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Development of cellulosic polymer based gel of novel ternary mixture of miconazole nitrate for buccal delivery

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ABSTRACT

Aim of the present investigation was to develop cellulosic polymer based mucoadhesive antifungal gel comprising novel ternary mixture of miconazole nitrate (MN) for buccal delivery. Crosslinking of gel was made by adjusting pH with triethanolamine (TEA) and gel formulation was optimized on the basis of flux of MN (0.562–1.751 mg/cm^2/h) calculated from *ex vivo* permeation study. Based on statistically validated polynomial equation and plotted response surfaces, B17 was found to be the optimum batch. Texture profile in terms of adhesiveness (3.24 ± 0.012 g), firmness (10.83 ± 0.067 g), spreadability (3.63 ± 0.033 mJ) and extrudability (35.6 ± 0.1 mJ) of B17 was evaluated using a novel instrumental approach. The texture parameters were found to be consistent over 90 days. Ternary mixture containing gel showed broader zone of growth inhibition (32.67–47.33 mm) in comparison to marketed formulation containing pure MN (17.50–40.33 mm) against selected strains of fungi. In conclusion, consistent and effective mucoadhesive antifungal gel of MN with extended residence time in oral mucosa was developed.

1. Introduction

Drug delivery *via* buccal mucosa is a very challenging task, especially when the drug is very slightly soluble in water and has no/very low systemic availability when administered orally. Permeability of the drug through biological membrane is the second point of consideration, in order to get the desired systemic availability. A BCS class II drug could possibly be a good choice for buccal delivery. One of the well-known member of BCS class II drugs is imidazole derivative miconazole nitrate (MN) which is a potent antifungal drug used in various ailments like foot lesions (Sharma et al., 2011), cutaneous mycosis (Ghaninejad et al., 2009), diaper dermatitis (Eichenfield & Bogen, 2007), angular cheilitis (Cross & Short, 2008; MacFarlane, Ferguson, & MacKenzie, 1978) and oropharyngeal candidiasis (Lalla & Bensadoun, 2011). Variety of topical formulations comprising MN is available in the market however its oral/oropharyngeal delivery is not attempted yet which is of utmost importance (Kimbleton, Liu, Sun, & Wang, 1999). The possible reason could be its very less aqueous solubility; however it has very high permeability (Nafee, Ismail, Boraie, & Mortada, 2003). Recently, a novel ternary mixture (SolD-IC4) of MN, β-CD and lactose with enhanced aqueous solubility of MN was reported by us. Owing to the presence of β-CD and lactose, this ternary mixture enhanced the aqueous solubility of MN *i.e.* from 110.4 to 57,640.0 μg/ml (316 times higher). The remarkable upsurge in solubility of MN by ternary mixture was possibly due to blending of water soluble polymers, *i.e.* lactose with β-CD which enhanced the solubilizing nature of β-CD (Rai, Dwivedi, Yadav, Chanotiya, & Saraf, 2013). Therefore, ternary mixture (SolD-IC4) of MN was used to develop the buccoadhesive antifungal gel formulation.

Cellulosic mucoadhesive polymers are recognized as a smart strategy to extend the residence time and to enhance explicit localization of drug on several membranes (Khan, Gajbhiye, Singhavi, & Yeole, 2012; Pathak, Chhabra, & Pathak, 2013). Study of mucoadhesive properties of various polymers revealed that the bioadhesive potential in hydrophilic macromolecules is due to abundant hydrogen bond forming groups that help macromolecules to hydrate and swell in aqueous solutions (Lefnaoui & Moulai-Mostefa, 2011) under some specific stimuli like change in pH, temperature, light, electric field and osmotic pressure

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(Marcombe et al., 2010). Cellulosic polymers such as hydroxyl ethyl cellulose (HEC), hydroxypropyl methyl cellulose (HPMC) and sodium carboxymethylcellulose (SCMC) (Schmaljohann, 2006) are some of the well-known mucoadhesive polymers which have been used in the development of oral, nasal, anal and vaginal dosage forms (Bansal et al., 2009; Gonjari et al., 2009). These polymers are biodegradable and have a high degree of mucoadhesive properties with swelling in aqueous solvents (Gross & Kalra, 2002). A combination of HEC and carbopol was used to get escalated residence time of the drug and optimum mucoadhesiveness of gel in oral mucosa (Lefnaoui & Moulai-Mostefa, 2011). Therefore, the aim of the present investigation was to develop cellulosic polymer based mucoadhesive antifungal gel incorporating novel ternary mixture (SolD-IC4) in miconazole nitrate for buccal delivery.

2. Materials and methods

2.1. Materials

A novel ternary mixture of miconazole nitrate (SolD-IC4) was formulated using the methodology reported by Rai et al. (2013). The above ternary mixture was used to develop the mucoadhesive antifungal gel. Hydroxypropyl methyl cellulose (HPMC), carbopol 940, methyl paraben (MP) and propyl paraben (PP) were purchased from HiMedia Pvt. Ltd. (Mumbai, India). Glycerol was purchased from Qualigens Fine Chemicals (Mumbai, India). Polyethylene glycol 400 (PEG 400), propylene glycol (PG), hydroxyethyl cellulose (HEC) and sodium carboxy-methyl cellulose (SCMC) were purchased from CDH Ltd. (New Delhi, India). Tween 20 and Tween 80 were purchased from Thomas Baker Pvt. Ltd. (Mumbai, India). Triethanolamine was purchased from Fisher Scientific (Mumbai, India). Cultures of fungus *viz. Candida albicans* (CA) and *Sporothrix schenckii* (SS) were obtained from CSIR-Institute of Microbial Technology, India and *Cryptococcus neoformans* (CN) was received from All India Institute of Medical Sciences, New Delhi. All the chemicals used in the study were of analytical grade and double distilled water was used throughout the study.

2.2. Methods

2.2.1. Selection of polymer

Polymers were selected on the basis of their adhesiveness and gelling behavior. Pilot batches of gel were prepared using different grades of polymers such as HPMC, SCMC, HEC in various permutations and combinations for instance HEC and HPMC, HEC and SCMC, HPMC and SCMC, carbopol and HEC, carbopol and HPMC. A combination of carbopol and HEC was selected on the basis of its optimum adhesiveness and good consistency. At different concentrations of carbopol and HEC various prototype were prepared. Finally 1:1 (w/w) ratio of these polymers was found to be best which was selected as a final polymer combination to develop the desired gel.

2.2.2. Preparation of gel

Propyl paraben (0.05%, w/v) and methyl paraben (0.1%, w/v) were taken as a preservative in a beaker containing propylene glycol (1.0%, v/v as penetration enhancer). These were mixed properly by gentle stirring (100 rpm) and heating (50 °C) on a hot magnetic plate (Magnetic Stirrer IKA RCT basic). A sufficient quantity of water was added in a beaker. Carbopol 940 (0.75%, w/v; polymer) was dispersed slowly into the solution and allowed to soak for 24 h. After hydration, HEC (0.75%, w/v; polymer) was dispersed into soaked carbopol 940 mixture and stirred for about 30 min at 600 rpm followed by the addition of glycerin (10%, v/v; humectant) and polyethylene glycol 400 (5%, v/v; humectant). Ternary mixture equivalent to 1% (w/v) of miconazole nitrate (SolD-IC4; antifungal agent), Tween 20 (4%, w/v; surfactant), Tween 80 (1%, w/v; surfactant) and ethanol (4%, v/v) were taken in a 15 ml centrifuge tube which was vortexed (Tarsons Spinix 2883) for 15 min. The SolD-IC4 solution was mixed in previously prepared polymer solution by vigorous stirring at 1200 rpm for 15 min with the help of a mechanical stirrer (IKA® Eurostar, IKA India Pvt. Ltd., India). Triethanolamine (pH adjuster) was added drop by drop to the final mixture and stirred thoroughly until clear viscous homogeneous gel was obtained. The pH of the prepared buccoadhesive antifungal gel was kept in the range of 6.0–8.0.

Table 1
Coded levels of the independent variables used in the experimental design for the formulation of buccoadhesive antifungal gel of ternary mixture of MN.

Variables	Coded X_i	Coded level					ΔX
		$-\alpha$	−1	0	+1	$+\alpha$	
Concentration of polymer blend	X_1	0.66	1.00	1.50	2.00	2.34	0.50
Concentration of TEA	X_2	0.17	0.20	0.25	0.30	0.33	0.05
Concentration of Tween 20	X_3	0.64	2.00	4.00	6.00	7.36	2.00

ΔX is the increment of each experiment factor value corresponding to one unit of the coded variables.

2.2.3. Experimental design

Based on the flux of MN, a response surface methodology using a three-factor-three-level central composite design was employed, in order to optimize the formulation parameters of buccoadhesive antifungal gel. Quadratic equation was demonstrated by Design Expert® (trial version 8.0.7.1 Stat Ease Inc. USA). The concentration of 1:1 (w/w) ratio of HEC and carbopol 940 (X_1; weight %), TEA concentration (X_2; weight %) and Tween 20 concentration (X_3; weight %) were selected as the independent variables and analyzed at three coded levels *i.e.* low, medium and high (−1, 0 and +1 respectively). The dependent variable investigated was the flux of MN (Y, μg/cm^2/h). The selected factor combinations indicating the actual and coded levels as per the design are represented in Tables 1 and 2. The experimentally determined data was fitted to the least square regression model to get the optimum value of the independent variables (Chaudhary, Rohilla, Rathee, & Kumar, 2013; Solomon, Sahle, Gebre-Mariam, Asres, & Neubert, 2012). The regression model equation for Y (flux of MN) was evaluated using quadratic models (Eq. (1)) generated for each response parameter.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{j=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j-i=1}^{2}\beta_{ij} X_i X_j \quad (1)$$

where Y is the level of the measured response; β_0, β_i, β_{ii} and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction coefficients, respectively; X_i and X_j stand for coded independent variables, the main effects and X_{ij} is the interaction between the main effects.

2.2.4. Characterization and evaluation of gel

2.2.4.1. Drug content. Gel equivalent to 10 mg of MN was dissolved in 100 ml distilled water and measured spectrophotometrically using UV–visible spectrophotometer (Shimadzu 1800) at 220.8 nm. Experiments were performed in triplicate.

2.2.4.2. pH evaluation. About 10% (w/v) solution of buccoadhesive antifungal gel in distilled water was used to measure the pH using a digital pH meter (Mettler Toledo LE-438) for all the experimental formulations (B1–B20). Experiments were performed in triplicate.

Table 2
Central composite design for the optimization of flux of MN of buccoadhesive antifungal gel.

Experimental run	Coded variables			Process variables			Response
	X_1	X_2	X_3	X_1	X_2	X_3	Y
1	−1	−1	−1	1	0.2	2	1.399
2	1	−1	−1	2	0.2	2	1.229
3	−1	1	−1	1	0.3	2	0.979
4	1	1	−1	2	0.3	2	0.666
5	−1	−1	1	1	0.2	6	1.409
6	1	−1	1	2	0.2	6	1.286
7	−1	1	1	1	0.3	6	0.997
8	1	1	1	2	0.3	6	0.685
9	−1.68	0	0	0.66	0.25	4	1.229
10	1.68	0	0	2.34	0.25	4	0.675
11	0	−1.68	0	1.5	0.17	4	1.483
12	0	1.68	0	1.5	0.33	4	0.562
13	0	0	−1.68	1.5	0.25	0.64	1.568
14	0	0	1.68	1.5	0.25	7.36	1.751
15	0	0	0	1.5	0.25	4	1.666
16	0	0	0	1.5	0.25	4	1.672
17	0	0	0	1.5	0.25	4	1.703
18	0	0	0	1.5	0.25	4	1.627
19	0	0	0	1.5	0.25	4	1.645
20	0	0	0	1.5	0.25	4	1.650

X_1, concentration of polymer blend (wt%).
X_2, TEA concentration (wt%).
X_3, Tween 20 concentration (wt%).
Y, Flux of MN (mg/cm^2/h).

2.2.4.3. Viscosity. Viscosity of buccoadhesive antifungal gels was measured by Brookfield Viscometer (DVLV-II+ pro model). The viscosity of the experimental formulations (B1–B20) was measured at 25 ± 1 °C and at 10 rpm speed using a spindle no. 61. Measurements were performed in triplicate.

2.2.5. Ex vivo drug permeation studies

To study the release behavior and to have an insight on the barrier properties of stratum corneum, an *ex vivo* permeation study was carried out for all the batches using buccal mucosa of goat with modified USP II type dissolution apparatus, enhancer cell and 200 ml glass jar instead of 900 ml merlon jar.

2.2.5.1. Preparation of mucous membrane. For *ex vivo* permeation studies, excised goat buccal mucosa was collected from the slaughter house on the day of the experiment, and kept in ringer's solution (pH 7.3) for further use. Adhering subcutaneous fat was cleaned using isopropyl alcohol, and immediately kept in phosphate buffer solution (pH 7.4), for its integrity to remain intact.

2.2.5.2. Permeation studies. Prepared buccal mucosa was used to perform *ex vivo* permeation studies of MN from buccoadhesive gel. A modified USP dissolution apparatus type II (Electrolab, Dissolution tester, EDT-08Lx) using enhancer cell and 200 ml capacity flasks instead of 900 ml were used in the study. About 200 mg of each testing sample (from B1 to B20) was placed in the donor enhancer cell, maintaining a complete and intimate contact with the mucosa (previously trimmed to the appropriate size) in such a manner that the stratum corneum faced the enhancer cell and dermis faced the receiving cell. The mucosa was backed by a hollow circular plate (exposing 1.77 cm^2 area of the mucosa). The receptor compartment enclosed phosphate buffer of pH 7.4 to allow 'sink' condition and to withstand MN solubilization. This compartment was constantly stirred and thermostated at 37 ± 0.5 °C with the help of the water jacket. The system was allowed to equilibrate for 30 min prior to collection of the first sample. An aliquot of 5 ml from receptor medium (200 ml) was collected for a duration of 10 h followed by replenishment by the same volume of fresh, preheated receptor medium at each sampling interval *i.e.* 0.5, 1, 2, 3, 4, 6, 8 and 10 h. The collected samples were analyzed spectrophotometrically.

The flux was determined by Fick's law of diffusion considering the transport of drugs across the skin barrier as a process of passive diffusion. The flux of MN (μg/cm^2/h) was calculated by the method reported elsewhere (Chaudhary et al., 2013). The cumulative drug release was calculated as the total concentration of drug in total volume divided by the surface area of mucosa. The flux (mg/cm^2/h) was calculated from the slope of the linear portion of the cumulative amount permeated per unit area *versus* time plot. Permeability coefficient (*Kp*) was calculated using the following equation:

$$Kp = \frac{J}{C} \quad (2)$$

where J is the flux and C is the initial concentration of MN.

2.2.6. Response analysis for optimization

The statistical polynomial equation generated by Design Expert® (Trial version 8.0.7.1) was established in terms of ANOVA. Total 20 runs (B1–B20) with triplicate center points were generated and the model was evaluated in terms of statistically significant coefficients and r^2 values. The compositions of optimized formulation over the whole experimental region were found by accompanying validated RSM results.

2.2.7. Texture analysis

Texture analysis of optimized batch (B17) was carried out against marketed formulations using the automatic CT3 Texture Analyzer (Brookfield Engineering Laboratories, USA) (Meher et al., 2013). Hardness/firmness, spreadability, extrudability and adhesiveness were evaluated for developed buccoadhesive gel using recommended specifications given elsewhere in supplementary materials (Brookfield, 2011). Texture analysis was performed in triplicate.

2.2.8. Antifungal activity

Qualitative antifungal activity of optimized batch of SolD-IC4 gel (B17), marketed antifungal formulation (BM) and placebo gel (B0) was performed against different pathogenic fungi employing agar well diffusion assay (Luqman, Diwivedi, Darokar, Kalra, & Khanuja, 2007; Zhang, Cui, Zhu, Feng, & Zheng, 2010). About 100 μl of the inoculum suspension of each test organism was distributed evenly over the surface of Sabouraud dextrose agar plate. A 7 mm well was bored in the center of each plate and 200 mg of each formulation was filled into it. The plates were incubated (LT-X, Labtherm, Kuhner) for 3 days at 28 °C (Javed, Shoaib, Mahmood, Mushtaq, & Iftikhar, 2012). Experiments were performed in triplicate and zone of growth inhibitions (ZGI) were measured in terms of millimeter (mm).

2.2.9. Stability studies

Stability analysis of the optimized formulation was carried out in accordance to the International Conference on Harmonization (ICH) guidelines. The optimized gel formulation (B17) was stored in well closed glass containers for a period of 90 days at 40 °C temperature and 75% relative humidity in a humidity chamber. At predetermined intervals; 0, 30, 60 and 90 days, samples were collected and their physicochemical evaluation parameters such as color, consistency, phase separation, texture analysis, pH and drug content evaluated.

3. Results and discussion

In our previous study, enhanced aqueous solubility of MN using a ternary mixture approach (SolD-IC4) was reported (Rai

Table 3
Ex vivo MN permeation through buccal mucosa of goat.

Batches	*Ex vivo* permeation (mg/cm^2) at different time points (h)								Flux of MN (mg/cm^2/h)	Permeability coefficient
	0.5	1	2	3	4	6	8	10		
1	0.79	1.060	1.24	2.30	4.10	6.20	9.74	14.16	1.389	0.046
2	0.24	1.160	1.60	2.10	4.10	6.60	8.82	12.12	1.229	0.041
3	0.15	0.840	1.56	2.60	3.40	5.26	7.95	9.32	0.979	0.033
4	0.14	0.650	1.57	2.70	3.22	4.08	5.38	6.75	0.666	0.022
5	0.37	1.880	2.87	3.04	4.50	9.60	11.45	13.37	1.409	0.047
6	0.34	1.850	2.87	3.76	4.01	6.54	10.05	13.51	1.286	0.043
7	0.37	1.376	2.18	2.95	3.78	5.89	7.67	10.39	0.997	0.033
8	0.19	0.755	1.15	1.93	2.50	3.88	5.34	6.82	0.685	0.023
9	0.78	1.940	2.87	3.94	5.88	8.58	10.36	12.39	1.229	0.041
10	0.27	0.793	1.24	2.10	3.28	4.45	5.40	6.66	0.675	0.023
11	0.07	0.333	1.22	3.50	4.46	7.36	10.67	13.93	1.483	0.049
12	0.26	0.840	1.35	1.88	2.57	2.72	3.570	6.72	0.562	0.019
13	0.65	1.845	2.87	3.80	5.70	9.88	13.11	14.90	1.568	0.052
14	0.35	0.690	4.70	6.97	10.29	13.73	15.62	15.60	1.751	0.058
15	0.09	0.365	1.25	2.10	3.40	7.02	11.20	15.98	1.666	0.056
16	0.08	0.349	1.45	2.20	3.40	6.98	11.40	16.03	1.672	0.056
17	0.09	0.370	1.27	2.12	3.46	7.19	11.40	16.34	1.703	0.057
18	0.07	0.413	1.56	2.35	3.12	6.82	11.02	15.78	1.627	0.054
19	0.07	0.424	1.57	2.30	3.15	6.83	11.44	15.89	1.645	0.055
20	0.09	0.347	1.28	2.10	3.40	6.98	11.20	15.77	1.650	0.055

et al., 2013). This mixture showed enhanced aqueous solubility of MN from 110.4 to 57,640.0 μg/ml which changed the solubility category of MN from very slightly soluble to soluble (Indian Pharmacopoeia, 2007). To ensure successful delivery of MN *via* buccal route buccoadhesive antifungal gel using SolD-IC4 was developed and characterized.

3.1. Characterization of gel

The pilot batches of prepared gel of various polymers were characterized for their elegance, adhesion and consistency based on sensorial evaluation. Adhesiveness and smooth consistency were the main point of concern because these points are in direct correlation of mucoadhesiveness. Carbopol 940 and HEC combination was finally selected to develop a gel owning to its good consistency and adhesiveness. Various prototypes of gel were prepared to get the optimum formulation.

Adhesiveness was found to decrease on decreasing HEC concentration. HEC alone produced very sticky gel in the concentration range of 0.75–1.0% (w/v) while carbopol 940 produced sticky gel with loose consistency. About 1.5% (w/v) of 1:1 ratio of carbopol 940 and HEC were found to show the optimum adhesiveness. Central composite design was employed in various ranges of concentration of the polymer blends, TEA and Tween 20. Statistically designed batches were evaluated for drug content, pH, viscosity and *ex vivo* drug permeation.

3.1.1. Drug content

Drug content of all twenty batches were found to be in the range of 89.10 ± 0.30 to 99.76 ± 0.09% as indicated in the supplementary table ST1.

3.1.2. pH value

pH value of 10% solution of all the prepared batches were determined and found to be in the range of 5.54 ± 0.02 to 7.93 ± 0.01 (represented in ST1). An increase in pH value was observed by increasing the concentration of TEA from 0.2 to 0.3% (v/v). This lies in the range of pH of the buccal mucosa. Formulated batches with 0.25% (v/v) TEA concentration was found to have a pH about 7.2 which could be administered easily *via* buccal mucosa.

3.1.3. Viscosity

Viscosity of all the 20 gel formulations was found to be in the range of 21,086.67 ± 45.96 to 45,468.00 ± 47.72 cP (ST1). The viscosity was found to increase by increasing the concentration of polymer (from 1 to 2%, w/v) and TEA (0.2–0.3%, v/v). This could be attributed to the enhancement in cross linking of polymer which occurs maximally at about neutral or alkaline pH and was found to increase when the pH of medium increased from acidic to neutral.

3.2. Evaluation of ex vivo permeation

Permeation studies were carried out for all the batches of SolD-IC4 loaded gel and the results are recorded in Table 3. Amongst all the batches prepared, B14 was found to display highest cumulative amount of drug permeated (15.6 mg/cm^2), flux (1.751 mg/cm^2/h) and permeability coefficient (0.058367). The permeation of MN was found to decrease by increasing the concentration of 1:1 (w/w) ratio of carbopol 940 and HEC from 1.0 to 2.0% (w/v) *i.e.* due to increase in crosslinking between SolD-IC4 and polymers. An increase in the concentration of TEA from 0.2 to 0.3% (v/v) showed a decrease in permeation of MN through mucosal barrier.

3.3. Model fitting to the data

A total of 20 runs were generated with the help of three factors, three level central composite design and responses of all the independent factors over dependent factor were observed. Response Y was found to be in the range of 0.6659–1.751 mg/cm^2/h at various independent parameters such as concentration of 1:1 (w/w) ratio of carbopol 940 and HEC (X_1), TEA concentration (X_2, wt%) and Tween 20 concentration (X_3, wt%) which were used in the range of 1–2%, 0.2–0.3% and 2–6% respectively. The best-fitted model was found to be quadratic and the values of goodness of fit (R^2), standard deviation (SD), and percentage coefficient of variation (%CV) have been recorded in Table 4 along with the regression equation generated for each response. All statistically significant ($p < 0.05$) coefficients are included in the equations. A positive value confirmed that a change in independent variable would lead to a considerable change in flux of MN and *vice versa.* The influence of the interaction between individual independent parameter over response Y is given in Table 4.

Table 4
Values of regression coefficients calculated for flux of MN.

Independent variable	Regression coefficient	Standard error	Significance level (p)
Constant	−6.08779	0.030	<0.0001
Linear			
X_1	3.40831	0.020	<0.0001
X_2	47.07347	0.020	<0.0001
X_3	0.064918	0.020	0.1530
Quadratic			
X_1^2	−1.09012	0.026	<0.0001
X_2^2	−98.98569	0.026	<0.0001
X_3^2	−5.56710E−003	0.026	0.2777
Interaction			
X_1X_2	−1.70800	0.019	0.1320
X_1X_3	4.85000E−003	0.019	0.8559
X_2X_3	−0.049000	0.019	0.8545
r^2			0.9821
Adjusted r^2			0.9660
Predicted r^2			0.8716
F			61.03
Probability > F			0.0001
Lack of fit F-value			14.75
Std. dev.			0.074
Mean			1.29
CV (%)			5.69
PRESS			0.39
Adequate precision			21.805

3.4. Two dimensional plots and response analysis of polynomial equations

Two dimensional contour plots and three dimensional response surface plots prepared for the response are shown in Fig. 1. Interaction between independent parameters, as shown by contour plot, is very useful to study the cumulative effect of the two factors at a particular point. Interaction terms are found to be very prominent as shown in counter plot plotted among X_1X_3 and X_2X_3 (Elliptical plot; Fig. 1) while X_1X_2 term revealed no interaction (circular plot).

3.5. Effect of independent variables on flux of MN

Final equation of response in terms of actual factors is shown below:

$$\begin{aligned} \textit{Flux of MN} = {} & -6.08779 + 3.40831X_1 + 47.07347X_2 + 0.064918X_3 \\ & - 1.7080X_1X_2 + 0.00485X_1X_3 - 0.049X_2X_3 - 1.09{X_1}^2 \\ & - 98.9856{X_2}^2 - 0.005567{X_3}^2 \end{aligned} \quad (3)$$

where $Y1$ is the flux of MN, X_1, X_2 and X_3 are the concentration of 1:1 (w/w) ratio of carbopol and HEC, concentration of TEA and concentration of Tween 20 respectively. The model F-value of 61.03 implies that the model is significant. F-value about 14.75 implies the significant lack of fit ($p < 0.0051$). In this case X_1, X_2, X_3 and X_1X_3 had more pronounced effect on the flux of MN. The predicted r^2 value 0.8716 is found to be in reasonable agreement with the adjustable r^2 (0.9660) (Table 4). Ratio 21.805 indicates an

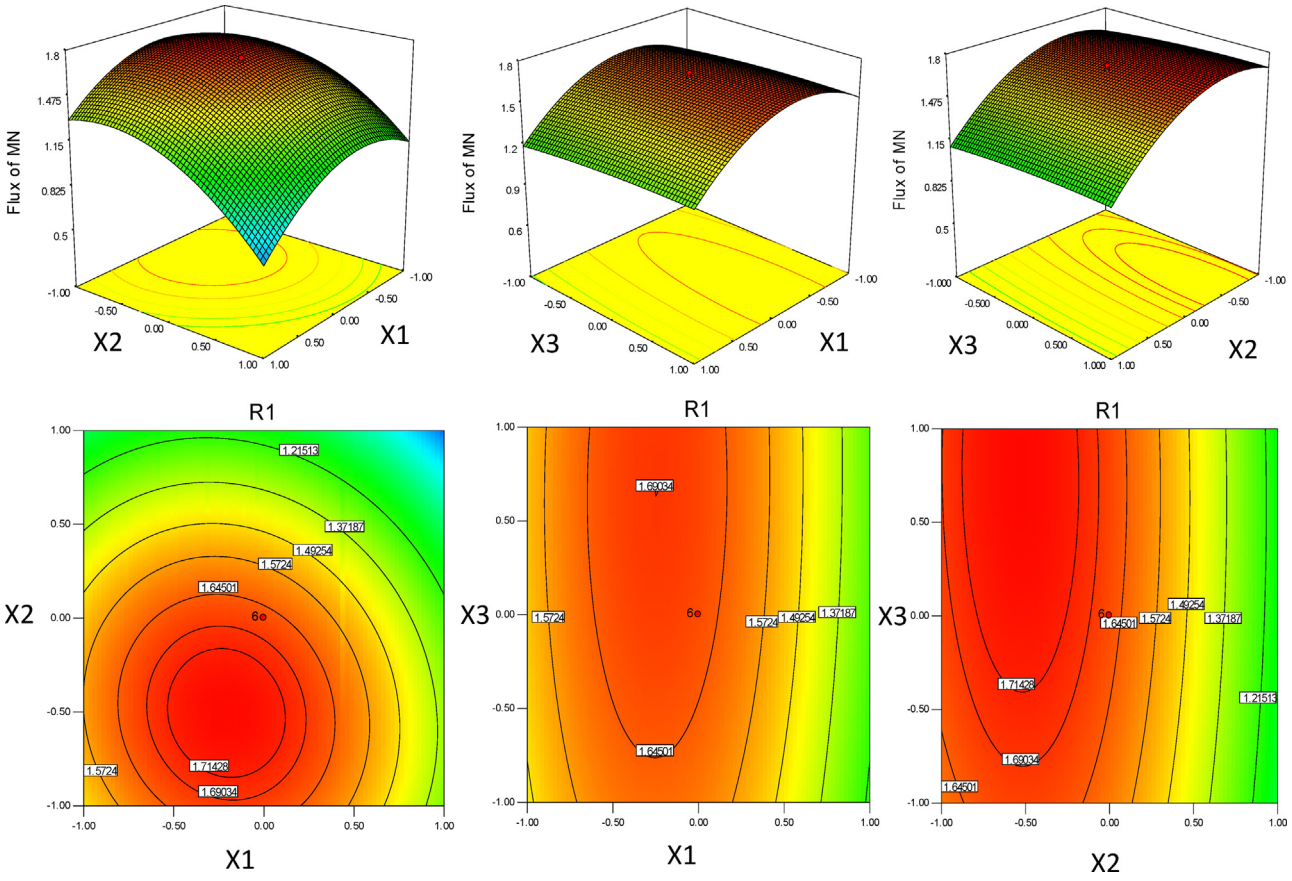

Fig. 1. Graphical representation of response surface plot and 2D contour plot showing effect of concentration of 1:1 (w/w) of carbopol 940 and HEC (X_1), concentration of TEA (X_2) and concentration of Tween 20 (X_3) on flux of MN ($Y1$).

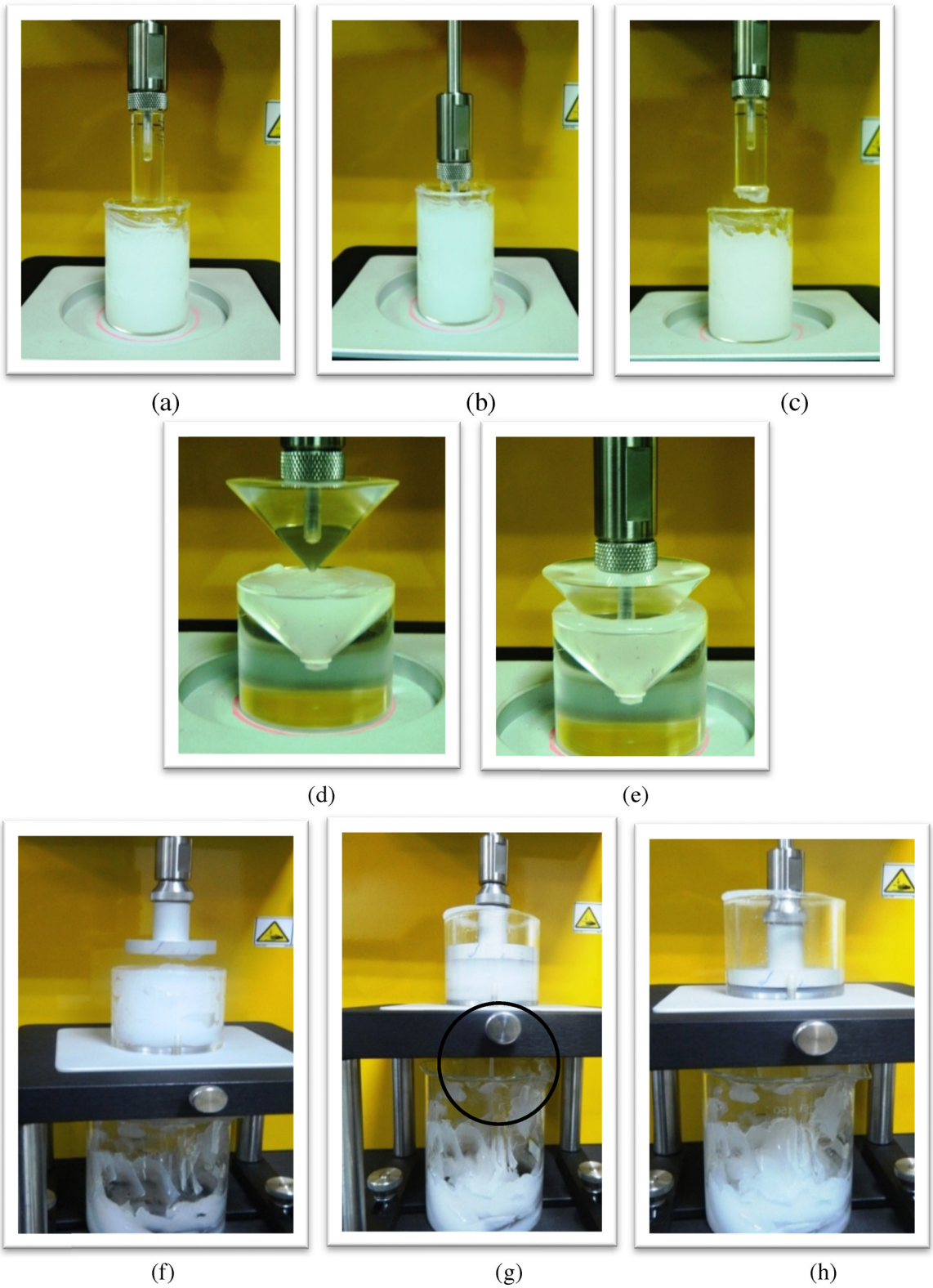

Fig. 2. Evaluation of texture profile of the developed gel using CT3 texture analyzer. (a), (b) and (c) are representing the various steps of hardness/firmness and adhesiveness testing where TA 10 probe is moving toward the TA-BT-KIT fixture equipped with gel filled beaker; (d) and (e) are depicting the steps of spreadability testing, where male probe is moving toward the female fixture; (f), (g) and (h) are showing the steps of extrudability testing with TA-DEC probe moving toward the fixed extrusion cell.

adequate signal. Contour plots show the effect of different independent variables on the flux of MN (Y). Increasing amount of polymer as well as TEA leads to decrease in the size of the channels (drug remains entrapped in the gel complex of the polymer) which results in decreased permeation of MN owing to the decreased degree of swelling of the gel. Owing to pH dependent gelling behavior of carbopol, increased crosslinking was observed in the gel as the concentration of TEA increased from 0.2 to 0.3% (v/v) which implies increase in the pH of the gel from 5.58 to 7.95. Effect of Tween 20 concentration in the formulation of the gel was found to be less as

Table 5
Texture profile and antifungal activity of optimized SolD-IC4 buccoadhesive gel and marketed oral formulation.

Parameters		Formulations		
		B 0	B 17	B M
Texture profile	Firmness (g)	13.33 ± 0.409	10.83 ± 0.067	9.2 ± 0.322
	Spreadability (mJ)	4.6 ± 0.153	3.63 ± 0.033	2.88 ± 0.053
	Extrudability (mJ)	39.767 ± 0.99	35.6 ± 0.1	33.93 ± 0.23
	Adhesiveness (g)	2.87 ± 0.041	3.24 ± 0.012	3.093 ± 0.064
Zone of growth inhibition in mm (percentage inhibition[a])	*Cryptococcus neoformans* (AIIMS, New Delhi)	Nil (0)	32.67 ± 1.45 (39.36)	17.50 ± 0.41 (21.08)
	Candida albicans (MTCC 227)	Nil (0)	36.50 ± 1.22 (43.98)	31.33 ± 0.33 (37.75)
	Sporothrix schenckii (MTCC 1359)	Nil (0)	47.33 ± 0.88 (57.03)	40.33 ± 1.20 (48.59)

Values are mean ± SEM, $n = 3$. B0, placebo gel; B17, optimized gel; BM, marketed formulation.
[a] Values in the parenthesis indicate percent zone of growth inhibition against the respective micro-organisms.

Table 6
Stability study of optimized buccoadhesive antifungal gel (B17).

Formulations characteristics		Days			
		0	30	60	90
Color[a]	Acceptable	Acceptable	Acceptable	Acceptable	
Separation[a]	None	None	None	None	
Consistency[a]	Acceptable	Acceptable	Acceptable	Acceptable	
Viscosity (cP)[b]	30,150.7 ± 81.50	30,131 ± 51.48	30,134.3 ± 49.65	30,121 ± 43.132	
Texture profile[b]	Firmness (g)	10.83 ± 0.067	10.83 ± 0.033	10.83 ± 0.088	10.883 ± 0.017
	Spreadability (mJ)	3.63 ± 0.033	3.83 ± 0.067	3.8 ± 0.057	3.85 ± 0.029
	Extrudability (mJ)	35.6 ± 0.100	35.567 ± 0.088	35.7 ± 0.200	35.77 ± 0.067
	Adhesiveness (g)	3.24 ± 0.012	3.26 ± 0.021	3.297 ± 0.012	3.3 ± 0.009
pH[b]	6.71 ± 0.007	6.707 ± 0.009	6.72 ± 0.003	6.75 ± 0.012	
Drug content[b]	97.55 ± 0.029	97.53 ± 0.017	97.53 ± 0.020	97.51 ± 0.006	

Stable: change < 10%; acceptable: 10% < change < 20%; unstable: 20% < change <40%; unacceptable: change > 40%.
[a] Based on sensorial evaluation.
[b] Tolerance of stability after one freeze/thaw cycle.

compared to other independent parameter. At the highest concentration of TEA (0.334%, v/v; B12) flux of MN was found to be very less (0.5621 mg/cm^2/h) due to increased cross-linking of gel and hence viscosity.

3.6. Optimization and data analysis

Based on physical evaluation and skin permeation study of prepared batches (B1–B20), formulation B14 to B20 exhibited good results and among these, highest flux of MN was observed for B14. Contour plots and three dimensional response surfaces of the central composite design confirmed B17 to be the optimized batch because of presence of interaction between independent parameters such as among X_1 and X_3 as well as among X_2 and X_3 while no interaction was revealed by X_1 and X_2 terms. Therefore, 1.5% (w/v) of polymer, 0.25% (v/v) of TEA and 4% (v/v) of Tween 20 were found to be optimum parameters for permeation of MN from SolD-IC4 gel *i.e.* the optimized batch (B17).

3.7. Texture profile

A novel instrumental technology was used to emulate human sensorial perception in terms of firmness, adhesiveness, spreadability and extrudability while applying semisolid formulations on skin (Aime, Arntfield, Malcolmson, & Ryland, 2001; Brookfield, 2011; Meher, Yadav, Sahu, & Sinha, 2012). These properties are collectively used to evaluate texture profile of the formulations. The texture of B17 was analyzed and compared with marketed formulation (BM) and gel base (B0). Pictures of texture evaluation are presented in Fig. 2 and the values of the evaluated parameters are recorded in Table 5. Firmness of B17 was found to be 10.83 ± 0.067 g while of BM and B0 were 9.2 ± 0.322 g and 13.33 ± 0.409 g respectively. Firmness is the maximum positive force required to deform the gel sample by a finger. B17 was found to be comparatively harder than BM *i.e.* it required slightly higher but acceptable force to deform its surface. Spreadability of B17 (3.63 ± 0.033 mJ) was found to be better than that of B0 (4.6 ± 0.153 mJ) while BM (2.88 ± 0.053 mJ) showed the best spreadability amongst all batches. Work done required to extrude B17 gel from the tube was found to be 35.6 ± 0.1 mJ while to extrude BM and B0 it was found to be 33.93 ± 0.23 and 39.767 ± 0.99 mJ respectively. Spreadability and extrudability of semisolid formulation are some of the important texture parameters which are preferred for an organoleptically esthetic product (Mahalik & Nambiar, 2010). Technically certain amount of work-done (mJ) is required in order to spread the sample (B17) at the surface and to uniformly extrude it from a packaging tube (Contreras & Sanchez, 2002; Mahalik & Nambiar, 2010; Meher et al., 2012). Higher spreadability and extrudability of B17 than that of BM revealed that greater work done is required to spread and extrude B17 than BM. This is due to the presence of slightly higher concentration of TEA and polymer. About 3.24 ± 0.012 g force was required to separate B17 from surface which reveals its higher adhesiveness than that of BM (3.093 ± 0.064 g) and B0 (2.87 ± 0.041 g). Adhesiveness of the formulation is the key parameter for buccoadhesive character and thereby an increased time of contact leading to higher absorptivity. Adhesiveness is defined as the maximum force required in overcoming the attractive force between any surface and formulation and is characterized as a measure of stickiness of the sample (Brookfield, 2011; Inoue et al., 2012). Adhesiveness of the B17 gel was found to be more as compared to BM because of the presence of optimum concentration of blend of a mucoadhesive polymer HEC and carbopol 940.

3.8. Antifungal activity

Antifungal evaluation of B17, marketed formulation (BM) and placebo (B0) indicated that B17 and BM have potential fungicidal activity against the tested pathogenic fungi (Table 5).

Placebo of the developed antifungal gel showed no sign of activity. Comparatively broader zone of growth inhibition (ZGI) *i.e.* 32.67 ± 1.45–47.33 ± 0.88 mm was observed in case of B17 (containing SolD-IC4) compared to BM (17.50 ± 0.41–40.33 ± 1.20 mm). This may occur due to the existence of enhanced aqueous solubility of MN in ternary complex which lead to the deep penetration of MN in to the growth media.

3.9. Stability study

Color, consistency, viscosity, texture profile, pH and drug content, of B17 were found to be consistent and no signs of separation and deterioration were observed over a period of 90 days (Table 6). This revealed the reproducibility of the physical and chemical parameters which ensures a consistent quality of the developed gel formulation (B17) over a longer duration.

4. Conclusion

A stable, consistent, mucoadhesive antifungal gel of ternary mixture (SolD-IC4) of MN was developed successfully. *Ex vivo* permeation studies revealed proficiency of developed gel toward efficient absorption of drug through buccal mucosa. Combination of hydroxyethyl cellulose and carbopol 940 (1:1, w/w ratio) played a key role in providing optimum adhesiveness of the gel. A simple, reproducible and precise instrumental method was established for the evaluation of different texture parameters of the gel such as adhesiveness, firmness, spreadability as well as extrudability. Prominent fungicidal action of ternary mixture containing gel (ZGI; 32.67–47.33 mm) as compared to pure MN containing formulation (ZGI; 17.50–40.33 mm) revealed the efficient diffusion of MN into the growth media due to the enhanced aqueous solubility of MN.

Conflict of interest

The authors state no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.12.019.

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