RESEARCH ARTICLE

Formulation and evaluation of clotrimazole transdermal spray

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Abstract

Context: Transdermal spray (TS) of clotrimazole (CTZ) was formulated to improve the drug transport through the skin up to 12 h to achieve the antifungal efficacy.

Objective: The aim of present study was to formulate and evaluate antifungal transdermal spray to improve the permeation of clotrimazole across the skin and to decrease the dosing frequency in fungal infection.

Materials and methods: Different ratios of ethanol and acetone and various grades of eudragit and ethyl cellulose were evaluated according to six criteria: viscosity, drying time, stickiness, appearance and integrity on skin and water washability. Propylene glycol (PG) and polyethylene glycol 400 (PEG 400) were used in the study as plasticizer and solubilizer. The TS was evaluated for in vitro drug release, spray angle, spray pattern, average weight per dose, pH, drug content, evaporation time, leak test and antifungal efficacy study.

Results and discussion: Eudragit E100 and blend of ethanol and acetone (80:20) satisfied the desired criteria. The selection of optimized batch was based on the results of in vitro drug release, spray pattern and spray angle. The optimized batch showed the spray angle <85° and uniform spray pattern. The formulation containing PG showed higher drug release than PEG 400. The inclusion of eutectic mixture consisting of camphor and menthol (1:1) showed improved drug transport through the rat skin and larger mean zone of inhibition indicating the improved antifungal efficacy.

Conclusion: The TS of CTZ can be an innovative and promising approach for the topical administration in the fungal diseases.

Keywords

Antifungal activity, clotrimazole, eudragit, eutectic mixture, polyethylene glycol 400, propylene glycol, solvent and polymer screening, transdermal spray

Introduction

Administration of the drug through the human skin (topical drug delivery) has received increased attention due to several advantages. However, topical drug delivery is challenging since the skin behaves as a natural barrier to the complex process of drug transport.

The fungi are common causes of skin infections around the world. The risk of fungal infections in the human skin during the whole life is 10–20%². The stratum corneum is the target organ of antymycotic drug treatment. Clotrimazole (CTZ) (1-[(2-chlorophenyl) diphenylmethyl]-1H-imidazole) is relatively nontoxic synthetic imidazole derivative with broad-spectrum of activity, first described in 1969³. CTZ, a wide spectrum triazole-based antifungal agent, works primarily by inhibiting the enzyme cytochrome P450 14x-demethylase. This enzyme converts lanosterol to ergosterol, and is required in the fungal cell membrane synthesis. CTZ also blocks steroid synthesis in humans. CTZ has marked in vitro activity against all the Candida spp. and Cryptococcus spp., against almost all strains of dermatophytes and against Aspergillus spp. and other fungal genera responsible for systemic mycoses⁴.

Topical therapy is often preferred to oral drug administration in the treatment of cutaneous fungal infections because CTZ is practically insoluble in water⁵ and poorly and erratically absorbed when administered orally. In some cases, this drug shows failure of treatment, side effects and high relapses of disease. Adverse effects of CTZ are abnormal liver function, nausea and vomiting, mild burning, irritation, stinging of skin. The transdermal patch has the occlusive nature which contributes to the irritation. Semisolid dosage forms like gels, creams and ointments are applied over the affected area with the help of fingers. Hence, there are chances of cross-infection to other body parts. The patients do not like to touch the skin infected by fungus. Semisolid dosage forms get easily rubbed off by the clothing and during the day-to-day activities. Physical stability of semisolid preparation containing aqueous and oily components is a serious concern.

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Therefore, an alternative approach to patches and other semisolid preparations is the concept of a quick-drying nonocclusive spray. Such a system would be easy to use, welltolerated and allow for application over a fixed area of skin. Eudragits, acrylic resins, have been widely used as coating material in the pharmaceutical industry. It has been reported that these polymers are well tolerated by the skin and have a high capacity for incorporating drugs. Eudragit may be useful for the development of transdermal drug delivery system. However, only a few studies have reported acrylic resin as a transdermal drug delivery polymer. Eudragit E100 was selected because of its good skin tolerance in clinical trials. Ethanol and propylene glycol (PG) are two of the permeation enhancers cum co-solvent to solubilize drugs at the same time. PG is widely used as a co-solvent in pharmaceutical preparations and has been employed as the basic vehicle in several studies on the percutaneous penetration of indomethacin. Gohel and Nagori reported that camphor and menthol are well known dermal penetration enhancers. Therefore, it can also be used as the permeation enhancer.

The aim of the present research work was to formulate functional and a patient friendly transdermal spray (TS) to facilitate permeation of CTZ. The volatile constituents evaporate rapidly leaving a highly concentrated, thin layer of drug in a residual vehicle that is rapidly taken up into the stratum corneum. The drug is then released slowly from the reservoir created in this way over a prolonged period.

**Methods**

**Materials**

CTZ was received as gift sample from Shreya Lifesciences Pvt. Ltd. (Mumbai, Maharashtra, India). Eudragits (E100, RS100, RLPO and S100) were received as gift sample from Evonik Degussa Pvt. Ltd. (Mumbai, Maharashtra, India). Ethyl cellulose, camphor and menthol were purchased from Astron Chemicals (Ahmedabad, Gujarat, India). All other ingredients and solvents used were of analytical or pharmaceutical grade.

**Preliminary solvent and polymer screening**

**Preparation of the polymeric solutions**

Film-forming solutions were prepared by adding 10% w/v eudragit (E100, RS100, RLPO or S100) or ethyl cellulose to the different blends of ethanol and acetone (100:0, 80:20, 50:50, 0:100). Each solution was stirred for about 6–8 h to ensure complete dissolution of the polymers. The formulations were stored in glass vials sealed tightly with an aluminum cap before using for the evaluation test. Each solution was evaluated for viscosity, drying time, stickiness and cosmetic appearance. Integrity on the skin and water washability of film were evaluated after 12 h of application of polymer solution. The formulations containing Eudragit E100, RS100, RLPO, S100 and EC were labeled as P1–P5, respectively.

**Evaluation of different polymeric solutions**

For a first assessment of the suitability of film forming solutions, the obtained formulations were evaluated according to a rating system for six characteristics. The rating criteria for film forming solution are summarized in Table 1. The formulations were applied with a micropipette in a very small quantity in the specific area of the skin (so as to form a film on the skin surface) to the inner sides of the forearm of a six healthy human volunteers of 21–27 years of age. The purpose of the study was fully explained to them and volunteers’ written consent was taken.

The viscosity of the solution was evaluated visually and rated as low (water-like), medium (glycerol-like) or high (syrup-like). The quantitative measurement of polymeric solutions was also performed using Brookfield viscometer (Dial Viscometer Model LVT, Middleboro, MA). The viscosity of the solutions was measured at 25 ± 1°C using LV spindle no. 62. The spindle was rotated at 30 rpm.

For the assessment of the drying time, the formulation was applied to the inner sides of the forearm of a volunteer. The applied volume was 10 μl/cm² as this amount was small enough to be applied without flowing away from the application site. After 5 min, a glass slide was carefully placed on the film without application of pressure. If the traces of the liquid were not visible on the glass surface after removal, the film was considered dry. If the traces of the liquid were visible on the glass surface, the experiment was repeated with a drying time of 7 min instead of 5 min and observations were recorded.

The stickiness of the dried film was tested by gently pressing cotton wool on the film. Depending on the quantity of cotton fibers retained by the film, the stickiness was rated high (dense accumulation of fibers on the film), medium (thin fiber layer on the film) or low (occasional or no adherence of fibers).

The cosmetic appearance of the films was assessed by visual examination of the dry films. From the appearance of the film, it was rated as low (shiny and transparent), medium (shiny and translucent) or high (dull-and opaque).

To test the integrity of the skin, the formulation was applied to the forearm of a volunteer as described for the assessment of the drying time. The dry film was then worn for 12 h by the test subject. After 12 h, the test area was examined visually for completeness of the film, appearance of cracks or flaking.

To check the water wash ability of the film, the film was washed with water after 12 h of application. Depending on the ease of washing, the film was rated as easily washed, moderately washed and poorly washed.
Three rating scores were assigned to each criterion with 1 representing the most positive evaluation (meaning that the film characteristic closely matched the target) and 3 the most negative result. The formulations were considered acceptable when all the six criteria were rated as 1. These formulations showed a low viscosity, short drying time, low stickiness, high cosmetic appearance, stayed intact on the skin for a prolonged time and easily washed out from the skin. Formulations with one or more criteria rated 2 were considered as acceptable with limitations. Formulations with one or more criteria rated 3 were not acceptable11. The selected polymer and solvent system were used for the further studies. The score of formulations are summarized in Table 2.

**Formulation development of TS**

_Determination of eutectic composition of camphor and menthol_

Camphor and menthol were mixed in different ratios, 1:1, 1:9, 2:8, 3:7, 4:6, 6:4, 7:3, 8:2 and 9:1 at 35 ± 2°C for 15 min in a glass mortar and pestle. The undissolved solids, if remained, were carefully separated from the liquid and then weighed. The presence of only one phase, i.e. liquid indicates eutectic composition1.

_Determination of solubility of drug and polymer in various solvents_

The solubility of CTZ was determined in a mixture containing ethanol and acetone and eutectic mixture of camphor and menthol. An excess amount of sample was added to 25 ml of ethanol:acetone blend (80:20) as solvent and the blend was stirred on a magnetic stirrer at 60–80 rpm for 30 min at 35 ± 2°C in a closed vessel. The mixtures were then filtered through 0.45 μm membrane filter and the weight of undissolved solids was recorded. The filtrate was critically observed for clarity1.

_Formulation of TS_

The polymer content, type and amount of permeation enhancer were varied in the formulations. The CTZ was dissolved in the blend of ethanol and acetone. Eudragit E100 weighed polymer was dissolved in the eutectic mixture of camphor and menthol (1:1). PG/polyethylene glycol 400 (PEG 400) was added to the polymeric mixture. This polymeric solution was added to the solvent mixture containing CTZ and stirred on a magnetic stirrer at 100–120 rpm at 35 ± 2°C for 20 min1. The resulting drug solution was filled in a refillable container containing plastic dip tube of 78 mm length and 2.6 mm internal diameter (Figure 1). The aperture size of the tube was 0.2 mm. The composition of formulations is shown in Table 3.

**Characterization and evaluation of developed TS**

The quantitative tests performed for the TS formulations were drug content, average weight per dose, in vitro drug release and pH. The qualitative tests include the evaluation of spray pattern, spray angle, evaporation time and effectiveness of pump seal13. The findings are shown in Table 4.

**pH of formulations**

The pH of the formulations was determined using a pH strip1.

**Drug content**

Drug content of each formulation was determined by mixing 1 ml solution of formulation with the acetate buffer (pH 6.0) containing 35% of dioxan and the blend was stirred on magnetic stirrer at 60–80 rpm at 35 ± 2°C for 1 h for complete drug extraction. The samples were then analyzed by spectrophotometric analysis (Shimadzu UV-1601, Duisburg, Germany) at 240 nm. Drug content was calculated from the linear regression equation obtained from a standard curve in acetate buffer pH 6.014.

**Evaporation time**

Evaporation time is the time required for the spray film to dry and it was estimated by spraying the formulation on a white paper and then the drying time was noted of each formulation13.

**Spray pattern**

Spray pattern was assessed by delivering the spray through the TS on white paper. Methyl orange (1%) was dissolved in all formulations to facilitate visualization. The paper was clipped to a board and the formulation was sprayed on it at a distance of 2.5–3.0 cm from the plate. The spots, formed as a result of spray testing, were observed and their diameters were measured. This was repeated three times and the average was taken of each reading13.
The initial weight of the containers was recorded. Five successive test were placed in the upright position at 30°C. The product was evaluated by this test. The filled containers under the TS were sprayed from the TS and the containers were weighed after 3 days to check the leakage of the formulation from the container.

Formulation code

| Formulation code | Composition (Eudragit E100:PG/PEG 400) | Drug content (% ± SD) | Average weight per dose (g ± SD) | Evaporation time (s ± SD) | Spray pattern | Spray angle (° ± SD) | pH | Leakage rate (%) |
|------------------|----------------------------------------|-----------------------|----------------------------------|--------------------------|---------------|---------------------|----|------------------|
| M1               | 4:3                                    | 95.23 ± 1.07          | 0.35 ± 0.02                      | 83.33 ± 1.52             | Spherical, not uniform | 82.05 ± 0.63 | 5.0 | 0.01            |
| M2               | 6:3                                    | 95.83 ± 0.56          | 0.33 ± 0.02                      | 81.33 ± 1.53             | Non-spherical, uniform | 83.07 ± 0.78 | 5.0 | 0.01            |
| M3               | 8:3                                    | 97.83 ± 0.97          | 0.34 ± 0.01                      | 79 ± 1                   | Spherical, uniform   | 84.85 ± 0.06 | 6.0 | 0.01            |
| M4               | 10:3                                   | 95.53 ± 1.01          | 0.35 ± 0.02                      | 73.33 ± 0.58             | Spherical, less uniform | 85.98 ± 0.22 | 6.0 | 0.03            |
| M5               | 10:5                                   | 95.4 ± 1.24           | 0.35 ± 0.03                      | 72.66 ± 0.58             | Spherical, less uniform | 87.39 ± 0.55 | 6.0 | 0.01            |
| N1               | 4:3                                    | 97.8 ± 0.2            | 0.35 ± 0.02                      | 87 ± 1.73                | Non-spherical, Not uniform | 79.11 ± 0.23 | 5.0 | 0.02            |
| N2               | 6:5                                    | 93.76 ± 0.25          | 0.34 ± 0.02                      | 84.33 ± 1.15             | Spherical, Not uniform | 82.32 ± 0.65 | 6.0 | 0.01            |
| N3               | 8:5                                    | 94.9 ± 0.26           | 0.33 ± 0.02                      | 80.33 ± 1.53             | Spherical, uniform   | 84.78 ± 0.16 | 5.0 | 0.02            |
| N4               | 10:5                                   | 95.96 ± 0.45          | 0.33 ± 0.01                      | 75.66 ± 0.57             | Spherical, less uniform | 85.41 ± 0.46 | 6.0 | 0.01            |
| N5               | 10:3                                   | 95.8 ± 0.6            | 0.34 ± 0.01                      | 74.33 ± 0.75             | Spherical, less uniform | 86.84 ± 0.24 | 6.0 | 0.03            |

Spray angle

The method of impingement of spray on a piece of paper was used for the study. Methyl orange (1%) was dissolved in all the formulations. The spray was actuated in horizontal direction onto a white paper mounted at a distance of 15 cm from the nozzle. The radius of the circle which was formed on the paper was recorded from different directions. The test was repeated three times and the average was taken. Spray angle was calculated from the following equation:

\[
\text{Spray angle} = \tan^{-1}(h/r),
\]

where \(h\) is the distance of paper from the nozzle and \(r\) is the average radius of the circle.

Average weight per dose

The initial weight of the containers was recorded. Five successive deliveries were sprayed from the TS and the containers were weighed again. The difference in the initial and final weight of the containers was divided by the number of deliveries to determine the average weight per dose

\[
\text{Average weight per dose} = \left\{ \frac{\text{Initial weight}(W_0) - \text{Final weight}(W_f)}{\text{Number of deliveries}(N)} \right\}.
\]

Leak test

Effectiveness of pump seal and its ability to store the contents of the product was evaluated by this test. The filled containers under test were placed in the upright position at 30°C for three days and then the containers were weighed before and after 3 days to check the leakage of the formulation from the container.

In vitro release study using nylon membrane

A nylon membrane having a pore size of 0.2 μm was used in the study. The nylon membrane was mounted on the Franz diffusion cell. The formulation solution (1 ml) was placed in the upper reservoir (donor compartment) and the receptor compartment was containing 20 ml of acetate buffer pH 6.0 having 35% dioxan. During the 12 h of the experiment, the temperature was kept at 35 ± 2°C. Aliquots of 5 ml samples were collected from a receptor compartment at different time interval (0, 1, 2, 12 h). After each sampling, fresh dissolution medium was replaced to receptor compartment in the same quantity. The drug concentration in each sample was determined by UV spectroscopy at 240 nm in acetate buffer pH 6.0. The cumulative amount of drug (%) penetrating per unit surface area (cm²) is shown in Figure 2.

In vitro skin permeation study

In vitro skin permeation study of antifungal TS was carried out using rat skin as biological barrier. The excised rat skin was obtained from Research Centre, Anand Pharmacy College, Anand (Approved by Institutional Animal Ethics Committee (IAEC), No. 9006). Wistar albino rats (previously shaved) having a weight of about 180–200 g was selected in the study. The animals were sacrificed and the dorsal part (abdominal region) of rat skin was immediately excised and was cleaned with normal saline. To remove the fat material, dermis part was wiped 3–4 times with the wet cotton swab soaked in the isopropanol. Then subcutaneous fat was removed and washed with normal saline. The tissue was cut in appropriate size and stored in liquid paraffin at −18°C until use. At the time of study, skin was clamped in the Franz diffusion cell. The receptor compartment was containing acetate buffer (pH 6.0) containing 35% of dioxan. During the 12 h of the experiment, the temperature of the skin was kept at 35 ± 2°C. The
donor compartment (upper reservoir) was filled with the solution of antifungal TS. Samples were collected from a receptor compartment at different time intervals (0, 1, 2, ..., 12 h). The drug concentration in each sample was determined by UV spectroscopy at 240 nm. The cumulative amount of drug (%) penetrating per unit surface area (cm²) is shown in Figure 3.

Antifungal efficacy studies

The antifungal efficacy study against Candida strain, i.e. *Candida albicans* (turbidity 0.5 on McFarland scale) was determined by agar diffusion methods employing "cup plate technique". Sterile solutions of CTZ in standard solution, i.e. ethanol:acetone (80:20), eutectic mixture in solvent, solvent alone and the developed formulation having the pH adjusted as required, was poured into cups (10 μl) bored into sterile Sabouraud dextrose agar previously seeded with the test organism (Table 5). After allowing diffusion of the solutions for given time, the agar plates were incubated at 37°C for 24 h. The zone of inhibition (ZOI) was measured and compared with that of pure drug. The entire operation was carried out in an aseptic condition. Figure 4 shows the outcome of the study.

Results and discussion

Preliminary solvent and polymer screening

Preliminary screening of solvent using various polymers showed that the higher solubility of polymers and faster film formation (<5 min) were obtained in the case of ethanol and acetone blend (80:20). Ethyl acetate, acetone and primary alcohol are preferred solvents in the formulation of topical solutions. As per the US Department of Health and Human Services—Food and Drug Administration (US FDA) inactive ingredient guide, limit of alcohol and acetone in topical solutions is more than 83% and 12%, respectively. Acetone was used along with alcohol in the vehicle blend to facilitate faster film formation (<5 min) on

Table 5. Composition and results of selective batches for evaluating antifungal activity.

| Batch code | % w/w of ingredient | M3 | M11 | M12 | M13 |
|------------|---------------------|----|-----|-----|-----|
| CTZ        |                     | 1  | 1   | –   | –   |
| Eudragit E100 |                 | 8  | –   | –   | –   |
| Propylene glycol |             | 3  | –   | –   | –   |
| Eutectic mixture |             | 10 | –   | 10  | –   |
| Ethanol:Acetone (80:20) (q.s.) | | q.s. | q.s. | q.s. | q.s. |
| Zone of inhibition (mm) | | 20 | 18  | 10  | 8   |

q.s., quantity sufficient.
the skin. This is preferred combination of solvents wherein the components have good solubility for the active principles and film forming agent(s). At the same time, the solvent must be sufficiently volatile to provide drying times of the topical solutions of a few minutes or less. Other important factors besides drying time are flow characteristics in application, rate of film forming, etc. The polymer concentration in the solution studied was 10% because it is reported the highest concentration of film forming polymer for topical spray.

The polymer selection was performed on the basis of evaluation tests of the preliminary polymeric solutions. Table 2 shows the scores for various tests. Ex-in vivo film formation of placebo batches (P1–P5) passed the desired criteria of drying time which varied from 89 to 110 s, indicating the suitability of all the polymers as film forming polymer with respect to drying time. The low stickiness and low viscosity (by visual observation) were observed in batch P1 containing Eudragit E100 as compared to other batches which might be due to the low viscosity of Eudragit E100 (3–12 mPa s) among all the other polymers. The viscosity of all the polymeric solutions (P1–P5) measured by Brookfield viscometer ranged from 10 to 80 cps. The low viscosity is required to be low to enable an application of the dosage form as spray, which would ensure an accurate, but at the same time flexible dosing and would be most convenient for the patient.

The appearance of batches P1–P3 was shiny and transparent, which is attributed to their viscosity and complete dissolution of polymers in the solvent system and hence rated as 1. Batch P4 was shiny and translucent which can be attributed to higher viscosity and higher concentration of Eudragit S100 in the solvent system while P5 was dull and opaque, which might be due to incomplete dissolution of EC in the solvent system. The integrity of the films after 12 h from the batches (P1–P3) was rated 1 as they showed no crack or not rubbed off during the wearing period indicating their sufficient strength and flexibility. The films of batches P4 and P5 were cracked or sporadic flaking during the integrity test indicating insufficient strength or flexibility of the skin due to incomplete dissolution of polymers in the solvent system. Water washability of batches P1, P3 and P5 was rated as 1 which might be due to their higher affinity to water as compared to other batches. None of the placebo formulations resulted in irritation, rashes and itching in any of the volunteers.

Based on the overall performance in the screening study, batch P1 containing Eudragit E100 was selected as the most promising film former as it was rated 1 in all the evaluation parameters and also it is considered preferable because of its good skin tolerance in clinical trials. Therefore, Eudragit E100 was used for the formulation of TS.

Determination of eutectic composition of camphor and menthol

When the camphor and menthol ratios were 6:4, 7:3, 8:2, 9:1, the % w/w of undissolved solids was 18 ± 3%, 44.9 ± 3%, 71 ± 3% and 93 ± 3% w/w, respectively. Complete liquefication was obtained in the ratio of camphor and menthol 1:1, 4:6, 3:7, 2:8 and 1:9 at 35 ± 2°C. The results show that percentage menthol and percentage undissolved solids are inversely related to menthol concentration up to 40%. Therefore, the eutectic mixture having camphor:menthol in the lower level (1:1) was selected for the formulation of Eudragit E100 and CTZ considering their antifungal activity, permeation enhancement and cost factor.

Determination of solubility of drug and polymer in solvents

The solubility of CTZ in a mixture of ethanol and acetone (80:20) (% w/v) was 140 mg/ml and in the eutectic mixture was less than 4 mg/ml. The solubility of Eudragit E100 in eutectic mixture was >80 mg/ml. So CTZ was dissolved in vehicle blend of ethanol and acetone whereas Eudragit E100 was dissolved in a eutectic mixture of camphor and menthol.

Characterization and evaluation of TS formulations

The drug content and average weight per dose in all the formulated batches ranged from 94% to 98% and 0.33–0.35 g, respectively. Insignificant variation in the amount of liquid emitted per actuation indicated the effectiveness of the pump system in delivering reproducible amounts of the formulation per actuation. Evaporation time for formulation P1, being a simple polymeric solution required 98 s to dry results into a film. The other formulations required 73–83 s. The reason may be attributed to the presence of other excipients added to the formulations. The evaporation time was inversely correlated with the polymer concentration. In addition, PG and PEG 400 also affects the evaporation time. The presence of polyethylene glycols in film results in greater evaporation time. Spray pattern for the formulation batches (M3–M5) and (N3–N5) exhibited good spray patterns in terms of uniform and spherical spots due to the flexible and cohesive film forming nature of the Eudragit E100 and higher concentration of polymer used. Spray angle from all the formulations ranged from 79° to 87°. This can be correlated with the polymer concentration. The spray angle should be less than 85° for easy actuation of drug solution from the container and to cover a maximum surface area. The spray angle of batches (M4, M5) and (N4, N5) was greater than 85° and thus failed to meet the desired criteria. Batches M3 and N3 showed the spray angle very close to 85°. The pH of formulated batches ranged in between 5 and 6 which is appropriate as the pH of the human skin is 5.5–6.5. No leakage or negligible leakage (0–0.03%) from all the TS containers was observed when placed in the upright position at 30° for 3 days.

In vitro release study using nylon membrane

The UV spectrum remained unchanged during the in vitro study, indicating the stability of CTZ during the analytical procedure. Table 4 and Figure 2 display the % amount of drug release over a period of 12 h. Batches M1, M2, N1 and N2 which were formulated using lower concentration of Eudragit E100 (less than or equal to 6% w/v) showed the complete drug release within 9 h. The problem of initial higher drug release and lack of sustained drug transport was rectified by increasing the concentration of Eudragit E100 in the subsequent batches (M3–M5) and (N3–N5). The batches containing 10% of Eudragit E100 required greater time for complete drug release (≥12 h) while batches M3 and N3 containing 8% of Eudragit E100 showed drug release up to 12 h. Thus, they satisfied the desired criteria of drug release (up to 12 h). Hence, considering the overall results of spray pattern, spray angle, % drug release of 12 h, the highest level of Eudragit E100, which can be incorporated in the formulation, was 8% w/w as in batches M3 and N3.
The effect of PG and PEG 400 as a permeation enhancer on the release of CTZ was investigated by taking different concentrations (3% and 5% v/v) in the formulations (Table 4). The steady state flux values for batches M1–M5 ranged from 0.173 to 0.249 mg/cm²/h and for batches N1–N5 it was 0.167 to 0.244 mg/cm²/h. In M series, marked effect was observed in batch M3 (3%) while in the N series, batch N3 (5%) showed a marked effect as they fulfilled the desired criteria of in vitro drug release up to 12 h and film formation. It may be stated from the results that as the concentration of PG and PEG 400 was increased from 3% v/v to 5% v/v, the steady state flux was decreased due to higher concentration of polymer. At the 5% v/v concentration of PG, it produced the sticky film so the desired criteria of film formation were not satisfied while at the 5% v/v concentration of PEG 400, it fulfilled the desired criteria of film formation, but not improved the in vitro drug release. Moreover, %, cumulative drug release and steady state flux were higher in batch M3 containing 3% PG than batch N3 containing 5% PEG 400 as shown in Table 4.

Therefore, it is concluded that the formulation M3 containing 3% w/w of PG as a permeation enhancer is suitable for the transdermal delivery of the CTZ. The higher in vitro drug release in case of PG might facilitate topical permeation which causes solvation of α-keratin within the stratum corneum and the occupation of proteinaceous hydrogen bonding sites, reducing drug–tissue binding and thus promoting permeation. So PG acts as a solubilizer as well as permeation enhancer while PEG 400 did not readily penetrate the skin when applied topically so resulted in lower drug release. Thus, on the basis of % cumulative drug release, evaporation time and spray angle, batch M3 was selected as the optimized batch from all the formulation batches.

**In vitro skin permeation study**

The effect of eutectic mixture on drug release was checked using rat skin as a biological membrane in the Franz diffusion cell (Figure 3). The UV spectrum remained unchanged during the in vitro drug transport study, indicating the stability of CTZ during the analysis. The excipients or membrane constituents did not show absorbance at 240 nm. Batch M0 was formulated without eutectic mixture. It exhibited incomplete drug transport (less than 50%). Batch M3 exhibited drug transport greater than 80%. The probable reason for this difference could be the presence of camphor and menthol in batch M3. It is reported that camphor and menthol work as penetration enhancers. Camphor and menthol cause leaching of the lipids present in the skin and cause pore formation.

It is reported that by the addition of eutectic mixture, the melting point of the drug is depressed to around or below skin temperature thereby enhancing drug solubility. However, it is also likely that the interaction of the penetration enhancer with stratum corneum lipids also contributed to the increased drug flux. Hence, it can be concluded that a eutectic mixture of camphor and menthol drastically affects the drug transport.

**Antifungal efficacy studies**

As shown in Figure 4, the mean zone of inhibition (antifungal activity) in batch M13 was least (8 mm) indicating the effect of solvent (ethanol:acetone). In batches M11 (drug in solvent) and M12 (eutectic mixture in solvent), zone of inhibition was 18 and 10 mm, respectively. Batch M3 (optimized formulation) shows the highest zone of inhibition (20 mm) indicating the synergism of activity by CTZ and eutectic mixture.

**Conclusion**

The TS of CTZ was effectively formulated for topical fungal infections. The ethanol:acetone (80:20) as solvent system and the Eudragit E100 as film forming agent fulfilled the desired criteria of the film formation and integrity of the skin. TS with good spray pattern in terms of uniform and spherical spots was obtained indicating flexibility and cohesive nature of the polymer. The spray angle was correlated with concentration of film forming agent. The highest concentration of polymer did not show good spray ability. The average weight per dose showed the reproducibility of the pump system to deliver approximately same amount per actuation. The optimized formulation showed the in vitro drug release up to 12 h. PG (3%) was effective for enhancing the in vitro drug release and it also acts as solubilizer and plasticizer. The eutectic mixture inclusion improved the drug release through the rat skin. The antifungal efficacy of the optimized formulation was higher than the other control batches. From the results obtained in the present work, it can be concluded that the TS of CTZ can be innovative and promising approach for the topical administration in the fungal diseases. This study can be performed in the broader population involving human subjects for the assessment of antifungal efficacy to treat the fungal diseases.

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**Declaration of interest**

The authors report no declarations of interest.

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Supplementary material available online