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Original Article

Fungal Infections: Effect of Eudragit RL-100 Based Miconazole Film Forming Spray

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ABSTRACT

Background: Miconazole nitrate, a BCS class II drug which is used for treatment of fungal infections. To minimize the disadvantages of sticky creams and ointments and their tendency to rub off, an intelligent dosage regimen needs to be designed as a film-forming spray formulation. The aim of this investigation was to develop a film-forming spray formulation containing 0.5% Miconazole nitrate for the treatment of fungal infections on the skin. **Materials and Methods:** The Miconazole nitrate film-forming spray formulation was prepared using different concentrations of Eudragit RL-100, Ethylcellulose, Camphor and Menthol crystals (Eutectic mixture), Polyethene glycol 400 and Ethanol by simple solvent dissolving method using a statistical tool, 2^3 full factorial design to optimize the film forming spray formulation. The film-forming spray underwent thorough testing to evaluate its formulation characteristics, including pH, viscosity, evaporation time, density, drug release and container-related factors such as spray angle, spray diameter and amount of spray solutions released per actuation at valve assembly. To carry out an antifungal activity test on an optimized spray formulation and compare it with a commercially available cream. **Results and Discussion:** From the study, the ethyl cellulose concentration has a greater influence on the viscosity and density, 45 cps and 0.8472 g/mL of the spray formulation respectively. Eudragit RL-100 and the eutectic mixture have a higher impact on the drug release 92.44% at 8 hr in pH 6.8 phosphate buffer. **Conclusion:** The topical treatment of fungal infections proved to be suitable for the Miconazole nitrate film-forming spray formulation. Longer, continuous medication administration is facilitated by the skin's longer retention period. The medication exhibits good antifungal effectiveness in preventing fungal infections, according to antifungal investigations. The Miconazole nitrate film-forming spray is a considerably better alternative to current dosage forms for treating topical fungal infections, as evidenced by its good stability, simplicity of administration and action.

Keywords: Fungal infections, Film forming spray, Miconazole nitrate, Eudragit RL-100, PEG 400, Ethylcellulose, Eutectic mixture.

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INTRODUCTION

The most accessible organ in the body is the skin as well and because of its poor susceptibility to environmental micro and macromolecules, functions as a barrier against them. The skin's complex structure acts as the body's main defence, creating a strong barrier that protects from infections, UV rays, toxins and physical injury. The thickness of each layer varies, but they all work together to support the skin's defences and robustness.[1]

Hospitals and physicians are facing a major issue as a result of the startling rise in the prevalence of fungal infections. Most fungi are ubiquitous and can reproduce in their natural environments without the assistance of humans or other animals. It's estimated that about 33% of all fungal skin infections and 50% of all nail diseases develop from nail infections, or onychomycoses. They impact anywhere from 2% to 13% of the global population as well as up to 30% of high-risk populations, including the elderly. Diabetes sufferers have an especially high frequency of onychomycosis; they are almost three times more likely to have the disease than the general population and run the danger of experiencing severe consequences if the infection is not treated.[2] Numerous fungal infections are also known to be caused by other fungus, such as *Aspergillus* and *Candida*. Although topical fungal infections often only cause mild, non-life-threatening symptoms, they can still significantly lower the life quality of the patient. These infections can spread throughout the body if they are not treated, therefore patients need to get suitable treatment and medication as soon as possible.

Miconazole nitrate, a BCS class II drug is used for the treatment of fungal infections.[3] For the treatment of fungal infections on the skin, a wide variety of formulations are available; the majority

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of them are traditional formulations. Among the present dosage forms' many drawbacks are their patches, ointments, lotions and other formulations. Occlusive patches, which block sweat ducts and prevent water vapour from leaving the skin's surface, can irritate the skin, be difficult to apply to curved surfaces, be difficult to peel off and be inconvenient. Moreover, the main drawback of patches is that they cannot be fitted into the application area due to their restricted size and form.[4] Semisolid treatments like ointments and lotions also have disadvantages. They do not remain in touch with the skin for very long since they are rapidly removed by the patient's clothes. This means that for chronic illnesses like ringworm, athlete's foot and candidiasis, repeated application is required. The main drawback of these traditional formulations is that they cannot be applied to the ridges and grooves of the nails.[5]

A novel dosage form that may be administered less often and for a longer time while yet retaining intimate contact with the skin is needed to enhance patient compliance due to the issues with existing dosage forms, such as semisolids and patches. Innovative methods that potentially take the place of conventional topical and transdermal formulations are the Film-Forming Spray (FFS). It is a non-solid dose form that forms a film as soon as it comes into contact with the skin or any other bodily surface. In these systems, the drug and excipients that produce the film are combined with a solvent that evaporates when it comes into contact with the skin, leaving the medication and excipients in a film. The resulting film may be a residual liquid film that is rapidly absorbed in the stratum corneum or a solid polymeric layer that functions as a matrix for drug release to the skin over time.[6]

MATERIALS AND METHODS

Materials

A gift sample of Miconazole nitrate was obtained from SKN Organics (P) Ltd., located in Puducherry. Eudragit RL-100 (Otto Chemie Pvt. Ltd., Mumbai), Ethyl Cellulose (Loba Chemie Pvt. Ltd., Mumbai), Camphor (Oxford Lab Fine Chem. LLP., Maharashtra), Menthol crystal (Loba Chemie Pvt. Ltd., Mumbai), PEG 400 (SD fine chem. Ltd., Coimbatore), Ethanol (Yarrow Chem products, Mumbai) were used.

Methods

Preparation of the Formulation

A straightforward solvent-dissolving technique is used to produce the Miconazole nitrate Film-forming spray solution. To prepare the camphor-menthol eutectic mixture, equal amounts of camphor and menthol are weighed and then allowed 10 min to liquefy in a bath sonicator. Take another beaker and mix ethanol and ethyl cellulose. Add Eudragit RL-100 to this eutectic mixture and stir at room temperature for 30 min using a magnetic stirrer. A solvent is used to dissolve miconazole nitrate individually. Miconazole nitrate was progressively added to the solvent system. Before being sonicated for 10 min, the solution was agitated for 15 min at 80 to 100 rpm. After adding PEG-400 to the mixture, it was stirred mechanically for 20 min to ensure a uniform dispersion. If there are any polymer clumps, the produced solution is maintained in a bath sonicator for dispersion. Transferring this finished solution into a suitable spray container.[7] Figure 1 displays the general preparation method for film forming sprays.

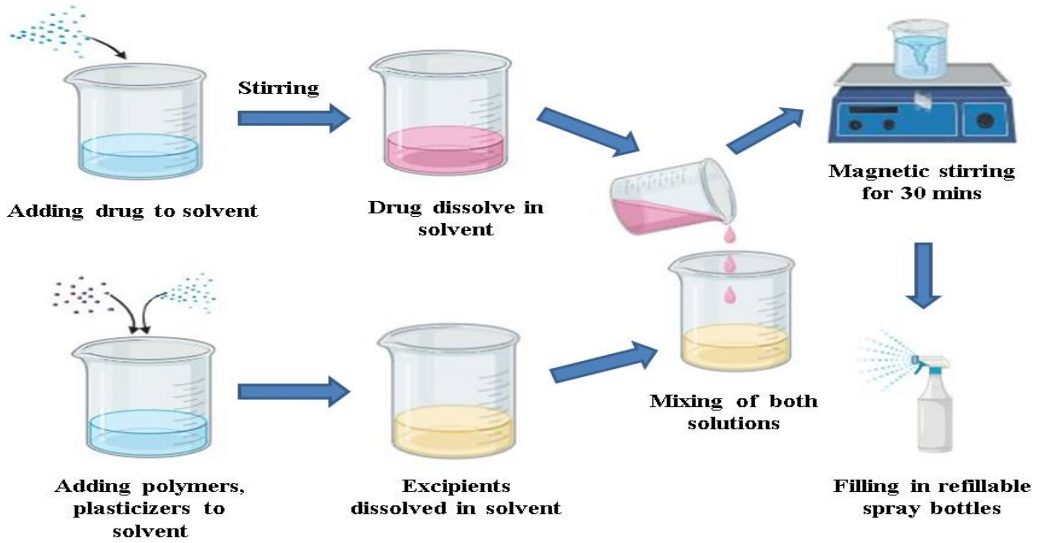

Figure 1: General preparation method of film-forming sprays.

Table 1: Optimization table.

Formulation	Run	Eudragit RL-100 (g)	Ethyl cellulose (g)	Eutectic mixture (g)
F1	1	10	2.5	12
F2	2	10	5	8
F3	3	10	2.5	8
F4	4	5	5	12
F5	5	5	2.5	12
F6	6	5	2.5	8
F7	7	5	5	8
F8	8	10	5	12

Experimental Design

A 2^3 full factorial design was used to optimize the formulation parameters of Miconazole Nitrate FFS with the least number of tests possible.[8] The polymer Eudragit RL-100 (X1), ethyl cellulose (X2) and camphor-menthol eutectic combination (X3) were selected and investigated at two different levels denoted as -1 and +1. These constituents are the main factors influencing the formulation's physicochemical properties. Viscosity (Y1), drug release (Y2) and density (Y3) were the dependent response variables that needed to be optimized. The experiment investigates the influence of three independent variables, labelled as A, B and C on three dependent variables.

A=Eudragit RL-100 (mg) with low level (-1) at 5 mg and high level (+1) at 10 mg, B=Ethyl cellulose (mg) with low level (-1) at 2.5 mg and high level (+1) at 5 mg, C=Eutectic mixture (mg) with low level (-1) at 8 mg and high level (+1) at 12 mg. The experiment aims to Minimize the viscosity (cps) of the resulting formulation, Maximize the drug release (%) from the formulation, and Minimize the density (g/mL) of the formulation. Table 1 depicts the Optimization Table 1.

Evaluation Studies

Formulation Related Evaluation

Viscosity

The solution's spray pattern and abilities are mainly affected by its viscosity. The area of spray ability will decrease with increasing viscosity. When the formulation was at room temperature, its viscosity was measured using a Digital Brookfield viscometer. The sample size was in the LA cylinder and the LA 31 spindle was set at 6 rpm.[9]

Density

For every actuation, density has a major effect on the spray pattern, spray angle, spraying area and volume of delivery. Use the specific gravity bottle, also called the density bottle, to find the spray solution's density. The empty weight of the 50 mL specific gravity bottle is mentioned. The optimized batch solution is then added and the weight is determined once more. Then, the formula is used to determine the density.[10]

Density (D)=(Weight of bottle filled with sample solution-Weight of empty bottle)/50

pH

The formulation's pH is assessed to eliminate skin irritation. A formulation that is similar to the skin's pH will be less likely to cause skin irritation. The formulation's pH is measured using a digital pH meter that has been calibrated. The pH of the film-forming solution being tested is dipped into the rod of the pH meter and the reading is recorded.[11]

Evaporation Time

The drying time is another name for the evaporation time. The rate at which the film forms after the solution is sprayed can be determined by measuring the film's evaporation. To find the drying time, the cleaned petri dish was sprayed with the optimal batch film-forming solution. After a certain period, there is no longer any moisture visible on the glass slide, the film is considered dry. The rate at which films form is determined by the drying time. After completing this procedure three times, the average evaporation time was determined.[7]

In vitro Drug Release

An open-ended cylinder with a cellophane membrane was used to carry the drug release from the formulation. Then 1 mL of the formulation was taken out and placed inside the cellophane membrane, which was placed in a beaker containing 200 mL of pH 6.8 phosphate buffer. To keep the sink condition, 5 mL of sample were extracted from the beaker at regular intervals of one hour and replaced with a new buffer. The sample absorbance was analyzed using a UV-visible spectrophotometer at 272 nm.[12]

In vitro Anti-fungal Activity

The anti-fungal activity of the formulations is determined by using the cup-plate method for common skin infection caused by fungi, namely Candida albicans and Aspergillus niger.[13,14] Sabouraud's dextrose agar is used to make the plates and an autoclave set at

121°C for 60 min is used for sterilizing the medium. Following sterilization, the agar is filled with the sterilized medium in a laminar airflow and allows becoming solid. Subsequently, 100 µL of fungal culture is poured onto the plates, covering the agar medium. For every fungal species listed above, repeat this process, dividing the plates into four sections, one for each fungal species labelled as A (control group), B (standard), C (MCN optimized formulation) and D (marketed cream). The plates are inoculating the fungi immediately after spraying the optimized formulation, standard drug and marketed formulation in the wells. After two days of incubation, the growth on the plates is observed. Next, the zone of inhibition and fungal inhibition were compared.[7]

Valve Performance

Spray Diameter

When sprayed, the spray solution falls according to the spraying pattern in the region. This was determined by horizontally spraying the solution onto a white sheet that is held 10 cm away. The sheet is then wet and visible due to the spray solution droplets. The wet area of the sheet is promptly marked with a dotted line. Using a ruler, measured the diameters of the circles that were produced in three different directions. The average of 3 diameters was computed.[10]

Spray Angle

The solution is sprayed horizontally onto a white sheet held 10 cm distant (d). Three measurements were made of the circle's diameter on the paper, each from an alternate angle. Calculating the radius (r) from the diameter. The formula is used to calculate the spray angle (θ).[15]

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

Where,

L=distance between sheet and spray nozzle,

R=radius of spray region.

Amount of Spray Solution Released Per Actuation

The volume of spray solution discharged would be measured to find out how much medicine was delivered with a single actuation. The amount of spray solution delivered for each actuation is calculated by measuring the initial weight of the spray container containing the spray solution (W1). Next, the solution is reweighed after being sprayed once with a single actuation (W2). The formula determines how much spray solution gets delivered with each actuation.[9]

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

Where,

D=density of the spray solution.

RESULTS AND DISCUSSION

Formulation and Optimization of Miconazole Nitrate FFS

The experimental runs for the 2^3 full factorial designs are included in Table 2. The Y1 response (viscosity) values have been determined to be between 42 cps and 57 cps, indicating that the viscosity has increased as the concentration of ethyl cellulose has increased, Y2 response (drug release) in the range of 80.78% to 92.02% which indicate the drug release has increased with the decreased Eudragit RL-100 concentration and increasing the concentration of eutectic mixture, Density (Y3) response ranges from 0.84 to 0.90 g/mL, indicating that density is increasing as ethyl cellulose concentration has increased.

Characterization of Optimized Miconazole Nitrate FFS

Viscosity

Using the Brookfield digital viscometer at LA 31 spindle, the optimized Formulation's (F9) viscosity is determined. The optimized Formulation (F9), which is composed of up to 5 g of Eudragit RL-100, 2.5 g of ethyl cellulose and 10 g of the eutectic mixture, then provides 45 cps at 6 rpm.

Table 2: Experimental runs using 2^3 full factorial design.

Formulation	Viscosity (cps)	Drug release (%)	Density (g/mL)
F1	47	83.25	0.84
F2	55	80.78	0.86
F3	43	81.85	0.85
F4	52	91.85	0.87
F5	44	92.02	0.85
F6	42	92.18	0.85
F7	49	89.42	0.87
F8	57	81.85	0.90

Table 3: Comparison of the marketed cream (2% Miconazole) and the optimized formulation (0.5% Miconazole) anti-fungal activity.

Formulation	Zone of inhibition (mm)	
	Aspergillus niger	Candida albicans
A (positive control)	0 mm	0 mm
B (standard)	22 mm	24 mm
C (optimized formulation)	27 mm	30 mm
D (marketed formulation)	30 mm	32 mm

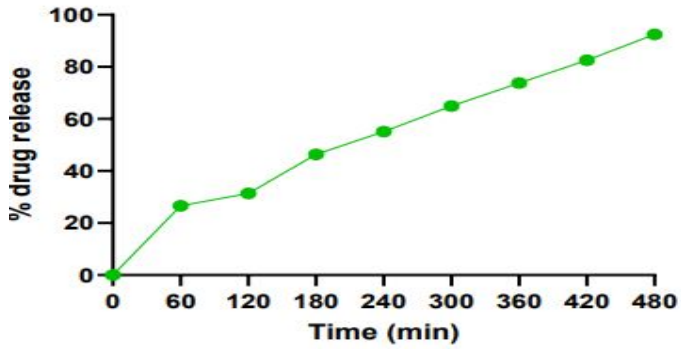

Figure 2: *In vitro* release study of MCN FFS.

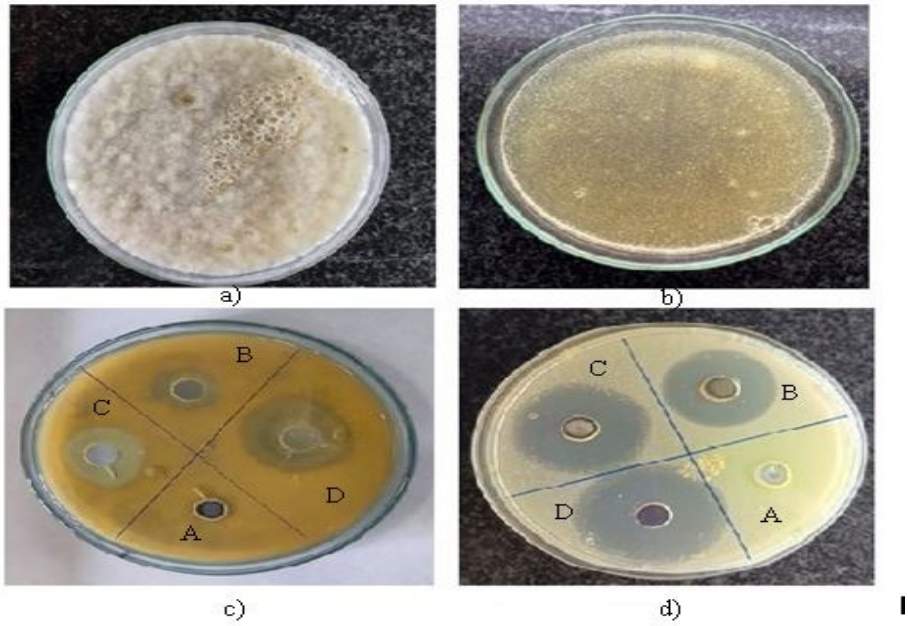

Figure 3: a) Positive control group of *Aspergillus niger* b) Positive control group of *Candida albicans* c) Anti-fungal activity of *Aspergillus niger* d) Anti-fungal activity of *Candida albicans*.

Density

The optimized batch's density is determined using a 50 mL specific gravity bottle with a weight of 13.85 g. The density bottle filled with a solution weighs 56.21 g. The spray solution's density was found to be 0.8472 g/mL.

pH

The pH of the optimal batch formulation (F9) can be ascertained by using a calibrated pH meter at room temperature to evaluate 20 mL of the optimized spray solution. It is found that the solution has a pH of 5.8, which is suitable for topical application.

Evaporation Time

The amount of time required for the spray solution to evaporate its solvent and create a film is known as the evaporation time. The glass slide is kept on the film to determine the evaporation time; the slide is considered dry if no moisture remains to it. After three calculations, the more effective formulation's evaporation time finds out to be 76, 79 and 80 sec, respectively. The film-forming or drying times have been relatively short due to the volatile nature of the solvent systems used. The average evaporation time was found to be 78.33 sec.

In vitro Drug Release Studies

Figure 2 depicts the optimized formulation (F9) of Miconazole Nitrate FFS, which at the end of 8 hr showed drug release of 92.44% in phosphate buffer with a pH of 6.8. The order of release for the optimized formulation (F9) was first-order. It indicates the drug's initial burst release during the first hour and its sustained release until the conclusion of the 8 hr. To determine the release constant and regression coefficient (R2) in the drug release kinetics, the released data were fitted to a variety of kinetic models. Regression coefficients (0.9940) and n value of 0.691 indicate that, among the evaluated models, the Korsmeyer-Peppas model best suited the drug release profile for the optimized formulation. The drug release kinetics result suggested that the release profile follows Non-Fickian movement. The drug transport mechanism is anomalous Non-Fickian transport and drug release mechanism follows both diffusion and erosion.

Anti-fungal Studies

Studies on the antifungal activity of the optimized formulation (F9) of Miconazole nitrate film-forming spray are conducted on a variety of fungi that cause cutaneous infections, including *Aspergillus niger* and *Candida albicans*.

Once the formulation has been poured into the wells and incubated for 2 days, the fungi are immediately inoculated to assess the formulation's anti-fungal activity. It shows that the fungal growth was inhibited in set C (optimized formulation) when compared with set A (control). Even after incubation, the test group showed no growth, indicating the fungicidal action, while the control group kept growing. The fungicidal activity of Optimized formulation listed in the Figure 3 .

Based on a comparative analysis of the anti-fungal activity of the optimized film-forming spray formulation and the marketed semi-solid formulation given in the Table 3. It can be concluded that the anti-fungal activity of the optimized Miconazole nitrate film-forming spray solution is almost identical to that of the marketed formulation.

Valve Performance

Spray Diameter

The average diameter of the spray pattern developed when the optimized batch Formulation (F9) is sprayed onto the sheet at a distance of 10 cm from the nozzle is found to be 8.6 cm.

Spray Angle

Based on the spray pattern analysis, the pattern's average diameter (d) and radius (r) are found to be 8.6 cm and 4.3 cm, respectively. A measurement of 10 cm (L) is made between the nozzle and the sheet. The spray angle (θ) is then determined to be$66.68^{o}\pm32$.

Amount of Spray Solution Released Per Actuation

It was found that the spray solution and container weight were 56.21 g. After one actuation, the weight of the formulation and the container was determined to be 56 g. The optimal batch solution has a density of 0.8472 g/mL. It is determined that 0.247 mL of solution gets delivered with each actuation.

CONCLUSION

In conclusion, the topical treatment of fungal infections proved to be suitable for the Miconazole nitrate film-forming spray formulation. The order of release for the Miconazole nitrate film-forming spray formulation was first-order. It indicates the drug's initial burst release during the first hour and its sustained release until the conclusion of the 8 hours. The Korsmeyer-Peppas release kinetics model has been shown by employing a miconazole nitrate film-forming spray formulation. The drug transport mechanism is anomalous non-fikian transport and the drug release mechanism follows both diffusion and erosion. The improved formulation has good spray properties that make it appropriate for topical administration. Longer, continuous medication administration is facilitated by the skin's longer retention period. The medication exhibits good antifungal action by effective control in the progression of fungal infections, according to antifungal investigations. The Miconazole nitrate film-forming spray is a considerably better alternative to current dosage forms because of its simplicity of administration, retention and action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MCN: Miconazole nitrate; **FFS:** Film forming spray; **PEG:** Poly ethylene glycol; **MC:** Marketed cream.

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