

# Formulation and Characterization of Clotrimazole Microemulsion for Topical Drug Delivery

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**Abstract:** Clotrimazole is a broad - spectrum antimycotic drug, *Candida albicans* as well as other fungal infections are typically treated with this drug. Clinical clotrimazole seems to be a topical therapy treating tinea pedis (athlete's foot), vulvo vaginal candidiasis, and oropharyngeal candidiasis. That is a synthetic azole antimycotic. It inhibits fungal growth by decreasing the production of ergosterol. Clotrimazole has become a medication of interest for a variety of illnesses, including sickle cell anaemia, malaria, and some cancers, in addition to its antimycotic properties. It's been mixed with other molecules as well, to create clotrimazole compounds with enhanced pharmacological effectiveness, including such metals. Numerous novel pharmaceutical formulations enabling variable releases are also being developed. Clotrimazole is a well - tolerated, little - adverse drug, however certain immunocompromised individuals are developing treatment resistance. Clotrimazole's pharmaceutical chemistry, use, and pharmacology are discussed in this study.

**Keywords:** Antifungal drug, topical drug delivery system, clotrimazole microemulsion gel

## 1. Introduction

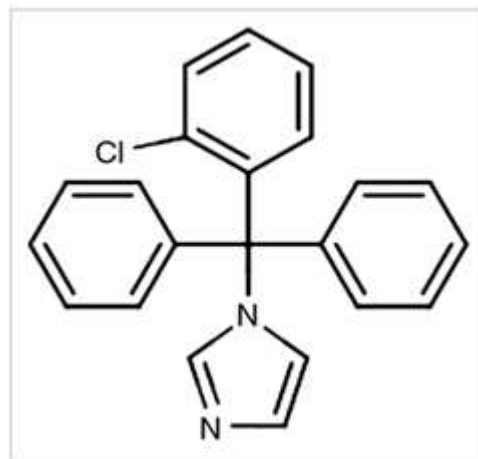
Clotrimazole, a synthetic imidazole derivative, is most often used locally to treat yeast and dermatophyte infections of both the vaginal and skin. This works well against *Candida* spp., *Trichophyton* spp., *Microsporum* spp., and *Malassezia furfur* in vitro (*Pityrosporon orbiculare*). It also shows modest in vitro action against Gram - positive bacteria and, at extremely high doses, activity against Gram - positive bacteria *Trichomonas* spp.

Clotrimazole also has been known to be successful in individuals who've already failed to react to certain other antifungal medications like nystatin and amphotericin B. In the case of trichomonal vaginitis, the results really aren't promising.

Topical application of clotrimazole has shown to be helpful in treating skin infections caused by *Candida* or dermatophytes.

Clotrimazole cream has been shown to be equally efficient as Whitfield's ointment or tolnaftate with in treatment of dermatophytoses, and even as efficient as nystatin inside the treatment of cutaneous candidiasis in clinical studies. Clotrimazole topical formulations are typically well tolerated, although in a few cases, local discomfort has prompted therapy discontinuation.<sup>(13)</sup>

**Chemical structure & Molecular formula:** - Clotrimazole has a molecular formulation of C<sub>22</sub>H<sub>17</sub>ClN<sub>2</sub> as well as a molecular weight of 344.8 g mol<sup>-1</sup>. Figure 1 illustrates the structure of clotrimazole.



**Figure 1:** The chemical structure of clotrimazole (1 - [(2 - chlorophenyl) diphenylmethyl] - 1H - imidazole)<sup>(1)</sup>

## 2. Materials and Methods

### 2.1 Materials

Polyethylene 20 sorbitanmono oleate (PSMO) and sorbitanmono oleate (SMO) P. C. Drug Center Co., Ltd. was acquired. RCI Labscan Limited has acquired isopropyl - alcohol (IPA). These are bought from the Orbit pharmaceutical, Gujarat Isopropyl palmitate (IPP). Sigma Aldrich bought fumed silica. During the tests distilled water was utilised. All chemical products were medicinal and utilised without further purification.

### 2.2 Preformulation study

#### 2.2.1 Solubility

Solubility of clotrimazole was determined in different oils, surfactant and co - surfactant. Clotrimazole was added in excess to different oils, surfactant and co - surfactant and

stirred for 24 h on a magnetic stirrer. After stirring, samples were centrifuged at 1500 RPM for 10 min and drug in the supernatant was analyzed at  $\lambda_{\max}$  261 nm.<sup>[8]</sup>

### 2.2.2 pH

A standard solution of clotrimazole, 1mg/ml in solution was prepared. It was further diluted with USP buffers of Ph 1.2, 4.5 and 6.8, each upto 10ml. These solution were incubated for 2 hrs at 37° C. In order to achieve adequate solubility level; aqueous samples were prepared with acetonitrile as a cosolvent at an effective final concentration of 10% (v/v). The samples were assayed for drug content by validated HPLC method.

### 2.2.3 FTIR Analysis

Test solution dissolved in 50mg of the substance to be examined in ethanol (96%) R and dilute to 5ml with the same solvent. Reference solution dissolve in 50mg of clotrimazole CRS in ethanol (96%) R and dilute to 5ml with the same solvent. Plate thin layer chromatography F<sub>254</sub> PLATE R. Mobile phase concentrated ammonia R1, propanol R, Toluena R (0.5: 10: 90 v/v/v)

### 2.2.4 Solubility of drug in different solvents

Solubility was calculated using the following protocol. Partially insoluble in water, soluble in ethanol (96%) and in methylene chloride. In different oils including oleic acid, lemon oil, olive oil and menthane oil, solubility of clotrimazole was studied. Maximum solubility of clotrimazole in mentha oil has been discovered among the oils that have been tested. The analgesic and cooling effects of mentha oil itself are sensory. Mentha oil has thus been used for the oil phase for the clotrimazole microemulsion.

### 2.2.5 Stability

Based on visual identification, microemulsion with Clotrimazole remained as clear liquid for a period of two months without the occurrence of phase separation or flocculation at the room temperature and refrigerator temperature. The results of various studies performed on ME & ME gel were found to be satisfactory so both were found to be stable for period of two months.<sup>[45]</sup>

### 2.3 Drug excipient study

Accurately weighed amounts of clotrimazole (100 mg) and each of selected excipient (500mg) were placed in 5ml glass vial and mixed thoroughly. Closed vials containing blends were stored in ovens at 60° C and at 40° C for 14 days. A standard clotrimazole sample without mixing with excipient clotrimazole sample kept under similar condition. The amount of drug substance in blends was determined on the basis of expected drug to excipient ratio in final formulation. Duplicate samples of drug – excipient blends were analyzed after 14 days by validated HPLC methods.

### 2.4 Preparation of CTM Microemulsion and CTM Microemulsion - based - gels

The desired microemulsion and microemulsion - based gels with 1 percent w/w of clotrimazole have been selected. In order to obtain clotrimazole microemulsions, the medicine has been dissolved. For clotrimazole based gel, fumed silica

has been dispersed in clotrimazole microemulsions. They have been produced.

### 2.5 Preparation of blank CTM Microemulsion and CTM Microemulsion gel

Microemulsion components in the preceding report, the region of microemulsion was chosen. The simple blending of IPP, 2: 1 water and IPA and 1: 1 PSMO and SMO mixtures at 20% of concentrations produced two microemulsions. 30% and 50% w/w, respectively for ME1 and in those of 20%, 40% and 40% w/w, respectively for ME2. Afterwards, 2.0 %, and 5 % of fumed silica was added into ME1 and ME2 to obtain microemulsion - based gel designated as MBG1 - 1 to MBG1 - 2 and MBG2 - 1 to MBG2 - 2, respectively.

**Table 1:** Composition of studied microemulsion – based - gel systems

Microemulsion	Fumed silica 2.5% w/w	Fumed silica 5.0% w/w
ME 1	MBG1 1	MBG1 2
ME2	MBG2 1	MBG2 2

### 2.6 Characterization of blank CTM Microemulsion and CTM Microemulsion based gel: -

Important characters of CTM Microemulsion and CTM Microemulsion based gel are

- The look has been seen visually.
- Spread ability was achieved by the spreading of a low to high skin stretchable and retainable quantity of each formulation over the skin with sensational consideration in the + to +++ range.
- Dilution test and conductivity measurement have established the kind of microemulsions.
- The dilution inspection should be carried out by the dropping into water of each microemulsion, classified for miscibility or immiscibility.
- The conductivity meter CM - 115 was tested (Orbit pharmaceutical, Gujarat).

### 2.7 Determination of Transmittance of clotrimazole microemulsions: -

The %Transmittance was checked against distilled water using UV - visible spectrophotometer at  $\lambda_{\max}$  630 nm.<sup>[12]</sup>  
T % = Antilog (2 - Absorbance)

### 2.8 Drug release kinetics

The drug release kinetic study was performed to find drug release mechanism from dissolution parameter by using various kinetic model equations. The zero - order, first - order, Hixon Crowell, Korsmeyer Peppas and Higuchi Plot models were tested.

### 2.9 Methods for Antifungal activity

Following protocol was followed for measuring anti - fungal activity

- Cup - plate method was used for anti - fungal formulation.
- Candida albicans suspension was poured into sterilized dextrose agar media (cooled at 40°C) and was mixed

thoroughly.

- 20ml of the above mentioned suspension was poured aseptically in a pre-sterilized petridish and was allowed to solidify. The surface of agar plate was pierced via sterile cork borer.
- These wells were filled with equal volume of the optimized batch of microemulsion based gel and marketed 1% clotrimazole gel followed by incubation at 18 - 24 °C, for 72 h.
- Fungal growth was detected and the zone of inhibition was measured using antibiotic zone reader.

### 3. Results and Discussion

#### 3.1 Preformulation studies

##### 3.1.1 Chemical properties

It is white powder or colorless crystalline powder. It has a melting point of 147 - 149 ° C. It is soluble in ethanol, acetone, and chloroform, but almost insoluble in water. It is odorless, tasteless and subject to rapid decomposition in an

acid solution. Clotrimazole hydrochloride has a melting point of 159° C.

##### 3.1.2 pH

The pH of clotrimazole in different solutions at initial and 2hrs. it was observed that, at pH 1.2, pH 4.5 and pH 6.8. at initial time  $98.94 \pm 1.55$ ,  $98.85 \pm 1.02$  and  $99.58 \pm 1.72$ , respectively. The value observed at 2 hrs  $98.25$ ,  $98.25$  and  $99.35$ , respectively.

Table 2: pH observed

TIME	pH 1.2	pH 4.5	pH 6.8
Initial	$98.94 \pm 1.55$	$98.85 \pm 1.02$	$99.58 \pm 1.72$
2 hrs	98.25	98.25	99.35

##### 3.1.3 FTIR Analysis

The FTIR Spectra of the clotrimazole and optimized clotrimazole microemulsion gel were recorded with KBr on infrared spectrophotometer as shown in figure.



Figure 2: FTIR Spectroscopy of clotrimazole drug

##### 3.1.4 Solubility in different solvents

CLT experimental solubility values in buffers pH 2.0 and 7.4, 1 - octanol and hexane expressed in molarity (S) in the temperature range (293.15 - 313.15) K. The temperature dependences of the drug solubility in the studied solvents are shown in table.

Stated that studied solvents the compound solubility increased at higher temperatures. Clotrimazole is stable in the buffer solution pH range of 1.2 - 7.5, but it degrades in strongly acidic and basic media and at high temperatures.

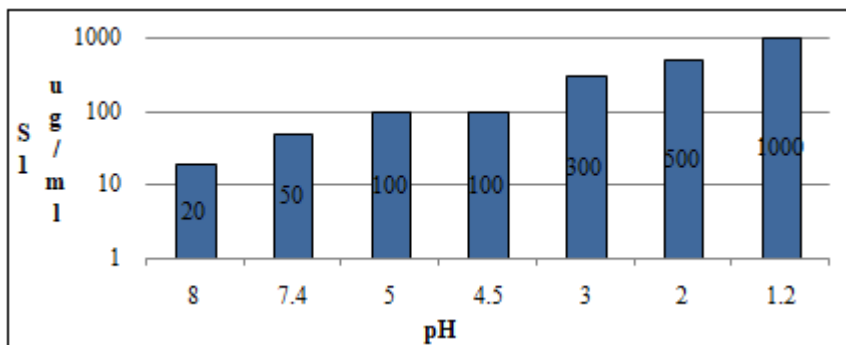


Figure 3: Solubility data of CLT in buffer solutions at different pH and temperature

The greater clotrimazole solubility in the oil phase is essential since clotrimazole is a low water soluble medicine. In different oils including oleic acid, lemon oil, olive oil and menthane oil, solubility of clotrimazole was studied. Maximum solubility of clotrimazole in mentha oil has been discovered among the oils that have been tested. The analgesic and cooling effects of mentha oil itself are sensory. Mentha oil has thus been used for the oil phase for the clotrimazole microemulsion. The maximal solubility of

clotrimazol in tween 80 was shown. Tween 80 was therefore used as the surfactant for formulation of clotrimazole.

Microemulsion. For other co - surfactants including IPA and ethanol, clotrimazole demonstrated highest solubility of propylene glycol. The skin is very well permeated by propylene glycol. The co - surfactant is therefore propylene glycol.

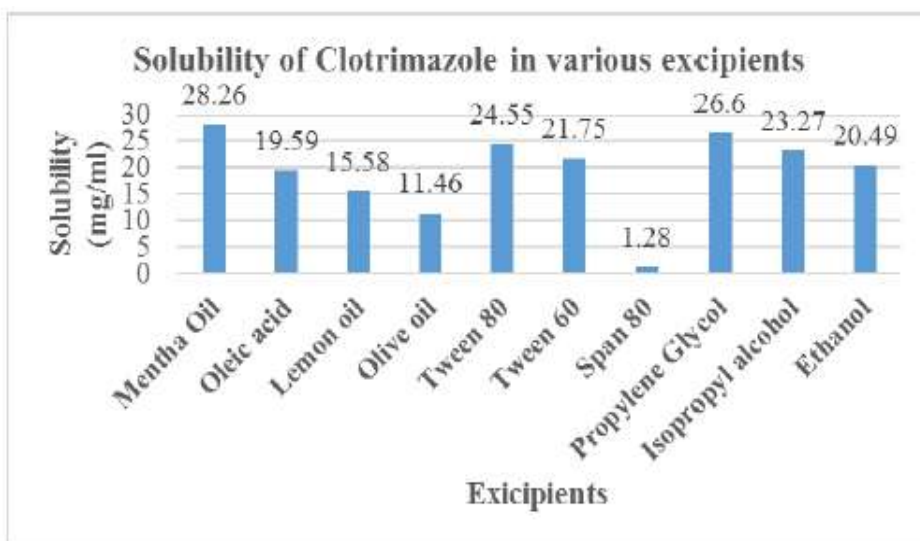


Figure 4: Solubility of clotrimazole in various oils, surfactants and co - surfactants

Clotrimazole o/w microemulsion by the titration technique throughout their experiment. Surfactants and co - surfactants have been combined and applied wisely to the water drop. The medicine was dissolved in the oil phase and stirred continually into the aforementioned solution. The solution allowed for clear and transparent liquid microemulsion to be formed. The phase titration technique was used to manufacture clotrimazole loaded o/w Microemulsion. Surfactants and co - surfactants have been combined and applied wisely to the water drop. The drug were dissolved in the oil phase and stirred continually into the a for mentioned solution. All located clear and transparent liquid solutions.

### 3.1.5 Stability

Optimized microemulsion & microemulsion Gel were subjected to stability study for a period of two months at room temperature and refrigeration condition (2 - 8°C). During the period of storage the ME was subjected for % transmittance, % assay & pH, while ME gel was subjected for % transmittance, % assay, pH, consistence & viscosity

(physical). Results are shown in Table 3, Table 4, Table 5, & Table 6.

Table 3: Result of stability study of microemulsion at room temperature

TEST	Initial time	1month	2month
Transmittance %	99.6 ± 0.06	99.6 ± 0.21	99.4 ± 0.18
Assay %	99.1 ± 0.26	98.8 ± 0.15	98.82 ± 0.13
Ph	5.50 ± 0.19	5.50 ± 0.14	5.50 ± 0.14

Table 4: Result of stability of Microemulsion gel at room temperature

TEST	Initial	1 month	2 month
Assay %	99.26 ± 0.68	99.2 ± 0.28	99.2 ± 0.26
pH	6.10 ± 0.25	5.92 ± 0.38	98.82 ± 0.13
Transparency	Transparency & clear	Transparency & clear	Transparency & clear
Viscosity	Very good	Very good	Very good



**Table 5:** Result of stability study of microemulsion at refrigeration temperature

TEST	Initial time	1month	2month
Transmittance %	99.9 ± 0.10	99.5 ± 0.10	99.4 ± 0.09
Assay %	99.6 ± 0.22	99.1 ± 0.30	99.0 ± 0.30
pH	5.46 ± 0.22	5.4 ± 0.20	5.42 ± 0.20

**Table 6:** Result of stability of Microemulsion gel at refrigeration temperature

TEST	Initial	1 month	2 month
Assay %	99.6 ± 2.42	98.9 ± 1.23	98.6 ± 1.13
Ph	5.99 ± 0.20	5.99 ± 0.14	5.97 ± 0.12
Transparency	Transparency & clear	Transparency & clear	Transparency & clear
Viscosity	Very good	Very good	Very good

**3.2 Drug excipient chemical compatibility:**

The total number of drug excipient blends in the study may be very high; therefore, excipient rank ordered with their solubility for CLZ were selected primary screening. For example, oils such as capryl 90, lauroglycol 90, and capmul MCM C8 exhibiting higher solubility for clotrimazole were selected. As summarized in table, every excipient clotrimazole had degraded approximately 5 - 15% in 14 days at both storage conditions. The rate of degradation increased with increase in temperature. similar degradation peak of clotrimazole was evident in chromatograms of all samples. The representative chromatograms of sample stored at 60° C for 14 days is shown in figure. Which shows well resolved degradation product of clotrimazole. In solution state, stability of clotrimazole is pH dependent.

**3.3 Preparation of CTM microemulsion & CTM ME gel**

Following steps were followed in preparing CTM microemulsion and CTM ME based gel: The microemulsions and microemulsion based - gels which had desirable appearance were selected and were added with 1% w/w of clotrimazole. The drug was dissolved in microemulsions to obtain clotrimazole microemulsions. Fumed silica was dispersed in clotrimazole microemulsions for the preparation of clotrimazole microemulsionbased gel.

**3.4 Preparation of blank microemulsions and microemulsion based - gels**

Following steps were followed in preparing blank microemulsion and ME based gel: Microemulsion components in the preceding report, the region of microemulsion was chosen. The simple blending of IPP, 2: 1 water and IPA and 1: 1 PSMO and SMO mixtures at 20% of concentrations produced two microemulsions.30% and 50% w/w, respectively for ME1 and in those of 20 %, 40 % and 40 % w/w, respectively for ME 2 1. Afterwards, 2.0 %, and 5 % of fumed silica was added into ME1 and ME2 to obtain microemulsion - based gel designated as MBG1 - 1 to MBG1 - 2 and MBG2 - 1 to MBG2 - 2, respectively.

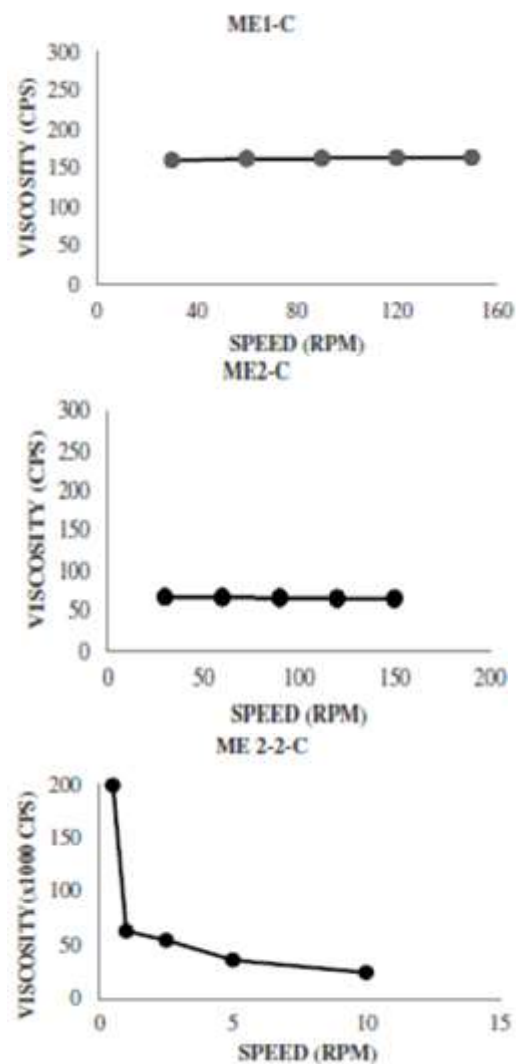
**3.5 Characteristics of clotrimazole microemulsions and microemulsion - based gels**

Following characteristics were observed:

- 1%w/w clotrimazole was incorporated in ME1, ME2, andMBG2 - 2 and ME1 - C, ME2 - C, and MBG2 - 2 - C, were obtained respectively. No significant visual changes were observed.
- However, conductivity values of clortimazole - loaded samples were low in comparison to their blank counterparts, while pH and spreadability showed remarkable change.
- The samples were water - in - oil type; therefore, clotrimazole located in the external oil phase, that resulted in lower conductivity. The rheological behavior ofME1 - C andME2 - C still showed as Newtonian flow, also the MBG2 - 2 - C still were shear thinning like their blank counterparts. The viscosity of MBG2 - 2 - C was raised slightly in comparision to its blank counterpart.

**Table 7:** Physical properties of clotrimazole microemulsions and microemulsion based - gels.

Formulation	Conductivity (µS/cm)	pH	Spreadibility
ME1C	16.70±0.17	6.92 ±0.03	+
ME2C	53.37±1.96	6.84 ±0.01	+
MBG - 2 - 2C	31.37 ±0.15	6.78 ±0.02	+++



**Figure 7:** Characterstis clotrimazole microemulsions and microemulsion based – gel

3.6 Characteristics of blank microemulsions and microemulsion - based gels

The obtained microemulsions (ME1 and ME2) were clear, pale yellowish liquids with little smell of alcohol and were immiscible with water. The results of dilution and conductivity exhibited that both ME1 and ME2 were water - in - oil type since their HLB value was 9.65.

Table 8: Characteristics of blank microemulsions and microemulsion - based gels

Formulation	Conductivity (μS/cm)	pH	Spreeradibility
ME1	20: 60 ± 0: 20	6.96 ±0.07	+
MBG1 - 1	26.83 ±0.15	6.99 ±0.02	+
MBG1 - 2	23.73± 0.55	6.90± 0.06	++
ME2	63.63± 2.90	6.98 ±0.09	+
MBG2 - 1	74.77 ±1.05	6.86± 0.03	++
MBG2 - 2	50.43 ±1.42	6.88 ±0.14	+++

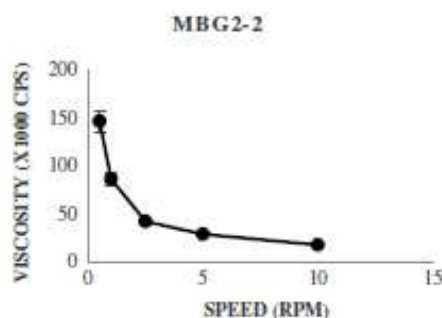
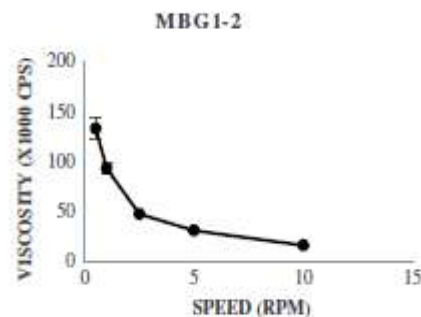
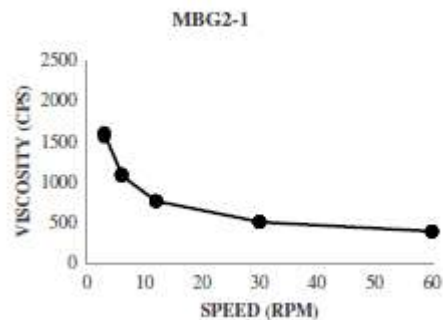
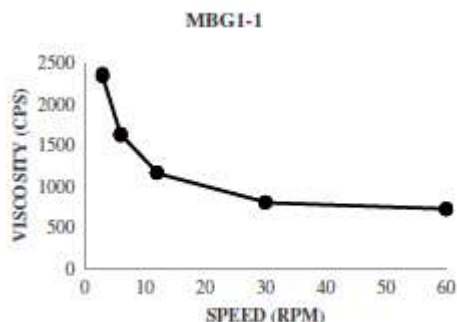
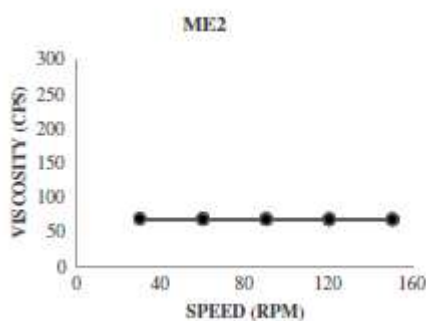
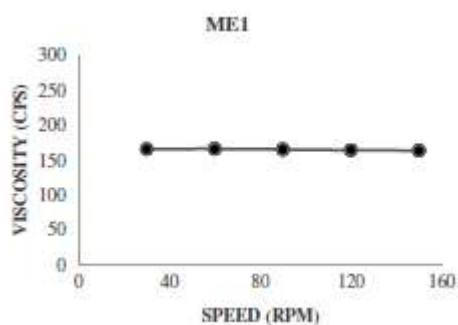


Figure 9: Characteristics of blank microemulsions and microemulsion based – gels



(Left) Figure 10 (a): Clotrimazol formulation assay based results FA



(Right) Figure 10 (b): Clotrimazole formulation software based results FB

The pH of the formulations FA and FB were found to be  $6.6 \pm 0.07$  and  $6.1 \pm 0.4$  respectively. The pH has been adapted to the appropriate physiological pH of 6.1 - 6.6. In USP, clotrimazole results were determined to be not less than 90.0 percent and not more than 110.0 percent 20. Test findings using the established analytical technique. The F2 formulation of F2 drugs discovered  $98.9 \pm 0.46\%$  whereas, the F3 formulation produced  $100.3 \pm 0.71\%$ . Fig. shows FB as optimal formulation based on the desired assessment. Researchers also determined that clotrimazole is often a suitable phenomenon for the hydrogel integration. It also shown that the solubility problem of hydrophobic drugs also isn't addressed by alcoholic content. A hydrogel without any hard disolvents that may cause irritation can be produced by co - disolvents. It was discovered to be compatible with solvents as Carbomer was used as a gelling agent. The approach devised does not require the removal of polymer over the day and so saves time. Has an increased propensity and is much more patient adaptive, therefore more clinical studies are necessary.

### 3.7 Drug release kinetics study

The kinetic research on drug release. The formulation of hydrogel based on microemulsion is an effective promoter of the localisation of clotrimazole to the skin. It was shown that the drug permeability of the optimised formula based on microemulsion (in vitro) was below (92.04 percent) its optimal hydrogel formulation based on microemulsion (ex vivo) (96.12 percent). This might be because of the drug partitioning into the oil phase of the hydrogel based on microemulsion that lowers drug release.

Table 8: Drug Kinetics release study

Kinetic model		Zero order	First order	Hixon crowell	Corse mayer papers	Higuchi plot
In vitro study	R <sup>2</sup> Value	0.920	0.965	0.985	- 0.229	0.831
	Slope	0.104	0.002	0.011	1.338	0.247
	Intercept	- 9.810	0.310	0.626	- 4.522	10.00
Ex vivo study	R <sup>2</sup> Value	0.929	0.948	0.981	- 0.269	0.836
	Slope	0.108	0.002	0.011	1.158	0.241
	Intercept	- 8.798	0.482	0.918	- 4.008	10.200

### 3.8 Antifungal activity

The values of mean zone of inhibition (in vitro antifungal activity) of optimum microemulsion based hydrogel batch

and marketed formulation. For topical antifungal medicines, such as clotrimazole, an effective formula is required. Their thermodynamically and isotopically stable characteristics are caused by surfactants and cosurfactants that lower interfacial tension from oil to water phase.

Table 9: Zones of inhibition by various gels

Sample	Zone of Microbial growth inhibition
Placebo	0
CLT - ME	$43.67 \pm 2.31$
CLT - ME gel	$41.67 \pm 2.88$

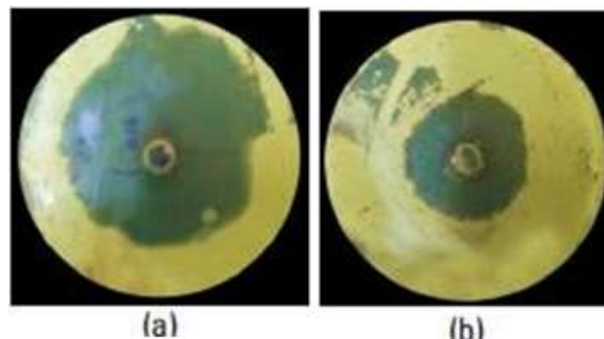


Figure 11: Zone of inhibition of (a) optimized batch & (b) marketed product

## 4. Conclusion & Summary

Clotrimazole is a broad - spectrum antimycotic drug mainly used for the treatment of *Candida albicans* and other fungal infections. A synthetic, azole antimycotic, clotrimazole is widely used as a topical treatment for tinea pedis (athlete's foot), as well as vulvovaginal and oropharyngeal candidiasis. It displays fungistatic antimycotic activity by targeting the biosynthesis of ergosterol, thereby inhibiting fungal growth. As well as its antimycotic activity, clotrimazole has become a drug of interest against several other diseases such as sickle cell disease, malaria and some cancers. It has also been combined with other molecules, such as the metals, to produce clotrimazole complexes that show improved pharmacological efficacy. Moreover, several new, modified - release pharmaceutical formulations are also undergoing development. Clotrimazole is a very well - tolerated product with few side effects, although there is some drug resistance appearing among immunocompromised patients. Here, we review the pharmaceutical chemistry, application and pharmacology of clotrimazole and discuss future prospects for its further development as a chemotherapeutic agent.

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