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Transdermal Itraconazole for the Treatment of Basal Cell Carcinoma; Effect of Chemical Enhancers on Skin Permeation

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ABSTRACT

The objective of this study was to prepare and evaluate itraconazole transdermal gel with different permeation enhancers. Itraconazole transdermal gels were prepared by dispersion method using natural polymer (Tara gum 2% and Kondagogu gum 4%) as gelling agents. Different permeation enhancers such as fatty acids (oleic and stearic), organic acids (citric, acetic, maleic and succinic), sulfoxides (dimethyl sulfoxide) with 2 different concentrations 1% and 2.5% were used. The prepared gels were evaluated for physicochemical properties, in vitro, ex vivo, skin irritation and stability studies. All formulations have shown good physicochemical properties. In vitro diffusion studies concluded that ITC1, ITM1, ITSU1, IKC1, IKM1 and IKSU1 have shown more than 90% drug release for 4-7 h. Ex vivo permeation studies of the ITM1 has shown better release of itraconazole in 8 h with Q8 of 2319.109 ± 5.91 µg/cm^2; flux of 281.12 ± 0.98 µg/cm^2/h; permeability coefficient of 110.199 ± 0.98 cm/h × 10^{-3} and enhancement ratio of 6.428 ± 0.12. Skin irritations studies have shown ITM1 to be non-irritant. ITM1 formulation was found to be stable for one month at room temperature. Based on results, it can be concluded that ITM1 formulation with maleic acid (1%) as permeation enhancer can provide good permeation of itraconazole for the treatment of basal cell carcinoma.

Keywords: Itraconazole, Tara gum, Kondagogu gum, Basal cell carcinoma, Organic acids

INTRODUCTION

Transdermal Drug Delivery System (TDDS) has been emerged as a novel tool over conventional routes as it avoiding the fluctuation in drug levels, minimizing undesirable side effects, increases the patient compliance and avoids the first pass hepatic metabolism [1]. Transdermal drug delivery system provides a means to sustain drug release as well as reduce the intensity of action and thus reduce the side effects associated with its oral therapy [2]. An essential prerequisite for the development of this system is that the drug must be capable of penetrating the skin at adequately high rate to reach therapeutic plasma concentrations. However, the outer most layer of skin, the stratum corneum, forms a major barrier to most exogenous substances, including drugs. To reduce the barrier function of the skin, one popular approach to deliver an effective dose of drug through skin with the aid of physical or chemical penetration enhancement techniques [3]. Topical application of gels at affected area offers great advantage in a faster release of medication directly to the site of action [4]. Gel formulation shows better application property like spreadability, extrudability and has more stability when compared to cream and ointment [5].

Basal Cell Carcinoma (BCC) is a non-melanocytic skin cancer that arises from basal cells (i.e., Small, round cells found in the lower layer of the epidermis). It is the most common skin cancer in humans, which typically appears over the sun-exposed skin as a slow-growing, locally invasive lesion that rarely metastasizes [6].

The azole antifungal agents represent a major advance in the treatment of both superficial and systemic fungal infections. These drugs can be divided in two main groups: the imidazoles and the triazoles [7]. Itraconazole, a US Food and Drug Administration (FAD) approved antifungal drug, inhibits the Hedgehog (HH) signaling pathway, a crucial driver of BCC tumorigenesis [8]. Itraconazole is a weak base with a pKa of 3.7, and relatively insoluble in water [9].

A topical itraconazole-containing formulation is preferred to use for some reasons like the opportunity to provide high local tissue levels and lower systemic concentration of the drug [10]. The objective of present work is to develop transdermal gel of the very poorly water soluble drug itraconazole which is useful in the treatment of BCC by enhancing its permeation using different chemical permeation enhancers. The transdermal gel formulations should provide delivery of an effective concentration of a triazole-triazolone compound such as itraconazole sufficient to inhibit, reduce or eliminate the tumor.

MATERIAL AND METHODS

Materials

Itraconazole was received from Suven Life Sciences limited, Hyderabad, Telangana, India. Tara gum, kondagogu gum, oleic acid, stearic acid, citric acid, maleic acid, succinic acid acetic acid and Dimethyl Sulfoxide (DMSO) were purchased from S.D. Fine Chemicals Ltd, Mumbai, Maharashtra India. The chemicals and reagents used were of analytical grades.

Preformulation studies

Drug excipient compatibility studies

The spectrum analysis of itraconazole and excipients used in the preparation of transdermal gels were determined by Fourier Transform Infra-Red (FTIR) spectroscopy. FTIR spectrums were obtained by preparing Potassium Bromide (KBr) disks using a Shimadzu Corporation (Kyoto, Japan) facility (model-8400S). KBr disks were prepared by mixing few milligrams of sample with potassium bromide by compacting in a hydrostatic press under vacuum at 6-8 tons pressure. The resultant disc was mounted in a suitable holder in IR spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 200 cm^{-1}. The resultant spectrum was compared for any spectral changes. They were observed for the presence of characteristic peaks for the respective functional group in the compound [11]. FTIR study was carried out to check compatibility of drug and excipients used in the formulations.

Solubility studies

Solubility studies were done using orbital shaker bath. Saturated solutions of itraconazole were prepared by adding an excess amount of drug to 10 ml distilled water and to a solvent mixture of propylene glycol, methanol, and water mixture (0.96:3:6.04) including appropriate quantity of chemical permeation enhancers which represent each gel formulations. Saturated solutions were kept in an orbital shaker for 24 h at room temperature; later it was centrifuged for 15 min at 3000 rpm. Aliquots were filtered through Whatman No. 41 filter paper. The filtrates were diluted appropriately in distilled water and assayed spectrophotometrically at 263.6 nm. The experiment was repeated three times in the same medium and a calibration curve was determined from the mean value [12].

Preparation of itraconazole transdermal gel formulations with different chemical permeation enhancers

Transdermal gels were formulated by dispersion method [13]. The required quantities of polymer tara gum & Kondagogu gum, were weighed and soaked in a beaker containing distilled water for 2-3 h. The swollen polymers were kept for stirring at 400-600 rpm. Propylene glycol (0.96 ml) was added slowly with stirring. Accurately weighed itraconazole was dissolved in 3 ml of methanol and this solution of drug was added slowly with stirring (400-600 rpm) in the previously prepared gel. Permeation enhancers were added with stirring either directly (oleic acid, acetic acid and DMSO) or after dissolved in water (citric acid, maleic acid and succinic acid) or after dissolved in methanol (stearic acid). The final quantity was made upto 10 g with distilled water. The composition of each gel formulations was tabulated in Table 1.

Table 1: Formulation of itraconazole transdermal gels

Formulation code	Ingredient (%w/w)								
	Tara gum	Kondagogu gum	Oleic acid	Stearic acid	Citric acid	Maleic acid	Succinic acid	Acetic acid	DMSO
ITCON	2	-	-	-	-	-	-	-	-
ITO1	2	-	1	-	-	-	-	-	-
ITO2.5	2	-	2.5	-	-	-	-	-	-
ITS1	2	-	-	1	-	-	-	-	-
ITS2.5	2	-	-	2.5	-	-	-	-	-
ITC1	2	-	-	-	1	-	-	-	-
ITC2.5	2	-	-	-	2.5	-	-	-	-
ITM1	2	-	-	-	-	1	-	-	-
ITM2.5	2	-	-	-	-	2.5	-	-	-
ITSU1	2	-	-	-	-	-	1	-	-
ITSU2.5	2	-	-	-	-	-	2.5	-	-
ITA1	2	-	-	-	-	-	-	1	-
ITA2.5	2	-	-	-	-	-	-	2.5	-
ITD1	2	-	-	-	-	-	-	-	1
ITD2.5	2	-	-	-	-	-	-	-	2.5
IKCON	-	4	-	-	-	-	-	-	-
IKO1	-	4	1	-	-	-	-	-	-
IKO2.5	-	4	2.5	-	-	-	-	-	-
IKS1	-	4	-	1	-	-	-	-	-
IKS2.5	-	4	-	2.5	-	-	-	-	-
IKC1	-	4	-	-	1	-	-	-	-
IKC2.5	-	4	-	-	2.5	-	-	-	-
IKM1	-	4	-	-	-	1	-	-	-
IKM2.5	-	4	-	-	-	2.5	-	-	-
IKSU1	-	4	-	-	-	-	1	-	-
IKSU2.5	-	4	-	-	-	-	2.5	-	-
IKA1	-	4	-	-	-	-	-	1	-
IKA2.5	-	4	-	-	-	-	-	2.5	-
IKD1	-	4	-	-	-	-	-	-	1
IKD2.5	-	4	-	-	-	-	-	-	2.5

Note: All gels were prepared using 500 mg of itraconazole, 24% (w/w) methanol, 10 % (w/w) propylene glycol and distilled water upto 10 g

Physicochemical evaluation of transdermal gels

Determination of pH

The pH of itraconazole gel formulations was determined by using digital pH meter. One gram of prepared gel was dispersed in 100 ml of distilled water and stored for 2 h at room temperature. The measurement of pH for each formulation was done in triplicate and average values were calculated [14].

Drug content

Itraconazole content in prepared gel was done by dissolving 100 mg of gel (equivalent to 5 mg of drug) in 10 ml solvent (Methanol) by sonication. The solution was passed through Whatman filter paper no.42 and filtered. Absorbance was measured after suitable dilution at 263 nm in UV-Visible spectrophotometer. The experiment was done in triplicate and average values were calculated [14].

Homogeneity

It was observed by visual inspection for the appearance of gel to check the presence of any aggregates [15].

Extrudability

It was carried out using Pfizer hardness tester. A 15 g of prepared gel was filled in aluminum tube. The plunger was adjusted to hold the tube properly. The pressure of 1 kg/cm² was applied for 30 sec. The quantity of gel extruded was weighed. The experiment was repeated at three equidistance places of tube. Test was carried in triplicate [16].

Spreadability

It was calculated by measuring the spreading diameter of 1 g of gel between 20 × 20 cm glass plates after 1 min. The mass of the upper plate was standardized at 150 g. The spreadability was calculated by using the formula [17].

$$S = \frac{ml}{t}$$

Where, S=Spreadability, m=Weight tied to the upper glass slide, l=Length of the glass slide, t=Time taken in seconds.

Determination of viscosity

Viscosity of prepared gels was determined by VISCOlab 3000 viscometer that contains a piston style electromagnetic sensor and integrated thermometer that provides continuous viscosity and temperature reading. The sample 1-2 ml was applied in the measurement chambers and the results were displayed on the screen of VISCOlab 3000. The determination of viscosity for each formulation was done in triplicate and average values were calculated.

***In vitro* diffusion studies**

Diffusion studies were performed using Franz diffusion cell. The cell was locally fabricated and the volume of receptor compartment was 25 ml. The dialysis membrane used for diffusion studies was placed between donor and receptor compartment. Gel formulation was uniformly applied on membrane and clamped together. The receptor compartment was filled with pH 7.4 phosphate buffer saline and maintained by continuous stirring with a magnetic bead. At predetermined time intervals, 1 ml samples were withdrawn and replaced with an equal volume of buffer. The samples were analyzed after appropriate dilution using spectrophotometer. Release rate was calculated by plot the amount of drug permeated *versus* square time. The slope is release rate (µg/cm²/$h^{½}$) [18].

***Ex vivo* permeation studies**

The experimental protocol was approved by the institutional animal ethical committee (IAEC) in G. Pulla Reddy College of Pharmacy Registration number 320/CPCSEA and student ID number: GPRCP/IAEC/20/16/2/PCE/ACE-8. Male Wister rats (150-180 g) were used for permeation study. The animal was sacrificed by cervical dislocation and hair was removed from abdomen using an animal hair clipper. Abdominal skin section was excised and observed for existence of cuts and wounds. The fat adhering on dermis was removed using scalpel and finally it was washed under tap water. The skin was stored at −20°C and used within a week [19].

Permeation studies

For the permeation studies locally fabricated Franz diffusion cells with an area of 4.9 cm^2 and 25 ml receptor volume were used. The thawed rat skin was mounted on to diffusion cell such that the dermis side was in constant contact with receptor solution. 250 mg of gel was applied to the stratum corneum facing the donor compartment and the hydrodynamics in the receptor compartment were maintained by stirring on magnetic stirrer at 600 rpm. 1 ml sample was withdrawn at predetermined time intervals for 12 h and drug content was analyzed by UV-visible double beam spectrophotometer at 263 nm [19].

Calculation of permeability parameters

Steady state flux (µg/cm²/h)

Steady state flux **(**Jss) is defined as the rate of diffusion or transport of a substance through a permeable membrane. After reaching the steady state of drug permeation, flux was calculated using the following equation

$$\text{Steady state flux (Jss)} = \frac{dM}{S}.dt$$

Where, dM=Amount of drug permeated, S=Unit cross-section area, t=Time (t). The steady state flux obtained by plotting the cumulative amount of drug permeated in micrograms per square centimeter *versus* time in hours and the slope is the flux. Lag time is X intercept of this graph [20].

Permeability coefficient (cm/h)

The permeability coefficient (Kp) was calculated with the following Equation:

$$\text{Permeability coefficient (Kp)} = \frac{\text{Jss}}{\text{Cv}}$$

Where, cv is the total donor concentration of the formulation [20].

Enhancement ratio

Enhancement ratio (ER) used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules and is calculated by:

$$\text{Enhancement ratio (ER)} = \frac{\text{Jss of drug with enhancer}}{\text{Jss of drug alone}}$$

Where, Jss=Steady state flux [20].

Drug kinetics

Regression coefficient (r^2) was calculated for all the formulations. Release component "n" was calculated from Korsmeyer-peppas equation. These calculations were carried out using MS-office excel [19].

Skin irritation studies

Skin irritation studies were performed on rabbits after the approval by the Institutional animal ethical committee (IAEC) in G. Pulla Reddy College of Pharmacy Registration number 320/CPCSEA and student ID number: GPRCP/IAEC/20/16/2/PCE/ACE-8. A primary skin irritation test was performed since skin is the vital organ through which the drug is transported. The test was carried out on three healthy rabbits weighing between 1.5-2 kg. The test was conducted on an unbraided skin of rabbits. Before placing the formulations, the unbraided skin was cleaned with rectified spirit. The control was kept on the right dorsal surface of first rabbit, whereas tara gum placebo gel and first optimized formulation (with drug and chemical enhancer) was placed on the left dorsal surface of the same rabbits. The kondagogu gum placebo gel was placed on the right dorsal surface of second rabbit, whereas second and third optimized formulation (with drug and chemical enhancer) was placed on the left dorsal surface of the same rabbits, and the third rabbit was kept as control. The experiment was carried out for 3 days and the application sites were graded according to a visual scoring scale [12]. Skin irritation was scored according to the Draize method [21].

Stability studies

Stability studies were carried out by keeping optimized formulations in glass containers with polypropylene closure for one month at room temperature. Known amount of gel was taken out at different time intervals like 0, 1st, 2nd, 4th week and was analyzed for appearance, pH, drug content and viscosity [22].

RESULTS AND DISCUSSION

Drug excipients compatibility studies

From FTIR spectrums of the pure itraconazole (Figure 1A), polymer (Figure 1B), blend of drug with polymer (Figure 1C) and optimized transdermal gel formulation ITM1 (Figure 1D), the peaks identified in the pure drug were relatively same when compared with the blend and optimized transdermal gel formulation indicating no drug excipients interaction i.e., the pure drug was not altered functionally and compatible with polymers.

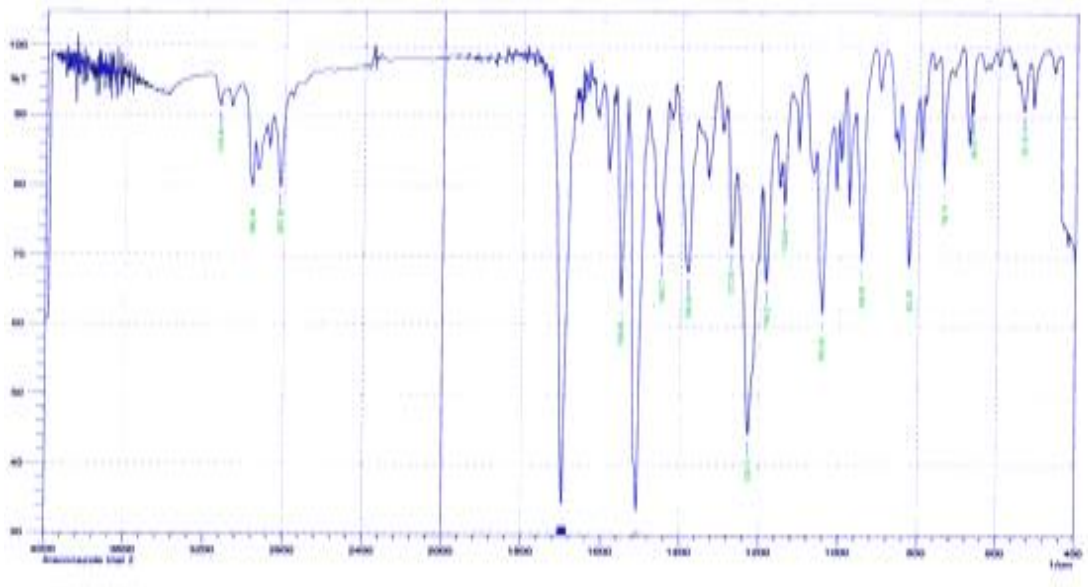

Figure 1A: FTIR spectrum of the pure itraconazole

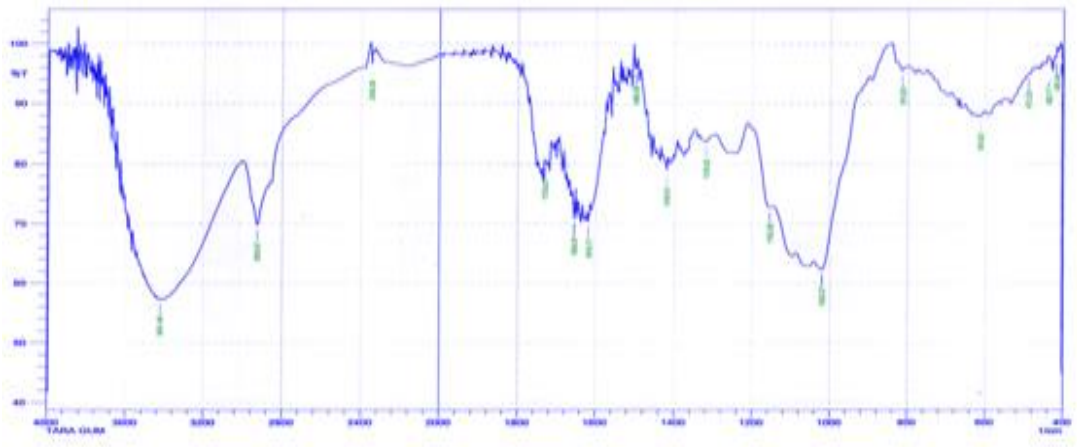

Figure 1B: FTIR spectrum of tara gum

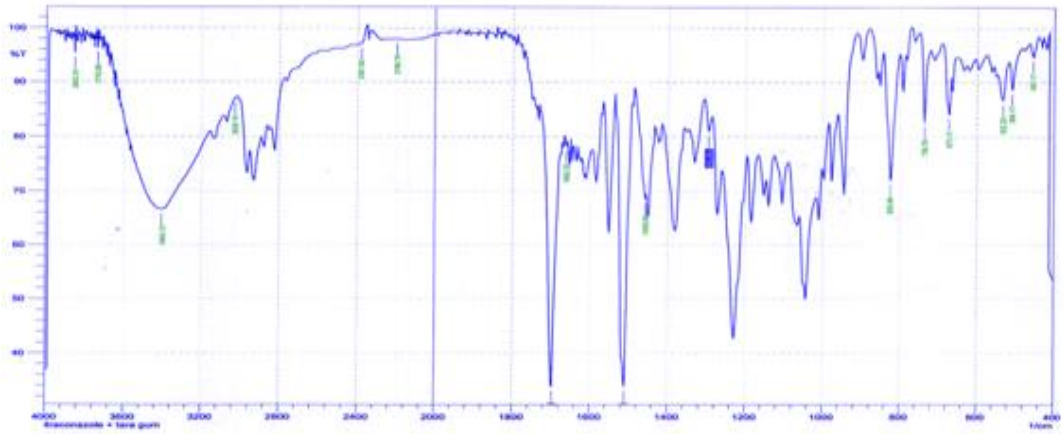

Figure 1C: FTIR spectra of physical mixture of pure drug and tara gum

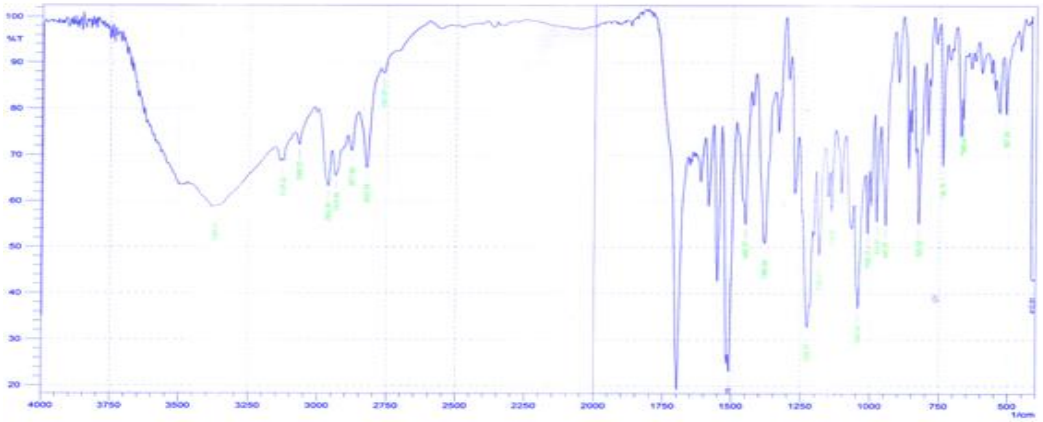

Figure 1D: FTIR spectrum of optimized formulation (ITM1)

Solubility studies

The solubility studies of itraconazole (Table 2 indicated that the drug solubility increase with increase in the concentration of the organic acid, also increase in the solubility was shown with the decrease in the pH of organic acid. Maleic acid having lower pH than other organic acid (citric acid<succinic acid<acetic acid). Thus, the trend of the solubility was shown as (Maleic acid>citric acid>succinic acid>acetic acid), the higher solubility of drug was observed in the medium containing maleic acid 2.5% of 17.98 ± 0.35 mg/ml, the reason could be that, the weakly basic drug (itraconazole) is more soluble in the lower pH while its solubility significantly decreases with an increase in the pH.

The effect of organic acids on the solubility profile of weakly soluble drugs is influenced by many factors connected with the drug used (solubility, molecular weight and pKb), the composition of the dosage form and the physico-chemical features of the incorporated acid such as the chemical structure, molecular weight, solubility and pKa [23].

Table 2: Solubility studies for itraconazole

Medium	Quantity	Solubility (mg/ml)	Medium	Quantity	Solubility (mg/ml)
Water	10 ml	0.00235 ± 0.0001	Propylene glycol (10%) Methanol (24%) Maleic acid (2.5%) Water upto	0.96 ml 3 ml 250 mg 10 g	17.98 ± 0.35
Propylene glycol (10%) Methanol (24%) Water upto	0.96 ml 3 ml 10 g	2.73 ± 0.08	Propylene glycol (10%) Methanol (24%) Succinic acid (1%) Water upto	0.96 ml 3 ml 100 mg 10 g	9.21 ± 0.17
Propylene glycol (10%) Methanol (24%) Citric acid (1%) Water upto	0.96 ml 3 ml 100 mg 10 g	10.47 ± 0.13	Propylene glycol (10%) Methanol (24%) Succinic acid (2.5%) Water upto	0.96 ml 3 ml 250 mg 10 g	12.33 ± 0.5
Propylene glycol (10%) Methanol (24%) Citric acid (2.5%) Water upto	0.96 ml 3 ml 250 mg 10 g	13.86 ± 0.15	Propylene glycol (10%) Methanol (24%) Acetic acid (1%) Water upto	0.96 ml 3 ml 100 mg 10 g	8.23 ± 0.04
Propylene glycol (10%) Methanol (24%) Maleic acid (1%) Water upto	0.96 ml 3 ml 100 mg 10 g	13.64 ± 0.07	Propylene glycol (10%) Methanol (24%) Acetic acid (2.5%) Water upto	0.96 ml 3 ml 250 mg 10 g	10.53 ± 0.6

Note: Values are expressed as mean ± SD, n=3

Physicochemical evaluation of transdermal gels

The formulated transdermal gels were evaluated for physicochemical properties and the results were obtained as shown below.

pH of prepared transdermal gels

The pH was found to be in range from 5.61-6.89 thus indicating suitability for skin application along with good extrudability and spreadability. Among all the formulations PH was less in case of IKM2.5 (5.61) formulated using kondagogu gum as gelling agent and maleic acid as permeation enhancer this may be due to the lower pH of maleic acid than other organic acid used while pH was more in case of ITD2.5 (6.93) formulated using tara gum as gelling agent and DMSO as permeation enhancer. The results in the present study showed a minimal decrease in the pH of gel when fatty acid (oleic acid and stearic acid) used as permeation enhancers this may be due to that the longer-chain fatty acids have minimal effect on the pH of an aqueous solution.

Drug content

The content of drug per 100 mg of gel ranged from 96.16% to 99.73% which indicates that efficient loading and uniform distribution of drug in the formulations.

Homogeneity

All formulated transdermal gel showed good homogeneity without lumps. The physical appearances of all gel formulations were opaque in nature.

Spreadability

The value of spreadability varies from 10.1-12.9 g.cm/s indicating that the gels are easily spreadable by small amount of shear. All gel preparations indicated a good spreadability.

Extrudability

The extrusion of the gel from the tube is important during its application and in patient acceptance. The extrudability of all formulations was found to be good and compatible.

Viscosity

Viscosity is an important parameter for characterizing the gels as it affects the extrudability and release of drug. The viscosity of the formulations ranged between 17600-22900 cps. Among all the formulations IKS 2.5 prepared using kondagogu gum as gelling agent in the concentration of 4% and stearic acid in concentration of 2.5% has shown highest viscosity of 22900 cps, while ITM2.5 prepared using tara gum as gelling agent in the concentration of 2% and maleic acid in concentration of 2.5% has shown lowest viscosity of 17600 cps. The evaluation studies of different gels were performed and were found to have desired physicochemical properties.

***In vitro* diffusion studies**

From the *in vitro* diffusion studies, the control gels of tara gum and kondagogu gum have show 32.72%, 28.48% of drug release for 8 h respectively. The formulation ITC1, ITM1, ITSU1, IKC1, IKM1 and IKSU1 have shown more than more than 90% drug release for 4-7 h with lowest percentage of permeation enhancers. This was dependent on the nature of polymer used, as well as type of permeation enhancers used and their concentrations.

It was found that the organic acids (Maleic, citric, succinic) have more permeation activities than fatty acids (oleic, stearic) and sulfoxide compound (DMSO). The reason may be that organic acid increase the solubility of the weakly basic drug (itraconazole) which is more soluble in the lower pH while its solubility significantly decreases with an increase in the pH [23].

The formulations containing oleic acid (unsaturated fatty acid) as permeation enhancers have more percentage of drug release than the formulations containing stearic acid (saturated fatty acid) as permeation enhancers; this could be due to that the effects of fatty acids as permeation enhancers have been shown to be dependent on their structure, alkyl chain length, and degree of saturation. Unsaturated fatty acids have been shown to promote higher magnitudes of permeation enhancement when compared to saturated fatty acids of the same chain length. (Ibrahim and Kevin, 2010) Incorporation of dimethyl sulfoxide as permeation enhancer enhanced the solubility and release rate. DMSO is known to cause erythema at higher concentrations. So, lesser concentrations were selected for investigating its effect. 1% and 2.5% concentrations were found to enhance permeation of itraconazole from gel formulations [18].

***Ex vivo* permeation studies**

Based on *ex vivo* permeation studies, the amount of drug permeated through the rat abdominal skin was less when compared to dialysis membrane. The *ex vivo* permeation studies on rat abdominal skin showed varied results from the *in vitro* studies because the itraconazole transdermal gel formulations containing fatty acids, organic acid and dimethyl sulfoxide act as permeation enhancers show effect on the surface of lipophilic skin by interacting with skin or by rupturing skin integrity which does not show on dialysis membrane [24].

ITM1, IKC1 and IKM1 have shown highest permeated amount and more than 90% of itraconazole was permeated from the transdermal gel formulations, this is could be due to the improved itraconazole solubility by organic acids and also due to the effect of these acids on the lipid layer of stratum corneum. The lipids in the stratum corneum consist of ceramides, cholesterol and fatty acids, as well as a small quantity of cholesterol sulfate. It has been known that ceramides are the main components responsible for the barrier effect of the stratum corneum [25]. Maleic acid showed highest permeated amount with tara and kondagogu gum this is may be due to that maleic acid had the polar head with the smallest size and that with the lowest H-bonding ability, which further confirmed the hypothesis that decreasing the size or H-bonding ability of the polar heads of ceramide analogs would contribute to their transdermal enhancement activity. Thus, the enhancer molecules could insert themselves between the hydrophobic tails of the ceramide bilayers and weaken the continuity of the lipid barrier which is responsible on the permeation of itraconazole through the skin to systemic circulation [25].

Permeability parameters of optimized gel formulations

From Table 3, it was concluded from the permeability parameters that the maleic acid has more permeation enhancement than other organic acids (citric and succinic). The enhancement of itraconazole permeability was shown in the trend maleic acid>citric acid>succinic acid. This could be due to that itraconazole solubility increase with decrease in the pH of organic acid. Another reason for these results may be that the maleic acid has polar head with the smallest size and that with the lowest H-bonding ability, which further confirmed the hypothesis that decreasing the size or H-bonding ability of the polar heads of ceramide analogs would contribute to their transdermal enhancement activity. Thus, the enhancer molecules could insert themselves between the hydrophobic tails of the ceramide bilayers and weaken the continuity of the lipid barrier which is responsible on the permeation of itraconazole through the skin to systemic circulation [26].

Table 3: Permeability parameters of optimized gel formulations

Formulation	Lag time (h)	Q_8 (µg/cm²)	Flux(µg/cm²/h)	Permeability coefficient (cm/h × 10^{-3})	Enhancement ratio
ITC1	0.45 ± 0.007	2118.120 ± 9.32	200.27 ± 0.99	78.506 ± 0.79	4.519 ± 0.08
ITM1	0.25 ± 0.003	2319.109 ± 5.91	281.12 ± 0.98	110.199 ± 0.98	6.428 ± 0.12
ITSU1	0.54 ± 0.004	1700.680 ± 6.76	188.72 ± 2.23	73.978 ± 0.85	4.315 ± 0.05
IKC1	0.37 ± 0.008	22210.884 ± 4.02	251.88 ± 0.56	98.737 ± 0.65	5.684 ± 0.04
IKM1	0.34 ± 0.001	2257.267 ± 7.84	252.39 ± 3.43	98.936 ± 0.99	5.696 ± 0.02
IKSU1	0.73 ± 0.006	1530.612 ± 8.99	176.08 ± 1.03	69.023 ± 0.45	3.973 ± 0.07

Note: Values are expressed as mean ± SD, n=3

Model dependent kinetics of optimized itraconazole gel formulations

From the results of *ex vivo* drug release kinetics for optimized gel formulations Table 4, it was found that all formulations follow zero order drug release kinetics except ITC1 which follow first order drug release kinetics; the drug release mechanism for ITC1, ITM1 and IKM1 was found to be follow Higuchi drug release mechanism while ITSU1, IKC1 and IKSU1 was found to be follow Kosmeyer-Peppas drug release mechanism. From the values of release component "n" it can be concluded that all formulations have anomalous diffusion release mechanism.

Table 4: *Ex vivo* drug release kinetics of optimized formulations

Formulation code	r^2				n	Drug transport mechanism
	Zero	First	Higuchi	Peppas		
ITC1	0.9533	0.9755	0.9825	0.9772	0.748	Anamolous transport
ITM1	0.9628	0.9555	0.9895	0.9882	0.970	Anamolous transport
ITSU1	0.9787	0. 9759	0.9602	0.9741	0.972	Anamolous transport
IKC1	0.9529	0.9453	0.9793	0.9815	0.936	Anamolous transport
IKM1	0.9646	0.9505	0.9892	0.9613	0.985	Anamolous transport
IKSU1	0.9944	0.9784	0.9826	0.9969	0.918	Anamolous transport

Skin irritation studies

The formulation ITM1, tara gum placebo gel and kondagogu gum placebo gel showed irritation potential of "0", thus proving to be non-irritant. The "0" value in an irritancy test indicates that the applied formulations are generally non-irritant to the human skin. No obvious erythema and edema was observed after 72 h of the application of the formulations.

Stability studies

The stability studies were conducted for one month at room temperature and the formulation ITM1 was found to be stable, with insignificant change in the appearance, drug content, viscosity and pH.

CONCLUSION

Itraconazole is a potent triazole antifungal agent used in the treatment of systemic, superficial fungal infections and used in the treatment of basal cell carcinoma. This study attempts to demonstrate the influence of various permeation enhancers at 1% and 2.5% concentrations of fatty acids (oleic acid and stearic acid), organic acids (citric acid, acetic acid, maleic acid and succinic acid), and sulfoxides (DMSO) on the percutaneous permeation of itraconazole transdermal gel. *In vitro* diffusion studies of the prepared formulations were performed to determine drug release rate from transdermal gel. It was concluded that ITC1, ITM1, ITSU1, IKC1, IKM1 and IKSU1 have shown more than 90% drug release for 4-7 h, in comparison to control gels (ITCON and IKCON) which have shown 32.72%, 28.48% of drug release for 8 h respectively. *Ex vivo* permeation studies revealed that the ITM1 has shown better release of itraconazole in 8 h with Q8 of 2319.109 ± 5.91 µg/cm^2/h; permeability coefficient of 110.199 ± 0.98 cm/h × 10^{-3} and enhancement ratio of 6.428 ± 0.12 in comparison to control gel (ITCON which has shown Q8 of 401.979 ± 3.54 µg/cm^2 and flux of 43.735 µg/cm^2/h. Skin irritation studies proved that the ITM1 formulation was non-irritant. ITM1 formulation was found to be stable for one month at room temperature. It can be concluded that the ITM1 formulation using maleic acid (1%) as permeation enhancers can provide good permeation of itraconazole for the treatment of basal cell carcinoma.

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