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Original Article

# Terbinafine HCl Film-Forming Spray for the Treatment of Topical Fungal Infections

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**ABSTRACT**

Terbinafine HCl is an allylamine used to treat fungal infections. It has substantial side effects that can be mitigated by using a topical semisolid dose form. The objective of this study was to develop a 1% Terbinafine HCl film-forming spray formulation to treat topical fungal infections. The formulation was developed by combining polymers, penetration enhancer, plasticizer, and a suitable solvent system. The central composite design with 3 independent variables and 2 dependent variables is implemented to optimize the formulation. The film-forming spray was put through its tests to assess formulation and container-related parameters such as pH, spray angle, spray pattern, density, volume delivered for one actuation and evaporation time. From the study, it was observed that the concentration of ethyl cellulose and Eudragit RSPO has a greater influence on the viscosity of the spray solution, whereas the eutectic mixture has a greater influence on the drug permeation followed by the polymers. It also tested with various fungi, and it was found that formulation showed better fungicidal activity than the marketed product achieved by triple action. The stability studies have shown that the optimized formulation was stable with temperature of 25 ± 2°C and RH 60 ± 5(six months) on the pH, viscosity and visual appearance of the formulation. The study has concluded that the formulated film-forming spray formulation is highly efficient in treating topical and transdermal fungal infections when compared to the traditional dosage forms.

**Keywords:** Terbinafine HCl, Formulation, Fungal infection, Solid dosage forms, Ethyl cellulose.

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## INTRODUCTION

The skin is the most readily accessible organ of the body and acts as a barrier against the micro and macromolecules of the environment because of its low permeability to such substances. A normal individual's skin has a surface area of roughly $2m^2$ and gets about one-third of the entire blood running throughout the body.[1] Fungal infections are becoming more common at an alarming rate, posing a significant challenge to hospitals and doctors. Fungal infections involving the skin, nails, hair, and mucous membranes are all examples of topical fungal infections. Onychomycoses, or nail infections, are considered to account for about 33% of all fungal skin infections and 50% of all nail problems. Fungal infections of the toenails and feet can act as a reservoir for organisms that can spread to other parts of the body or to other people.[2] Direct contact with infected individuals, animals, soil, or fomites causes fungal transmission. Dermatophytes such as Trichophyton, Microspores and *Epidermophyton* are responsible for most topical fungal infections. Other fungi like Candida and Aspergillus are also known to cause various fungal infections.[3] The symptoms of topical fungal infections are usually minor and not life-threatening, but they can have a significant influence on the patient's quality of life. These infections can lead to systemic when untreated and should be concerned immediately with proper medication and treatment.

There are many formulations available for treating topical fungal infections, such as creams, powders, patches, ointments and gels. Patches have several drawbacks, including skin irritation caused by their occlusive qualities, which block sweat ducts, prevent water vapour loss from the skin surface, difficulties placing on curved surfaces, discomfort while peeling off, and a lack of aesthetic appeal. Moreover, patches have restricted size and shape that cannot fit the area of the application, which stands as the major drawback.[4] Semisolids, which have been important dosage forms for various diseases, had their own limitations. They do not maintain long-term touch with the skin and are quickly wiped away by the patient's clothing. As a result, chronic disorders such as athlete's foot, ringworm, and candidiasis require frequent application. Furthermore, they leave a sticky, oily remnant on the skin after application, resulting in poor patient compliance.[5] The film-forming spray (FFS) is a revolutionary technique that can replace traditional topical and transdermal formulations. It's

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**Table 1: Factor Level for Central Composite Design.**

| Factors | Coded Levels | |
|---|---|---|
| Independent Variables | Low Level (-1) | High Level (+1) |
| A= Eudragit | 5 | 15 |
| B = Ethyl cellulose | 2.5 | 7.5 |
| C = Eutectic mixture | 7.5 | 12.5 |
| Dependent Variables | Goals | |
| Viscosity (cps) | Minimize | |
| Drug permeation (%) | Maximize | |

a non-solid dosage form that forms a film *in situ* or after being applied to the skin or any other body surface. These systems contain the drug and film-forming excipients in a solvent that evaporates when comes in contact with the skin, leaving a film of excipients and the drug behind. The produced film can be a solid polymeric material that works as a matrix for drug release to the skin over time or a residual liquid film that is quickly absorbed in the stratum corneum.[1]

Terbinafine HCl, also called Lamisil, is a widely used drug that is proven to have broad-spectrum antifungal activity. Squalene epoxidase, also called squalene monooxygenase, is a fungal enzyme that converts the squalene to 2,3-oxydosqualene, a stage in ergosterol synthesis pathway. Terbinafine HCl inhibits the squalene epoxidase, thus preventing the conversion of squalene to 2,3-oxydosqualene, which in turn prevents the synthesis of the ergosterol that will reduce the fungal membrane integrity. As there is no membrane integrity is observed for the fungi, thus fungicidal activity can be achieved. Terbinafine HCl has demonstrated excellent fungicidal activity against the dermatophytes and variable activity against yeasts and non-dermatophyte moulds *in vitro*.[6,7] The objective of this study is to formulate, optimize and characterize Terbinafine HCl Film-forming spray formulation (FFS).

## MATERIALS AND METHODS

### Materials

Terbinafine Hydrochloride was purchased from BDL Pharmatech Pvt. Ltd., (Hyderabad, India), DL-Camphor, Ethyl cellulose, Polyethylene glycol 4000 (PEG 4000) were purchased from S.D.Fine Chemicals Ltd. (Mumbai, India), Menthol crystals was obtained from Micro Fine Chemicals (India), Eudragit RSPO was purchased from Evonik Industries Pvt Ltd., (Mumbai, India), Ethanol and ethyl acetate used were of analytical lab grade.

### Selection of drugs by Docking studies

The protein squalene epoxidase of PDB ID 6C6N, plays a major role in synthesis of ergosterol in fungi. Inhibiting this enzyme induces fungicidal activity. Allylamine drugs were proven to be efficient inhibitors of the squalene epoxidase/ squalene monooxygenase. The drug selection among all the allylamines for the antifungal activity was done by the docking using Pyrx virtual screening tool. Depending on the binding energy, the drug among the allylamines was selected.

### FTIR- Compatibility studies

The drug and excipients have been subjected to FTIR studies for conforming the compatibility between them.

### Preparation of the formulation

The Terbinafine HCl Film-forming spray solution were prepared by a simple solvent dissolving method. To prepare the camphor-menthol eutectic mixture, equal quantities of the camphor and menthol were weighed and then kept in a bath sonicator for 10 min to liquify. In another beaker Ethanol and ethyl cellulose blend was taken to this drug (Terbinafine HCl) and plasticizer (Polyethylene glycol 4000) was added and stirred for 30 min on a magnetic stirrer at room temperature to get a clear solution. Then the liquified camphor-menthol eutectic mixture was added to the solvent mixture under stirring. After that, the polymers (Eudragit RSPO, Ethylcellulose) were weighed and added to the above mixture. Then, this was kept under mechanical stirring for 20 min to get dispersed homogeneously. The prepared solution was kept in a bath sonicator to disperse if there are any polymer aggregates. This final solution was transferred into a refillable suitable spray container.

### Experimental Design

A response surface technique based on a Three-factor; three-level Central Composite Design (CCD) was used to optimize the formulation parameters of Terbinafine HCl FFS with the least number of tests possible. The primary elements impacting the formulation's physicochemical qualities, namely the polymer Eudragit RSPO ($X_1$), the concentration of polymer ethyl cellulose ($X_2$), and the concentration of camphor-menthol eutectic mixture ($X_3$), were chosen and examined at two distinct levels designated as -1 and +1. The dependent response variables to optimize were viscosity ($Y_1$) and Drug permeation ($Y_2$). In the Table 1 lists the dependent and independent variables and their actual values at their high and low levels. A total of 15 runs were needed based on the CCD matrix provided by the Design-Expert® program (Version 13, M/s Stat-Ease, Minneapolis, USA) in Table 2.

**Table 2: Central Composite Experimental Design.**

| Std | Run | Eudragit | Ethylcellulose | Eutectic mixture |
|---|---|---|---|---|
| 5 | 1 | 5 | 2.5 | 12.5 |
| 7 | 2 | 5 | 7.5 | 12.5 |
| 8 | 3 | 15 | 7.5 | 12.5 |
| 1 | 4 | 5 | 2.5 | 7.5 |
| 15 | 5 | 10 | 5 | 10 |
| 13 | 6 | 10 | 5 | 5.79552 |
| 12 | 7 | 10 | 9.20448 | 10 |
| 11 | 8 | 10 | 0.795518 | 10 |
| 2 | 9 | 15 | 2.5 | 7.5 |
| 4 | 10 | 15 | 7.5 | 7.5 |
| 14 | 11 | 10 | 5 | 14.2045 |
| 9 | 12 | 1.59104 | 5 | 10 |
| 10 | 13 | 18.409 | 5 | 10 |
| 3 | 14 | 5 | 7.5 | 7.5 |
| 6 | 15 | 15 | 2.5 | 12.5 |

## Evaluation studies

### *Formulation-related evaluation*

### *Viscosity*

The viscosity plays a major role in deciding the spray pattern and spray ability of the solution. The more viscosity, the less will be the area of spray ability. A Digital Brookfield viscometer was used to determine the viscosity of the formulation at room temperature. The ULA S00 spindle was set at 4 rpm, and the sample size was in the ULA cylinder. The torque level was set at ten while measuring the viscosity.

### *pH*

The pH of the formulation was evaluated to omit skin irritation. The formulation with a similar pH of the skin will have less chance of showing skin irritation. The pH of the optimized formulation is evaluated by the use of calibrated digital pH meter 335 (Systronics, Pvt. Ltd., India). The pH meter's rod is dipped in the Film-forming solution being tested, and the meter's pH were recorded.

### *Density*

The density plays a major role in the spray angle, amount of delivery per actuation and the spray pattern, and the spraying area. The density of the spray solution can be determined by using the specific gravity bottle (density bottle). The empty weight of the 25 mL capacity-specific gravity bottle was recorded. Then it is filled with the optimized batch solution and then reweighed. Then the density was calculated by the formula.

Density (D) = (Weight of bottle filled with sample solution-Weight of empty bottle) / 25

## *In-vitro* Drug permeation studies

The *in-vitro* drug permeation was carried out using the cellophane membrane arranged on the Franz diffusion cell. The membrane used for the permeation studies was cut with the shape and size to fit the size of the diffusion cell. The 10 mL of the 5.5 pH phosphate buffer were filled in the receptor compartment of the diffusion cell. The cellophane membrane with a 2.4 cm diameter were arranged in between the donor and receptor compartments. 1 mL (equivalent to 10mg of drug) of the film-forming solution was taken and added to the receptor compartment. The circulation of 37± 2°C water in the outer jacket of the diffusion cell was performed using a water pump to mimic the body temperature. Then the stirrer was set at the rpm of 200. Exactly, 0.5 mL of sample were withdrawn at periodic intervals of time from the receptor chamber for up to 7 hr. These samples were made up to 10 mL in a volumetric flask, and absorbance is recorded using a U.V spectrophotometer at 283 nm. The concentration of the drug was calculated using the absorbance recorded and the calibration curve line equation.[8] Then the release kinetics of the optimized formulation for evaluating the release model were performed for Zero-order, first-order, Higuchi and Korsmeyer-Peppas. The regression ($R^2$) of all the models was calculated.

## Evaporation time

The evaporation time is also termed drying time. To detect how rapidly the film develops after the solution is sprayed, the evaporation of the film is determined. The optimized batch film-forming solution was sprayed on the clean petri dish to

determine the drying time. Then, a glass slide was laid on the film without stress after a certain period. The film is said to be dry if no moisture remains observed on the glass slide. The drying time defines the rate of film formation. This process is repeated 3 times, and the average evaporation time was calculated.

## Anti-fungal studies

The anti-fungal activity of the formulations and excipients were determined by using the cup-plate method for six common skin infection-causing fungi, namely *Candida albicans, Candida krusei, Aspergillus fumigatus, Epidermophyton, Trichophyton mentagrophytes* and *Cryptococcus neoformans*. The plates were prepared with the Sabouraud's dextrose agar; the medium is sterilized using an autoclave at 121°C, 15 pounds per inch pressure for 20 min. Then the sterilized medium was poured into the agar in the laminar airflow and allowed to solidify. Then 100 µL of fungal culture was poured on the plates and spread on the agar medium. This is repeated for all the fungal species mentioned above, making 5 sets of plates for each fungal species labelled sets A, B, C, D, E. One set of the plates is labelled as the control (set A) and immediately kept for incubation. Another set of 6 fungi plates (set B) were inoculated with fungi immediately after spraying the optimized formulation. Then sets A and B were compared with the fungal growth. If no colonies are observed in set B, then it is stated that formulation prevents the growth of fungi.

In set C, the plates were kept for incubation for 2 days, when the growth is observed, the formulation was sprayed once on each plate and kept for incubation again. After two days, then set C and set A plates were compared for the growth. If the growth is seized, that indicates that the fungi are killed, and no replication is going on, and it proves that the formulation has fungicidal activity.

In set D, after spreading the fungi culture on the plates, then 2 wells were made on each plate and naming one as the MC (marketed cream) and another as the TF (test formulation). 100 mg of marketed cream was weighed and placed in the MC wells of 6 plates, and 100µl of formulation solution was poured into the TF wells of 6 plates. Then all 6 plates of set D were incubated for some days, and inhibition of fungal growth around the wells is observed and recorded.

Four wells were made on each plate after spreading fungi on the 6 plates of set E. Wells were named Sol for solvent alone, EM for eutectic mixture, E+S for Eudragit dissolved in the solvent, and EC+S for ethyl cellulose dissolved in the solvent. Then 100 µL of each sample was poured into the wells according to the naming on all the plates of set E. These plates were then incubated for 3 days to check the anti-fungal activity of the excipients.

## Container-related evaluations

### *Volume of spray solution delivered per each actuation*

The volume of spray solution released can be used to calculate the amount of drug-delivering for one actuation. To calculate the amount of spray solution released for each actuation, the initial weight of the spray container with spray solution is checked (W1). Then the solution was sprayed once with a single actuation, and then it is again reweighed (W2). The amount of spray solution delivered for each actuation was calculated by the formula

$$\text{Amount delivered for one actuation (A)} = (W1-W2) / D.$$

Where,

D = density of the spray solution

### *Spray pattern*

The spraying pattern in the area and pattern of the spray solution falls when sprayed. This was determined by spraying the solution horizontal on a white sheet held at a distance of 10cm. Then, the droplets of the spray solution wet the sheet and make it visible. Immediately, a dotted line was drawn around the wet region of the sheet. Then the diameters of the circle formed in three directions were measured with a ruler. The average of the 3 diameters was calculated.

### *Spray angle*

The solution was sprayed horizontally onto a white sheet held at a distance of 10 cm (d). The diameter of the circle drawn on the paper was measured three times from different angles. From the diameter, radius (r) is calculated. The spray angle (θ) is calculated by the formula

$$\text{Spray angle } (\theta) = \tan^{-1} (L/r)$$

Where,

L = distance between sheet and spray nozzle

R = radius of spray region

### *Short-term stability studies*

The optimized batch's short-term stability was tested using a photostability chamber for two months at 25±2°C and RH 60±5%. The goal of stability studies was to see how the quality of a formulation changes over time as a result of a variety of factors like pH, viscosity, the volume of solution released for each actuation and homogeneity of the optimized batch stayed constant throughout the experiment. If nothing has changed before and after, then the formulation is said to be stable.

**Table 3: Binding energy of antifungal allylamine drugs with squalene epoxidase.**

| Drug | Binding energy (kcal/mol) |
|---|---|
| Terbinafine HCl | -10.3 |
| Naftifine | -10 |
| Amoralfine | -9.2 |
| Butenafine | -8.5 |
| Co-crystal | -7.9 |

# RESULTS AND DISCUSSION

## Selection of drug by docking

By the docking results, it was found that the Terbinafine HCl had a good affinity with the enzyme squalene epoxidase of PDB ID 6C6N. The binding affinity was seen better for the terbinafine HCl compared to that of the co-crystal. The binding energy values of antifungal allylamine drugs with the protein of interest are given in Table 3.

## FTIR- Compatibility studies

The FTIR results for the drug and the physical mixture has been performed, and no addition or deletion of the major functional groups such as C-H–3040.23, C=C-H–2967.91, C=C–2222.56, C-N–1132.97 was observed, which confirms no compatibility between drug and excipients. The data has been given in Figure 1.

## Formulation and optimization of Terbinafine HCl Film-forming spray

The general preparation of the Terbinafine HCl Film-forming spray formulation is by dissolving the excipients in the blend of solvents and sonicating it until a homogenous solution forms. The formulation optimization was performed using the statistical tool, namely the design of experiments. The 3-factorial Central composite design was chosen for the optimization by selecting the formulation factors Eudragit, Ethyl cellulose and Eutectic mixture and the response variables Drug permeation and viscosity. The total number of the batches is 15 batches, which includes 8 corner point runs, 6 axial point runs and 1 center point run, which includes a total of 15 non-repeating combinations of excipients. The effect of the different concentrations of the excipients used on the drug permeation and viscosity can be determined by the 15-run study. In Table 4 summarizes all experimental runs of 3-a factorial central composite design. The values of $Y_1$ response (Viscosity) were found to be in the range of 9.81 cps to 27.14cps, and $Y_2$ response (Drug permeation) in the range of 73.53% to 97.19%, respectively.

To demonstrate the effects and relationships between the excipients, computer design tools were used to create polynomial equations and interactive charts. The sequential model sum of squares, the lack of fit test, and model summary statistics were used to choose the response analysis models. ANOVA was used to verify the polynomial equations statistically, with model terms deemed significant when 'Prob>F' 0.0500 and non-significant when 'Prob>F'>0.1000. For the observed data, three-dimensional response surface charts were constructed to determine the effect of the chosen independent factors on the responses.

## ANOVA analysis for each model selected for each response

### *ANOVA for Linear model for response 1*

The 44.01 Model *F*-value shows that the model is valid. *F*-value of this value has a 0.01 percent probability of happening due to noise (Table 5). Model terms with *P*-values less than 0.0500 are valid. A is a crucial model term in this situation. The model terms are not useful if the value is more than 0.1000. Model reduction may improve the model if there are numerous insignificant model terms.

### *ANOVA for Linear model for response 2*

The *F*-value of 10.55 for the model shows that it is valid. *F*-value of this level has a 0.14 percent probability of happening due to noise (Table 6). Model terms with *P*-values less than 0.0500 are significant. A is a crucial model term in this situation. The model terms are not useful if the value is more than 0.1000. Model reduction may improve the model if there are numerous insignificant model terms.

## Response surface plot

The response surface plots, such as the 3D response graphs, are a great tool for evaluating the interactions and influence of the independent variables. From the interpretation of the 3D plots in Figures 2 and 3, it is also observed that the viscosity has increased with the increase in the polymer concentration, particularly ethyl cellulose. It was seen that the drug permeation had increased with the increased eutectic mixture from Figures 4 and 5.

## Perturbation plots

The perturbation plot is useful for comparing the effects of all variables at a certain point in the design space. Over the response's range, just one variable was modified, while all other parameters stay constant. More the deviation of a factor from the centre point indicates more influence of factor on the response and vice-versa. In Figure 6 it was observed that both factor A (Eudragit) and factor B (Ethyl cellulose) has an influence on the viscosity when compared to factor C (Eutectic mixture). In both factors A and B, it is observed that Factor B has more effect on the viscosity. In Figure 7 it is observed that factor C (eutectic mixture) has more influence on the drug permeation.

## Desirability Criteria and overlay plot

The desirability factor generated by the software was 0.912, demonstrating the rationale for selecting the optimized formula.

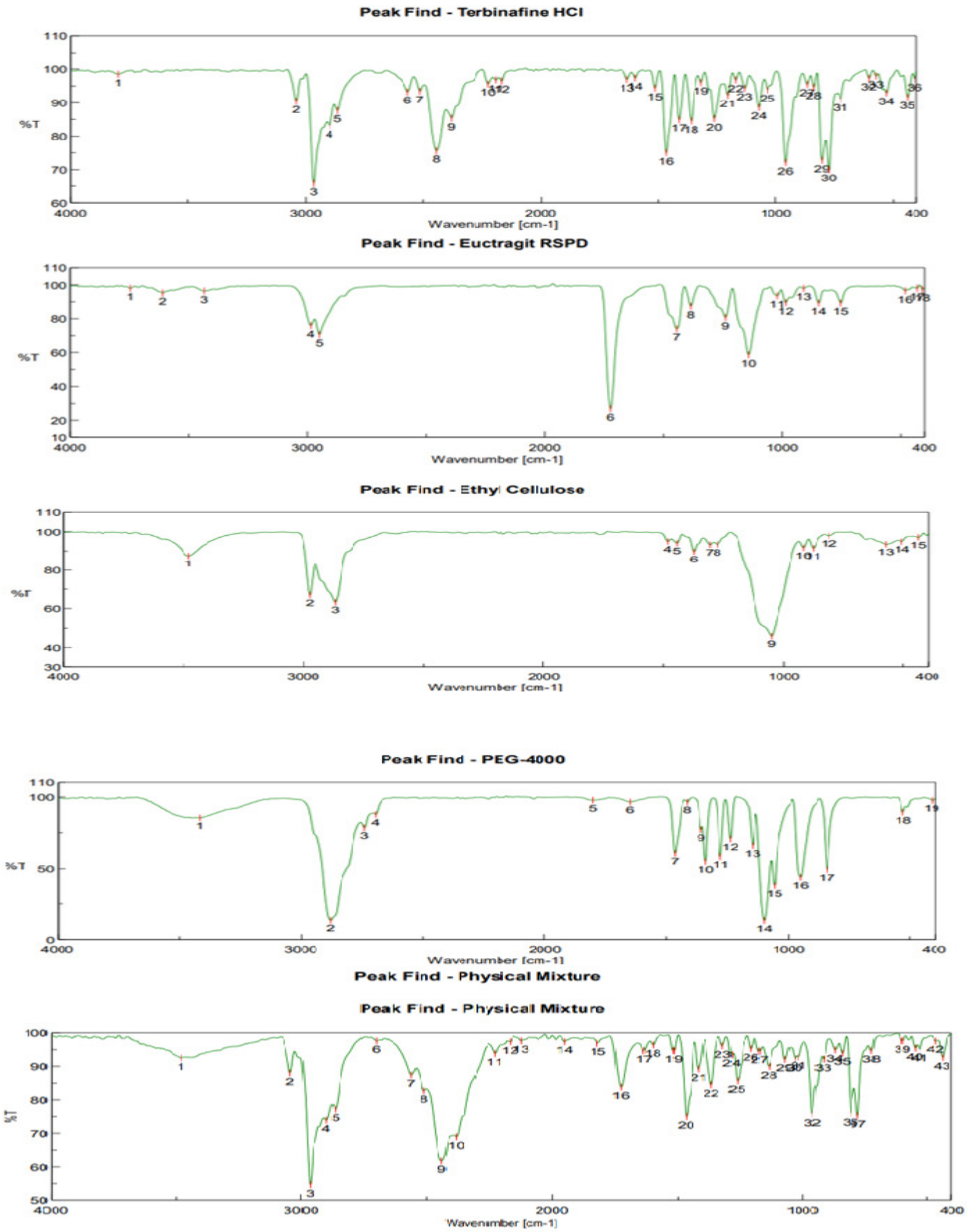



**Figure 1:** FTIR compatibility studies of drug and excipients.

**Table 4:** Experimental runs using 3-factorial Central composite design.

| Run | Eudragit | Ethylcellulose | Eutectic mixture | Viscosity (cps) | Drug permeation (%) |
|---|---|---|---|---|---|
| 1 | 5 | 2.5 | 12.5 | 11.94 | 96.66 |
| 2 | 5 | 7.5 | 12.5 | 21.04 | 92.46 |
| 3 | 15 | 7.5 | 12.5 | 27.14 | 80.37 |
| 4 | 5 | 2.5 | 7.5 | 9.81 | 92.98 |
| 5 | 10 | 5 | 10 | 18.07 | 86.125 |
| 6 | 10 | 5 | 5.79552 | 16.5 | 73.53 |
| 7 | 10 | 9.20448 | 10 | 26.1 | 81.42 |
| 8 | 10 | 0.795518 | 10 | 9.34 | 88.255 |
| 9 | 15 | 2.5 | 7.5 | 15.12 | 79.31 |
| 10 | 15 | 7.5 | 7.5 | 25 | 79.84 |
| 11 | 10 | 5 | 14.2045 | 19.52 | 97.19 |
| 12 | 1.59104 | 5 | 10 | 14.01 | 94.56 |
| 13 | 18.409 | 5 | 10 | 16.2 | 84.5 |
| 14 | 5 | 7.5 | 7.5 | 19.8 | 89.83 |
| 15 | 15 | 2.5 | 12.5 | 17.27 | 81.94 |

**Table 5:** ANOVA for Response 1 (Viscosity)

| Source | Sum of squares | $d_f$ | Mean square | *F*-value | *P*- value | |
|---|---|---|---|---|---|---|
| Model | 388.92 | 3 | 129.64 | 44.01 | < 0.0001 | significant |
| A-Eudragit | 48.07 | 1 | 48.07 | 16.32 | 0.0019 | |
| B-Ethyl cellulose | 328.96 | 1 | 328.96 | 111.68 | < 0.0001 | |
| C-Eutectic mixture | 11.88 | 1 | 11.88 | 4.03 | 0.0698 | |
| Residual | 32.40 | 11 | 2.95 | | | |
| Cor Total | 421.32 | 14 | | | | |

**Table 6:** ANOVA for Response 2 (Drug permeation).

| Source | Sum of squares | $d_f$ | Mean square | *F*-value | *P*- value | |
|---|---|---|---|---|---|---|
| Model | 539.17 | 3 | 179.72 | 10.55 | 0.0014 | significant |
| A-Eudragit | 332.53 | 1 | 332.53 | 19.52 | 0.0010 | |
| B-Ethyl cellulose | 28.95 | 1 | 28.95 | 1.70 | 0.2190 | |
| C-Eutectic mixture | 177.69 | 1 | 177.69 | 10.43 | 0.0080 | |
| Residual | 187.42 | 11 | 17.04 | | | |
| Cor Total | 726.59 | 14 | | | | |

The predicted response provided by the software for the selected optimized formula was 11.93 Cps of viscosity and 96.59% of drug permeation for the factors using 5gm of Eudragit and 2.5gm of ethyl cellulose and 12.5gm of the eutectic mixture. Terbinafine HCl film-forming spray formulation was prepared using these values in run 1 of the design, and the actual responses obtained were 11.94 Cps viscosity and 96.665 drug permeation after 7 hr as shown in Table 7. This shows that the Formulation prepared is in very close accordance with the predicted values. Figure 8 shows the overlay plot for the optimized variables for the formulation.

## Evaluation studies

### *Formulation-related evaluation studies*

### *Viscosity*

The viscosity was determined for all the 15 batches using the Brookfield digital viscometer at the torque of 10. Then the results are found to be highest for batch 3 (27.14), which was formulated with 15gm Eudragit, 7.5 gm of ethyl cellulose and 12.5gm of the eutectic mixture. Similarly, the lowest viscosity was found for batch 5 (9.81), formulated with Eudragit 5gm, ethyl cellulose 2.5gm and eutectic mixture 7.5gm. This indicates

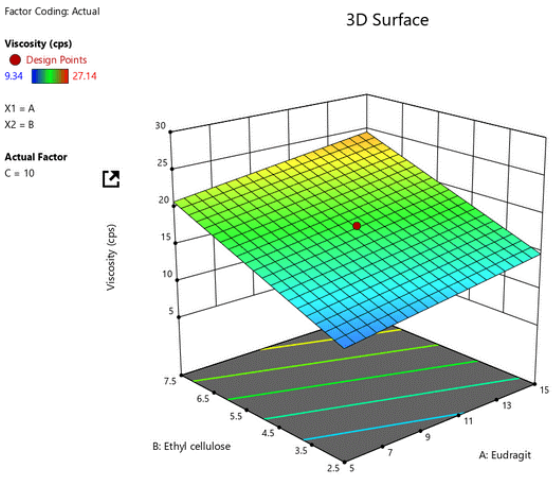



**Figure 2:** 3D response surface plot showing the influence of the Eudragit, ethylcellulose on Viscosity.

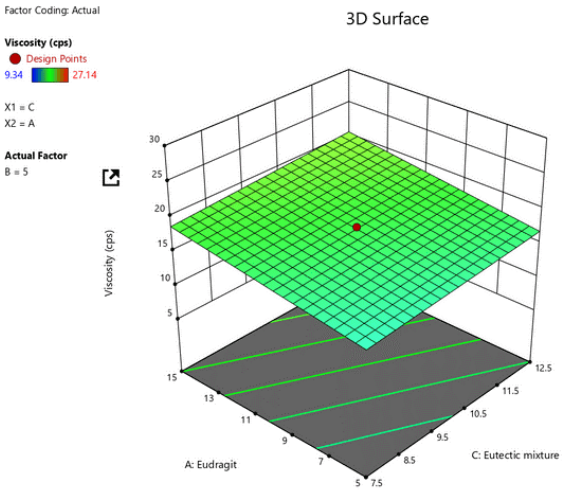



**Figure 3:** 3D response surface plot showing the influence of the Eudragit, Eutectic mixture on Viscosity.

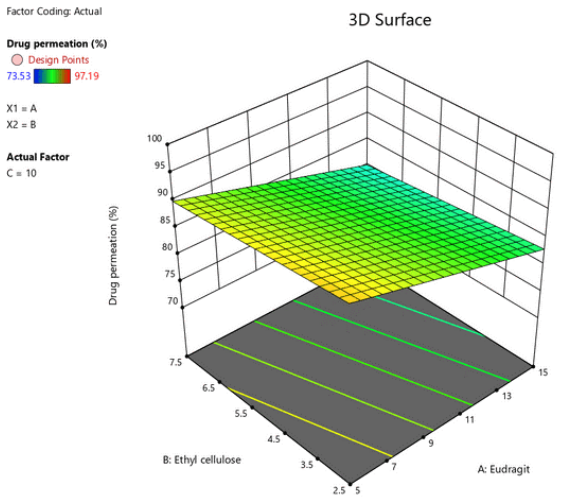



**Figure 4:** 3D response surface plot showing the influence of Eudragit, ethylcellulose on Drug permeation.

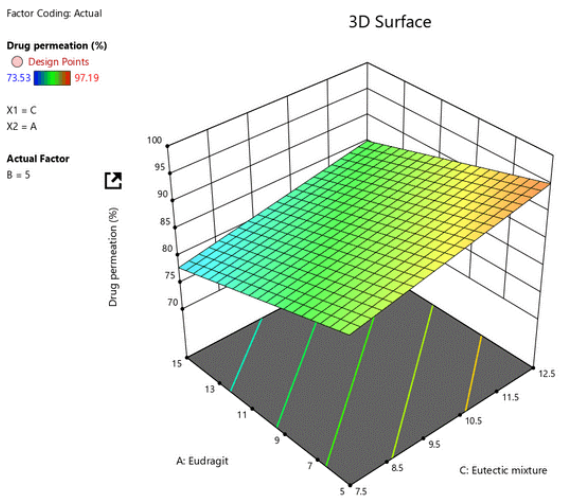



**Figure 5:** 3D response surface plot showing the influence of Eudragit, Eutectic mixture on Drug permeation.

that the polymer concentration is influencing the viscosity of the formulation, particularly ethyl cellulose. The viscosity of all the batches is presented in Table 8 as follows.

### *pH*

The pH of the optimized batch formulation was determined by analyzing the 20 mL of the optimized spray solution with the calibrated pH meter at room temperature. The pH of the solution was found to be 5.16, which is safe for topical application.

### *Density*

The density of the optimized batch was determined using the 25 mL capacity-specific gravity bottle of weight 20. 519 gm. The weight of the density bottle filled with a solution is found to be 42.298 gm. The density of the spray solution was found to be 0.87116 gm/mL. The following formula was used to calculate the density.

Density (D) = (Weight of bottle filled with sample solution-Weight of empty bottle) / 25

## Evaporation time

The evaporation time is the time taken for the spray solution to form a film by solvent evaporation. The evaporation time is calculated by keeping the glass slide on the film; if no wetness sticks to the slide, it is said to be dry. The evaporation time of the optimized formulation was calculated three times, and it is found to be 75 sec, 80 sec, and 75 sec respectively. As the solvent systems used are volatile in nature, the drying time or film-forming time is low comparatively. The average evaporation time is found to be 76.66 sec.

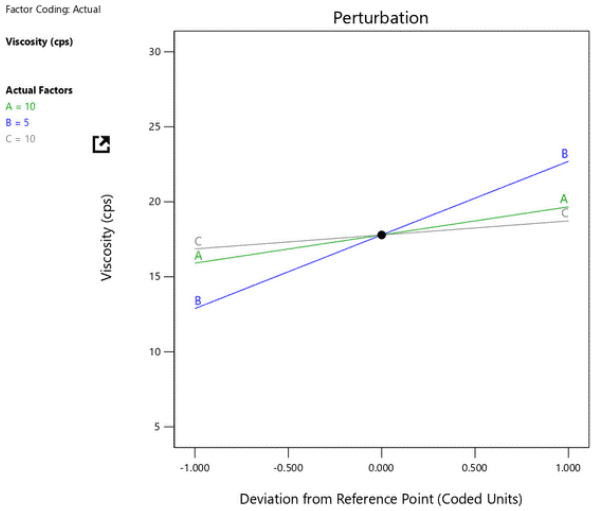



**Figure 6:** Perturbation plot showing the influence of Eudragit, Eutectic mixture, and ethyl cellulose on Viscosity.

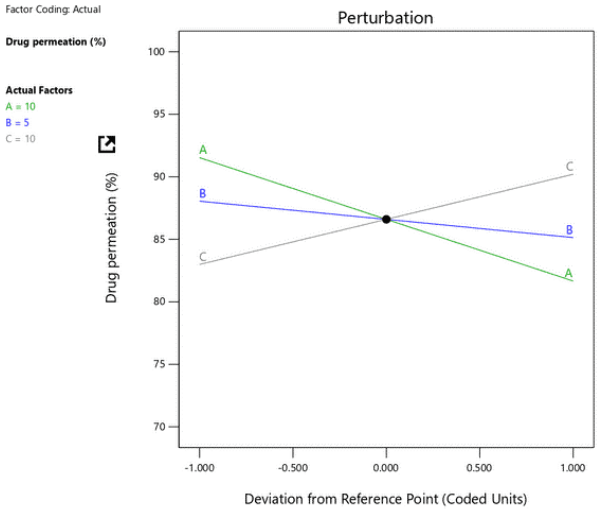



**Figure 7:** Perturbation plot showing the influence of Eudragit, Eutectic mixture, and ethyl cellulose on Drug permeation.

## *In-vitro* drug permeation studies

The drug permeation studies are conducted using the Franz diffusion cell with the cellophane membrane. The permeation studies of all 15 batches were performed using the 5.5 pH phosphate buffer. As the fungal infections spread up to the deeper layers of the skin, more dug permeation is needed. Batch 10 was found to have the greater permeability with 97.19% in 7 hr, while batch 5 got the lowest permeability with 73.53% drug permeation after 7 hr. From the results, it was observed that the drug permeation has increased with the permeation enhancer (eutectic mixture) concentration and reduced with the increase in polymer concentration. The cumulative drug permeation results are given in Table 9, and the drug release profile of the optimized batch is given in Table 10. The $R^2$ (regression) value is found to be highest for the zero-order model; thus, the drug permeation from the optimized batch is found to follow the zero-order kinetics,

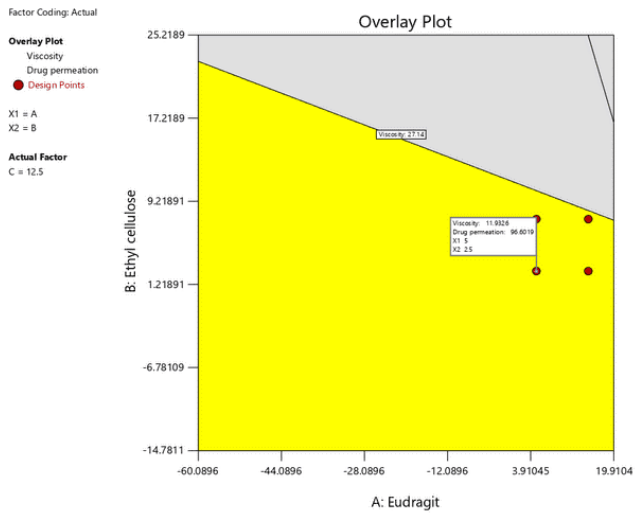



**Figure 8:** Overlay plot for the optimized variables for the formulation.

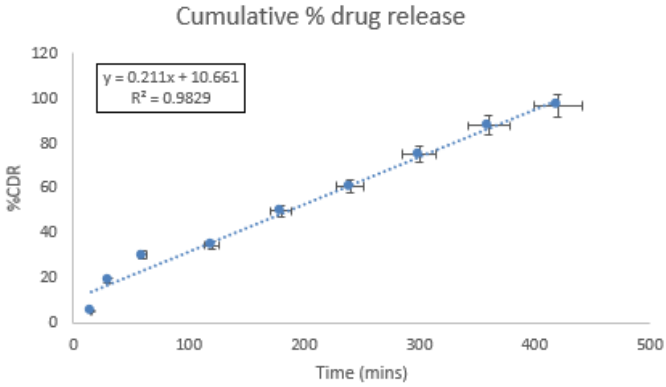



**Figure 9:** *In-vitro* drug permeation profile.

**Table 7: Predicted and actual responses for Terbinafine HCl film-forming spray formulation.**

| Response | Viscosity (Cps) | Drug permeation (%) |
|---|---|---|
| Predicted | 11.93 | 96.59 ± 0.07 |
| Actual | 11.94 | 96.66 ± 0.43 |

as shown in Figure 9. The $R^2$ values of all the models are given in Table 11.

## Anti-fungal studies

The antifungal studies of the optimized Terbinafine film-forming spray formulation were performed in different phases for the various topical infection-causing fungi such as *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Candida krusei, Trichophyton mentagrophytes* and *Epidermophyton*.

The anti-fungal activity of the formulation was evaluated by inoculating the fungi immediately after spraying the formulation. There is no single colony of the fungi observed after incubation for 4 days. This proved that the prior administration of the

**Table 8: Viscosities of all the batches.**

| Run | Eudragit | Ethylcellulose | Eutectic mixture | Viscosity (cps) |
|---|---|---|---|---|
| 1 | 5 | 2.5 | 12.5 | 11.94 ± 1.33 |
| 2 | 5 | 7.5 | 12.5 | 21.04 ± 0.44 |
| 3 | 15 | 7.5 | 12.5 | 27.14 ± 3.21 |
| 4 | 5 | 2.5 | 7.5 | 9.81 ± 1.86 |
| 5 | 10 | 5 | 10 | 18.07 ± 0.54 |
| 6 | 10 | 5 | 5.79552 | 16.5 ± 3.42 |
| 7 | 10 | 9.20448 | 10 | 26.1 ± 2.21 |
| 8 | 10 | 0.795518 | 10 | 9.34 ± 2.41 |
| 9 | 15 | 2.5 | 7.5 | 15.12 ± 0.48 |
| 10 | 15 | 7.5 | 7.5 | 25 ± 1.44 |
| 11 | 10 | 5 | 14.2045 | 19.52 ± 2.54 |
| 12 | 1.59104 | 5 | 10 | 14.01 ± 2.09 |
| 13 | 18.409 | 5 | 10 | 16.2 ± 1.76 |
| 14 | 5 | 7.5 | 7.5 | 19.8 ± 2.76 |
| 15 | 15 | 2.5 | 12.5 | 17.27 ± 0.55 |

**Table 9: Cumulative % drug permeation profile of terbinafine HCl of all batches.**

| Run | Eudragit | Ethylcellulose | Eutectic mixture | Drug permeation (%) |
|---|---|---|---|---|
| 1 | 5 | 2.5 | 12.5 | 96.66 ± 2.55 |
| 2 | 5 | 7.5 | 12.5 | 92.46 ± 1.87 |
| 3 | 15 | 7.5 | 12.5 | 80.37 ± 0.75 |
| 4 | 5 | 2.5 | 7.5 | 92.98 ± 1.54 |
| 5 | 10 | 5 | 10 | 86.125 ± 3.21 |
| 6 | 10 | 5 | 5.79552 | 73.53 ± 2.22 |
| 7 | 10 | 9.20448 | 10 | 81.42 ± 0.89 |
| 8 | 10 | 0.795518 | 10 | 88.255 ± 0.74 |
| 9 | 15 | 2.5 | 7.5 | 79.31 ± 1.32 |
| 10 | 15 | 7.5 | 7.5 | 79.84 ± 1.66 |
| 11 | 10 | 5 | 14.2045 | 97.19 ± 2.21 |
| 12 | 1.59104 | 5 | 10 | 94.56 ± 2.86 |
| 13 | 18.409 | 5 | 10 | 84.5 ± 3.21 |
| 14 | 5 | 7.5 | 7.5 | 89.83 ± 2.09 |
| 15 | 15 | 2.5 | 12.5 | 81.94 ± 3.21 |

film-forming solution prevents fungal infections. This was compared with the control group, which was not administered with any formulation. Figure 10 (a-e) shows that there was no growth of the fungi on the sprayed plates in comparison with the control groups.

The fungal cultures of the 6 species mentioned above spread on the agar plates and incubated for 2 days along with the control plates. Then the 6 test plates which show the growth is sprayed with the formulation and incubated for another 2 days. The control group is kept undisturbed after being spread with cultures in an incubator. The cessation of the growth in the sprayed plates proved that the formulation had fungicidal activity (as the dead fungi can't replicate in incubation). Figure 11 (a-e) shows the growth of fungi in the control group and test group at the time of spraying the formulation on the test group.

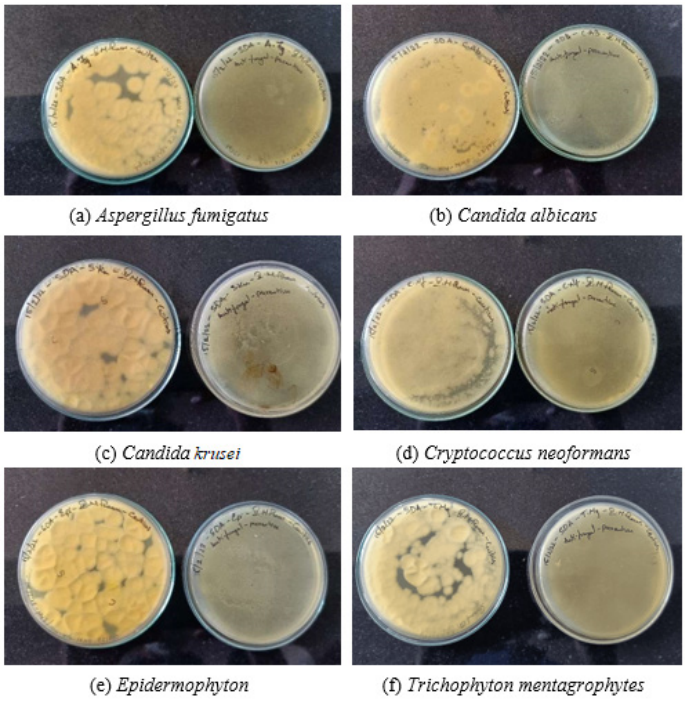
The control group continued to grow, but the test group didn't show any growth even after incubation indicating fungicidal

**Table 10: Cumulative % drug permeation profile of Terbinafine HCl from optimized formulation.**

| Sl. No. | Time (mins) | % Drug Release |
|---|---|---|
| 1 | 15 | 5.58974359 ± 0.05 |
| 2 | 30 | 18.87051282 ± 0.65 |
| 3 | 60 | 30.43461538 ± 1.55 |
| 4 | 120 | 34.63974359 ± 1.76 |
| 5 | 180 | 49.88333333 ± 2.11 |
| 6 | 240 | 60.92179487 ± 1.86 |
| 7 | 300 | 75.11410256 ± 2.13 |
| 8 | 360 | 87.72948718 ± 0.66 |
| 9 | 420 | 96.66538462 ± 3.22 |

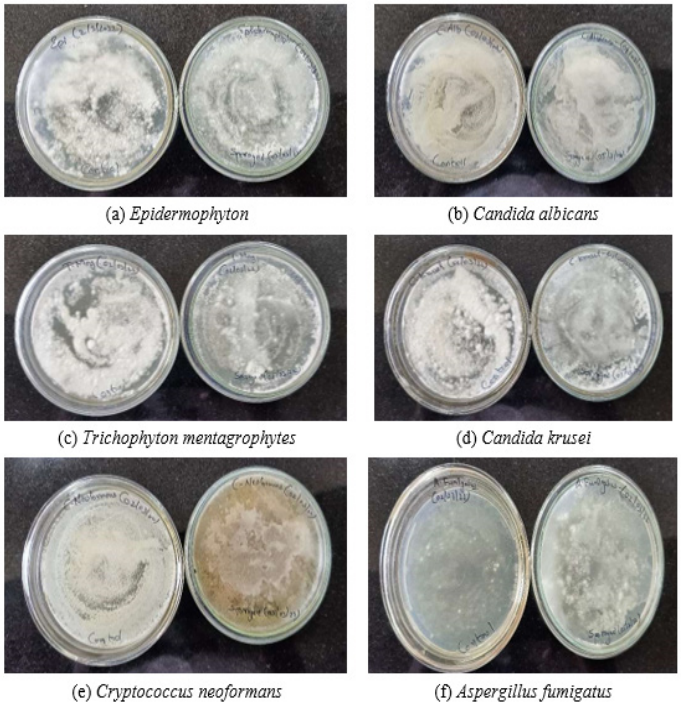
**Table 11: Models and the regression values of the optimized formulation.**

| Sl. No | Model | Regression value ($R^2$) |
|---|---|---|
| 1 | Zero-order | 0.9829 |
| 2 | First-Order | 0.7655 |
| 3 | Higuchi | 0.9752 |
| 4 | Korsmeyer-Peppas | 0.9477 |



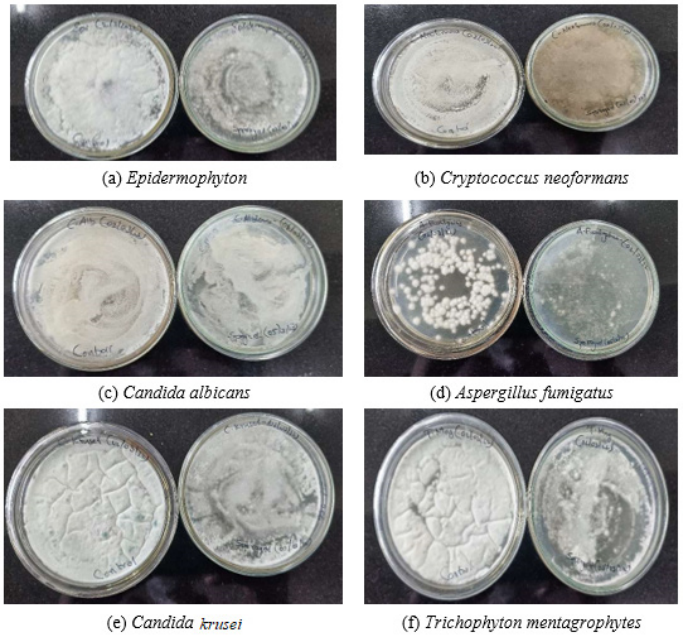
(a) *Aspergillus fumigatus* (b) *Candida albicans* (c) *Candida krusei* (d) *Cryptococcus neoformans* (e) *Epidermophyton* (f) *Trichophyton mentagrophytes*

**Figure 10:** Anti-fungal activity of the optimized Terbinafine HCl film-forming formulation.



(a) *Epidermophyton* (b) *Candida albicans* (c) *Trichophyton mentagrophytes* (d) *Candida krusei* (e) *Cryptococcus neoformans* (f) *Aspergillus fumigatus*

**Figure 11:** Comparison of control and test group fungi after at the time of spraying the formulation.



(a) *Epidermophyton* (b) *Cryptococcus neoformans* (c) *Candida albicans* (d) *Aspergillus fumigatus* (e) *Candida krusei* (f) *Trichophyton mentagrophytes*

**Figure 12:** Comparison of control and test group fungi after two days of spraying the formulation.

activity. The fungicidal activity of the optimized formulation is illustrated in Figure 12 (a-e).

The anti-fungal activity of the excipients and solvents is evaluated by the cup-plate method. Only the Eutectic mixture has restricted the growth of the fungi. The rest of the excipients and the solvent has shown no anti-fungal activity. The anti-fungal activity of excipients and the solvent is illustrated in Figure 13 (a-d).

A comparative study among the marketed semi-solid formulation and the Optimized film-forming spray formulation for the anti-fungal activity has been performed, and the results have shown that the optimized Terbinafine HCl film-forming spray solution has greater anti-fungal activity when compared to that of

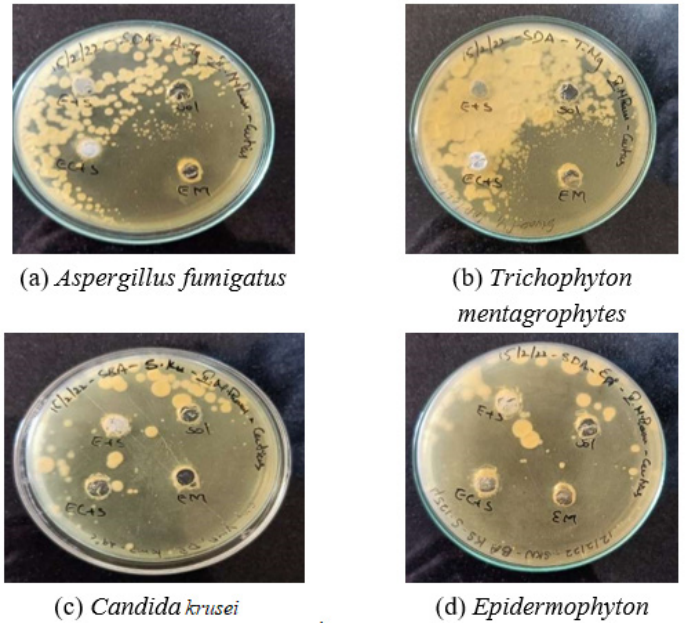



**Figure 13:** Anti-fungal activity of solvent and excipients.

**Table 12: Parameters before and after the stability studies.**

| Parameter | Before stability testing | After stability testing |
|---|---|---|
| pH | 5.16 | 5.19 |
| Viscosity | 11.94 Cps | 12.07 Cps |
| Visual appearance | No floccules/ aggregates of polymer were observed | No floccules/ aggregates of polymer were observed |

the marketed formulation. The antifungal activity comparison of the marketed antifungal cream and the test formulation is shown in Figure 14 (a-e).

## Container-related evaluation

### *The volume of spray solution delivered per each actuation*

The initial weight of the container and the spray solution was found to be 201.527. The weight of the container and the formulation after one actuation was found to be 201.342. From the above studies, the density of the optimized batch solution is found to be 0.87116 gm/mL.

Amount delivered for one actuation (A) = (W1-W2) / D.

The volume of the solution delivered per actuation is found to be 0.212 mL.

### *Spray pattern*

The average diameter of the spray pattern formed by the spraying of optimized batch formulation on the sheet at a distance of 10cm from the nozzle was found to be 15.41 cm.

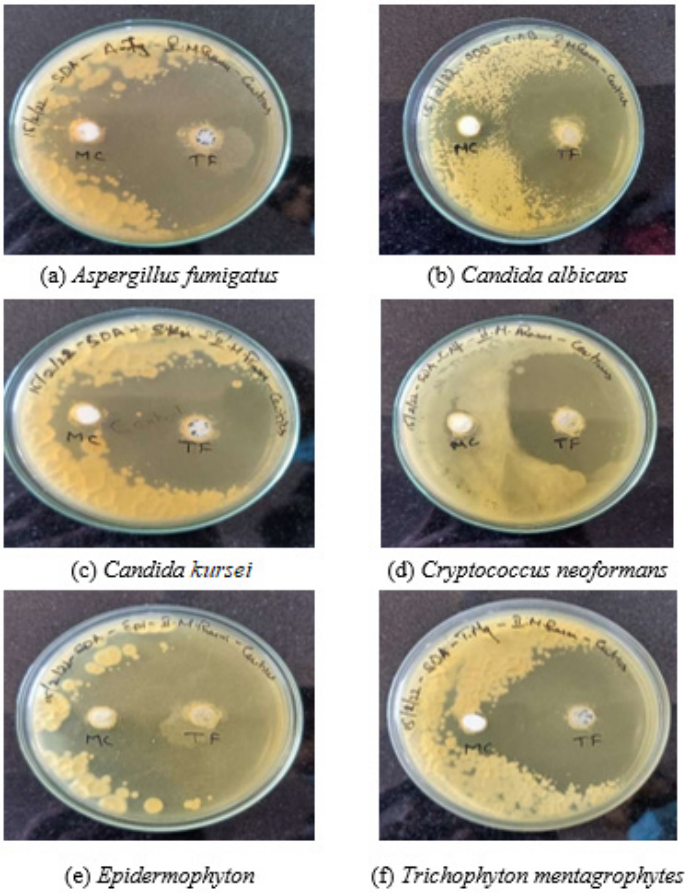



**Figure 14:** Comparison of anti-fungal activity of marketed cream and test formulation.

### *Spray angle*

The average diameter of the spray pattern was found to be 15.41 cm from the spray pattern analysis, and then the radius (r) is calculated to be 7.705cm. The length (L) from the nozzle to the sheet is taken as 10 cm. Then the spray angle (θ) is found to be

The spray angle for the optimized batch is found to be 52.38°.

### *Stability studies*

After two months of stability studies of the optimized Terbinafine HCl film-forming formulation at the temperature of 25±2°C and RH 60±5%, it is observed that there were no significant changes in the pH, viscosity and the appearance of the formulation. The parameters evaluated before and after the stability studies are shown the Table 12.

## CONCLUSION

The Terbinafine HCl film-forming spray was optimized using the Central composite design. The optimized formulation containing 5% Eudragit RSPO, 2.5% Ethyl cellulose and 12.5% camphor menthol eutectic mixture has good spray ability, viscosity and drug permeation. The viscosity of the optimized batch was found to be 11.94cps and *in-vitro* drug permeation after 7 hr was found

to be 96.66%. The pH was found to be 5.16 with a spray angle of 58.38° and a good spray pattern. The stability studies at 25±2°C and 60±5% RH for 2 months has revealed that the formulation is stable with no change in pH, viscosity and appearance. Thus, the prepared optimized formulation may be considered a promising approach for the treatment of topical fungal infections.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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