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DESIGN AND EVALUATION OF PRONIOSOMAL GEL OF ECONAZOLE NITRATE FOR ENHANCED TOPICAL DELIVERY

Chandhana Krishnan* and Sujith S. Nair

Department of Pharmaceutics, Crescent College of Pharmaceutical Sciences, Payangadi P O Kannur-670358, Kerala, India.

***Corresponding Author: Chandhana Krishnan**

Department of Pharmaceutics, Crescent College of Pharmaceutical Sciences, Payangadi P O Kannur-670358, Kerala, India.

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ABSTRACT

The application of topical drug delivery system is gaining momentum due to their adaptability and widespread usability. The feasibility of a proniosomal gel of econazole nitrate for topical delivery has not been widely explored. The present study aims to design and optimize a proniosomal gel formulation of econazole nitrate for enhanced topical delivery in antifungal therapy. Econazole nitrate belongs to the BCS class II with a half-life 4 hours. Econazole nitrate proniosomes were formulated by coacervation phase separation method consisting of span 60, cholesterol and other ingredients. The formulated proniosomes were characterized for various parameters such as entrapment efficiency, vesicle size, *in vitro* drug release etc. The optimization based on central composite design assessed the effect of varying ratios of span 60 and cholesterol on percentage entrapment efficiency, mean vesicle size and *in vitro* drug release as responses. Evaluation of the optimized formulation indicated a prolonged release of 12 hours with a higher entrapment efficiency of 95.41% at optimal combination of span 60 and cholesterol in the ratio 6.7:1 and vesicles were found as discrete and spherical. Moreover, the optimized formulation exhibited an excellent antifungal activity characterized by greater zone of inhibition compared to marketed preparation. With sustained drug release and higher entrapment efficiency, proniosomal gel of econazole nitrate could be considered a suitable candidate for enhanced skin permeation, serving improved patient compliance and better therapeutic uses. The present study concludes on the feasibility of econazole-based proniosomal gel in antifungal therapy.

KEYWORDS: Econazole, Proniosomal gel, Span 60, Antifungal-Therapy.

INTRODUCTION

The most challenging issue encountered in the advancement of transdermal drug delivery system is to overcome the physical, biochemical and immunological barriers of the skin. The rate of transdermal drug transport is mostly restricted by the stratum corneum, the outer skin layer. Several physical as well as chemical strategies have been estimated to improve the transdermal permeation such as ultrasound, electroporation, heat, drug delivery carriers like liposomes, ethosomes, niosomes etc. The application of drug delivery vesicle could remarkably modify the physicochemical properties of the entrapped compound and thus assist in percutaneous transfer of large hydrophilic or electrically neutral molecules. Transdermal administration can maintain consistent plasma levels for an extended period of time after a single dose. To enhance the percutaneous transfer of drugs, several formulation strategies, including liposomes, niosomes, proniosomes, microemulsion has been explored. During the last few decades, the transdermal permeation of various drugs employing niosomes have been assessed and proven as an excellent transdermal nanocarrier.

Proniosomes are dry, free flowing pro-vesicles with a liquid crystalline consistency up on hydration readily forms niosomes. The components present in proniosomes namely phospholipids and non-ionic surfactants have the ablity to diffuse into the stratum corneum, interrupting the fluidity of the lipid bilayers causing defective permeablity barrier function to act as penetration enhancers.[1] Non-ionic surfactant and cholesterol ratio could influence on entrapment efficiency and on release characteristics of the incorporated drug.[2] Proniosomal gel when applied to skin under occlusive condition get hydrated with the skin moisture and converted to niosome.[3] Proniosomal gel offer great potentialality to reduce the side effects of drugs and increase the therapeutic effectiveness of transdermal drug delivery.[4] Proniosomes are assumed to prevent numerous formulation related issues associated with liposomes, like physical and chemical instabilities, difficulties faced in sterilization, purity related issues of phospholipids and large-scale production process.[1] Proniosomal gel are becoming more favourable in the area of semisolid dosage forms due to their ease of application and better percutaneous absorption.[4]

Econazole Nitrate belongs to the class of imidazole broad spectrum antifungal agent categorised under BCS class II.[5] It has a terminal half-life of about 4 hours.[6] The plasma protein binding is about 98% and the absorption is very poor when administered topically.[7] It is commonly given by topical route for the controlling of superficial candidiasis, dermatophytosis, ptyriasis versicolor and skin contagions.[8] It interacts with 14α-demethylase, a cytochrome p-450 enzyme required to convert lanosterol to ergosterol. Econazole inhibits the synthesis of ergosterol which is an essential component of fungal cell membrane, increasing the cellular permeability resulting in leakage of cellular contents, causing fungal cell death.[5]

The present study aims at developing a new topical formulation for econazole nitrate defined by safety and high therapeutic efficacy, through designing an optimum proniosomal gel formulation with a subsequent enhancement in patient compliance. The objective of the study was to design and optimize econazole nitrate loaded proniosomal gel by central composite design to improve the skin penetration and residence time of the drug. Optimization was performed by evaluating concentration effect of formulation components on entrapment efficiency, vesicle size and *in vitro* drug release. The optimized proniosomal gel was compared with marketed preparation for antifungal activity. Stability studies of the optimized formulation were also conducted for a period of 3 months.

MATERIALS AND METHODS

Materials

Chemicals and Instruments used

Econazole nitrate (Yarrow chem products, Mumbai), Span 60 (Sisco Research laboratories Maharashtra), Lecithin (Sisco Research laboratories Maharashtra), Cholesterol (Sisco Research laboratories Maharashtra), Carbopol 934 (Loba Chemie). Double beam UV Spectrometer, Electronic weighing balance (Price scale industries, Ahmedabad), Bruker Alpha-Attenuated Total Reflectance FTIR.

Methods

Preformulation Study

Preformulation may be described as a phase of research and development process that describes the physical, chemical and mechanical properties of new drug substances in order to develop stable, safe and effective dosage forms. These studies are intended to determine the compatibility of initial excipients with the active substance for a biopharmaceutical, physicochemical and analytical investigation in support of promising experimental formulations.[9]

Preparation of standard calibration curve of econazole nitrate in phosphate buffer pH 7.4

Accurately weighed 50 mg of econazole nitrate was dissolved in 25 ml of methanol and made up to 50 ml with phosphate buffer pH 7.4 in 50 ml volumetric flask to get the stock solution of 1000 µg/ml. From the above stock solution 5 ml was pipetted out and further diluted up to 50 ml with buffer to get 100µg/ml solution. From 100µg/ml solution take 5 ml and diluted to 50 ml to get 10µg/ml solution. From these aliquots of 2, 4, 6, 8, 10ml was withdrawn and diluted to 10 ml with phosphate buffer pH 7.4 to obtain concentration range of 2-10µg/ml. The absorbances of the solutions was measured at 272 nm by using UV-Visible spectrophotometer. A graph of concentration versus absorbance was plotted.[10]

Physicochemical properties of drug

Organoleptic properties

Drug was tested for colour, odour, and taste and compared with official monograph.

Solubility of drug

For the purpose of solubility, beyond saturation additional amount of drug is added in the solvent (either aqueous or non-aqueous) at room temperature and kept for 24 hours with rare shaking. The supernatant was taken and evaluated by using UV double beam spectrophotometer.[5] Solubility of econazole nitrate was tested in different solvents such as distilled water, phosphate buffer pH 7.4, ethanol (95%v/v), methanol.

Drug- excipient compatibility

FTIR spectroscopy method was used to carry out drug-excipients compatibility study. FTIR spectra of pure drug, span 60, cholesterol, lecithin and their physical mixture were taken by KBr pellet technique between 400- 4000cm^{-1}. Once spectra were recorded, the peaks of pure drug, polymer and physical mixtures of polymers and drug were compared for incompatability.[2]

Determination of melting point of pure drug

Determined by using capillary method. Drug is filled into capillary tube up to the height of 3mm by sealing its one end. The capillary tube is introduced into the digital melting point apparatus and the point at which the drug starts melting note that point until the entire sample get melted.[5]

Method of preparation of proniosomal gel of Econazole nitrate

Econazole nitrate loaded proniosomal gel were prepared by coacervation phase separation method. A clean glass beaker was taken in that drug, surfactant, lecithin and cholesterol were added and mixed it. A measured amount of ethanol was added to beaker to dissolve all the ingredients. The glass beaker was enclosed with a closure to avoid the solvent loss. The beaker was then taken into a water bath, warmed at 60-70°C for nearly five minutes, till the surfactants were dissolved completely. In that phosphate buffer pH 7.4 was added and warmed on a water bath till clear solution was formed. The beaker was set aside in a dark place for allowed to cool at room temperature till the dispersion was converted to proniosomal gel.[2]

Table 1: Formulation design of proniosomal gel of Econazole nitrate.

Formulation code	Drug (mg)	Span 60 (mg)	Cholesterol (mg)	Lecithin (mg)	Ethanol (ml)	Phosphate Buffer pH 7.4 (ml)
F1	50	3000	300	1250	5	5
F2	50	2000	300	1250	5	5
F3	50	1000	100	1250	5	5
F4	50	1000	300	1250	5	5
F5	50	2000	100	1250	5	5
F6	50	3000	100	1250	5	5
F7	50	2000	300	1250	5	5
F8	50	2000	300	1250	5	5
F9	50	2000	500	1250	5	5
F10	50	2000	300	1250	5	5
F11	50	1000	500	1250	5	5
F12	50	3000	500	1250	5	5
F13	50	2000	300	1250	5	5

Percentage entrapment efficiency

The entrapment efficiency was determined by centrifugation method. 10 ml of phosphate buffer (pH 7.4) was added to proniosomal gel (0.2g) in a small glass tube. The dispersion was agitated in an ultrasonicator for 30 minutes. The niosomes entrapped with econazole nitrate were separated from the free drug using high speed refrigerated centrifuge run at 9000 rpm, 4°C for 45 minutes. The supernatant layer was filtered, suitably diluted with phosphate buffer, and analysed by UV method.[1] The percentage of drug encapsulation (%EE) was calculated.[11] by

$$\% EE = [(C_t - C_f) / C_t] \times 100$$

Where C_t = total concentration of drug, C_f =concentration of free drug.

Vesicle size analysis

A small quantity of proniosomal gel was added in a 10ml of phosphate buffer (pH 7.4). The dispersion of proniosome was manually shaken for few seconds so that lumps of proniosomes are disintegrated into individual proniosomes. A drop of dispersion was placed onto the slide and examined under the microscope at 100x magnification, circular vesicles bodies were observed with uniform small size.[12]

***In vitro* drug release studies**

In vitro release studies of proniosomal gel were carried out using locally manufactured Franz-diffusion cell. The capacity of receptor compartment was 15ml. The dialysis cellophane membrane was mounted between donor and receptor compartment. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment contained phosphate buffer pH 7.4 containing 10%V/V methanol and in the upper donor compartment 10 mg of proniosomal gel was spread uniformly on the membrane. The receptor phase was constantly stirred with the help of magnetic stirrer and was surrounded by a water jacket to maintain the temperature at 37.5±0.5°c. 1 ml of the sample were withdrawn for a time period of 12 hours and were replaced by equal volume of fresh buffer to maintain sink condition of the receptor phase. Samples withdrawn were analyzed spectrophotometrically at 272 nm. By determining the amount of econazole nitrate released at various time intervals, the cumulative percentage drug release versus time was plotted.[2]

Optimization by Design Expert Stat Ease Software

Statistical design of experiment, a computer aided optimization technique, was used to recognize critical factors, their interactions and ideal process conditions that accomplish the targeted response. The best formulation was established using Design Expert State Ease Software (version 13). Central composite design was employed for the optimization. In this study span 60, cholesterol was chosen as the two factors and percentage entrapment efficiency, mean vesicle size and *in vitro* drug release were assessed as the responses. Hence, thirteen experimental trials were conducted. Contour plots were drawn and optimum formulation based on optimization criteria was selected.[13]

Table 2: Factor combination by Central Composite Design for the formulation of proniosomal gel.

Formulation code	Point type	Coded factor level	
		X1	X2
F1	Axial	+1	0
F2	Central point	0	0
F3	Factorial	-1	-1
F4	Axial	-1	0
F5	Axial	0	-1
F6	Factorial	+1	-1
F7	Central point	0	0
F8	Central point	0	0
F9	Axial	0	+1
F10	Central point	0	0
F11	Factorial	-1	+1
F12	Factorial	+1	+1
F13	Central point	0	0

X_1- Span 60, X_2- Cholesterol.

Scanning electron microscopy (SEM)

The surface morphology of proniosomes was studied by scanning electron microscopy (SEM). The shape of the formulation and sizes of the vesicles were determined by SEM. A drop of proniosomal suspension was placed on the specimen stub which was coated with carbon and then with gold vapour appeared using hitachi vacuum evaporator. The samples were examined under a scanning electron microscope for vesicular shape and then photographed.[14]

Drug release kinetics

The exact mechanism of drug release from the dosage form, was understood from the study of drug release kinetics, the data of *in vitro* drug release study of the optimized formulation was integrated into various kinetics equations (Zero order, First order, Higuchi model and Korsmeyer-Peppa's model).[2]

***In vi*tro antifungal study**

In a 500 ml of conical flask required amount of saboured dextrose agar was taken and 250 ml of purified water is added. Heat is applied to dissolve the saboured dextrose agar completely. Sterilized for 15 minutes at 121°C at 15lb pressure in autoclave for about 20 minutes. Then cooled it at room temperature and the fungal strain(Candida albicans) was dispersed in the medium and then the medium was poured into the required petridish and allowed it to cool until it get solidified at room temperature and then the cups are bored in agar plate by using cork borer having 6mm diameter and calculated concentration of the optimized proniosomal gel, control(Proniosomal gel without econazole nitrate) and marketed preparation were placed in the bores and incubated the petri plates for 72 hours at 28°C in incubator. The zone of inhibition was measured and the mean radius of the zone of inhibition was calculated.[2]

Stability studies

Stability studies were carried out at temperature and humidity conditions as per ICH guidelines and the test were carried out in a stability chamber. The ability of vesicle to retain drug was assessed by keeping the proniosomal gel at three different temperature conditions, that is, Refrigeration temperature 4-8°c, Room temperature 25±2°c at 60%±5% RH and oven temperature 40±2°c at 75% ±5% RH. Throughout the study the proniosomal formulation were stored in aluminium foil-sealed glass vials. The sample were withdrawn at different time intervals over a period of 3 months and evaluated for percentage entrapment efficiency, mean vesicle size and *in vitro* drug release.[15]

RESULTS AND DISCUSSION

Calibration curve of Econazole nitrate

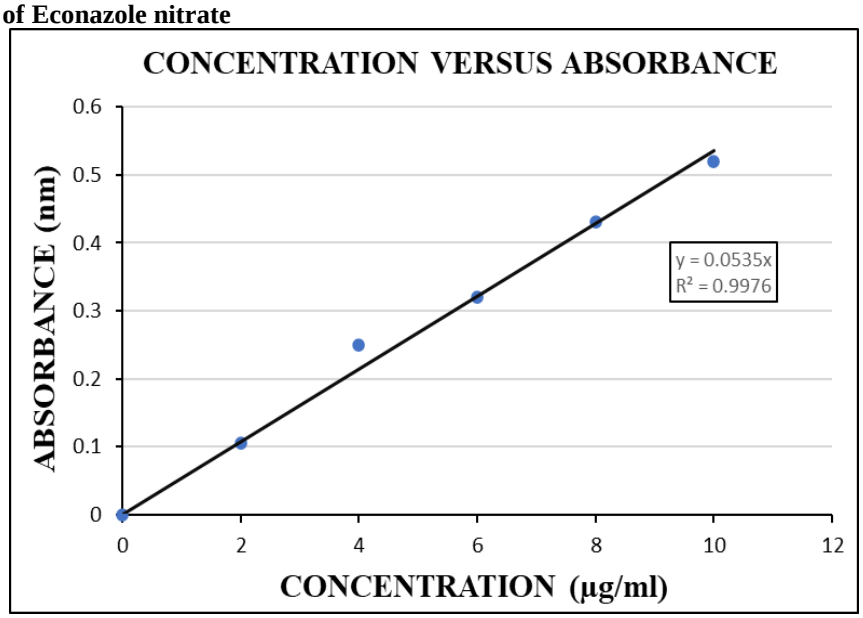

Fig. 1: Standard calibration curve of Econazole nitrate in phosphate buffer pH 7.4 at 272 nm.

The drug was scanned in UV region (200-400nm) by preparing 1000µg /ml solution using phosphate buffer pH 7.4 to find out wavelength of maximum absorption (λ_{max}). The λ_{max} was found to be 272nm. So, the standard calibration curve of econazole nitrate was developed at this wavelength. Standard calibration curve of econazole nitrate was determined in phosphate buffer pH 7.4 by plotting concentration against absorbance at 272nm. The calculation of entrapment efficiency, drug release and stability studies are based on this calibration curve.

Physicochemical properties of drug

Organoleptic properties

Organoleptic properties of the drug were studied and it was found that, econazole nitrate is a white crystalline powder and bitter in taste.

Solubility profile

Solubility studies were carried out in different solvents and it was found that econazole nitrate is very slightly soluble in distilled water, sparingly soluble in phosphate

buffer pH 7.4, slightly soluble in ethanol and freely soluble in methanol. The result complies with the pharmacopoeia specification.

Identification of compatibility by FTIR studies

FTIR studies were conducted in pure econazole nitrate, span 60, cholesterol, lecithin and physical mixture of drug with span 60, cholesterol and lecithin. The FTIR spectra is shown below in fig 2. Drug identification is done by performing FTIR studies. During FTIR studies, the peaks of econazole nitrate was obtained at 3105cm^{-1}(C-H stretching aromatic), 1545 cm^{-1}(C=C stretching), 3668cm^{-1}(O-Hstretching), 1584cm^{-1}(O=N=O stretching), 632cm^{-1}(C-Cl stretching) etc. There were no significant changes in the peaks of the pure drug in the FTIR spectrum of physical mixture of pure drug with the polymers, ie., the span 60, cholesterol and lecithin. It indicates that there is no chemical interaction between the drug and the polymers. This shows that econazole nitrate was compatible with other excipients.

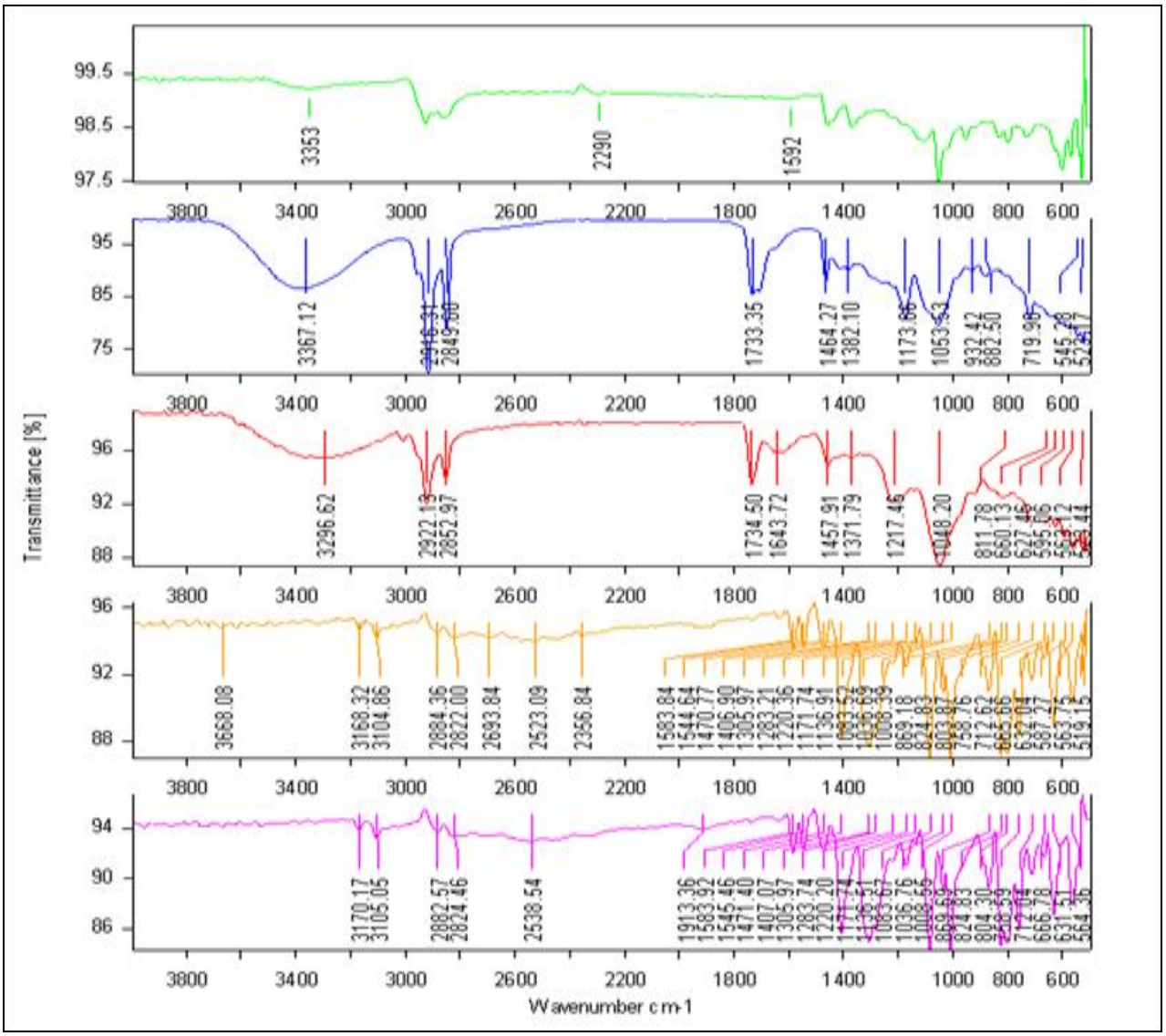

Fig. 2: FTIR spectrum of Econazole nitrate, Span 60, Cholesterol, Lecithin, Physical mixture (Econazole nitrate+ Span 60+ Cholesterol+ Lecithin).

Melting point determination

Melting point of econazole nitrate was found to be 164°C (n=3).

Percentage entrapment efficiency

The entrapment efficiency of econazole nitrate in different proniosomal gel is summarised in table 3. Percentage entrapment efficiency of vesicles was mainly influenced by the fundamental properties of surfactant like HLB, phase transition temperature, structural orientation and critical packing parameter. Entrapment efficiency of proniosomes prepared using span 60 was moderately higher compared to other non-ionic surfactants. This could be explained by the fact that span 60 has higher phase transition temperature (Tc, 53°C) and longer saturated alkyl chain(C16). To prevent the leaky and permeable proniosome, cholesterol was added as a stabilizing agent. Raising cholesterol above specific

concentration may alter the linear vesicular membrane structure and hence inhibit the drug to be within the[1] bilayers. Entrapment efficiency is an important parameter that conveys the stability of vesicles and this depends upon the amount of surfactant and cholesterol used. when the concentration of span 60 varied from 1000 mg to 3000 mg, maximum and minimum entrapment efficiency was found. Variation in the concentration of span 60 from 1000 to 2000 mg showed significant increase in entrapment efficiency, whereas further increase in concentration from 2000 mg to 3000 mg decreased the entrapment efficiency. The entrapment efficiency was increased significantly when cholesterol amount increased from 100mg to 300mg, but further increasing the cholesterol decreased the entrapment efficiency.

Table 3: Percentage entrapment efficiency of formulations F1 - F13.

Formulation code	Percentage entrapment efficiency (%)
F1	90.47±1.321
F2	95.41±0.653
F3	63.32±0.341
F4	60.16±0.359
F5	86.67±0.873
F6	84.38±0.528
F7	95.41±0.653
F8	95.41±0.653
F9	82.05±1.711
F10	95.41±0.653
F11	54.87±1.101
F12	69.26±0.493
F13	95.41±0.653

All values expressed as mean of ± SD, n = 3

Vesicle size analysis

All prepared proniosome formulations displayed vesicle size ranging from 5.20µm-10.37µm. The proniosomes were found as discrete and spherical shaped vesicles without any aggregation. Both span 60 and cholesterol had predominant effect on vesicle size. Small vesicle size[1] diameter is beneficial since it minimizes irritation and improves the transdermal permeation of the vesicles. Increase in the surfactant concentration led to an increase in vesicle size and was related to increase in the overall degree of hydrophilicity. Raising cholesterol content diminished vesicle size since it caused a reduction in the hydrophilicity of bilayers, thus restricting the entry of water to the core of the vesicles. High surfactant/lipid ratio showed vesicles with large diameter because of rise in the overall field of hydrophilicity.

Table 4: Mean vesicle size of formulations F1 -F13.

Formulation code	Mean vesicle size(µm)
F1	7.41±0.54
F2	5.20±0.18
F3	5.83±0.47
F4	7.84±0.71
F5	9.62±0.35
F6	10.37±0.72
F7	5.20±0.18
F8	5.20±0.18
F9	6.15±0.74
F10	5.20±0.18
F11	8.34±0.63
F12	6.74±0.44
F13	5.20±0.18

All values expressed as mean of ± SD, n = 3

***In vitro* drug release studies**

The percentage of econazole nitrate released from various proniosomal gel formulations is presented in figure 3. All the formulation was found to have a linear release and most of the formulations were found to provide approximately 90% release for a period of 12hours. Among all the formulations, F2 showed significant prolonged *in vitro* drug release (96.78±0.44) over a period of 12hrs. A fast drug release has been detected in the initial phase, about 40-60% of the drug entrapped was released within the first few hours and in the second phase, a prolonged release of econazole nitrate was observed from the different proniosomal formulations. Immediate release.[1] in the earlier phase

was because of removal of drug from the surface of vesicles, while the slow drug release at the later stage was due to the controlled diffusion through the swollen niosomal bilayers structure. This type of release behaviour could be beneficial as the initial fast release of drug saturate epidermal which create enough concentration gradient essential for better delivery of drug to the blood.

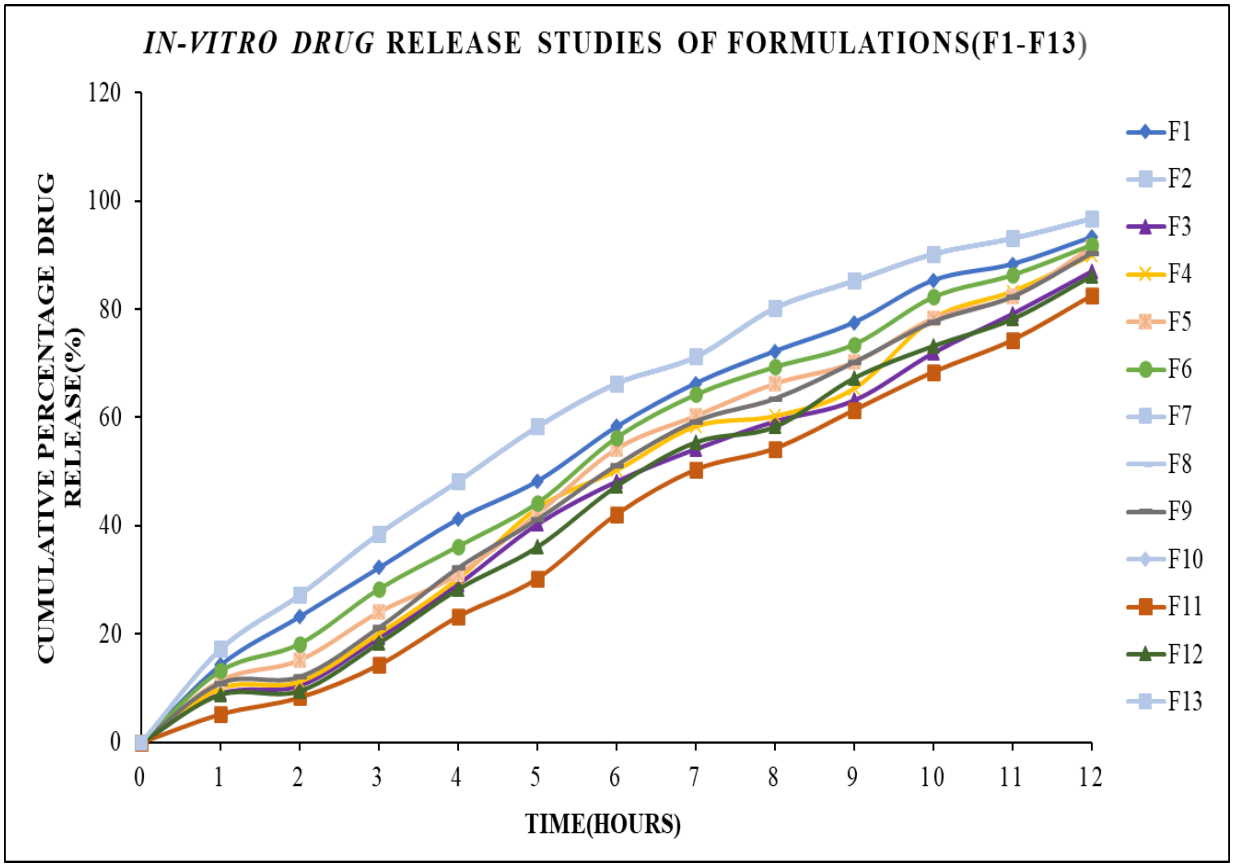

Fig. 3: *In vitro* drug release studies of formulations F1-F13.

3.2.8. Optimization by Design Expert Software

Optimization was done by Design Expert Stat Ease Software version 13.0.7.0. Two factors were selected for optimizing the formulation. The factors selected were span 60 and cholesterol. Central composite design was used for optimization. To determine the best formulation, 3 responses that is Percentage entrapment efficiency, mean vesicle size and *in vitro* drug release were considered. 13 formulations were suggested by the software. The average values were submitted to multiple regression analysis using Design Expert Software. Polynomial models were generated for all response variables. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the coefficient variation (CV), the multiple correlation coefficient (adjusted R^2) and predicted residual sum of square as shown in Table 5.

Table 5: Numerical test results of model adequacy checking for influence of independent variables on response variables.

Response	Model	Sequential P Value	R^2	Adjusted R^2	Predicted R^2	Adequate precision	%CV
Y1	Quadratic	<0.0001	0.9831	0.9982	0.9414	47.2848	1.04
Y2	Quadratic	<0.0256	0.7758	0.6156	-0.8954	7.1241	16.32
Y3	Quadratic	<0.0001	0.9922	0.9867	0.9276	37.2152	0.5957

Y1: Percentage entrapment efficiency, Y2: Mean vesicle size, Y3: *In vitro* drug release

The fit of the model was evaluated using the R^2 values. As observed from the table 5 predicted R^2 value was in reasonable agreement with the adjusted R^2 value (the difference is less than 0.2), indicating reliability of the models. Based on the fit summary quadratic model was chosen as best fit for percentage entrapment efficiency, mean vesicle size and *in vitro* drug release as suggested by software. Adequate precision (which measures signal to noise ratio) was greater for all responses showing that the proposed models can be used to navigate the space.

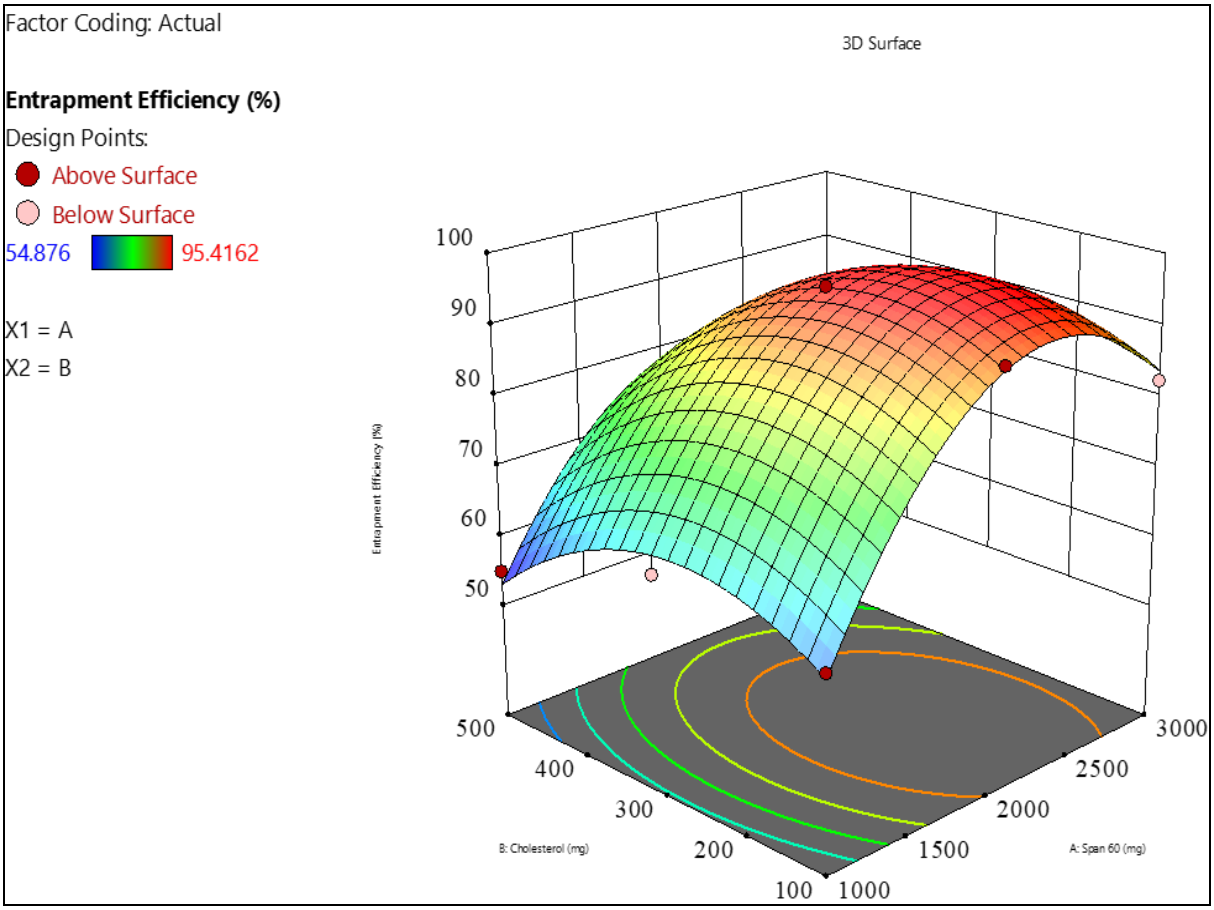

Fig. 4: 3-D response surface plot for the effect of amount of span 60 and cholesterol on percentage entrapment efficiency (%).

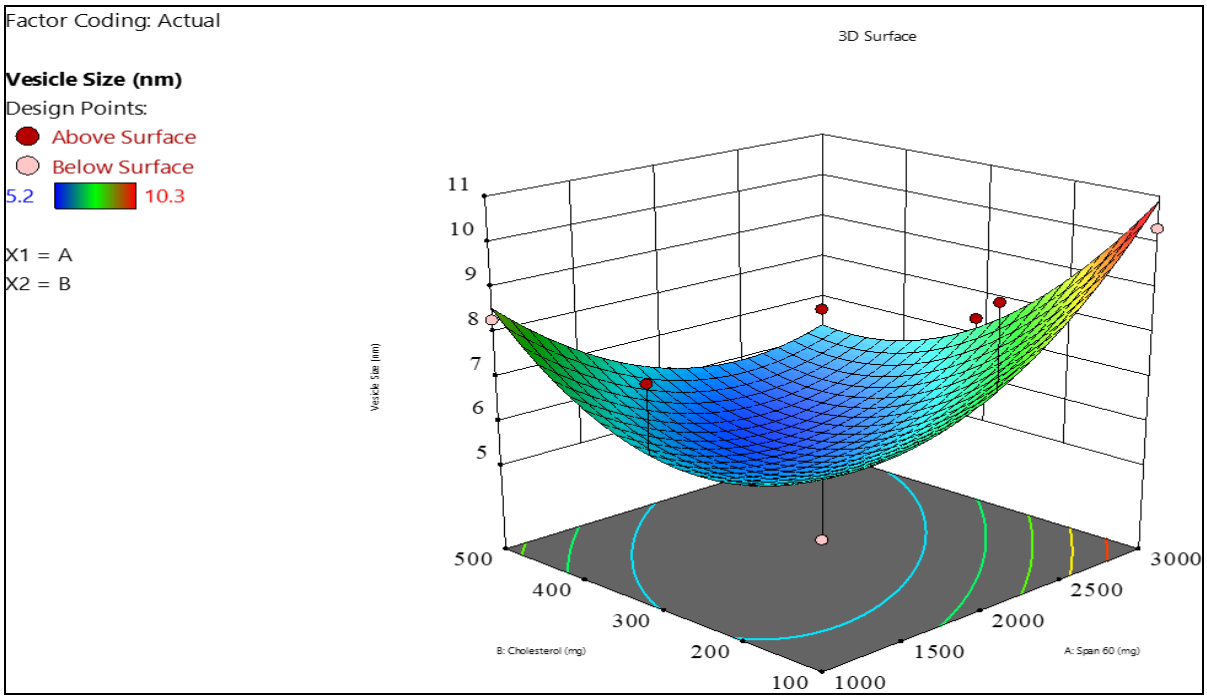

Fig. 5: 3-D response surface plot for the effect of amount of span 60 and cholesterol on mean vesicle size (µm).

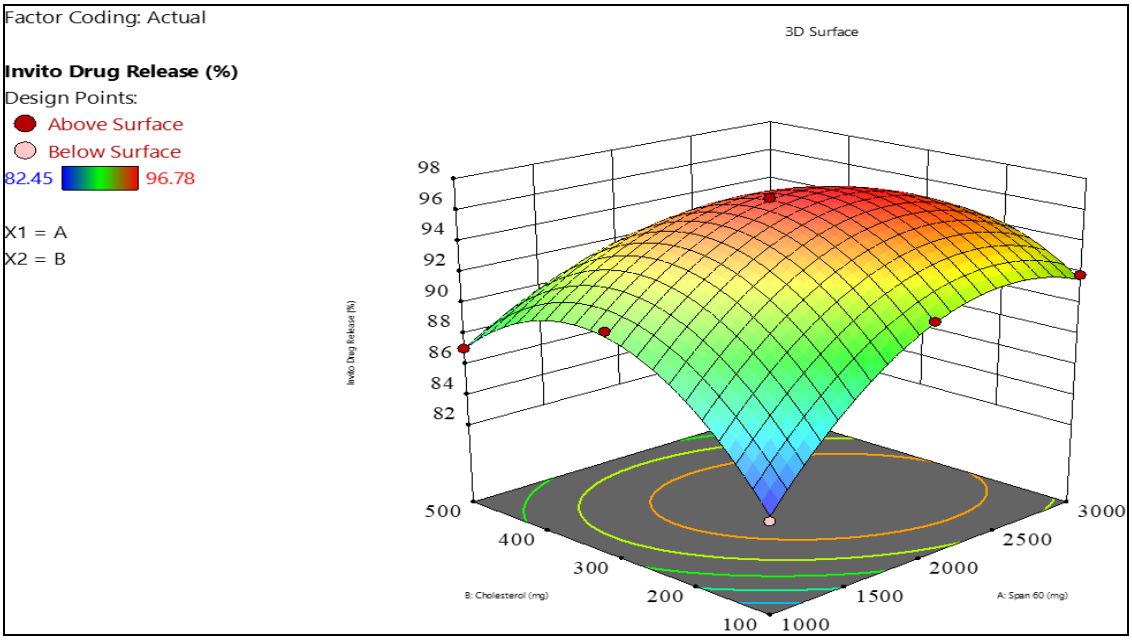

Fig. 6: 3-D response surface plot for the effect of amount of span 60 and cholesterol on *in vitro* drug release (%).

The desirability function approach is one of the most widely used method for optimization of multiple responses. Overall desirability function is a measure of how well the combined goals for all responses are satisfied. Desirability function ranges from 0 to1, with value closer to 1 indicating a higher satisfaction of response goal. The numerical optimization tool provides 3 set of optimum solution as shown in table 6 among which 2083.165 mg of span 60 and 312.989 mg of cholesterol were selected by the software as optimized concentration with desirability of 0.976. The area of optimized formulation was also ratified using overlay plot as shown in fig 9 in which yellow region represents the area satisfying the imposed criteria.

Table 6: Desirability table.

Sl. no	Span 60(mg)	Cholesterol (mg)	Y1	Y2	Y3	Desirability	
1	2083.165	312.989	95.416	5.538	96.713	0.976	**Selected**
2	2051.549	310.289	92.309	6.534	92.710	0.834	
3	2089.328	309.356	90.401	5.021	90.708	0.812	

Y1: Percentage entrapment efficiency, Y2: Mean vesicle size, Y3: *In vitro* drug release.

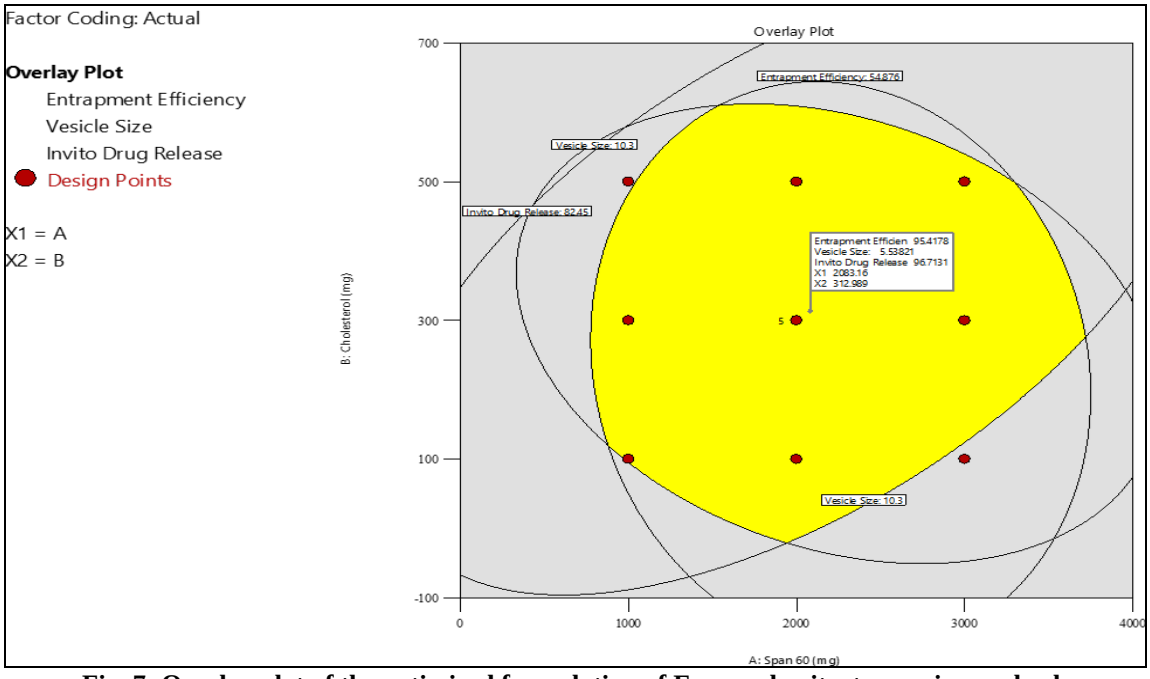

Fig. 7: Overlay plot of the optimized formulation of Econazole nitrate proniosomal gel.

The experiment was carried out in triplicate at the selected optimum concentrations (2083.165mg of span 60 and 312.989mg of cholesterol) and the resulting proniosomal gel were evaluated for entrapment efficiency, vesicle size and drug release. The results are shown in table 7.

Table 7: Response values of predicted, experimental and percentage error obtained at optimal levels of the factors.

Solution 1 of 1 Response	Predicted value	Observed value	% error
YI	95.416	95.368	-0.0503
Y2	5.538	5.522	-0.0028
Y3	96.713	96.682	-0.0320

Y1: Percentage entrapment efficiency, Y2: Mean vesicle size, Y3: *In vitro* drug release.

Scanning Electron Microscopic examination

The fig 8 shows the scanning electron microscopic (SEM) image of the proniosomal gel. It has nearly spherical shape with a smooth surface indicating that encapsulated drug vesicles shows no sign of aggregation, signifying the homogeneity of the produced proniosomal gel.

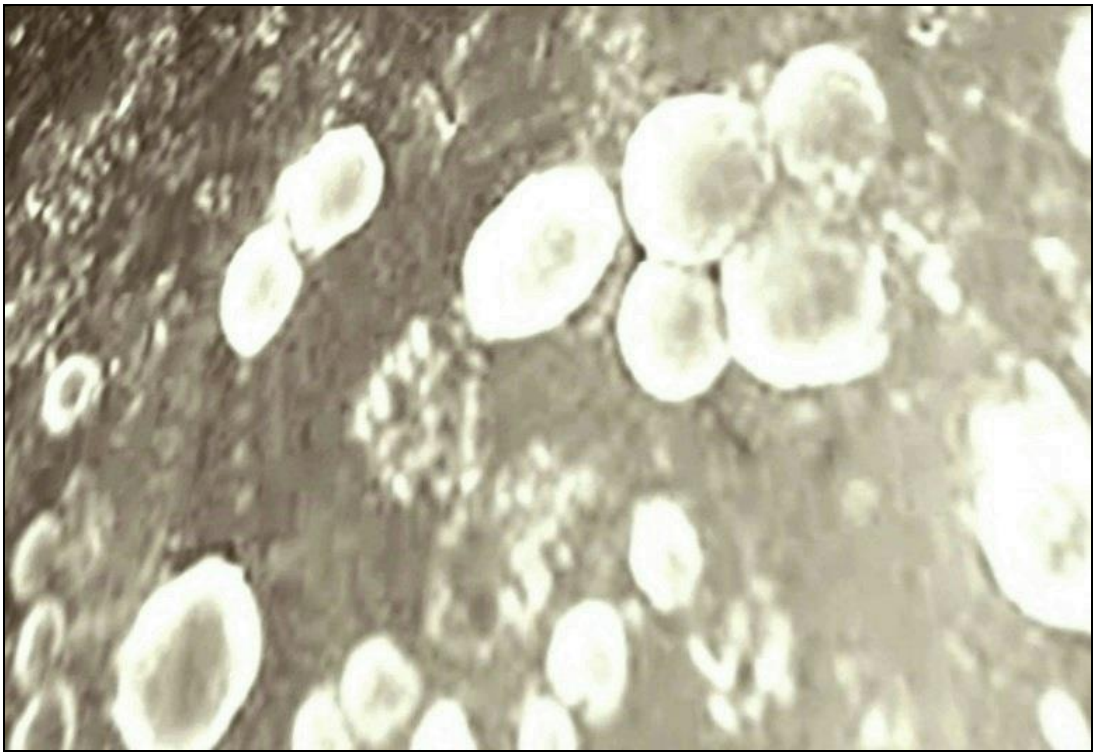

Fig. 8: Scanning electron microscopic image of the optimized formulation.

Drug release kinetics

In optimized formulation, correlation coefficient of zero order kinetics was found to be 0.9397, first order release kinetics was 0.9821 and Higuchi plot was found to be 0.9065. Hence the formulation follows first order kinetics. To confirm the exact mechanism of drug release from the proniosomal gel, data was fitted according to Korsmeyer-Peppa's plot. The value of slope of plot n gives indication of release mechanism when n=1, release is independent of time that is zero order. If n= 0.5, then release is fickian diffusion. If n= 0.5 -1, diffusion is non-fickian and n>1 then it is super case transport. The 'n' exponent value of best batch was 0.7951. Hence it shows non-fickian diffusion.

Table 8: Kinetic studies of the optimized formulation.

Zero order	First order	Higuchi	Korsmeyer-Peppas
R^2	R^2	R^2	R^2
0.9397	0.9821	0.9065	0.9618

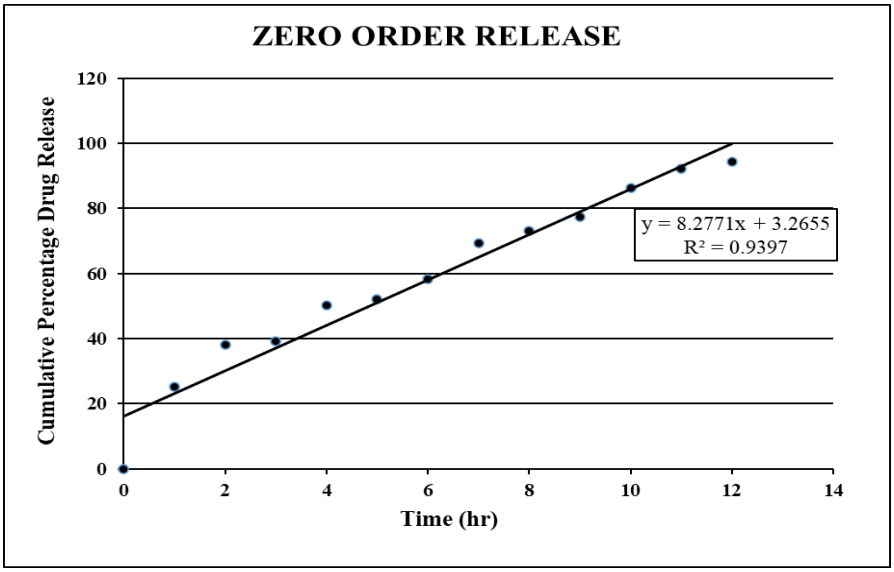

Fig. 9: Zero order release kinetics of the optimized formulation.

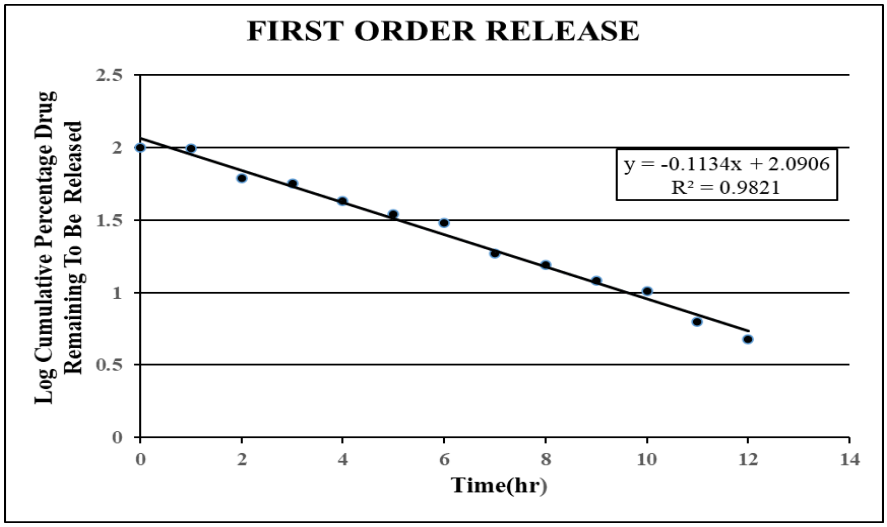

Fig. 10: First order release kinetics of the optimized formulation.

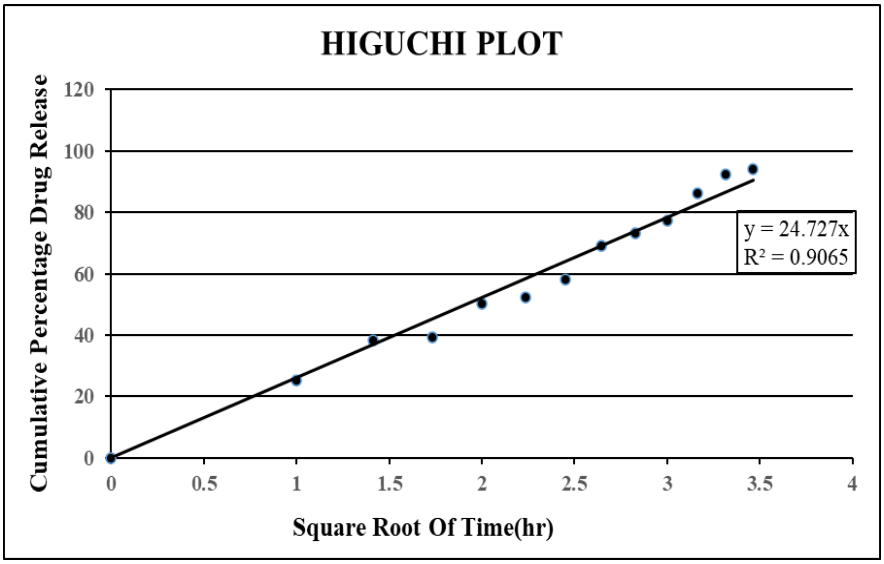

Fig. 11: Higuchi Plot of the optimized formulation.

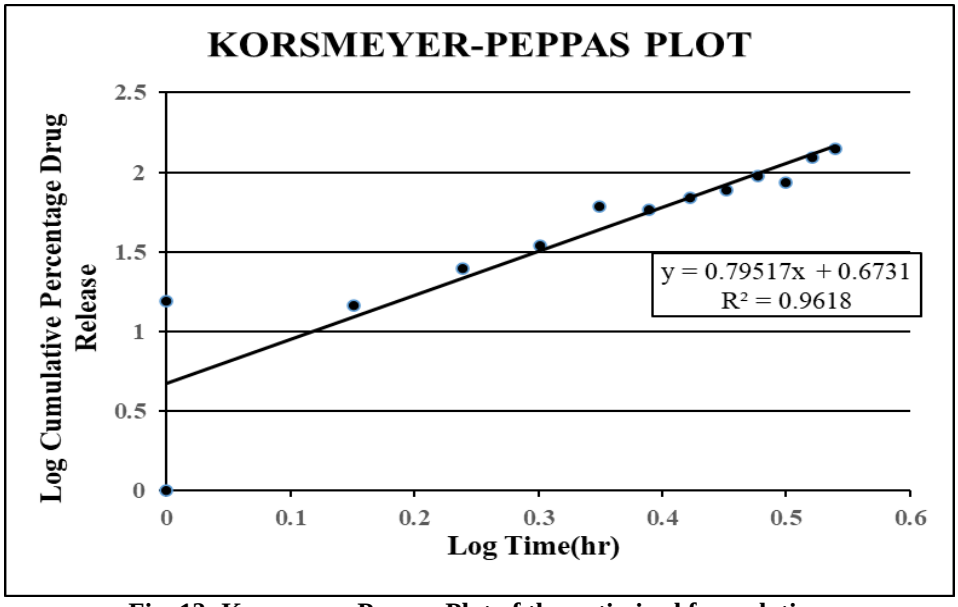

Fig. 12: Korsmeyer-Peppas Plot of the optimized formulation.

***In vitro* antifungal study**

The radius of zone of inhibition (ZI) obtained with the three samples was shown in table 9. The optimized formulation showed more promising and efficient antifungal efficacy against candida albicans in concentration 1%w/w compared to control (proniosomal gel without drug) and marketed preparation(1%w/w). Optimized formulation showed ZI of 18.43mm compared to ZI of 13.05 mm obtained for the marketed preparation and ZI of 1.01mm for control proniosomal gel without drug. The reason[16] for the significant increase of ZI of optimized formulation is that ZI largely depends on the solubility and diffusion of the drug through the agar media and exert its fungistatic effect against candida albicans, by inhibiting the cytochrome P-450-dependent enzyme lanosterol demethylase which is required for the conversion of lanosterol to ergosterol.

Table 9: Mean of radius of zone of inhibition (mm) of (a) control (proniosomal gel without drug), (b) optimized formulation in concentration (1%w/w), (c) marketed preparation (1%w/w) against Candida Albicans.

Sl.no	Formulation code	Mean of radius of zone of inhibition (mm) against candida albicans.
(a)	Sample 1	1.01±0.21
(b)	Sample 2	18.43±0.76
(c)	Sample 3	13.05±0.38

All values expressed as mean of ± SD, n = 3

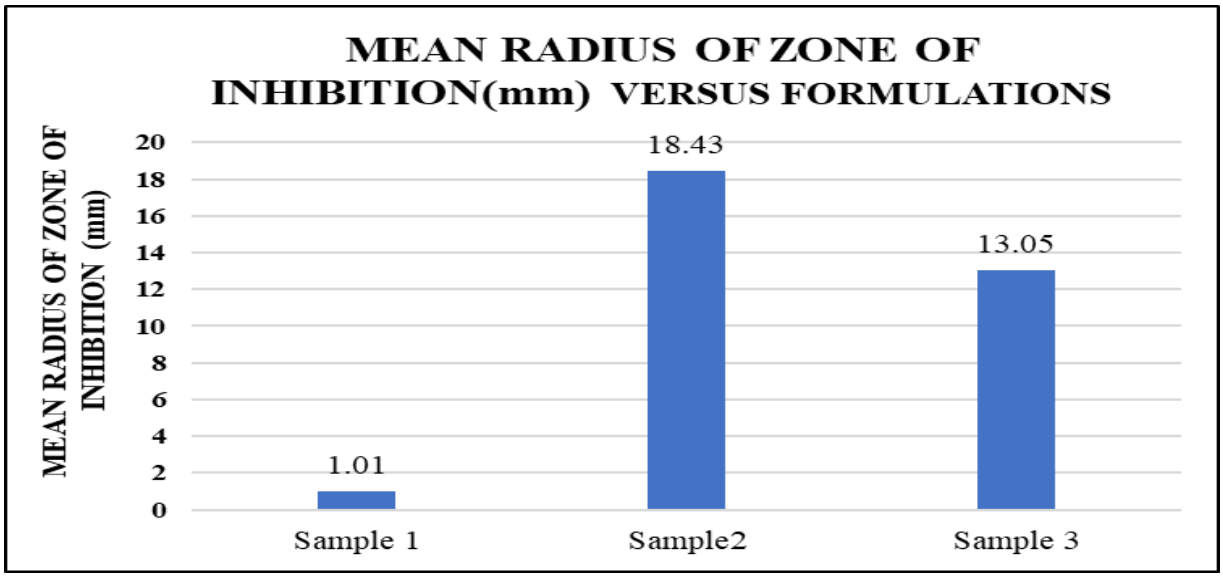

Fig. 13: Bar graph representing antifungal activity of Sample 1- control (proniosomal gel without drug), sample 2- optimized formulation (1%w/w), sample 3- marketed preparation (1%w/w).

Stability studies

From the prepared 13 formulations, the optimized formulation was used for stability studies as per the ICH guidelines for a period of 3 months. It indicates that prepared proniosomal gel passed stability studies without much significant changes in the percentage entrapment efficiency, mean vesicle size and *in vitro* drug release.

Table 10: Stability data of the optimized formulation.

Storage condition	Sampling interval	Percentage entrapment efficiency (%)	Mean vesicle size (µm)	*In vitro* drug release (%)
40ºC ± 2ºC at 75% ± 5%RH	Initial study	95.41±0.65	5.20±0.18	96.78±0.48
	30 days	94.76±0.85	5.18±0.33	95.46±0.48
	90 days	94.41±0.55	5.16±0.56	94.78±0.48
25ºC ± 2ºC at 60% ± 5% RH	Initial study	95.41±0.15	5.20±0.11	96.78±0.48
	30 days	94.15±0.63	5.20±0.74	95.55±0.48
	90 days	93.03±0.79	5.15±0.09	95.12±0.48
5ºC ± 3ºC	Initial study	95.41±0.43	5.20±0.12	96.78±0.48
	30 days	95.12±0.63	5.14±0.22	95.29±0.48
	90 days	94.05±0.81	5.11±0.44	94.07±0.48

All values expressed as mean of ± SD, n = 3

CONCLUSION

A proniosomal gel of econazole nitrate containing span 60, cholesterol, and lecithin was successfully formulated by the coacervation phase separation method. The study showed the suitability of the proniosomal gel in achieving the desired sustainment effect for topical delivery of econazole nitrate for the management of fungal infections. The optimized formulation selected for enhancing percentage entrapment efficiency, *in vitro* drug release and spherical shaped vesicle size incorporated 2083.165mg of span 60 and 312.989mg of cholesterol.

The optimization studies recommended that the percentage entrapment efficiency, vesicle size, and drug release were mainly influenced by the concentration of both non-ionic surfactant and cholesterol. The prepared proniosomal gel of econazole nitrate is considered a successful topical delivery system providing sustained release of the encapsulated drug and maintaining better therapeutic efficacy.

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