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Original Article

Topical Film Forming Clotrimazole Emulgel

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ABSTRACT

Introduction: Clotrimazole is a weak base and is practically insoluble in water. To enhance drug loading of hydrophobic Clotrimazole in the formulation and, to circumvent greasiness, poor retention time and easy wipe off associated with conventional topicals, it was formulated as a Film Forming Emulgel. **Materials and Methods:** Preformulation studies were performed to evaluate solubility and emulsification of drug in oils and surfactants, and screening of gelling and film forming agents. Capryol 90 and Tween 80 were selected as the oil and surfactant, respectively. Sepineo P 600 and HPMC E4M were selected as the gelling agent and film forming agent respectively. The films were evaluated for peelability, tackiness, folding endurance, swelling index and cosmetic attractiveness. **Results:** The optimized formulation formed clear, uniform and adhesive films and exhibited similar *in vitro* and *ex vivo* release profiles, to that of the marketed product (α = 0.05, *p*=0.605 for *in vitro* diffusion). Dry and wet swab studies performed on the optimized formulation presented that the film formed exhibits good adhesion, lowered drug loss associated with wipe off and enhanced retention. *In vivo* dermal irritation test on Sprague-Dawley rats was also carried out. Release profiles were compared using multiple models with first order release being the best suited model for the optimized formulation (R^2= 0.9932) and marketed product (R^2=0.9859). Stability studies performed helped conclude the optimized formulation was stable. **Conclusion:** The developed formulation is an excellent alternative to emulgel and patches, overcoming the drawbacks of each by giving longer retention on the skin for prolonged therapy.

Keywords: Adhesive film, Wipe-off resistant, Longer retention, Long term therapy, Antifungal.

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INTRODUCTION

Clotrimazole (CTZ) is one of the first drugs of the imidazole class developed for the treatment of mycoses in humans. It has good *in vitro* activity at very low concentrations against a large variety of fungi like *Candida albicans*, Trichophyton (spp rubrum, mentagrophytes), Malassezia furfur.[1]

CTZ is sparingly soluble in water but soluble in ethanol and other organic solvents. CTZ has poor oral bioavailability and is absorbed less than 3% from the mucosa and less than 0.5% through the Skin.[2] Extensive protein binding besides hepatic metabolism attributes to the poor bioavailability of CTZ. Oral administration of CTZ causes rapid and potent induction of microsomal liver enzymes causing increased metabolism and elimination of its antimycotic activity.[3,4] Hence even troches administered for oral thrush have to be dissolved completely in the mouth since swallowed troche will be ineffective.[5] Troches or oral formulations of CTZ have been associated with asymptomatic and transient serum aminotransferase elevations during therapy in about 15% of of patients.[6] To elude demerits presented by the systemic route of administration and to enhance the therapeutic efficacy of CTZ, topical formulations have been employed. CTZ formulations are available as creams, lotions, solutions, tinctures, oral and vaginal tablets, and troches for the treatment of superficial mycoses.[7] It is also available as an intranasal infusion for the treatment of nasal aspergillosis.[2] CTZ has been incorporated into nanoemulsions and microemulsions, to enhance drug loading for hydrophobic CTZ,[8] but these have low viscosity and poor ease of application. CTZ has also been formulated as a mucoadhesive *in-situ* gel forming preparation using aqueous synthetic polymers like Hydroxypropyl Methyl Cellulose (HPMC) and thermoresponsive polymers like poloxamer with good syringeability for vaginal delivery,[9] and a microemulsion formulated as a gel.[10] Solid dispersion incorporated gels using Carbopol 940 containing CTZ has also been formulated.[11] A microemulsion gel containing isopropyl myristate, Tween 80, n-butanol, and water also exhibited promising results for topical delivery of CTZ.[12] Ufosomes of CTZ have been reported to enhance drug loading and exhibited ~84% entrapment efficiency. This formulation exhibited high accumulation in the viable epidermis and dermis layer as

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Table 1: Advantages of FFE over conventional topical semi-solids, patches, etc.

Wipe off resistant, thereby increasing residence time and reducing the frequency of application
Can be used as a protective barrier over gentle or compromised skin, since it exhibits gentle adhesion
Provides suitable tack for quick bonding, including wet skin
Higher patient compliance since the application hassle-free, unlike in patches wherein adhesive needs to be peeled off
Non-greasy (unlike creams, lotions, ointments) and easy to remove (no residual glue-like in case of patches)
Since films are clear and invisible, they exhibit higher cosmetic attractiveness and patient acceptability
Convenient to apply on contoured areas of the skin, such as nail beds, ankles, elbows, webbings of feet, etc.
Provides complete drug delivery, no wastage of drug-like in case of patches or semi-solids
Irritation caused by adhesives used in patches is circumvented, and painless removal thereby
Non-occlusive films are formed thereby preventing moisture entrapment

compared to a commercial product, indicating its potential to be used for targeted drug delivery.[13]

Topical therapy minimizes systemic effects, offers localized and targeted effects, and provides excellent levels of patient compliance. However, along with the potential advantages, there are some drawbacks in the case of semi-solid and solid topical preparations such as creams, ointments, lotions, dusting powders, which include poor penetration of drug across the skin/mucosa/nail, limiting local bioavailability and drug efficacy, formulation demerits such as greasiness and stickiness allied with creams and lotions which lead to lower residence time of the medicament at the site of infection.[14] A major challenge for efficacious topical drug delivery is the targeted biologic membrane which presents substantial impediments to drug transport. Moreover, easy wipe-off of medicaments like creams, lotions, solutions, and tinctures, owing to mechanical rubbing with clothes or skin-to-skin contact reduces the efficacy of the drug.

For long-term therapy of localized infections, topical formulations are an attractive alternative to oral/parenteral antimycotics, where long-term systemic administration may increase the risk of exposure to drug-drug interactions,[15] enzymatic interactions, and other adverse events like skin rash, visual abnormalities, photosensitivity reactions (photophobia and blurred vision) and elevated hepatic transaminase or serum bilirubin levels.[16] Clotrimazole therapy involves applying the formulation to the infected area once or twice a day for two to four weeks, depending upon the intensity and type of infection. Effective treatment rates for 1% CTZ cream was 58.7% (p = 0.0001) at 4 weeks and 73.1% (p = 0.002) at 6 weeks.[17] This study indicates that for effective treatment by CTZ, a minimum therapy of 4 weeks is essential. Therefore, the most imperative considerations for topical CTZ are, the delivery and, maintenance of the effective drug concentration at the site of infection for long-term therapy. Film-forming systems can thereby be explored to circumvent the issues of low residence time and poor applicability feel on the skin associated with conventional topical Film-forming systems can be defined as liquid or semi-solid formulations that form films *in situ* after application on the skin on evaporation of the solvent. Films formed on the skin or nail are occlusive and prevent fungal adhesion and reduce risks of re-infection. Since films are formed by evaporation of the solvents in the formulation, it increases the degree of drug saturation thereby facilitating partitioning of the drug from the vehicle to the biologic membrane. Film-forming systems therefore can also be of vital use for transungual drug delivery in case of onychomycosis.[15] Film-forming spray containing CTZ was formulated in an ethanol-acetone solvent system since CTZ is a hydrophobic drug and requires organic solvents for solubilizing it.[18] Ethanol and acetone both are flammable solvents and are also expensive. Selection of spray pump and actuator type for a spray solution is important for a propellant-free system, which adds to production costs. Chances of leaks due to an inefficient pump system may be hazardous causing an explosion due to the volatile solvent systems employed in the spray. For formulating Film-Forming Emulgels (FFE), no flammable solvents are employed, need for specialized pumps and actuators is excluded, thereby reducing the cost of production, and eliminating the need for specialized scale-up facilities for handling flammables.

Emulgels are biphasic systems with a gel base forming the outer phase, with an oil layer entrapped in the gel matrix.[19-20] The gelling agent along with imparting thixotropy also facilitates a controlled release of the lipophilic active ingredient from the oil layer of the emulgel system. The oil layer forms a depot system, by encapsulating CTZ in it and thereby gives a controlled release of the drug. Emulgels are also greaseless, easily spreadable, emollient, and non-staining.[21] Combining a gelling agent and a film former in the aqueous phase of a classical emulsion forms a film-forming emulgel, which provides the pros of a gel by increasing patient compliance due to low incidences of wiping-off of the formulation. It also reduces the greasiness and offers the advantage of incorporation of the drug in the oily phase of the emulsion. These favorable properties of emulgels (Table 1[22]) amalgamated into a film-forming system presents an ideal formulation to circumvent demerits of conventional topicals, difficulties in the incorporation of a hydrophobic drug, overcoming poor patient compliance, and inclusion of nano and microparticulate systems to enhance drug loading and stability (Figure 1).

Since CTZ is a hydrophobic drug, an oily phase in a formulation will enhance drug loading and prevent precipitation of the drug

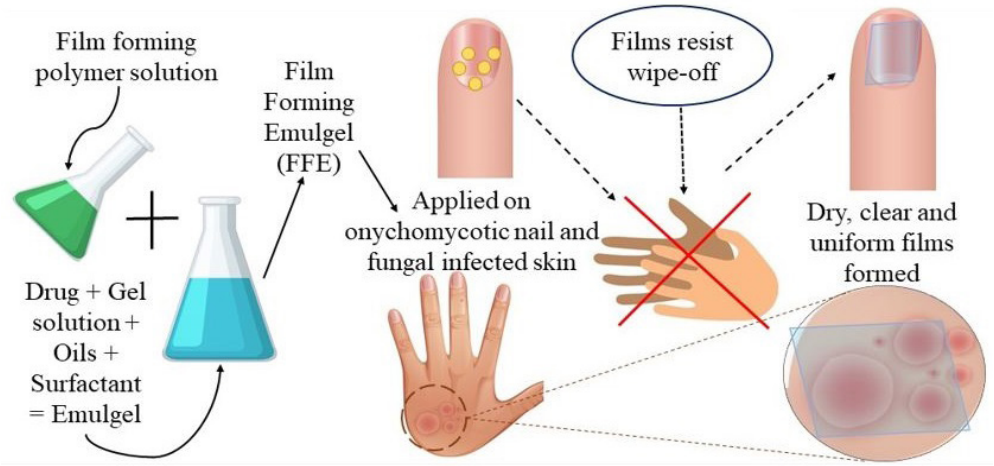

Figure 1: Film forming emulgel with prolonged retention.

in the formulation. Hence emulsion formulation is suitable for CTZ. The present investigation aimed to formulate a film-forming gel of CTZ to impart wipe-off resistance and longer action of the drug for the topical treatment of fungal infections which require prolonged therapy.

MATERIALS AND METHODS

Clotrimazole was gifted by Cipla Ltd., Mumbai, and Lincoln Pharma, Gujarat. Vinylpyrrolidone-vinyl acetate copolymer (Kollidone® VA 64), polyvinyl alcohol/polyethylene glycol graft copolymer (Kollicoat® IR), Polyoxyl castor oil (Kolliphor® EL), were obtained as gift samples from BASF Ltd., Mumbai. Propylene glycol monocaprylate- type II (Capryol™ 90), Propylene glycol monocaprylate- type I (Capryol™ PGMC), Caprylic triglyceride (Labrafac™ lipophile WL 1349), Propylene glycol monolaurate (Lauroglycol™ 90), Glyceryl monooleate (Peceol™), Acconon® and Caprylocaproyl macrogol-8-glyceride (Labrasol®) were provided by Gattefosse, Mumbai. Glyceryl monocaprylate (Capmul® MCM C8) was gifted by Abitec. Hydroxypropyl methyl cellulose (HPMC E4M, K4M, K100) were gifted by Colorcon, Mumbai. Polyacrylic acid polymers (Carbopol® ETD 2020, 974P and 971P) were supplied by Lubrizol. Dispersion of acrylamide/sodium acryloyldimethyl taurate copolymer in isohexadecane (Sepineo™ P 600) was gifted by Seppic, Mumbai. Polysorbate 80 (Tween 80- Extra Pure), Sodium carboxy methyl cellulose (Extra Pure) and Triethanolamine (Extra Pure) were purchased from Loba Chemie. Sabouraud Dextrose Agar and Cellophane membrane (mol. wt. cut of 12000-14000) were purchased from HI Media Ltd, Mumbai. Marketed reference product was purchased from the local market.

Quantification of Clotrimazole by UV-Spectroscopy

UV-Spectroscopy method was developed for the analysis of clotrimazole by employing a Shimadzu-1800 Double Beam Spectrophotometer. A standard stock solution was prepared by dissolving 100 mg of Clotrimazole in Citrophosphate buffer pH 5.5 with 2% SLS to obtain a solution of 1000 µg/ml. Aliquot of 1ml of this solution was diluted to 10 ml using the buffer to obtain a working standard solution of 100 µg/ml. Aliquot of 0.5 ml of working stock solution was diluted with the buffer to obtain a concentration of 50 µg/mL. The resulting solution was analysed at 264.4 nm.

Preformulation studies

Solubility profiling of Clotrimazole in oils and surfactants

An excess amount of clotrimazole was added to each glass vial containing selected oils and surfactants and mixed using a cyclomixer (Remi CM-101 Plus, Remi Labs. The vials were kept on a shaker incubator (Hally Instruments) at 37°C and shaken for 48 hr. Samples were then centrifuged (Remi 120-C, REMI Labs) at 5000 rpm for 10 min. Aliquot of 1 ml supernatant was diluted with Citrophosphate buffer pH 5.5 with 2% SLS and analyzed by UV spectrophotometer at 264.4 nm.

Emulsification studies of oils by surfactants

Surfactant and water were taken in the ratios of 7:3, 6:4 and 5:5 and was mixed vigorously with the help of a cyclomixer (Remi CM 101 Plus, Remi Labs). These mixtures were titrated against the selected oils. The amount of oil required to turn the clear surfactant and water mixture turbid, was considered as the end point. The surfactant which incorporated maximum amount of oil was selected as the best emulsifying agent.

Selection of gelling agents

Semi-synthetic gels were prepared by dispersing the calculated amount of gelling agent in warm water with constant stirring using a magnetic stirrer at 60°C. Weighed quantities of synthetic gelling agents were dispersed in distilled water with the help of magnetic stirrer for 30 min. Triethanolamine was added to Carbopol gel to adjust pH to 7.0.

Selection and Screening of film-forming agents

Polymers were dissolved in water with heating, on a magnetic stirrer. The solution thereby formed was reaerated by sonication and poured onto a petri plate. The solvent was allowed to dry by exposing it to high temperature to form dry films.

Preparation of film forming emulgel

Drug solution was prepared by dissolving CTZ in the selected oil and surfactant mixture at 60°C. Gel solution was prepared by dispersing the gelling agent in water under constant magnetic stirring for 30 min. The polymer solution was prepared by dissolving the film-forming agents in water at 60°C. The CTZ solution prepared was added to the gel solution with stirring, to which the polymer solution was added gradually to form a homogenous solution. The emulsification process was carried out at 60°C. Triethanolamine was added to Carbopol containing formulation to adjust pH to 7.0.

Evaluation of Clotrimazole film forming emulgel

Appearance

The formulation prepared was observed visually for its appearance. It was noted as clear, opaque, or white.

pH

The pH of the emulgel was measured using a digital pH meter (DBK Instruments) by dissolving 1 g of emulgel in water to form a clear solution.

Viscosity

Viscosity measurements were carried out using a Viscometer (Brookfield, DV-E model) by employing spindle S94 (T-shaped spindle).

Spreadability

Spreadability study was performed using two tiles lined with butter paper. Weighed quantity (0.5 g) of the emulgel was placed between the two tiles and the initial diameter was noted. For 2 min, a constant weight of 2 kg was kept on the assembly and the increase in diameter was noted.[23]

Extrudability

The tubes were crimped at the end and the extruded strand of the emulgel was observed for continuity. Continuous strand extruded is graded as 1, and further strands were rated as 2 if strands were broken intermittently and rated as 3 for completely discontinuous strands.

Drug content

Weighed quantity of the emulgel (0.5 g) was dissolved in 50 mL of Citrophosphate buffer pH 5.5 with 2% SLS, and then filtered through a Whatman filter paper. The concentration of the CTZ was determined spectrophotometrically at 264.4 nm using the same buffer as the blank. Drug content was calculated from the linear regression equation obtained from a standard curve in Citrophosphate buffer pH 5.5 with 2% SLS.

In vitro diffusion studies through dialysis membrane

In vitro diffusion studies were carried out using the vertical type of Franz Diffusion cell (Remi Labs), using cellophane membrane (molecular weight cut of 12000-14000, HI Media Ltd, Mumbai, India). The emulgel (0.5 g) was applied on the cellophane membrane which was placed between the donor and receptor compartment containing 13 ml Citrophosphate buffer pH 5.5 with 2% SLS. The temperature of the diffusion medium was maintained at 37 ± 0.5°C and stirred continuously at 100 rpm on a magnetic stirrer. Aliquots of 2 ml were withdrawn at specified time intervals and replaced with equal amounts of fresh buffer. Samples were analysed spectrophotometrically at 264.4 nm and the cumulative % drug release was calculated. The release profiles of the Optimized FFE and the Marketed Product were compared using Hixon Crowell model, Zero order release kinetics, First order release kinetics, Higuchi release model and Korsemeyer Peppas model.

Evaluation of film formed from Clotrimazole FFE

Film forming time

Film forming time of the formulation was noted by applying 0.5 g of the formulation as a 2x2 cm^2 patch on the forearm of five volunteers, whose written consent was taken after complete intimation of the procedure. The time required for the emulgel to completely dry and form a film on the hands of the volunteers was recorded. Complete drying was checked by placing a glass slide on the film without pressure. The film was considered dry when no liquid residues were visible on the glass slide after removal of the slide.

Peelability

Peelability was checked by slightly peeling the films from the skin and checking its uniformity. The peeled films should be continuous and non-flaky.

Folding endurance

Folding endurance was determined by repeatedly folding the peeled film at the same place until it broke. The number of times the film could be folded at the same place without breaking denoted the folding endurance.

Tackiness

The tackiness of the outer surface was tested by pressing cotton wool on the dry film under low pressure. Depending on the amount of cotton fibers that were retained by the film the stickiness was rated high (dense accumulation of fibers on the

film), medium (thin fibre layer on the film) or low (occasional or no adherence of fibres).

Cosmetic attractiveness

The cosmetic attractiveness of the film was assessed by visual examination of the dry films. The films were rated on a scale of 1-3, with 1 for transparent films that exhibited high attractiveness as they were almost invisible. Opaque films were considered less attractive as they formed translucent films and a slight wrinkling of the skin and were rated as 2. Whitish films and films causing heavy wrinkling of the skin, which displayed a low attractiveness, were rated as 3.

Swab studies

Swab test was carried out by applying the optimized FFE and marketed cream on a glass plate marked with six squares of 3x4 cm^2. Dry and wet cotton swabs of the same volume were used for dry and wet swab studies, respectively. The applied formulations were removed with the cotton swabs at 0 min, 30 min, 2 hr, 4 hr, 6 hr, and 8 hr. The swabs were kept in vials containing 10 ml methanol and sonicated for 10 min for complete extraction of the CTZ into methanol.

Antifungal efficacy studies

Antifungal efficacy of the optimized FFE and marketed cream was determined by the agar well diffusion method. A sterile 10 mm cork borer was used to make wells on the Sabouraud Dextrose Agar plate. The standard *Candida albicans* ATCC No 60193 strain was inoculated on the plate by streaking method. Marketed cream and the optimized FFE were dissolved in methanol to obtain 10 µg/mL concentration of each formulation. These samples were poured into the previously bored plates and were incubated at 37°C for 24 hr in aseptic conditions.

Animal studies

Ex vivo permeation through pork skin

Ex vivo diffusion was carried out using the vertical type of Franz Diffusion cell using porcine ear skin. The excised porcine ear skin was cleaned using normal saline. The emulgel (0.5 g) was applied on the section of skin and placed in the space between the donor and receptor compartment containing 13 ml Citrophosphate buffer pH 5.5 with 2% SLS. The temperature of the diffusion medium was maintained at 37 ± 0.5°C and stirred continuously at 100 rpm on a magnetic stirrer. Aliquots of 2 ml were withdrawn at specified time intervals and replaced with equal amounts of fresh buffer. Samples were analysed spectrophotometrically at 264.4 nm and the cumulative % drug release was calculated.

In vitro antifungal efficacy

In vitro antifungal efficacy of the optimized FFE and marketed cream was determined by the agar well diffusion method. A sterile 10 mm cork borer was used to make wells on the Sabouraud Dextrose Agar plate and zone of inhibition was evaluated.

In vivo dermal irritation test

Healthy female Sprague-Dawley rats weighing 220 ± 20 g were used in this test, which was carried out as per the OECD guideline 431, Test No. 404. The test was carried out using three rats, in two stages. The fur on the dorsal part of the trunk of the rats were clipped closely, 24 hr before each test. In the initial test, 0.5 g of the formulation was applied on one rat as approximately 2x2 cm^2 patches and covered with a gauze patch. The formulation was applied three times sequentially on the animal, and the responses were graded. Since no corrosive effect was observed in the initial test, the irritant response was confirmed using two additional animals, each with one patch and were exposed for a period of four hours. Responses for erythema and oedema were scored at 60 min, 24 hr, 48 hr and 72 hr after removal of the patch. The skin reactions were graded as 0 for 'No erythema/oedema', 1 for 'Very slight erythema/oedema' (barely perceptible), 2 for 'Well defined erythema/slight oedema' (edges of area well defined by definite raising), 3 for 'Moderate to severe erythema/moderate oedema' (raised approximately 1 mm), 4 for 'Severe erythema (beef redness) to eschar formation' preventing grading of erythema/severe oedema (raised more than 1 mm and extending beyond area of exposure).[24]

Fourier Transform Infrared (FT-IR) spectroscopy for Drug-excipient Compatibility Studies

FTIR was carried out to investigate the thermodynamic compatibility between the excipients and the drug. This was determined by FTIR for optimized FFE as compared to pure drug and placebo formulation. The FTIR spectra of the samples were recorded (Shimadzu IR infinity) over a range of 4,000-400 cm^{-1}.

Stability studies

The formulated emulgels were packed in collapsible aluminium tubes and subjected to stability studies at 25±5°C /60% RH and 40±5°C /75% RH for 1 month. The emulgels were evaluated for their physical properties, *in vitro* and *ex vivo* release properties.

RESULTS AND DISCUSSION

UV-spectroscopic method of analysis of CTZ

The method was validated according to the ICH guidelines, Q2 (R1). The linearity ($R^2 = 0.9978$) was observed in the concentration range of 100–250 µg/ml. Precision and repeatability studies indicated a percent relative standard deviation of < 2%, thereby complying with requirements of the validated method.

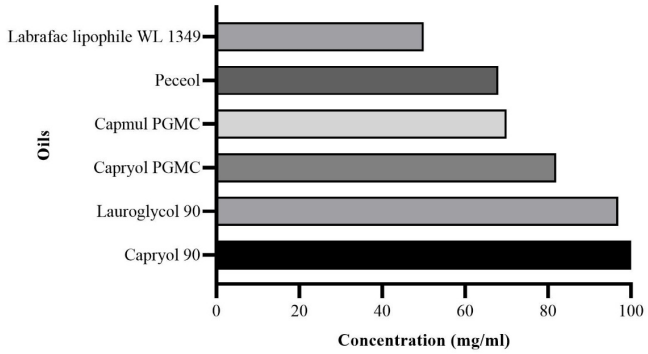

Figure 2: Solubility profile of CTZ in oil.

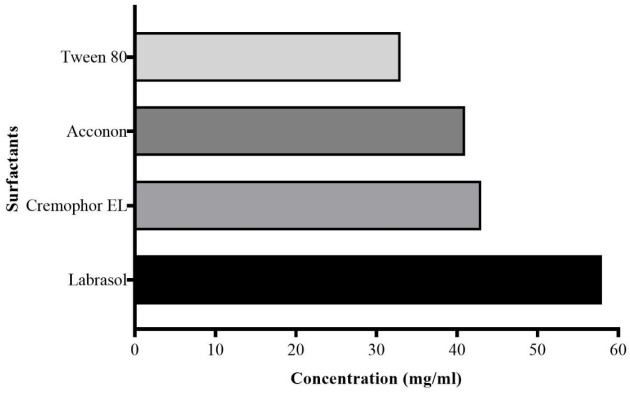

Figure 3: Solubility profiles of CTZ in surfactants.

Solubility profile of Clotrimazole in oils and surfactants

To identify suitable oil and surfactant having maximal solubilizing potential for CTZ, solubility profile of lipidic excipients and surfactants were evaluated. Unmodified edible oils are the most preferred choice of oils but have been reported to exhibit relatively poor emulsification efficiency. CTZ exhibited comparatively lower solubility in unmodified edible oils such as soybean oil and medium chain triglycerides but was found to have good solubility in semi-synthetic oils like Capryol 90, Capryol PGMC, Lauroglycol 90, Capmul MCM C8, Peceol and Labrafac lipophile WL 1349. The selected oil must solubilize maximum amount of CTZ, which is important to prevent precipitation of the drug. Maximum solubilizing potential is also important for achieving optimal drug loading. Capryol 90 was selected as the oily phase since it exhibited maximum solubility of the drug (Figure 2).

Surfactants evaluated were Labrasol, Tween 80, Cremophor EL and Acconon. CTZ does not exhibit any interaction with the selected oils and surfactants and therefore was compatible.[25] Surfactants were graded for their relative solubilizing ability of the selected oil phase as shown in Figure 3. Labrasol exhibited the highest solubilizing potential for CTZ followed by Labrasol > Cremophor EL > Acconon > Tween 80. However, emulsions

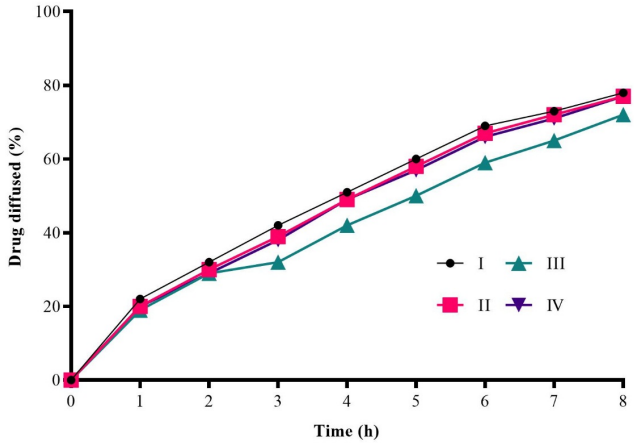

Figure 4: *In vitro* diffusion studies of CTZ FFE.

prepared with Labrasol, Cremophor EL and Acconon exhibited phase separation on standing. Since these exhibited poor emulsifying ability of Capryol 90, Tween 80 was chosen as the surfactant. An emulsion was formulated with Tween 80, with no phase separation on standing.

Evaluation of gelling agents

The gels were selected based on the concentration of gelling agent required to form a gel with optimal viscosity as well compatible with the film former to give a gel with film forming property. Semi-synthetic and synthetic gelling agents were evaluated based on their appearance, concentration required for gelling, viscosity, spreadability and pH.

Semi-synthetic gelling agents like Sodium CMC, HPMC (K100M, K4M, and E4M) were evaluated. All gelling agents formed clear, smooth and easily spreadable gels. HPMC E4M exhibited the most acceptable viscosity and formed a gel at low concentrations (Table 2). Synthetic gelling agents Carbopol (ETD 2020, 974 P, 971P) and Sepineo P 600 formed clear, smooth, and easily spreadable gels. It was employed as a thickening agent in topicals. It is advantageous as it does not require neutralization since it exhibits stability over pH ranges of 3-11 and it can also be employed for topicals with higher oil phase composition.[26]

Carbopols are cross-linked together and form a microgel structure that makes them optimal to be used as a gel base for dermatological formulations. The microgel structure makes it viable for these systems to endure mechanical stresses on the area of application. This property of Carbopols is advantageous for formulating film forming emulgels with good folding endurance and thereby preventing cracks by forming intact films. Carbopol 974P formed gels at 0.3% w/w which was more easily dispersed as compared to Carbopol 971P and exhibited acceptable viscosity as compared to Carbopol ETD 2020. Since Sepineo P 600 formed gels of acceptable consistency at 2% w/w, Carbopol 974P and Sepineo P 600 were finally chosen as gelling agents.

Table 2: Evaluation of semi-synthetic and synthetic gelling agents.

Parameters	Sodium CMC	HPMC K100M	HPMC K4M	HPMC E4M	Carbopol ETD 2020	Carbopol 974P	Carbopol 971P	Sepineo P 600
Concentration (% w/w)	4.5	2.5	3	0.5	0.3	0.3	0.5	2
Viscosity (cps)	93090	85090	71850	66410	51070	46360	36000	80060

Table 3: Compositions of CTZ loaded film forming emulgels (% w/w).

Ingredients	I	II	III	IV
Clotrimazole	1	1	1	1
Capryol 90	5	5	5	5
Tween 80	2.5	2.5	2.5	2.5
Carbopol 974P	0.8	0.8	-	-
Sepineo P 600	-	-	3	3
Kollicoat IR	10	-	10	-
Kollidon VA 64	5	-	5	-
HPMC E 4M	-	3	-	3
Triethanolamine	q.s.	q.s.	-	-
Distilled water	q.s. 100 g	q.s. 100 g	q.s. 100 g	q.s. 100 g

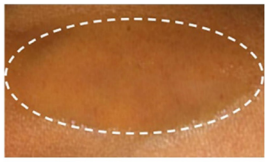

Figure 5: FFE IV on application.

Figure 6: Dried film formed by FFE IV.

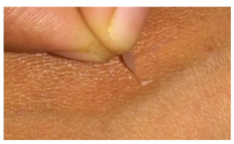

Figure 7: Peelability of the film formed by FFE IV.

Selection of film forming agents

Film forming agents HPMC E4M, Kollicoat IR, Kollidon VA 64 were evaluated by solvent casting method. HPMC E4M acts as a rheology modifier as well as a film-forming agent. The concentration of polymer which formed clear, non-tacky and complete film with good folding endurance was selected for the formulation of CTZ loaded film forming gels. HPMC E4M and Kollicoat IR formed films at 3% w/w and 10% w/w, with folding endurance of 100 and 89, respectively. Kollidon VA 64 formed a non-uniform film, with high tackiness and drying as irregular patches, but exhibited good adhesivity.

Compositions of film-forming emulgel batches

Kollicoat IR formed intact films with low adhesivity. To enhance adherence of the films, 5% Kollidone VA 64 was added. After incorporation of other excipients for formualting the emulgel, concentration of Carbopol 974P was increased from 0.5% w/w to 0.8% w/w to obtain a proper consistency. (Table 3).

Evaluation of Clotrimazole film forming emulgel

Formulation IV exhibited comparable diffusion results to films I and II (Figure 4, Table 4). Formulation IV was chosen for further study since it formed clear, adhesive, uniform films (Figure 5,6, 7). The films exhibited good adhesivity with long retention time on the applied surface and were not very easily perceptible for peeling (Table 5). Therefore, formulation IV was selected as the optimized FFE and was selected for further comparison studies with the marketed cream (Table 6).

Comparison of marketed cream with film-forming emulgel

Comparison of in vitro diffusion studies by graphical method

The optimized FFE was compared with the marketed cream to primarily assess CTZ release from the formulation and % CTZ retention at the site of application. The graphs of *in vitro* release of the optimized FFE, dried optimized film and the marketed cream (Figure 8) were compared for evaluating the diffusion pattern and concentration of CTZ at each point which signifies extent of CTZ release from the optimized formulations. If the curves are

Table 4: Evaluation of Clotrimazole FFE.

Parameters	I	II	III	IV
Appearance	White	White	White	White
pH	7.1	6.9	6.9	6.8
Viscosity (cps)	36000	66000	73500	68600
Spreadability (cm)	4.20 ± 0.20	5.83 ± 0.55	5.77 ± 0.91	4.30 ± 0.20
Extrudability	1	2	1	1
CTZ content (%)	99.98±1.47	101.43±1.87	101.87±3.19	99.70±0.61
In vitro diffusion studies (%)	80.30±0.29	78.48±1.16	72.74±2.97	75.77±0.74

Table 5: Evaluation of film formed from Clotrimazole FFE.

Parameters	I	II	III	IV
Film forming time (min)	5	5	3	3
Adhesiveness and Peelability	Peelable, but poor adhesivity due to flakiness	Peelable, but poor adhesivity due to flakiness	Peelable, but poor adhesivity due to flakiness	Peelable and uniform films, with good adhesivity and no flakiness
Folding endurance	22	47	21	53
Tackiness	Non-tacky	Non-tacky	Non-tacky	Non-tacky
Cosmetic attractiveness	2	2	2	1

Table 6: Comparative evaluation of marketed cream and optimized FFE.

Parameters	Marketed cream	Optimized FFE
Appearance	White	White
pH	7.4	6.8
Viscosity (cps)	58100	68600
Spreadability (cm)	4.07 ± 0.12	4.30 ± 0.20
Extrudability	1	1
CTZ content (%)	100.3±1.01	99.70±0.61
In vitro diffusion studies (%)	82.67±0.63	75.77±0.74
Flux (mg/cm^2/h)	0.165±0.002	0.151± 0.002
Permeation coefficient (cm/h)	0.033	0.031

overlapping, then the dissolution profiles are comparable. With increasing differences in the curves, the dissolution profiles are disparate.

Comparison of *in vitro* diffusion studies by model independent method

Generally, dissimilarity factor (f_1) values up to 15 (0-15) and similarity factor (f_2) values greater than 50 (50-100) ensures equivalence of the two *in vitro* diffusion profiles. Similarity factor (f_2) of the optimized FFE was 62.5 and the dissimilarity factor (f_1) value was 9.34, hence the test and reference release profiles are similar.

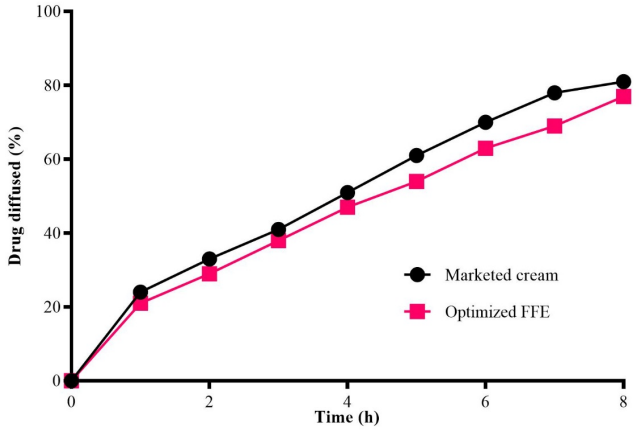

Figure 8: *In vitro* release comparison of marketed cream and optimized FFE.

Comparison of *in vitro* diffusion studies by Student *t*-test

The unpaired *t*-test compares two mean values to arbiter similarity between two values. The student's *t*-test is the most sensitive test for interval data, but it also requires the most appropriate assumptions. The variables or data are assumed to be normally distributed. For comparison of diffusion profile of the optimized FFE and marketed cream, GraphPad Prism 6 was employed. The significance level (α) value was determined as α= 0.05. The *p* value

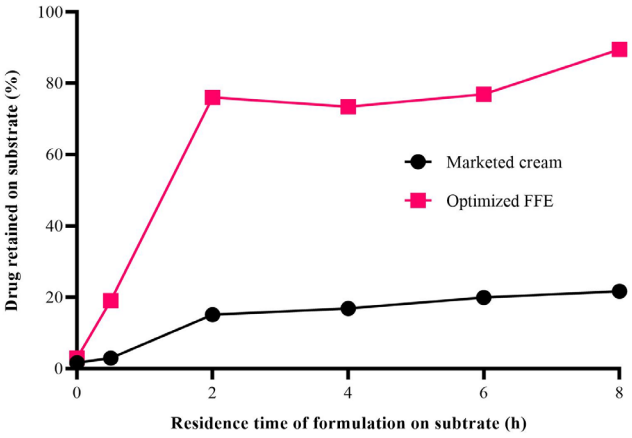

Figure 9: Dry swab studies of marketed cream and optimized FFE.

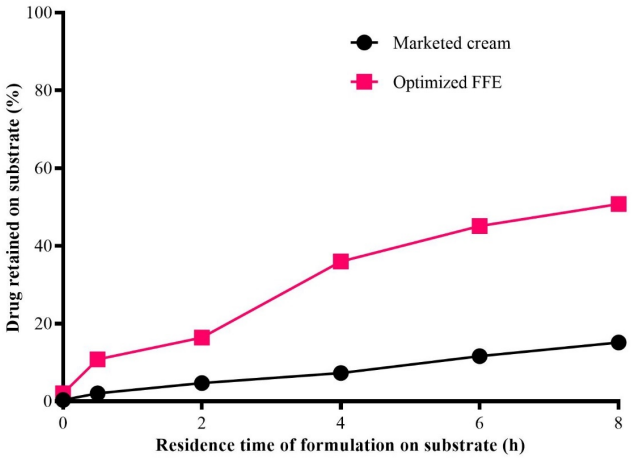

Figure 10: Wet swab studies of marketed cream and optimized FFE.

is 0.605, which is higher than 0.05, thereby accepting the null hypothesis (H_0), wherein the diffusion profiles of the optimized FFE and the marketed cream were found to be similar. The *t* and d_f values were 0.5276 and 16, respectively.

Comparison of release kinetics models of *in vitro* diffusion studies

First order release kinetics for Optimized FFE (R^2= 0.9932) and Marketed product (R^2=0.9859) gave the best fit model results for *in vitro* diffusion studies indicating initially an immediate release followed by slow release over a period of time (Table 7).

Swab Studies

Swab test was performed to compare the residence time of the film-forming emulgel with the marketed formulation. Since residence time and application frequency are associated with wipe off, swab studies help evaluate these parameters which determine the effectiveness of FFE over the marketed cream. Dry and wet swab test depicts the behavior of developed formulation on the dry skin and when it comes in contact with water or sweat, respectively.

From dry swab studies (Figure 9) and wet swab study results (Figure 10), it can be concluded that drug loss is more in case of marketed cream when compared to the optimized FFE, concluding that the film formed on drying holds more drug in

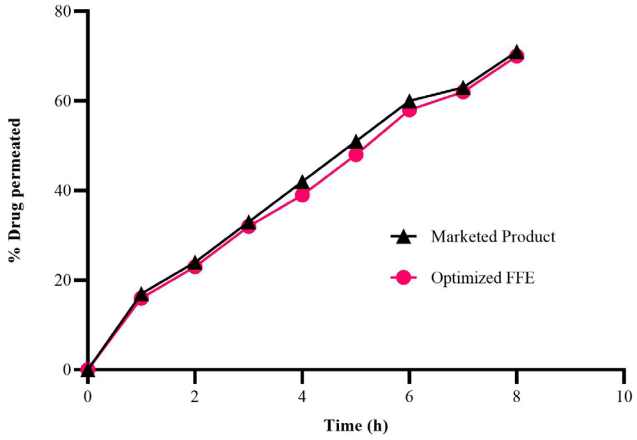

Figure 11: *Ex vivo* release comparison of marketed cream and optimized FFE.

Table 7: Release kinetic models comparison for *in vitro* diffusion studies.

	Optimized FFE	Marketed Product
Hixon Crowell	y = -0.213x + 4.562 R^2 = 0.9129	y = -0.2433x + 4.8233R^2 = 0.9926
Zero Order release	y = 8.9333x - 0.4444R^2 = 0.9753	y = 9.6667x + 0.4444R^2 = 0.9667
First Order release	y = -0.0677x + 2.0554R^2 = 0.9932	y = -0.0897x + 2.0994R^2 = 0.9859
Higuchi release	y = 8.9333x - 0.4444R^2 = 0.9753	y = 9.6667x + 0.4444R^2 = 0.9667
Korsemeyer Peppas	y = 0.6161x + 1.3013R^2 = 0.9898	y = 0.6048x + 1.3553R^2 = 0.9814

Table 8: *Ex vivo* permeation studies.

Parameters	Marketed cream	Optimized FFE
Ex vivo permeation studies (%)	70.64±0.41	70.82±0.115
Flux (mg/cm^2/hr)	0.141	0.151
Permeation coefficient (cm/h)	0.021	0.030
Similarity factor (f_2)	-	83.98
Dissimilarity factor (f_1)	-	3.6

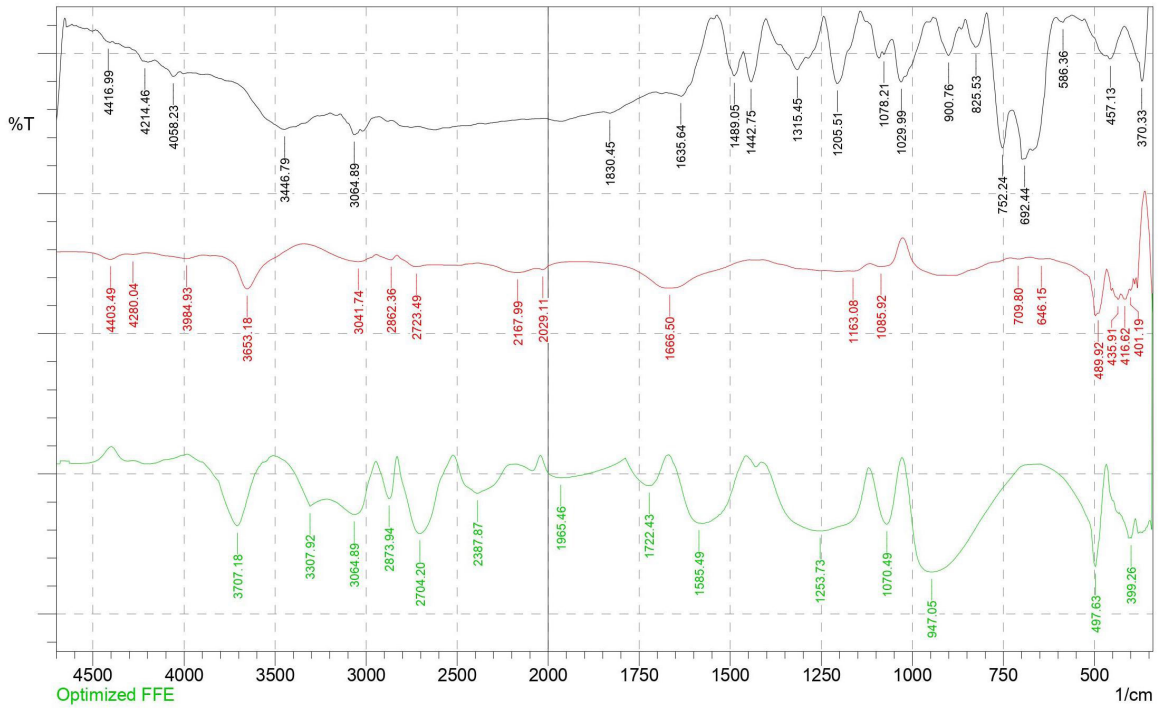

Figure 12: FTIR data for Drug-Excipient compatibility studies: (a) CTZ, (b) Placebo, (c) Optimized FFE.

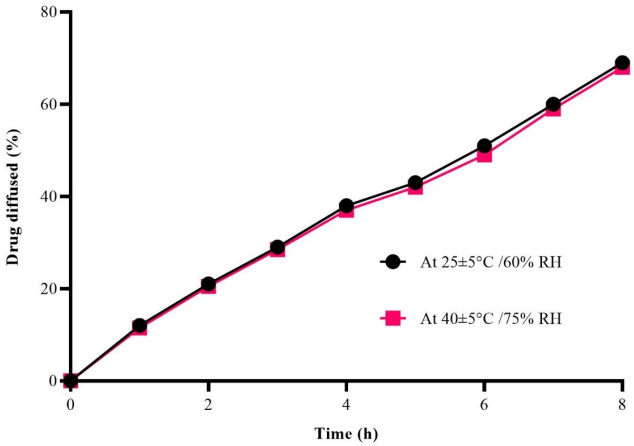

Figure 13: *In vitro* diffusion of FFE stability batches.

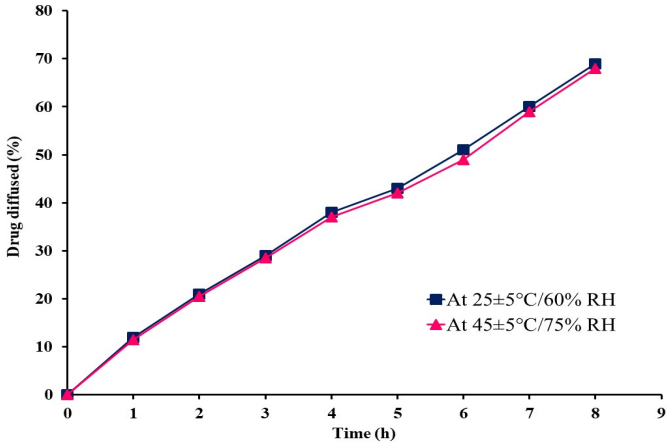

Figure 14: *Ex vivo* diffusion of FFE stability batches.

it because of wipe-off resistance. Therefore, the optimized film acts as a depot for the slow release of the drug than the marketed cream. Enhanced retention on the site of application also reduces application frequency and can improve drug availability at the site of action.

Evaluation of Antifungal efficacy

The mean zone of inhibition values of the marketed cream and optimized FFE was found to be 23±0.047 mm and 21±0.047 mm, respectively. This indicates that the optimized FFE formulation is as effective as the marketed product.

Animal studies

Evaluation of ex vivo permeation through pork skin

The study showed the release of CTZ from the marketed cream and optimized FFE formulation were similar (Figure 11, Table 8). On comparison of *ex vivo* diffusion data by model independent method, f_2 and f_1 value of the optimized formulation was found to be 83.98 and 3.6, respectively, indicating that the test and reference release profiles are similar.

Skin Irritation studies

Since no corrosive effect was observed in the initial and confirmatory test after removal of patches at the prescribed intervals, animals were observed for reversibility after 14 days. All animals showed a '0' score for erythema and oedema formation, indicating that there are no signs of skin irritation.

FTIR for Drug-Excipient Compatibility Studies

The FTIR overlay spectrum of CTZ, placebo formulation and the optimized FFE is shown in Figure 12, which indicates a lack of a significant shift in the wavenumber (cm^{-1}) or functional groups of the drug in the optimized FFE vis-a-vis the pure drug. This corroborates the absence of any physiochemical interaction(s) and incompatibility(ies) between the drug and the excipients during processing.

Stability studies

Evaluation of optimized emulgel of stability batches

The samples of stability batches exhibited identical physical properties as well as *in vitro* (Figure 13) and *ex vivo* (Figure 14) diffusion profiles to that initial formulation.

CONCLUSION

The film forming emulgel was formulated effectively using Capryol® 90 as oil, emulsified by Tween 80 with 3% Sepineo™ P 600 as gelling agent and HPMC E4M as the film former incorporated in the emulgel. It exhibited good *in vitro* antifungal results, with promising drug content, flux, and permeation coefficient results. The FFE exhibited good resistance to wipe off by exhibiting low drug content in dry and wet swabs of the optimized formulation. The release profiles of the optimized FFE and marketed product were found to be similar. The release profiles of the optimized FFE and the marketed product were compared and first order release kinetics was the best fit model for *in vitro* diffusion studies. With no corrosive effects observed in skin irritation tests, the optimized FFE is a stable formulation forming clear, adhesive, and uniform films and exhibited similar *in vitro* and *ex vivo* release profiles to that of marketed cream of CTZ. Therefore, the film forming emulgel is a potential formulation to overcome the drawbacks of conventional semi solid topical products and can be successfully employed for patience compliance for long term topical applications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CTZ: Clotrimazole; **FFE:** Film Forming Emulgel; **HPMC:** Hydroxypropyl methyl cellulose; **CMC:** Carboxymethyl cellulose.

SUMMARY

Clotrimazole is a weakly basic hydrophobic drug marketed as conventional topicals. CTZ formulated as a Film Forming Emulgel improved residence time and enhanced patience compliance. Films formed after application were invisible and uniform with good adhesion on skin. The FFE formulation exhibited *in vitro* and *ex vivo* release profiles similar to the marketed product. The dry and wet swab studies of the film exhibited enhanced retention and lowered drug loss, with no corrosive effects observed. Thereby, the FFE can efficiently circumvent demerits of conventional topicals and prove to be a patient compliant formualtion for long-term use.

Human and animal rights

No humans were used for this research. The authors declare that the animal study protocols have been approved by Institutional Animal Ethics Committee, VES College of Pharmacy, Chembur, Mumbai- 400 074 (Approval No. VESCOP/08/2015).

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