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FORMULATION DEVELOPMENT AND IN-VITRO EVALUATION OF POSACONAZOLE LOADED TRANSFEROSOMES GEL

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ABSTRACT

The goal of the current research was to create Posaconazole (PSZ) transferosome gel that would be effective against fungal infections. The gel was created by thin film hydration method. A study of the interactions between drugs and excipients was then conducted using the Fourier transform infrared (FTIR) spectroscopy method. The formulations were prepared and evaluated for measurement of pH, viscosity, spreadability, % entrapment efficiency, drug content estimation and *in vitro diffusion* study. Eight formulations were developed(PF1-PF8). In a Franz's diffusion cell, *in vitro diffusion studies* were carried out. The PF7 batch demonstrated the highest drug release after 24 hours. The developed formulation was stable, non irritant and provided sustained release over 24 hrs.

KEYWORDS: Posaconazole, gel, FTIR, Franz's diffusion cell.

INTRODUCTION

Transdermal drug delivery systems (TDDS), also known as medicated adhesive patches applied to the skin to administer a precise dose of medication via the skin and into the bloodstream, are dosage forms created to transport a therapeutically effective amount of drug across a patient's skin.[1] Since frequent medication intake is not required, transdermal treatment devices may create prolonged, steady, and controlled levels of drug in the plasma, enhancing patient compliance.[2]

The perfect penetration booster diminishes the stratum corneum's barrier resistance in a reversible manner without endangering the skin. The ability to avoid issues with stomach irritation, pH, and emptying rate impacts; avoid hepatic first pass metabolism[3]; and increase the bioavailability of the drug is the safest and most commonly utilized penetration enhancer.

Posaconazole (PSZ) is a triazole antifungal drug of BCS Class-II medication with a high lipid solubility and low water solubility. Posaconazole is an antifungal medication that comes in a variety of forms, including injections, oral suspensions, and delayedrelease tablets. When taken orally, these formulations can cause patient incompliance,

bioavailability, site specific administration, poorer stability, constipation, and stomach pain.[4] Hence, preparing Posaconazole loaded nanostructured lipid carriers gel for topical delivery to avoid such side effects of drugs and to improve the bioavailability, patient compliance for different topical fungal infections. The current research was to develop PSZ transfersome gel.

MATERIALS AND METHODS

Materials

PSZ was a gift sample from Chandra Labs Hyderabad, India, Telangana. Soya Lecithin was obtained from Bright Lab. Hyderabad. HPMC Span 80, and Tween 80 obtained from Standard Chemical Reagents. All other ingredients used were of pharmaceutical grade.

Methods

Drug polymer interaction

FTIR study

Fourier Transform Infrared Spectroscopy (FTIR) Study

The FTIR spctroscopy allows identification of functional groups in various chemicals as well as incompatibilities between the drug and excipients. Infrared spectrum of drug and excipients were determined on Fourier Transform Infrared spectrophotometer (8400 S Shimadzu) using KBr dispersion method.

Calibration curve

The standard solution was created by combining 10 mg of PSZ with 10 ml of phosphate buffer pH 6.8, and then increasing the amount to 100 ml. A series of dilutions containing 0.5, 1, 1.5, 2, and 2.5 ml from this standard solution were pipetted out and subsequently diluted to 10 ml with phosphate buffer pH 6.8 to produce 5, 10, 15, 20, and 25 µg/ml respectively. When using phosphate buffer pH 7.4 as a blank solution, the absorbances of these dilutions were determined using a UV spectrophotometer at 260 nm.

Preparation of Posaconazole (PSZ) transfersome gel

Transferosomes were prepared by thin film hydration method using posaconazole, Soya Lecithin, and different concentrations of surfactants (Span-20, Tween80). The amount of drug is kept constant (100mg) in all the formulations. Different formulations were prepared by using different ratios of phospholipid and surfactants in different ratios. The details about the surfactants used and amount of lecithin and surfactant used in each formulation are given in the Table No. 1. Lecithin, surfactants and the drug are dissolved in 10ml of organic solvent (Chloroform: Methanol 1:1). The organic solvent is then removed by evaporation while hand shaking above lipid transition temperature (430c). 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: Formulation of Posaconazole Transferosomes gel.

Formulation	Drug (mg)	Lecithin (mg)	Span 20 (mg)	Tween 80 (mg)	Chloroform (ml)	Methanol (ml)
PF1	100	90	10	--	5	5
PF2	100	85	15	--	5	5
PF3	100	80	20	--	5	5
PF4	100	75	25	---	5	5
PF5	100	90	--	10	5	5
PF6	100	85	--	15	5	5
PF7	100	80	--	20	5	5
PF8	100	75	---	25	5	5

EVALUATION OF TRANSFERSOME GEL[5-8]

pH

1gm of gel formulation were dissolved in 10ml of distilled water (pH 7) was prepared. The pH of the gel was determined by using digital pH meter (Digisun electronics), measured by bringing the probe of the pH meter in contact with the samples.

Viscosity

Viscosities of the gels were determined by using Brookfield viscometer .Spindle type, S-64 at 100 rpm.

Spreadability

It was determined by modified wooden block and glass slide apparatus. A measured amount of gel was placed on fixed glass slide, the movable pan with a glass slide attached to it and was placed over the fixed glass slide, such that the gel was sandwiched between the two glass slides for 5min. The weight was continuously removed. Spreadability was determined using the formula.

Entrapment efficiency[9]

The amount of Posaconazole entrapped in transfersomes was estimated by centrifugation method. 1gm of Transfersome gel was taken and diluted with 10ml phosphate buffer (pH 6.8). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted approximately and absorbance was measured using UV spectrophotometer. Entrapment efficiency is expressed as the percentage of drug trapped.

Drug content

In a shaker incubator, a precisely measured quantity of film (about 100 mg) is dissolved in 100 mL of phosphate buffer pH 6.8 in which the medication is soluble. The solution is then agitated continuously for 24 hours. The entire solution is then sonicated after that. Drug concentration is determined spectrophotometrically by suitable dilution after sonication and subsequent filtration.

In-vitro diffusion drug release studies

In-vitro drug release studies from posaconazole transferosomal gel were performed by using Modified Franz diffusion cell on egg membrane in phosphate buffer solution (pH 6.8).Egg membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was $2cm^2$ and capacity of receptor compartment was 30ml of phosphate buffer (pH 6.8) maintained at

37± 0.5^{0}C and stirred by a magnetic bar at 100rpm. Transfersomal gel formulation equivalent to 5mg drug was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 6.8) to maintain sink conditions. The samples were analyzed spectrophotometrically at λ max.

Scanning electron microscopy (SEM)

The morphology of the posaconazoletransferosomal gel was studied using scanning electron microscopy (SEM).The samples for SEM were prepared by lightly sprinkling on a double adhesive tape stuck to an aluminum stub. The stubs were then coated with gold film under reduced pressure. The stub containing the coated samples was placed in the scanning electron microscope (Hitachi S3400N) chamber. The samples were then randomly scanned, and photomicrographs were taken at the acceleration voltage of 5 kV. Microphotographs were taken on different magnification and higher magnification was used for surface morphology.

Accelerated stability studies

The optimized formulation was stored in sealed glass ampoules at stability test chamber (Remi, India), temperature (4±2^{o}C), and room temperature (25±2^{o}C) and body temperature (37±2^{o}C) for a period of at least 3 monthsthrough accelerated stability studies in accordance with ICH requirements. The percentage entrapment of the drug and % drug content was determined. Physical parameters for the optimized PF7 patches were compared before and after the expedited stability analysis.

RESULTS AND DISCUSSION

Analytical methods for the estimation of PSZ

The calibration curve of pure drug was developed in phosphate buffer pH 6.8 for use *in vitro* quantification of PSZ during drug content investigations and dissolution studies. The values of absorbance to corresponding concentration for PSZ were found in various buffers. Drug content uniformity and in vitro dissolution experiments both employed the formulae for straight lines derived from calibration curves. Table 2 provided the analytical parameters for the UV-Visible spectroscopic technique.

Table 2: Analytical parameters of PSZ for the development of UV method.

Parameters	Values for phosphate buffer pH 7.4
λmax (nm)	260
Beer's law limit (µg/ml)	5-25
Regression equation	Y = 0.030X+0.001
Slope	0.030
Intercept	0.001
Correlation coefficient (R)	0.998

Fourier Transform Infrared Spectroscopy (FTIR) Study

FTIR is used to identify distinctive peaks that show whether a medicine and its excipients are compatible. Various FTIR spectra are studied to determine the compatibilities between drugs and excipients. It is clear from the drug-excipient combo that there is no conflict between PSZ and this substance.

Formulation of gel

The transferosome gel of PSZ was prepared by thin film hydration method.

Evaluation of Transferosomal gel

All the formulations were observed and the results are tabulated in Table 3 and found that all are in accepted pharmacopoeial limits. The pH of all topical transfersomal gels were found to be in the range of 6.1±0.34 to 6.7±0.54. The viscosity of the gel was found to be range of 3300±1.27 cps to 4500±2.24 cps. Spreadability of gel was found to be in range of 2.8±0.16 to 3.6±0.19 cm/s. % Entrapment Efficiency of gel was range of 80.26±0.27 to 86.21±0.20. % drug content of transferosome formulations were determined according to procedure described. The results obtained shows in range of 91.48 -96.41% in the formulations.

The percentage entrapment of Posaconazole was found to be maximum with formulation PF7 because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, the efficiency increased with increasing surfactant concentration and thus increased with increasing lipid concentration.

Table 3: Evaluated parameters of Transferosomal gel.

Formulation Code	pH ± SD	Viscosity (cps) ± SD	Spreadability (cm/sec)* ± SD	% Entrapment Efficiency(%) ± SD	% Drug content ± SD
PF1	6.2 ± 0.31	3300±1.27	3.2±0.11	81.26±0.18	92.34±0.12
PF2	6.4±0.19	3550	2.8±0.16	82.14±0.14	91.48±0.27
PF3	6.2±0.26	4100	3.2±0.22	84.28±0.25	93.24±0.16
PF4	6.4±0.52	3750	3.5±0.14	82.14±0.12	92.48±0.24
PF5	6.2±0.27	3700	3.4±0.46	84.12±0.36	94.24±0.19
PF6	6.4±0.28	3600	3.4±0.21	80.26±0.27	94.16±0.21
PF7	6.6±0.74	4500±2.24	3.6±0.19	86.21±0.20	96.41±0.36
PF8	6.5±0.58	4000	3.4±0.22	84.28±0.22	94.24±0.23

N.B. All the values are represented as Mean ± SD (n=3)

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

The *In-vitro* drug release from the formulations was studied using franz diffusion cell for a period of 24 hours. The % cumulative drug release (% CDR) was calculated. The % CDR for PF1, PF2, PF3,PF4,PF5,PF6, PF7, and PF8 were 80.24±0.7, 82±0.9, 84.8±0.2, 73.28±0.1, 74.14±0.2, 88.35±0.4, 96.18±0.4 and 89.46±0.2% respectively of PSZ at the end of 24 h. Comparison of results obtained from diffusion studies for all eight formulations have been done. It was found that formulation PF7 shows higher drug release rate than other formulations. The formulation PF7 having lecithin (80mg) and tween 80 (20mg) had the drug highest release of 96.18% in 24 hours. This result of diffusion profile showed slight initial burst release. This is probably caused by the release of drug absorbed on the transferosome surface or precipitated from the superficial lipid layer. Prolonged release in the later stage can be attributed to the slow diffusion of the drug from the lipid vesicle. The % CDR is shown in Figure 1 (PF1 to PF4) and Figure 2 (PF5 to PF8).

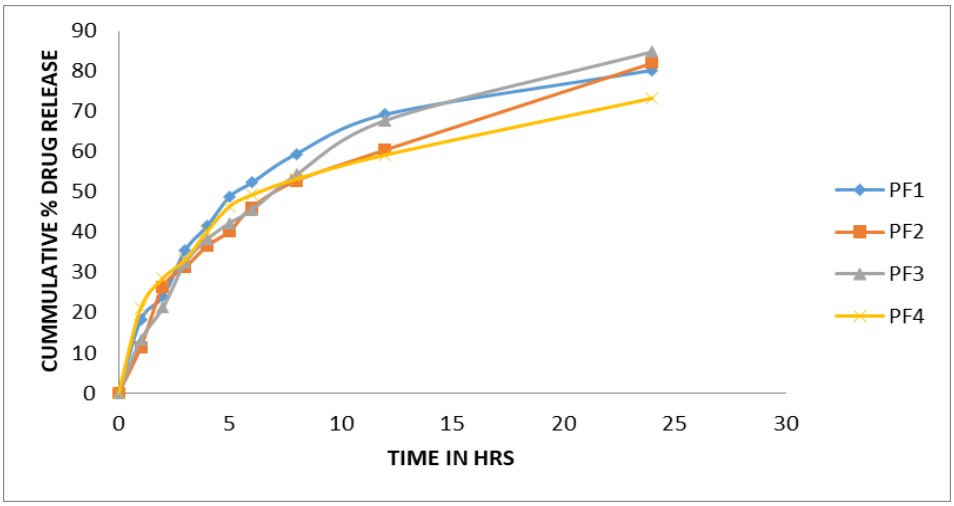

Figure 1: Comparative *In-vitro* Drug Release of Formulations PF1-PF4.

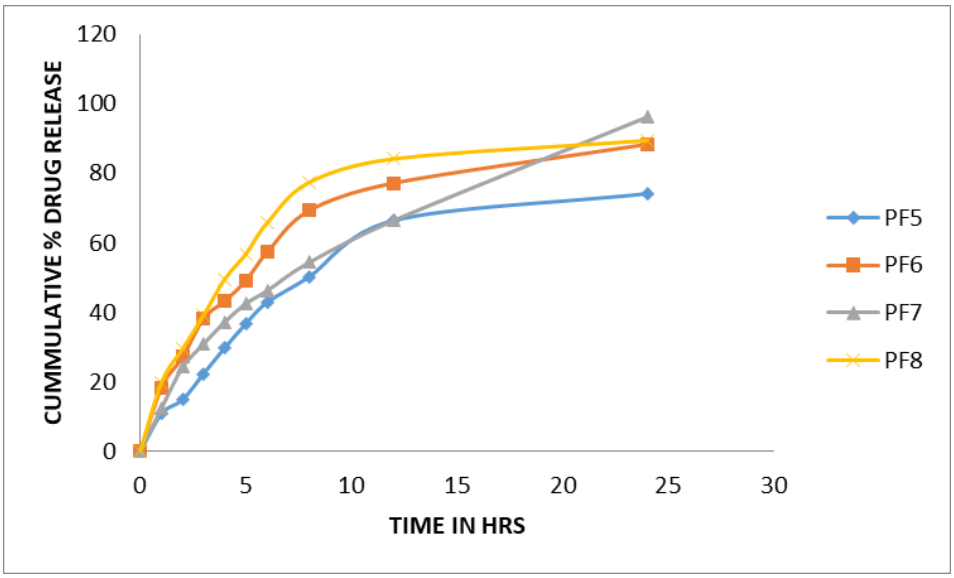

Figure 2: Comparative *In-vitro* Drug release of Formulations PF5-PF8.

Surface morphology of optimized formulation

The transferosomes were subjected to microscopic examination (S.E.M) for characterizing size and shape of the transferosomes. Microscopic examination revealed, spherical small unilamellar esicles size. SEM photograph is shown in Figure 3.

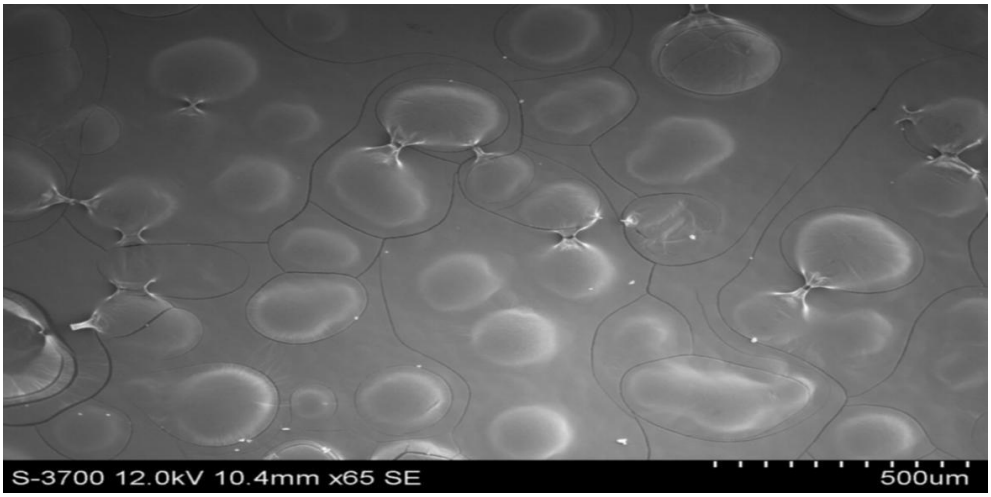

Figure 3: SEM Analysis of Optimized Formulation (PF7).

Accelerated Stability Studies

For the optimized formulation (PF7) of PSZ gels, various parameters that were measured at various time intervals during stressful settings. No discernible alteration in drug content, *in vitro* drug permeability, etc., was seen.

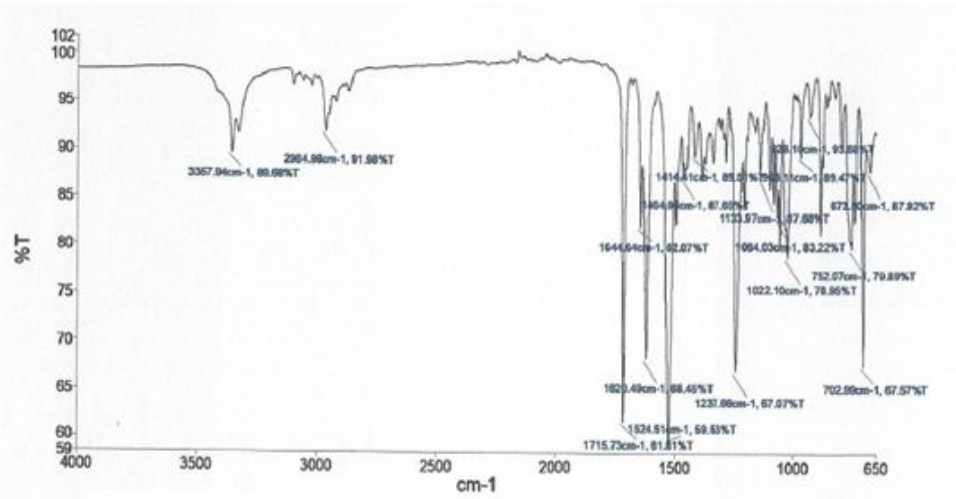

Figure No. 4: FTIR Spectra of Posaconazole Pure Drug.

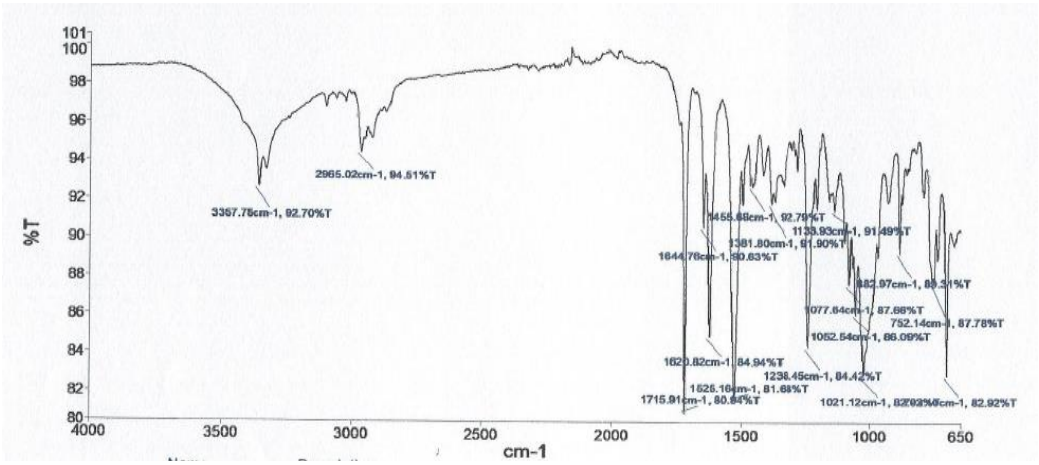

Figure No. 5: FTIR Spectra of Optimized Posaconazole Transferosome Formulation.

Table No. 4: FTIR Interpretation Table.

Characteristic peak	Literature Values	OBSERVED VALUES	
		Pure drug	Optimized formulation
C –O	1275-1200	1237.66	1238.45
C –N	1250-1020	1022.10	1021.12

FTIR studies were performed to understand the compatibilities between the drug with different excipients. The figures above illustrate that the functional groups like **C-O** Stretching with the observation range of 1275-1200 has peaks at 1237.66 in pure drug and 1238.45 in optimized formulation. Similarly the functional group C–N Stretching has a peak range of 1250-1020 has peaks at 1022.10 in pure drug and 1021.12 in optimized formulation. The functional groups in both the pure drug and optimized formulation are found. Hence it can be concluded that the pure drug is compatible with the excipients used in the study.

CONCLUSSION

The results of present study shows that transfersome Gel of optimized batch PF7 prolong the drug release, and improve the site specificity of the drug Posaconazole. Transfersomes formed from Lecithin: Tween80 in the ratio 80:20 (%w/w) is a promising approach to improve the permeability of Posaconazole in period of time. 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. Transfersomes are formulated to deliver Posaconazole across skin and target drug to synovium or specific tissues which in turn increase drug efficacy with minimum extra synovial toxicity.

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