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Design, evaluation and optimization of fluconazole trandermal patch by 2^2 factorial method

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ABSTRACT

Transdermal drug delivery system (TDDS) has been an increased interest in the drug administration via the skin for both local therapeutic effects on diseased skin (topical delivery) as well as for systemic delivery of drugs. Fluconazole is a synthetic antifungal agent belonging to the group of triazole. It is one of the commonly used antifungal agents for most kinds of fungal infections including superficial and invasive fungal infections. Preliminary trials were conducted with differ polymer combination (PVA:PVP, HPMC K4M:PVP, HPMC K15M:PVP, HPMC K100M:PVP and EC: PVP). In this F2 & F4 were selected depending on their parameters such as tensile strength and folding endurance. The selected polymers (factors) were of HPMC and PVP, by using two different grades of HPMC and PVP concentrations as levels incorporated in 2^2 factorial design by using Design of Experiments software. The drug release through the transdermal patches of fluconazole follows first order kinetics with diffusion controlled mechanism.

Key words: Fluconazole, Transdermal patch, 2^2 factorial design

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INTRODUCTION

Transdermal drug delivery systems (TDDS) are adhesive drug-containing devices of defined surface area that delivers a predetermined amount of drug to the intact skin at a preprogrammed rate [1]. FDA approved the first transdermal patch products in 1979 [2.3]. Fungal infection of skin is now a day's one of the common dermatological problems. The physicians have a wide choice for treatment from transdermal and to liquid formulations. Amongst the topical transdermal formulations have been widely accepted in both cosmetics and pharmaceuticals. Transdermal therapeutics systems are defined as self contained discrete dosage forms when applied to the intact skin, deliver the drugs through the skin, at controlled rate to the systemic circulation. The advantages of delivering drugs across the skin for systemic therapy are well documented.

Fluconazole is a water soluble triazole antifungal drug used for the treatment of superficial fungal infections. The mechanism of action of triazoles they inhibit the fungal cytochrome P450 enzyme lanosterol 14-demethylas and thus impair ergosterol synthesis leading to a cascade of membrane abnormalities in the fungus [4]. It is available as tablets for oral administration, as a powder for oral suspension and as a sterile solution for intravenous use. It is widely used in vaginal candidia oropharyngeal and esophageal candidiasis and cryptococccal meningitis. It is also effective for the treatment of candida urinary tract infections peritonitis and systemic candida infections.

The main objectives of the study:

- To design and formulate fluconazole transdermal patch
- To screen out the suitability of polymers for the transdermal patch formation
- To evaluate the selected polymer ratio patch for drug incorporation to form medicated transdermal patch
- Optimization of patch by simple 2^2 factorial design to obtain optimal transdermal patch

MATERIALS AND METHODS

Materials: Felodipine was purchased from Yarrow chemicals PVT Ltd, Mumbai, India. HPMC K4M, HPMC K100, and HPMC E15 LV were purchased from Yarrow Chem, Mumbai, India. PVP was obtained from SD fine—Chem. Ltd, Mumbai. Polyethylene glycol 400 was obtained from Merck Specialities Private Ltd. (Mumbai). All other materials and chemicals used were of either pharmaceutical or analytical grade.

Analytical method for the estimation of fluconazole:
A spectrophotometric method based on the measurement of absorbance at 276 nm in a phosphate buffer of pH 7.4 was used in the present study for the estimation of fluconazole.

Reagents:
Phosphate buffer of pH 7.4:
Phosphate buffer of pH 7.4 was made by dissolving 6.8g of potassium dihydrogen phosphate and 1.56g of sodium hydroxide in 1000 ml with distilled water. The pH was adjusted to 7.4.

Standard solution:
Weigh accurately 25 mg of fluconazole was dissolved in distilled water in 25ml volumetric flask and the solution was made up to volume with distilled water.

Method:
The standard solution of fluconazole was subsequently diluted with phosphate buffer of pH 7.4 to obtain series of dilutions containing 100, 200, 400, 600 and 800 µg of fluconazole per ml of solution. The absorbance of the above dilutions was measured in Elico UV-Vis. Spectrophotometer at 276 nm using phosphate buffer of pH 7.4 as blank. The concentration of fluconazole and corresponding absorbance were given in Table 1. The absorbance values were plotted against concentration of fluconazole as shown in Fig. 1.

Validation:
The method was validated for linearity, precision and accuracy. The method obeyed Beer's Law in the concentration range 100-800 µg/ml. When a standard drug solution was assayed repeatedly (n=6), the mean error (accuracy) and relative standard deviation (precision) were found to be 0.6 and 0.8 %, respectively. Thus the method was found suitable for the estimation of fluconazole contents in dissolution fluids.

Table 1: Data for standard calibration plot

Concentration (µg/ml)	Absorbance
0	0.000±000
50	0.085±0.02
100	0.113±0.09
200	0.222±0.07
400	0.461±0.04
600	0.646±0.04
800	0.825±0.03

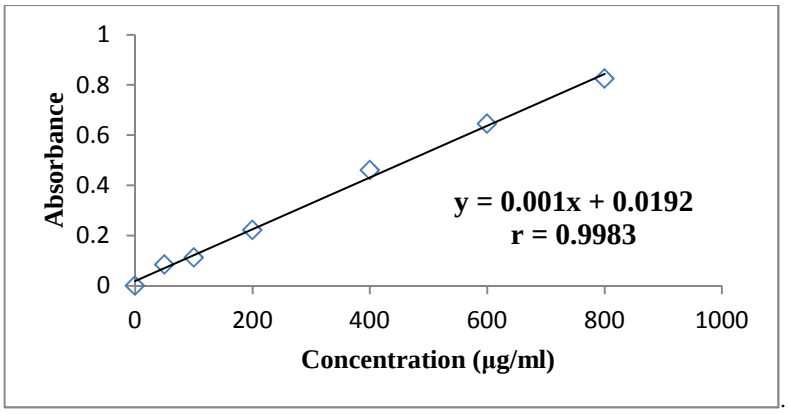

Fig 1: Standard calibration plot of fluconazole in phosphate buffer pH 7.4

Methods
Preparation of Transdermal Patch: Drug-loaded matrix- type transdermal patches of fluconazole were prepared by using solvent casting method. A petridish with a total area of 44.15 cm^2 was used. Polymers were accurately

weighed and dissolved in 10mL of water, methanol (1:1) solution and kept aside to form clear solution. Drug was dissolved in the above solution and mixed until clear solution was obtained. Polyethylene glycol 400 (10% w/w of total polymer) was used as plasticizer. The resulted uniform solution was cast on the petri dish, which was lubricated with glycerin and dried at room temperature for 24 h. An inverted funnel was placed over the petridish to prevent fast evaporation of the solvent. After 24 h, the dried patches were taken out and stored in a desiccator for further studies [5].

Preliminary Screening*:* Preliminary study was carried out to check effect of various polymer combinations on transdermal patch formulation. Composition of preliminary trial batches F1 to F5 is shown in Table 2.

Table 2: Preliminary trial batches

Formulation code	Polymer	Polymer proportions	Solvent	Plasticizer (10% w/w)
F1	PVA:PVP	1:1	Water	PEG 400
F2	HPMC K4M:PVP		Water	
F3	HPMC K15M:PVP		Water	
F4	HPMC K100M:PVP		Water	
F5	EC:PVP		Methanol	

Optimization of Variables Using Full Factorial Design

A 2^2-randomized full factorial design was used in the present study. In this design, 2 independent factors were evaluated, each at 2 levels, and experimental trials were performed for all 4 possible combinations. The different grades of HPMC (X_1) and concentration of PVP (X_2) were chosen as independent variables in 2^2 full factorial designs. Tensile strength, cumulative % drug release at 1 h (Q_1), cumulative % drug release at 12 h (Q_{12}), and drug content were taken as dependent variables. The formulation layout for the factorial design batches (F21, F22, F41 and F42) are shown in Table 3.

Table 3: Independent factors selected for TD patch formulation as per 2^2 factorial design

Factors	Levels (-)	Levels (+)
HPMC different grades	HPMC K4M	PVP (1%)
PVP different concentrations	HPMC K100M	PVP (2%)

Evaluation Parameters of Transdermal Patch

Folding Endurance [6] A strip of specific area (2 cm×2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film was folded at the same place without breaking gave the value of the folding endurance.

Tensile Strength [5] The tensile strength of the patch was evaluated by using the tensiometer (Erection and instrumentation, Ahmedabad). It consists of two load cell grips. The lower one was fixed and upper one was movable. Film strips with dimensions of 2×2 cm^2 were fixed between these cell grips, and force was gradually applied till the film broke. The tensile strength was taken directly from the dial reading in kg.

Percentage Elongation Break Test [7] The percentage elongation break was determined by noting the length just before the break point, the percentage elongation was determined from the below mentioned formula.

$$\text{Elongation percentage} = \left[\frac{(L1-L2)}{L2}\right]\times 100$$

where $L1$ is the final length of each strip, and $L2$ is the initial length of each strip.

Thickness [6] Patch thickness was measured using digital micrometer screw gauge at three different places, and the mean value was calculated.

Drug Content [1] A specified area of patch (2 cm×2 cm) was dissolved in 100mL methanol and shaken continuously for 24 h. Then the whole solution was ultrasonicated for 15 min. After filtration, the drug was estimated spectrophotometrically at wavelength of 276 nm and determined the drug content.

Percentage Moisture Content [5] The prepared films were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 24 h. After 24 h, the films were reweighed and determined the percentage moisture content from the below mentioned formula:

Percentage moisture content=$\frac{(initial\ weight-final\ weight)}{final\ weight}\times 100$

Percentage Moisture Uptake [5] The weighed films were kept in a desiccators at room temperature for 24 h containing saturated solution of potassium chloride in order to maintain 84% RH. After 24 h, the films were reweighed and determine the percentage moisture uptake from the below mentioned formula:

Percentage moisture uptake=$\frac{(final\ weight-initial\ weight)}{initial\ weight}\times 100$

***In vitro* drug release**

By using USP type 1 dissolution test apparatus, Drug release from the prepared TD patch was studied using 8 station dissolution rate test apparatus (Labindia, DS 8000) employing a paddle stirrer at 50 rpm and at 37±1°C. Phosphate buffer of pH 7.4 (500 ml) was used as dissolution fluid. Samples of 5 ml of each were withdrawn at different time intervals over a period of 12 h. Each sample withdrawn was replaced with an equal amount of fresh dissolution medium. Samples were suitably diluted and assayed at 276 nm for fluconazole using Elico SL159 double beam UV-spectrophotometer. The drug release experiments were conducted in triplicate.

Data analysis

Release data were analyzed as per zero order, first order, Higuchi [8] and Peppas [9] equation models to assess the drug release kinetics and mechanism from tablets.

RESULTS

Preliminary Study.

All the batches of transdermal patch showed thickness variation range from 0.12±0.01 to 0.20±0.01 mm as shown in Table 4. High thickness of batch F5 was found, it may be due to low solubility of ethyl cellulose in solvent render uneven distribution of polymer layer. All the batches of transdermal patch showed tensile strength and % elongation in uniform range from 0.12 to 0.30 and 5.1 to 23.5, respectively, except batch F5 may be due to poor solubility of ethyl cellulose and weak bond formation. Formulation F1 formed very rigid film having less flexibility and also has less folding endurance and tensile strength. Formulation F3 patch was prepared by HPMC K15M which showed less tensile strength and percent elongation. Whereas F5 was not suitable for TDD patch as all the parameters were not optimum.

Hence F1, F3 & F5 prepared formulation were eliminated and F2 and F4 were having good tensile strength and folding endurance was selected for further studies.

Table 4: Results for Preliminary trial batches

Formulation code	Thickness (mm)	Tensile strength (kg/cm^2)	% elongation	Folding endurance
F1	0.12±0.01	0.12±0.12	20.5±0.21	180±0.02
F2	0.17±0.01	0.26±0.15	22.5±0.13	320±0.04
F3	0.14±0.02	0.09±0.13	10.2±0.16	260±0.02
F4	0.15±0.04	0.30±0.12	23.5±0.21	310±0.01
F5	0.20±0.01	0.10±0.14	5.1±0.09	100±0.01

Folding Endurance, Tensile Strength, % Elongation and Thickness.

The results of folding endurance, tensile strength, % elongation and thickness of factorial design batches are shown in Table 4. The folding endurance values of all the factorial design patches were found satisfactory which indicates that the patches prepared using PEG 400 in a concentration of 10% w/w of polymer were having optimum flexibility and were not brittle. The tensile strength of the patches prepared with HPMC K4M and PVP were found in between 0.15 ± 0.01 kg/cm^2 to 0.12 ± 0.05 kg/cm^2, which were 0.17 ± 0.04 kg/cm^2 to 0.18 ± 0.07 kg/cm^2 for the patches composed of HPMC K100. It was observed that with the increase of PVP concentrations and HPMC grade, the tensile strength of the patches gradually increased. The % elongation was found to be in the range of 28.95±0.015% to 41.2±0.015%.

The thickness ranges were 0.12 ± 0.025 to 0.25 ± 0.022 mm. The results showed (Table 5) that the patches were uniform, as it was evidenced by SD value, which were less than 0.01 for all the factorial design batches.

Table 5: Formulation and evaluation of TD patches by 2^2 factorial design batches

Runs	Combinations	Tensile strength (kg/cm^2)	Folding endurance	Drug release (%) Q_1hr	Drug release (%) Q_{12}hr	Drug content (%)
F21	HPMCK4M:PVP(1%)	0.15±0.01	310±0.11	30.42	98.9	96.7±0.73
F22	HPMCK4M:PVP(2%)	0.12±0.05	300±0.21	34.25	97.8	98.7±0.84
F41	HPMCK100M:PVP(1%)	0.17±0.04	300±0.04	37.23	95.2	97.5±0.65
F42	HPMCK100M:PVP(2%)	0.18±0.07	300±0.07	35.41	99.1	98.5±0.22

Moisture Content, Moisture Uptake and Drug Content Studies.

The moisture content in the patches ranged from 4.14 ± 0.17 to 5.12 ± 0.01%. The moisture content in the formulations was found to be increased by increase in the concentration of PVP and also with increasing the grade of HPMC. The moisture uptake in the patches ranged from 4.27 ± 0.01 to 6.89 ± 0.09%. The lower moisture content in the formulations helps them to remain stable and become a completely dried and brittle film. Again, low moisture uptake protects the material from microbial contamination and bulkiness. The drug content ranged from 96.73 to 98.84%.

***In vitro* drug release**: Fluconazole release from al the prepared TDD patch was slow and spread over more than 12h and depended on the polymer concentration used as shown in Fig. 2. The release data were analyzed as per zero order, first order, Higuchi and Peppas equation models. The correlation coefficient (r) values in the analysis of release data as per various models are given Table 6. Analysis of the release data as per zero order and first order kinetic models indicated that the drug release from TDD patches formulated followed first order kinetics. The correlation coefficient (r) values were higher in first order model when compared to zero order model. The r-values were also more than 0.950 in the Higuchi and Peppas equation models indicating that the drug release from the TDD patches prepared also obeyed these two models.

When the release date were analyzed as per Peppas equation the release exponent 'n' was found in the range 0.416-0.490 in the case of TDD patches indicating non-fickian (anomalous) diffusion as the release mechanism from these tablets. Plots of % released vs. Square root time were found to be linear (r > 0.952) with all the TDD patches prepared indicating that drug release from the TDD patches prepared was diffusion controlled.

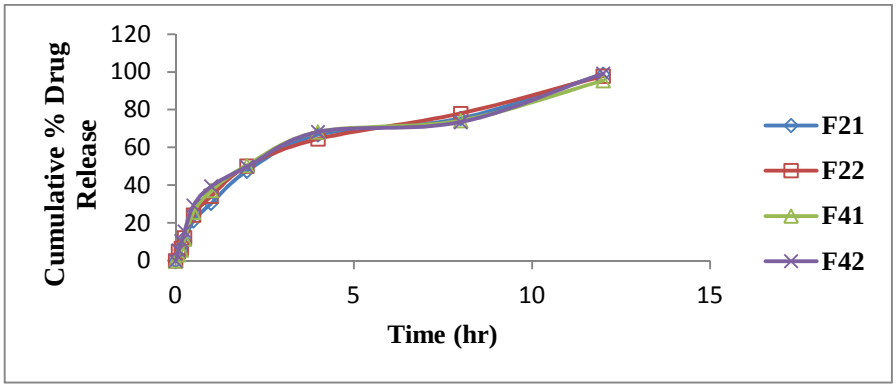

Fig 2: Drug release profiles from different prepared TDD patches

Table 6: Drug release kinetics data

F. code	Zero order		First order			Higuchi	Korsmeyer's peppas	
	r	K_0 (mol.L^{-1}h-1)	r	K_1 (hr^{-1})	$t_{1/2}$ (hr)	r	r	n
F21	0.932	9.573	0.988	0.281	2.412	0.988	0.989	0.490
F22	0.928	9.626	0.987	0.314	2.261	0.988	0.991	0.459
F41	0.906	9.454	0.982	0.328	2.164	0.978	0.983	0.440
F42	0.913	9.638	0.979	0.345	2.043	0.981	0.986	00.416

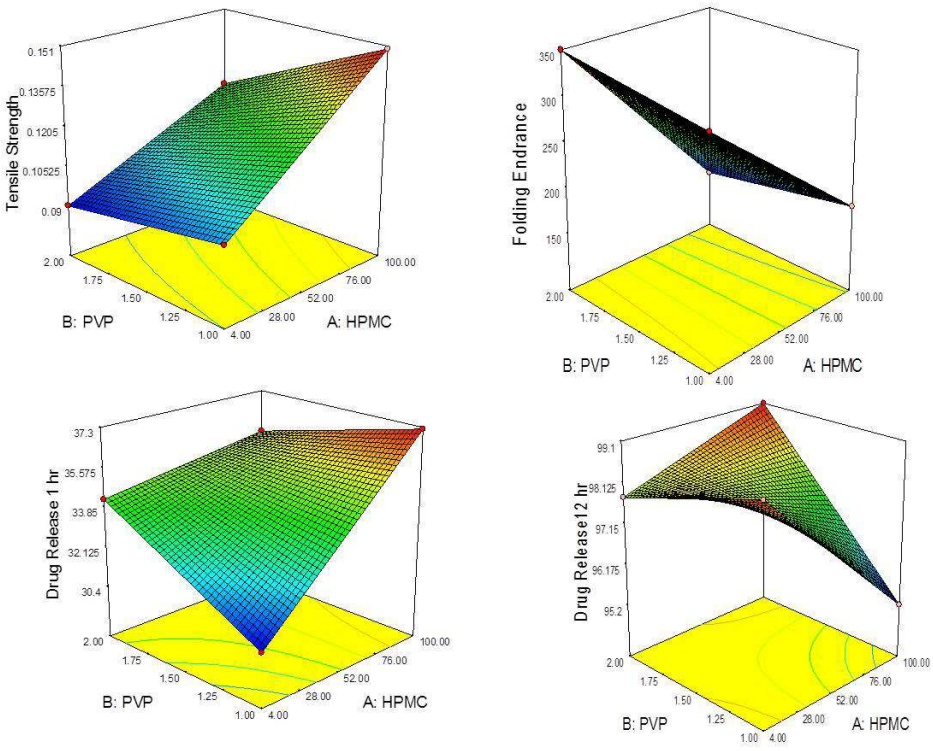

Fig 3 : Response surface plots

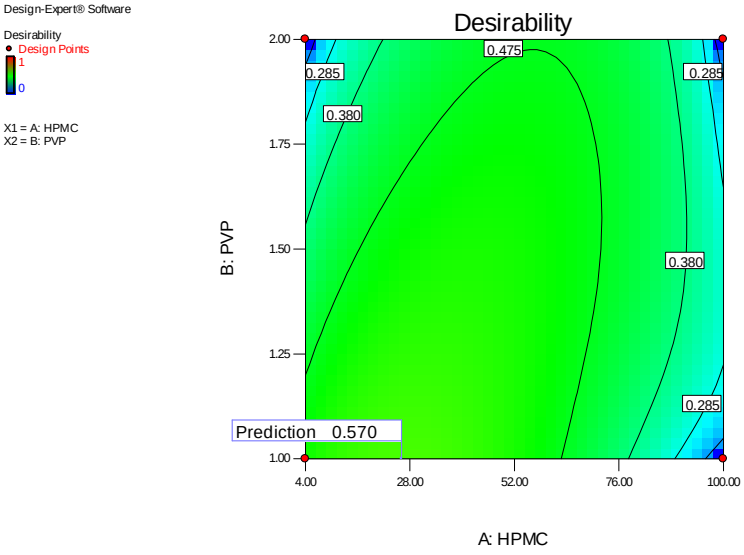

Fig 4. Desirability plot

DISCUSSION

The transdermal delivery has gained importance in recent years. Transdermal delivery of drugs is a novel drug delivery system and this system breaks many barriers in drug therapy like need of assistance, intermediate dosing and uncomfortable administration. Transdermal delivery has many advantages over conventional modes of drug administration;

- ✓ it avoids hepatic first pass metabolism
- ✓ potentially decreases side effects
- ✓ improves patient compliance

Fluconazole is a synthetic antifungal agent belonging to the group of triazole. It is one of the commonly used antifungal agents for most kinds of fungal infections including superficial and invasive fungal infections. Fluconazole differs markedly from other imidazole in its pharmacokinetic properties. The presence of two triazole rings (bis-triazole) makes this compound less lipophilic and more hydrophilic when compared with other azoles antifungal agents [10]. The presence of halogenated phenyl ring increases its antifungal activity. Commercially available products are oral dosage forms only. So, in this study we attempted to formulate the fluconazole in the form of transdermal patch, even though it has long half life and minimum dose is of 50 mg. however, it has susceptible to first pass metabolism, in order to prevent hepatic metabolism and also to elicit local antifungal action, drug was designed to incorporate in transdermal patch to enhance better therapeutic action.

As the drug comes under BCS class I (high solubility and high permeability), practically we observed that the drug was not freely soluble in water. Drug solution was made by keeping in bath sonicator for 5 minutes. Preliminary studies were conducted to screen out the polymers for suitable of patch formation. In this case, polymers such as PVA, PVP, HPMC (K4M, K 15M & K 100M) and EC were used. F1 to F5 formulations were prepared with constant polymer to polymer ratio (1:1) i.e. PVA:PVP, HPMC K4M:PVP, HPMC K15M:PVP, HPMC K100M:PVP and EC: PVP. Among all combinations HPMC K4M:PVP and HPMC K100M:PVP gave better tensile strength, elongation and folding endurance. Hence these two formulations were selected and incorporated in 2^2 factorial design and evaluated for further studies. The design shows only four runs, the two factors were HPMC and PVP and two levels (different HPMC grades and different concentrations of PVP) as independent factors. The dependent factors or responses selected were tensile strength, folding endurance, drug content, drug release for 1 hour and drug release for 12 hours. The regression equation for all the four responses shows that they follow linear equation without any interaction effects. As the model is simple showing the main effects responsible for responses. Increase in concentration of both HPMC and PVP increases or accommodates more amount of drug and indicating a positive effect. In case of drug release, PVP effect is more than that of HPMC, means high concentration of PVP more amount of drug release was observed. Whereas for tensile strength and folding endurance were inversely proportional to PVP concentration, mainly HPMC plays key role in maintaining these two responses. As the desirability value was 0.570 indicating the suitability of this model is 50% shown in Fig 4.

Regression equations for the responses
Tensile strength (Kg/cm^2)= 0.12+0.020A-0.010B-13.75AB
Folding endurance (%)=251.25-86.25A-1.25B-13.75AB
Drug release $Q_{1(}$hr)= 34.33+1.99A+0.50B-1.41AB
Drug release $Q_{12(}$hr)=97.75-0.60A+0.70B+1.25AB
Drug content= 97.85+0.15A+0.75B-0.25AB

Three different formulations were optimized by selecting HPMC K 100M and PVP (1%) combination showing the optimal responses for the prepared fluconazole transdermal patches as given Table 7.

Table 7: Prepared TDD patches according to 2^2 factorial design method

Desirability	HPMC (mg)	PVP (%)	Tensile strength (kg/cm^2)	Folding endurance	Drug release Q_{12}hr(%)	Drug content (%)
1	126.08	1	0.11	291.64	96.88	98.04
2	224.77	1	0.11	293.62	96.87	98.09
3	328.58	1	0.11	287.86	96.90	97.95

The drug release through the transdermal patches of fluconazole follows First order kinetics with diffusion controlled mechanism. The finding of this result revealed that the problems of fluconazole on oral administration like dissolution rate limited absorption and gastric side effects can be overcome by applying fluconazole topically in the form of transdermal patch.

CONCLUSION

The prepared transdermal drug delivery system of fluconazole using different grades of HPMC and PVP had shown good promising results for all the evaluated parameters. It was concluded that HPMC K100 and PVP of moderate level useful for preparation of sustained release matrix transdermal patch formulation.

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