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Formulation Development and Evaluation of Topical Film Forming Spray of Ketoconazole

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Abstract: Topical formulation has currently gained increasing patronage by formulation scientist in development of dermal drug delivery systems, particularly since they help in reducing the systemic side effect and aid in enhancing local drug concentration at a site of application, thus increasing drug effectiveness. In recent decades, various innovations have continued to be developed to obtain efficient and effective spray preparations. One of them is a film-forming spray (FFS) which has been applied in multiple fields, such as the food industry, cosmetics, pharmaceuticals, plantations, etc. The objective of the present work was the development of film forming spray of Ketoconazole to improve the patient acceptability by increasing cosmetic attractiveness and drug effectiveness by reformulating in a novel drug delivery vehicle. Ketoconazole is an antifungal drug marketed formulations are available in conventional dosage form (topical cream, lotion).

Keywords: Topical drug delivery, Film forming spray, Ketoconazole.

INTRODUCTION

Topical routes of drug delivery aim for systemic or local effects and offer various advantages, including avoiding first-pass metabolism and the effect of low pH and enzymes in the gastrointestinal tract, as well as a large available surface area. To improve therapeutic efficiency or pharmacokinetic profiles, drugs administered via the topical route are generally made in a dosage system, such as a patch, gel, lotion, cream, ointment, or spray.

In recent decades, various innovations have continued to be developed to obtain efficient and effective spray preparations. One of them is a film-forming spray (FFS) which has been applied in multiple fields, such as the food industry, cosmetics, pharmaceuticals, plantations, etc. FFS generally consists of active substances, enhancers, and polymers that are dissolved inorganic solvents. A thin, non-sticky film forms that can increase the contact time and permeability of the drug, resulting in continuous drug release, and can prevent crystallisation so that more drug is available to provide therapeutic effects compared to other conventional topical preparations.

Ketoconazole is a Topical and oral antifungal agent with activity against many species of yeast and candida albicans, which used to treat infections caused by a fungus or yeast. It works by killing the fungus or yeast or preventing its growth. Ketoconazole is used to treat skin infections such as athlete's foot, jock itch, ringworm, and certain kinds of dandruff. This medication is also used to treat a skin condition known as pityriasis (tinea versicolor), a fungal infection that causes a lightening or darkening of the skin of the neck, chest, arms, or legs.

MATERIALS AND METHODS

MATERIALS

Ketoconazole was obtained from Yarrow Chem. Pharmaceuticals Ltd, Mumbai. PVP K 30 and Eudragit RS 100 used as film forming agent. Propylene Glycol used as Plasticizer, Ethanol and Acetone used as solvent. All the chemicals used were of analytical grade.

METHODS

1. Preformulation Study

1.1 Characterization of Ketoconazole:

Various test was performed on the obtained drug sample to establish its identity and purity and the results were compared with specification reported in literature survey. Wherever possible. The parameters studied include;

Description:

The drug sample was analysed for physical appearance, colour, and odour.

Melting Point:

The melting point of ketoconazole was recorded by Digital Melting Point apparatus and was compared with the literature reported data

pH Range:

The pH of a 10% solution in water was recorded.

Solubility:

Ketoconazole is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of ketoconazole in these solvents is approximately 1, 2, and 5 mg/ml, respectively. Ketoconazole is sparingly soluble in aqueous buffers. Insoluble in water.

FTIR Analysis:

The identification of Ketoconazole was done by FTIR spectroscopy. The FTIR spectra (FTIR 1-S Affinity), in transmittance mode, were obtained in the spectral region of 4000- 400 cm-1 using a resolution of 4 cm^{-1}

1.2 Characterization of Excipients:

Polymer and other excipients used in the study were standardized as per USP 2004 NF and Handbook of Pharmaceutical Excipients for their physicochemical characteristics such as appearance, solubility, melting point, and viscosity.

2. Drug Excipient Compatibility Study:

FTIR Analysis:

FTIR Analysis of following mixture was taken

1. Physical mixture comprising of Ketoconazole and PVP K 30 in the ratio of 1:1
2. Physical mixture comprising of Ketoconazole and Eudragit RS 100 in the ratio of 1:1

The sample was stored at 60 ºC for 6 days to accelerate the interaction between drug and excipients.

DSC Analysis:

Differential scanning calorimetry (DSC) analysis was performed for pure drug, PVP K 30, Eudragit RS 100and physical mixture using a DSC (DSC-60Plus), instrument. The physical mixtures in the ratio 1:1 Ketoconazole + PVP K 30, Ketoconazole + Eudragit RS 100 first 5 mg of each processed sample was meticulously weighed and placed in sealed aluminium pans, then the thermal attitudes were investigated at a scan rate of 20°C/min (25-220°C) and analysed by TA60 software. The indium and aluminium oxide powders were served as standard and reference models, respectively.

Preparation of Film Forming Spray:

The best blank composite (EP 8) obtained from preliminary studies using EVOP was selected for further studies. FFS of Ketoconazole was prepared by dissolving film former PVP K 30, Eudragit RS 100 and Plasticizer Propylene glycol (20% of W/V of polymer weight) in 50 ml ethanol with continuous starring using electric stirrer, drug was added in vehicle blend and kept for ultra Sonicator for 30 min. to have clear

solution, measured quantity of acetone (20ml) was added, remaining volume was made up to 100ml with ethyl alcohol. The resultant solution was filled in a refillable glass container having screw on pump spray.

3. Evaluation of Film Forming Spray:

Drying Time of FFS Solution:

Time required for film formation was measured by spraying a formulation on to a glass slide, the weight change every 30sec till the constant weight was determined by using Shimadzu electronic digital balance at 25ºC ± 1ºC.

Viscosity Measurement of Film Forming Spray Solution:

The Viscosity of the solution was determined at 25ºC ± 1ºC using a Brookfield Rheometer R/S plus Rheo 3000 (100 rpm).

pH Measurement of FFS Solution:

The pH of the FFS was measured using digital pH meter and was made sure that the pH is accurate so does not cause skin irritation.

Spray Angle of FFS Solution:

First, the distance 8 cm from nozzle between papers was fixed. Methylene blue was dissolved in formulation to facilitate visualization. The sprays were actuated in horizontal direction after that, one actuation was sprayed onto paper and the circle size was measured. Spray angle is calculated as:

Spray angle (Θ) =tan-1 (r/h)

Where,

h = are the paper's distance from the nozzle.

r = is average circle radius.

Drug Content:

The drug content per ml was determined by firing sprays in a beaker containing 50 ml 6.8 phosphate buffer. This solution was shaken for 10 min and it is made up to volume 100 ml with methanol in volumetric flask. 1 ml of above solution is diluted further with 10 ml of 6.8 phosphate buffer, filtered. The concentration of dissolved drug was measured by U-V visible spectrophotometer at 244 nm.

4. Evaluation of Film

Drug content of films:

Prepared film was put into 100 ml phosphate buffer solution pH 6.8 and stirred vigorously for 2 hours. Then the whole solution was sonicated for 15 min. the above solution was filtered and drug was estimated spectrophotometrically at λ max.

Weight variation test:

For each formulation, three film samples (10 × 40 mm) were used. Each film sample was weighed individually and the average weighed was calculated.

Folding Endurance:

Folding Endurance was measured manually for the prepared film. A strip of film (10 × 40mm) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without broking or cracking gave the value of folding endurance.

Tensile strength and % Elongation:

Films were evaluated for tensile strength and % elongation using an apparatus assembled in the laboratory. Films of dimension 10 × 40 mm were attached to a support that was inextensible but flexible and this support was in turn held between two clamps separated by a distance of 3 cm. clamps were designed to secure the patch without crushing it during the test. These were supported on a metal base. One of the clamps was fixed; the other one was weights could be added to the movable clamp. During measurement, the films were pulled by the movable clamp with the addition of weights. The strength and elongation were measured when the films broke and tensile strength and % elongation were calculated using the following formulae;

$$\text{Tensile Strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}}$$

$$\% \text{ Elongation} = \frac{\text{Maximum length recorded at break- original length}}{\text{Original length}} \times 100$$

Water Vapour Permeability:

Films were produced with a solvent evaporation technique as described earlier. Circular samples with a diameter of 2.0 cm were cut from the dry film sheets with the help of a scalpel. For the sample preparation 10 ml glass vials with an opening of 1.4cm diameter (A= 1.53 cm2) were filled with approximately 8g of distilled water, covered with the circular film samples and the vial was sealed tightly with an aluminium foil. To start the experiment, the top of the vial cap was opened and the weight of the vial was determined with an analytical scale. The vials (three replicates per formulation) were ten placed into desiccators containing a desiccant to create a climate of low relative humidity (approximately 0%). They were kept at a determined temperature (37ºC) for 72 hours and weighed. From the weight loss of the vials W (g) the WVP was calculated as the amount of water that had permeated through the film in relation to the surface area (A cm2) and the time (t, 24 hours) using the following formula; [62] WVP = W/(A*t) (g cm-2 24 hrs-1)

In - vitro Drug Release Study (Diffusion study)

Laboratory assembled apparatus resembling a Franz diffusion cell was used to determine the release profile of drug from film forming Solution. The cell consisted of two chambers, the donor and receptor compartment between which a cellophane membrane was mounted. The donor compartment, with inner diameter 24 mm was open i.e. exposed to the atmosphere at one end and the receptor compartment was such that it permitted sampling. The diffusion medium used was phosphate buffer solution pH 5.5 (PBS).

100mg 1% of the drug containing film was placed in the donor compartment over the drug release membrane and was separated from the receptor compartment by the cellophane membrane. The donor and receptor compartments were held together using a clamp. The position of the donor compartment was adjusted so that cellophane membrane just touches the diffusion medium. The whole assembly was fixed on a magnetic stirrer. The receptor compartment with 22 ml of PBS was placed on a thermostatically controlled magnetic stirrer. It was maintained at 37± 0.5 ºC and stirred constantly at 50 rpm. Samples of 1 ml were collected at predetermined time intervals and analysed for drug content by UV Spectrophotometer at λ max against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal.

Stability studies:

The best formulation was kept for accelerated stability in a stability chamber (Thermolab) for a period of three months at temperature 40 °C+2 °C and RH 75±5%. Any changes in clarity, drying time, spray angle, viscosity, in-vitro antifungal activity, drug content was observed after intervals of one month.

RESULTS AND DISCUSSION

Evaluation of Drug Standard:

Table 1: UV calibration curve of Ketoconazole

Sr.no	Concentration (µg/ml)	Absorbance(nm)
1	10	0.115
2	20	0.186
3	30	0.261
4	40	0.354
5	50	0.441
6	60	0.523

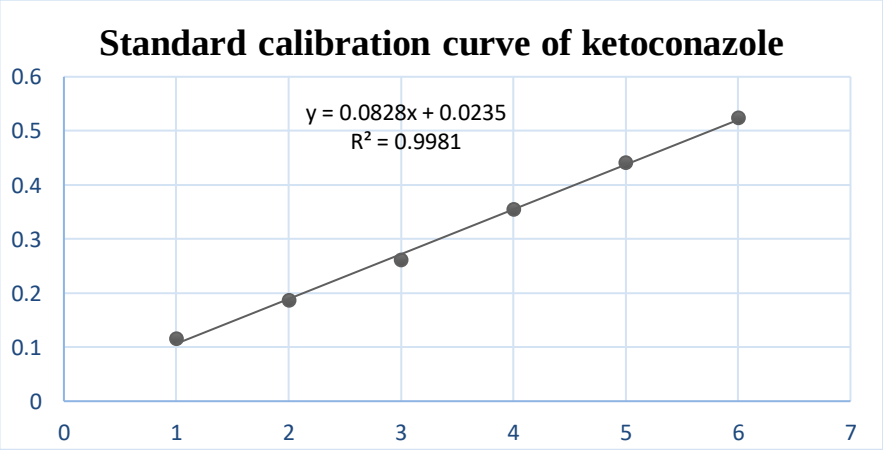


Fig 1: Standard Calibration Curve of Ketoconazole

Preformulation Study

Table 2: Characterization of Ketoconazole

Test	Specification	Results
Color	White Powder	Confirm
Odor	Odor suggestive of cereals	Confirm
Identification	FTIR	Positive
Melting Point	148-152 ºC	152 ºC
Solubility	Soluble in Methanol Ethanol, Dimethyl sulfoxide (Dmso) & Dimethyl formamide (Dmf). Insoluble in water	Confirm

Table 3: Characterization of Eudragit RS 100

Test	Specification	Results
Appearance	Granules	Confirm
Odor	Characteristics Odor	Confirm
Solubility	Soluble in Methanol, Ethanol, Acetone and insoluble in water	Confirm

Table 4: Characterization of PVP K 30

Test	Specification	Results
Color	Off- White color	Complies
Odor	Odorless	Complies
Solubility	Soluble in water, Acetone and Ethanol	Complies
pH (5%) sol,	6.5-7.5	7

FTIR Spectra Analysis:

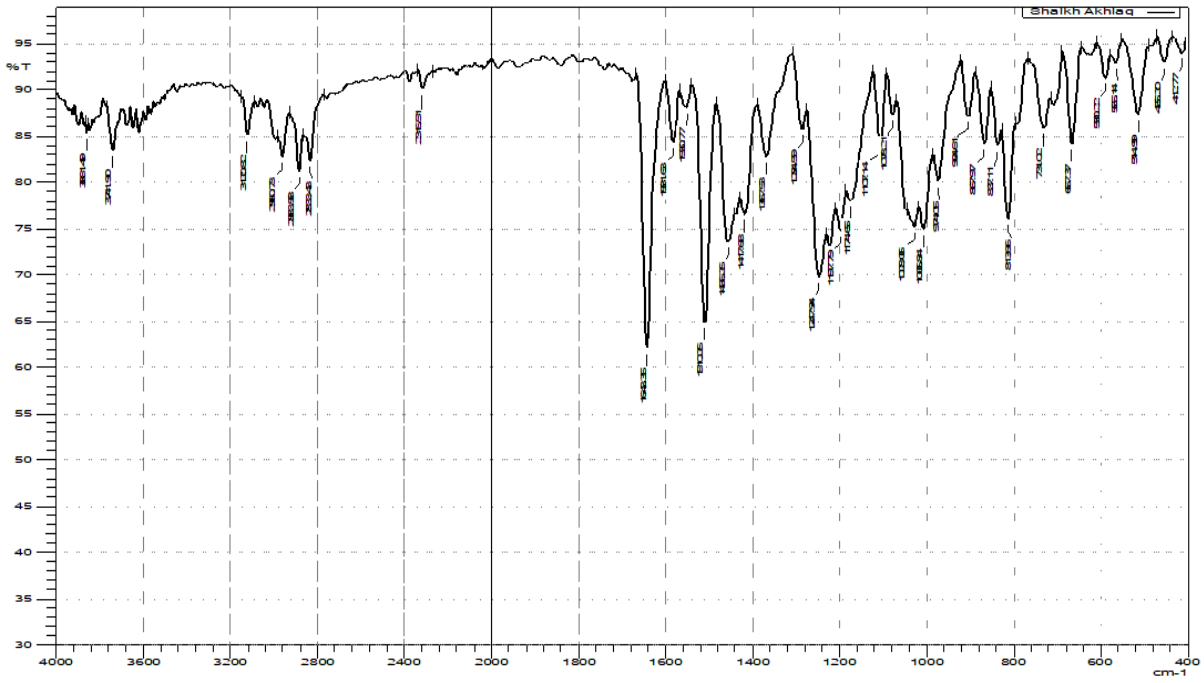


Fig. 2: FTIR Spectrum of Ketoconazole

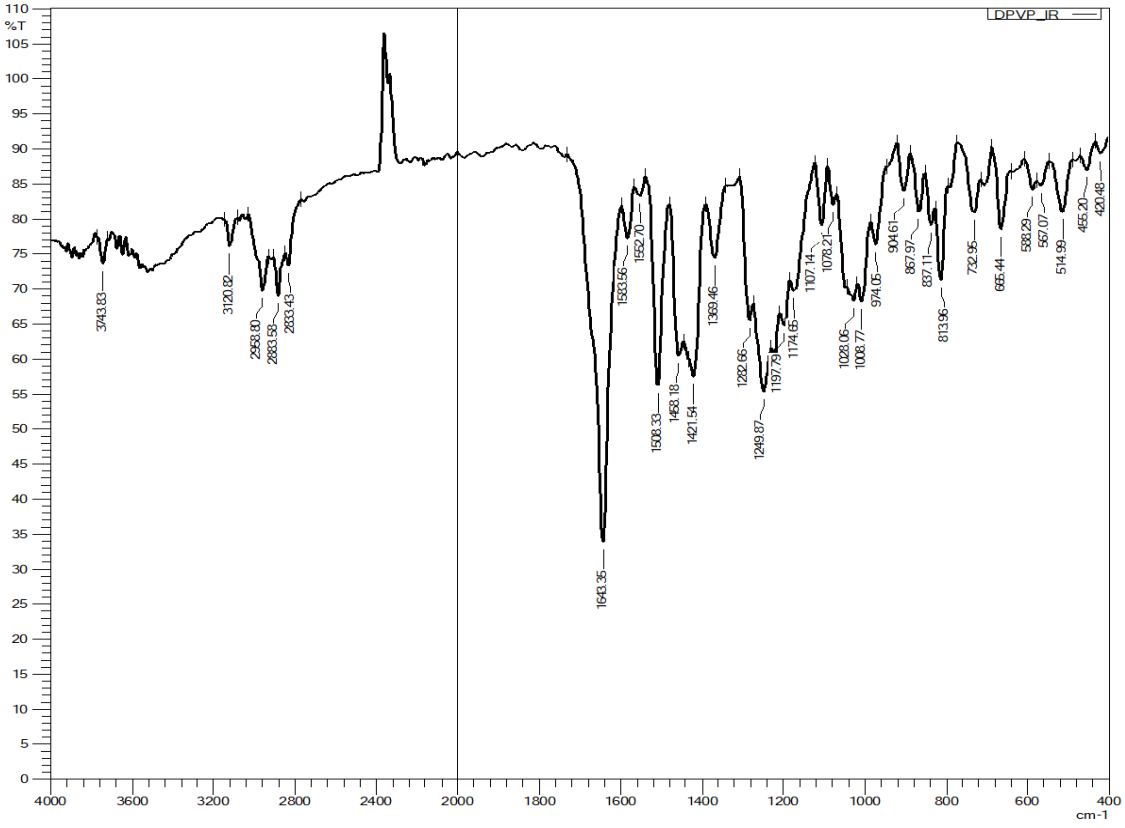


Fig 3: FTIR Spectrum of Ketoconazole: PVP K 30

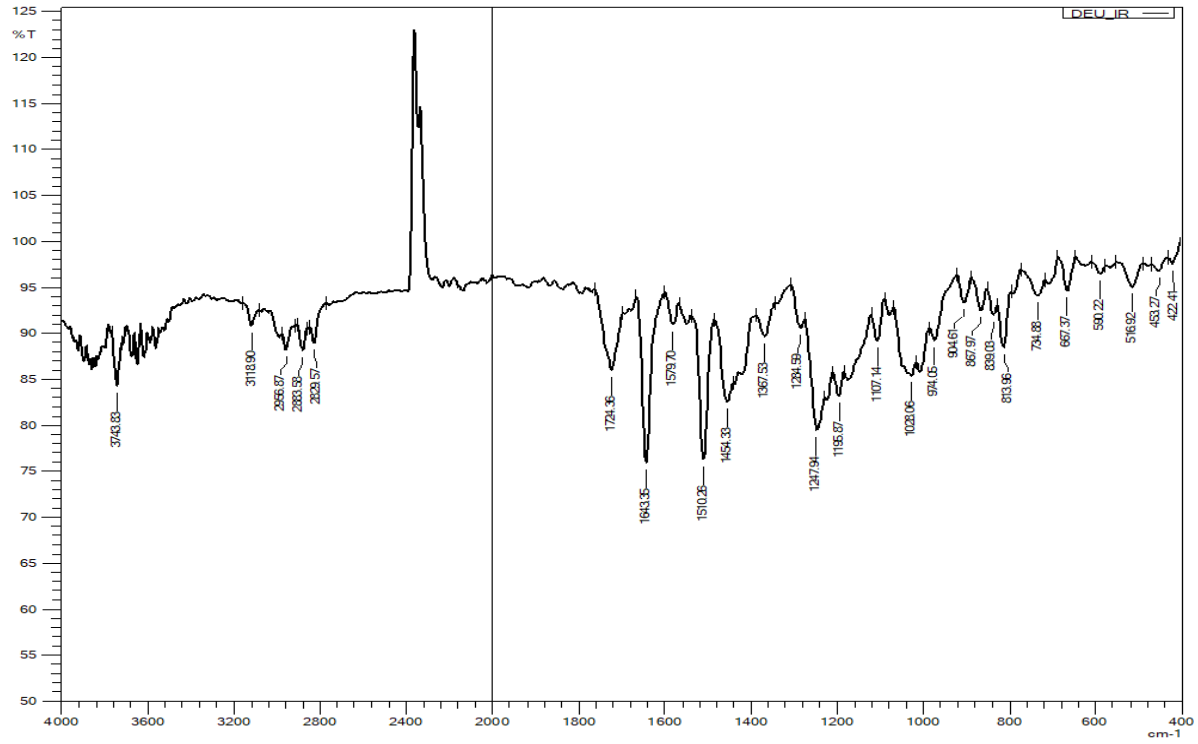


Fig 4: FTIR Spectrum of Ketoconazole: Eudragit RS 100

Table 5: Evaluation of Film Forming Spray

Sr. No.	Formulation Code	pH ± SD	Drying Time (Sec)± SD	Viscosity Measurement (cps) ± SD	Spray angle (Degree)± SD	Drug content per Spray (mg/ml)± SD
1	F1	5.26±0.10	170±4.78	16.24±0.55	78.69±1.01	9.51±0.70
2	F2	5.61±0.15	180±5.29	20.55±0.42	77.22±1.12	8.95±0.69
3	F3	5.70±0.14	202±3.05	23.32±0.63	78.94±0.80	9.72±1.06
4	F4	5.64±0.11	235±3.60	26.78±0.51	78.90±1.04	9.79±0.40
5	F5	5.77±0.15	258±4.04	28.13±0.74	80.76±0.61	9.86±0.70
6	F6	5.50±0.12	272±6.50	30.23±0.86	80.06±1.16	9.44±1.06
7	F7	5.37±0.16	280±6.65	31.91±0.33	76.84±0.85	9.93±1.45
8	F8	5.96±0.17	288±6.77	32.86±0.34	76.29±1.04	9.51±0.70
9	F9	5.62±0.14	297±7.37	34.89±0.52	75.40±0.79	9.44±1.06

Table 6: Evaluation of Film

Sr. No.	Formulation Code	Film Thickness (mm) ± SD	Folding Endurance± SD	Tensile Strength(N/m²) ±SD	% Elongation± SD
1	F1	0.11±0.01	4±1	12666±38	34.48±0.69
2	F2	0.13±0.01	3±1	11054±58	53.57±0.61
3	F3	0.12±0.01	2±1	16843±77	50±0.57
4	F4	0.14±0.01	5±1	13399±59	61.90±1.02
5	F5	0.11±0.01	7±1	20624±41	82.60±1.25
6	F6	0.15±0.01	6±1	17076±42	64±0.55
7	F7	0.13±0.01	4±1	13918±97	51.85±0.93
8	F8	0.15±0.01	5±1	14219±45	50±1.09
9	F9	0.16±0.01	6±1	17473±52	46.15±0.62

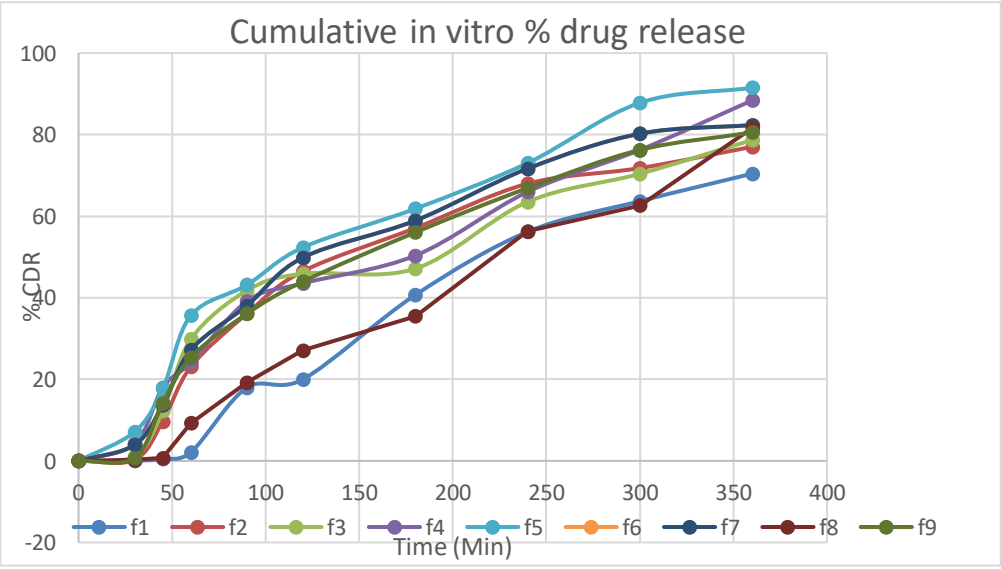
Table 7: Evaluation of Film

Sr. No.	Formulation Code	Weight Variation Test (mg) ± SD	Drug Content ± SD	Water Vapor Permeability (**g cm^{-2} 24 hrs^{-1}**) ±SD
1	F1	125±0.5	89.51±1.39	0.032±0.003
2	F2	132±1	93.0±1.45	0.040±0.002
3	F3	146±1.15	95.1±1.61	0.050±0.004
4	F4	154±0.57	97.2±1.85	0.033±0.007
5	F5	158±1.52	97.9±1.21	0.020±0.011
6	F6	165±0.57	95.8±1.85	0.047±0.003
7	F7	172±1	94.4±1.21	0.053±0.009
8	F8	177±1.5	90.9±0.40	0.056±0.003
9	F9	183±1.15	91.60±1.45	0.048±0.010

Table 8: Cumulative Drug release (%) of formulations (mean ± SD, n=3)

Time (min.)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
30	0.44±0.03	0.26±0.95	1.16±0.48	4.24±0.15	7.15±0.32	3.93±0.38	3.93±0.38	0.22±0.01	0.70±0.15
45	2.09±0.07	9.68±0.62	12.21±0.20	18±0.15	17.73±0.35	13.7±0.33	13.7±0.33	0.70±0.08	14.19±0.30
60	18.01±0.09	23.09±0.31	29.83±0.48	24.15±0.30	35.64±0.31	27.21±0.54	27.21±0.54	9.28±0.11	25.38±0.40
90	20.02±0.07	36.10±0.55	41.77±0.17	39.16±0.38	43.16±0.08	38.10±0.40	38.10±0.40	19.25±0.22	36.10±0.31
120	40.70±0.10	46.53±0.38	45.91±0.49	43.56±0.40	52.36±0.40	49.89±0.55	49.89±0.55	27.06±0.52	44±0.34
180	56.18±0.06	57.09±0.40	47.14±0.31	50.35±0.31	61.84±0.55	58.93±0.17	58.93±0.17	35.48±0.23	56.03±0.08
240	63.69±0.10	67.98±0.39	63.53±0.46	65.97±0.32	73.04±0.08	71.68±0.24	71.68±0.24	56.32±0.08	66.90±0.32
300	70.43±0.08	71.80±0.46	70.40±0.09	76.25±0.23	87.81±0.34	80.23±0.23	80.23±0.23	62.61±0.23	76.25±0.23
360	75.79±0.08	77.02±0.42	78.69±0.23	88.40±0.08	91.50±0.17	82.36±0.38	82.36±0.38	81.62±0.31	80.54±0.26

It can be deduced from *in vitro* diffusion study that formulations F1 to F3 did not completely release the drug over 360 min. This may be attributed to low levels of drug release modulating polymers and low viscosity of the formulations. Formulations F6 to F9 did not sustain the drug release. On the other hand, Formulations F4 and F5 sustained drug release over 360 min. Drug release was found to be sustained at intermediate levels of hydrophobic polymer, Eudragit and hydrophilic polymer. PVP K30. of the nine formulations, maximum release was found to be for formulation F5 after 360 min. 91.50% of the drug in the formulation was available for antifungal activity. The composite film had hydrophobic and hydrophilic portions which provide competition for drug release as both the polymers have different release properties. Therefore, as the polymer ratio varies, competition to release drug also varies. Formulation F4 showed steady state release up to 24 hours which also indicates that this formulation would show better contact with biological membrane.



In vitro Antifungal activity

The study indicates that Ketoconazole retained its antifungal efficacy when formulated as a film forming spray and drug was active against selected strain of micro-organism (***candida albicans***). F5 formulation showed a zone of inhibition **30.2 mm** and Zone of inhibition for ethanol as a control was also calculated to determine the influence of its inherent antifungal activity.

The study indicates that Ketoconazole retained its antifungal efficacy when formulated as a film forming spray and drug was active against selected strain of micro-organism (*Aspergillus niger*). F5 formulation showed a zone of inhibition **33 mm.** Zone ofinhibition for ethanol as a control was also calculated to determine the influence of its inherent antifungal activity

Table 9: Results of in vitro anti fungal activity

Sr. no.	Formulation Code	*Candida albicans*	*Aspergillus niger*
		Zone of Inhibition (mm)±SD	
1	F1	24±0.51	22.4±0.32
2	F2	26±0.34	24.8±0.70
3	F3	28±0.57	26±0.72
4	F4	29.5±0.76	30.7±0.40
5	F5	30.2±0.86	33±0.28
6	F6	29.9±0.90	31±0.23
7	F7	27±0.57	29.5±0.25
8	F8	26.6±0.30	26±0.15
9	F9	22±0.17	24±0.20
10	Ketz (Ketoconazole) Lotion	22±0.32	19±0.55

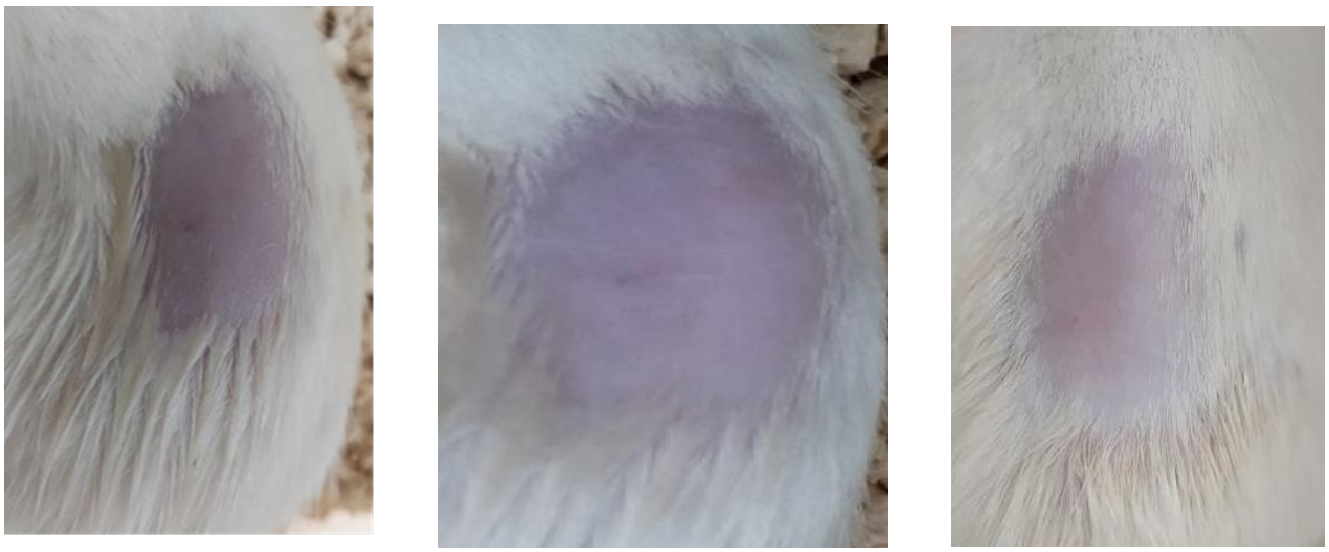
Evaluation of Optimized batch: Skin irritation study on rats showed that after application of optimized formulation there was no evidence of irritation (erythema and oedema). Hence, the optimized formulation F5 was found to be safe.

Table 10: Draize Test Score Obtained After Application On Rat Skin.

Formulation	Score after 24 hrs.
Control	0
Formalin	1
Optimized batch	0

Table 11: Draize Test Score System for Skin Reaction

Reaction erythema and oedema	Score
No erythema and oedema	0
Very slight erythema and oedema	1
Well defined erythema and oedema	2
Moderate to severe erythema and oedema	3
Severe erythema and oedema	4



Formalin **F5** **Control**

Fig.: 5 Skin Irritation Study Carried On Rat Skin By Applying

Stability Study

The optimized formulation was evaluated after storage at room temperature and after accelerated stability study at elevated temperature (40º C/75% RH) in stability Chamber.

Table 12: Stability study data

Sr. No.	Observation	Before study	Duration of Study		
			1 month	**2 month**	**3 month**
1	Clarity	Clear	Clear	Clear	Clear
2	Drying time (sec ± SD)	272 ±6.65	258 ±4.04	280 ±2.77	288 ±6.50
3	Viscosity (cps)Mean ±SD	28.13 ±0.74	28.02 ±0.25	28.10 ±0.17	28.12 ±0.16
4	Spray angle (degree ± SD)	80.76 ±0.61	80.54 ±0.24	80.98 ±0.42	80.94 ±0.12
5	Drug content (%±SD)	91.50 ±0.17	91.12 ±0.22	91.05 ±0.37	91.10 ±034
6	In vitro antifungal activity (mm ± SD)	31.5 ±0.86	30 ±0.32	31.2 ±0.35	31.4 ±0.26

CONCLUSION

The materials were characterized and were found to comply with standards. The FFS were formulated with the help of 3^2 factorial designs. The formulation was formulated so as to obtain desired cosmetic attractiveness, optimum drying time, drug content, film forming characteristics and maximum antifungal activity. The formulations were studied for drying time, viscosity, pH, spray angle ex-vivo physical evaluation, mechanical properties of film and in-vitro antifungal activity. Finally, we conclude that F5 formulation comprising of a combination of PVP K30 (10%w/w) and Eudragit RS 100 (1%w/w) and Propylene Glycol (20% w/v of polymer weight) used as a plasticizer in a solvent constituted by a unique combination of ethanol: acetone (8:2) has a potential for use as a film forming spray (FFS) for topical delivery of Ketoconazole.

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