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Research Article

Formulation And Characterization Of Provesicular Based Drug Delivery System For Effective Transdermal Delivery Of Antifungal Drug

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ABSTRACT

Oral intake of Itraconazole (ITZ) causes severe side effects in the gastrointestinal tract and has low bioavailability ~55%. Owing to these disadvantages, Transdermal drug delivery system (TDDS) of such antifungal agents can overcome such issues and can improve the efficacy and bioavailability. The aim of this study was to evaluate the potential of proniosomes as a transdermal drug delivery system for ITZ by encapsulating the drug in various formulations of proniosomal gel by Coacervation-phase separation method. Among all the formulated proniosomal gel formulation TF5 containing tween 20 was found to be the most appropriate surfactant and was selected for preparing Transdermal proniosomal hydrogel which was formulated using different concentrations of Carbopol and HPMC. These gels were characterized for pH, spreadability, drug content and in-vitro diffusion study and stability studies wherein the optimized formulation (G2) showed the values of 7.01, 33.70 g.cm/sec, 99.5% and 87.83% ($p<0.05$). The drug release kinetics studies revealed that release was prolonged up to 8hrs and release pattern was found to be diffusion controlled which was confirmed by Higuchi's plot. The results of this study suggest that proniosomes are promising nano vesicular carriers to enhance the transdermal delivery of ITZ.

INTRODUCTION

ITZ is an orally active triazole, synthetic lipophilic antifungal agent, used in the treatment of a broad spectrum of fungal infections. The presently available oral therapies involve problems such as low bioavailability and side effects. TDDS is a painless method of delivering drugs systemically by applying a drug formulation onto intact and healthy skin. Provesicular drug delivery system can be used for encapsulation of the drug in vesicular structures which can be expected to extend the duration of the drug in the systemic circulation and reduce toxicity by selective uptake. Proniosomes are an inactive form of niosomes, they are transformed into their active forms niosomes upon hydration. This project work

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involved the formulation of a Itraconazole proniosomal gel and to evaluate its efficacy as transdermal drug delivery system to avoid the oral side effects associated with the drug and to prepare vesicles with highest drug entrapment efficiency required for achieving high concentration of drug at site of action and reduced frequency of administration.

MATERIALS AND METHOD

Materials

Itraconazole was obtained as a gift sample from Srijan Medi Solution Private Limited (India). Lipoid P 75 3 was obtained as gift sample from Lipoid GmBh (India), HPMC K4M was obtained as a gift sample from Colorcon (Goa, India), Cholesterol, Span 20, 40, 60, 80, Tween 20, 40, 80, Triethanolamine was obtained from Molychem (India), Propylene Glycol, Methyl Paraben, Propyl Paraben was obtained from Lobachemei (India), Ethanol was obtained from Changshu Hongsheng Fine Chemical (India), Carbopol 974P was obtained from Lubrizol (India).

Preformulation studies

Preformulation study is a preliminary step in the development of a dosage forms. It is the process of characterization of drug as well as excipients used in the formulation in order to develop a safe and efficacious dosage form.

Determination of solubility of Itraconazole: The solubility studies were performed by shake flask method. The solubility of Itraconazole was studied in various formulation related solvents such as methanol, phosphate buffer pH 7.4. An excess amount of drug was added to 10 ml of each solvent in different volumetric flasks, which were subjected to agitation with the help of a mechanical shaker for 24 h. Solutions were then filtered and aliquots were made with respective medium. The samples were analysed spectrophotometrically at 262 nm and concentrations were determined.

Determination of Melting Point of the drug: Melting point of Itraconazole was determined using melting point apparatus.

Compatibility studies

Infrared Spectroscopy

Infrared spectroscopy technique was used to determine the compatibility between Itraconazole and other formulation excipients. Infrared spectra of pure drug i.e. Itraconazole, and physical mixtures of drug-excipients (1:1) were taken by using Fourier Transform combined with Attenuated Total Reflectance Spectrophotometer between 4000-400 cm. Interaction between Itraconazole and excipients was determined by comparing the spectra of pure drug with that of the physical mixtures.

Preparation of proniosomal gel

Itraconazole Proniosomes were prepared by coacervation phase separation method. Required quantity (**Table 1**) of drug and surfactant mixture was weighed, (ratio of surfactants: alcohol was kept 1:1). After mixing lipid, surfactant, Itraconazole and cholesterol, and then warmed on a water bath at 60–70°C for 5min until the surfactants dissolve completely. The aqueous phase (Phosphate Buffer 7.4) was then added and warmed on a water bath till a clear solution was obtained. This dispersion was allowed to cool to room temperature until it was converted to proniosomal gel. The formed gel was preserved in dark until characterization. In case of incomplete dissolution of the drug in the prepared formulations, the drug and surfactants were dissolved first in chloroform or ether, followed by vacuum evaporation of the solvent [1].

Table 1: Composition of proniosomal formulation

Sample code	Drug (mg)	Lipoid P 75-3	Cholesterol (mg)	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	Span 80 (mg)	Tween 20 (mg)	Tween 40 (mg)	Tween 60 (mg)	Tween 80 (mg)
SF1	100	900	100	900							
SF2	100	900	100		900						
SF3	100	900	100			900					
SF4	100	900	100				900				
TF5	100	900	100					900			
TF6	100	900	100						900		
TF7	100	900	100							900	
TF8	100	900	100								900

Evaluation of Proniosome Gel

Appearance: All the proniosomal gel formulations SF1-TF8 were evaluated for appearance by visual observation for colour and to check the presence of any foreign particles.

Determination of particle size, polydispersity index and zeta potential: All the proniosomal gel formulations SF1 to TF8 were analysed for particle size, polydispersity index (PDI) and zeta potential using light scattering by Laser Diffraction method using Mastersizer by Malvern instrument. The samples were measured after dispersing in water for hydration and subsequent ultrasonication for 5 min[1].

Determination of Entrapment Efficiency:[1] 0.2g Proniosomal gel was hydrated with 10ml of phosphate buffer 7.4 and sonicated further for 5min. Niosomes containing drug were isolated from free drug by centrifugation at 15,000rpm for 45 min at 4°C. The supernatant was recovered and assayed spectrophotometrically using UV spectrophotometer at 262 nm using phosphate buffer pH 7.4 as blank. The percentage encapsulation efficiency of drug was calculated using the equation

$$\%EE = [(Ct-Cr)] / Ct \times 100$$

Where, Ct - Concentration of total Itraconazole, Cr - Concentration of free Itraconazole

Statistical Analysis: All data was expressed as mean ± SD. For all continuous data that was normally distributed, one-way analysis of variance test (ANOVA) followed by the least significant difference test (LSD) or unpaired t-test were performed to compare two or more groups. Statistical analysis was performed using SPSS® software.

Evaluation of optimised proniosomal gel formulation

Light microscopy of proniosomal gel

0.2g of gel was hydrated with phosphate buffer pH 7.4 (10ml) and few drops were placed on a glass side and observed under microscope. Photomicrographs were taken at suitable magnifications [2].

Morphological study by scanning electron microscope (SEM)

The optimised proniosomal gel formulation was visualised by transmission electron microscopy in order to study morphology of niosomes.

Differential scanning calorimetry (DSC) analysis

Study DSC experiments were performed using DSC instrument (DSC-60 Plus Shimadzu, Kyoto, Japan). About 5 mg of Itraconazole, Tween 20, Lipoid P75-3, Blank and Itraconazole loaded proniosome sample were individually placed in aluminium pan, sealed and heated under atmosphere of nitrogen (50 ml/ min) with heat flow rate of 10°C/min from 30 to 300°C and the corresponding spectra of heat flow (w/g) on Y-axis and temperature on X-axis were obtained. The heat flow as a function of temperature was measured.

Determination of particle size, polydispersity index and zeta potential of optimised proniosomal formulation

The optimised proniosomal gel formulation was analysed for particle size, polydispersity index (PDI) and zeta potential by using Malvern zeta sizer as previously stated.

***In-vitro* drug release studies of optimised proniosomal formulation**

In-vitro diffusion study of optimised proniosomal gel was performed using modified Franz diffusion cell of 2.5 cm in diameter which was fabricated in house. It was performed by using dialysis membrane-70 (Hi media) was soaked in phosphate buffer pH 7.4 for 12 h before use. Dialysis membrane was secured to the donor compartment was mounted between donor and receptor compartments. The receptor compartment consisted of 50 ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer at 100 rpm and temperature was maintained at 37 ± 1°C for 8 h. The donor compartment consisted of 1g of proniosomal gel formulation. Aliquots of 5 ml of samples were withdrawn from the receptor compartment which was replenished with equal volume of phosphate buffer pH 7.4 at specified time intervals of 0.5, 1, 2, 4, 6, and 8 h. The aliquots of samples withdrawn were analysed by UV-Visible spectrophotometer at 262 nm and % cumulative drug release (% CDR) at the end of 8 h was calculated [3].

Formulation of Proniosome Hydrogel

Proniosomal gels were prepared by dispersion method using HPMC K4M and Carbopol 974P. A specific amount (Table 2) of Carbopol and HPMC was soaked in distilled water overnight. The required amount of Itraconazole proniosome equivalent to 1% drug was dissolved in soaked Carbopol or HPMC by stirring at 500 rpm on magnetic stirrer for 1h. Carbopol was neutralised with 0.5% triethanolamine to obtain pH in a range of 5.5 -7.5. Propyl paraben (0.05% w/v) and methyl paraben (0.1%w/v) were taken as a preservative in a beaker containing propylene glycol (10%). These were then mixed properly by gentle stirring at 100 rpm to obtain viscous hydrogel [4].

Table 2: Composition of proniosomal hydrogel

Formulation Code	G1	G2	G3	G4	G5	G6
Proniosomal Gel	Equivalent to 1% of drug	Equivalent to 1% of drug	Equivalent to 1% of drug	Equivalent to 1% of drug	Equivalent to 1% of drug	Equivalent to 1% of drug
Carbopol 974P (%)	0.5	1	1.5	-	-	-
HPMC K4M (%)	-	-	-	1	2	3
Methyl Paraben (%)	0.1	0.1	0.1	0.1	0.1	0.1
Propyl Paraben (%)	0.05	0.05	0.05	0.05	0.05	0.05
Triethanolamine	q. s	q. s	q. s	q. s	q. s	q. s
Distilled water	q. s	q. s	q. s	q. s	q. s	q. s

Evaluation of Proniosomal Hydrogel

Organoleptic characterisation

The Proniosomal hydrogels were evaluated for its organoleptic properties such as colour homogeneity and presence of any foreign particles.

Determination of pH

Gels should not cause any irritation on application to the skin and therefore pH plays an important role in topical formulations. The pH of 1% aqueous solution of proniosomal hydrogel was determined by using a digital pH meter (Genial, BioEra Life science). The pH was then measured by dipping the electrode of pH meter in the solution.

Spreadability

One of the ideal criteria of a gel is that it should possess good spreadability. Spreadability refers to the extent of area to which gel readily spreads on application to the skin. For determining spreadability, a glass slide marked previously with a circle of 1 cm was taken and 0.5 gm of gel sample was placed in the centre of that circle, upon which a second glass slide of similar size was placed. Further, 50 gm of weight was allowed to rest on the upper slide for about 1 min and increase in the diameter of gel placed within the centre circle due to spreading was measured.

$S = (M \times L) \div T$

Where, S = Spreadability, M = Weight tide to the upper slide, L = Length of a glass slide, T = Time taken to separate the slide completely from each other. [5]

Viscosity

The viscosity holds a major contribution in deciding the drug content and the release of the drug from gels. This parameter also affects the spreadability and the flowability of the gel. Viscosity of formed hydrogels was determined by Brookfield viscometer (Brookfield Engineering Laboratories, Inc., USA, DVE) using T-bar spindle (Spindle S-63). Spindle was placed perpendicular to hydrogel in beaker and the speed was maintained at 5 rpm at 25°C [6,7].

Drug content

The drug content of proniosomal hydrogels was determined by dissolving 1 g of Proniosomal hydrogel in 10 ml of methanol. The resultant solution was sonicated for 20 mins for total extraction of the drug. The sample after suitable dilution was analysed by UV-Visible spectrophotometer against blank at 262 nm.

***In-vitro* drug release studies**

In-vitro diffusion study of Hydrogels was performed using modified Franz diffusion cell which was fabricated in-house. It was performed by using dialysis membrane-70 (Hi media) which was soaked in phosphate buffer pH 7.4 for 12 hours before use. Dialysis membrane was secured to the donor compartment by rubber band and was mounted between donor and receptor compartments. The receptor compartment consisted of 50 ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer at 100 rpm and temperature was maintained at 37 ± 1°C for 8h. The donor compartment consisted of 1 ml of Proniosomal gel formulation. The whole assembly was fixed in such a way that the lower end of the tube containing Proniosomal gel just touched (1-2 mm deep) the surface of diffusion medium. Aliquots of 5 ml of samples were withdrawn from the receptor compartment which was replenished with equal volume of phosphate buffer pH 7.4 at specified time intervals of 0.5, 1, 2, 4, 6, and 8 h. The aliquots of samples withdrawn were analysed by UV-Visible spectrophotometer at 262 nm and % cumulative drug release (% CDR) at the end of 8 h was calculated [3].

***In-vitro* skin permeation studies**

Preparation of rat skin

Institutional animal ethics committee permission was granted for completion of this study with reference No: IAEC/10/xxx. Healthy male Wistar albino rats weighing about 300- 350 g were selected from animal house, Department of Pharmacology, Goa College of Pharmacy, Panaji – Goa. All the instruments used were sterilised before use by wrapping them in a clean dry cotton cloth and sterilised in an autoclave for 30 min at 15 psi pressure at 121°C. Simultaneously rats were weighed and euthanized by giving high dose of thiopental solution and sacrificed by cervical dislocation. Then were placed on a clean dry cotton cloth and shaved on the dorsal side using razor to remove all the hair. The skin was properly cleaned to remove the subcutaneous fat and other blood vessels. Further, it was equilibrated in

phosphate buffer solution (pH 7.4) for 1 h prior to the experiment.

Skin permeation study

In-vitro diffusion studies of Proniosome loaded hydrogels using modified Franz diffusion cell which was fabricated in house. The rat skin was sandwiched between the two compartments such that the stratum corneum side and the dermis of the skin were towards the donor and receptor compartments of the Franz diffusion cell (diffusion area: 2.5 cm^2 and volume: 50ml). The receptor compartment consisted of 50 ml of phosphate buffer pH 7.4 which was stirred continuously using magnetic stirrer at 100 rpm and temperature was maintained at 37 ± 1°C for 8h. The donor compartment consisted of 1gm of Proniosomal gel formulation. Aliquots of 5 ml of samples were withdrawn from the receptor compartment which was replenished with equal volume of phosphate buffer pH 7.4 to maintain skin condition at specified time intervals of 0.5, 1, 2, 4, 6, and 8 h. Absorbances of the withdrawn samples were recorded spectrophotometrically using UV-Vis spectrophotometer at 262 nm. Cumulative amount of drug permeated through rat skin as a function of time was calculated.

Skin Irritation Study

Skin irritation test was performed on Wistar albino rats in accordance with guidelines and permission taken from Institutional Animal Ethics Committee (IAEC) Goa College of Pharmacy. Each rat was shaved on its dorsal side using a trimmer and sterilized razor, an area of 4 cm^2 was marked on it. Study was conducted 24 h after shaving was done. The rats were divided into three groups (n= 3). Group 1: No application (control); Group 2: Itraconazole Proniosomal gel; Group 3: Marketed gel preparation. The selected medicated formulation (1 gm) was applied on the shaved area. Control and proniosomal gel were applied to the respective groups. For 7 days, daily the animals were observed for any sign of erythema or oedema [8].

The skins were scored according to erythema and oedema scale as

0: No erythema and oedema

1: Very slight erythema

2: Well defined erythema

3: Moderate to severe erythema

4: Severe erythema to slight eschar formation.

Antifungal activity

Antifungal studies are carried out to ascertain the biological activity of optimised Proniosomal hydrogel and compared with the marketed Itraconazole gel formulation against Candida albicans. Stock inoculum suspension for Candida albicans was prepared from overnight culture containing approximately 107cfu/ml. Antifungal activity was determined by sabourauds dextrose agar diffusion test using 'Spread plate technique' on petri plates of uniform size which were pre sterilized in an autoclave. The 20 ml of molten sabourauds dextrose agar medium was allowed to cool down on levelled surface until solidification and this solidified agar plates were spread with 100 µl of the fungal inoculum with help of sterile spreader and were further dried for 15 min at room temperature. Wells were made with help of sterile borer of 1cm in diameter on solidified agar layer.100 mg of the prepared gel was placed into each well. Standard Itraconazole proniosomal hydrogel (1%) and marketed Itraconazole gel (1%) acting as positive controls were used to compare the inhibition zone of optimized formulation. Gel without the drug was used as a negative control. After keeping the petri plates at room temperature for 1h, the plated were incubated at 37°C for 2 days in an incubator and later the responses were observed by measuring the zones of inhibition surrounding the wells and compared with that of standard. All the determinations were recorded in triplicate [9].

Stability studies

Optimized formulation was tested for stability for 3 months at temperature 25 ± 2 °C and 2- 8°C which was evaluated for physical stability, drug content and in-vitro diffusion study using semipermeable dialysis membrane- 70 in phosphate buffer pH 7.4.

RESULTS AND DISCUSSION

Preformulation Studies

Determination of solubility of Itraconazole

Solubility of Itraconazole in different solvents in given in Table 3

Table 3: Solubility of Itraconazole in formulation.

Solvent	Concentration (mg/mL) ± SD
Ethanol	1.607 ± 0.1010
Phosphate buffer 7.4	0.572 ± 0.1401
Distilled water	0.023 ± 0.1023

Determination of Melting Point of the drug

The observed melting point of Itraconazole was found to be 168ºC.

Compatibility studies of Itraconazole and excipients by Infrared spectroscopy

The Fig. 1 shows FTIR spectra of itraconazole which various stretches at 3126 cm-1 (C=C stretch), 2962-2823.79 cm-1 (C-H stretch), 1697 cm-1 (C=O stretch), 1600-1550 cm-1 (C-C aromatic stretch), 1379-1271 cm-1 (C-N stretch), 1271-1184 cm-1 (C-O stretch), 736-530 cm-1 (C-Cl stretch).

The IR spectra of Itraconazole was taken with all excipients (Fig. 2) to identify any possible drug-excipient interaction, it was observed that there was no significant interaction as there was no new characteristic peak other than the pure drug spectra. Hence it was evident that the drug is compatible with the excipients used in the design of Itraconazole proniosomal gel.

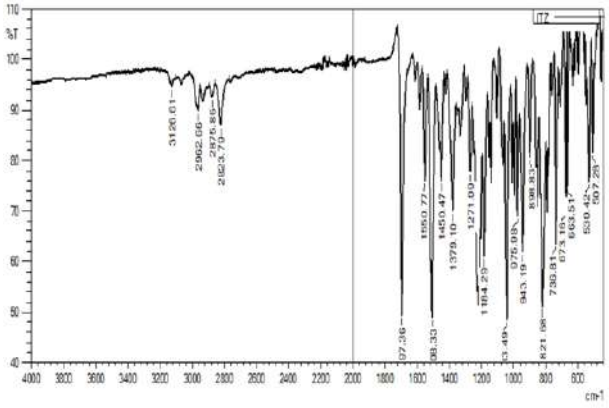

Figure 1: IR spectra of pure Itraconazole

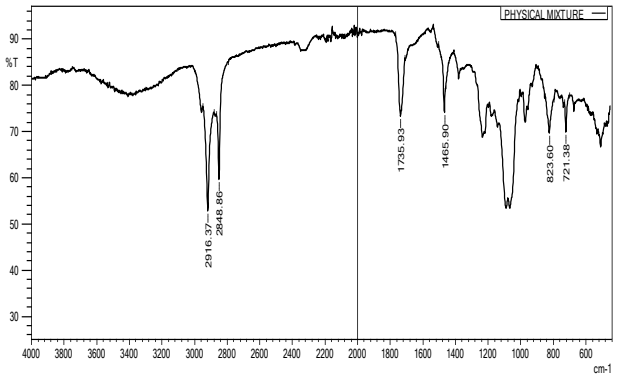

Figure 2: IR spectra of physical mixture of itraconazole, tween 20, phospholipid and cholesterol.

Evaluation of Proniosome Gel

Appearance

All the proniosomal gels were found to be white to off-white in colour and were opaque in nature (Fig. 3)

Figure 3: General appearance of proniosomal gel formulations.

Determination of particle size, polydispersity index and zeta potential

As shown in Table 4 the Proniosome vesicles formed after hydration were found to have particle size ranging from 194.2 to 717.6nm. The zeta potential of Proniosomal gel were found to be in the range of -10.5 mV to -58.6 mV and niosomes ranged from -53.7 to -10.5. From the data given in table 12 it can be seen that zeta potential value of those proniosomes prepared from tween (TF5 to TF8) was significantly lower (-30.5 to -10.5) than those prepared from spans (-58.6 to -32.7) i.e., SF1 to SF4. Thus, it was observed that with increased hydrophilicity of surfactants zeta potential increased. All formulations were found quite stable. All the formulations showed PDI from 0.169 to 0.58 which indicates that the formed niosomes were uniform and homogenous.

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Table 4: Observed values of particle size, zeta potential and PDI

Formulation code	Particle size	Zeta potential	Polydispersity index
SF1	267.2	-53.7	0.550
SF2	208.9	-50	0.586
SF3	194.2	-32.7	0.384
SF4	456.1	-58.6	0.598
TF5	517.50	-10.5	0.169
TF6	498.46	-14.23	0.237
TF7	717.65	-29.28	0.226
TF8	580.5	-30.5	0.456

Determination of entrapment efficiency

The entrapment efficiency of proniosomes as shown in (**Table 5**) ranged from 74 – 94.67%. The observed responses were statistically analysed by SPSS software by One-way ANOVA followed by Least Significance difference (LSD) test to determine statistical significance.

From statistical analysis (**Table 6**) the conclusion was drawn that EE% of the formulations were statistically different. In case of niosomes prepared from tween (TF5, TF6, TF7, TF8) TF5 has the highest EE: 94.63% ($p < 0.05$), followed by TF6 92.5% and then TF7: 91.35% i.e., EE% increases as Tween 20> Tween 40> Tween 60> Tween 80.

Table 5: Observed value for entrapment efficiency

Formulation code	Entrapment efficiency
SF1	77.5±1.01
SF2	74.06±1.36
SF3	81.26±0.64
SF4	71.96±1.41
TF5	94.63±0.65

TF6	92.5±0.43
TF7	91.35±2.33
TF8	86.43±1.25

Table 6: Results of ANOVA with post hoc test for entrapment efficiency

	Sum of squares	Df	Mean square	F	Sig
Between groups	1514.239	7	216.320	32.566	.000
Within groups	106.280	16	6.642		
Total	1620.519	23			

Evaluation of Optimised Proniosome Gel

Morphological study by light microscopy

Microscopic examination of the vesicles formed after hydration of TF5 proniosomes displayed formation of multilamellar spherical vesicles with a central core of entrapped ITZ (**Fig. 4**).

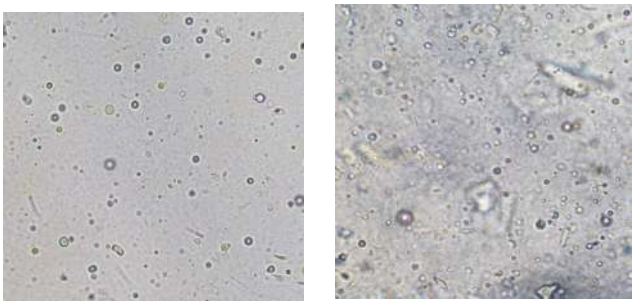


Figure 4: Light microscopy pictures of proniosome after hydration, 100x

Morphological study by Scanning Electron Microscope

The Scanning electron microscopy study images of the niosomes prepared from proniosomal gel after hydration of formulae TF5 are shown in **Fig. 5**. Electron micrographs exhibited spherical nano vesicles with well-identified outline and core.

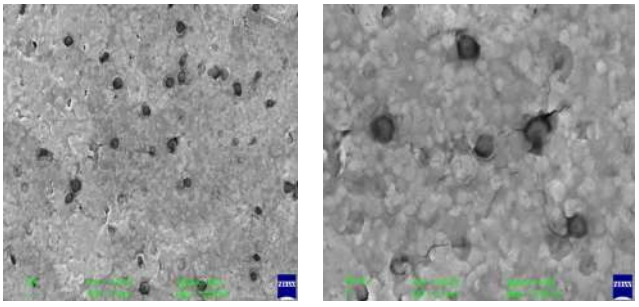


Figure 5: Scanning electron microscopy image of proniosomal gel TF5

DSC study

In Fig. 6, DSC thermogram showed the characteristic peak of Itraconazole at 167.49°C corresponding to its melting point. The empty proniosomes and drug loaded proniosomes showed the peak at 42-52 °C and 82.99 – 88.32 which confirmed the absence of any chemical interactions between the Itraconazole and Proniosome components. The thermogram of TF5 (Fig. 7) exhibit sharp single peak at 82.99°C which is different from the peak observed in Itraconazole and other formulation components thermogram peak.

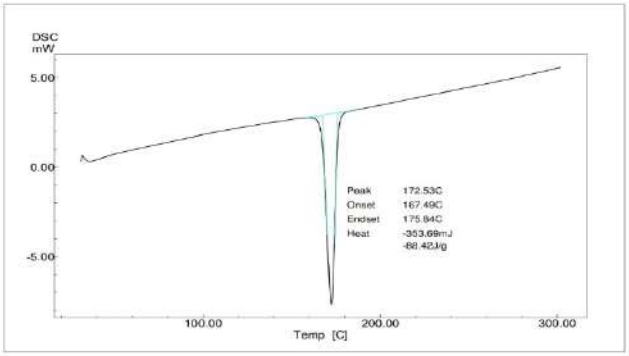


Figure 6: DSC thermogram of itraconazole

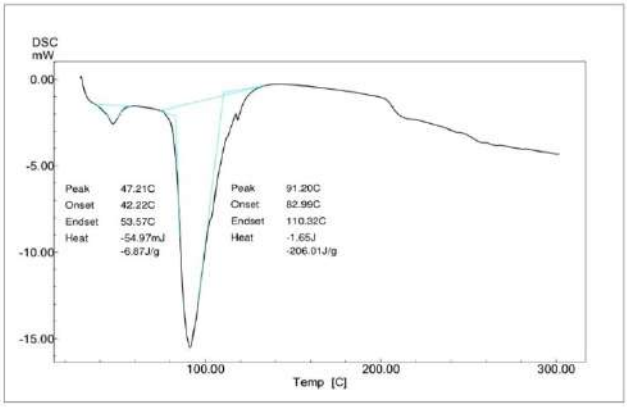


Figure 7: DSC thermogram of formulation TF5

Determination of Particle size, polydispersity index and zeta potential of optimised proniosomal formulation

The optimised formulation was found to have particle size of 517.50 nm, zeta potential - 10.50 and PDI 0.169 as shown in **Fig. 8** and **Fig. 9**.

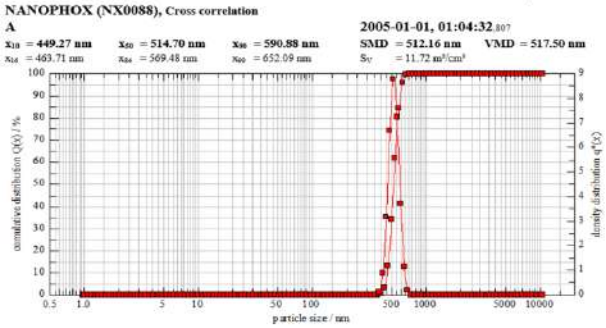


Figure 8: Particle size and PDI of optimised proniosomal gel formulation

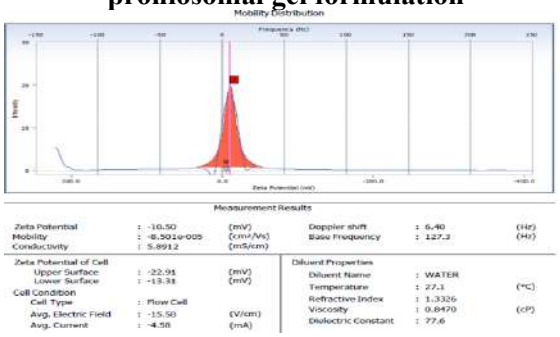


Figure 9: Zeta potential of optimised proniosomal gel formulation

Determination of Entrapment Efficiency

The entrapment efficiency of the optimized formulation TF5 containing tween as non-ionic surfactant showed highest EE% i.e., 94.63±0.65 than other formulations of span and tween as shown in **Table 4**. ($P<0.05$).

***In- vitro* drug release study**

The % CDR of the optimised proniosomal gel formulation TF5 at the end of 8 hours was found to be 35.72% as shown in **Table 7**. The *in-vitro* release data of optimised formulation was fitted into four kinetic models that are Zero order, First order, Higuchi plot and Peppa's plot. Based on the results obtained from the release profile the best fit model for the Proniosome were first order and Higuchi's (Matrix) model that is evident in **Table 8**, which shows highest linearity values (R2 = 0.9853 for first order and R2 = 0.9903 for Higuchi's model). The n value of the optimised formulation was found to be 0.622 therefore, it can be concluded that the drug release from optimised formulation followed non-fickian type of diffusion.

Table 7: Kinetics of drug release of optimised proniosomal gel *in- vitro* drug release study

Time (hrs)	Square root time	Log time	% CDR	% Drug remaining	Log % CDR	Log of % drug remaining
0	0	-	0	100	-	2
0.5	0.70	-0.3	6.44	93.56	0.80	1.97
1	1	0	9.45	90.55	0.97	1.95
2	1.41	0.30	14.63	85.37	1.16	1.93
3	1.73	0.47	19.41	80.59	1.28	1.90
4	2	0.60	22.6	77.4	1.35	1.88
5	2.23	0.69	26.63	73.37	1.42	1.86
6	2.44	0.77	29.52	70.48	1.47	1.84
7	2.64	0/84	32.31	67.69	1.50	1.83
8	2.82	0.90	35.72	64.28	1.55	1.80

Table 8: Values of regression coefficient and kinetics for optimised formulation

Formulation code	Zero order plot		First order plot		Higuchi plot		Peppa's plot	
	R^2	K	R^2	K	R^2	K	R^2	K
TF5	0.968	4.465	0.9853	-0.0833	0.9903	22.726	0.9883	0.622

Stability studies of optimized formulation

The optimized Proniosomal formulation was subjected to stability studies under three different conditions i.e. -20°C, 2-8°C and at room temperature. At the end of three months the proniosomal formulation was found to be physically stable as there was no change in its appearance. The results of % entrapment efficiency and in-vitro release as illustrated in Table 9.

Table 9: Results of stability studies of optimised proniosomal gel formulation.

Temperature	%EE	*In-vitro* drug release
-20°C	94.7	35.47
2-8°C	95.1	35.7
Room Temperature	93.6	34.3

Evaluation of Proniosomal Hydrogels

Organoleptic Characterisation

The evaluated proniosomal Hydrogels prepared were homogenous in nature, smooth in consistency non-gritty, free from any foreign particles. and were white in colour as shown in **Fig. 10**.

Figure 10: Proniosomal hydrogels formulations

Determination of pH

The pH of proniosomal hydrogel containing Carbopol was found in range of 6.89 to 7.03 and pH of HPMC Gels were found in range of 6.75 to 7.16 which were equivalent to skin pH and was considered non-irritant and fit for topical application. The pH of proniosomal hydrogel is illustrated in **Table 10**.

Table 10: pH of proniosomal hydrogels

FORMULATION CODE	pH
G1	6.89±0.035
G2	7.01±0.101
G3	7.03±0.16
G4	6.75±0.19
G5	7.16±0.098
G6	6.86±0.24

Spreadability

Spreadability of Proniosomal Hydrogels was determined on the basis of 'Slip' and 'Drag' characteristics of gels. It was observed that proniosome loaded hydrogels exhibited good spreading property.

Viscosity

The viscosities of Proniosomal Hydrogels determined using Brookfield viscometer were tabulated in **Table 11**. Formulations G1-G3 containing Carbopol were found to have viscosities ranging from 12550 cP to 24516 cP whereas proniosomal gels G5-G6 containing HPMC were found to have viscosities ranging from 16890 cP to 21306 cP, which is considered as adequate viscous for transdermal application.

Table 11: Viscosity of proniosomal hydrogels

Formulation code	Viscosity (cP)
G1	21490±729
G2	24516.67 ±835
G3	12550±655
G4	16890.33± 408
G5	21140±346
G6	21306.67±1154

Drug Content

The drug content of proniosomal hydrogels was estimated and the data are tabulated in **Table 12**. The drug content for hydrogels containing Carbopol (G1-G3) was found the range of 94.76% to 98.92% whereas for hydrogels comprising of HPMC (G4-G6) were found to have slightly lower drug content i.e., 93.68% to 98.34%. The drug content of all hydrogels was found satisfactory.

Table 12: Drug content of proniosomal hydrogels

FORMULATION CODE	Drug content (%)
G1	94.76 ± 0.29
G2	98.92 ± 0.92
G3	96.2 ± 0.5
G4	98.34 ± 0.73
G5	93.68 ± 0.73
G6	94.76667 ± 1.05

***In-vitro* Drug Release**

The drug release characteristics of the ITZ proniosomal hydrogel were studied in in vitro conditions using modified Franz diffusion cell with semipermeable dialysis membrane in phosphate buffer pH 7.4.

From the *in-vitro* release studies data and plots, it can be observed that the drug release gradually increased over the period of 8 h of study and release was more than 70% in all the formulation at the end of 8 h. Formulation G1 to G3 showed drug release of 83.03 to 86.53% at the end of 8 h, while formulation G4 to G6 showed drug release 87.22 to 79.15%. The Carbopol gel showed better release than the HPMC gels the may due to increase in the viscosity of the hydrogel. Among all six batches the Carbopol gel G2 and HPMC gel G4 showed highest drug release i.e., 86.53 to 87.22% respectively, as shown in **Table 13, Fig. 11.**

Formulation G2 (**Fig. 12**) was selected as the optimized batch, as it showed highest drug content (98.92 ± 0.92%) and maximum in-vitro drug release (87.83 ± 0.52%). Also, the optimised proniosome loaded hydrogel G2 was found to have pH 7.01 ± 0.11, which is almost close to that of the skin. It was found to have a viscosity of 24516.67 ± 835cP and the spreadability was found to be 24.06 ± 0.69 g.cm/sec.

% Drug release at time intervals (hrs)	Pure drug	Marketed preparation	G1	G2	G3	G4	G5	G6
0	0	0	0	0	0	0	0	0
0.5	47.97 ± 0.631	43.98 ± 0.728	24.26 ± 1.19	23.71 ± 0.80	15.98 ± 0.92	10.93 ± 0.62	10.93 ± 0.62	9.73 ± 0.34
1	60.66 ± 0.960	61.46 ± 0.969	32.15 ± 0.98	33.81 ± 1.63	21.14 ± 0.51	19.01 ± 1.07	19.01 ± 1.07	14.54 ± 0.47
2	73.89 ± 0.357	79.12 ± 0.952	44.2 ± 1.13	42.97 ± 1.50	29.67 ± 1.7	28.77 ± 1.88	28.77 ± 1.88	22.57 ± 1.04
3	92.92 ± 0.967	86.63 ± 1.590	51 ± 0.288	51.49 ± 1.64	36.63 ± 0.81	40.25 ± 1.68	40.25 ± 1.68	33.62 ± 1.1
4	98.47 ± 1.51	93.37 ± 1.610	57.48 ± 1.58	60.62 ± 0.30	47.41 ± 1.20	49.93 ± 1.13	49.93 ± 1.13	44.88 ± 1.2
5	-	106.48 ± 0.672	64.02 ± 1.17	67.23 ± 0.32	55.31 ± 0.99	60.46 ± 1.56	60.46 ± 1.56	55.17 ± 0.63
6	-	-	71.6 ± 1.50	73.8 ± 0.44	63.93 ± 0.56	68.72 ± 0.56	68.72 ± 0.56	64.81 ± 0.38
7	-	-	78.59 ± 1.68	80.26 ± 0.74	73.23 ± 0.78	75.25 ± 1.26	75.25 ± 1.26	73.4 ± 0.66
8	-	-	85.5 ± 0.84	87.83 ± 0.52	81.69 ±1.15	80.58 ± 0.80	80.58 ± 0.80	79.59 ± 0.19

Table 13: *In-vitro* release profile

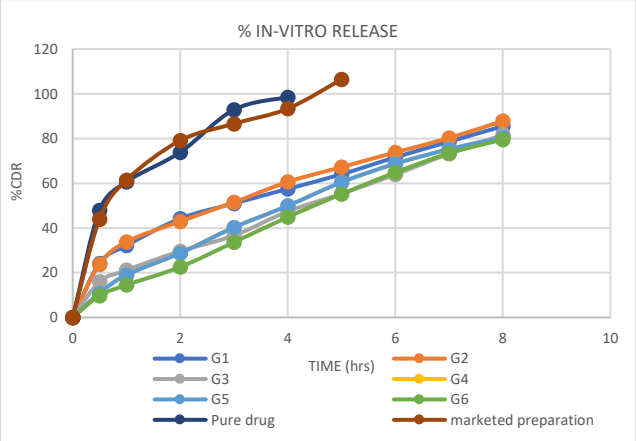


Figure 11: Percentage *in-vitro* release of G1-G6, pure drug and marketed preparation

Figure 12: Optimised itraconazole proniosomal hydrogel (G2)

Comparison of Release Profile of Optimised Proniosomal Hydrogel and Pure Drug Gel Formulation

In-vitro drug release study of the optimised Itraconazole Proniosome gel was compared with marketed formulation (**Fig. 13**) in order to check the efficiency of the Proniosome gel as compared to the Itraconazole Carbopol gel formulation prepared using dispersion method.

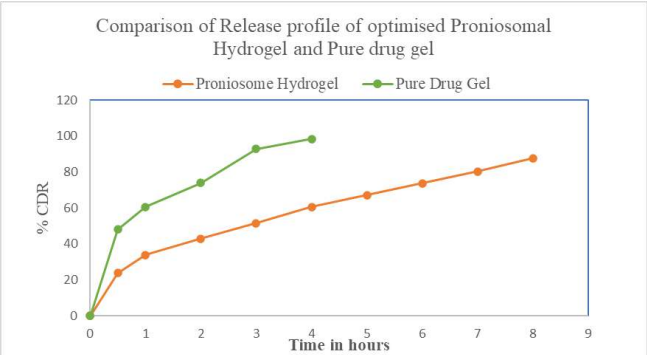


Figure 13: *In-vitro* release profile of optimised proniosomal hydrogel and pure drug gel

Comparison of Release Profile of Optimised Proniosomal Hydrogel and Marketed Formulation

In-vitro drug release study of the optimised Itraconazole proniosome gel was compared with marketed formulation in order to check the efficiency of the proniosome gel as compared to the marketed Itraconazole gel formulation shown in **Fig. 14**.

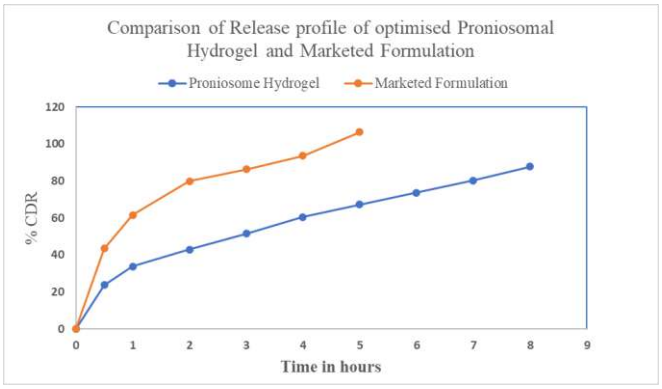


Figure 14: Comparison of *in-vitro* release profile of optimised proniosome hydrogel and marketed formulation.

On comparison of the release profiles of optimised formulation with that of marketed formulation, it was observed that % CDR of optimised hydrogel was 87.83% at the end of 8 h than that of the marketed formulation were complete release of the drug was observed within 5 h ($p > 0.05$). Thus, conclusion can be drawn that since the proniosomal hydrogel exhibits drug release for longer period of time this reduces the repeated application.

In-vitro Skin Permeation Studies

Proniosomes are hydrated to niosomes vesicles before the penetration through the skin. *In vitro* permeation studies were conducted on wistar albino rat skin membrane as a semipermeable barrier using Franz diffusion cell for optimised proniosomal gel and pure Itraconazole Carbopol gel. Further, to confirm release mechanism Peppa's plot was analysed and it was found that the drug release from optimised formulation followed non-fickian type of diffusion.

In-vitro Skin Permeation Release Profile of Itraconazole Proniosomal Hydrogel, Pure Drug Gel and Marketed Gel

In-vitro skin permeation study showed that permeation of drug through rat skin for pure Itraconazole gel and marketed formulation in 4 h was 98.68 and 100.5% as it is having higher permeation and maximum cumulative percent drug release (% CDR) from donor in to the receptor medium (**Fig. 15**). Whereas, the proniosomal Hydrogel formulation (Optimized Itraconazole Hydrogel G2) shows up to 90.89 % of drug permeation through skin at the end of 8 h as shown in figure 68. Therefore, it is concluded that Itraconazole proniosomal hydrogel showed increased retention time of the drug as compared to Pure Itraconazole Gel and Marketed formulation. It is evident that drug permeated through skin is prolonged for up to 8 h in optimized hydrogel formulation and the drug release was sustained.

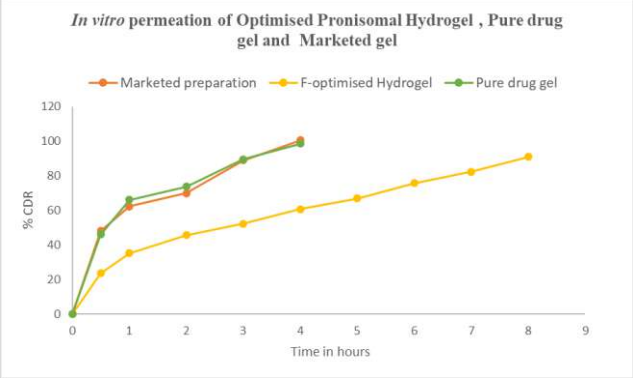


Figure 15: Comparison of *in-vitro* release profile of optimised proniosome hydrogel and marketed formulation

Skin Irritation Studies

All three groups of animals were checked for the presence of erythema or oedema for the period of 7 days. There was no evidence of any signs of erythema or oedema up to 7 days at the site of application as seen in figures 66-69. Results indicated (**Fig. 16**) that Itraconazole proniosomal hydrogel skin irritation was absent. The values obtained are reported in **Table 14**.

Table 14: Skin irritation observation data of ITZ proniosomal hydrogel

Duration	Response control	ITZ proniosomal gel	Marketed preparation
1st day	0	0	0
3rd day	0	0	0
7th day	0	0	0

A **B**

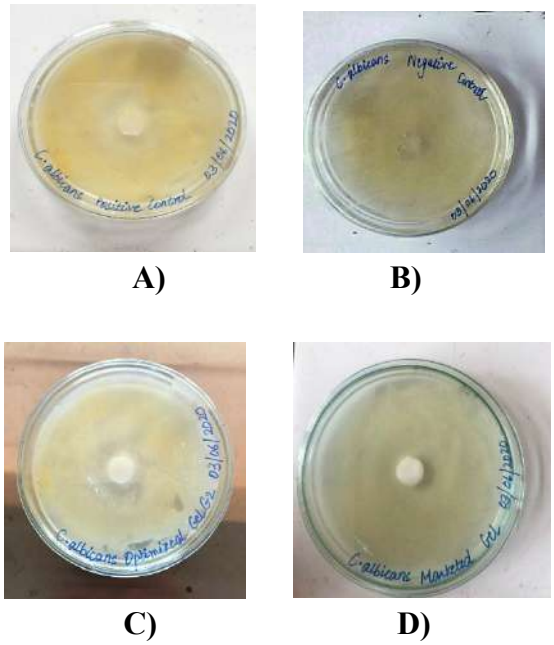
Figure 16: Images of rat at the end of seventh day. (A) is ITZ proniosomal hydrogel; (B) indicates marketed ITZ gel

Antifungal Activity

The optimized formulation was subjected to antifungal studied against Candida albicans. There was no significant difference between zone of inhibitions of pure drug and marketed cream. The optimized gel exhibited larger zone indicating strong antifungal activity as seen from **Fig 17**. The zone of inhibitions observed against Candida for different formulation is given in the **Table 15**.

Table 15: Results of zone of inhibition against Candida Albicans species

Sample	Zone of inhibition (cm)
Positive Control (pure drug gel)	2.4
Itraconazole Proniosome Hydrogel	2.6
Marketed Itraconazole Gel	2.0
Negative control (Gel without drug)	0



A) B)

C) D)

Figure 17: Antifungal activity test of itraconazole formulations against *candida* species. (A) is positive control (pure drug gel); (B) indicates negative control; (C) is Itraconazole proniosome hydrogel; (D) shows marketed itraconazole gel.

Stability Studies

The optimised proniosomal hydrogel was subjected to stability studies under two different conditions i.e., 2-8°C and at room temperature. At the end of three months, the proniosomal hydrogel was found to be physically stable as there was no change in its appearance and no phase separation was observed. The result of drug content and *in-vitro* diffusion is tabulated in **Table 16**.

Table 16: Results of stability studies

Temperature	Drug content	In-vitro release (%CDR)
2-8°C	98.45	86.87
Room temperature	95.66	85.53

The optimised proniosomal hydro gel stored at 2-8°C showed good drug content and in-vitro release the results observed after three months stability period were similar to that of the fresh proniosomal hydrogel. The proniosomal hydrogel stored at room temperature showed a decrease in drug content and decreased % CDR.

CONCLUSION

A transdermal proniosome formulation of an antifungal agent Itraconazole in a hydrogel base was developed with an aim of overcoming the oral side effects associated with the drug. The optimised itraconazole-loaded proniosomal gel formulation containing tween 20 showed highest entrapment efficiency of 94.63% as compared to other non-ionic surfactant and showed good particle size of vesicles (517nm), zeta potential (-10.5) and polydispersity index (0.169). The optimised proniosomal gel was then incorporated into Carbopol and HPMC gel bases of varying ratios. The optimised hydrogel was characterised for antifungal activity, skin irritation and skin permeation.

Thus, it was concluded that the Itraconazole proniosomal hydrogel can significantly improve the therapy against skin fungal infection via transdermal route as proniosomes forms depot in deeper skin layers and continuously release drug for a prolonged period. Transdermal route of delivery overcomes limitation of oral therapy of Itraconazole and increasing the efficacy of treatment by improving patient compliance by avoiding first pass metabolism with reduced side effects.

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