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Research Article

Formulation and Evaluation of Proniosomal Topical Antifungal Gel of Miconazole Nitrate

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ABSTRACT

Proniosomal gel formulations of miconazole nitrate (MCZ) were prepared by using combinations of different grades of (non-ionic surfactant) span, cholesterol, and lecithin by coacervation phase separation method. Developed 10 proniosomal gel formulations were characterized for particle size, shape, % entrapment efficiency, drug content, *in vitro* drug permeation, scanning electron microscopy (SEM), DSC, stability study. The fourier transform infrared spectroscopy (FTIR) studies confirmed the compatibility of the drug with excipients. The results showed that all the formulations were pale yellow to pale brown in color, pH was is in the range of 5.60 to 7.20, and encapsulation efficiency was found is in the range of 83 to 91.25% and particle size in between 5.81 ± 0.2 to 07.52 ± 0.07. Among the ten formulations MF2, MF3, MF5, MF6, and MF8 showed maximum drug release in a controlled manner at 12 hours of study and developed into carbopol proniosomal topical gel and evaluated for ex-vivo drug permeation. Formulation-optimized formulation C5MF8 showed higher drug permeation 74.19 ± 0.16% at 12 hr. with a flux value of 6.829 ± 0.12 μg/cm^2/hr. The permeability coefficient of 0.341 ±0.08 cm^2/hrs., higher correlation coefficient R2 0.9944 for zero-order drug release kinetic model, and follows zero-order release kinetics.

Among the 5 formulations, optimized carbopol proniosomal topical gel formulation C5MF8 drug release and *in-vitro* antifungal activity was compared with marketed formulation cream.C5MF8 showed sustain drug release and zone of inhibition value was very near to marketed preparation. Hence it was concluded that developed carbopol proniosomal topical gel had the potential to act as a controlled release drug carrier, which sustains the drug release for many hours and exhibit good antifungal activity

Introduction

For topical drug delivery, skin acts as a main barrier, and the stratum corneum has a major role in barrier function for topical drug delivery. The low permeability of the stratum corneum limits the application of topical drug delivery. A novel drug delivery system is used to overwhelm these limitations, offering control of drug release in the body. Sustain drug release at a pre-determined rate is occur through novel drug delivery systems, and relatively constant effective drug level is maintained with minimization of undesirable side effects. Such novel drug carriers are niosomes, liposomes, lipoproteins, and microcapsules, which can degrade slowly to target specific sites.[1] Drug administration through topical is a localized system of drug delivery anywhere in the body through ophthalmic, rectal, vaginal, and the skin as topical routes. Skin is one of the most readily easily reached organs on the human body for topical administration and is the main route of topical drug delivery.[2]

Miconazole nitrate is an antifungal agent of an azole class and lipophilic in nature. It is a weak base with pKa 6.7, high log octanol/water partition coefficient, and poor aqueous solubility.[3] Miconazole is an imidazole antifungal agent used as miconazole base or miconazole nitrate to treat superficial candidiasis and skin infections dermatophytosis pityriasis Versicolor. The drug has also

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been given intravenously by infusion for the treatment of disseminated fungal infections. Miconazole nitrate is usually applied twice daily as a 2% cream, lotion, or powder in the treatment of fungal infections of the skin, including candidiasis, dermatophytosis, and pityriasis Versicolor.[4-5]Various novel approaches were studied to improve the solubility and drug permeation through the skin such as liposomes,[6] niosomes,[7-8] solid lipid nanoparticles,[9] microemulsion.[10]

Proniosomes are either anhydrous free-flowing formulations or liquid crystals with a jelly-like consistency of water-soluble carrier coated with suitable noisome-forming surfactants. They can be hydrated in body compartments to form niosomal vesicles or easily be reconstituted with an aqueous phase before administration. These proniosomes-derived niosomes are better than conventional niosomes.[11] Nowadays, proniosomes are used to enhance drug delivery in addition to conventional niosomes.[12] Proniosomal system aids as a rate-limiting barrier for the absorption of drugs. These systems can overcome the permeation barrier of the skin and act as a penetration enhancer for the drugs.[13]

The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle while the hydrophobic chains face each other within the bilayer.[14] Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while the hydrophobic drugs are embedded within the bilayer itself.[15] Addition of cholesterol results in an ordered liquid phase formation which provides rigidity to the bilayer and results in less leaky niosomes. Dicetyl phosphate is known to increase the size of vesicles, give charge to the vesicles, and enhance entrapment efficiency. Other charge-inducers are stearyl amine and diacylglycerol that also help in electrostatic stabilization of the vesicles. Niosomes have unique advantages over liposomes. Niosomes are quite stable structures, even in the emulsified form. Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency.[16-18]

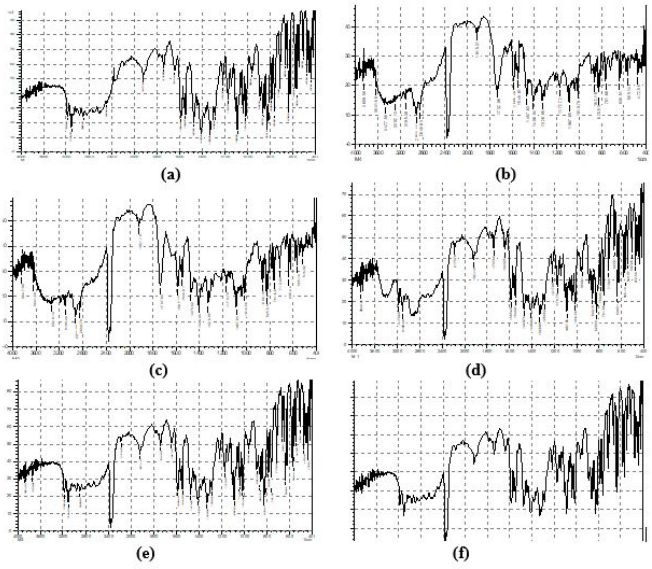


Fig. 1: FTIR spectrum of (a) Miconazole nitrate; (b) Miconazole nitrate with span 60; (c) Miconazole nitrate with lecithin; (d) Miconazole nitrate with cholestrol; (e) Miconazole nitrate with carbopol; (f) Miconazole nitrate with excipients

In the present study, proniosomal gel was prepared by coacervation phase separation method using different grades of non-ionic surfactants such as span 20, span 40, span 60, span 80, and cholesterol and lecithin and characterized by optical microscopy, encapsulation efficiency, SEM, *in-vitro* drug release. Further to enhance the stability and increase the viscosity of the system, the selected proniosomal gel formulation was mixed with carbopol gel, and their ex-vivo study, stability study was performed, and *in vitro* antifungal activity was compared with marketed preparation.

MATERIALS AND METHODS

Materials

Miconazole nitrate, Soya lecithin (Phospholipid), Cholesterol, Non-ionic surfactants span 20,40,60,80 were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Ethanol and other reagents and solvents were purchased from Research Lab., Fine Chem Industries, and Mumbai. Dialysis membrane (Himedia Laboratories Pvt. Ltd, Av. flat width: 32.34 mm; Av. diameter: 21.5 mm, Molecular Cut off: 14000 Da)

Methods

Drug–excipients Compatibility Study by FTIR Spectroscopy

The drug excipient compatibility studies were performed to check whether any interaction between drug and excipients are observed. Drug–excipient interaction was studied by FTIR spectroscopy. The spectra were recorded for pure Miconazole Nitrate and with different excipient mixture individually using FTIR-spectrophotometer (Model-FTIR 8400S, Shimadzu, Japan). The scanning range was 400 to 4000 cm^{-1}, and the resolution of 4 cm^{-1}. The FTIR spectra of drug with a non-ionic surfactant (span), drug with lecithin, drug with cholesterol, drug with carbopol, and the drug with excipients mixture were compared with the FTIR spectrum of Pure drug which was shown in Figs.: 1a-f and the observed peaks are represented in Table 1.[19-21]

Method of preparation of Proniosomal Gel (Proniosomes) of Miconazole Nitrate

Miconazole proniosomes were prepared by coacervation phase separation method reported by Vora et al. A clean, dry wide-mouth glass vial was taken in that drug, surfactant, lecithin, and cholesterol were added and mixed it well. A measured amount of ethanol (1.0 mL) was added to a glass vial to dissolve all the ingredients. All the

Table 1: Composition of proniosomal gel formulations of miconazole nitrate

Formulation Code	*MF1*	*MF2*	*MF3*	*MF4*	*MF5*	*MF6*	*MF7*	*MF8*	*MF9*	*MF10*
Miconazole (mg)	80	80	80	80	80	80	80	80	80	80
Span20 (mg)	1800				900	900	900			
Span40 (mg)		1800			900			900	900	
Span60 (mg)			1800			900		900		900
Span80 (mg)				1800			900		900	900
Cholestrol (mg)	200	200	200	200	200	200	200	200	200	200
Lecithin (mg)	1800	1800	1800	1800	1800	1800	1800	1800	1800	1800
Ethanol (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Phosphate Buffer (pH6.8) (mL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 2: Interpretation of FTIR spectrum of miconazole nitrate

Characteristic functional group	*Observed peak (cm^{-1})*
C-cl (785–540cm^{-1})	761.88, 786.96
C = C (1600–1475cm^{-1})	1585.49–1473.62
Aromatics out of plane blend (900–690cm^{-1})	862.18
C – O (1300–1000cm^{-1})	1286.52–1014.56
C – N (1350–1000cm^{-1})	1328.95
C – H (2900–2800cm^{-1})	2897.08
C = O (1670–1820cm^{-1})	1737.86
O – H (3500–3000cm^{-1})	3105.39

ingredients are mixed well. After mixing the open end of the glass vial was covered with a lid to prevent the loss of solvent. Carefully all the vials are then warmed on a water bath at 60-70°C for about 5 minutes until the surfactants were dissolved completely. The aqueous phase (phosphate buffer) was added and warmed on a water bath until a clear solution was formed. The glass vials were set aside in a dark place to cool at room temperature until the dispersion was converted to proniosomal gel. The composition of proniosomal gel formulations were given in Table 2.[12,22,23]

Characterization of Proniosomal Gel of Miconazole Nitrate

Physical Appearance of Proniosomal Gel

The prepared gel was watched through the naked eye to characterize the color and physical state of gel. Proniosomal gel was also observed by optical microscope at 10X and 40X magnification to note crystal characteristics of gel. The appearance of each formulation was checked for its color, consistency, and fluidity by spreading a thin layer of gel on a glass slide and placing the coverslip on it.[23, 24]

pH Determination

Digital pH meter was used to determine the pH of proniosomal gel. The pH meter was calibrated before use with buffered solution pH 4.0, 7.0, 10.0. One gram of gel was dissolved in 25 mL of distilled water and the electrode was then immersed in to gel formulation for 30 minutes until the constant reading was obtained. The measurement of pH of every formulation was replicated two times, and then the average value was calculated.[23, 24]

Vesicle Size Determination

For Size and size distribution studies, the proniosomal gel (100 mg) was hydrated first in a small glass test tube holding 10 mL of phosphate buffer (pH 6.8) with manual shaking for 10 minutes. After hydration, the aqueous dispersion of niosomes was observed under an optical microscope at 10X and 40 X magnification. The particle sizes of vesicles were measured using a calibrated ocular and stage micrometer built-in optical microscope.[23-24]

Entrapment Efficiency Determination

In a glass tube, 0.2 gm of proniosomal gel was taken and 10 mL of phosphate buffer (pH 6.8) was added. This aqueous suspension was sonicated in a sonicator bath for 10 minutes followed by centrifugation at 18000 rpm at 5° C for 40 minutes. The supernatant was collected and assayed by UV method. The percentage of drug encapsulation (EE%) was calculated by subtracting the amount of un-encapsulated (free) drug in the supernatant from the total drug incorporated.[23,24]

$$EE\ \% = C_t - C_f / Ct \times 100$$

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

Differential Scanning Colorimetry (DSC) Studies

The differential calorimetric scanning analysis of Miconazole and Miconazole loaded proniosomal gel samples was carried out using DSC 1 Mettler Toledo. The drug sample and Miconazole loaded proniosomal gel sample were closed in aluminium pan and the DSC thermograms were recorded at a heating rate of 10°C/min in the range of 30 to 300°C under constant purging of nitrogen at 50 mL/min. An empty closed pan was used as a reference. DSC graphs for Miconazole nitrate

and its formulation were recorded as shown in Fig. 4, respectively.[25-26]

The *in-vitro* Drug Release Studies

The *in-vitro* drug release studies of the Miconazole proniosomal gel formulations were carried out in Franz Diffusion cell using Dialysis membrane (Himedia Laboratories Pvt. Ltd, Av. flat width: 32.34 mm; Av. diameter: 21.5 mm; Molecular cut off: 14000 Da). The volume of receptor compartment was 15 ml. The area of donor compartment exposed to receptor compartment was 3.14 cm^2. Before the experiment, the membrane was soaked in phosphate buffer (pH6.8) and methanol (7:3) for overnight. The membrane was mounted between the donor and receptor compartment which were seized together with a clamp. The receiver compartment contained phosphate buffer (pH6.8) and methanol (7:3), and in the upper donor compartment, a weighed amount of proniosomal gel equivalent to 20 mg of drug was spread uniformly on the membrane. The receptor phase phosphate buffer (pH6.8) and methanol (7:3) were constantly stirred with magnetic stirrer help and was surrounded by a water jacket maintained at a temperature of 37 ± 0.5ºC during the experiments. Sample of 1-mL was removed from the receiver compartment at time intervals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 hours. and the same amount of fresh buffer solution was added to maintain the sink condition in the receiver compartment. Caution was taken to ensure that no air bubbles were lodged beneath the diffusion membrane during the experiments. The samples were diluted up to 10 ml with phosphate buffer (pH 6.8) and methanol (7:3) and were analyzed spectrophotometrically. By determining the amount of Miconazole Nitrate released at various time intervals, the Cumulative %drug release (Miconazole Nitrate) versus time was plotted. This experiment was carried out for 12 hours.[22,27]

Drug Release kinetics:

To study the release kinetics, data obtained from *in vitro* drug release studies was fixed in various kinetic models: zero-order as a cumulative percent of drug release *vs.* time, first-order as cumulative log percentage of drug remaining *vs.* time, Higuchi's model as a cumulative percent of drug release *vs.* square root of time. To determine the mechanism of drug release, the data was fixed into Korsmeyer Peppas equation as log cumulative percentage of drug released *vs.* log time, and calculated the exponent 'n' from the slope of the straight line. The value of *n* characterizes the release mechanism of the drug; if the exponent is 0.5, then diffusion mechanism is Fickian; if $0.5 < n < 0.89$, mechanism is non- Fickian, $n = 1$ to Case II (relaxational) transport, and $n > 1$ to super case II transport.[28-29]

Surface Morphology by SEM

For scanning electron microscopy, take 0.2 gm. Miconazole proniosomal gel in a glass tube was diluted with 10 mL of phosphate buffer (pH6.8). The niosomes were mounted on an aluminum stub using double-sided adhesive carbon tape and the vesicles were examined using Model No. S-4800 TYPE-II, HITACHI, JAPAN.[30]

Formulation of Carbopol Topical Proniosomal Gel

Based upon entrapment efficiency and *in vitro*, drug release studies proniosomal gel formulation MF2, MF3, MF5, MF6, and MF8 were selected for ex-vivo permeation studies. Before ex-vivo permeation studies, further topical applicability of the selected formulation was enhanced by the development of gel formulation. Carbopol 934 was designated as a polymer for gel preparation.

1.0 g of carbopol- 934 powder was dispersed into distilled water which was strongly stirred by a magnetic stirrer, taking caution to avoid the formation of in dispersible lumps and allowed to hydrate for 24 hours. In that methylparaben was mixed well. Triethanolamine was added into dispersion to neutralized and adjust the pH 7.4 by using pH meter. An appropriate amount of Miconazole proniosomal gel was then incorporated into gel-base with continuous stirring until homogenous formulation was attained. Similarly, all remaining formulation batches were prepared.

Evaluation of Carbopol Proniosomal Gel

Physical examination

Prepared topical proniosomal gel formulations were observed for their color, appearance, presence of any aggregate or lumps by visual inspection.[31]

pH Determination

The pH of gel formulations was determined by using a digital pH meter. One gram of gel was dissolved in 100 mL of distilled water and stored for 2 hours. The measurement of pH of all formulation was done in triplicate, and average values were calculated.[32]

Viscosity Determination

The measurement of viscosity of the prepared gel was completed by using Brookfield Viscometer. The gel was rotated at 20 and 30 rpm using spindle no.64 at each speed; the corresponding dial reading was noted.[33-35]

Drug Content

Drug content was studied by an accurately weighing 100 mg of gel and was dissolved in 100 ml of phosphate buffer (pH 6.8) and methanol (7:3 ratio). Then the solution was stirred continuously for 24 hours on a magnetic stirrer. Then the whole solution was sonicated. After sonication and subsequent filtration, the drug in solution was estimated spectrophotometrically by appropriate dilution.[35,36]

Spreadability Study

Two glass slides of standard dimensions were taken for this study. The gel was placed over one slide and the other

slide was placed over the top of the gel such that the gel sandwiched between the two slides was pressed uniformly to form a thin layer. The pressure was removed, and the excess of the gel adhering to the slides was scrapped off. The two slides were fixed to a stand without disturbance in such a way that only the lower slide was held firmly by the opposite fangs of the clamp allowing the upper slide to slip off freely by the force of weight tied to it. 20 gm weight was tied to the upper slide carefully. The time taken for an upper slide to travel the distance of 6cm and separate away from the lower slide under the direction of weight was noted. The spreadability of the gel formulation was calculated by using a formula,[37]

$$S = ML/T$$

Where, S - Spreadability
M –Weight tied to upper slide
L-Length of slides and T-Time taken

Extrudability Study

It is a usual empirical test to measure the force required to extrude the material from the tube. It consists of a wooden block inclined at an angle of 45° fitted with a thin metal strip at one end. While the other end was free. The aluminium collapsible tube containing 10 gm of gel was positioned on an inclined surface of the wooden block; 30 gm weight was placed on a free end of the aluminum strip and was just touched for 10 seconds. The quantity of gel extruded from each tube was noted. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was measured.[21] The results are shown in Table 5.

Ex vivo Permeation through Wistar Rat Skin

The selected formulations based on entrapment efficiency, drug content, and permeation through cellophane membrane were subjected to permeation studies through rat skin using Franz Diffusion cell in a similar manner as through cellophane membrane.

Preparation of wistar rat skin for permeation Studies

Ex vivo skin permeation studies were performed with Institutional Animal Ethical Committee approval. In the present study, hairless Wistar rat abdominal skin was used. Hair on the dorsal skin of the sacrificed animal was removed with an animal hair clipper. Subcutaneous tissue was surgically removed, and the dermis side was wiped with isopropyl alcohol to remove residual adhering fat. The skin was washed with PBS pH 6.8 and methanol (7:3) ratio. The skin so prepared was wrapped in aluminum foil and stored in a deep freezer at -20°C till further use. The skin was defrosted at room temperature when required.[29]

Ex vivo Permeation Through Wistar Rat Skin

The rat skin was mounted between the donor and receptor compartment with the stratum corneum facing the upper side on the diffusion cell. To maintain sink conditions, phosphate buffer (pH 6.8) and methanol ratio (7:3) was taken in the receptor compartment. The temperature was maintained at 37 ± 1°C. Carbopol proniosomal topical gel formulation equivalent to 20 mg of Miconazole nitrate was spread over the rat skin. The receptor compartment content was stirred with the help of magnetic beads. The samples were collected at different time intervals and were immediately replaced with fresh media. The samples were analyzed for drug content using a UV spectrophotometer at 270 nm. The cumulative amount of drug permeated (Q) at different time intervals and various parameters like steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio (ER) were calculated using following equations.[25]

Steady state flux (J_{ss}) = amount of drug permeated / (time × area of membrane)

$$= Q / (t \times A)$$

Permeability Coefficient (K_p) = flux /initial concentration of drug in the donor chamber

$$= J_{ss} / D_0$$

Enhancement ratio (ER) = Jss of proniosomes/Jss of control

Stability Studies

The carbopol proniosomal gel formulations MF2, MF3, MF5, MF6, and MF8 have filled in tightly closed glass vials and been subjected to stability testing. The formulations were kept at refrigerated conditions (4 ± 2°C) and at room temperature (25 ± 2°C) and were analyzed for vesicle size and entrapment efficiency after 3 months.[29,38,39]

In-vitro 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 15 lb pressure in autoclave for about 20 minutes. Then cooled at room temperature and the fungal strain (Candida albicans) was dispersed in the medium. The medium was poured into the required Petri dish and allowed it cool it until it got solidified at room temperature. The cups are then bored in agar plate by using a cork borer with 6mm diameter and calculated concentration of the gel formulation (C5MF8), and marketed preparations were placed in the bores[40] and incubated the Petri plates for 72 hours at 28°C in incubators.[41] Then the zone of inhibition was measured and calculated the radius of the zone of inhibition. The photographic images of gel formulation (C5MF8) and marketed preparations were shown in Fig.10.

Results And Discussion

Drug-Excipients Compatibility Studies

The FTIR spectra of pure Miconazole Nitrate and physical mixture of drug with different excipients were shown in

Figs. 1a-f. The observed peaks of the drug are shown in Table 2. All these peaks were characteristic of the pure miconazole nitrate. These peaks were not affected and prominently observed in IR spectra of miconazole nitrate along with polymers. This specifies that there was no known chemical interaction between drugs and excipients.

Physical appearance of proniosomal gel

Proniosomal gel of Miconazole Nitrate prepared by using span (20, 40, 60) have pale yellow semi solid gel like appearance and span 80 have light brownish gel like appearance. As shown in Fig. 2. and the results are reported in Table 3.

pH determination

Skin compatibility is the most important requirement for a good topical formulation. It was found that the pH of all the formulations was in the range of 5.60 to 7.20, which suits the skin pH, so compatible with skin. The results of pH determination are reported in Table 3.

Vesicle Size Determination

The mean vesicle size of Miconazole Nitrate proniosomes ranged from 2.70 ± 0.42 to 6.34 ± 0.30 µm and was presented in Table 3. The mean Vesicle size of Miconazole Nitrate proniosomes was detected, indicating that vesicles formed with spans were smaller in size, having nanometric size,[30] multilamellar vesicles. Photomicrographs were taken for niosomes by using an optical microscope. The optical microscopic images of hydrated MF5, MF6, MF3, and MF8 proniosomal gel formulations were shown in Figs 3a-d, respectively. The photomicrograph is shown that the niosomes are multilamellar, having a spherical shape, and no aggregation or agglomeration is observed.

Drug Encapsulation Efficiency Determination

Encapsulation efficiency of proniosomal gel formulations ranged from 83 to 91.25%. The encapsulation efficiency of formulations batches MF1 to MF10 was shown in Table 3. Among the span combination batches, batch containing a combination of span 40 and 60 have higher encapsulation efficiency due to the longer saturated alkyl chain compared to that of span 20 and 80. The entrapment efficiency depends upon the surfactant used, their alkyl chain length, HLB values and the phase transition temperature of surfactant[26] Spans have low HLB value and highly

Fig. 2: Proniosomal gel of Miconazole Nitrate

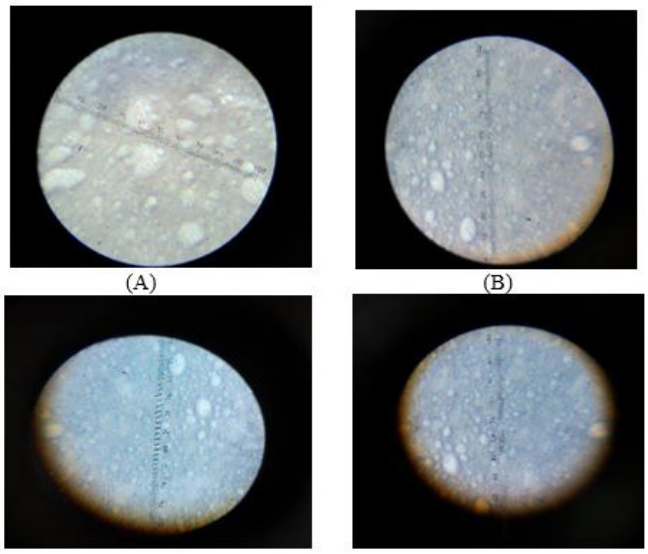


Fig. 3: Optical microscopic examination of formulations MF5 (A), MF6 (B), MF3(C) and MF8 (D)

Table 3: Characterization of proniosomal gel formulations for physical appearance, ph, vesicle size and encapsulation efficiency

Formulation code	*Physical appearance**	*pH**	*Mean Vesicle size (µm)**	*EE%**
MF1	Pale yellow semi solid gel	6.31 ± 0.26	6.11 ± 0.30	84
MF2	Pale yellow semi solid gel	6. 52 ± 0.24	5.80 ± 0.35	86.13
MF3	Yellow semisolid gel	6.83 ± 0.14	3.40 ± 0.15	86.56
MF4	Light brownish gel	6.90 ± 0.34	4.75 ± 0.66	83
MF5	Pale yellow semisolid gel	7.24 ± 0.05	3.10 ± 0.56	87.28
MF6	Pale yellow semisolid gel	5.81 ± 0.20	3.90 ± 0.45	89.35
MF7	Light brownish semisolid gel	6.74 ± 0.12	2.70 ± 0.42	84.23
MF8	Pale yellow semisolid gel	6.82 ± 0.18	3.14. ± 0.12	91.25
MF9	Light brownish semisolid gel	7.21 ± 0.03	4.10 ± 0.69	85.12
MF10	Light brownish semisolid gel	7.52 ± 0.07	6.34 ± 0.30	85.47

*All value represent mean ± standard deviation (SD), n=3

lipophilic in nature and formed closed identical bilayer structure in that Miconazole nitrate gets embedded.

Alkyl Chain Length

The HLB value and the alkyl chain length play an important role in the %EE. As the length of the saturated alkyl chain increases from formulation MF1 to MF10 in span series, a significant increase in %EE was observed. The order of entrapment was Span 60 > Span 40 > Span80 > Span 20.[25] Span 80 and span 60 had the same polar head, but in span 80, the introduction of double bonds give rise to more permeable bilayer and lower entrapment efficiency.[25,42]

Phase Transition Temperatures

Span 60 and span 40, due to their high phase transition temperatures, formed a uniform bilayer structure within which lipophilic drug got completely entrapped to give the high entrapment efficiency. Span 20 and span 80 had low phase transition temperature and were liquid at room temperature, and showed comparatively low entrapment efficiency.[25,43]

Surfactant Concentration

To increase the entrapment efficiency appropriate amount of surfactant concentration will require to form the bilayer structure. A significant increase in niosomes number resulted in the volume of hydrophobic bilayer domain, and hence the available space is more for lodging of hydrophobic drug miconazole nitrate was increased with increased entrapment efficiency.[25,44]

Effect of Cholesterol

Cholesterol gets intercalated between bilayers of non-ionic surfactants to formless leaky and rigid bilayers.[45] All the formulation contain appropriate amount (200 mg) of cholesterol, so all formulation shows good entrapment efficiency with less difference. A higher concentration of cholesterol may compete with the drug for space within bilayers, thus excluding the drug and disrupting ordered bilayer structure.[25,45]

Effect of Lecithin

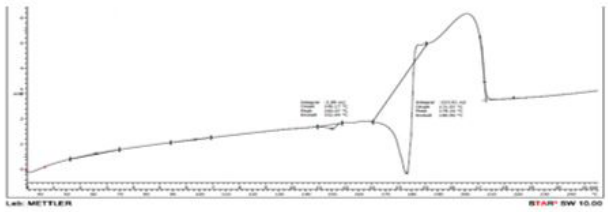
The entrapment efficiency was increased when lecithin was added in proniosome formulations, as it increases the rigidity of bilayer structure similar to cholesterol, which was attributed to the high phase transition temperature of the soy lecithin. However, double bonds in phosphatidylcholine allow the chain to bend, so the adjacent molecule was not tightly close enough. When they assemble with non-ionic surfactants, it leads to the formation of a more permeable bilayer. If the saturation of a double bond occurs, it forces the bilayer molecules to get arranged to form a less permeable bilayer.[13]

Differential Scanning Colorimetry (DSC)

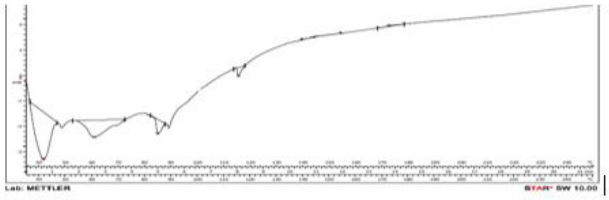
DSC is one of the best widely used colorimetric techniques to characterize the solubility and physical state of drug in lipid vesicles. The DSC analysis of the pure miconazole nitrate and the miconazole nitrate proniosomal gel formulation were studied as shown in Figs 4a-b, respectively. The DSC analysis of Miconazole Nitrate showed a large endothermic peak at 178.16°C, which denotes the melting point of Miconazole Nitrate. This prominent peak was not observed in the DSC thermogram of Miconazole proniosomes prepared with a combination of span 40 and span 60 (Formulation MF8). The broadening of peak and very small peak are observed suggested the presence of the drug in a more soluble amorphous state. This was due to inhibition of its crystallization and solubilization in proniosomes. The physical state transformation of a drug to an amorphous or partially amorphous state leads to a high energy state and high disorder resulting in improved solubility.[46]

In-vitro Release Study

The cumulative amount of drug permeated for each formulation was shown in Figs 5 and 6. The result indicated that formulation MF8 containing span 40 and span 60



(a)Differential Scanning Colorimetry (DSC) thermogram of Miconazole nitrate



(b)Differential Scanning colorimetry (DSC) thermogram of Proniosomal gel Formulation of Miconazole nitrate

Fig. 4: Differential scanning calorimetry (DSC) thermogram

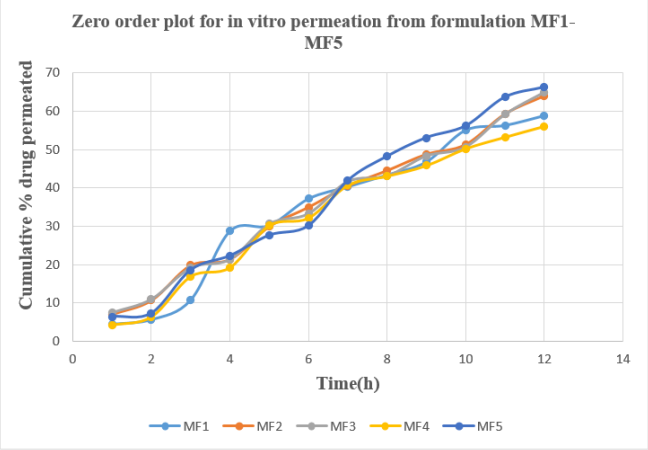


Fig. 5: Cumulative percent drug permeation of proniosomal gel formulation (MF1 to MF5)

combination was shown higher drug release 72.03% up to 12 hours. Among the ten formulations, the first four formulations from MF1 to MF4 prepared using single non-ionic surfactant (Span), and the remaining six formulations was prepared using a combination of different grades of span. The proniosome formulation MF2, MF3, MF5, MF6 and MF8 shows higher drug release up to 12 hrs. The order of decreasing percentage of drug release was MF8 > MF6 > MF5 >MF3 > MF2>MF7> MF10> MF9 > MF7>MF1>MF4.

Proniosomal gel formulation MF1, MF4,MF7 which formulated using span 20 and 80 either alone or in combination, showed less entrapment efficiency and drug release as compared to formulation MF9 and MF10, which contain span 40 and span 60 in combination with span 80. The MF2 and MF3 formulated with single non-ionic surfactant span 40 and 60 respectively had more entrapment efficiency and drug release when they were compared with batch MF9 and MF10. This was due to Span 20 and span 80 had low phase transition temperature and were liquid at room temperature, and showed comparatively low entrapment efficiency. Span 40 and 60 had high phase transition temperatures and longer alkyl chain lengths.

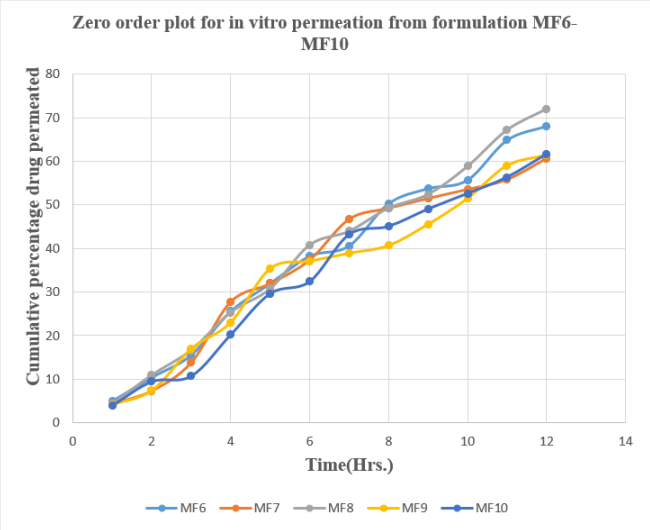


Fig. 6: Cumulative percent drug permeation of proniosomal gel formulation (MF6 to MF10)

Among the proniosomal gel formulation the batch MF8 (40:60) showed higher drug permeation up to 12 hours. This might be due to the synergistic emulsification effect of the surfactant after the hydration of the proniosomes by the dissolution medium and formation of elution channels within the gel structure due to loss of lipid bilayers. The same results have also been observed by Singla S. et al., 2012.[29]

Alsarra *et al*. (2005) has already reported that increasing the cholesterol content resulted in a more intact lipid bilayer as a barrier for drug permeation and decreased its leakage by improving the fluidity of the bilayer membrane and reducing its permeability, which runs to lower drug elution from the vesicles.[30]

Drug release Kinetics

In-vitro drug release for all formulations of the proniosomal gel was fixed into different equations and kinetic models to explain the release kinetics of Miconazole nitrate from proniosomal gel. Calculated regression coefficient(R^2) of formulations for different kinetics models were shown in Table 4. When the R^2 values of the regression coefficient for a first-order and zero-order were considered. R^2 values of zero-order graphs were higher for Formulating MF2, MF3, MF5, MF6, and MF8 than first-order graphs. Hence it is evident that the drug release from optimized batch MF8 proniosomal gel formulation followed zero-order kinetics.

By incorporating release data in Higuchi and Erosion models, the R2 values of all the formulations were found to be greater for Higuchi model. So all the formulations in this study were best expressed by Higuchi's classical diffusion equation. The linearity of plot indicated that the release process was diffusion controlled. To know the further mechanism of drug release, the drug release data was fitted into the Korsmeyer Peppas model and from the

Table 4: Release kinetics of formulations in *in-vitro* drug release

	Correlation coefficient of model fitting (R^2)			
Formulation Code	*Zero-order*	*First-order*	*Higuchi*	*Peppa's*
MF1	0.9534	0.9795	0.9700	0.9328
MF2	0.9925	0.9841	0.9808	0.9891
MF3	0.9908	0.9904	0.9744	0.9880
MF4	0.9708	0.9791	0.9844	0.9688
MF5	0.9886	0.9755	0.9675	0.9639
MF6	0.9892	0.9850	0.9846	0.9918
MF7	0.9458	0.9768	0.9757	0.9634
MF8	0.9926	0.9759	0.9841	0.9857
MF9	0.9660	0.9740	0.9763	0.9680
MF10	0.9783	0.9889	0.9768	0.9810

value of release exponent 'n' concluded the mechanism of drug release. All the formulations show release exponent 'n' in between 0.5 to 0.89. This showed that the drug released from all the formulations followed non-Fickian diffusion. Hence drug release was controlled by a diffusion mechanism.

Surface Morphology

Proniosomes converted into niosomes after hydration, and the surface morphology was studied using SEM. SEM photograph Figs 7a and b showed that niosomes formed were nearly spherical, multilamellar, and homogeneous

Evaluation of carbopol topical proniosomal gel

Physical examination: The prepared carbopol proniosomal gel was examined visually for their color and appearance. All developed gel formulations showed good homogeneity with no aggregates or lumps.

All the five formulations were evaluated for PH, viscosity, drug content, spreadability, extrudability results were shown in Table 5.

Ex-Vivo Drug Permeation Studies

The *ex vivo* permeation of Miconazole nitrate through rat abdominal skin from carbopol topical proniosomal gel formulations was determined using Franz diffusion cell. MF2, MF3, MF5, MF6, and MF8 proniosomal gel formulations were selected depending on its high encapsulation efficiency and in vitro drug release. These selected proniosomal gel formulations developed into carbopol topical proniosomal gel, and they were characterized for their drug permeation through rat abdominal skin. The cumulative amount of miconazole nitrate permeated per unit area across excised rat skin as the function of time, flux, and permeability coefficient were determined.

Zero-order plots for carbopol proniosomal topical gel formulations were presented in Fig. 8. The calculated

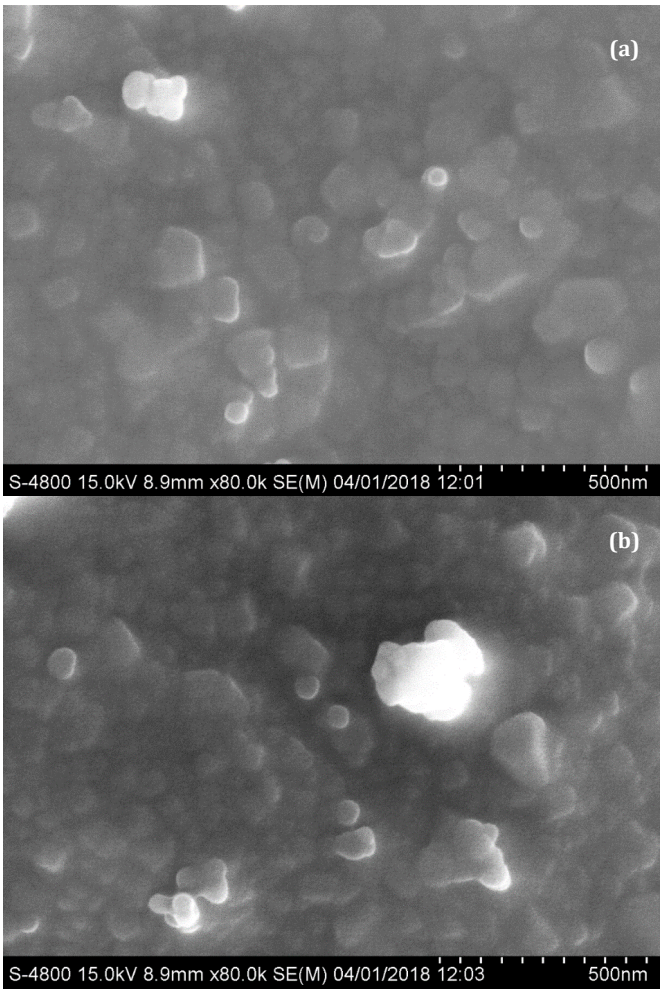


Figure 7: (a) and (b) Scanning electron images of proniosomal gel formulation of MF8

Table 5: pH, viscosity, drug content, spreadability, and extrudability

	*pH **	*Viscosity **			
Formulation batch No.	*cps*		*Drug content**	*Spreadability **	*Extrudability **
C1MF2	6.71 ± 12	1023 ± 1.28	94.05	11.54 ± 0.26	81 ± 0.45
C2MF3	6.56 ± 03	1103 ± 0.50	95.10	13.33 ± 0.34	83 ± 0.65
C3MF5	6.07 ± 11	1178 ± 1.14	96.20	15.34 ± 0.14	85 ± 0.22
C4MF6	6.78 ± 45	1206 ± 2.10	96.33	12.11 ± 0.37	84 ± 0.42
C5MF8	6.89 ± 16	1312 ± 1.05	97.23	15.49 ± 0.18	89 ± 0.72

*All values represent mean ± standard deviations (SD), n = 3

Table 6: Release kinetics of formulations in ex-vivo drug release

	Correlation coefficient of model fitting (R^2) Peppa's mechanism					
Formulation code	*Zero-order*	*First-order*	*Higuchi*	*Peppa's*	*'n' value*	*Release*
C1MF2	0.9773	0.9954	0.9905	0.977	0.44	Non-Fickian
C2MF3	0.9875	0.9458	0.9744	0.9827	0.45	Non-Fickian
C3MF5	0.9886	0.9790	0.9744	0.9734	0.59	Non-Fickian
C4MF6	0.9894	0.9784	0.9846	0.9688	0.64	Non-Fickian
C5MF8	0.9944	0.968	0.9860	0.9929	0.69	Non-Fickian

regression coefficient of carbopol proniosomal topical gel formulations for different kinetic models were presented in Table 6. The regression coefficient R2 value of zero-order plot was higher for all the formulations, indicating that the drug release rate from all the formulation was independent of its concentration and, hence, follows zero-order release kinetics. Flux value and permeability coefficient of all carbopol topical gel formulations were shown in Table 7. Among all the formulations, C5MF8 showed higher R^2 value 0.9944, higher drug permeation ($p < 0.05$,t-test) 74.19 ± 0.16% in 12 hours. with a flux value of 6.829 ±0.12µg /cm^2/hr. and permeability coefficient of 0.341 ±0.08 cm^2 /hrs. C5MF8 had a diffusion exponent 'n' value 0.69 confirm non Fickian diffusion.

In the present study, a fixed ratio of cholesterol was used (200mg), which results in the formation of a rigid bilayer. That acts as a barrier for drug permeation and decreases its leakage by improving the fluidity of the bilayer membrane and reducing its permeability, which runs to lower drug elution from the vesicles as the release rate is dependent on alkyl chain length saturation or cholesterol content. The addition of lecithin at optimum concentration has played an important role in drug permeation.[30]

Mixing of two surfactant results in the formation of new type of mixed micelle and the synergistic penetration enhancement effect is concentration-dependent which occurs when equal fractions of the surfactant are present in mixture.[47]

Comparative Study of Carbopol proniosomal gel with Marketed Preparation

Further cumulative percentage of drug release from optimized batch C5MF8 were compared with marketed formulation their ex-vivo drug release plot was shown in Fig. 9. The cumulative percentage of drug release was found to be 81.89 ± 0.16 % for marketed preparation and 74.19 ± 0.10% for carbopol proniosomal gel formulation at the end of 12 hours. similar findings that is proniosome showed prolong drug release than marketed preparations

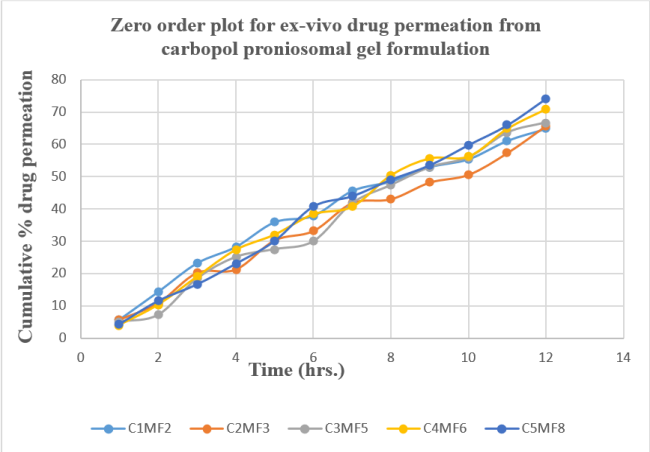


Fig. 8: *Ex-vivo* drug permeation plot from carbopol proniosomal gel formulation

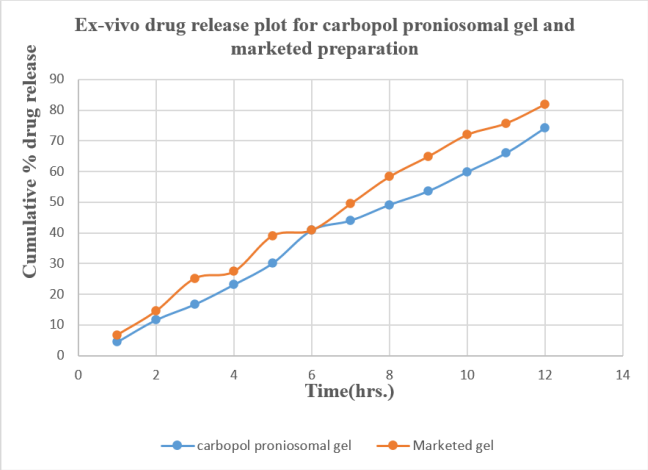


Fig. 9: Comparative *ex-vivo* drug permeation plot for carbopol proniosomal gel and marketed preparation

Table 7: Flux, permeability coefficient and enhancement ratio of miconazole nitrate carbopol proniosomal gel

Formulation code	*Flux (Jss) (µg/cm^2/hr.)**	*Permeability coefficient (K_p) (cm^2/hr.)**	*Enhancement ratio*
C1MF2	5.96 ± 0.10	0.298 ± 0.03	0.79
C2MF3	6.043 ± 0.13	0.302 ± 0.05	0.80
C3MF5	6.146 ± 0.08	0.307 ± 0.06	0.81
C4MF6	6.528 ± 0.05	0.326 ± 0.06	0.86
C5MF8	6.829 ± 0.12	0.341 ± 0.08	0.90

Table 8: Stability data of optimized carbopol proniosomal gel formulation C5MF8

	Refrigerated temperature (4 ± 2°C)		*Room temperature (25 ± 2°C).*	
Time	*Vesicle size**	*%EE**	*Vesicle size**	*%EE**
0 month	3.14 ± 0.10	91.30	3.14 ± 0.10	91.30
1-month	3.34 ± 0.12	89.97	4.19 ± 0.15	88.08
2-month	4.25 ± 0.20	88.20	5.45 ± 0.16	86.10
3-month	4.85 ± 0.30	87.89	6.13 ± 0.12	82.23

*All Values are expressed as mean ± SD, (n=3).