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Design, development and evaluation of Itraconazole loaded transfersomal gel

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ABSTRACT

The main objective of the present study was to enhance a transdermal penetration of a water-insoluble anti-fungal drug, Itraconazole, by encapsulating it into a transfersomal gel. The transfersomal gel of itraconazole was prepared firstly by preparing transfersomes by modified hand shaking method using surfactants (Tween 80 and Span 80), Lecithin in various concentrations and chloroform and methanol added in 3:1 ratio in each formulation and evaluated for their vesicle shape and size, entrapment efficiency, % drug content and %entrapment efficiency and in vitro permeation studies. Later carbopol gels such as carbopol 940 along with distilled water was used in the aqueous dispersion of transfersomes to prepare topical transfersomal gel. The characterization was carried out and in vitro permeation studies carried out. The results were obtained which showed that transfersomal gel was a promising candidate for transdermal delivery with targeted and prolonged release of a drug. It also enhances skin permeation of many drugs.

Keywords: Transfersomal gel, Entrapment efficiency, Transdermal delivery.

INTRODUCTION

Transdermal delivery

Transdermal delivery of drugs is a convenient route of administration for a variety of clinical indications. Transdermal delivery has many advantages over the conventional drug delivery [1, 2, 3]. Transdermal delivery faces significant barrier across the skin, associated mainly with outermost stratum corneum layer of epidermis which limits it to fewer drugs [4, 5]. The skin structure consists of stratum corneum cells are embedded within intercellular lipid lamellae [6]. These lamellae are also responsible for imparting barrier properties to the stratum corneum [7, 8]. As a result, very less quantity (mg) of drug can be delivered by this route. This limits its application to only potent drugs. Work is going on to overcome this limitation. These include augmenting skin permeability using penetration enhancers, using iontophoresis, electroporation, phonophoresis, microneedles, jet injectors, etc., (forces which are independent of concentration gradient) and many more. Transfersomes or vesicles belong to the latter category.

Advantages of TDDS [9]

Transdermal drug delivery offers several advantages over conventional dosage forms, which includes

- The steady permeation of drug increases consistent serum drug levels across the skin.
- Transdermal drug delivery is less invasive than intravenous infusion which also results in consistent plasma level.
- It reduces risk of side effects due to lack of peaks in plasma concentrations. Thus, for transdermal

drug delivery, drugs that require relatively consistent plasma levels are preferable.

- Toxicity is limited to a certain area and can be prevented by removing the patch.
- Transdermal drug delivery is very convenient, especially with patches that require application once in a week. Such simple dosing regimen can aid in patient adherence to drug therapy.
- It is very useful for patients who cannot tolerate oral dosage form.
- It does not result in nausea or unconscious.
- Transdermal delivery avoids direct effects on the stomach and intestine and thus can be a route for drugs causing gastrointestinal upset.
- It can target the gastrointestinal system drug that degrades by the acid and enzymes present in it.
- Transdermal administration avoids first pass metabolism.
- It allows continuation of drug administration with short biological half-life.

Disadvantages of TDDS [9]

- One of the greatest disadvantages to transdermal drug delivery is the possibility of local irritation that may develop at the site of application.
- In patch formulation, the drug, the adhesive, or other excipients, can cause erythema, itching and local edema. For most patients, changing site can minimize irritation.
- The skin's low permeability limits its use for fewer drugs.
- Many drugs with a hydrophilic structure permeate the skin too slowly resulting in no therapeutic benefit.

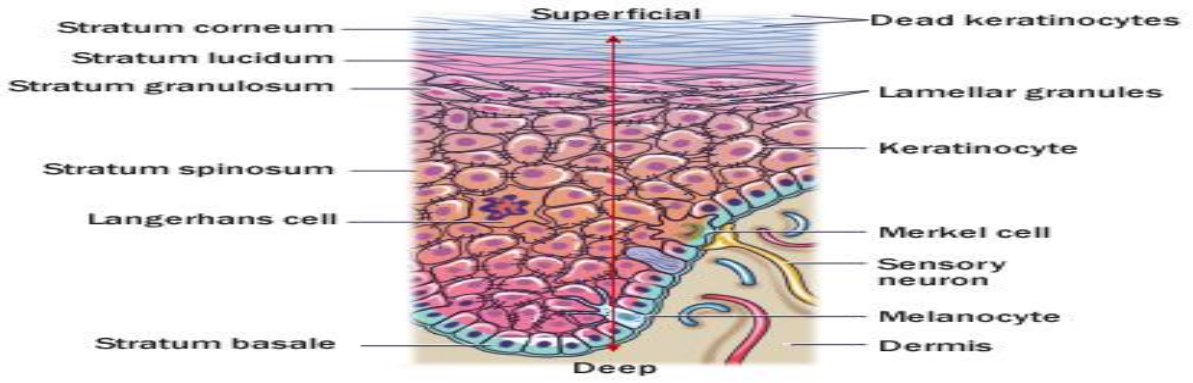

Figure 1: Epidermis

Transfersomes

Transfersomes are flexible or deformable vesicles and hence also called as elastic vesicles. Gregor Cevc in 1991 introduced the concept and term of elastic vesicles. Since then, extensive work is going on worldwide on these elastic vesicles under different titles like flexible vesicles, ethosomes', etc. Transfersome is a term '/registered as a trademark by a German company IDEA AG, and uses it to refer its proprietary drug delivery technology. Transfersome is derived from the Latin word 'transferre', meaning 'to carry across', and the Greek word 'soma', meaning 'body'. A transfersome carrier is an artificial vesicle that resembles the natural cell vesicle. Thus it is suitable for both targeted and controlled drug delivery. Functionally, it may be described as lipid droplet with such deformability that permits its easy penetration through the pores much smaller than the droplet size. Transfersome is a highly adaptable and stress-responsive complex aggregate. On topical application, the carrier search and exploits hydrophilic pathways i.e. 'pores' in the skin, which it opens wide enough to permit it to pass through with its drug cargo, deforming itself to accomplish this without losing its vesicular integrity. The vesicle is both self-regulating and self-optimizing due to its interdependency on local composition and shape of the bilayer. This allows the transfersome to cross different transport barriers efficiently. Transfersome penetrates the stratum corneum either via intracellular route or the transcellular route [24].

Transfersomes takes advantage of phospholipid vesicles as transdermal carrier. These self-optimized aggregates deliver the drugs reproducibly and efficiently into/or through the skin, due to their ultra flexible membrane. The drug delivery depends upon the choice of administration or application. The vesicular transfersomes are more elastic than the standard liposomes and thus well suited for skin penetration. Transfersomes squeezes itself along the intracellular lipid sealing of stratum corneum overcoming the skin penetration difficulty as shown in fig 1.4. Flexibility of transfersomes membrane is achieved by mixing suitable surface-active components in the proper ratios [25].

EXPERIMENTAL WORK

Preformulation studies

Preformulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of the new drug substance, to check for its stability, safety and effective dosage form. Ideally, the Preformulation phase begins early in the discovery process such as the appropriate physical and chemical data is available to aid the selection of new chemical entities that enters the development process during this evaluation possible interaction with various inert ingredients intended for use in final dosage form are also considered in the present study.

Organoleptic properties

The color, odor and taste of the drug were recorded using descriptive terminology.

Solubility

The solubility of the drug sample was carried out in different solvents (aqueous and organic) according to I.P. The results are then compared with those mentioned in the official books and Indian Pharmacopoeia.

Melting point

The melting point of Itraconazole was determined by capillary method using digital melting point apparatus.

Analytical methods

Standard curve

Preparation of standard solution

Stock solution-I

100mg of itraconazole was accurately weighed into 100ml volumetric flask and dissolved in small quantity of buffer. The volume was made with 6.8 pH Phosphate buffer to get a concentration of 1000µg/ml (SS-I).

UV Absorption Maxima (λ_{max}) of Itraconazole sample in pH 6.8 Phosphate buffer

Stock II

10ml of above solution was then further diluted to 100ml with 6.8 pH Phosphate buffer to get a stock solution of 100µg/ml. UV scanning was done for 100 µg/ml drug solution from 200-400 nm using pH 6.8 Phosphate buffer as a blank in Shimadzu, UV 2450 spectrophotometer. The wavelength maximum was found to be at 262 nm.

Preparation of working standard solutions:

Further, from SS-II aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipetted out into 10ml volumetric flasks. The volume was made up with 6.8 pH Phosphate buffer to get the final concentrations of 2, 4, 6, 8, and 10µg/ml respectively. The absorbance of each concentration was measured at 262nm.

Calibration curve for the estimation of Itraconazole

Calibration curve of Itraconazole was estimated in 6.8 pH buffer.

Compatibility studies

IR spectroscopy can investigate and predict any physicochemical interactions between different components in a formulation and therefore it can be applied in selection of suitable chemically compatible excipients. The aim of the present study was to test whether there are any interactions between the carriers and the drug. One part of the sample and three parts of potassium bromide were taken in a mortar and triturated. A small amount of triturated sample was taken into a pellet maker and compressed at 10kg/cm^2 using hydraulic press. The pellet was kept on the sample holder and scanned from 4000cm^{-1} to 400cm^{-1} in Bruker IR spectrophotometer. Then it was compared with the original spectra.

IR spectrum was compared and checked for any shifting in functional peaks and non-involvement of functional groups. From the spectra it is clear that

there is no interaction between the selected carriers, drug and mixtures. Hence the selected carrier was found to be compatible in entrapping the Itraconazole with carriers without any mutual interactions.

Formulation of transferosome gel

- Preparation of transfersomes containing Itraconazole
- Preparation of topical transfersomal gel.

Preparation of Transfersomes by Modified Hand shaking lipid film hydration technique

Six transfersome formulations were prepared by thin film hydration method using Itraconazole, Soya lecithin and different concentrations of surfactants (Span-80, Tween80). The amount of drug is kept constant (80mg) in all the formulations. Different formulations were prepared by using different ratios of phospholipid and surfactants. This was tabulated with amount used. Lecithin, surfactants and the drug are dissolved in 5ml of organic solvent (Chloroform: Methanol; 3:1). The organic solvent is then removed by evaporation with hand shaking above lipid transition temperature (43^0C). Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the phosphate buffer (pH 6.8) by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated using sonicator for 30 minutes.

Table 1: Quantity of substances taken for preparation of transfersomes

Formulation	Itraconazole (mg)	Lecithin (mg)	Tween 80 (mg)	Span 80 (mg)
T1	80	95	5	--
T2	80	90	10	--
T3	80	85	15	--
T4	80	95	--	5
T5	80	90	--	10
T6	80	85	--	15

In each formulation, 5ml of chloroform and methanol were added separately in 3:1 ratio.

Preparation of topical transfersomal gel

Carbopol gels were prepared to be used as vehicle for incorporation of transfersomes for topical delivery. Aqueous dispersion of transfersomes was utilized for the topical gel formulation. Gel polymer such as carbopol 940 was utilized to prepare transfersomal gel. 2g powder of carbopol 940 was dispersed into vigorously stirring (stirred by magnetic stirrer Remi 5MLH) distilled water (taking care to avoid dispersible lumps formation) and allowed to hydrate for 24 hrs. The dispersion was neutralized with tri ethanolamine to adjust the pH [6.8] by using pH meter (Lab India Sab 5000).

Melting Point

This test was performed and the result was illustrated in the following table.

Table 2: Table showing the melting point of API's

Material	Melting Point
Itraconazole	166^0C

RESULT

The Result was found to be within the limit.

PREFORMULATION STUDIES

Standard calibration curve

In the pre-formulation studies, the λ max of Itraconazole by spectrophotometric method in phosphate buffer pH 6.8 was found to be 262nm.

Table 3: Calibration Curve of Itraconazole in Phosphate Buffer pH6.8

S. no.	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.12
3	4	0.26
4	6	0.38
5	8	0.44
6	10	0.57

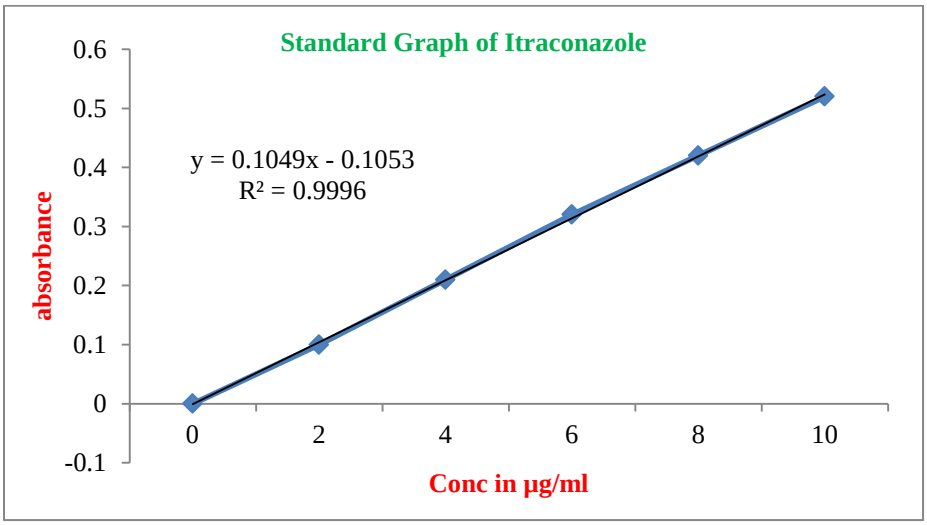

Fig 2: Standard Graph of Itraconazole in Phosphate Buffer pH6.8

Drug excipients compatibility study

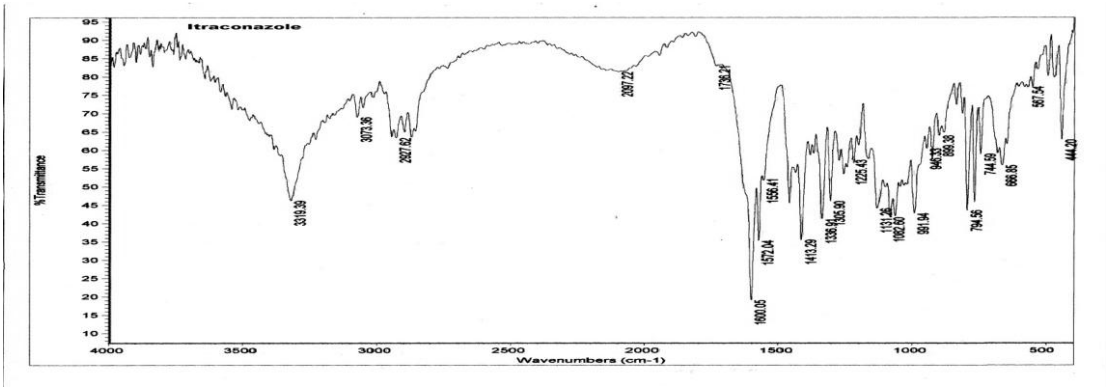

Fig 3: FTIR spectra of Itraconazole

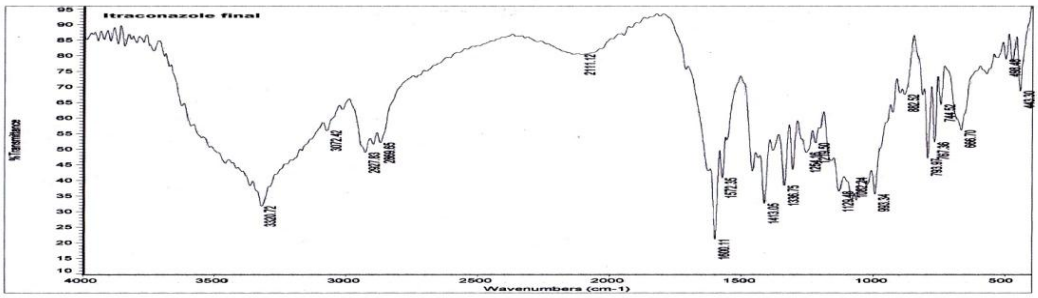

Fig 4: FTIR spectra of Itraconazole optimizedformulation

Evaluation of transfersomal gel

Vesicle shape and type

The surface morphology was studied by Optical Microscopy. A drop of transfersome suspension was placed over the slide and Photomicrograph was taken at 10x resolution. The shapes of most of the containing Itraconazole transfersomes were found to be spherical from SEM analysis, as shown in figures.

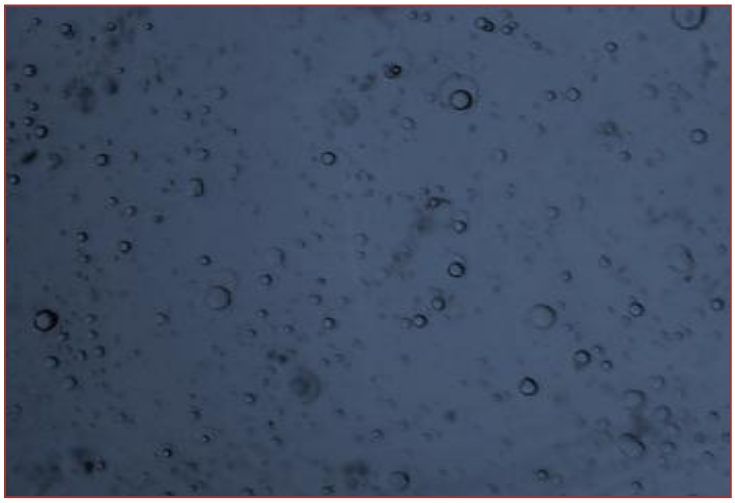

Fig 5: Photomicrograph of Itraconazole loaded transfersome (ET6) at 10X

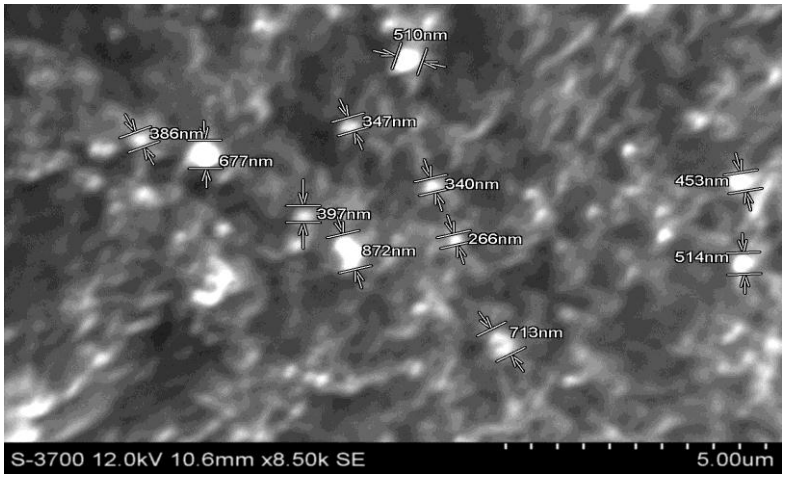

Fig 6: SEM analysis of optimized formulation (T6)

% Entrapment efficiency

The amount of Itraconazole entrapped in transfersome gel was estimated by centrifugation. 1gm of Transfersome gel was taken and diluted with 10ml phosphate buffer (pH 7.4). Later it was sonicated using bath sonicator for 20 minutes. The solution was then placed in a centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted 20 times before taking its absorbance using UV Spectrophotometer at 262nm. This gives us the total amount of unstrained drug. Entrapment efficiency is expressed as the percent of drug trapped. The % entrapment efficiency of deformable vesicles formulations were found to be in the range of 83.15 to 88.33 (Table 1.8). Entrapment efficiency of the T6 formulation was high (maximum 88.33).

% Drug content

1gm of a transfersome gel formulation was taken and the vesicles were lysed with 25 ml of Ethanol by sonication for 15 min. Later this solution was placed in a centrifugation tube and centrifuged at 14000 rpm for 30 minutes. The clear solution was diluted to 100 ml with methanol. Then 10 ml of the solution was diluted to 100 ml with phosphate buffer pH7.4.

Aliquots were withdrawn and drug content was calculated for Itraconazole by using UV spectrophotometer at 262 nm. % drug content of transfersome formulations (T1to T6) were determined. The results obtained shows 87.78 – 93.09% drug content in the formulations. The results obtained are shown in table.

Table 4: % Drug entrapped and % Drug content in transfersomes

Formulations	% Entrapment Efficiency	% Drug content
T1	83.15	87.78
T2	86.56	92.70
T3	83.75	90.65
T4	86.09	90.14
T5	87.54	92.74
T6	88.33	93.09

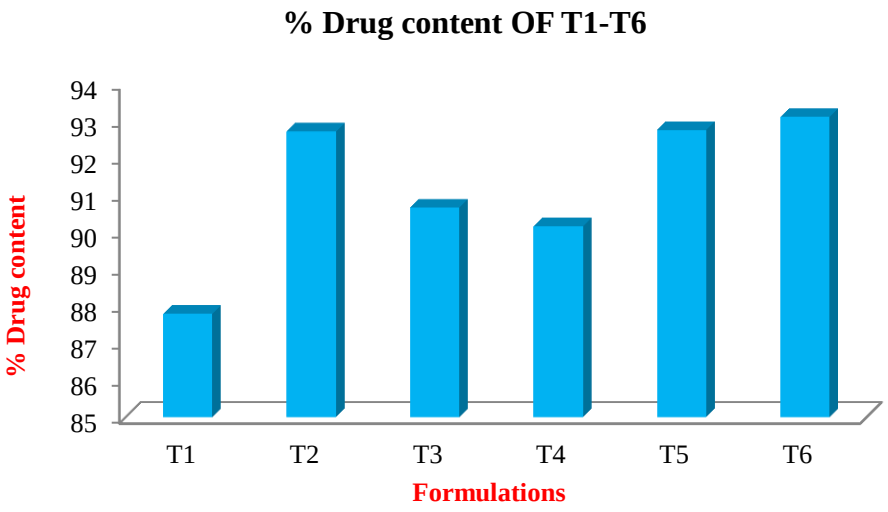

Figure 7: % Drug content of different formulations

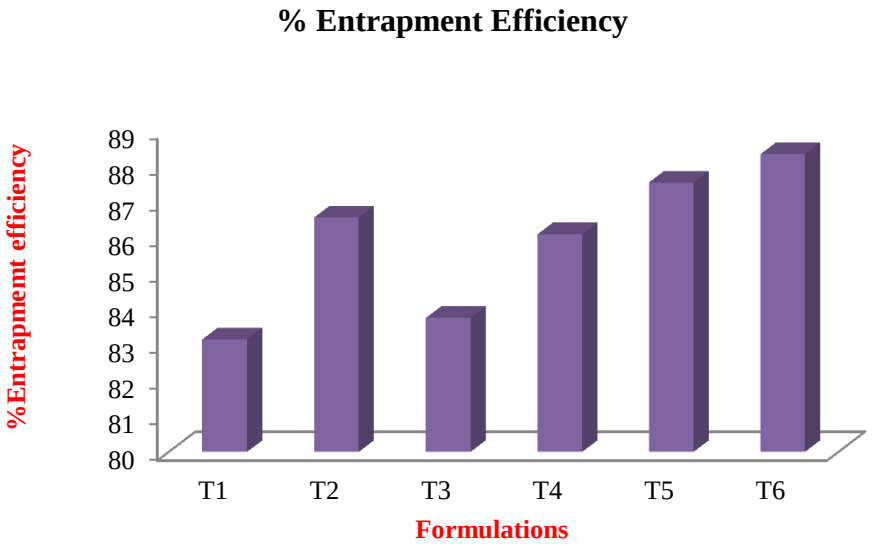

Figure 8: Entrapment efficiency of different formulation

pH value of topical transfersome gel

The value of pH of topical transfersome gels was measured by using digital pH meter (Lab India Sab 5000 pH meter) at the room temperature. The pH of all topical transfersomal gels were found to be in the range of 6.2±0.02 to 6.2±0.08.

In-vitro drug release study

The *in-vitro* diffusion study in phosphate buffer pH 6.8 was carried out using Franz diffusion cell according to procedure explained. For drug release studies, 2 ml of drug loaded Transfersomal gel/ simple gel were put into donor compartment. Dialysis membrane was mounted between donor and receptor compartment of the Franz diffusion cell with an effective diffusion area of 2.26 cm2 and a cell volume of 25 ml. Initially the donor compartment was empty and the receptor compartment was filled with methanolic phosphate buffer saline (methanolic PBS), pH 7.4 (30:70%, v/v). The diffusion cell was maintained at 37°C and receiver fluid was stirred continuously using a magnetic stirrer at a speed of 100 rpm. The formulation was gently placed in the donor compartment. The samples were withdrawn periodically for 24 hours. The samples were then analyzed spectrophotometrically at 255 nm for presence of the drug [21]. Each preparation was studied three times and the result of each preparation is the average value of three experiments. The results are shown in table 1.9.

Table 5: *In-Vitro* drug release of transfersome gel (T1to T6)

Time(hr)	% Drug Release					
	T1	T2	T3	T4	T5	T6
0	0	0	0	0	0	0
1	20.15	22.5	24.1	23.1	27.3	21.6
2	23.92	32.68	28.65	32.5	31.1	27.6
3	28.05	40.05	31.08	47.4	36.7	29.6
4	34.15	44.66	35.55	51.1	45.9	36.9

5	40.92	50.25	43.68	57.2	53.6	45.6
6	46.81	55.65	45.54	69.6	66.5	57.1
8	58.62	58.95	52.92	75.6	71.6	78.6
12	61.17	68.64	64.58	87.6	88.7	95.2

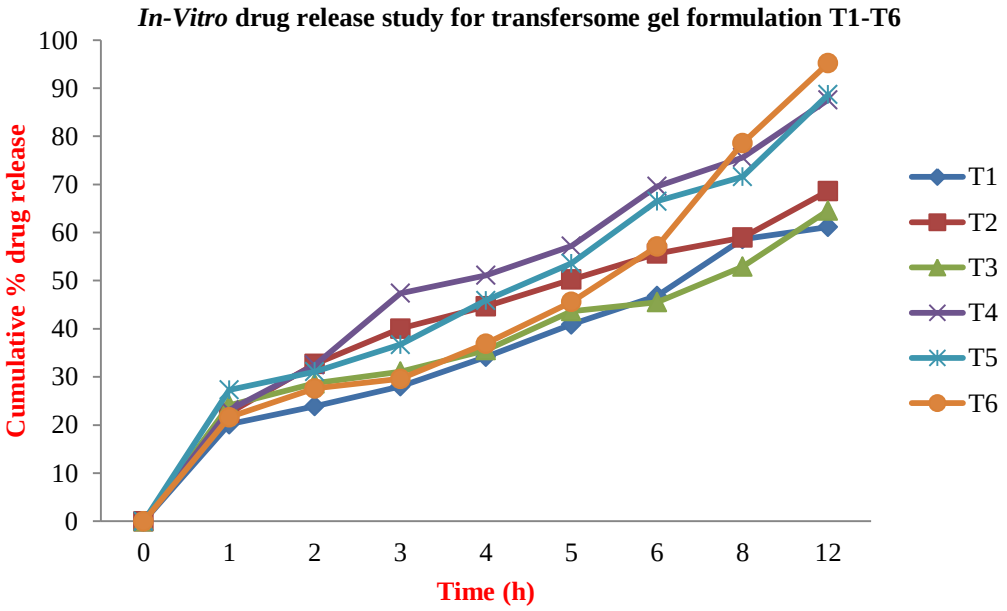

Fig 10: *In-Vitro* drug release study for transfersome gel formulation T1-T6

KINETIC STUDIES

Table 6: Release Kinetics for Optimized formulation T6

	ZERO	**FIRST**	**HIGUCHI**	**PEPPAS**
	% CDR Vs T	**Log % Remain Vs T**	**%CDR Vs √T**	**Log C Vs Log T**
Slope	7.734819802	-0.09829940	27.49438141	1.227010029
Intercept	8.329565347	2.106917318	-9.69149256	0.785192357
Correlation	0.986404902	-0.95856296	0.962567596	0.797432479
R 2	0.965952473	0.918842965	0.926536377	0.635898558

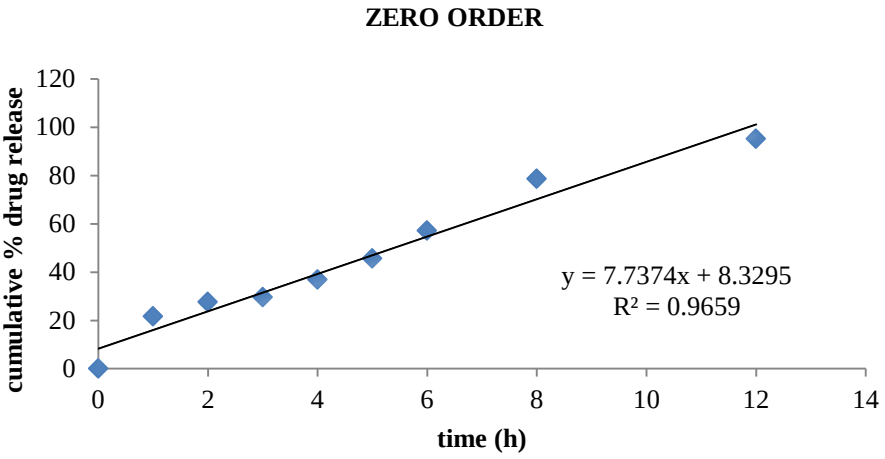

Fig 11: Zero order plot for optimized formulation

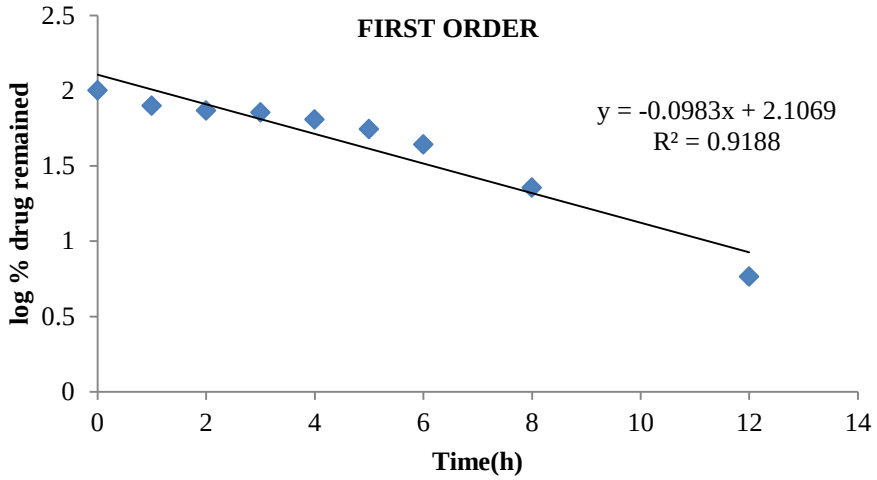

Fig 12: First order plot for optimized formulation

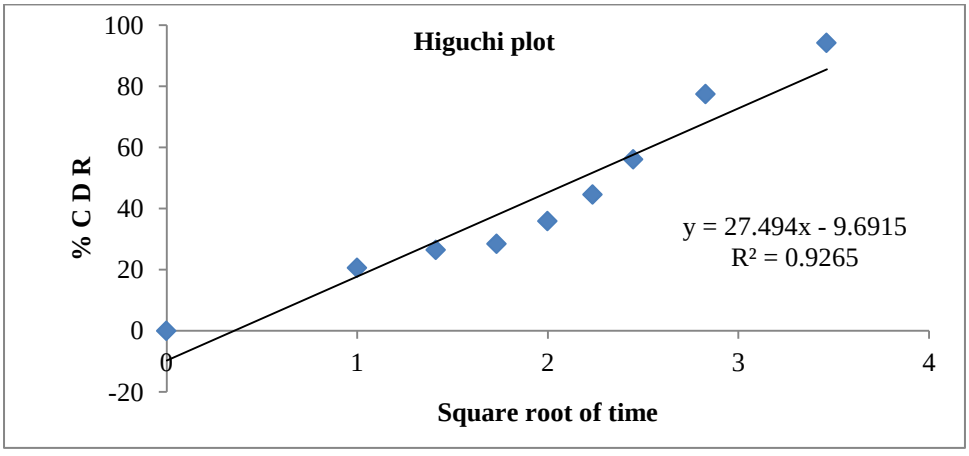

Fig 13: Higuchi plot for optimized formulation

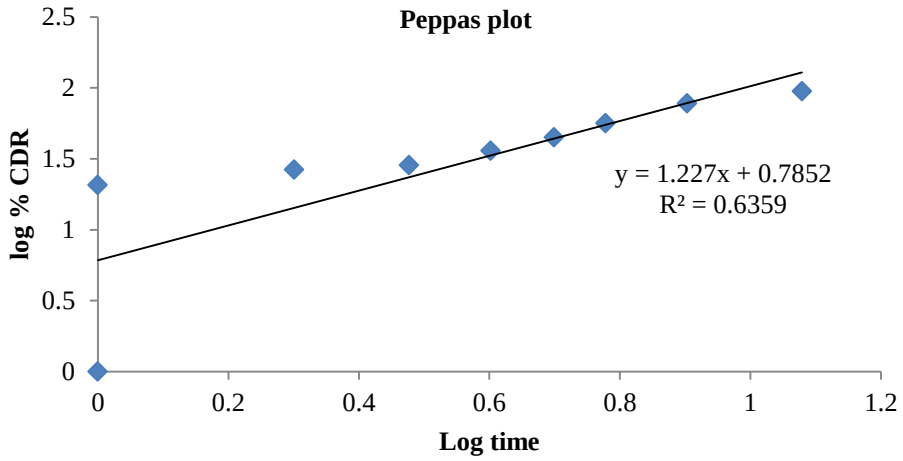

Fig 14: Peppas plot for optimized formulation

Stability studies

The stability studies were carried out according to the procedure described in the previous section. The results were shown in the table below:

Table 7: % Entrapment efficiency and % Drug content after stability studies (T6)

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	88.14	87.82	88.83	93.6	93.1	93.6
30	87.3	86.71	88.05	93.1	92.5	92.6
90	87.0	86.18	87.70	92.6	92.7	91.2

SUMMARY AND CONCLUSION

The work was carried out to prepare Itraconazole transfersomal gel to achieve controlled release effect at the site of administration**.** The FTIR spectra revealed that there were no interactions between the drug and the carriers. Transfersome formulations were prepared by thin film hydration technique and were incorporated into 2% carbopol gel. The Formulation T6 containing Lecithin: Span-80 in ratio 85:15(%w/w) has higher entrapment efficiency and maximum drug release. In-vitro skin permeation studies showed that, transfersomal gels were found to increase the skin permeation and deposition showing a controlled effect. Stability studies performed for optimized transfersome gel formulations indicates that the prepared transfersomes have more stability at freezing temperature than that of room temperature. Based on the above data, it was confirmed that prepared Itraconazole transfersome gel (T6) can be considered as one of the promising approach to reduce the dosing frequency and to maintain drug concentration at the desired site for longer time. Transfersomes gel improves the transdermal delivery, prolong the drug release, and improve the site specificity of the drug Itraconazole. Transfersomes creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

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