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ORIGINAL ARTICLE OPEN ACCESS

Formulation and Evaluation of Econazole Transfersomal Gel

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ABSTRACT
The aim of the present research work is to formulate a transfersomal gel of Econazole for deeper penetration into skin via topical route. Optimization of transfersomes and their characterization for different parameters are performed. The optimized preparation is evaluated for in vitro efficacy. The selected research work was divided into three phases. The first phase comprised of selection of drugs and excipients, Preformulation studies, preparation, optimization and in vitro characterization of selected carriers, nano vesicular transfersome. Drugs selected were Econazole and nano vesicular carriers selected. In the second phase of work, preparation and characterization of transfersomal gel formulation containing selected novel carrier was carried out. In third phase, prepared delivery system was evaluated for in vitro studies to ensure the behavior of delivery system. The entrapment efficiency percent of deformable vesicles was detected to be in the range of 75.76±5.27% to 91.17±3.84%. The formula F3 showed the small particle size (160.19 nm), and good release pattern. Accordingly, the formula F3 was used to be incorporated to formulate gel. Use of certain skin permeation enhancers with transferosomal Econazole gel is available and potentiates the permeation of the drug. This technique can serve as a potential tool for delivery of various topical drugs without altering the skin structure.
Key words: *Econazole, Transfersomes, Cholesterol, Lecithin, Span 80, Poloxamer 407 and HPMC k15.*

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INTRODUCTION

Nanoparticles are one or several types of systems known collectively as colloidal drug delivery systems. Also included in this group are microcapsules, nanocapsules, macro molecular complexes, polymeric beads, micropspheres and liposomes.

A nanoparticle is a particle containing dispersed drug with a diameter of 200to 500 nm. Materials used in the preparation of nanoparticles are sterilisable, non- toxic and biodegradable. They usually are prepared by a process similar to the coacervation method of micro encapsulation. Nanoparticles are also called as nanospheres or nanocapsules depending upon whether the drug is in a polymer matrix or encapsulated in a cell. The polymers used are the usual bio degradable ones. The main advantage of this system is that it can be stored for up to one year and can be used for selective target via reticuloendothelial system to liver and to cells that are active phagocytically.

Nonionic surfactant vesicles known as niosomes are used as carriers to delivery drugs to target organs and modify drug disposition.

Niosomes are found to improve therapeutic efficacy of drugs in cancer therapy, parastic, viral and microbial diseases. Many non- ionic surfactants like cetrimide, sodium dodecyl sulphate are used with cholesterol to entrap drugs invesicles.

Livers can act as a depot for many drugs where niosomes containing drug maybe taken up by the liver where they are broken down by lysosomal lipase slowly to release the free drug to the circulation. Niosomes slowly degraded providing a more sustained effect.

Niosomes are capable of releasing entrapped drug slowly. Niosomes are found to have selective drug delivery potential for cutaneous application of 5- α – dihydro testerione triamcinolone acetamide and intravenous administration of methotraxate for cancer treatment and sodium stilbogluconate in the treatment of lishmaniasis etc.

When erythrocytes are suspended in a hypotonic in a hypotonic medium, they sell to about one and half times their normal size and the membrane ruptures resultingin the formation of pores with diameters of 200- 5000A^0. The pores allow equilibration of the medium then is adjusted to iso-tonicity and the cells

are incubatedat 37^{0}C, the pores will close and cause the erythrocytes to reseal. Using this techniquewith a drug present in the extra cellular solution, it is possible to entrap up to 40% of the drug inside the resealed erythrocyte and to use this system for targeted delivery via intravenous injection. The advantage of using resealed erythrocytes as drug carrier is that they are biodegradable, fully biocompatible and non -immunogenic, exhibit flexibility in circulation time depending on their physiochemical properties, the entrapped drug is shielded from immunologic detection and chemical modification of drug is not required. Resealed erythrocytes can be targeted selectively to either the liver or spleen, depending on their membrane characteristics.

The ability of resealed erythrocytes to deliver drugs to the liver or spleen can be viewed as a disadvantage in that other organs and tissues are inaccessible.

Microspheres

Microspheres are free flowing powders consisting of spherical particles of size ideally less than 125 microns that can be suspended in a suitable aqueous vehicle and injected. Each particle is basically a matrix of drug dispersed in polymer from which release occurs by a first order process. The polymers used are biocompatible and biodegradable ex. Polylactic acid, poly lactidecoglycolide etc. Drug release is controlled by dissolution / degradation of matrix. The system is ideally suited for controlled release of peptide/ protein drugs.

In order to overcome uptake of intraveneousaly administered microspheres by the reticuloendothelial system and promote drug targeting to tumours with good perfusion, magnetic microspheres were developed. They are prepared from albumim and magnetite and have size of 1µ g to permit intravascular injection.

Monoclonal antibodies

Monoclonal antibodies are exceptionally high quality antibodies which consistof one molecular species and which may be obtained in a virtually homogeneousstate.

Kohler and Milstein in 1975 showed that somatic cell hybridization could be used to produce a continuous hybrid cell line producing a single type of anti body. The basic principle was to b-lymphocyte from an antigen primed mouse, having the ability to secrete a specific antibody and to fuse this with a suitable mouse derived plasmacytoma (often called myleom) line. The outcome was hybrid cell line (hybridoma) which had the phenotypic properties of both parental cells, that is malignancy and specific antibody secretion indefinitely one b- lymphocytes or plasmacell is committed to one antibody specificity. The discovery of hybridoma technology has been more dramatic than arrival of new scientific theory and has revolutionized immunology in a matter of few years.

Liposomes

It is defined as spherule vesicle of lipid bilayers enclosing an aqueous compartment. The lipid most commonly used is phospholipids, sphingolipids, glycolopids and sterols have been used to prepare liposomes.

In recent year, liposomes have been extensively studied for their potential to serve as carriers for delivery of drugs, antigens, hormones, enzymes and other biologicals. Because liposomes are composed of naturally occurring substance they have the distinct advantage of being nontoxic and biodegradable. Biologically active materials encapsulated withing liposomes are protected to various extends from immediate dilutions or degradations *in vivo*. This protective property promotes the delivery of entrapped drugs to the target organ by preventing a premature drug releaseafter administration.

Liposomes have two standard forms.Multilamellar vesicles (MLV's) made up of several lipid bilayers separated by fluid. Unilamellar vesicles (ULV's) consisting of single bilayer surrounding an entirely fluid core. The ULV's are typically characterized as being small (SUV's) or large (LUV'S).

MATERIAL AND METHODS

Econazole Provided by SURA LABS, Dilsukhnagar, Hyderabad. Cholesterol Procured from Gattefosse Pvt. Ltd., Mumbai. Lecithin Purchased from Merck Limited, Mumbai (India). Span 80 Purchased from SD Fine-Chem Limited, Mumbai. Sodium Cholate Purchased from Loba Chemie Pvt Ltd. (Mumbai, India). Brij 35 Purchased from SD Fine- Chem Limited, Mumbai. Poloxamer 407 Purchased from S. D. Fine. Chemicals Ltd. (Mumbai, India). HPMC K15 Purchased from Merck Limited, Mumbai (India). Propylene glycol Purchased from Merck Limited, Mumbai (India). DMSO Purchased from Merck Limited, Mumbai (India). Methanol Purchased from Merck Limited, Mumbai (India). Chloroform Purchased from Merck Limited, Mumbai (India). Ethanol urchased fromMerckLimited, Mumbai (India).

Analytical Method Development

Identification and Characterization of Drug

Preparation of reagents:

Preparation of 0.2M NaOH Solution

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Dissolved 4g of Sodium hydroxide pellets in to 1000mL of Purified water and mixed

Preparation of pH 6.8 Phosphate buffer

Dissolved 6.805 g of Potassium dihydrogen phosphate in to 800mL of purified water and mixed added 112mL of 0.2M NaOH solution and mixed. Diluted to volume 1000mL with purified water and mixed. Than adjusted the pH of this solution to 6.8 with 0.2M NaOH solution.

a) Determination of absorption maxima

A solution containing the concentration 10 µg/ ml drug was prepared in 6.8 phosphate buffer UV spectrum was taken using Lab India Double beam UV/VIS spectrophotometer (Lab India UV 3000+). The solution was scanned in the range of 200 – 400 nm.

b) Construction of standard graph

100 mg of Econazole was dissolved in 100 mL of pH 6.8 phosphate buffer to give a concentration in 1mg/mL (1000µg/mL) 1 ml was taken and diluted to 100 ml with pH 6.8 phosphate buffer to give a concentration of 0.01 mg/ml (10µg/ml). From this stock solution aliquots of 10 ml, 20 ml, 30 ml, 40 ml, 50 ml, were pipette out in 10 ml volumetric flask and volume was made up to the mark with pH 5.5 phosphate buffer to produce concentration of 1, 2, 3, 4 and 5 µg/ml respectively. The absorbance of each concentration was measured at respective (λ_{max}) i.e., 230 nm.

Organoleptic properties:

Take a small quantity of sample and spread it on the white paper and examine it visually for color, odour and texture.

Determination of Econazole Melting point

The melting point of Econazole was determined by capillary tube method according to the USP. A sufficient quantity of Econazole powder was introduced into the capillary tube to give a compact column of 4-6 mm in height. The tube was introduced in electrical melting point apparatus and the temperature was raised. The melting point was recorded, which is the temperature at which the last solid particle of Econazole in the tube passed into liquid phase.

Determination of Econazole Solubility

Determination of solubility of drug by visual observation. An excess quantity of Econazole was taken separately and adds in 10 ml of different solutions. These solutions were shaken well for few minutes. Then the solubility was observed and observations are shown in the Table.

Preformulation Studies

The objective of preformulation studies is to develop an elegant, stable, effective and safe dosage form by establishing kinetic rate profile, compatibility with the other ingredients and establish physicochemical parameter of new drug substances. It is the determination of those physical and chemical properties, which are considered as important factor in development of a newer dosage form. A preformulation study is a phase which is initiated once the new entity is introduced. It deals with studies of physical characteristics, analytical properties and its methods, chemical properties, and pharmaceutical behavior related to molecule. It is a very important aspect of developing medicines. This study gives idea about of new molecule properties, related to interactions with different solvent medium, ingredients used in developments of dosage form and some intrinsic chemical reactivity. At various stages of development, it is essential to understand the physicochemical characteristics of compounds. Data obtained from such studies forms an important fact for understanding the potential pharmacokinetics and pharmacological behavior of a drug in humans and animals. Such studies are useful during manufacture, transport and storage. The different parameters i.e. the measurement of solubility and dissolution rate. It also involves such as IR, UV, and DSC etc. according to the nature of compound. This study also gives idea about morphological characteristics of molecule or compounds by using SEM studies (Khan et al., 2013). Thus, in order to establish optimum condition for developing suitable drug delivery system preformulation studies are important. In the present work, preformulation studies were conducted on the drug Econazole by adopting following methods.

PREPARATION OF TRANSFERSOMES

Methods of preparation of transfersomes

Transfersomes formulations were prepared by a thin film hydration method. Soybean phosphatidylcholine, cholesterol, sodium cholate, span 80, and Brij 35 with different molar ratios were dissolved in 10 mL of a mixture of three organic solvents (Methanol:chloroform:ethanol) at (2:1:2) v/v/v ratio.

Using rotary evaporator, thin lipid film on the internal surface of the round-bottomed flask was formed. Econazole (100 mg) was dissolved in 20 mL of an isotonic phosphate buffer (pH 5.8). Econazole solution was used to hydrate the prepared thin film by rotation at 100 rpm for 2 hours. To form large multilamellar vesicles, the resulting suspensions were kept for 24 hours at 25°C. To form smaller vesicles, the transferosomal dispersions were sonicated for 30 minutes.

The Econazole transfersomes were separated from the entrapped Econazole by high-speed centrifugation at 20,000 rpm for 3 hours at −5°C using cooling ultracentrifuge. To separate the untrapped Econazole, clear supernatant was carefully taken out after the centrifugation. The transfersomes remained as precipitate containing the entrapped Econazole. The precipitate was resuspended in 10 mL of isotonic phosphate buffer (pH 5.8) in order to be evaluated. The transferosomal dispersions (free from the untrapped Econazole) were kept at a constant temperature of 4 °C within glass vials. Laminar air flow hood was used for conducting experimental procedures under aseptic conditions.

Table 1: Formulation chart

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Econazole (%)	1	1	1	1	1	1	1	1	1
Cholesterol (mg)	4	4	4	4	4	4	4	4	4
Lecithin	2	2	2	2	2	2	2	2	2
Span 80	5	10	15	-	-	-	-	-	-
Sodium Cholate	-	-	-	5	10	15	-	-	-
Brij 35	-	-	-	-	-	-	5	10	15
Methanol:chloroform:ethanol (mL) (2:1:2)	10	10	10	10	10	10	10	10	10

CHARACTERIZATION OF TRANSFERSOMES

Particle Sizes, PDI and Zeta Potential:

The mean particle length and polydispersity index (PDI), that's a degree of the distribution of transfersomes, was decided the usage of dynamic light scattering (Delta Nano C, Beckman counter), and Zeta capability becomes anticipated on the premise of electrophoretic mobility under an electric powered field, the use of zeta Sizer Nano ZS (Malvern Instruments, UK). Samples had been diluted with the distilled water before measurement and measure at a hard and fast angle of 165^{0}c for the particle size and poly dispersity index (PDI) analysis. For the Zeta ability measurement, Samples have been diluted as 1:40 ratio with filtered water (v/v) before analysis. Average particle size, PDI, and zeta potential have been then measured in triplicate

ENTRAPMENT EFFICIENCY

The entrapment efficiency was determined by using direct method. Detergents are used to break the transfersome membranes1 ml of 0.1% Triton X- 100(Triton X-100 dissolved in phosphate buffer) was added to 0.1 ml Transfersomes preparations and made up to 5 ml with phosphate buffer then it was incubated at 37°C for 1.5 hrs to complete breakup of the transfersome membrane and to release the entrapped material. The sample was filtered through a Millipore membrane filter (0.25) µm. and the filtrate was measured at 230 nm for Linagliptin. The amount of Econazole was derived from the calibration curve.

The entrapment efficiency is expressed as:

$$\text{Percentage Entrapment Efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100$$

Drug content

A specific quantity of Transfersomes which is equivalent to drug was taken and dissolved in 100ml of phosphate buffer of pH 6.8. The volumetric flask containing dispersion was shaken for 2hr in bath sonicator in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically at 230 nm using phosphate buffer (pH 6.8) as blank.

Table 2:Preparation of Topical Transfersome Gel Formulation

Formulation code	Ingredients			
	Poloxamer 407 (%)	HPMC k15 (mg)	Propylene glycol	DMSO
F3	0.5	20	10	10
F3	1	30	10	10
F3	2	40	10	10

The gel was prepared by the same procedures described In brief, in 10 mL distilled water, a required quantities of Poloxamer 407 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.

TRANSFERSOMES GEL EVALUATIONS

Physical appearance:

All prepared gel formulations have been observed for their visual appearance, such as transparency, colour, texture, grittiness, greasiness, stickiness, smoothness, stiffness and tackiness. The prepared gels were also evaluated for the presence of any particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

P^H of formulation:

pH measurement of the gel was carried out of the formulation was measured by using a digital pH meter (Lab India SAB 5000), dipping the glass electrode completely into the gel system. The observed pH values were recorded for all formulations (F3) in triplicates.

Determination of viscosity

Viscosities of the gels were determined by using Brookfield Viscometer (model- RVTP).Spindle type, RV-7 at 100 rpm. 100gm of the gel was taken in a beaker and the spindle was dipped in it and rotated for about 5 minutes and then reading was taken.

Extrudability

It is useful empirical test to measure the force required to extrude the material from the tube. The formulations were filled in a collapsible metal tubes with a nasal tip of 5mm opening tube extrudability was then determined by measuring the amount of gel, extruded the tip when a pressure was applied on tube gel. The extrudability of the formulation was checked and the results were tabulated.

Skin Irritation test

The developed formulation was tested for primary skin irritation on albino mice of either sex weighing 20-22gm. The hair was far away from the mice 3 days before the experiments. The animal was divided into two batches each batch was used on the test animal. A bit of cotton soaked during a saturated drug solution was placed on the rear of albino mice taken as controls. The animals were treated daily up to 7 days and eventually the treated skin was examined visually for erythema and edema.

Spreadability: For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1kg weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability.

$$S = M.L / T$$

M- Weight tied to the upper slide
L - Length moved on the glass.
T - Time Taken

Homogeneity:

The homogeneity of Econazole Transfersomal gels were checked by visual inspection. In this regard the gels were filled into narrow transparent glass tubes and were checked in light for the presence of any particulate or lump.

***IN VITRO* DRUG RELEASE**

Diffusion Study for Econazole Transfersomes

The *in vitro* release of Econazole from the transfersome formulations were studied by open ended cylinder method. This diffusion cell apparatus consist of a glass tube with inner diameter of 2.5cm, open at both ends. One end tied with artificial membrane, which serves as a donor compartment.

This study is performed for determining the permeation rate. The time needed to attain permeation flux at steady state and the information from *in vitro* studies was used to optimize the formulations. Studies of drug release from transfersomes gel formulation were performed using the *in vitro* diffusion method at 37^0C, 100 rpm, within a period of 24hr. A weighed amount of prepared transfersomes gel formulation was poured in to the glass cell and diffused against phosphate buffer pH 6.4 as a diffusion medium. Aliquots were taken at regular intervals and analyzed spectrophotometrically at 230nm using phosphate buffer pH 6.4 as blank.

Application of Release Rate Kinetics to Dissolution Data:

Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into
zero-order, first order, Higuchi, and Korsmeyer-Peppas release model.

Zero order release rate kinetics:
To study the zero–order release kinetics the release rate data ar e fitted to the following equation.
$F = K_o t$
Where, 'F' is the drug release at time't', and 'K_o' is the zero order release rate constant. The plot of % drug release versus time is linear.
First order release rate kinetics: The release rate data are fitted to the following equation
Log (100-F) = kt. A plot of log cumulative percent of drug remaining to be released vs. time is plotted then it gives first order release.
Higuchi release model: To study the Higuchi release kinetics, the release rate data were fitted to the following equation.
$F = k t^{1/2}$
Where, 'k' is the Higuchi constant.
In higuchi model, a plot of % drug release versus square root of time is linear.
Korsmeyer and Peppas release model:
The mechanism of drug release was evaluated by plotting the log percentage of drug released versus log time according to Korsmeyer- Peppas equation. The exponent 'n' indicates the mechanism of drug release calculated through the slope of the straight Line.
$M_t / M_\infty = K t^n$
Where, M_t / M_∞ is fraction of drug released at time 't', k represents a constant, and 'n' is the diffusional exponent, which characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.5 and 1.0; while in case of Fickian diffusion, n = 0.5; for zero-order release (case I I transport), n=1; and for supercase II transport, n > 1. In this model, a plot of log (M_t / M_∞) versus log (time) is linear.
Fourier Transform Infrared (FTIR) spectroscopy:
The formulations were subjected to FTIR studies to find out the possible interaction between the drug and the excipients during the time of preparation. FT IR analysis of the pure drug and optimized formulation were carried out using an FT IR spectrophotometer (Bruker FT-IR - GERMANY).
Differential Scanning Calorimetry:
The possibility of any interaction between the drug and the Excipients during preparation of SLN was assessed by carrying out thermal analysis of optimized formulation using DSC. DSC analysis was performed using Hitachi DSC 7020, on 5 to 15 mg samples. Samples were heated in sealed aluminum pan at a rate of 10°C/min conducted over a temperature range of 30 to 350°C under a nitrogen flow of 50 mL/min.
SEM (Scanning Electron microscope) studies
The surface morphology of the layered sample was examined by using SEM (Hitachi, Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs. These sample stubs were coated with a thin layer (30Å) of gold by employing POLARON-E 3000 sputter coater. The samples were examined by SEM and photographed under various magnifications with direct data capture of the images onto a computer.
Powder X-ray Diffraction (PXRD) Studies
The prepared mixtures were also analyzed using X-ray powder diffractometer (PXRD) which confirms the formation of the new solid phases. The difference in the 2 theta lines confirms the formation of the new solid phases as no two solids have same 2 theta lines, thus revealing the formation of new solid phases. It also reveals the information about the crystal structure, chemical composition, and physical properties of the material and also helps in structural characterization. This technique detects changes in the crystal lattice and is therefore a powerful tool for studying polymorphism, pharmaceutical salts, and cocrystalline phases. Spectra of PXRD were taken on a sample stage Spinner PW3064. The samples were exposed to nickel filtrate Cukœ radiations (40 KV, 30 mA) and were scanned from 10° to 40°, 2Θ at a step size of 0.045° and step time of 0.5 s.

RESULT AND DISCUSSION
Organoleptic properties

Table 3: Organoleptic properties

S NO.	Properties	Results
1	State	Solid
2	Colour	White
3	Odor	Odorless
4	Melting point	160°C

Solubility studies

Table 4: Solubility studies of drug in different solvents

S NO.	Solvents	Solubility of Econazole
1	Water	Slightly Soluble
2	Methanol	Freely soluble
3	Acetonitrile	Sparingly soluble
4	Dimethyl formamide	Soluble
5	pH 6.8 Phosphate Buffer	Soluble
6	Ethanol	Soluble
7	DMSO	Soluble

Initially the drug was tested by UV to know their significant absorption maximum which can be used for the diffusion study of the drug.

Analysis of drug:

A. UV scans:

The lambda max of Econazole was found to be 230 nm.

B. construction of calibration curve:

Table 5: Standard graph of Econazole

Concentration (µg/ml)	Absorbance (at 230 nm)
0	0
10	0.124
20	0.241
30	0.368
40	0.478
50	0.587

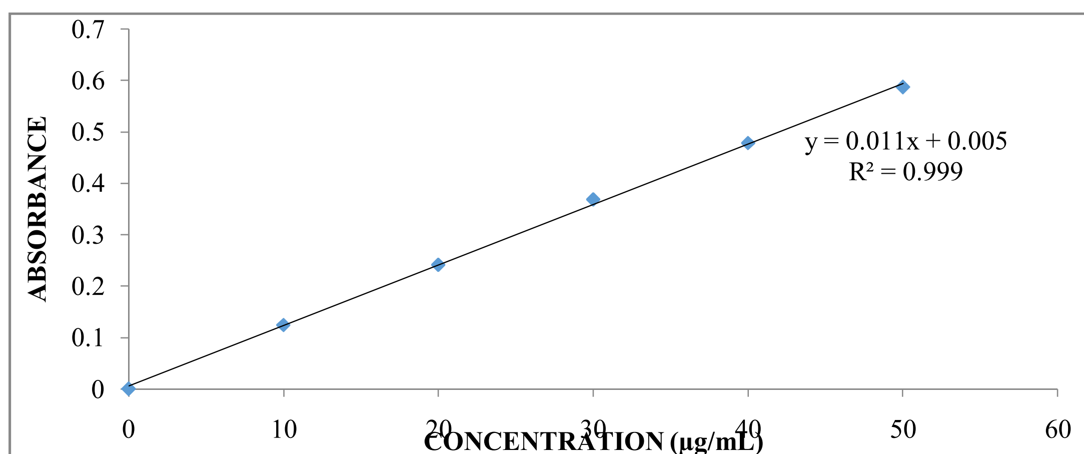

Figure 1: Standard calibration curve of Econazole

Standard graph of Econazole was plotted as per the procedure in experimental method and its linearity is shown in Table and Fig. The standard graph of Econazole showed good linearity with R^2 of 0.999, which indicates that it obeys "Beer- Lamberts" law.

Characterization of Transfersomes:

Table 6: Percentage yield, Drug Content, Entrapment Efficiency of all Transfersomes formulations

FORMULATION	PDI	Particle Sizes	Zeta Potential	Entrapment Efficiency	Drug content
F1	2.136	175.14±1.54	-34.04±2.27	85.91±4.63	82.02±1.39
F2	1.128	170.26±0.18	-42.92±1.35	90.35±6.47	91.29±0.21
F3	0.496	160.19±2.03	-55.62±3.65	91.17±3.84	98.01±2.23
F4	0.503	189.18±2.61	-26.88±1.45	75.76±5.27	76.99±0.69
F5	1.378	194.09±3.16	-31.23±4.61	80.42±7.59	82.16±0.01
F6	1.213	198.23±2.27	-37.01±2.72	82.30±6.19	89.22±1.32
F7	0.752	162.34±1.20	-24.89±1.16	76.91±5.44	62.90±3.29
F8	0.987	173.48±3.32	-35.18±3.57	79.35±5.95	78.10±0.11
F9	1.235	194.13±3.50	-41.66±1.42	83.56±4.65	83.91±0.36

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Transfersomes were subjected in to laser particle counter (L.P.C) for characterizing size distribution of transfersomes. Its shows that the particle size range 200-700nm, 200-600nm, and 200-700 nm range for Econazole transfersomes of 1:1, 1:2 and 1:3 ratios respectively. It is shown in Table.
The transfersomes were subjected to microscopic examination (S.E.M) for characterizing size and shape of the transfersomes. Microscopic examination revealed, spherical small uni-lamellar vesicles size.
Zeta potential results reveal that Span 80 transfersomes possess negative charge at pH 6.8 indicating that a weak electrostatic repulsive force exist in niosomal bilayer. Also, the inclusion Span 80 transfersomes found to have increased the zeta potential. Particles with zetapotential close to zero have been found less phagocytable in comparison with charged particles. The nature and density of charge on the surface of transfersomes influence the extent of biodistribution as well as interaction and uptake of transfersomes by target cells. F3 formulation highest zeta potential and it had good stability.

Fig 2: SEM Photograph of Econazole Transfersomes (Formulation-3)

ENTRAPMENT EFFICIENCY
The formulation variables were altered and optimized to obtain the transfersomes with maximum drug entrapment, desired transfersomal size and stability. Increased in the lipid concentration compared to drug entrapment with increase in quantity of lipid more number of transfersomes per ml of the transfersomal dispersion were formed, resulting in to an increased percent drug entrapment.
However, further increase in the lipid concentration had no proportionateincrease in percentage drug entrapment due to approaching system saturation.
Here 1:1, 1:2 and 1:3 ratios were used to prepare transfersomes. The percentage entrapment of transfersomes was found to be 75.76 to 91.17 respectively and 1:3 ratios found to have more entrapment efficiency compared to other two formulations. It is shown in Table.
Increasing the sonication time resulted in to reduction in percent drug entrapment; the decrease in percent drug entrapment is due to leakage of the drug during sonication.
Sonication brings about size reduction by breaking large transfersomes to smaller ones and in doing so, leakage of small quantities of drug from the transfersomes occur. Hence sonication time was optimized to 30 min, and further reduction in the size by increasing sonication time was not attempted.

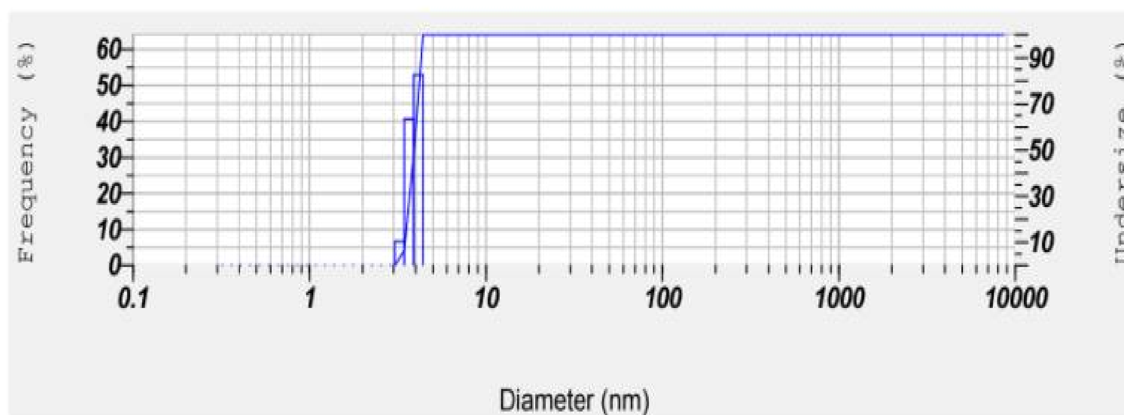

Fig 3: Particle size of F3 Formulation

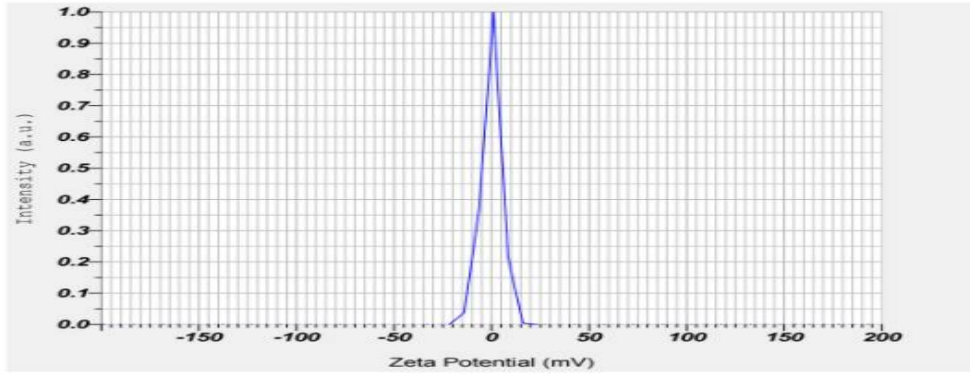

Fig 4: Zeta Potential of F3 Formulation

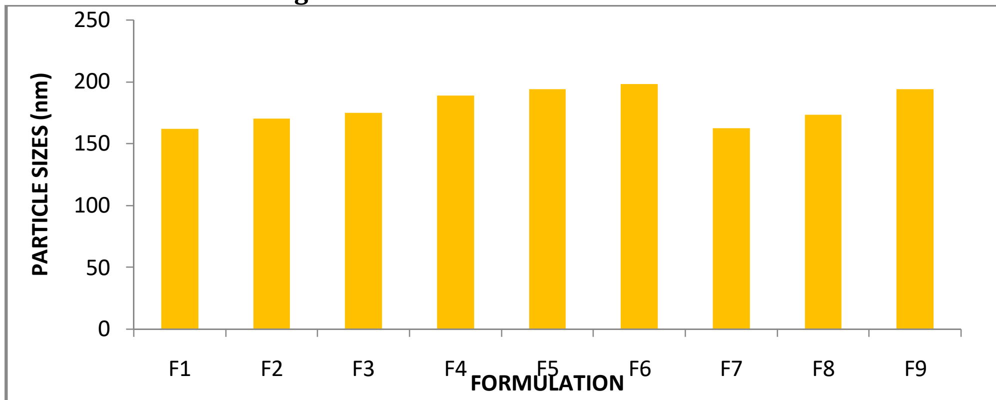

Fig 5: Particles size graph of Econazole Transfersomes (All Formulation)

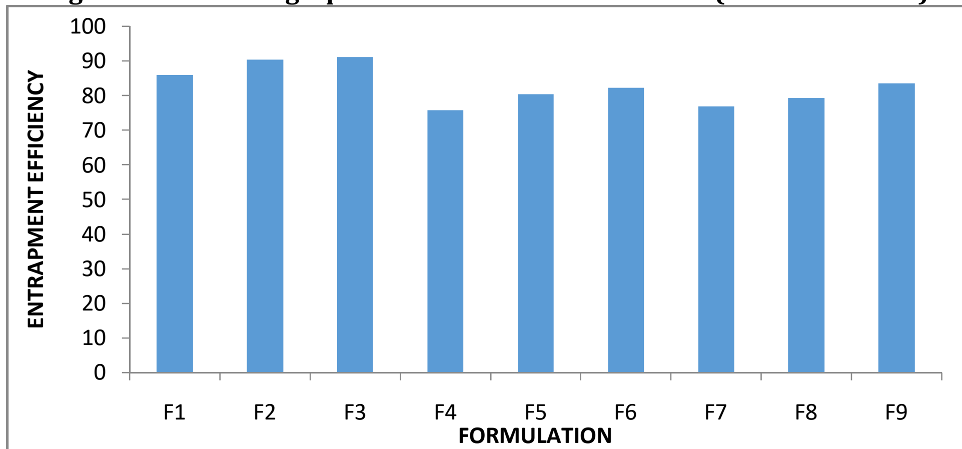

Fig 6: Entrapment efficiency graph of Econazole Transfersomes (All Formulation)

XRD

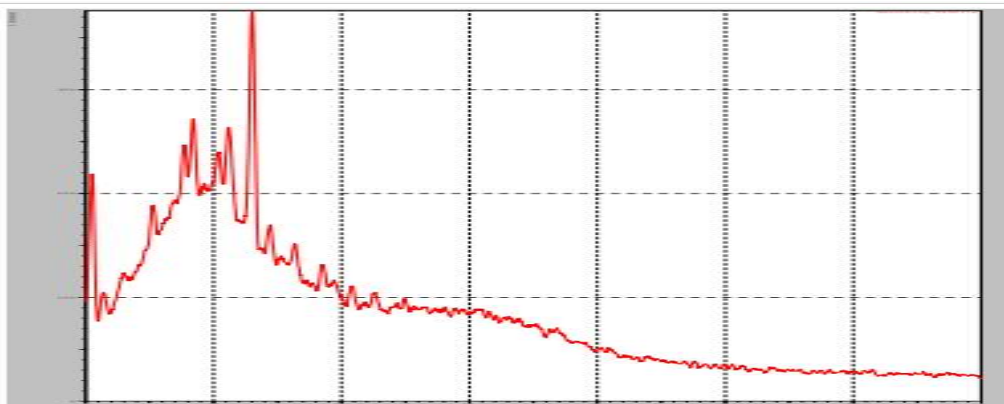

Figure 7: Econazole F3 optimized formulation

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Table 7: *In vitro* dissolution studies of F1-F9 Transfersomes formulations in percentage

TIME (H)	CUMULATIVE PERCENT DRUG DISSOLVED								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	10.26	16.83	20.31	21.60	25.43	09.91	16.12	20.52	18.75
2	18.80	20.95	26.14	26.94	29.82	16.58	20.90	26.33	21.63
3	22.96	31.61	32.67	36.56	34.97	24.82	36.56	31.98	26.98
4	29.57	36.15	46.52	43.13	38.69	31.94	42.35	38.36	32.76
5	33.34	45.75	51.74	48.75	46.28	36.56	56.92	42.61	40.12
6	47.21	48.56	68.61	54.82	50.15	42.71	63.84	47.18	46.34
7	54.93	56.90	73.96	61.34	57.67	48.38	72.27	55.15	53.18
8	60.76	61.38	77.81	68.95	63.75	62.17	77.16	62.22	57.65
9	66.83	68.19	88.18	72.26	69.41	67.49	85.26	67.64	61.21
10	76.54	75.21	96.42	79.15	75.25	72.24	96.33	76.63	69.17

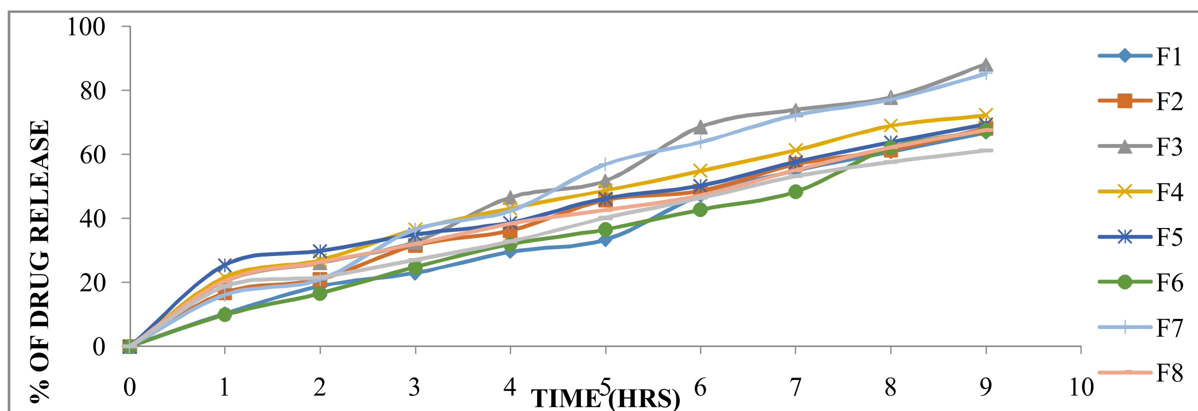

Fig 8: *In vitro* dissolution studies of F1-F9 Transfersomes formulations in percentage

In vitro drug release study of the selected Transfersomes (F1, F2, F3, F4, F5, F6, F7, F8 and F9) was carried out. The Transfersomes exhibited 10 hours sustained release pattern. Twenty six % of the incorporated amount of drugs was found to be released during the first 2 hours, followed by a slowed release of 96.42% of the drug up to 10 hours. The Econazole Transfersomes F3 showed a better release profile of 99.42 % by 10 hours. The prolonged release at 10 hours can be attributed to slow diffusion of drug from lipid matrix. The results of *in vitro* drug release are depicted in above Table.

Table 8: GEL EVALAUTION PARAMETERS

Formulation	pH	Viscosity (cp)	Extrudability	Homogeneity	Drug Content	Skin Irritation test
F3 optimized 0.5% Poloxamer 407 gel	5.64	5154	+	Satisfactory	93.19	No
F3 optimized 1% Poloxamer 407 gel	5.16	5597	+	Satisfactory	96.02	No
F3 optimized 2% Poloxamer 407 gel	5.01	5960	++	Excellent	97.29	No

Table 9: Physical evaluation of Econazole Pharmacosomal gel

Formulation	Colour	Spreadability (g.cm/sec)
F3 optimized 0.5% Poloxamer 407 gel	White	0.353±0.61
F3 optimized 1% Poloxamer 407 gel	White	0.326±1.30
F3 optimized 2% Poloxamer 407 gel	White	0.213±2.26

Table 10: *Ex vivo* permeation studies of Transfersomes gel

Time (hrs)	F3 optimized 0.5% Poloxamer gel	F3 optimized 1% Poloxamer gel	F3 optimized 2% Poloxamer gel
0	0	0	0
1	48.96	35.72	29.30
2	59.31	49.01	34.62
4	67.24	52.82	42.06
6	71.59	64.02	51.10
8	80.07	73.94	63.16
10	92.41	81.18	70.24
12		86.20	75.18
18		90.54	82.44
24			97.31

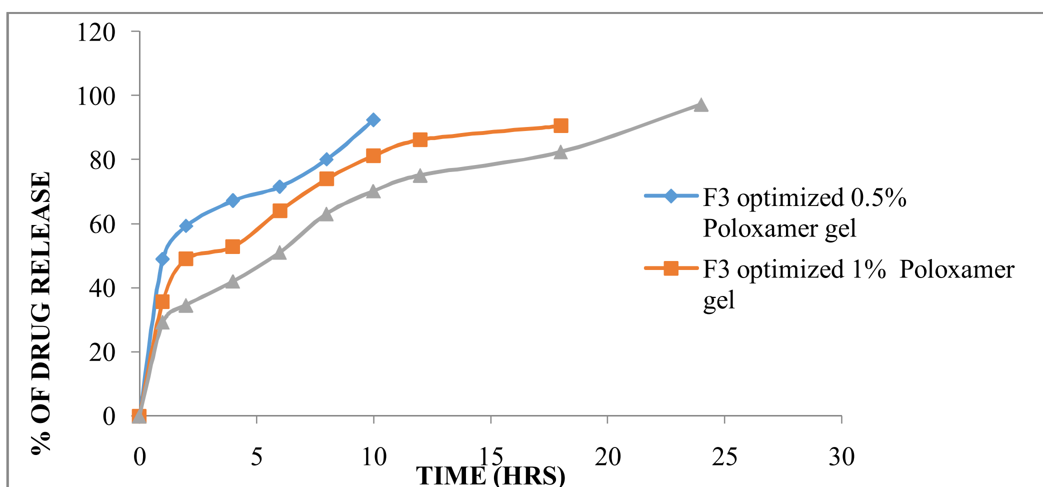

Figure 9: *Ex vivo* permeation studies for Transfersomes gel with different concentrations of Poloxamer.

F3 optimized 2% Poloxamer gel highest drug release (97.31% for 24 hours), good Homogenity, highest drug content, Proper viscosity. Hence it was considered as optimized formulation.

Table 11: Release kinetics of optimised formulation

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
29.3	1	1.000	1.467	0.000	1.849	29.300	0.0341	-0.533	70.7	4.642	4.135	0.507
34.62	2	1.414	1.539	0.301	1.815	17.310	0.0289	-0.461	65.38	4.642	4.029	0.613
42.06	4	2.000	1.624	0.602	1.763	10.515	0.0238	-0.376	57.94	4.642	3.870	0.772
51.1	6	2.449	1.708	0.778	1.689	8.517	0.0196	-0.292	48.9	4.642	3.657	0.985
63.16	8	2.828	1.800	0.903	1.566	7.895	0.0158	-0.200	36.84	4.642	3.327	1.314
70.24	10	3.162	1.847	1.000	1.474	7.024	0.0142	-0.153	29.76	4.642	3.099	1.543
75.18	12	3.464	1.876	1.079	1.395	6.265	0.0133	-0.124	24.82	4.642	2.917	1.725
82.44	18	4.243	1.916	1.255	1.245	4.580	0.0121	-0.084	17.56	4.642	2.599	2.042
97.31	24	4.899	1.988	1.380	0.430	4.055	0.0103	-0.012	2.69	4.642	1.391	3.251

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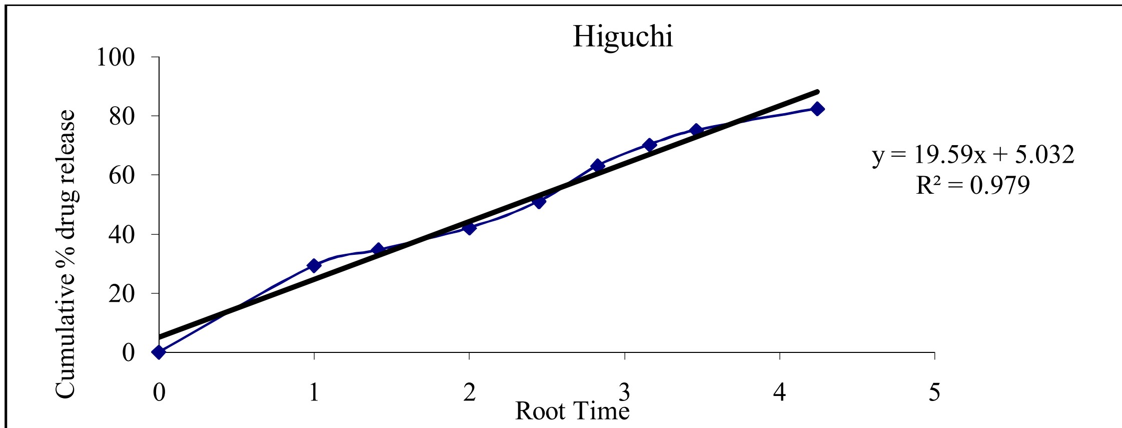

Figure 10: Higuchi release kinetics

The prepared F3 optimised 2% Poloxamer 407 Transfersomes gels were subjected to the drug release kinetics and release mechanism. The formulations were studied by fitting the drug release time profile with the various equations such as Zero order, First order, Higuchi and Korsmeyer pappas. The optimised formulation F3 optimised 2% Poloxamer 407 Transfersomes gel was analyzed for the drug release mechanism. The best correlation coefficient value (0.979) indicates the best release mechanism (Higuchi release kinetics).

FTIR

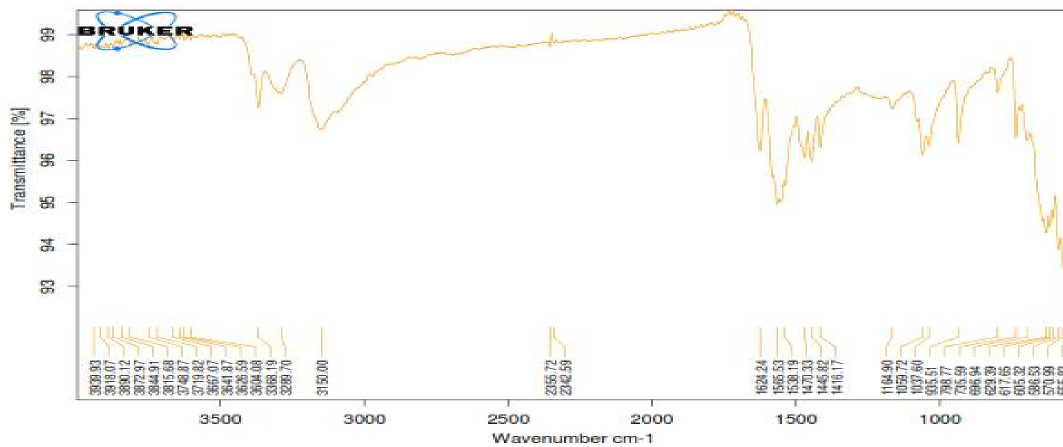

Figure 11: Econazole Pure drug FTIR

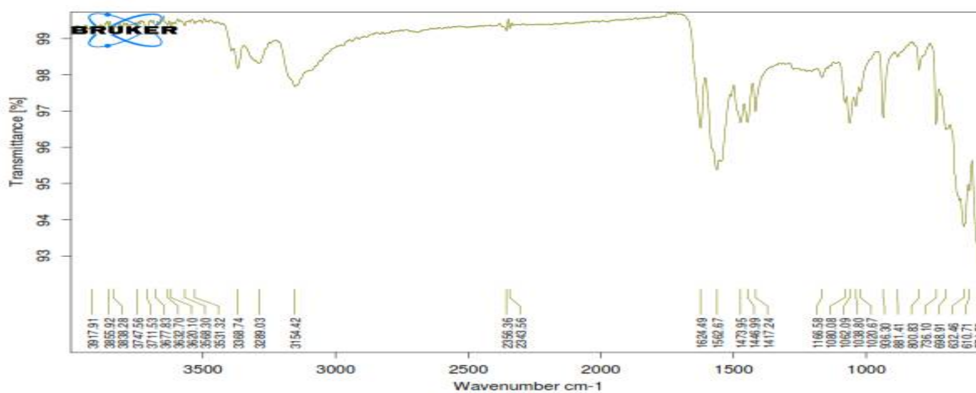

Figure 12: Econazole F3 optimized 2% Poloxamer 407 gel FTIR

Infrared studies were carried out to confirm the compatibility between the lipid, drug, and selected excipients. From the spectra it was observed that there was no major shifting, as well as, no loss of functional peaks between the spectra of the drug and transfersomes gel. This indicated no interaction between the drug and other excipients.

DSC:

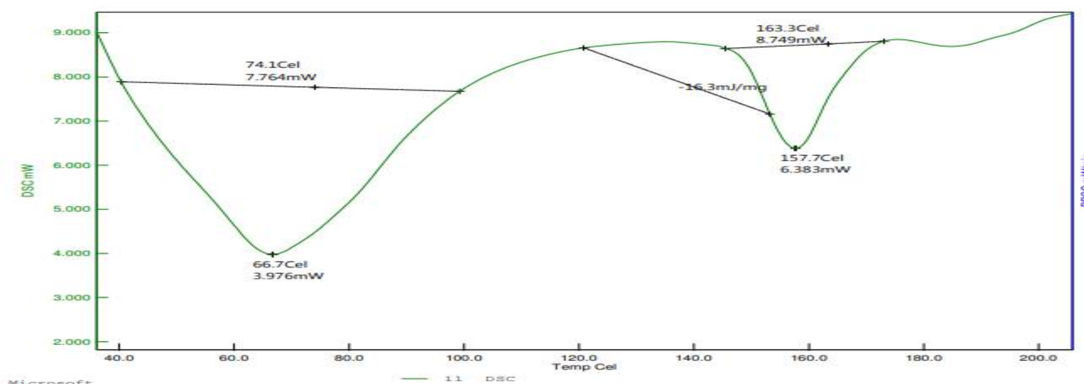

Figure 13: Econazole Pure drug

CONCLUSION

Transfersomes are excellent drug carrier to permeate skin tissues. Embedding of transferosomal Econazole to gel improves permeation of the drug. Moreover, stability of transferosomal vesicles is improved when they are embedded into gel dosage form. Use of certain skin permeation enhancers with transferosomal Econazole gel is available and potentiates the permeation of the drug. This technique can serve as a potential tool for delivery of various topical drugs without altering the skin structure.

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REFERENCES

1. Remington 'The science and practice of pharmacy' 20th edition, VOLI: 903-905, 2001.
2. Y.W. Chien, (1983). Drug Development And Industrial Pharmacy; 9:447-520, 1291-1330.
3. F. Teewes, (1983), Drug Development And Industrial Pharmacy; 9: 1331-1357.
4. J. M. Class., R.L.Stephan and S. C. Jacobson, (2018). Int. j. Dermatol, 19:519.
5. H.Sezahi and M. Hashida, Crc, (1984). Critical Reviews In "Therapeutic drugCarrier Systems", 1:1.
6. T.A Horbett, B.D Ratner, T.Kost and M.Sigh (1984). In " Recent Advances InDrug Delivery System" Plenum Press, New york. 209-220.
7. Joseph R. Robinson., Vincent H.L. Lee, (1987). "Controlled Drug Delivery",2nd edition, revised and expanded, 596-597.
8. S. P. Vyas, R.K. Khar. (2002). "Targeted and Controlled Druig Delivery Novel Carrier Systems", I edition, CBS Publishers, New Delhi, 39-40, 42-46.
9. Welling pG, Dobrinska MR, In Robinson JR, Lee VHL, eds: (1987).Controlled Drug Delivery", II edition, Marcel Dekker, Newyork.1987: 253.
10. Gregoriadis G, (1977). "Targeting of drugs", Nature. 407-411.
11. Gregoriadis G. In Gregoriadis C, Senior J, Trout A, eds: (1982). "Targeting ofdrugs", Plenum, New york.: 155.
12. Tretise A, D.M. Brahmankhar, Sunil B. Jaiswal, (2001). "Biopharmaceuticsand Pharmacokinetics": 360-363.
13. Wirth M, Hamilton G and Gabar F, "J. (1998). Drug Target", 6, 95-104.
14. Subheet Jain, Dipankar Bhadra, N. K. Jain. (2001)"Advances in Controlled and Novel Drug Delivery" 1st edition, CBS Publishers,New Delhi, 427.
15. Christophers, e.; Walff, H. H; Laurence, E.B. (1974) "The formationof epidermal cell columns", J. Invest. Dermatol., 62: 556-564.

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