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Research Article

A Novel Itraconazole Bioadhesive Film for Vaginal Delivery: Design, Optimization, and Physicodynamic Characterization

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Abstract. The purpose of this work was to design and optimize a novel vaginal drug delivery system for more effective treatment against vaginal candidiasis. Itraconazole was formulated in bioadhesive film formulations that could be retained in the vagina for prolonged intervals. The polymeric films were prepared by solvent evaporation and optimized for various physicodynamic and aesthetic properties. In addition, percentage drug retained on vaginal mucosa was evaluated using a simulated dynamic vaginal system as function of time. A polymeric film containing 100 mg itraconazole per unit (2.5 cm×2.5 cm) have been developed using generally regarded as safe listed excipients. The pH of vaginal film was found to be slightly acidic (4.90±0.04) in simulated vaginal fluid and alkaline (7.04±0.07) in water. The little moisture content (7.66±0.51% *w*/*w*) was present in the film, which helps them to remain stable and kept them from being completely dry and brittle. The mechanical properties, tensile strength, and percentage elongation at break (9.64 N/mm^2 and 67.56% for $ITRF_{65}$) reveal that the formulations were found to be soft and tough. The films ($ITRF_{65}$) contained solid dispersion of itraconazole (2.5)/hydroxypropyl cellulose (1)/polyethylene glycol 400 (0.5), which was found to be the optimal composition for a novel bioadhesive vaginal formulation, as they showed good peelability, relatively good swelling index, and moderate tensile strength and retained vaginal mucosa up to 8 h. Also, the film did not markedly affect normal vaginal flora (lactobacillus) and was noncytotoxic as indicated by the negligible decrease in cell viability.

KEY WORDS: bioadhesive films; factorial design; physicochemical properties; vaginal drug delivery.

INTRODUCTION

Vaginitis is one of the most frequent genital infections occurring in women of all age groups (1). It has been reported that 30–35% of vaginitis episodes are due to *Candida albicans*. About 75% of women experience an acute episode of vaginal candidiasis once in their lifetime, most commonly during pregnancy or after treatment with antibiotics (2). The increasing incidence of *Candida* vaginitis has highlighted an urgent need of appropriate therapeutic strategies aimed for successful eradication of infectious agent, short-term treatment, achievement of high drug levels at the target site, avoidance of first-pass metabolism, and safety (3). From such a perspective, topical antifungal chemotherapy could represent a rational choice for treatment of vaginal candidiasis because of the toxicity of antifungal drug after systemic administration (4,5). The introductions of potent imidazole and triazole antifungal agents have significantly altered the duration of treatment of acute vaginal candidiasis (6,7). Itraconazole (ITR) is a new triazole antifungal drug with a broad spectrum of activity (8,9). This agent appears to be an attractive alternative for treatment of vaginal candidiasis because of its enhanced activity against *Candida* species, leading to short courses of therapy (10–12).

Several drug delivery systems are used for treatment of vaginal infections (13). Indeed, conventional vaginal formulations (suspensions, cream, and solutions) cannot maintain effective drug concentration for prolonged period of time inside the vaginal cavity due to their short residence time at the site of administration. Tablets and gels associated with limitation of leakage and messiness cause inconvenience to users, leading to poor patient compliance (14). Bioadhesive vaginal drug delivery systems (BVDDS) may avoid these problems. In addition to effective treatment of vaginitis, the formulation should adhere to vaginal mucosa in order to bring drug in contact with target tissues for sufficient period of time and prevent expulsion of formulation (15,16). Bioadhesive polymers have the capability to adhere to mucous–epithelial surfaces and act as most promising candidate to be used in the design of BVDDS for topical application (17–19). Vaginal films are more preferred over gels (20) by women in different areas of the world due to its aesthetic appeal. In addition, the film formulation possesses several advantages of easy storage and handling, ease of application (applicator not required for administration), and improved stability of drug at tropical climate. Hence, efforts were made to develop a BVDDS in the form of film for ITR.

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Finally, the drug must be in a bioavailable form. Optimal action will be observed with the solution form of the drug. A colloidal dispersion may also be preferred for local action on vaginal mucosa (21). ITR possesses very poor water solubility (~4 μg/mL at acidic pH) that makes it difficult to formulate in bioadhesive delivery system (22). One approach, which has been applied for producing BVDDS of ITR, is the use of solid dispersion of ITR (SDITR) and hydroxypropyl methylcellulose (HPMC) E15 in which drug particles are homogeneously distributed throughout the hydrophilic polymer (23). SDITR can improve wettability of ITR, which may help in the development of the bioadhesive delivery system. Also, SDITR form colloidal dispersion in simulated vaginal fluid (SVF) is essential for local action on vaginal mucosa. SDITR was prepared by previously reported spray drying method and characterized by differential scanning calorimetry and X-ray diffraction technique.

In the present work, we are reporting the design and physicodynamic characterization of ITR containing bioadhesive vaginal film. The vaginal films were targeted to retain on vaginal mucosa up to 8 h and should possess aesthetic appeal such as flexibility, softness, and free of any sharp edges to avoid mechanical injuries during insertion. These desired features of film formulation can not only improve the patient's compliance but also provide good bioadhesion and retain in the vaginal cavity for prolonged time periods.

MATERIALS AND METHODS

Materials

ITR was procured as a gift sample from Intas Pharmaceutical Limited (Ahmedabad, India). HPMC E15 was gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Hydroxypropyl cellulose (HPC; MW, 140,000) was purchased from Innovative Chemicals (Mumbai, India). Carrageen and hydroxyethyl cellulose (HEC) were purchased from Himedia (Mumbai, India). Dichloromethane, methanol, and polyethylene glycol 400 (PEG 400) were purchased from S. D. Fine Chemicals (Mumbai, India). All other chemicals used were of analytical grade.

Drug–Excipient Compatibility Study

Compatibility of ITR with different polymers to be used for development of film formulation was studied by thermal analysis. In addition, samples of pure ITR, excipients, $ITRF_{65}$, and their physical mixture were characterized by differential scanning calorimeter (DSC 60, Shimadzu, Japan). Samples were crimped in aluminum pans and analyzed at a nitrogen flow of 30 mL/min and heating rate of 15°C/min from 35°C to 300°C.

Preparation of Films

Bioadhesive films (BF) were prepared by solvent evaporation technique (24), using specific amount of SDITR (250 mg) and various ratios of film-forming polymers to plasticizer. SDITR was added in distilled water, and sonication was done for 30 min to obtain an aqueous dispersion. Subsequently, film-forming polymer and plasticizer were added to this dispersion. This polymeric dispersion was stirred on magnetic stirrer (Remi equipments Ltd., Mumbai, India) for 30 min followed by sonication for 15 min and kept for 2 h to remove all the entrapped air bubbles. Polymeric dispersion of ITR was uniformly spread onto a plastic plate of defined area (50 cm^2) and dried in a vacuum oven at 50°C for 16 h. Dried films were carefully peeled off from the plate surface and cut into pieces of defined size (2.5 cm×2.5 cm). Film was sealed in sachets prepared from polyethylene laminated aluminum foil and stored at temperature of 30± 2°C and relative humidity 60±5% until further analysis.

Factorial Design and the Desirability Function

To study all possible combinations of all factors at all levels (two factors, three levels), full-factorial design was constructed and conducted in a fully randomized order (25). The dependent variables to be measured are tensile strength, percentage of elongation, and percentage of drug retained on the vaginal mucosa up to 8 h ($Y_{8\ h}$). The composition and responses of the 3^2 factorial designs are shown in Table I. Two independent factors, concentration of HPC (X_1) and PEG 400 (X_2), were set at three different levels as shown in Table II. High and low levels of each factor were coded as 1 and −1, respectively, and the mean value was zero. The range of a factor was chosen in order to adequately measure its effects on the response variables. This design was selected as it provides sufficient degrees of freedom to resolve main effects as well as the factor interactions.

The desirability function was used for optimization of formulation composition. In addition, the responses have to

Table I. Composition and Responses for 3^2 Factorial Designs

	Independent variables		Response values			
Batches	X_1	X_2	Tensile strength (N/mm^2)	% Elongation	$Y_{8\ h}$	Overall desirability
$ITRF_{61}$	−1	−1	7.83	51.42	15.14	0.00
$ITRF_{62}$	−1	0	7.23	54.37	12.25	0.00
$ITRF_{63}$	−1	1	6.99	55.91	9.77	0.00
$ITRF_{64}$	0	−1	10.46	61.28	18.63	0.81
$ITRF_{65}$	0	0	9.64	67.56	17.75	0.92
$ITRF_{66}$	0	1	9.15	70.08	14.06	0.78
$ITRF_{67}$	1	−1	10.31	59.84	15.03	0.65
$ITRF_{68}$	1	0	9.85	60.61	15.41	0.68
$ITRF_{69}$	1	1	9.27	63.64	13	0.62

Table II. Independent Factors Set at Three Different Levels

Independent variables	Levels		
	Low	Medium	High
X_1 = amount of hydroxypropyl cellulose (mg)	80	100	120
X_2 = amount of polyethylene glycol 400 (mg)	40	50	60

be combined in order to produce a product of desired characteristics. The applications of desirability function combine all the responses in one measurement and give the possibility to predict optimum levels for the independent variables (26). The combination of responses in one desirability function requires the calculation of individual functions. An ideal film should have a moderate tensile strength, high percentage elongation, and high percentage of drug retained on vaginal mucosa. The individual desirability for each response was calculated using the methods discussed below (25,26).

In this study, there were no specific requirements for tensile strength of optimum formulation. Therefore, the range of values of produced formulations was selected. As moderate tensile strength was desired, the formulations that have its value within the range of 7.0–10.0 have a desirability of 1, while the formulations which have values out of this range have a desirability of 0. These can be described by the following equations:

$$d_1 = 0 \ for\, Y_i < Y_{\min}$$
$$d_1 = 1 \ for\, Y_{\min} < Y_i < Y_{\max}$$
$$d_1 = 0 \ for\, Y_i > Y_{\max}$$

where d_1 is the individual desirability of tensile strength.

In addition, optimum vaginal film formulation should have high percentage elongation and high percentage of drug retained on vaginal mucosa. Desirability functions of these responses were calculated using the following equation:

$$d_2 \, or \, d_3 = \frac{Y_i - Y_{\min}}{Y_{target} - Y_{\min}} \ for\, Y_i < Y_{target}$$
$$d_2 \, or \, d_3 = 1 \ for \ \ Y_i > Y_{target}$$

where d_2 is the individual desirability of percentage elongation, and d_3 is the individual desirability of percentage drug retained on vaginal mucosa at 8 h. The values of Y_{target} and $Y_{\min}$ for percentage elongation are 70.08 and 51.42, and the values of Y_{target} and $Y_{\min}$ for percentage drug retained on vaginal mucosa are 18.63 and 9.77, and Y_i is the experimental result. The overall desirability values were calculated from individual values by using the following equation:

$$D = (d_1 d_2 d_3)^{1/3}$$

Pharmaceutical Characterization

Bioadhesive film formulation was characterized for various aesthetic (appearance, odor, color, flexibility, and peelability) and physicodynamic properties (27,28).

Thickness of each sample was measured using a thickness tester (Model 110, 0.01 mm capacity, Mitutoyo Manufacturing Corporation Ltd., Japan) at five locations (center and four corners) and mean thickness was calculated.

Viscosity of polymeric dispersion of film was determined by Brookfield cone and plate rheometer LVDVIII. For studying viscosity and pH of film, one unit of formulation was dispersed in 10 mL each of distilled water and SVF. Viscosity of BF was measured at 33.8°C by keeping speed at 5 rpm and shear rates of 10 s^{-1}. The pH of film dispersion was measured with pH meter (LABINDIA Pvt. Ltd., New Mumbai). Each parameter was measured in triplicates for each film.

Morphology Study

Morphology of prepared film was observed under a scanning electron microscope (Model JSM 5610LV, Jeol, Japan). The samples were attached to slab surfaces with double-sided adhesive tapes, and scanning electron photomicrograph was taken at ×1,000 magnification.

Moisture Content

For determination of moisture content, piece of film (2.5 cm×2.5 cm) was weighed and kept in desiccators containing calcium chloride at 40°C for 24 h. Films were removed from desiccator and reweighed until a constant weight was obtained. The percentage of moisture content was calculated as the difference between initial weight and final weight with respect to initial weight (28).

Measurement of Swellings Index

Film swelling study was carried out in a simulated vaginal environment using SVF as media. Each film sample with a surface area of 2.5 cm×2.5 cm was weighed (Wo) and placed in a preweighed stainless steel basket with 200 mesh aperture. Then, mesh containing film sample was submerged into 15 mL medium in a glass beaker. Basket was removed from beaker at preset time intervals and reweighed until no further change in weight of film (Wt). The degree of swelling was calculated as follows (29):

$$\text{Swelling index} = (\text{Wt} - \text{Wo})/\text{Wo}$$

Measurement of Mechanical Properties

Mechanical properties of film were evaluated using Instron Universal Testing Instrument (Model 1121, Instron Limited, UK) equipment with a 100-kg load cell. Samples with air bubbles, nicks, or tears and having mean thickness

variations of greater than 5% were excluded from analysis. Film was cut into narrow strips in dimension of 40 mm×10 mm. Film strip was placed between two clamps positioned at a distance of 10 mm in same plane. During measurement, the lower clamp was fixed, and the strip was pulled by a top clamp at a rate of 100 mm/min. The force and elongation at a moment of break were recorded, and tensile strength as well as percentage elongation was calculated by using the following equations (30):

$$\text{Tensile strength} = \frac{\text{Force at break (N)}}{\text{Initial cross} - \text{sectional area of the sample}(\text{mm}^2)}$$

$$\%\ \text{Elongation at break}\left(\%\text{mm}^{-2}\right) = \frac{\text{Increase in length}}{\text{Original length}} \times \frac{100}{\text{cross} - \text{sectional area}(\text{mm}^2)}$$

Results of film samples, which broke at and not between the clamps, were not included in the calculations. Measurements were run in triplicates for each film.

Determination of Average Drug Content in Films

To ensure uniformity of distribution of ITR in BF, average drug content in the film was measured. In addition, samples (2.5 cm×2.5 cm) were collected from five different locations (center and four corners) within film, weighed, and dissolved in methanol. After filtration, sample was analyzed spectrophotometrically at 262 nm against methanol as blank. The content of ITR was calculated using a preconstructed calibration curve for ITR (5–35 μg/mL) in methanol. No polymeric interference was observed under conditions of assay procedure.

Bioadhesion and Retention in Simulated Vaginal Environment

The bioadhesive property of vaginal film was assessed in simulated vaginal environment (31,32) using a texture analyzer equipped with 2.0-kg load cell (Model 1121, Instron Limited, UK). Isolated sheep vaginal mucosa free from supporting tissues was stored in a deep freezer at −20°C. For experiments, vaginal tube (thawed in normal saline with 0.1% *w/v* sodium azide preservative) was incised longitudinally and held on lower platform of the texture analyzer. Film was applied to the upper probe with the help of a double-sided adhesive tap. The vaginal mucosa was moistened with SVF. Mucosal membrane was kept in contact with film for 5 min to allow formation of adhesive bond. Upper probe of texture analyzer was moved at speed of 0.1 mm/s. The force required to detach the film from the mucosal surface was determined as bioadhesive strength.

Retention of ITR films on vaginal mucosa was measured by using simulated dynamic vaginal system shown in Fig. 1. The study is based on principle of measuring weight of dispersion falling down (or retained) as function of time. Sheep vaginal tube was obtained from a local slaughterhouse immediately after the animal was killed. Before commencement of the experiments, sheep vaginal tube was thawed in normal saline containing 0.1% (*w/v*) sodium azide as preservative. The

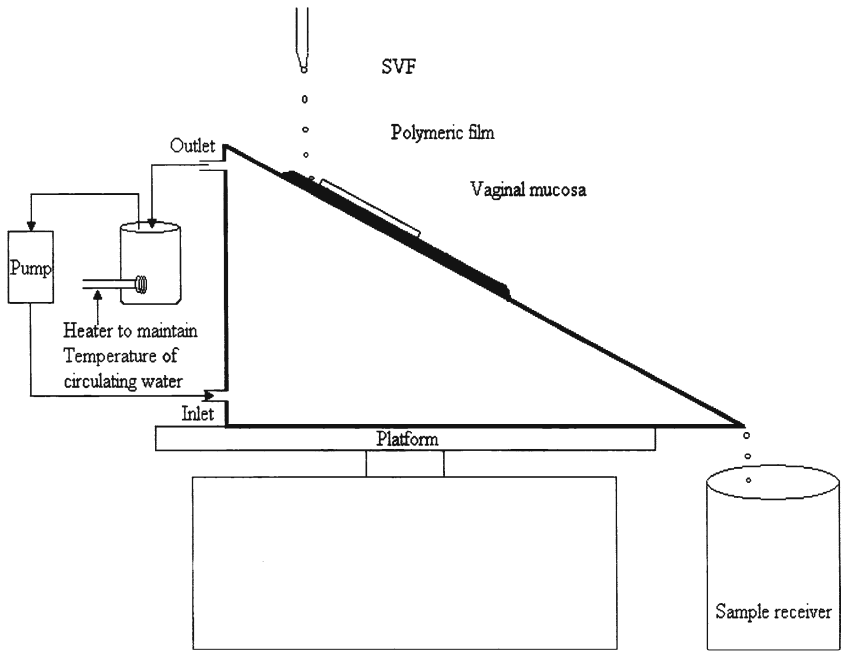

Fig. 1. Outline diagram of simulated dynamic vaginal system for *ex vivo* retention of drug measurement

sheep vaginal mucosa was cut into 5-cm×5-cm pieces and mounted on simulated dynamic vaginal system (30° angle slope) with mucosal side up. The polymeric film (2.5 cm× 2.5 cm) was placed on the mucosal membrane, and SVF was applied on the films with a flow rate of 5 mL/h. At predetermined time intervals, dispersion was collected into a receiver beaker, which was analyzed spectrophotometrically and recorded as percentage drug retained on vaginal mucosa.

In Vitro Lactobacillus Inhibition

In vitro activities exerted by ITR, placebo film, and BF against *Lactobacillus acidophilus* could be estimated by using cup plate method (33,34). The bacterial strains of *L. acidophilus* were obtained from MTCC (Microbial Type Culture Collection and Gene Bank, Chandigarh, India) and subcultured in MRS broth two to three times before commencement of experiment. After autoclaving, first base agar (3% *w*/*v*) was poured in to sterile petri dish (15 cm in diameter) and allowed to solidify. Five milliliter of standardized suspension of *L. acidophilus* (10^5 cells/mL) was uniformly mixed with 50 mL of 1% *w*/*v* MRS agar (top agar) and then plated on previously solidified base agar plate. Wells were made in the plate using an 8-mm borer. Test samples were poured into the wells, and plates were incubated at 37±2°C for 24 h.

Cellular Viability

The cytotoxicity (cellular viability) of the vaginal formulation is evaluated by 3-[4-5-dimethylthiazol-2-4]-2, 5-diphenyltetrazolium bromide (MTT) assay using HeLa-S3 cell lines. HeLa-S3 cell lines obtained from National Center for Cell Science (NCCS, Pune) and grown under 5% CO_2 in Ham's F12 K medium supplemented with 10% fetal bovine serum, 2.0 mM L-glutamine, and 1.5 mg/mL $NaHCO_3$. Exponentially growing HeLa epithelial cells are seeded into 96-well plate containing Ham's F12 K medium at a density of 10^6 cells/well. The cells are allowed to grow for 24 h at 37°C prior exposure to vaginal formulation. On the day of treatment, Ham's F12 K medium is replaced with fresh medium. Test samples were placed on top of the cells and allowed to incubate for 24 h at 37°C. After incubation, cells were washed with phosphate-buffered saline (PBS) to remove the formulation, and 100 µl of fresh medium with 10 µl of MTT solution (5 mg/mL in 0.1 M PBS, pH 7.2) was added to each well. Cells containing only medium and MTT were considered as negative controls (without formulation treated). Plates are then incubated for 4 h at 37°C in a CO_2 incubator. After incubation, MTT reaction medium was discarded and cells were washed with PBS. Then, 100 µl DMSO was added in each well to dissolve blue formazan crystals, and optical density was measured at 570 nm with a 96-well multiscanner ELISA reader with DMSO serving as blank. The percent viability was calculated by the following formula:

$$\text{Cell viability} = \frac{\text{OD of the test sample}}{\text{OD of the control sample}} \times 100$$

RESULT AND DISCUSSION

Drug–Excipient Compatibility

Drug–excipient compatibility studies are conducted with the objective of selecting a reasonable composition for vaginal bioadhesive film. Any kind of incompatibility between ITR and film-forming polymer affects its performance to a significant extent. Results of ITR–excipient compatibility study performed by DSC are shown in Fig. 2. DSC thermogram for pure ITR shows sharp endotherms at 166°C that corresponds to the melting point of ITR. In DSC thermogram of solid dispersion, ITR endotherm disappeared, indicating that the drug transformed from the crystalline to partially amorphous state. DSC thermogram of film formulation also shows complete absence of ITR peak, which indicates no risk of transforming the physical state of ITR from an amorphous to crystalline state during preparation of film. Amorphous state of drug leads to high-energy state resulting in enhanced solubility.

Formulation Design and Optimization

Selection of Plasticizer and Film-Forming Polymer

In preliminary experiments, various polymers and plasticizers were explored for preparation of ITR film formulation. The physical characteristics of ITR film prepared with various polymers and plasticizers are given in Table III. Different water-soluble plasticizer such as glycerol, PEG 400, and D-sorbitol were explored for development of BF. Film containing glycerol and D-sorbitol as plasticizers could not be

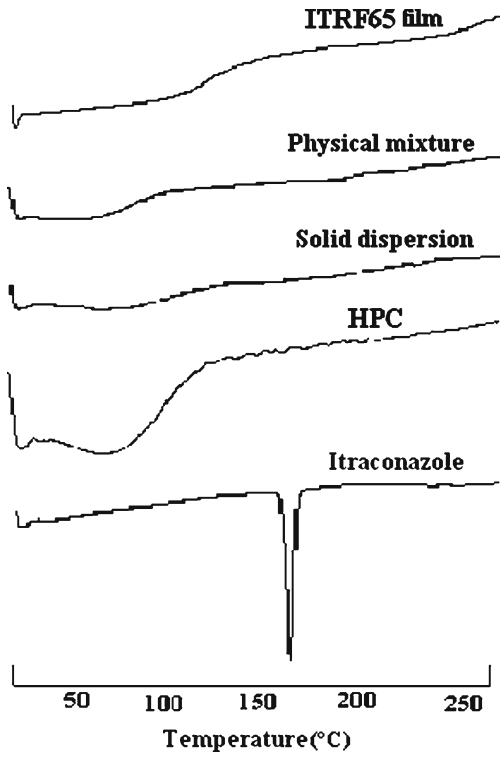

Fig. 2. Representative DSC thermogram for ITR–excipient compatibility study

Table III. Composition of ITR Film Prepared with Various Polymers and Plasticizers and their Physical Characteristics

Film	Polymer	Plasticizer	Composition (SDITR/polymer/plasticizer)	Physical characteristic of film
$ITRF_1$	Nil	D-Sorbitol	2.5:0:0.5	Could not be removed from casting surface
$ITRF_2$	Nil	Glycerol	2.5:0:0.5	Could not be removed from casting surface
$ITRF_3$	Nil	PEG 400	2.5:0:0.5	Could not be easy to removed from casting surface
$ITRF_4$	HEC	PEG 400	2.5:1.0:0.5	Nonhomogeneous surface, more soft, easy to peel
$ITRF_5$	Carrageenan	PEG 400	2.5:1.0:0.5	Brittle, hard to peel
$ITRF_6$	HPC	PEG 400	2.5:0.8:0.5	Homogeneous surface and easy to peel

SDITR solid dispersion of itraconazole and HPMC E15

removed from the casting surface. On the other hand, films containing PEG 400 as plasticizer were formed, but not easily peelable. It was concluded that HPMC-based SDITR with plasticizer could not form film, necessitating the addition of another film-forming polymer to increase peelability and mechanical strength.

Three, different, water-soluble, film-forming polymers such as HEC, HPC, and carrageen were investigated for preparation of ITR film. Film containing carrageen as film-forming polymer was hard to peel from the casting surface and is brittle in nature. In addition to HEC, high viscosity of polymeric dispersion causes difficulty to remove entrapped air bubbles. Nonhomogeneous surface of films with HEC was posing the problems, such as unequal distribution of drug in film. This problem was overcome by using HPC as film-forming polymer. Polymeric dispersion of SDITR with HPC was found to be less viscous and uniform in spreading on the casting surface. Physical characteristics and other mechanical properties of ITR film containing HPC and PEG 400 were acceptable. Hence, these two independent variables were selected for further systematic optimizations of formulation.

Mechanical Properties

The mechanical properties such as tensile strength and percentage elongation at break (%EB) indicate the strength and elasticity of the film. It depends on the ratio of polymer/plasticizer. For all the designed film formulation, tensile strength was found between 6.99 and 10.46 N/mm^2. The results of mechanical properties shown in Tables I and II indicate that an increase in HPC content resulted in a higher TS and %EB, while an increase in the amount of PEG 400 reflects a decrease in TS and an increase in %EB. An interesting finding was a decrease in TS and an increase in % EB of film as a function of plasticizer by weakening the intermolecular interactions between the polymer chains. Therefore, the optimum level of HPC and PEG 400 was desired because a high content of PEG 400 produced a film that is more flexible and softer. ITR films with HPC are tougher and softer than those without HPC.

The prediction profiles were obtained for the measured responses using JMP 5.1, statistical discovery software. The relationship between independent variables and dependent response value of ITR film can be further explained by using prediction profile as shown in Fig. 3. Among the tested variables, the HPC concentration seems to be the most prominent factor in determining response value of film. An interesting observation of these profiles was improved tensile strength, percent elongation, $Y_{8\ h}$, and swelling index as the content of HPC in film increased. The high concentration of PEG 400 can decrease $Y_{8\ h}$ and TS value of bioadhesive film and increase EB value of film.

Desirability function was utilized to find out the optimum level of HPC and PEG 400 out of nine batches. Desirability function was calculated for TS, EB, and percent drug retained on vaginal mucosa at 8 h. Batch $ITRF_{65}$ showed the highest overall desirability of 0.92. Therefore, this batch was considered to be optimized batch, and values of independent variables of this batch were considered to be optimum values for BF. The optimized composition of film containing ITR is given in Table IV. Excipients used in films are generally regarded as safe and listed and approved for vaginal use.

Characterization of Physicodynamic Properties of Film

Newly developed ITR films are colorless, odorless, flexible, uniform, and possesses a smooth surface. Films of three different batches of $ITRF_{65}$ (optimized batch) were found to have similar aesthetic, mechanical, and other

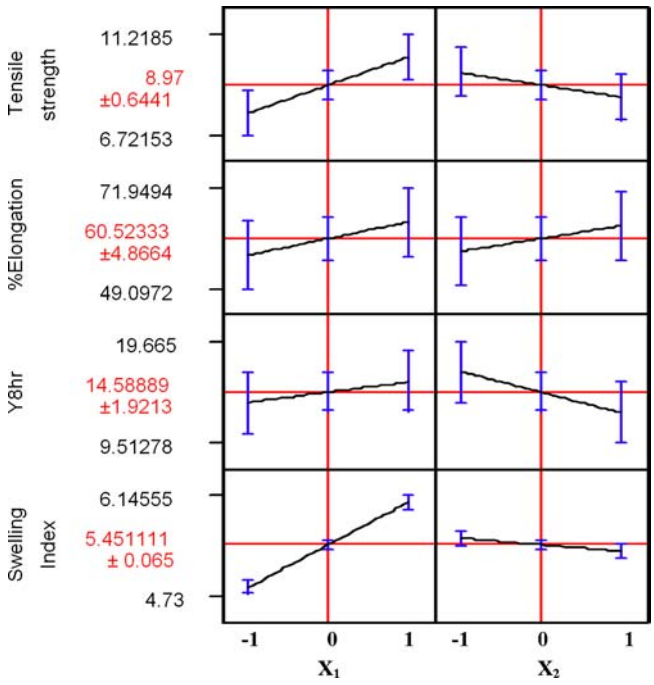

Fig. 3. Predictions profile for all the dependent variables against independent variables

Table IV. Optimized Composition of Film Formulation Containing Itraconazole

Independent variable	Optimum value (mg per film of 2.5-cm×2.5-cm area)
SDITR eq. to 100 mg of ITR	250
HPC	100
PEG 400	50

physicodynamic properties. This suggests that films with desired properties can be prepared consistently and reproducibly.

All the performance parameters of film ($ITRF_{65}$) have been given in Table V. Average drug content was found between 94.5% and 98.9% of added amount of ITR per film (2.5 cm×2.5 cm). The viscosity of polymeric dispersion of film was found to be more in SVF (7.84±0.25) as compared to water (7.57±0.31). Viscosity and dispersibility of formulation in vaginal environment after administration governs the spreading and retention of the formulations, which is essential to achieve desired efficacy. The developed film was dispersed rapidly in SVF and would form bioadhesive layer over vaginal mucosa in order to bring the drug in contact with the target tissue for sufficient periods of time. The pH of polymeric film was found to be slightly acidic (4.90±0.04) in SVF, and alkaline (7.04±0.07) in water reveals that vaginal pH as well as microflora may remain unaffected after administration of BF. The moisture content in the film was found to be 7.66±0.51% (*w*/*w*). The little amount of moisture content in formulations helps them to remain stable and prevent them from being a completely dry and brittle film.

The purpose of scanning electron microscopy was to obtain morphological characterization of film. Figure 4 illustrates the scanning electron photomicrographs of the film at 1,000× magnification confirm that film surface was free from any scratches or transverse striations.

Table V. Performance Parameter of Bioadhesive $ITRF_{65}$ Films

Parameter	Optimum values
Size (cm^2)	2.5×2.5
Weight[a] (mg)	431.5±8.78
Thickness[a] (mm)	0.46±0.026
Moisture content[a] (%*w*/*w*)	7.66±0.51
Tensile strength[b] (N/mm^2)	9.64±0.21
% elongation at break[c]	67.56±2.13
pH of dispersion[d]	
In water	7.04±0.07
In SVF	4.90±0.04
Viscosity (cp) of dispersion[e]	
In water	7.57±0.31
In SVF	7.84±0.25
Bioadhesive strength[f] (N)	0.368±0.02
Retention on isolated sheep vaginal mucosa	17.75% retained amount of drug on vaginal mucosa after 8 h

[a] Mean ± SD, $n=3$
[b] Determined by texture analyzer, mean ± SD, $n=5$
[c] Mean ± SD, $n=5$
[d] pH of dispersion (2.5×2.5 cm^2 film dissolve in 10 mL each of water and SVF at 30°C), mean ± SD, $n=3$
[e] Viscosity of dispersion measured by Brookfield Viscometer (2.5×2.5 cm^2 film dissolved in 10 mL each of water and SVF at 22°C), mean ± SD, $n=3$
[f] Bioadhesive strength determined by texture analyzer, mean ± SD, $n=5$

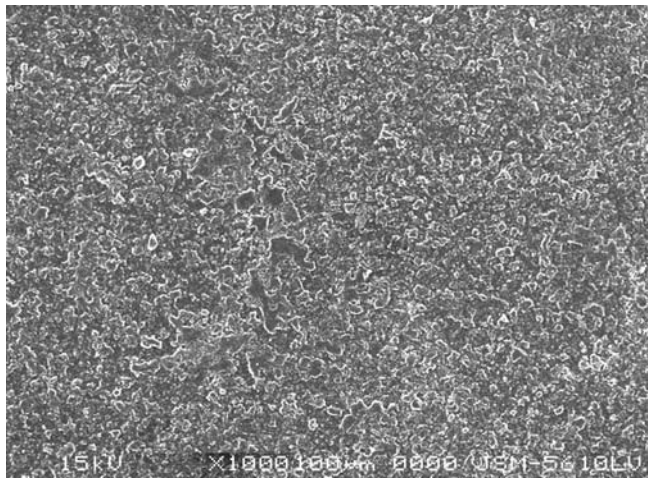

Fig. 4. Scanning electron photomicrographs of the ITR film

Swelling Capability of Films

Results of the swelling index of films with various compositions are shown in Fig. 5. Films were found to be rapidly swollen within 4 min and thereafter slowly reached to plateau. Maximum swelling was seen with film containing high content of HPC. The swelling index of film increased as the concentration of HPC increased. The swelling capability of polymer is crucial for its bioadhesiveness. Adhesion occurs shortly after the beginning of swelling but the bond formed is not very strong.

Bioadhesion and Retention in Simulated Vaginal Environment

Bioadhesion is a very important aspect for maintaining high drug levels at the site of administration and prevents expulsion of formulation. By maintaining effective drug concentration for longer time, one can achieve successful eradication of infectious agent. Figure 5 shows the effect of

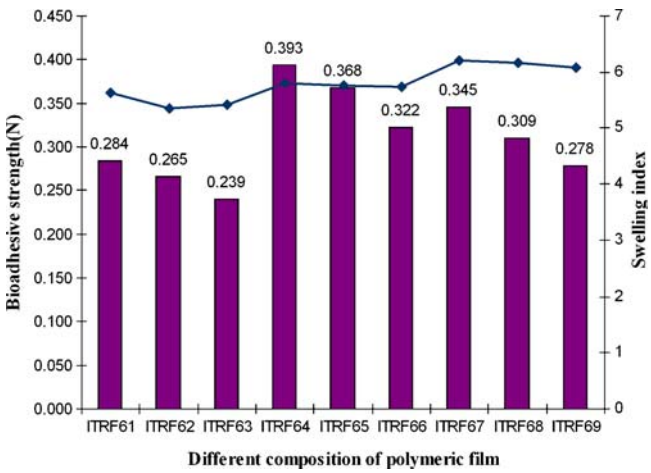

Fig. 5. Comparison of swelling capability and bioadhesive strength of polymeric films

Table VI. Percentage Retention of ITR on Vaginal Mucosa as a Function of Time

Time (h)	Cumulative percentage retained of itraconazole, mean ± SD (n=3)								
	$itrf_{61}$	$ITRF_{62}$	$ITRF_{63}$	$ITRF_{64}$	$ITRF_{65}$	$ITRF_{66}$	$ITRF_{67}$	$ITRF_{68}$	$ITRF_{69}$
1	93.38±0.13	93.03±0.11	92.72±0.09	93.79±0.16	93.59±0.10	93.31±0.10	94.40±0.16	94.03±0.13	93.79±0.11
2	86.07±0.11	85.02±0.14	83.21±0.12	88.80±0.13	87.93±0.08	86.32±0.08	89.67±0.11	88.45±0.07	86.37±0.10
3	68.37±0.16	67.83±0.12	66.64±0.12	72.24±0.11	71.15±0.13	66.67±0.23	73.34±0.17	71.90±0.14	70.05±0.19
4	52.72±0.16	49.71±0.20	50.68±0.12	55.97±0.25	55.15±0.2	52.61±0.12	57.52±0.26	55.72±0.18	53.48±0.21
5	39.00±0.30	35.82±0.26	39.18±0.26	39.43±0.44	42.20±0.27	39.83±0.20	42.87±0.30	42.45±0.26	40.49±0.28
6	28.45±0.27	27.47±0.27	25.72±0.30	33.20±0.30	31.71±0.32	28.08±0.29	34.07±0.31	30.26±0.23	27.58±0.34
7	21.34±0.32	20.84±0.28	17.46±0.46	26.05±0.32	24.07±0.43	21.42±0.31	25.87±0.38	22.55±0.36	17.47±0.39
8	15.14±0.44	12.25±0.60	9.76±0.55	18.63±0.41	17.75±0.32	14.06±0.36	15.03±0.39	15.41±0.40	13.00±0.48

various polymers/plasticizer ratios on bioadhesive strength of film. The bioadhesive strength of film was improved with increased content of HPC up to a certain extent and then decreased. As the concentration of PEG 400 in film increased, bioadhesive strengths of film were found to be decreased. This finding suggests that adhesion will improve with extent of hydration until an optimum point where overhydration leads to a decrease in adhesive force due to disentanglement at the polymer/tissue interface. Amongst nine films, $ITRF_{65}$ showed a good bioadhesion (0.368± 0.02 N) under simulated vaginal environment.

Retention performance of film was studied using simulated dynamic vaginal system, which mimics the physicodynamic conditions of the vagina. Initially, the film softened on the vaginal mucosa after absorbing SVF and became a swollen structure, helping it to adhere to the vaginal mucosa. The film would form a bioadhesive layer over the vaginal mucosa, which may help to achieve high drug levels at infectious site for longer time. Films were eroded over time. The remaining percentage of ITR on vaginal mucosa expressed as a function of time is shown in Table VI. Films were eroded slowly within the first 2 h and then gradually increased up to 8 h. Time required for entire removal of polymeric film from vaginal mucosa varied with the compositions of film. As the ratio of HPC to PEG 400 in film increased, the residence time of film increased until an optimum point and then it decreased. This indicates that retention properties of polymeric film can be controlled by varying HPC/PEG 400 ratio. Results of this study clearly indicate that amount of HPC and PEG 400 is an integral factor in retention of BF over vaginal mucosa.

Lactobacillus inhibition

However, ITR has an inhibitory effect against *C. albicans*; it does not affect the growth of lactobacillus, which is a normal component of vaginal flora. The results of lactobacillus inhibition against ITR bulk powder, $ITRF_{65}$ film, and placebo film are shown in Table VII. ITR bulk powder and $ITRF_{65}$ film at a concentration of 10 mg/mL did not show any significant inhibition on growth of *L. acidophilus* after 24 h.

Table VII. *In Vitro* Lactobacillus Inhibition Activity

Samples	Zone of inhibition, mm, mean ± SD (n=3)
ITR bulk powder	1.83±0.29
$ITRF_{65}$ film	1.67±0.29
Placebo film	Nil

Cytotoxicity

Amongst nine films, $ITRF_{65}$ was preferable for subsequent cellular viability studies owing to its good mechanical properties, moderate bioadhesion, desired pH, and viscosity in SVF, which was expected to present a prolonged residence time in vaginal cavity under normal physiological conditions. In order to determine the safety of film against epithelial cell, MTT assay was performed using HeLa-S3 cell lines. Figure 6 shows the cellular viability of HeLa-S3 cells, which was investigated over various concentrations of $ITRF_{65}$ film and placebo film prepared in PBS. These samples did not show any cytotoxic effect in HeLa-S3 cells at concentration up to 500 µg. Nevertheless, in the presence of a high concentration (1,000 µg) of $ITRF_{65}$ film and placebo, cell viability was decreased by 6% and 2%, respectively. The cytotoxicity studies concluded that HPMC- and HPC-based BF can be safely used intravaginally without affecting cell viability of vaginal mucosa.

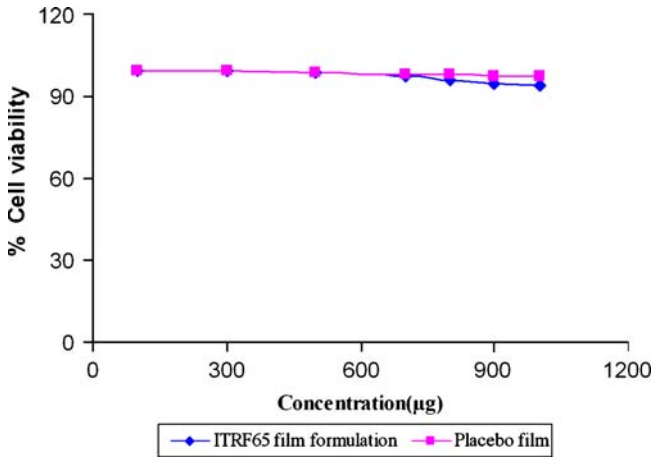

Fig. 6. Percentage viability of HeLa-S3 cells against various concentration of ITF_{65} and placebo film

CONCLUSION

In the present work, novel itraconazole bioadhesive vaginal films have been developed. The formulation was optimized by using 3^2 full-factorial designs and the desirability function. The applications of the desirability function combine all the responses in one measurement, which may help to predict optimum levels for the independent variables. The overall results obtained during this investigation suggest that ITR bioadhesive film possessed desirable aesthetic, pharmaceutical, and biological properties, making it a novel delivery system for effective and convenient treatment of vaginal candidiasis. This novel delivery system will offer substantial benefits for improving women's health.

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