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Research Article

Fabrication of Modified Transport Fluconazole Transdermal Spray Containing Ethyl Cellulose and Eudragit® RS100 as Film Formers

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Abstract. The present investigation was undertaken to fabricate modified transport fluconazole transdermal spray using ethyl cellulose and Eudragit® RS100 as film-forming polymers. Eudragit® RS100 (X_1) and ethyl cellulose (X_2) were selected as independent variables in 3^2 full factorial design, whereas drug transport in first hour (Y_1) and the time required for 50% drug transport (Y_2) were selected as dependent variables. Eutectic blend of camphor and menthol was used as permeation enhancer cum solvent for film-forming polymers. The pH, viscosity, volume of solution delivered upon each actuation, spray angle, *ex–in vivo* physical evaluation and *in vitro* drug transport of the formulated products were evaluated. The optimized batch B16 containing 5.25% *w/w* ethyl cellulose and 10.6% *w/w* Eudragit® RS100 was formulated by overlapping the contour plots of Y_1 and Y_2. The pH, viscosity, volume of solution sprayed upon each actuation and spray angle of the batch B16 was 6.3, 52.9 cPs, 0.24 ml and 82.6° respectively. The film of optimized batch was flexible and dermal-adhesive. The responses Y_1 and Y_2 of batch B16 were 7.91 μg/ml and 347 min respectively. The kinetics of drug transport was best explained by the Korsmeyer and Peppas model. The eutectic mixture consisting of equal parts of camphor and menthol showed improved drug permeation through shed snake skin. Short-term stability study demonstrated insignificant changes in performance characteristics.

KEYWORDS: fluconazole; modified transport; transdermal spray; factorial design and short term stability study.

INTRODUCTION

Tinea infections are caused by three species of fungi collectively known as dermatophytes. Tinea infections are named considering the affected area of human body, i.e., Tinea corporis (general skin), Tinea cruris (groin), and Tinea pedis (feet). Topical therapy is generally successful unless the infection covers an extensive area or is resistant to initial therapy. In these cases, systemic therapy may be required.

Fluconazole, a novel triazole antifungal drug, is used in the treatment of superficial and systemic fungal (Tinea) infection. The drug has moderate (8 mg/ml) solubility in water (1,2). Like other imidazole- and triazole-class antifungals, fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylase (3). Fluconazole is primarily fungistatic, however may be fungicidal against certain organisms in a dose-dependent manner. Ayub *et al.* investigated *in vitro* skin penetration and permeation of fluconazole from emulsions containing propylene glycol and isopropyl myristate as penetration enhancers (4). El-Laithy and El-Shaboury evaluated influence of vehicle on the release and permeation of fluconazole dissolved in jojoba oil (5). Rivera *et al.* prepared fluconazole-loaded poly(D,L-lactic-*co*-glycolic) acid microspheres by spray-drying process (6). Kavitha *et al.* formulated and characterized topical drug delivery systems of fluconazole in form of ointment, cream and gel using water-soluble and water-insoluble base in absence of penetration enhancer (7).

The aim of the present research work was to develop patient-friendly modified transport transdermal spray of fluconazole using ethyl cellulose and Eudragit® RS100 as film-forming polymers. A 3^2 full factorial design was employed for optimization. The formulation contained eutectic mixture of menthol and camphor (dermal penetration enhancers) that readily partition into the stratum corneum (8–14). Eutectic mixture of camphor and menthol also acted as a solvent for film-forming polymers,

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ABBREVIATIONS: f_2, similarity factor; MIC, minimum inhibitory concentration (μg/ml); PEG 400, polyethylene glycol; X_1, concentration of Eudragit® RS100 (% *w/w*); X_2, concentration of ethyl cellulose (% *w/w*); Y_1, the amount of drug transported (μg) per milliliter at the end of first hour; Y_2, the time (min) required to transport 50% of the drug ($t_{50\%}$); US FDA, US Department of Health and Human Services-Food and Drug Administration.

imparted cooling effect to the skin and possessed antifungal activity (15,16).

MATERIALS AND METHODS

Materials

Fluconazole USP and ethyl cellulose (Ethocel standard 10FP premium) were received as gift samples from Zydus Cadila (Ahmedabad, India). Eudragit® RS100 was received as a gift sample from Degussa Pvt. Ltd. (Mumbai, India). Camphor and menthol were purchased from Gem Corporation (Ahmedabad, India) and Shreeji Pharma International (Ahmedabad, India), respectively. Polyethylene glycol (PEG 400) and acetone were purchased from S. D. Fine Chemical Pvt. Ltd. (Ahmedabad, India). Alcohol I.P was purchased from Baroda Chemicals Industries Ltd. (Baroda, India). The other chemicals and reagents were of analytical grade.

Method

Determination of Solubility of Fluconazole and Polymer in Various Solvents

The solubility of fluconazole was determined in a mixture containing 80 parts of alcohol (80% *v/v*) and 20 parts of acetone. The drug solubility was also determined in a eutectic mixture of camphor and menthol (1:1). The solubility of ethyl cellulose and Eudragit® RS100 was determined in the eutectic mixture. An excess amount of sample was added to 35 ml of solvent and stirred at 100 rpm on a magnetic stirrer (Remi Electronics, Ahmedabad, India) for 30 min at room temperature (35±2°C) in a closed vessel. The mixtures were then filtered through 0.22 μm millipore filters and the weight of undissolved solid was recorded. The filtrate was carefully observed for clarity.

Preliminary Studies

Film formers (ethyl cellulose and Eudragit® RS100) and plasticizer (polyethylene glycol) were sequentially dissolved in the eutectic mixture consisting of equal proportion of camphor and menthol. Fluconazole was separately dissolved in vehicle blend consisting of 80 parts of alcohol (80% *v/v*) and 20 parts of acetone. The solution of ethyl cellulose/ Eudragit® RS100 in eutectic mixture was gradually added to the solution of fluconazole and mixed for 15 min at 80–100 rpm. The resulting solution was filled in a refillable container containing plastic dip tube of 75 mm length and 1.2 mm internal diameter. The aperture size of the tube was 0.3 mm. Table I displays the composition of the formulated batches (B1–B6). The sprays were analyzed for pH, viscosity, volume of solution delivered upon each actuation, spray angle, *ex–in vivo* physical characteristics and *in vitro* drug transport. The results of drug transport are expressed in Fig. 1.

Factorial Design

A 3^2 full factorial design was used for optimization of formulated product. The concentration of Eudragit® RS100 (X_1) and ethyl cellulose (X_2) were selected as independent variables, whereas the amount of drug transported in 1 h/ml (Y_1) and the time required to transport 50% of the drug ($t_{50\%}$, Y_2) were selected as dependent variables. Tables II and III show the composition, design layout for the optimization study and the responses. The method for preparation and evaluation of the formulated batches (B7–B16) was similar to

Table I. Composition and Evaluation of Fluconazole Sprays

Ingredient (% *w/w*)	Batch code					
	B1	B2	B3	B4	B5	B6
Fluconazole	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl cellulose	3	5	7.5	–	–	–
Eudragit® RS100	–	–	–	5	10	15
PEG 400	0.25	0.25	0.25	0.25	0.25	0.25
Eutectic blend	10	10	10	10	10	10
Alcohol and acetone blend (q.s.)	100	100	100	100	100	100
Tests	Average results (n=3)					
Viscosity (a±2.7 cPs)	15.0	26.4	43.5	7.6	20.2	51.6
Volume of solution delivered upon each actuation (b±0.03 ml)	0.32	0.30	0.26	0.39	0.31	0.24
Spray angle (c±0.4°)	78.0	79.1	81.0	76.1	78.3	82.2
Y_1[a]	21.07	17.07	12.55	23.44	14.12	11.73
Y_2[b]	94	115	196	120	160	210
Ex–in vivo film formation time[c] (d±35 sec)	130	160	210	110	152	230
Feeling of warmth and subsequent cooling sensation[c] (e±1 min)	12	14	15	12	14	16
Appearance of the film[c]	+	+	++	+	+	++
Dermal adhesion and flexibility of the film[c]	++	++	++	++	+++	+++
Water washability[c]	++	++	++	+++	+++	++

[a] Y_1 is the amount of drug transported (μg) per ml at the end of first hour
[b] Y_2 is the time (min) required to transport 50% of the drug ($t_{50\%}$)
[c] Measured for placebo batches replacing fluconazole with blend of alcohol and acetone

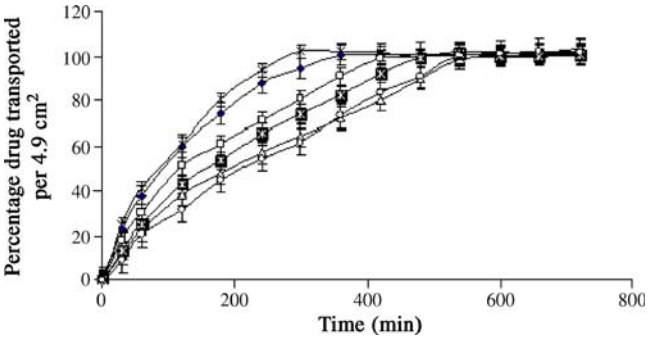

Fig. 1. *In vitro* drug transport from batches B1–B6 through nylon membrane; B1 (-◆-), B2 (-□-), B3 (-△-), B4 (- -※- -), B5 (- -■- -), B6 (-○-)

that described under preliminary studies. The results are expressed in Fig. 2 and Tables II and III.

Evaluations

1. Viscosity

The viscosity of the solutions was measured at 25±1°C using Brookfield viscometer (digital viscometer model DV-II+, Stoughton, MA, USA). The ULA spindle was rotated at 1 rpm.

2. Volume of solution delivered upon each actuation

The volume of solution delivered upon each actuation was calculated using eq. 1.

$$A_L = (W_t - W_o)/D_n \quad (1)$$

where A_L is the volume of solution delivered upon each actuation, W_t is weight of formulation after actuation, W_o is the initial weight of the formulation before actuation, and D_n is the density of the formulation.

3. Spray angle

The method of impingement of spray on a piece of paper was used for the study. Sudan red (10 mg) was dissolved in formulation to facilitate visualization. The sprays were actuated in horizontal direction onto a white paper mounted at a distance of 15 cm from the nozzle. The radius of the circle, formed on the paper, was recorded in triplicate from different directions. Spray angle (θ) was calculated by eq. 2.

$$Spray\ angle(\theta) = \tan^{-1}(l/r) \quad (2)$$

where l is the distance of paper from the nozzle, and r is the average radius of the circle.

4. *Ex–in vivo* physical evaluation

Placebo batches (B1P–B16P) were actuated on the left-hand palms of three healthy human volunteers of 21–27 years in age, four times every 10 s from a distance of 15 cm. The purpose of the study was fully explained and the volunteers gave written consent. The departmental review board approved the study. The time required for film formation, appearance of the film, *dermal adhesion* and flexibility of the film, feeling of warmth and subsequent cooling sensation, irritation potential and water washability were recorded. The appearance of the film was graded as shiny and transparent (+) or shiny and translucent (++) or dull and opaque (+++). Dermal adhesion, flexibility and water washability of the film were graded as poor (+), moderate (++) or good (+++). To check dermal adhesion of the film, the palms of each volunteer were rotated for 10 min in anti-clock wise direction with occasional opening and closing of palm, after 8 min of actuation of the spray. During the study (720 min), the nature of the film was carefully evaluated for any fracture, separation or removal. After 720 min, water washability of the film was checked.

Table II. Composition and Evaluation of Fluconazole Sprays Based on 3^2 Full Factorial Design

Ingredient (% *w/w*)	Batch Code									
	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Fluconazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ethyl cellulose	7.5	5	3	7.5	5	3	7.5	5	3	5.25
Eudragit® RS100	15	15	15	10	10	10	5	5	5	10.6
PEG 400	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Eutectic blend	10	10	10	10	10	10	10	10	10	10
Alcohol and acetone blend (q.s.)	100	100	100	100	100	100	100	100	100	100
Tests	Average results (n=3)									
Viscosity (a±2.7 cPs)	128.3	92.8	70.8	69.7	48.9	38.1	52.5	34.0	23.7	52.9
Volume of solution delivered upon each actuation (b±0.03 ml)	0.14	0.18	0.2	0.21	0.26	0.31	0.26	0.3	0.33	0.24
Spray angle (c±0.4°)	91.5	88.2	83.8	84.2	81.8	80.6	82.6	79.8	78.7	82.6
Ex–in vivo film formation time[a] (d±35 sec)	532	435	390	372	328	287	340	282	249	335
Feeling of warmth and subsequent cooling sensation[a] (e±1 min)	19	18	17	18	16	15	17	13	13	17
Appearance of the film[a]	+++	++	++	++	++	+	++	+	+	++
Dermal adhesion and flexibility of the film[a]	+++	+++	+++	+++	+++	+++	+++	++	++	+++
Water washability[a]	+	+	++	++	++	++	++	++	++	++

[a] Measured for placebo batches replacing fluconazole with blend of alcohol and acetone

Table III. Design Layout for 3^2 Factorial Design

	Real Values		Transformed Values		Dependent Variables	
Batch Code	X_1	X_2	X_1	X_2	Y_1	Y_2
B7	15	7.5	1	1	4.53	660
B8	15	5	1	0	6.15	540
B9	15	3	1	–1	10.7	292
B10	10	7.5	0	1	5.73	466
B11	10	5	0	0	8.49	320
B12	10	3	0	−1	12.85	165
B13	5	7.5	−1	1	10.56	240
B14	5	5	−1	0	16.15	165
B15	5	3	−1	−1	21.7	107
B16*	10.6	5.25	0.12	0.1	7.91	347

*B16** is the point batch, X_1 is the concentration of Eudragit® RS100 (% *w/w*), X_2 is the concentration of ethyl cellulose (% *w/w*), Y_1 is the amount of drug transported (μg) per milliliter at the end of first hour, Y_2 is the time (min) required to transport 50% of the drug ($t_{50\%}$)

5. *In vitro* drug transport

A nylon membrane with pore rating equal to 0.22 μm was mounted in a Franz cell. The surface area of membrane available for drug transport, was 4.9 cm^2. One milliliter of the drug formulation and 88 ml of solution (pH 7.4, 100 rpm) containing 0.9% *w/v* sodium chloride and 1% *w/v* sodium lauryl sulfate were filled in donor and receptor compartments, respectively. Throughout the experiment, the temperature of media present in the receptor compartment was maintained at 32±2°C using a hot-water jacket (37±2°C). Sodium lauryl sulfate was used to provide sink condition. Aliquots of 10 ml samples were withdrawn at different time intervals from the receptor compartment, and fluconazole was estimated spectrophotometrically at 264 nm (5). Equal amount of fresh dissolution medium was replaced after each withdrawal. Figures 1 and 2 show the drug transport profiles. The amount of drug transported in 1 h/ml (Y_1) and the time required to transport 50% of the drug (Y_2) were found. The UV spectrum of fluconazole was observed for fluconazole–formulation excipient interaction. The *in vitro* drug transport from the optimized batch B16 and batch B17 was also determined using shed snake skin as a membrane in Franz diffusion cell (Fig. 4). The shed snake skin was procured from the local snake habitat (Sundarvan, Ahmedabad, India). The composition of batches B16 and B17 was similar except that batch B17 was formulated without using the eutectic mixture.

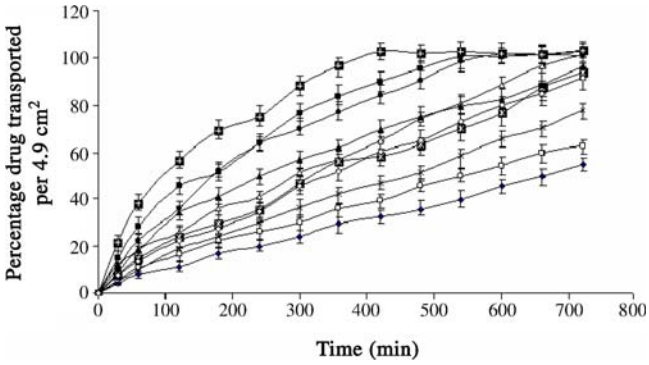

Fig. 2. *In vitro* drug transport from batches B7–B16-A; B7 (-◆-), B8 (-□-), B9 (-△-), B10 (-⊸-), B11 (-⊞-), B12 (-●-), B13 (-▲-), B14 (-■-), B15 (-⊕-), B16 (-◇-), B16-A (-○-)

Kinetics of Drug Transport

The method of Bamba *et al.* was adopted to ascertain kinetics of drug transport from the formulated batches B1–B16 (17). *In vitro* drug transport data of batches B1–B16 were analyzed by zero-order, first-order, Higuchi, Hixson–Crowell, Korsmeyer–Peppas and Weibull models (18–23). A FORTRAN software, developed in-house, was used. The least value of sum of square of residuals (SSR) and Fisher's ratio (F) were used to select the most appropriate kinetic model.

Similarity Factor

The similarity factor (f_2) was calculated by comparing test and reference *in vitro* drug transport profile using eq. 3 (24).

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{n=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \qquad (3)$$

where n is the number of pull points, R_t and T_t are percentage drug transported from reference and test products, respectively, at time "t."

Criteria for Optimized Batch

The film formation time was arbitrarily fixed as less than 8 min. The film should be mucoadhesive and flexible in nature to allow day-to-day activity yet should be water washable. Viscosity of the formulations should be less than 80 cPs. The selected limits for drug transport were: (1) Y_1:

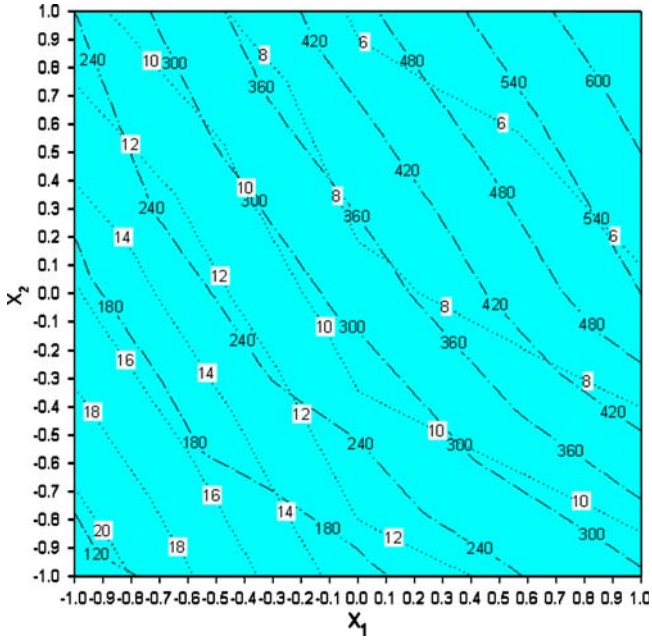

Fig. 3. Overlapped contour plots; *dotted line* Y_1, *dashes and dots* Y_2

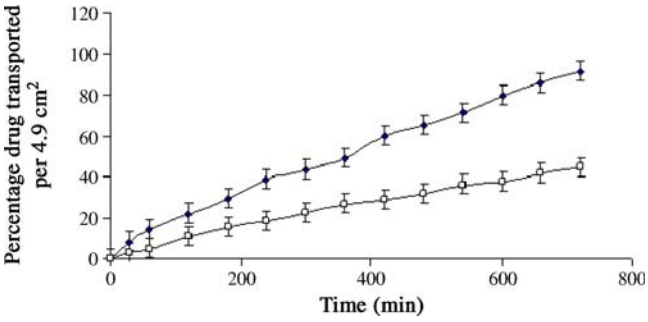

Fig. 4. *In vitro* drug transport from batches B16 and B17 through shed snake skin; B16 (*closed diamonds*), B17 (*open squares*-)

amount of drug transported in 1 h/ml should be equal to the minimum inhibitory concentration of fluconazole (minimum inhibitory concentration (MIC)=8 μg/ml)±5%, and (2) Y_2: time required to transport 50% of the drug ($t_{50\%}$) should be equal to 360 min±5%.

Statistical Analysis

Unpaired *t* test with equal variance was used to find any statistically significant difference in the *in vitro* drug transport profile between batches B16 and B17 through shed snake skin at 5% level of significance.

Stability Study

Optimized formulation (batch B16) was stored for 2 months at 25±2°C away from light. At the end of second month, the formulation was subjected to various tests like volume of solution delivered upon each actuation, pH, viscosity, solution delivered upon each actuation, spray angle, *ex–in vivo* physical characteristics and *in vitro* drug transport. The procedure employed for the study was identical to that described above. The results are shown in Table IV.

RESULT AND DISCUSSION

A eutectic mixture is a mixture of two or more solids which has lower melting temperature than any of its constituents. Various substances such as ibuprofen, menthol, chloral hydrate, beta naphthol, lidocaine, and prilocaine form eutectic mixtures. The primary criterion for eutectic formation is the mutual solubility of the components in the liquid. Camphor and menthol also forms a hydrophobic eutectic mixture. Camphor and menthol are powerful penetration enhancers (8–14). Camphor and menthol cause leaching of the lipids present in the skin and thus cause subsequent pore formation (25). In the present study, attempt was made to use eutectic mixture of camphor and menthol as multifunctional excipient (solvent for the film formers, powerful permeation enhancer and antifungal agent).

Preliminary Batches Containing Ethyl Cellulose

The solubility of fluconazole in a mixture consisting of 80 parts of alcohol (80% *v*/*v*) and 20 parts of acetone and the 1:1 eutectic mixture of camphor and menthol was 150 and less than 2 mg/ml, respectively. Acetone and primary alcohols are preferred solvents in concentration of 40–65% in formulation of topical solutions (26). As per US Department of Health and Human Services-Food and Drug Administration (US FDA) inactive ingredient guide, limit of alcohol and acetone in topical solutions is more than 83% and 12%, respectively (27). Acetone was used along with alcohol in the vehicle blend to facilitate faster film formation (<8 min) on skin. Ethyl cellulose, a film former, was soluble (>100 mg/ml) in the eutectic mixture. Hence, fluconazole was dissolved in vehicle blend of alcohol and acetone, whereas ethyl cellulose was dissolved in eutectic mixture of camphor and menthol.

The pH of formulated batches (B1–B3) ranged from 5 to 7. The pH of human skin is in between pH 5.5 and 6.5 (28). Hence, the pH adjustment was unnecessary. Table I shows that the viscosity of the formulated batches containing ethyl cellulose as film former (B1–B3) ranged from 15 to 43.5 cPs. The viscosity of the formulations increased with ethyl cellulose concentration. Formulated batches (B1–B3) showed good sprayability. The volume of solution delivered upon each actuation and spray angle ranged from 0.26 to 0.32 ml and 78° to 81°, respectively. The stated parameters were correlated with polymer concentration and viscosity of the formulation. *Ex–in vivo* film formation of placebo batches (B1P–B3P) passed the desired criteria of film formation, which varied from 130 to 210 s after actuation. The film turned from transparent to translucent on increase in viscosity. Dermal adhesion and flexibility of films were moderate. Feeling of warmth and subsequent cooling sensation were perceived after application of spray (around 12 min) because of camphor and menthol in the formulation. None of the placebo formulations resulted in irritation, rashes and itching in any of the volunteers. Water washability of the placebo batches (B1P–B3P) was moderate.

The formulation excipients did not show absorbance at 264 nm. The UV spectrum remained unchanged during *in vitro* drug transport study, indicating stability of fluconazole during study. The excipients or membrane constituents did not show absorbance at 264 nm. The MIC of fluconazole against *Candida* species is 8 μg/ml (29). The problem of dose

Table IV. Results of 2 Months Stability Study of Optimized Batch B16

Tests	Average results (n=3)
Viscosity (a±2.9 cPs)	54.7
Volume of solution delivered upon each actuation (b±0.06 ml)	0.24
Spray angle (c±0.4°)	83.4
Ex–in vivo film formation time[a] (d±30 s)	341
Feeling of warmth and subsequent cooling sensation[a] (e±0.5 min)	16
Appearance of the film[a]	++
Dermal adhesion and flexibility of the film[a]	+++
Water washability[a]	++

[a] Measured for placebo batches replacing fluconazole with blend of alcohol and acetone

dumping and lack of sustained drug transport was seen with formulated batches (B1–B3) with values of Y_1 greater than 12 μg/ml and Y_2 less than 200 min (Table I and Fig. 1). Considering the results of viscosity, the concentration of ethyl cellulose was not increased beyond 7.5% *w/w*.

Preliminary Batches Containing Eudragit® RS100

Eudragit® RS100 was soluble (>120 mg/ml) in the eutectic mixture. Hence, Eudragit® RS100 was dissolved in eutectic mixture of camphor and menthol. The pH of formulated batches (B4–B6) ranged from 5 to 7. Table I shows that the viscosity of batches B4–B6 increased with polymer concentration and ranged from 7.6 to 51.6 cPs. The formulated batches containing Eudragit® RS100 as a film former (B4–B6) showed good sprayability. The volume of solution delivered upon each actuation and spray angle ranged from 0.24 to 0.39 ml and 76° to 82°, respectively. The polymer concentration and viscosity were found to affect the stated two parameters. *Ex–in vivo* film formation of placebo batches (B4P–B6P) passed the desired criteria of film formation. The appearance of film changed from shiny transparent to translucent on increase in viscosity. Dermal adhesion and flexibility of films were good except batch B4P containing lower concentration of Eudragit® RS100. Eudragit® RS100 is a widely used polymer in formulation of mucoadhesive dosage forms (30). Feeling of warmth and subsequent cooling sensation were perceived after application of spray (around 12 min). None of the placebo formulations resulted in irritation, rashes and itching in any of the volunteers. Water washability of the placebo batches (B4P–B6P) was good except batch B6P containing higher concentration of Eudragit® RS100. Concentration of Eudragit® RS100 was not increased beyond 15% *w/w*, considering the results of water washability. The formulated batches containing Eudragit® RS100 as a film former (B4–B6) failed to meet the desired *in vitro* drug transport criteria with values of Y_1 greater than 11.75 μg/ml and Y_2 less than 215 min (Fig. 1). Considering the results of preliminary study, a combination of ethyl cellulose and Eudragit® RS100 was tried to achieve desired *in vitro* drug transport.

Factorial Design

A two-factor, three-level full factorial design was used for formula optimization. The levels of independent variables were determined from the results of preliminary batches. For Eudragit® RS100, the low, medium and high levels were 5%, 10% and 15%, respectively. Ethyl cellulose was used at low (3%), medium (5%) and high (7.5%) levels. The pH of formulated batches (B7–B15) ranged from 5 to 7. Table II shows that viscosity, volume of solution delivered upon each actuation and spray angle of formulated batches (B7–B15) ranged from 23.7 to 128.3 cPs, 0.14 to 0.33 ml and 78.7° to 91.5°, respectively. Viscosity of the liquids increased with total increase in polymeric concentration. All placebo batches (B7P–B15P) passed *ex–in vivo* film formation criteria (<8 min) except batch B7P containing high level of Eudragit® RS100 and ethyl cellulose. The appearance of film changed from shiny transparent to dull and opaque on increase in viscosity. Dermal adhesion and flexibility of films were good except batches B14P and B15P. Feeling of warmth and subsequent cooling sensation were perceived after application of spray (around 13 min). None of the placebo formulations resulted in irritation, rashes and itching in any of the volunteers. Water washability of the placebo batches (B7P–B15P) was moderate except batches B7P and B8P, which showed poor water washability because of presence of higher level of Eudragit® RS100 along with moderate to low level of ethyl cellulose.

The values of Y_1 (amount of drug transported at the end of first hour) and Y_2 (time required to transport 50% of the drug) for formulated batches (B7–B15) varied from 4.53 to 21.7 μg/ml and 107 to 660 min, respectively (Table III and Fig. 2). Hence, it can be concluded that selected independent variables exhibit significant influence on the values of Y_1 and Y_2. Further optimization was carried out by evolving mathematical models using linear regression analysis. Equation 4 shows the relationship between the amount of drug transported at the end of first hour (Y_1) and the independent variables.

$$Y_1 = 9.02 - 4.5X_1 - 4.07X_2 + 1.24X_1X_2 + 2.6X_1^2 \quad (4)$$

(Multiple R=0.994, p<0.05)

Equation 5 shows the relationship between the time required to transport 50% of the drug (Y_2) and the independent variables.

$$Y_2 = 328.33 + 163.33X_1 + 133.66X_2 + 58.75X_1X_2 \quad (5)$$

(Multiple R=0.992, p<0.05)

Contour plots of Y_1 and Y_2 were overlapped to locate the region of acceptability. The critical observation of the contour plot (Fig. 3) reveals that if X_1 and X_2 is varied from −0.5 to 0.12 and 0.1 to 1, respectively, the responses Y_1 and Y_2 are close to the target values of 8 μg/ml and 360 min, respectively. This limit may be considered for fine tuning of the formulation. Three formulations (A: X_1=−0.1; X_2=0.4, B: X_1=−0.45; X_2=1; and C=batch B16: X_1=0.12; X_2=0.1) theoretically satisfy the values of Y_1 and Y_2. A check-point batch (B16) was prepared. The experimental values of Y_1 and Y_2 for batch B16 were 7.91 μg/ml and 347 min, respectively (Table III). Batch B16 showed good sprayability. *Ex–in vivo* film formation of batch B16 passed the desired criteria of film formation (<8 min). The appearance of the film was shiny translucent. Dermal adhesion and flexibility of the film was good, whereas water washability was moderate. Thus, looking to overall results of sprayability, water washability and *in vitro* drug transport, batch B16 was ranked as one of the optimized batches and taken for further study.

A spray formulation (batch B16-A, Fig. 2) was prepared to evaluate the effect of acetone on *in vitro* drug transport by replacing acetone with alcohol (80% *v/v* ethanol) in batch B16. The Y_1 and Y_2 of batch B16-A was 16 μg/ml and 190 min, respectively. The probable reason for faster drug transport is delayed *ex–in vivo* film formation (720 s). The similarity factor (f_2=38.5) calculated using *in vitro* drug transport data of batches B16 and B16-A as reference and test profile, respectively, indicated significant difference in drug transport between both the batches (24). It is therefore concluded that acetone is indispensable in getting desired drug transport profile.

The *in vitro* drug transport data were analyzed for determining kinetics of drug transport. Model fitting was done using an in-house computer program, developed by the authors. Zero-order, first-order, Higuchi, Hixson–Crowell, Korsmeyer–Peppas and Weibull models were tested. Korsmeyer–Peppas model showed the least of SSR and therefore, it was used for further data analysis. The slope and intercept values were 0.706, 0.799, 0.737 and −2.031, −2.413, −2.161, respectively for the formulations B9, B10, and B11. Equations 6 and 7 for slope and intercept, respectively, were evolved using linear regression analysis taking slope or intercept as dependent variable and ratio of X_1 and X_2 in the formulation as independent variable. The values of multiple R were greater than 0.85 in both the equations indicating good correlation. The unified equation (eq. 8) was evolved by combining the equations of slope and intercept.

$$\text{Equation of slope} = 0.81 - (0.02 \times \text{ratio of } X_1 \text{ and } X_2) \quad (6)$$

$$\text{Equation of intercept} = -2.43 + (0.08 \times \text{ratio of } X_1 \text{ and } X_2) \quad (7)$$

$$\log f = (\text{equation of slope} \times \log t) + (\text{equation of intercept}) \quad (8)$$

Where f and t are fraction of drug transported and time, respectively. Model validation was done by comparing the values of observed and predicted drug transport for three formulations (batches B9–B11) as well as for a check-point batch B16 containing 10.6% Eudragit® RS100 and 5.25% ethyl cellulose. Similarity factor (f_2) was calculated considering calculated and observed drug transport data as reference and test profile, respectively (24). Batches B9–B11 and B16 showed insignificant difference in observed and predicted drug transport with similarity factor (f_2) greater than 64 in each case.

The effect of eutectic mixture on drug permeation was checked using shed snake skin as a biological membrane (31,32) in Franz diffusion cell. The UV spectrum remained unchanged during *in vitro* drug transport study, indicating stability of fluconazole during the analysis. Figure 4 shows that the amount of drug transported and present in the receptor compartment per milliliter at the end of first hour, Y_1 was 8.18 and 2.78 μg/ml for batches B16 and B17, respectively. The Y_2 values were 367 and >720 min, respectively. Incomplete drug transport (less than 45%) was seen from the batch B17 formulated without using eutectic mixture. The probable reason for this difference could be presence of camphor and menthol in batch B16. It is reported that camphor and menthol works as penetration enhancer (8). Camphor and menthol causes leaching of the lipids present in the skin and thus causes pore formation (24). The difference in drug transport between batches B16 and B17 through shed snake skin was found to be significant at 5% level with $t_{\text{calculated}} = 2.7 > t_{\text{critical two-tail}} = 2.05$. Hence, it can be concluded that eutectic mixture of camphor and menthol significantly improved the drug permeation. Batch B16 showed similar *in vitro* drug transport through nylon (reference profile) and biological membrane (test profile) with similarity factor (f_2) of 86. Probable reason for this behavior could be high concentration (10%) of eutectic mixture in the formulation.

Stability Study

Short-term stability study of the optimized batch B16 was carried out for 2 months at 25±2°C. Table IV shows that pH, viscosity, volume of solution delivered upon actuation, spray angle and *ex–in vivo* physical characteristics of optimized batch B16 remained unchanged during the study. Unpaired t test with equal variance indicated insignificant difference in the *in vitro* drug transport from the optimized batch B16 at $p=5\%$ with $t_{\text{calculated}} = 0.07 < t_{\text{critical one-tail}} = 1.71$. The amount of drug transported at the end of 1 h (Y_1) and time required to transport 50% of the drug ($Y_2, t_{50\%}$) of optimized batch (B16) at the end of stability study were 7.78 μg/ml and 364 min, respectively.

CONCLUSION

Modified transport transdermal spray of fluconazole was developed using 3^2 full factorial design. The optimized batch B16 containing 10.6% Eudragit® RS100 and 5.25% ethyl cellulose yielded mucoadhesive and flexible film on the human skin. The *in vitro* drug transport at the end of first hour was equal to the minimum inhibitory concentration (8± 0.4 μg/ml) with $t_{50\%}$ of (360±18 min). The eutectic mixture showed increased penetration of the drug through shed snake skin. Batch B16 passed short-term stability study, carried out at 25±2°C, with no change in performance characteristics of the product.

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