FORMULATION AND CHARACTERISATION OF ECONAZOLE NITRATE LOADEDNIOSOMES FOR ANTIFUNGAL TREATMENT

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ABSTRACT

Niosome is one of the synthetic nanoparticles which often used as drug delivery system for various applications. The aim of this study was to prepare niosomes encapsulated with ECN and characterized for various physico chemical parameters. Niosomes encapsulated with ECN were prepared by thin-film hydration technique, and encapsulation efficiency was evaluated. Niosome surface morphology and encapsulation efficiency was evaluated. The results show that ECN were successfully loaded in the formulations with encapsulation efficiencies of 79.25% to 91.88% respectively. The zeta potential of ECN encapsulated niosomes were in the range of - 14.5- -30.2 respectively. Niosomes had spherical shapes with rough surfaces. Niosomes were evaluated for invitro drug release studies, in first hour of diffusion studies the drug release was observed ranging from 10.31% to 12.86% for all the formulations and by 12 hrs it was found to be in the range of 78.98% to 94.05%. In conclusion, these ECN encapsulated niosomes have high potential for therapeutic applications.

Key words: Niosomes, Econazole nitrate, Cholesterol, Span 60, Tween 80.

DOI: 10.48047/ecb/2023.12.Si11.033

INTRODUCTION

Novel drug delivery systems have the potential to deliver drugs and othermolecules to the specific targets. Different carriers such as polymeric nanoparticles, liposomes, niosomes, and nano/micro emulsions are successfully applied to deliver therapeutic agents in this regard [6].

Transdermal drug delivery (TDD) is one of the most convenient systems for patients. TDD has many advantages such as being painless, easy to apply, avoiding the first-pass effect, and having a better pharmacokinetic profile for extendedperiods and fewer side effects.Permeation of drugs across the stratum corneum (SC) is therate-limiting step for transdermal delivery.Several nanocarriers have emerged and are being used to improve the pharmacokinetic and pharmacodynamics profiles of drugs. Therefore, vesicular systems have been used to increase the absorption of encapsulated drugs while also providing controlled and sustained therapeutic activity[2].Niosomes are nonionic surfactant vesicleswhich can entrap both hydrophilicand lipophilicdrugs, either in aqueous layer or in vesicularmembrane made of lipid materials. Niosomes areeither unilamellar ormultilamellar vesicles that have abetter stability than liposomes. Niosomes are formedon admixture of nonionic surfactant, cholesterol withsubsequent hydration in aqueous medium. In additionof cholesterol, which gives the rigidity to the bilayerand results in less leaky niosomes. Niosome behave invivo like liposomes prolonging thecirculation of entrapped drug and altering itsorgan distribution.

This study was to investigate the feasibility of using niosome as a transdermal drug delivery systemfor Econazole. Niosomesoffer a versatile vesicle delivery concept with potential for delivering drug via transdermal route.Topically appliedniosomes can increase residence time of drug in the stratum corneumand epidermis, while reducing the systemic absorption ofthe drug. It also improves the horny layer properties both by reducing transepidermal water loss and by increasing smoothness via replenishing lost skin lipids. Econazole is a imidazole antifungal agent used in the treatment of candida infections, fungal infections. Econazole is available as conventional dosage formslike powders, parenterals, gels, creams and ointments in the market. The conventional dosage formof this drug has several side effects like nausea, vomiting and abnormalities of liver enzymes.

Better targeting of drugs to the infected organs can be achieved by niosomal formulation surfactants increase the permeability of Econazole through the biological membrane and also reduces the systemic toxicity of anti-infective drugs. Thus the therapeutic index of the Econazole can be improved when given in niosomal formulation[4].

MATERIALS AND METHODS

MATERIALS

Econazole Nitrate powder was procured from Biophore India, India. Cholesterol, Span 60, PEG 400, Chloroform was obtained from S.D. Fine chemicals, Ahmedabad. Tween 60 & Chloroform was purchased from Finar Chemicals, Ahmedabad. All the chemicals used were of analytical grade[1].

METHODS

PREFORMULATION STUDIES

Preformulation study is the initial step in the development of both active pharmaceutical ingredient (API) and drug product. A preformulation study is an important mechanism for determination of physical and chemical properties of the drug before including it in formulation advancement. Preformulation studies are essential step for progress of safe, effective, and stable dosage form. Thus, in order to establish supreme condition for clinically useful delivery system, preformulation studies were executed. Valuable pharmaceutical dosage form development requires various preformulation guidelines. Preformulation studies include solubility, melting point, and partition coefficient. The drug and excipient interaction studies were carried out by FT- IR and DSC[3].

DETERMINATION OF MELTING POINT

Melting point of Econazole Nitrate was determined by open capillary method. Melting-point apparatus is most frequently used for the determination of the melting point of a solid. A few crystals of the compound are placed in a thin walled capillary tube 10-15 cm long, about 1 mm in inside diameter, and closed at one end.

FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROSCOPY

IR spectroscopy is most effective system for qualitative identification of compound. The necessary information regarding the group present in specific compound has been given by IR spectroscopy. IR study was executed by using Perkin Elmer Fourier transformed infrared spectrophotometer. The potassium bromide (KBr) disk technique was applied using 100 mg of spectroscopic grade dried KBr. KBr was ground into fine powder using a mortar/pestle and coagulate into discunder a hydraulic pressure at 10,000psi. Econazole nitrate and drug excipient mixture were deposited on the KBr disc withthe help of a capillary tube. Each KBr disc

was examined at a resolution of 400 cm-1 over the wavelength region of 4000 – 400 cm-1 and characteristics bands were reported[3].

CALIBRATION CURVE OF ECONAZOLE NITRATE

20mg of pure drug, econazole nitrate was dissolved in methanol and made upto 100ml with methanol in volumetric flask. In the similar way, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml are prepared by using methanol in respective volumetric flasks. These samples are measured for absorbance at 271.5nm in UV spectrophotometer against reagent blank.

PREPARATION OF ECONAZOLE NITRATE LOADED NIOSOMES

Niosomes were prepared by adopting the Thin Film Hydration technique. Briefly, an accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform (5 ml) and drug Econazole nitrate was dissolve in Chloroform (1 ml). Both the solvent mixture were mixed in 150 ml capacity round bottom flask. The solvent mixture was evaporated by rotary vacuum evaporator (Macro Scientific work Pvt. Ltd., Delhi) at 40ºC by maintaining vacuum pressure of 380-420 mm/Hg to evaporate the solvent leaving a thin film (lipid phase) on the wall of the flask at a rotation speed of 100 rpm. All criteria for rotary vacuum evaporation were selected from prior studies. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 10 ml Phosphate Buffer 7.4 pH at a temperature of 50 to 60o C for 1 hour until the formation of multi lamellar niosomes[7].

CHARACTERIZATIONS OF NIOSOMES

MORPHOLOGY

The prepared niosomal formulation was studied under the microscope for morphological evaluation. A drop of preparation was poured on a glass slide and placed under the pre-adjusted optical microscope. The morphology was then studied carefully[5].

DRUG CONTENT

100 mg gel was added and dissolved in 100 mL phosphate buffer pH 5.5. The gelsolution filled in the volumetric flask was shaken continuously with the help of a mechanical shaker for 2 hr to enhance the solubility of the drug. Then 1ml of gel solution was made up to 10 mL phosphate buffer pH 5.5 and was scanned under UV- Visual spectroscopy at λmax274 nm using phosphate buffer pH 5.5 as blank[1].

DETERMINATION OF ENTRAPMENT EFFICIENCY

The entrapment efficiency of the formulations was determined by centrifuging 1 ml of the suspension diluted to 10 ml with distilled water at 15,000 rpm for 60 minutes at 4°C using high speed cooling centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 210nm using UV- Visible Spectrophotometer after suitable dilution, and drug used in the formulation 100 mg used. The percentage of drug entrapment in niosomes was calculated using the followingformula[3].

ZETA POTENTIAL

Zeta potential is a key indicator for evaluating the stability of dispersed colloid system. The higher of the absolute value of zeta potential, the bigger the electrostatic repulsion between each niosomes in suspension and can result in more stable niosomes in suspension and maintains a homogeneous, transparent and stable state for a long time without deposition of nanoparticles. For Zeta potential

determination, the niosomal suspensions were suitably diluted with distilled water and dropped into the Zeta sizer electrophoretic cell which was carried out using a zeta potential analyzer (Nikissomicrotrac USA – Department of pharmaceutics, Saurashtra Uni., Rajkot, Gujarat)[7].

Table 01: Composition of Niosome of Econazole nitrate (F1 to F10)

S.NO	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	Econazole nitrate (mg)	10	10	10	10	10	10	10	10	10	10
2	Cholesterol (mg)	10	10	10	10	10	10	10	10	10	10
3	Cholesterol: Span 60	1:1	1:1.5	1:1.7	1:2.0	1:2.5	-	-	-	-	-
4	Cholesterol: Tween 80	-	-	-	-	-	1:0.5	1:1	1:1.5	1:2.0	1:2.5
5	DMSO(Di Methyl Sulfoxide)	10 ml	10 ml	10 ml	10 ml	10 ml	10 Ml	10 ml	10 ml	10 ml	10 ml
6	Phosphate Buffer	10 ml	10 ml	10 ml	10 ml	10 ml	10 Ml	10 ml	10 Ml	10 ml	10 ml

IN VITRO RELEASE STUDY FOR NIOSOMAL FORMULATIONS

Drug release was determined by dialysis method; one ml of each formulation (testand control) were poured into dialysis bags (cut off:8 kD, Sigma) and put into 25 ml phosphate buffer (pH 7.4, 20mM) and stirred (100 rpm, 37 h, room temperature). At predetermined time intervals, 2 ml of phosphate buffer was taken and then substituted by fresh phosphate buffer. Finally, the amounts of released silibinin in phosphate buffer were measured by spectrophotometer at 290 nm and the amount of released drug was estimated by the standard curve[8].

$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug loaded}}{\text{Unloaded drug}} \times 100$$

KINETIC ANALYSIS OF IN VITRO RELEASE PROFILES

To investigate the mechanism of drug release from prepared niosomes, the release data were analyzed by using different mathematicalmodels as follows

Zero order A graph of %CDR against time was plotted to study the release kinetics.

First order A graph of log %DR against time was plotted to study the release kinetics. *Higuchi model* A graph of %CDR against SQRT was plotted to study the release kinetics.

Korsmeyer–Peppas model A graph log %CDR against log time was plotted to study the release kinetics[5].

RESULTS AND DISCUSSION

DETERMINATION OF MELTING POINT

The melting point of Econazole nitrate was found to be 165^0C which complied with the BP standards.

FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROSCOPY

In the FT-IR drug -excipients interaction study, it was found that Econazole nitrate was compatible with all excipients used in the formulation. There were no extra peaks was observed. Thus the chosen excipients for the formulation were found to be compatible with the active ingredient and have nophysical interaction with the active pharmaceutical ingredient.

CALIBRATION CURVE OF ECONAZOLE NITRATE

From the calibration curve of Econazole nitrate it was observed that the drug obeys Beer's law in the range 20-100 µg/ml and the equation was generated, absorbance and concentration was used to calculate the drug content and % CDR of the dosage form.

MORPHOLOGY OF NIOSOMES

The prepared Noisome were undergone morphological studies by using Scanning Electron Microscopy (SEM) method. The shape of all formulations (F1 to F10) was found as smooth and spherical in shape.

DRUG CONTENT

The highest drug content was found to be as 95.26% for F5 formulation For all other formulations (F1 to F10), it was in the range of 86.25%-95.26%.

ENTRAPMENT EFFICIENCY

Highest entrapment efficiency of 91.88%was obtained with formulation F5. For all formulations (F1 to F10) it was in the range of 79.25%-91.88%.

ZETA POTENTIAL

Zeta potential for all the formulations was found to be in the range of -14.5- -30.2

IN VITRO DRUG RELEASE STUDIES

In first hour of diffusion studies the drug release was observed ranging from 10.31% to 12.86% for all the formulations and by 12 hrs it was found to be in the range of 78.98% to 94.05%.

KINETIC ANALYSIS OF IN-VITRO RELEASE PROFILE

The regression coefficient (R^2) values were approximated to follow First-order release. All equations including zero order, Higuchi model and Korsemeyer-Peppas equations, exhibited good linearity. Table -gives the values of the rate constants and regression correlations using rate equations for the release of Econazole nitrate from niosomes. The overall results revealed that the First-order R^2values for all formulations were higher than the Zero-order values, which suggests that niosomal formulations (F_1-F_{10}) can release the drug in sustained manner.

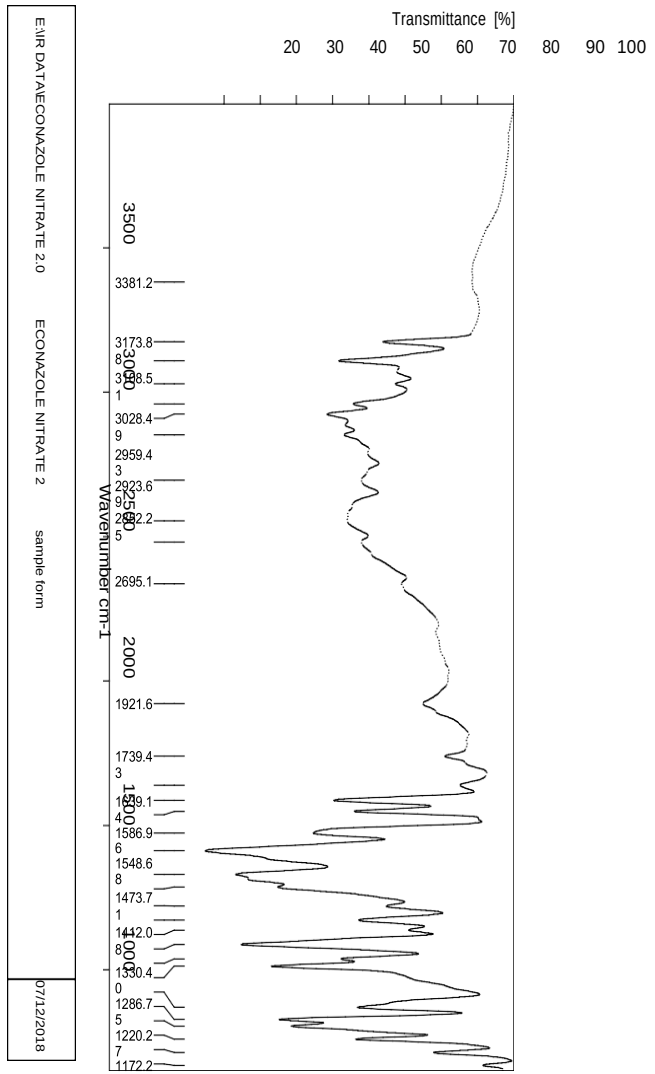

Figure 01:FTIR spectra of pure Econazole nitrate

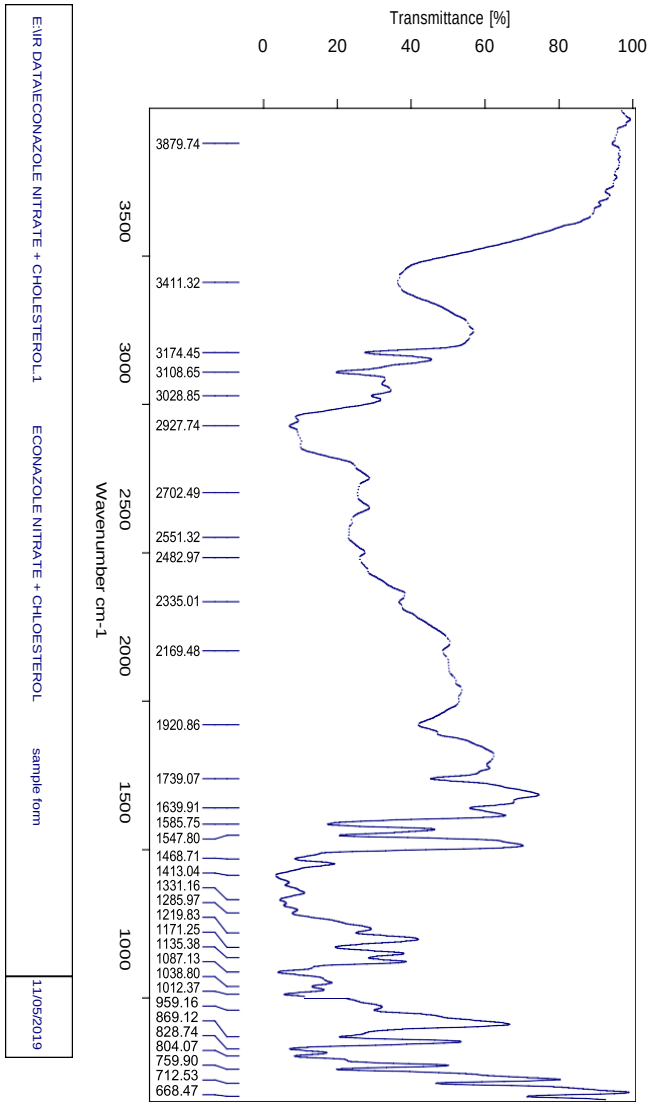

Figure 02: FTIR Spectra of Econazole nitrate + Cholesterol

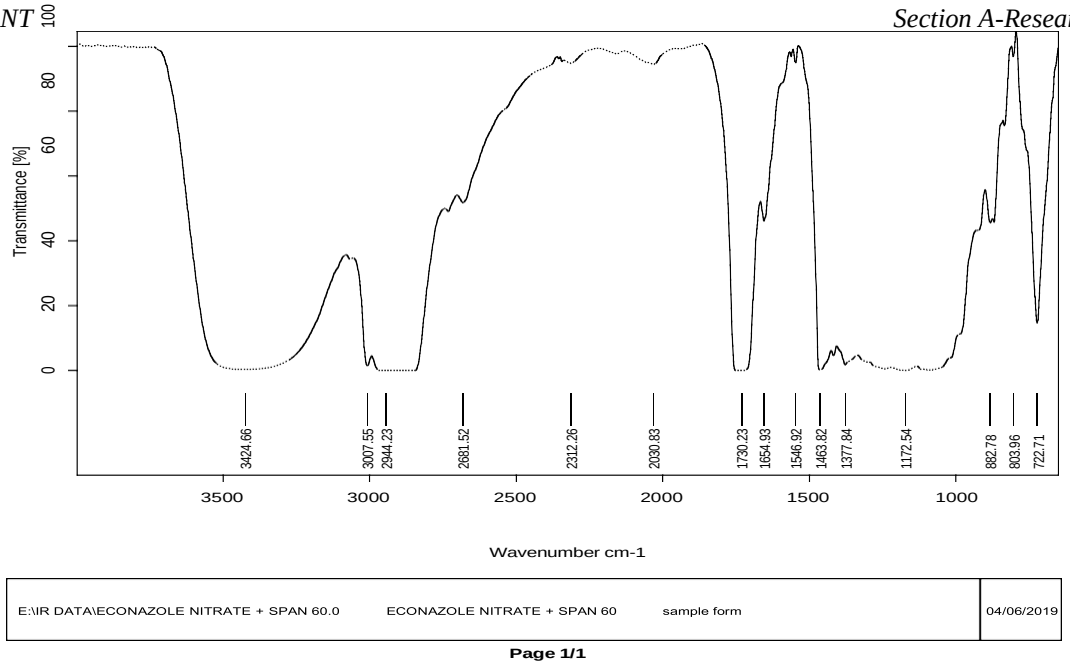

Figure 03:FTIR Spectra of Econazole nitrate + Span 60

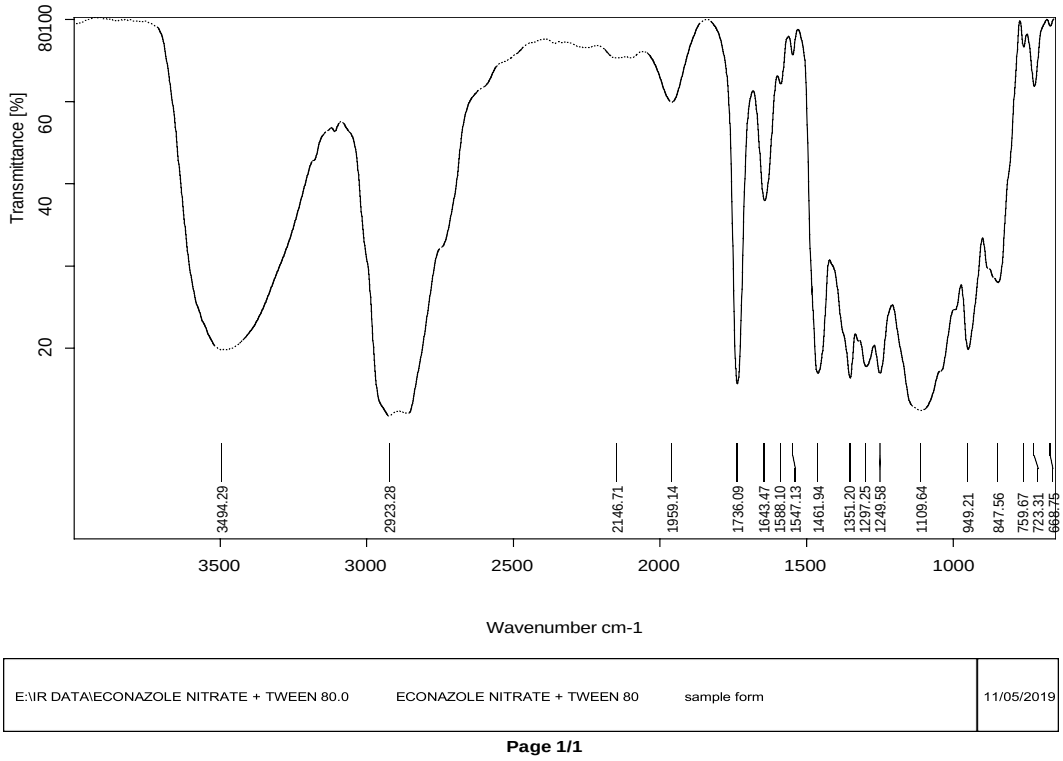

Figure 04: FTIR Spectra of Econazole nitrate + Tween 80

Table 02: Calibration Curve data for Econazole Nitrate at 271.5nm

S.No	Concentration in µg/ml	Absorbance at 271.5nm
1.	0	0
2.	20	0.214
3.	40	0.418
4.	60	0.596
5.	80	0.760
6.	100	0.931

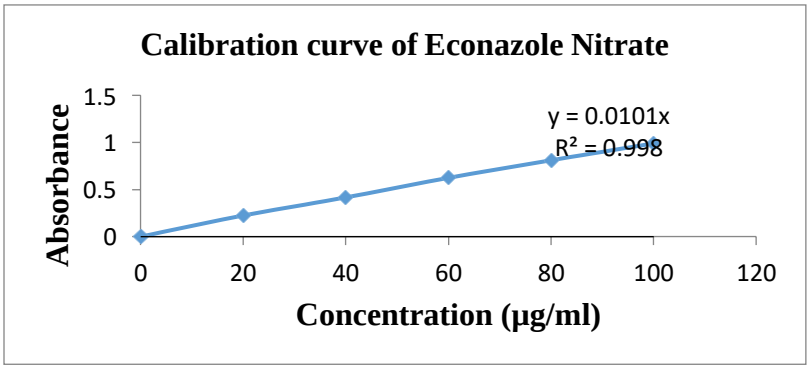

Figure 05: Calibration curve of Econazole Nitrate

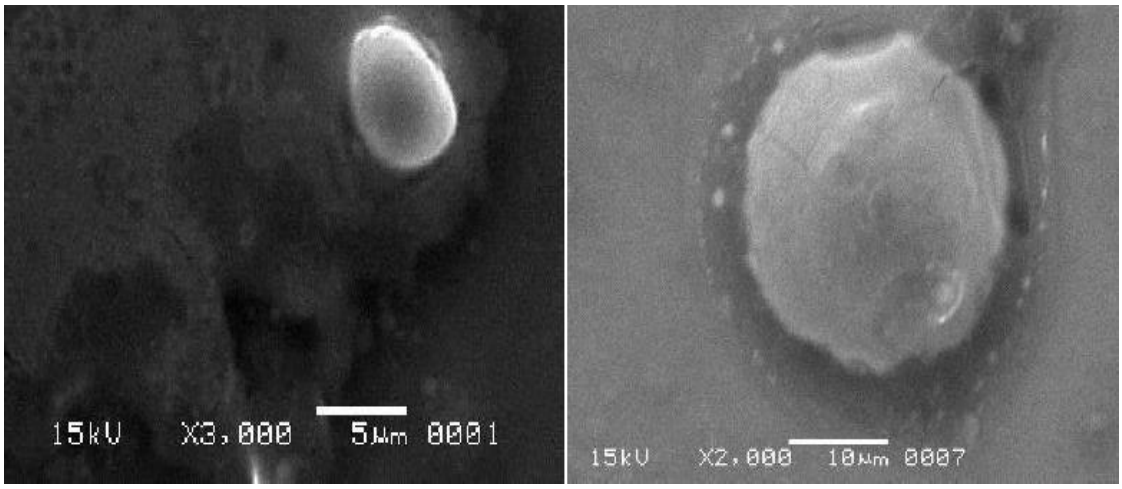

Figure 06: Morphology of Niosomes

Table 03:In vitro diffusion data for formulation F1 to F5

S. No	Time(h)	%cumulative drug release				
		F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	1	12.86	10.31	11.03	11.53	11.02
3	2	21.57	19.15	18.71	18.80	17.74
4	3	30.60	27.28	26.36	27.64	28.10
5	4	34.73	33.90	32.57	32.83	33.10
6	5	40.40	41.83	42.52	43.41	42.25
7	6	46.48	46.10	48.02	53.24	51.16
8	7	53.07	54.48	54.97	64.76	63.31
9	8	61.01	64.20	65.67	73.93	73.17
10	12	78.98	82.05	84.16	87.47	86.37

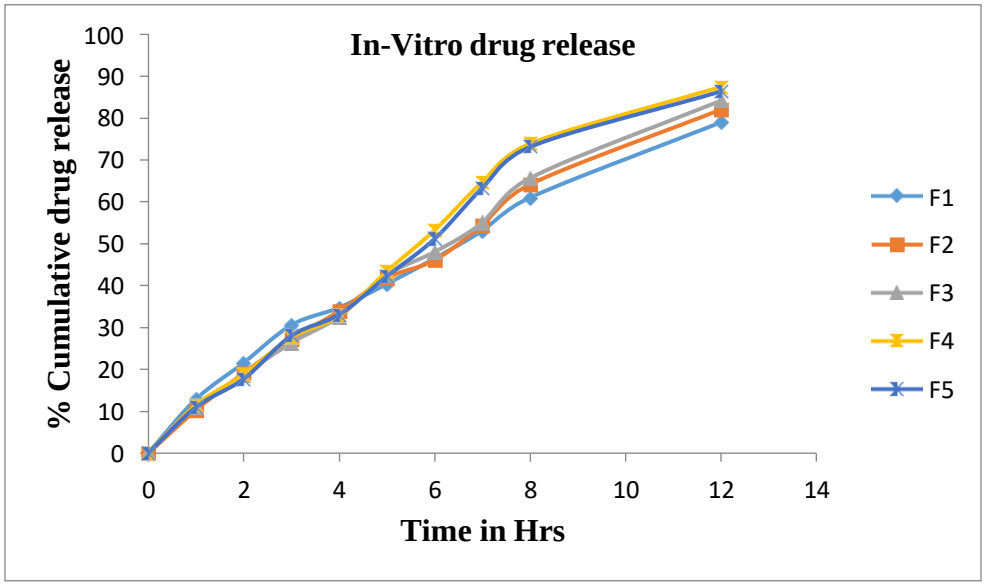

Figure 07: In vitro diffusion studies for formulations F1-F5

Table 04: In vitro diffusion data for Formulation F 6 to F10

S.No	Time(h)	% cumulative drug release				
		F6	F7	F8	F9	F10 `
1	0	0	0	0	0	0
2	1	10.27	11.51	11.91	12.34	12.60
3	2	16.45	17.00	16.93	17.16	20.30
4	3	27.12	29.81	30.61	32.08	30.87
5	4	33.47	35.47	34.96	36.11	39.61
6	5	41.20	43.20	41.47	42.04	46.19
7	6	51.66	56.04	51.88	51.41	56.15
8	7	64.10	65.53	61.54	63.27	65.56
9	8	71.47	73.66	71.08	70.44	76.12
10	12	83.43	87.24	88.79	90.34	94.05

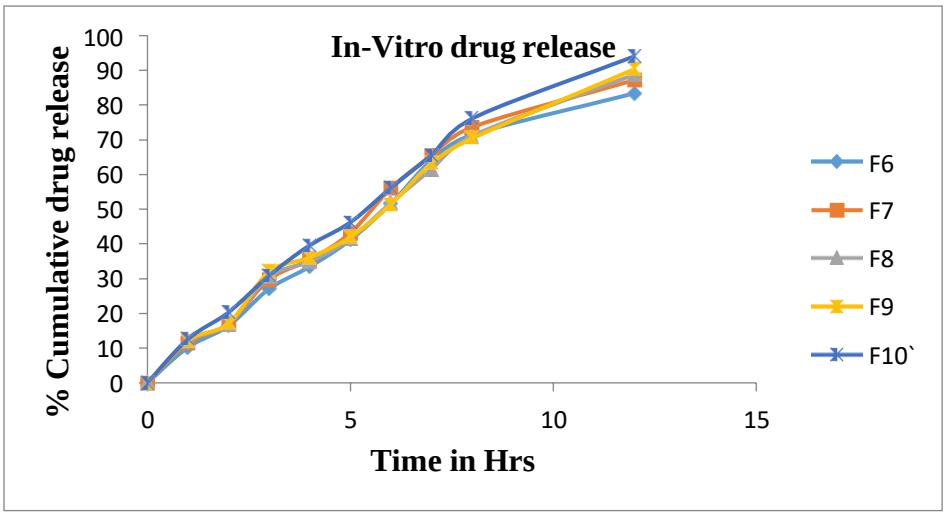

Figure 08: In-vitro diffusion studies for formulations F6-F10

Table 05: Release Kinetics Data of the formulation F1 to F10

Formulation Code	Zero order R^2	First order R^2	Higuchi R^2	Korsmeyer-peppas	
				n	R^2
F1	0.977	0.986	0.963	0.848	0.962
F2	0.984	0.976	0.949	0.856	0.991
F3	0.987	0.984	0.938	0.845	0.989
F4	0.969	0.976	0.943	0.853	0.986
F5	0.970	0.978	0.939	0.865	0.986
F6	0.962	0.989	0.966	0.759	0.983
F7	0.964	0.969	0.953	0.852	0.991
F8	0.980	0.985	0.965	0.828	0.926
F9	0.980	0.959	0.959	0.834	0.988
F10	0.979	0.986	0.954	0.858	0.976

CONCLUSION

The study performed on niosomes as a drug carrier for transdermal delivery of econazolenitrate.From the results of the present experiment it may be concluded that formulations containing high concentration of cholesterol showed better entrapment efficiency. All the niosomes showed good *in vitro* release indicating thesustained release of drug.Econazole nitrate is used as anti-fungal drug and carrier as niosomes can produce sustainedrelease,at specific site thereby reducing frequency of drug administration.

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