FORMULATION AND CHARECTERIZATION OF ITRACONAZOLE ETHOSOMAL GEL FOR TOPICAL APPLICATION

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ABSTRACT

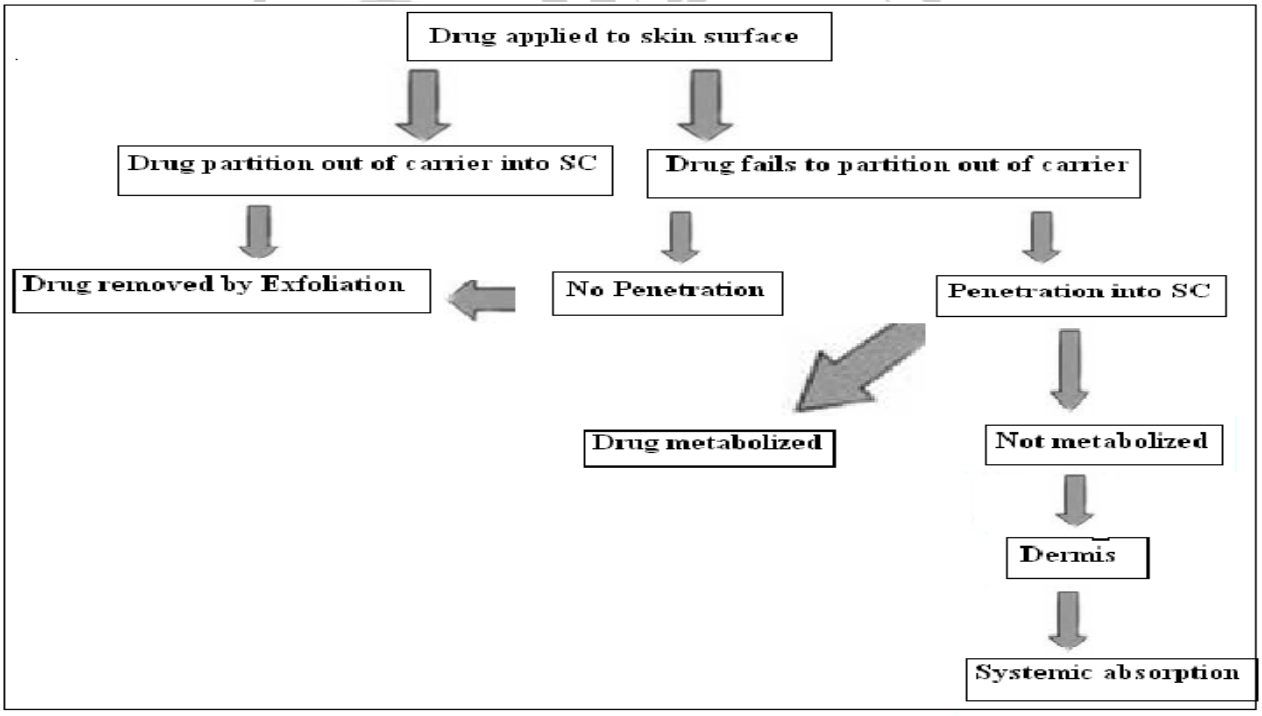
The aim of this research work involves the design and characterization of the drug being treated Itraconazole which is a Antifungal, Antiprotozoals, Antifungal Agents, Antiprotozoal Agents as a novel vesicular carrier system (transdermal drug delivery system) in the form of Ethosomes. These Ethosomes have been planned in order to overcome all the lacunae and various problems being confronted by the patients treated by Itraconazole namely half-life even though being a highly potent drug and also possessing very poor bioavailability (20-40%).The method of preparation of Itraconazole Ethosomes involves the use of various concentrations of Ethanol, phospholipids and polymers by the cold method (Sonication) which facilitates the suitable size reduction of vesicles. The designed Itraconazole Ethosomes have been characterized and validated by the parameters/techniques namely Visualization, Vesicle size and Zeta potential studies, Scanning Electron microscopy, Entrapment efficiency, Assay, Vesicle stability study, Solubility measurement, Penetration & Permeation studies and drug stability studies. Various Ethosomal formulations were prepared of different compositions namely IF1, IF2, IF3, IF4, IF5, IF6, IF7, IF8, and IF9. Among these the best formulation based on in-vitro drug release studies by dialysis membrane revealed that IF9 was adjudged the best from among the sonicated Ethosomes. However ethosomes prepared by sonication method were more uniform and small in size which is essential for skin penetration. While comparing the entrapment efficiency, ethosomes containing 30% w/w methanol and prepared by sonication showed highest value respect to all other formulation; so it is concluded ethosomal prepared by sonication and containing 30 % w/w methanol as the best formulation considering all other aspects. The highest value of transdermal flux for sonicated ethosomes containing 30% w/w methanol is the indication of complete and rapid penetration through the skin may be because of tiny vesicular size. This is an encouraging observation for drugs which are poorly absorbed from skin. When effect of sonication was compared on ethosomal formulation, IF8 formulation possessed better or suitable characteristics (smaller size, uniform size, distribution, highest entrapment efficiency).

Key words: Ethosomes, Itraconazole, Sonication.

No: of References: 8

INTRODUCTION

Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery[1]. Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action. Improved methods of drug delivery for biopharmaceuticals are important for two reasons; these drugs represent rapidly growing portion of new therapeutics, and are most often given by injection. Discovery of new medicinal agents and related innovation in drug delivery system have not been only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs. Throughout the past two decades, the transdermal patches has become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin. Since the first transdermal patch was approved in 1981 to prevent nausea and vomiting associated with motion sickness, the FDA has approved through the past 22 years more than 35 transdermal patch products spanning 13 molecules[2].



Proposed mechanism of drug absorption through skin

Structure of ethosomes

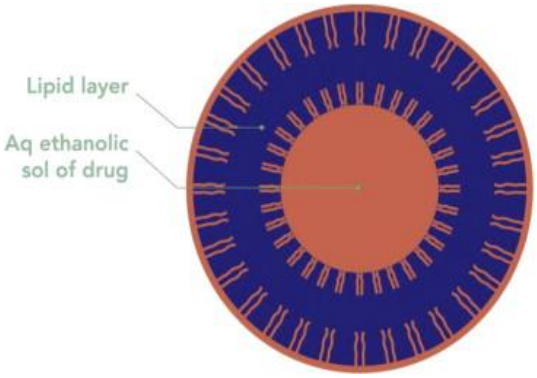


Figure 3: Structure of ethosomes

Mechanism of penetration

Although the exact process of drug delivery by ethosomes remains a matter of speculation, most likely, a combination of processes contributes to the enhancing effect. The stratum corneum lipid multilayer at physiological temperature are densely packed and highly conformationally ordered. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for it's disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicles have the ability to penetrate the stratum corneum. Also because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure, giving it more freedom and ability to squeeze through small places such as the openings created in disturbing the stratum corneum lipid[7].Ethanol interacts with lipid molecules in the polar hard group region, resulting in a reducing the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effect of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier[4].

Preparation: Formulation and preparation of ethosomes is reported by Touitou4 et al., according to which ethosomal system can be prepared from soyabeen phosphotidyl choline 2 – 5 % (Phospholipon 90), 20 – 50 % w/w ethanol, drug and water to 100% w/w. For preparation of ethosomes Phospholipon 90 and drug were dissolved in ethanol. Double distilled water was added slowly as a fine stream with constant mixing at 700 rpm in a well sealed container. Mixing was continued for additional 5 minutes. The system was kept

at 30 °C throughout the preparation and then stored in cool place.

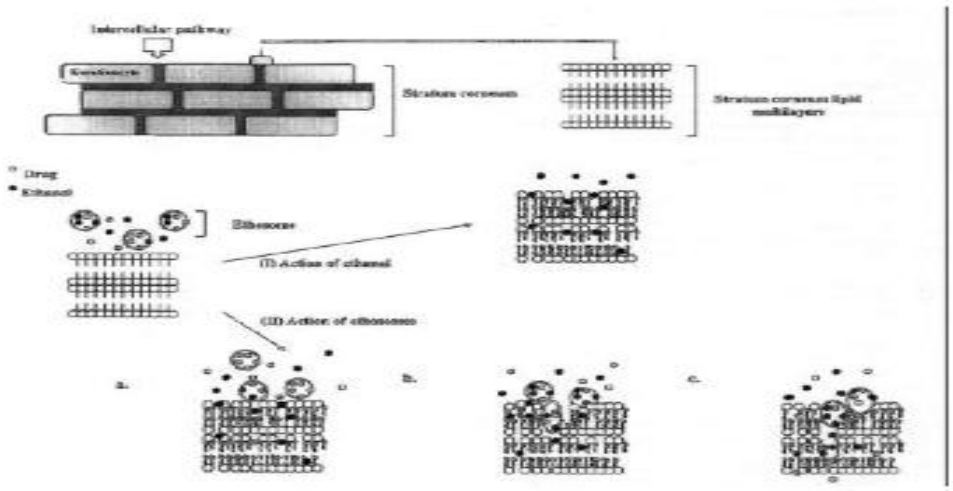


Figure 1.3: Proposed model for skin delivery ethosomal systems

MATERIALS AND METHODS:

MATERIALS: Itraconazole was obtained as a gift sample from Granules India ltd. Soya lecithin, Propylene glycol, Methanol, Cholesterol, Carbopol-934, Triethanol amine, Ultrapure water were obtained from Research lab fine chem. Industries(Mumbai). All the ingredients used were of analytical grade.

Composition of different ethosomal formulations

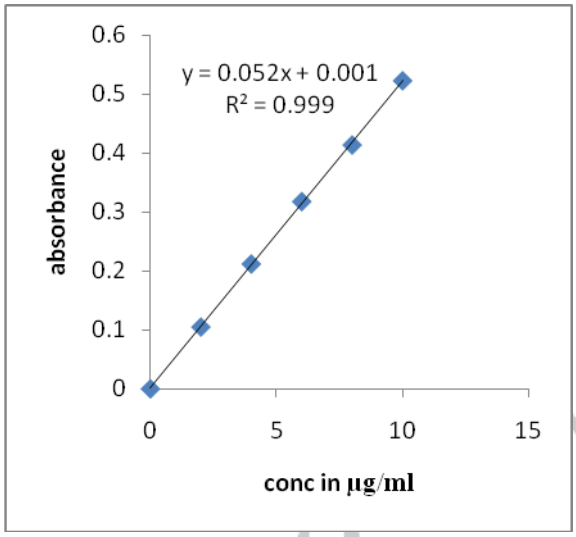
Ethosomal formulation	Lecithin (Soya lecithin%)	Methanol (%)	Propylene glycol (%)	Drug (g)	Cholesterol(g)	Water
IF_1	2	10	10	0.20	0.005	q.s
IF_2	2.5	10	10	0.20	0.005	q.s
IF_3	3	10	10	0.20	0.005	q.s
IF_4	2	20	10	0.20	0.005	q.s
IF_5	2.5	20	10	0.20	0.005	q.s
IF_6	3	20	10	0.20	0.005	q.s
IF_7	2	30	10	0.20	0.005	q.s
IF_8	2.5	30	10	0.20	0.005	q.s
IF_9	3	30	10	0.20	0.005	q.s

The interdigitated, malleable ethosome vesicle can forge paths in the disordered stratum corneum. In the case of ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles.

While encapsulated drug in classic liposomes remained primarily at the surface of the skin the ethosomal system was showed to be highly efficient carrier for enhanced drug delivery through the skin. The efficient drug delivery shown together with the long-term stability of ethosomes makes this system a promising candidate for transdermal delivery of drug.

RESULT: CALIBRATION CURVE IN pH 6.8 PHOSPHATE BUFFER

STANDARD CALIBRATION CURVE



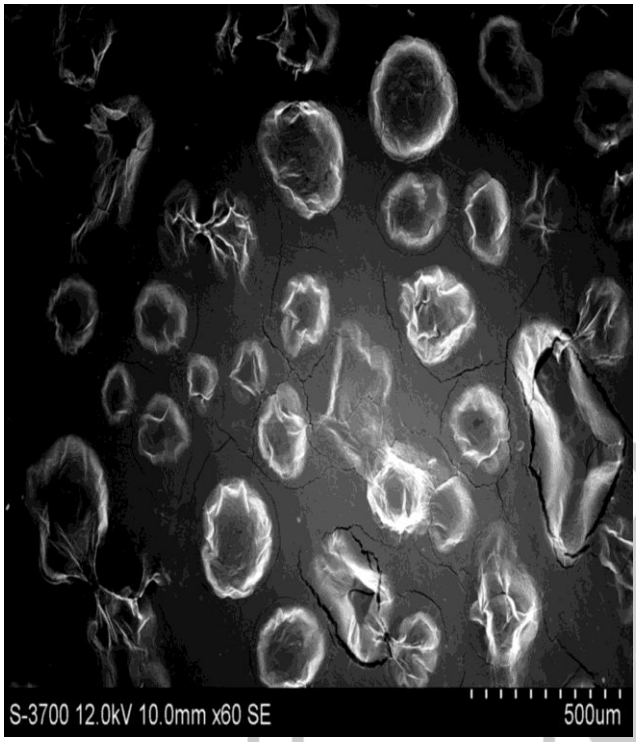
S.NO	Concentration µg/ml	Absorbance
1	0	0
2	2	0.105
3	4	0.212
4	6	0.318
5	8	0.414
6	10	0.523

PREPARTION OF ITRACONAZOLE ETHOSOMES

Ethosomal formulations composed of phospholipid, drug and ethanol were prepared using the method detailed in last chapter materials and methods and also according to literature with little modification. Ethosomal suspension obtained with sonication were slight yellowish in colour and hazy in appearance. Different characteristics of ethosomes and the effect of sonication were further evaluated and results are reported under the characterization.

CHARACTERIZATION OF ETHOSOMES:

SCANNING ELECTRON MICROSCOPE (SEM):



ENTRAPMENT EFFICIENCY:

Formulation code	Entrapment efficiency(%)
IF1	81.2
IF2	85.6
IF3	77.5
IF4	75.2
IF5	85.1
IF6	72.5
IF7	70.6
IF8	87.8
IF9	71.5
IF10	70.3

EVALUATION OF ETHOSOMAL GEL:

Organoleptic characteristics of ethosomal gel

Organoleptic Characteristics:	Color: golden yellow Greasiness: Non greasy Grittiness: Free from grittiness Ease of application: Easily/smoothly applied Skin irritation: No skin irritation
Washability:	Easily washable without leaving any residue on the surface of the skin.

PH measurements of Ethosomal gel:

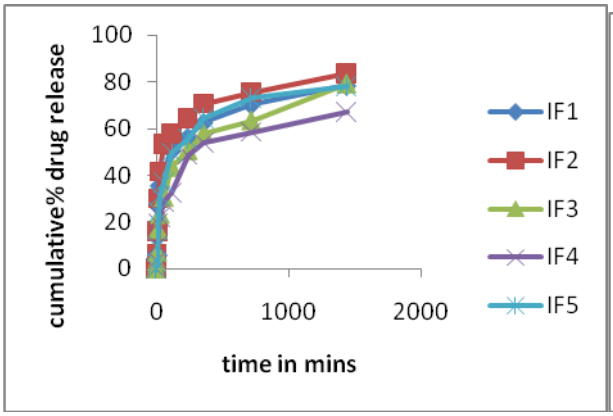
Formulation code	pH
IF1	6.92
IF2	6.7
IF3	6.88
IF4	6.95
IF5	6.89
IF6	6.83
IF7	6.97
IF8	6.91
IF9	6.89
IF10	6.88

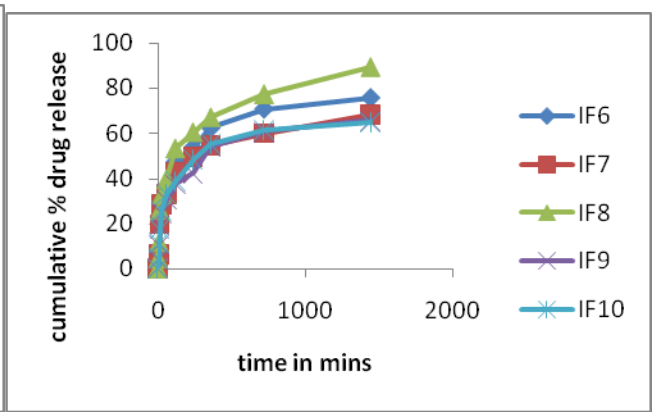
Drug content and content uniformity:

Formulation code	Drug content (%)
IF1	98.6
IF2	99.3
IF3	98.6
IF4	98.3
IF5	95.3
IF6	95.6
IF7	99.7
IF8	99.3
IF9	98.9
IF10	95.4

IN-VITRO RELEASE STUDIES:

Time (min)	IF1	IF2	IF3	IF4	IF5	IF6	IF7	IF8	IF9	IF10
5	5.42	6.26	3.25	3.6	2.31	1.46	0.75	4.57	4.20	4.59
10	13.5	15.95	7.2	10.08	9.42	8.4	6.26	11.8	10.6	11.3
15	27.15	30	16.94	19.2	24.4	22.97	19.68	26.04	19.3	17.3
30	35.68	41.68	23.42	22.04	31.24	30.01	28.48	33.02	24.7	25.7
60	41.68	53.3	30.6	28.7	37.02	35.68	33.02	39.6	30.5	32.9
120	50.5	57.7	43.5	32.4	49.7	47.1	42.6	53.3	37.49	38.6
240	56.22	64.4	50.68	48.6	55.5	53.5	49.7	60.44	42.36	48.2
360	62.5	70.6	57.9	53.7	64.4	62.22	54.77	67.1	53.83	54.8
720	70.2	75.6	63.4	58.5	73.3	70.6	60.1	77.3	60.39	61.36
1440	78.4	83.7	79.2	67.11	78.2	75.5	68.44	89.3	65.75	64.7

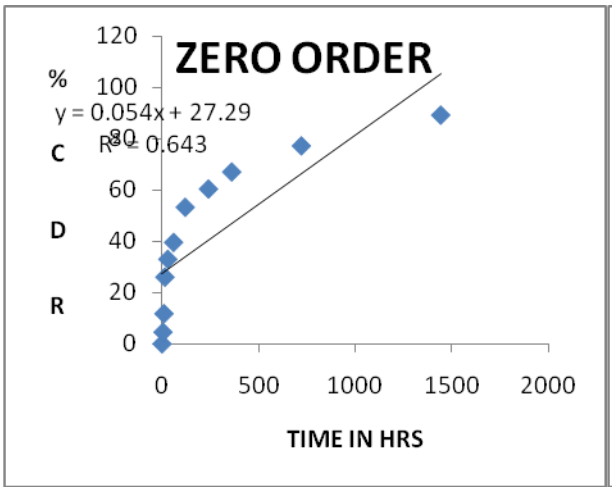


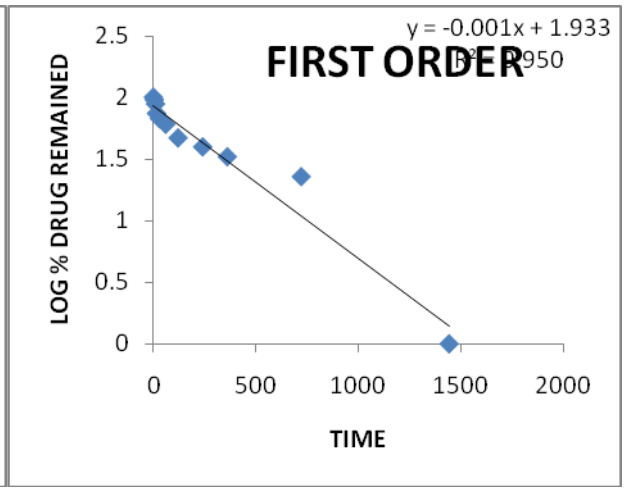


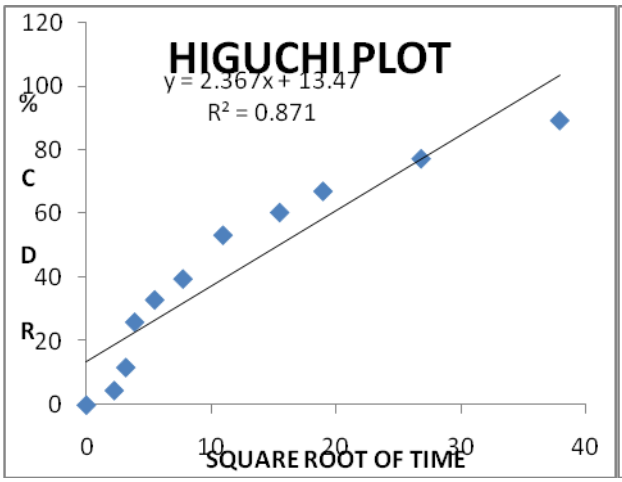
PHARMACOKINETIC PROFILES FOR IF8 ETHOSOMAL GEL:

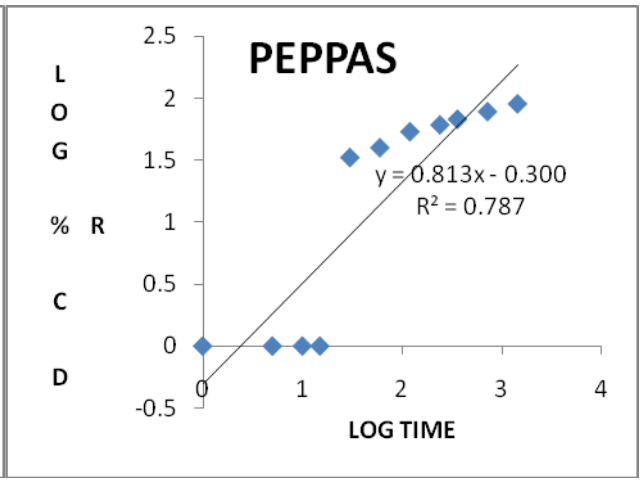
Release kinetics for optimized formulation

	ZERO	FIRST	HIGUCHI	PEPPAS
	% CDR Vs T	Log % Remain Vs T	%CDR Vs √T	Log C Vs Log T
Slope	0.054071196	-0.00124158	2.367889828	0.813927961
Intercept	27.29603757	1.933243954	13.47850215	-0.30054412
Correlation	0.802092707	-0.97509645	0.94532271	0.887456923
R 2	0.643352711	0.950813088	0.893635026	0.787579791









% Entrapment efficiency and % Drug content after stability studies

Number of Days	% Entrapment Efficiency at temperatures			% Drug Content at temperatures		
	4±2°C	25±2°C	37±2°C	4±2°C	25±2°C	37±2°C
15	87.7	87.63	87.59	99.3	99.19	99.81
30	87.6	86.82	86.76	98.16	98.94	98.77
45	87.27	86.67	86.56	97.23	98.48	98.58
90	86.93	86.40	85.84	97.45	98.39	98.06

Conclusion

It is well known that if drug molecules presenting any difficulties in its solubility and bioavailability along the GI tract, are candidates for other routes of administration and if the site of action for drug candidate is subdermal, an effective penetration enhancers are required to provide the drug molecule deeper into skin tissue for optimized therapeutic delivery of drug. It is generally agreed that classic liposomes are of little or no value as carriers for transdermal drug delivery because they do not penetrate the skin. Recently derived ethosomal system can deliver drug molecules into and through the skin. An attempt was made to formulate the highly efficient ethosomal drug delivery system using Itraconazole as model drug.When effect of sonication was compared on ethosomal formulation, IF8 formulation possessed better or suitable characteristics (smaller size, uniform size, distribution, highest entrapment efficiency). From the above observations it can be concluded sonicatation is an essential tool for the preparation of ethosomes. Thus, the specific objectives listed in the introduction

chapter of this thesis were achieved namely design, characterization and release studies of Itraconazole ethosomes. Certainly these finding can be applied for transdermal drug delivery of Itraconazole for treatment of fungal disorders. Further these findings may help the industry for development and scaling up a new formulation.

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