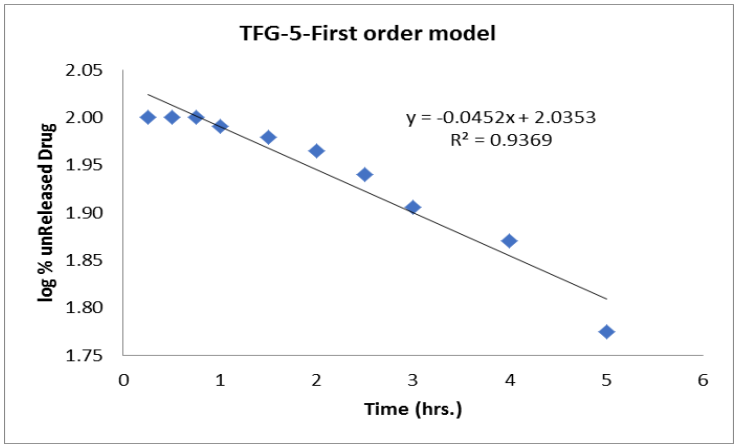

Figure 22: Kinetic model fitting of econazole nitrate loaded transferosome gel formulations (TFG-5) -First Order.

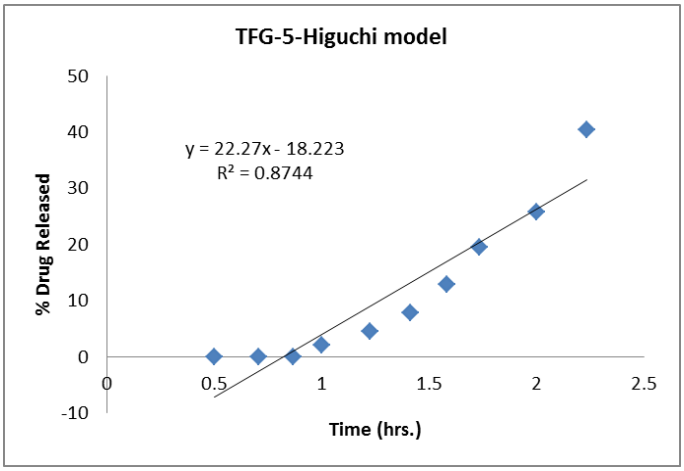

Figure 23: Kinetic model fitting of econazole nitrate loaded transferosome gel formulations (TFG-5) –Higuchi.

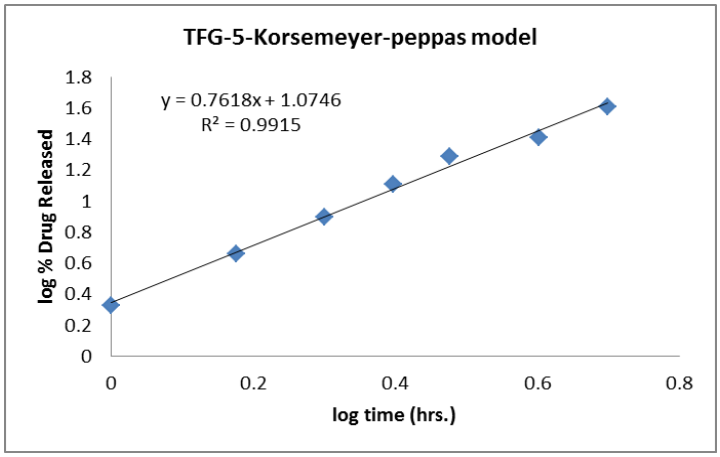

Figure 24: Econazole nitrate loaded transferosome gel formulations (TFG-5) - Korsemeyer-Peppas.

Table 12: Stability study of econazole nitrate loaded transferosome gel formulations (TFG-4 & TFG-5).

Time	Stability Condition/ Parameters					
	4°C± 2°C		25°C± 2°C		40°C± 2°C	
1.	**Appearance**					
	TFG-4	**TFG-5**	**TFG-4**	**TFG-5**	**TFG-4**	**TFG-5**
Initial	Off-white	Off-white	Off-white	Off-white	Off-white	Off-white
30 Days	Off-white	Off-white	Off-white	Off-white	Off-white	Off-white
60 Days	Off-white	Off-white	Off-white	Off-white	Off-white	Off-white
90 Days	Off-white	Off-white	Off-white	Off-white	Off-white	Off-white
2.	**pH**					
	TFG-4	**TFG-5**	**TFG-4**	**TFG-5**	**TFG-4**	**TFG-5**
Initial	6.76	5.26	6.76	5.26	6.76	5.26
30 Days	6.67	5.20	6.55	5.13	6.42	5.10
60 Days	6.62	5.15	6.45	5.09	6.35	5.04
90 Days	6.58	5.10	5.82	5.07	5.29	4.95
3.	**Viscosity(Pascal Second)**					
	TFG-4	**TFG-5**	**TFG-4**	**TFG-5**	**TFG-4**	**TFG-5**
Initial	20.68	17.85	20.68	17.85	20.68	17.85
30 Days	20.62	17.84	20.59	17.79	20.65	17.71
60 Days	20.60	17.80	20.55	17.72	20.62	17.68
90 Days	20.55	17.75	20.48	17.70	20.59	17.65
4.	**% Drug Content**					
	TFG-4	**TFG-5**	**TFG-4**	**TFG-5**	**TFG-4**	**TFG-5**
Initial	98.56	98.47	98.56	98.47	98.56	98.47
30 Days	98.52	98.42	97.50	96.16	96.77	95.05
60 Days	98.55	98.40	96.46	95.32	95.23	93.16
90 Days	98.50	98.38	95.41	94.29	93.14	91.68
5.	**% Drug Release (After 9 hr.)**					
	TFG-4	**TFG-5**	**TFG-4**	**TFG-5**	**TFG-4**	**TFG-5**
Initial	70.26	78.67	70.26	78.67	70.26	78.67
30 Days	70.12	78.62	70.10	77.95	69.65	77.12

60 Days	70.05	78.55	68.91	74.28	67.18	75.92
90 Days	69.15	78.46	67.86	73.53	65.25	72.89

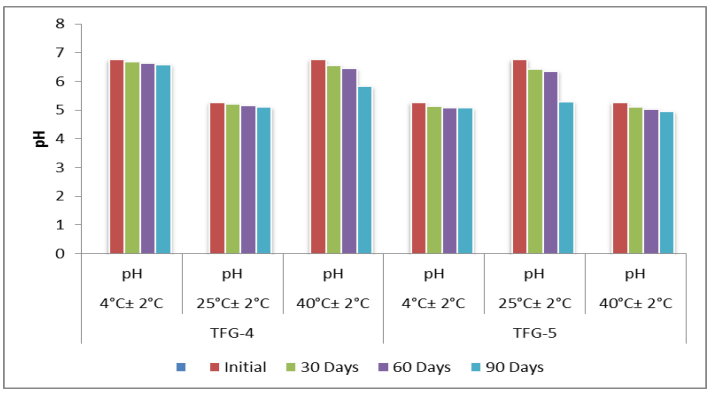

Figure 25: Stability Study (pH) of Econazole nitrate loaded transferosome gel formulations (TFG-4 & TFG-5)

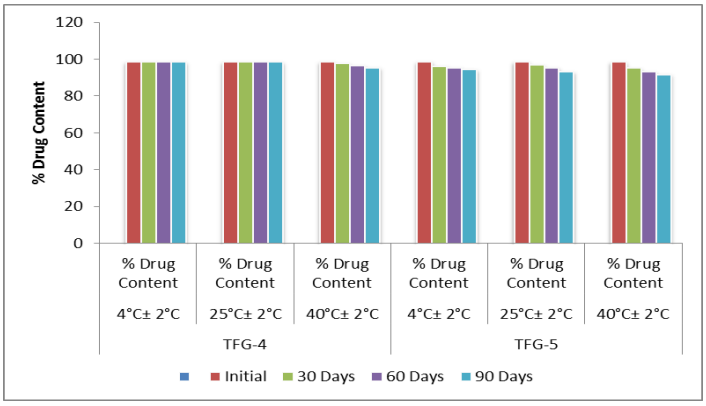

Figure 26: Stability Study (% Drug content) of econazole nitrate loaded transferosome gel formulations (TFG-4 & TFG-5).

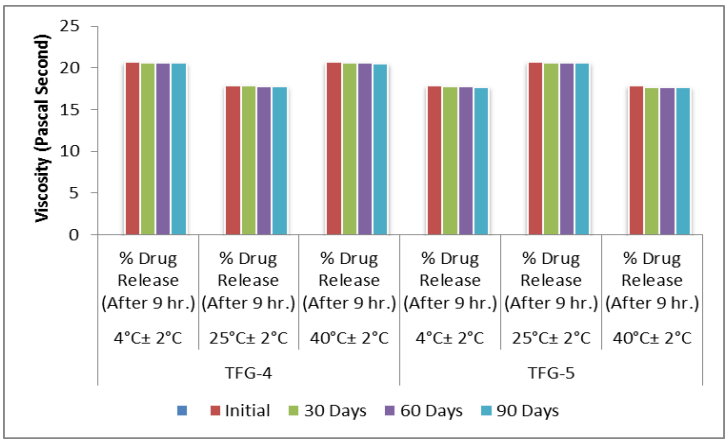

Figure 27: Stability Study (Viscosity) of econazole nitrate loaded transferosome gel formulations (TFG-4 & TFG-5).

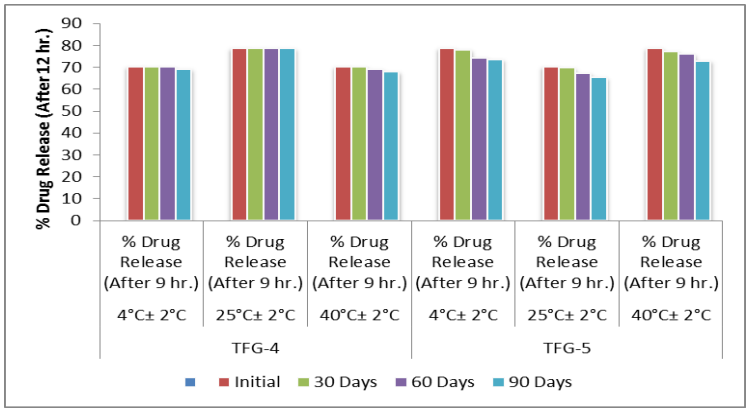

Figure 28: Stability study (% Drug release after 9 hr.) of econazole nitrate loaded transferosome gel formulations (TFG-4 & TFG-5).

SUMMARY

The research aimed to formulate a transferosomal gel of Econazole for enhanced penetration into the skin via the topical route, addressing issues related to compromised therapeutic efficacy of bioactives. Three phases were involved: preformulation studies and carrier optimization, preparation and characterization of transferosomal gel, and in vitro evaluation of the delivery system.

Key findings

- Econazole exhibited satisfactory physical and chemical properties for formulation.
- Transferosomal formulations demonstrated sub-micron to nanosize range, with high encapsulation efficiency and good release patterns.
- Formula F4 showed optimal characteristics and was incorporated into the gel.
- Gel formulations exhibited good viscosity, pH compatibility with skin, and drug content.
- Transferosomal gel formulations significantly enhanced the permeation of Econazole compared to plain gel.
- Release kinetics analysis indicated delayed drug release behavior, confirming diffusion and erosion mechanisms.
- Stability studies revealed refrigeration conditions maintained physical stability of transferosomal vesicles, while higher temperatures led to decreased entrapment efficiency due to lipid bilayer fluidity.

CONCLUSION

Transferosomes proved to be promising carriers for enhanced skin penetration, with embedding into gel further improving stability and drug permeation. Incorporating certain

skin permeation enhancers potentiated drug permeation, offering a potential strategy for topical drug delivery without altering skin structure.

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Conflict of interest

No authors declared Conflict of Interest

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