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Research

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.

Keywords: Econazole, Transfersomes, Cholesterol, Lecithin, Span 80, Poloxamer 407 and HPMC k15.

INTRODUCTION

Nanoparticles[1]

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, micropsheres 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.

Niosomes

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 likecetrimide, 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 foundto have selective drug delivery potential for cutaneous application of 5- α – dihydro testerione triamcinolone acetamide and intravenous administration of methotraxatefor cancer treatment and sodium stilbogluconate in the treatment of lishmaniasis etc.

Resealed Erythrocytes

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 37^0C, 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 sizeideally 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 goodperfusion, 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 upof 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).

MATERIALS 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 purchased from Merck Limited, Mumbai (India).

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[3]

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 165^0c 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[4]

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 37ºC 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.

RESULTS 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
UV scans:
The lambda max of Econazole was found to be 230 nm.

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

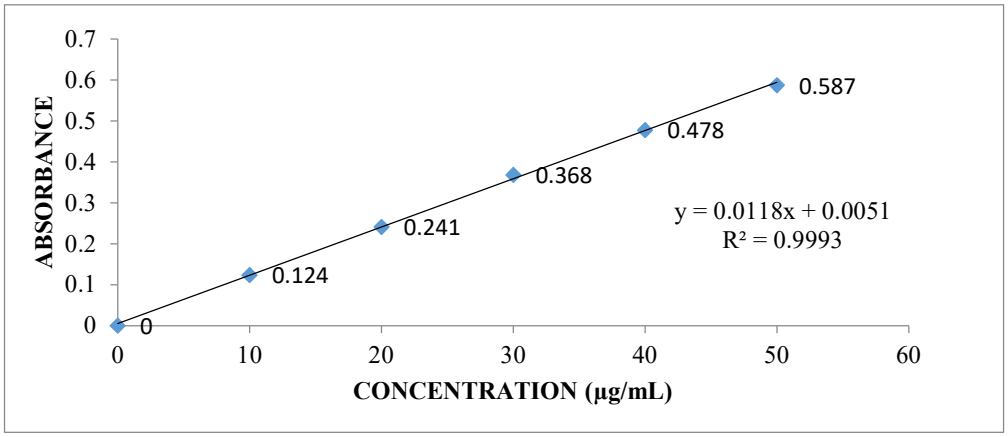

Fig 1: Standard calibration curve of Econazole

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

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.

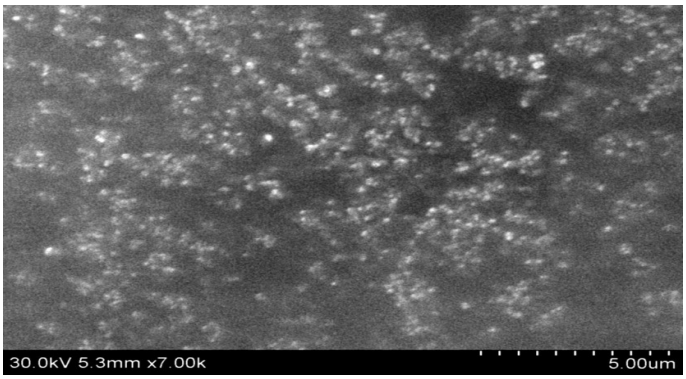

Fig 2: SEM Photograph of Econazole Transfersomes (Formulation-3)

Entrapment Efficiency[5]

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.

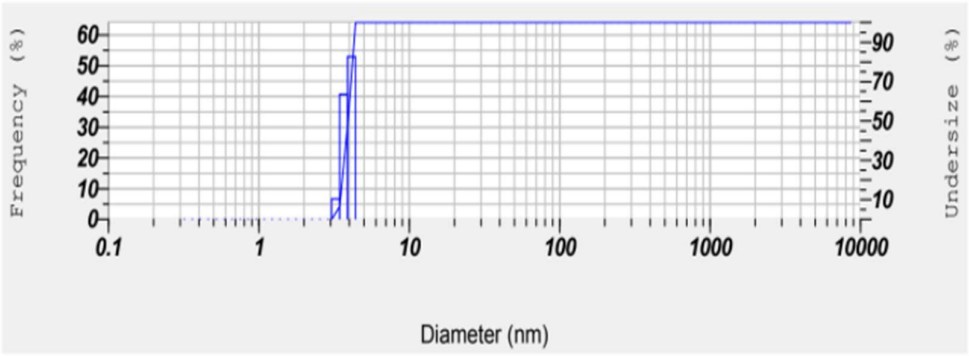

Fig 3: Particle size of F3 Formulation

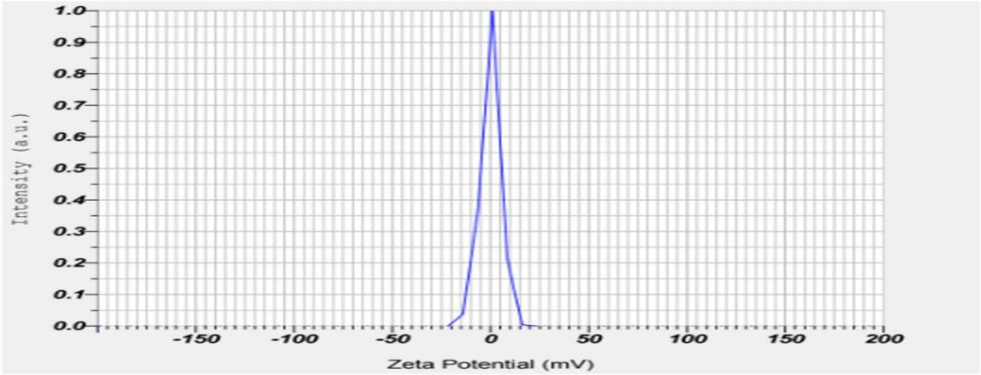

Fig 4: Zeta Potential of F3 Formulation

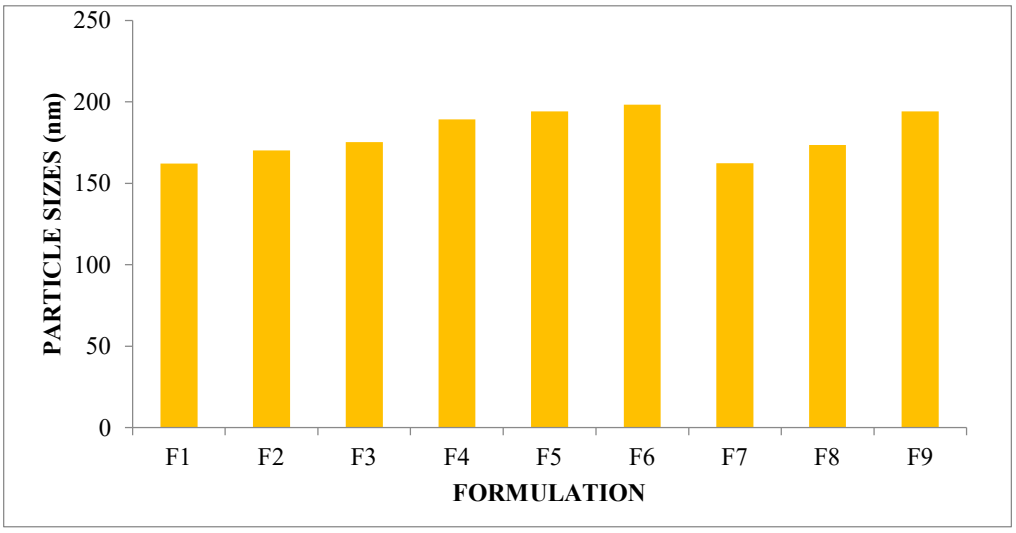

Fig 5: Particles size graph of Econazole Transfersomes (All Formulation)

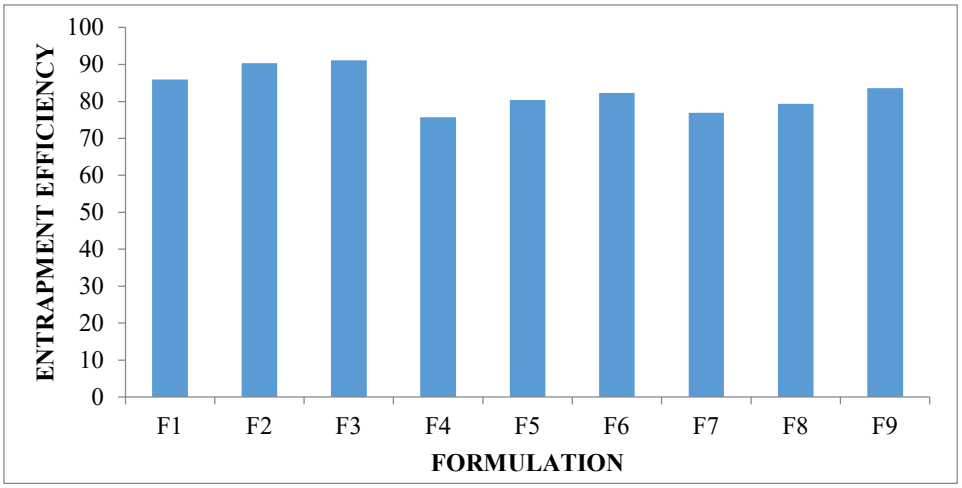

Fig 6: Entrapment efficiency graph of Econazole Transfersomes (All Formulation)

XRD

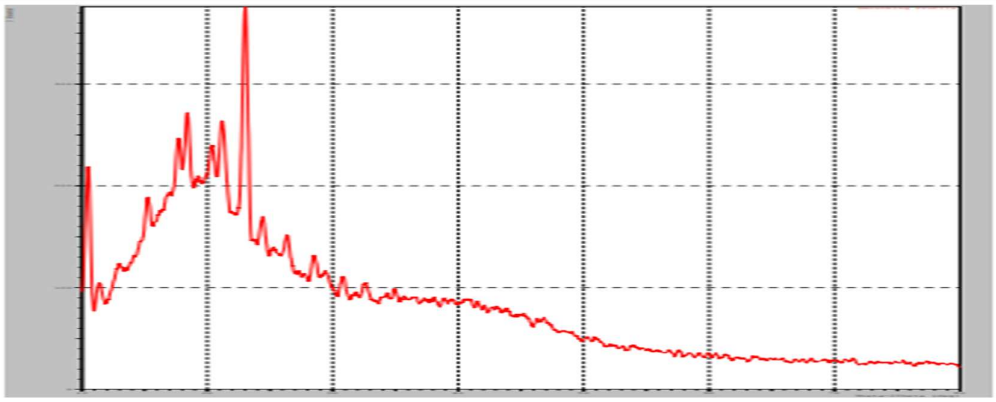

Fig 7: Econazole F3 optimized formulation

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

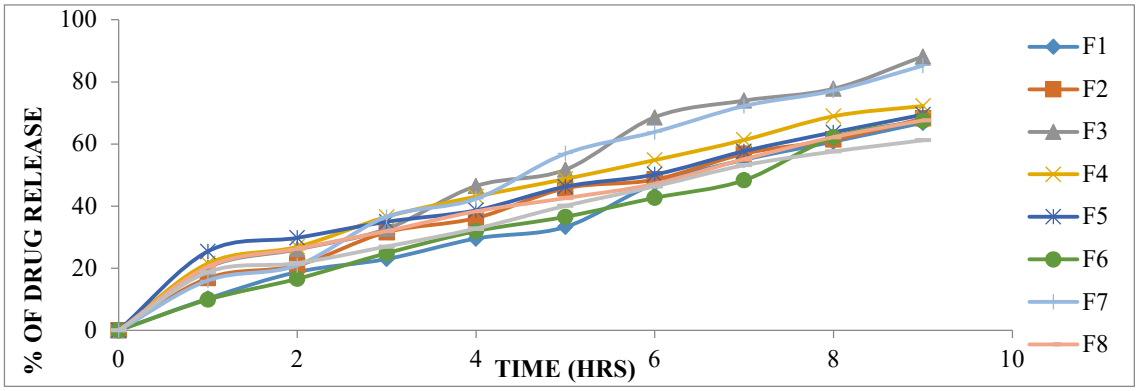

Fig 8: In vitro dissolution studies of F1-F9 Transfersomes formulations in percentage

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

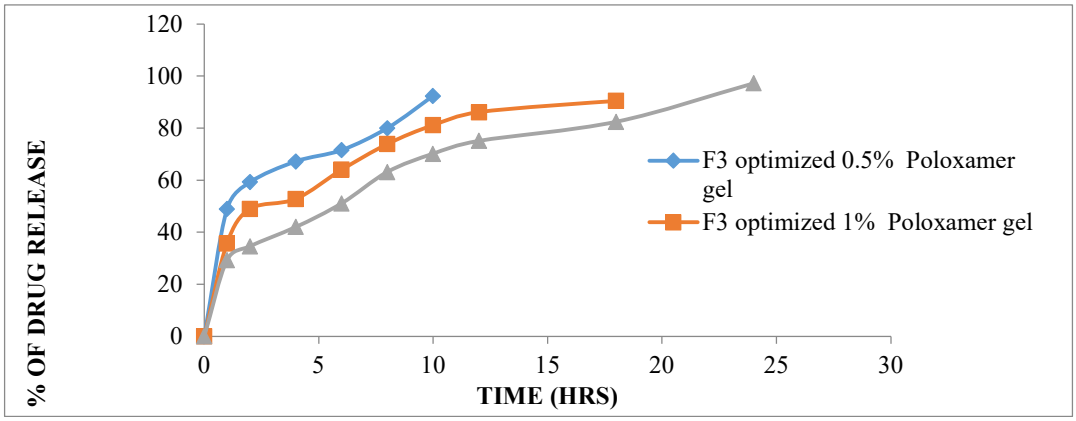

Fig 9: Ex vivo permeation studies for Transfersomes gel with different concentrations of Poloxamer.

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

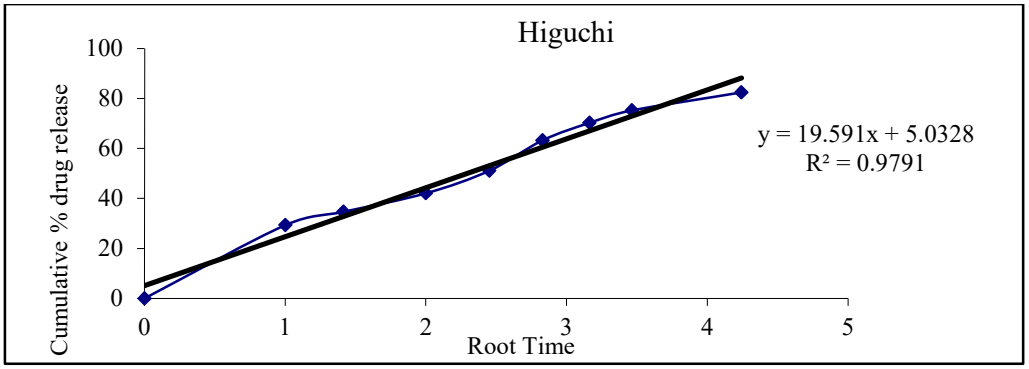

Fig 10: Higuchi release kinetics

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

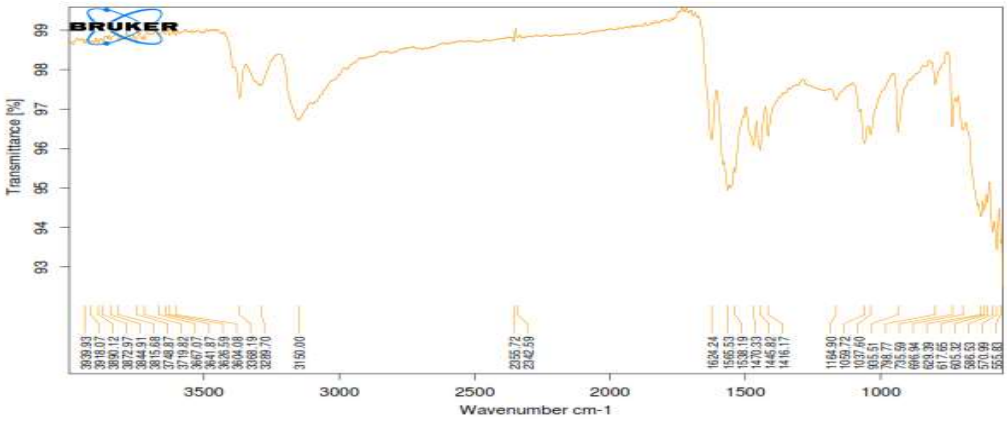

Fig 11: Econazole Pure drug FTIR

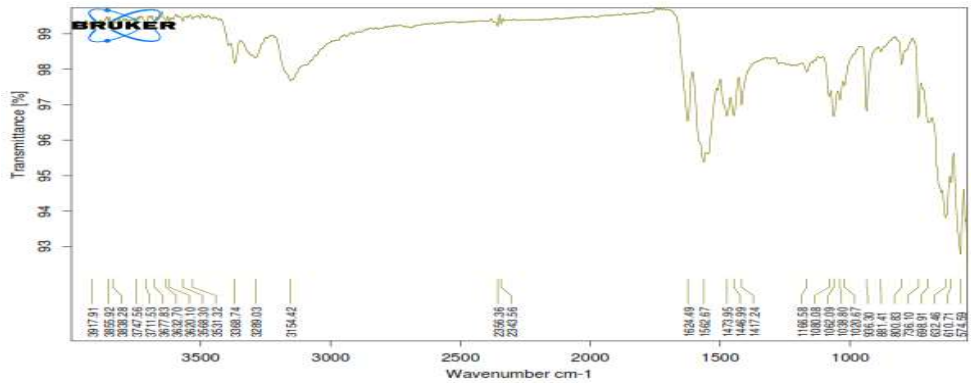

Fig 12: Econazole F3 optimized 2% Poloxamer 407 gel FTIR

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

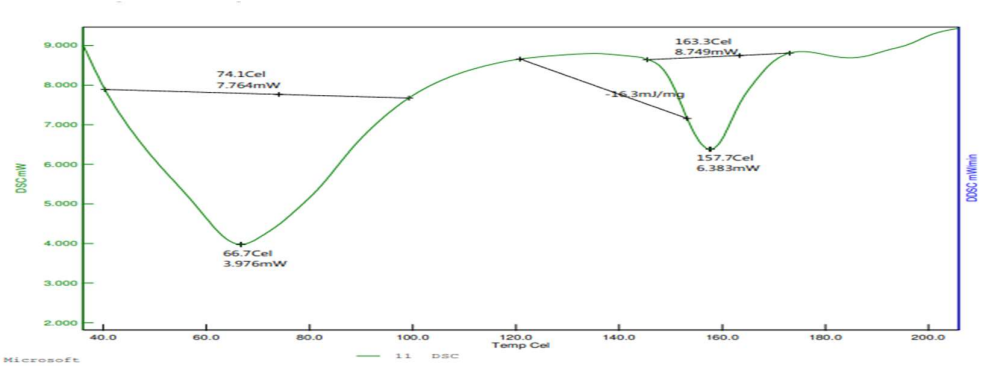

Fig 13: Econazole Pure drug

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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