Original Article

Formulation and evaluation of clotrimazole from pluronic F_{127} gels

Boushra M. El-Houssieny[1,*], Hayam M. Hamouda[2]

[1] *Department of Pharmaceutics, National Organization for Drug Control and Research (NODCAR), Giza, Egypt;*
[2] *Department of Microbiology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.*

ABSTRACT: Thermally reversible gels of poly(oxyethylene)-poly(oxypropylene)-poly(oxyethylene)-triblock copolymer, pluronic F_{127} (PF_{127}), were evaluated as a vehicle for topical administration of clotrimazole as a model of a broad spectrum antifungal agent against superficial fungal infections. The solubility of clotrimazole was significantly increased as a linear function of pluronic F_{127} concentration at four temperatures. Clotrimazole was highly trapped by the micelles as indicated by a large partition coefficient. The micellar solubilization was a spontaneous ($\Delta G < 0$) and exothermic ($\Delta H < 0$) process which resulted in a less orderly state ($\Delta S > 0$). Different additives were used to enhance drug release from preparations including propylene glycol, polyethylene glycol 400, glycerin, and dimethyl sulfoxide at concentrations of 5 and 10% and polysorbate 80 at concentrations of 1 and 2%. Different formulae were characterized in terms of drug content, pH and particle size measurement, spreadability, rheological properties, drug release, diffusion, and permeation. The formulae showing the best drug release were selected to study the effect of storage on various parameters over a period of 6 months and for microbiological evaluation. The best release enhancers were propylene glycol and polyethylene glycol 400 at a concentration of 10% and polysorbate 80 at a concentration of 2% and the formulae containing it were stable and proved to be effective in inhibition. Furthermore they were tested microbiologically against three fungi as well as yeast. The antimicrobial activities of the tested preparations were compared with the pure drug at the same concentrations and also tested for their antifungal activity. It was found to be effective against *Aspergillus niger*, *A. flaves*, *Candida albicans*, and *Sacharomyces cerevisiae* with inhibition zones of 39, 39, 35, and 32 mm, respectively.

Keywords: Clotrimazole, pluronic F_{127}, solubility, gel formulation, release enhancers, antimicrobial activities

1. Introduction

The delivery of drugs to the skin is recognized as an effective means of therapy for local dermatologic diseases, which is a desirable feature for the relief of local symptoms at a low dose, thereby reducing systemic side effects.

Clotrimazole is a synthetic imidazole derivative shown to be a potent well-tolerated topical antifungal agent. It is active against dermatophytes (the causative organisms of time infections) and yeast (*Candida albicans*) (*1,2*). Its *in vitro* spectrum includes yeasts, dermatophytes, dimorphic fungi, and dematiaceous species (*3*). Plempel *et al.* (*3,4*) reported that it was inhibitory *in vitro* at concentrations of 4 µg or less per mL for most susceptible fungi and that many species, particularly Trichophyton and Candida, were inhibited by 1 µg/mL or less. However, it was said to be fungicidal only at concentrations greater than 20 µg/mL (*3*). Published data regarding the clinical effectiveness of clotrimazole are limited. In one report (*5*), it was described as being effective in one patient with pulmonary aspergilloma, in another with bronchial infection due to *Candida krusei*, and in a third with tinea barbae due to *C. albicans*.

According to another report (*6*), it was effective in treatment of candidiasis in a limited number of pediatric patients. Unpublished data from clinical studies in 87 adults indicated that it was effective in approximately 80% of patients with acute or systemic candidiasis including septicemia, pneumonia, endocarditis, and renal infection.

Efficacy and safety of topical clotrimazole were investigated by El-Gibaly (*7*) who found that clotrimazole (1%) formulated in an oleaginous or water

*Address correspondence to:
Dr. Boushra M. El-Houssieny, Department of Pharmaceutics, National organization for Drug Control and Research (NODCAR), Giza, Egypt.
e-mail: dr.boushra@yahoo.com

soluble ointment base was clinically more effective for the treatment of tinea circinate as compared with emulsion bases or its respective vehicles.

Over the last few decades, gels formed from natural, semisynthetic or synthetic polymers have been confirmed as vehicles for different types of pharmaceutical applications. They have good viscosity and satisfactory bioadhesion without irritating or sensitizing actions. Generally, hydrogel bases can be easily washed out and adhered well to mucous membrane or skin, wet with secreting fluid, and thus these are applied to injured skin and also to eyes (*8*).

Pluronic F_{127} is one in a series of poloxamer ABA block copolymers, the members of which share the chemical formula:

$$H\text{-}(O\text{-}CH_2\text{-}CH_2)_a(O\text{-}\underset{\displaystyle CH_3}{\underset{|}{CH}}\text{-}CH_2)_b(O\text{-}CH_2\text{-}CH_2)_a\text{-}OH$$

The polymers are produced by condensation of ethylene oxide and propylene oxide. Pluronic F_{127} has a molecular weight of 11,500, 70-79% of which is accounted for by the hydrophilic ethylene oxide portion. It is more soluble in cold water than hot due to increased solvation and hydrogen bonding at lower temperatures. Aqueous solutions of between 20 and 30% (w/w) of PF_{127} have the interesting characteristic of reverse thermal gelation, that is, they are liquid at refrigerated temperatures (4-5°C) but gel upon warming to ambient levels. The gelation is reversible upon cooling (*9*).

Gelation of PF_{127} is thought to occur due to the hydration of the polymer leading to increased chain friction and entanglement, producing a hydrophilic association (*10,11*). Reverse thermal gelation and low toxicity have been the basis of research into the use of PF_{127} as a possible drug delivery system in man (*12-17*).

The present study is concerned with formulation of a topical clotrimazole gel using pluronic F_{127} as a polymer having surface active properties. The prepared gels were investigated to observe the physicochemical phenomena and thermodynamic properties of clotrimazole in PF_{127} and to determine the effects of different penetration enhancers on the micellar solubilization and release of clotrimazole from the vehicle into the receptor medium. Therefore, the aim of the present investigation was also to test the *in vitro* activity of the gel preparations of clotrimazole against pathogenic fungi.

2. Materials and Methods

2.1. *Materials*

Clotrimazole (molecular weight 344 g/mol, aqueous solubility: 5.5 µmol/L), was purchased from Sigma Chemicals (Steinheim, Germany). Pluronic F_{127} (PF_{127}), propylene glycol, polyethylene glycol 400, semi-permeable cellulose membrane (molecular weight cut off 12,000-14,000), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Citric acid anhydrous, disodium hydrogen phosphate, polysorbate 80, glycerol, dimethyl sulfoxide were from El-Nasr Chemical Industries (Cairo, Egypt). All other chemicals were of analytical reagent grade and used without further purification.

2.2. *Organisms*

Four isolates of pathogenic fungi, *Aspergillus niger*, *Aspergillus flaves*, *Candida. albicans* CAIM 22, and *Saccharomyces cerevisiae* CAIM 14, were used for determination of antifungal activity. All test organisms were kindly supplied by Microbiological Resource Center, Cairo, Mircen-Egypt (CAIM). Potato dextrose agar (PDA, Oxoid) was used as media for fungi and yeasts.

2.3. *Equipment*

The equipment used was: Ultra-violet Spectrophotometer (Schimadzu, UV 1601, Kyoto, Japan), Electric balance (Satorius, GMBH, Gottingen, Germany), Thermostatically controlled heater with magnetic stirrer (LABINCO BV, Netherlands), pH meter (Cyberscan 10, USA), Cone and plate viscometer (Brookfield Model DV 111, USA), Franz diffusion (Hanson Research Corporation (HRC), USA), Thermostatatically controlled shaking water bath (Gallenkamp. Co., Germany), Laser Scattering particle size distribution analyzer (LA-920, Horiba, USA), USP dissolution tester (Pharma test, type PTW2, Germany).

2.4. *Solubility test*

An excess amount of clotrimazole was added to 0.1 M cetrophosphate buffer (pH 5.5) containing different amounts of PF_{127} (0-10%, w/w) in glass vials which were continuously shaken for 48 h in a thermostated water bath set at 25, 30, 37, and 40°C (± 1°C). The properly diluted samples were assayed spectrophotometrically at 250 nm after filtration through 0.45 µm Millipore membrane filters. The solubility was established by analyzing serial samples. To minimize the effect of temperature change on solubility, filtration was performed in a temperature-controlled oven. PF_{127} did not interfere with the assay.

2.5. *Preparation of pluronic F_{127} gels*

Clotrimazole PF_{127} gels were prepared by the cold method as described by Schmolka (*9*). The required amount of PF_{127} (20%) was dissolved in a buffer solution (0.1 M citrophosphate buffer, pH 5.5) with the aid of a

magnetic stirrer and the solution was left in a refrigerator overnight. When the mixture became a clear solution, an ethanol solution of the drug: ethyl alcohol (1:20) was thoroughly mixed into the PF_{127} solution. The mixture was then left at room temperature until becoming a clear gel. To study the effect of penetration enhancers on the *in vitro* release (namely, propylene glycol, polyethylene glycol 400, glycerin, and dimethyl sulfoxide in concentrations of 5 and 10%, and polysorbate 80 in concentration of 1 and 2%), they were incorporated in the gel either separately or in combination namely, propylene glycol and polyethyelene glycol 400 (5% + 5%), propylene glycol and polysorbate 80 (5% + 1%), polyethylene glycol 400 and polysorbate 80 (5% + 1%). The compositions of the gels are summarized in Table 1.

2.6. *Evaluation of the physical properties of the prepared gel*

All prepared gels (with or without the penetration enhancers) were subjected to the following tests.

2.6.1. *Determination of actual drug content in the prepared clotrimazole gels*

The actual drug content was determined in each prepared formula as follows: 0.5 g of the gel was dissolved in 100 mL of citrophosphate buffer, pH 5.5, and then filtered through a 0.45 µm membrane filter (No. 40) (*18*). The concentration of the drug was determined spectrophotometrically at a λ_{max} of 250 nm using the same buffer as a blank.

2.6.2. *Determination of pH of the prepared formula*

pH of the prepared formulae was determined using the following method: one g of gel was diluted with 9 g distilled water and shaken well (*19,20*). pH measurement was repeated three times for each formula and the reading was the average of 3 replicates.

2.6.3. *Particle size measurement*

A particle size distribution analyzer was used, frequency distribution curve was done by plotting $q^3\%$ against diameter in µm, where $q^3\% = (V_d / V_t) \times 100$. V_d is the volume of particles corresponding to each diameter in µm and V_t is the total volume of particles in the examined sample.

2.6.4. *Test for spreadability*

A sample of 0.1 g of each formula was pressed between two slides (divided in squares of 5 mm sides) and left for about 5 min where no more spreading was expected (*21-24*). Diameters of spread circles were measured in cm and were taken as comparative values for spreadability. The results obtained were the average of three determinations.

2.6.5. *Rheological measurements and data analysis*

Steady shear measurements were conducted where the rheograms of all prepared gels were performed at 25 ± 0.1°C with spindle 52, with the shear rate ranging from 2 to 400 sec^{-1} corresponding to 1 to 200 rpm with 10 sec between each two successive speeds and then in a descending order. Equilibration of the sample for 5 min was made following loading of the viscometer. Ramp time for each viscosity stage was the reading after 20 sec. All studies were performed in triplicate and the average was taken.

Rheological data was fitted to different models (Bingham, Power law, Casson) to examine the pattern of flow and the presence of yield value (*25*).

Bingham: $\tau = \tau_o + \eta\gamma$
Power law: $\tau = \eta\gamma^n$
Casson: $\tau^{1/2} = \tau_o^{1/2} + \eta^{1/2}\gamma^{1/2}$

where τ is the shear stress, τ_o the yield value, η a constant called the apparent viscosity or the consistency index, γ the shear rate, and n is the flow index. In the case of Newtonian behavior $n = 1$ and $\tau_o = 0$, whereas in the case of pseudoplastic (shear thinning) behavior $0 < n < 1$ and $\tau_o = 0$, for plastic behavior, it is the same as pseudoplastic but with $\tau_o > 0$ while in the case of dilatant's flow (shear thickening) $n > 1$ (*26*).

Table 1. Composition of clotrimazole pluronic F_{127} gels

Components	Gel formulae													
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}	F_{13}	F_{14}
Clotrimazole	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Pluronic F_{127}	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Ethyl alcohol	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Propylene glycol	—	5	—	—	—	—	10	—	—	—	—	5	5	—
Polyethylene glycol 400	—	—	5	—	—	—	—	10	—	—	—	5	—	5
Polysorbate 80	—	—	—	1	—	—	—	—	2	—	—	—	1	1
Glycerin	—	—	—	—	5	—	—	—	—	10	—	—	—	—
Dimethyl sulfoxide	—	—	—	—	—	5	—	—	—	—	10	—	—	—
Buffer pH 5.5 to	100	100	100	100	100	100	100	100	100	100	100	100	100	100

2.6.6. *In vitro release*

The study was carried out using the modified USP dissolution apparatus. A sample of 3 g of the preparation was spread on a cellophane membrane previously soaked overnight in the release medium. The loaded membrane was firmly stretched over the edge of a 2 cm diameter glass tube; the membrane was tied with a rubberband to prevent leakage (*27*). Tubes were then immersed in the dissolution vessel which contained 200 mL of the release medium, citrophosphate buffer, pH 5.5, containing 1% polysorbate 80:ethyl alcohol (1:1) and maintained at 35 ± 0.5ºC (*28*). The shafts were rotated at 100 rpm and at appropriate intervals, 3 mL aliquots of the release medium were withdrawn and immediately replaced by an equal volume of fresh release medium. The sample was assayed spectrophotometrically at a λ_{max} of 250 nm and the concentration of the drug was determined from the previously constructed calibration curve. Experiments were carried out in triplicate, the results were averaged and blank experiments were carried using plain base.

2.6.7. *Diffusion tests*

The release of clotrimazole from PF_{127} gels was studied at 35 ± 0.5°C using a Franz diffusion cell apparatus with a permeation area of 1.77 cm^2. Cellulose membranes were previously soaked in the release medium consisting of citrophosphate buffer, pH 5.5, containing 1% polysorbate 80:ethyl alcohol (1:1). A constant weight (0.5 g) of each gel formula was added to the donor compartment and 7.5 mL of the release medium were placed in the receptor compartment with constant stirring. At appropriate intervals, 500 µL aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed spectrophotometrically at 250 nm after suitable dilution to determine the drug permeation per unit time.

2.6.8. *Kinetic analysis of drug release data*

Drug release data generated from the dissolution experiments were fitted to the following power law equation by Peppas, 1985 (*29*) using logarithmic transformations and least squares regression analysis:

$$M_t/M_x = kt^n$$

where M_t/M_x is the fraction of drug released up to time t, k is the kinetic constant and n is the release exponent indicative of the release mechanism.

The permeation parameters of clotrimazole from gels including permeability coefficient (p, cm/min), diffusion coefficient (D, cm^2/min), and apparent steady state flux (Jss, µg/cm^2/min) were calculated as follows:

The penetration profile conducted by plotting the cumulative amount of permeated drug (µg/cm^2) *versus* time (min), the flux (Jss) was calculated from the slope of the line.

P: permeability coefficient calculated by dividing (Jss) by the employed concentration of the drug Co according to the equation P = Jss/Co.

D: diffusion coefficient was calculated from the slope by plotting the cumulative amount of drug permeated versus square route of time ($\sqrt{t}$ min) and (D) was calculated from the equation: $D = (slope/2Co)^2\pi$.

2.7. *Stability study on the selected clotrimazole gels*

Based on the results from previous studies, formulae prepared with 10% (w/w) propylene glycol, 10% (w/w) polyethylene glycol 400, and 2% (w/w) polysorbate 80 (F_7, F_8, and F_9, respectively) were stored in well stoppered glass container for 6 months at room temperature. Gels were subjected to all the previous tests. The results obtained from the freshly prepared samples and after storage were compared using a Student's *t*-test and the software utilized was Graphpad Instant ver. 2.04 with a 5% level of significance.

2.8. *Evaluation of antimicrobial activity*

The antimicrobial activity of the gel preparation against four fungi, *Candida. albicans* CAIM 22 and *Saccharomyces cerevisiae* CAIM 14 (kindly supplied by Microbiological Resource Center, Cairo, Mircen-Egypt; CAIM). *Aspergillus niger* and *Aspergillus flaves* obtained from the Microbiology Laboratory, Department of Microbiology, NODCAR. The antimicrobial activity was determined by the pour diffusion method as described in CLSI 2006 (*30*) using potato dextrose agar plates (PDA, Oxoid) previously inoculated with 18 h spores (10^6 spores/mL fungi) suspension in potato dextrose broth (PDB, Oxoid) of the test organisms. Gel preparation as well as pure drug of the same concentration was applied over each of the culture plates previously seeded with the 10^6 spores/mL of fungi. The experiment was performed in triplicate. Incubations were at 37°C for 24-48 h for *C. albicans* and at room temperature for 72 h for the other filamentous fungi. Following incubation the zones of inhibition formed were measured and the mean diameter obtained. Overall, cultured fungi with 10 mm halos were considered susceptible to the tested compound.

3. Results and Discussion

3.1. *Solubility of clotrimazole in pluronic F_{127} solutions*

For the development of topical formulations, the solubility of the active ingredient in the vehicle is

often an important factor to determine the applied dose. Clotrimazole is practically insoluble in water, thus, limiting its use in aqueous preparations. Since surfactants have been successfully used to enhance the solubility of drugs in many pharmaceutical formulations, pluronic F_{127} was evaluated for its potential use in formulating an aqueous clotrimazole preparation in this study.

3.1.1. *Solubility profile*

Figure 1 shows a linear relationship between the amount of clotrimazole solubilized and the concentration of PF_{127} in the medium at four temperatures. The solution properties of the surfactant including the solubilizing activity are generally known to change drastically near the critical micelle concentration (CMC). In the presence of 10% PF_{127}, the solubility of clotrimazole increased nearly 100-fold at each of the four temperatures. However, due to the low CMC of PF_{127} (*31*), the solubilization of drugs in a surfactant solution has been suggested to occur due to the increased partitioning of solute molecules into the micelles which could be considered as a separate, pseudo-phase (*27*). The linear increase in the solubility of clotrimazole as a function of PF_{127} concentration observed in this study suggested that the total micellar volume into which clotrimazole partitioned was a linear function of the amount of PF_{127} present..

3.1.2. *Partition coefficient (17)*

The partition coefficient of clotrimazole (k_m) between micellar and aqueous phases was determined by the ratio of the clotrimazole solubility in the micellar phase (S_m) to that in the aqueous phase (S_w) as shown in Table 2. The solubility of clotrimazole in the extramicellar phase was assumed to be equal to that in water since colloidal surfactants usually do not change the chemical potential of the solute (*32*).

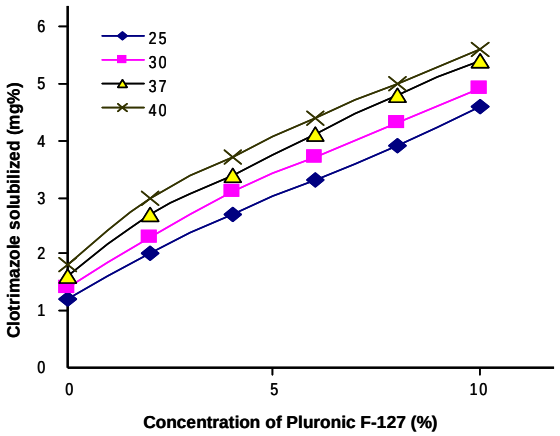

Figure 1. Solubility profile of clotrimazole as a function of pluronic F_{127} concentrations at different temperatures. ♦, 25°C; ■, 30°C; ▲, 37°C; ×, 40°C. *n* = 3.

The S_m values were estimated by extrapolating the solubility of clotrimazole to 100% (w/w) of PF_{127} as previously reported (*33*).

The large k_m values observed in this study indicated that clotrimazole had highly partitioned into the micelles.

The partition coefficients of clotrimazole were found to be inversely related to temperature. When temperature was increased from 25°C to 40°C, the $\log K_m$ values were decreased from 1.63 to 1.54, despite the higher solubility of clotrimazole in both the micellar and aqueous phases. According to Elworthy and McDonald (*34*), the size of micelles increased only slightly until a threshold temperature, approximately 20°C below the cloud point of the surfactants, was reached. They showed that beyond this temperature, the micellar volume expanded rapidly and asymmetrically, thus causing increased loading of the drug into the micelles. In this experiment, the highest temperature used was 40°C, far below the cloud point of PF_{127} which was reported to be beyond 100°C (*35*). Therefore, the reduction of k_m at 40°C as compared to that at 25°C could be attributed to a smaller increase in the solubility of clotrimazole in the micellar phase than to that in the external aqueous phase, probably due to a higher temperature coefficient of the aqueous solubility of clotrimazole.

3.1.3. *Thermodynamics*

Table 2 summarizes the thermodynamic parameters associated with the solubilization of clotrimazole in the PF_{127} solution. These parameters are practically useful in understanding the thermodynamic phenomena involved in the micellar solubilization of clotrimazole.

A typical Van't Hoff's plot, $\log K_m$ *versus* 1/T, which was nearly straight over the employed temperature range as shown in Figure 2, was used to calculate thermodynamic parameters. ΔH, ΔS, and ΔG (apparent enthalpy, entropy and free energy change, respectively) were calculated from temperature dependence of k_m values. The standard free energy change which indicates the spontaneity of the solubilization process was calculated using the equation: $\Delta G = -RT \ln K_m$, where ΔG is the free energy change for the transfer of one mole of clotrimazole from the aqueous phase into

Table 2. Partition coefficient (K_m) and thermodynamic parameters of clotrimazole in pluronic F_{127} at four different temperatures

Temperatures		K_m	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS(J/K·mol)
°C	K				
25	298	42.668	-9.29	-0.77944	30.07
30	303	39.82	-9.27	-0.91787	27.56
37	310	36.07	-9.25	-1.10435	26.28
40	313	34.71	-9.24	-1.18059	25.75

K_m, partition coefficient; ΔG, free energy change; ΔH, apparent enthalpy; ΔS, entropy.

the micellar phase; R, the gas constant; T, the absolute temperature, and k_m is the partition coefficient. All the values of ΔG obtained at the four temperatures were found to be negative, indicating that clotrimazole molecules spontaneously partitioned into the micelles.

The standard enthalpy change, ΔH, which was calculated from the slope of the Van't Hoff's plot representing d ln k_m/d (1/T) = –ΔH/R, was also negative, indicating that the micellar solubilization of clotrimazole was an energetically favored exothermic process. This is in accordance with the negative ΔH values for the solubilization of steroids in the nonionic polyoxyethylene surfactant solution as was previously reported (*36*).

The standard entropy change for the micellar solubilization of clotrimazole which was calculated using ΔS = (ΔH – ΔG)/T was a positive value (Table 2). Entrapment of clotrimazole molecules within micelles could restrict their activity, causing a decrease in entropy of the system.

3.2. *Evalution of the physical properties of the prepared gels*

3.2.1. *Clotrimazole content in the prepared gels*

Drug content of the prepared gels ranged from 98.9 to 101.3% of the labeled amount. These results revealed that no interference occurred between different components and clotrimazole in the assay which indicates homogeneity and uniformity of the prepared gels.

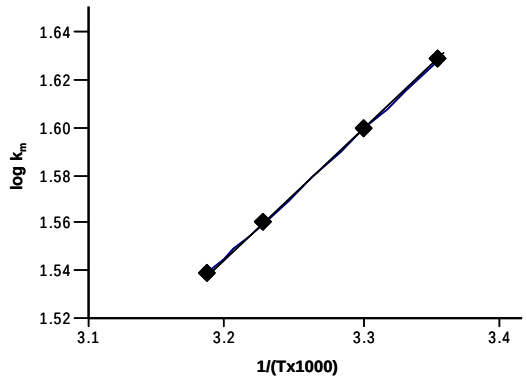

Figure 2. Van't Hoff's plot of clotrimazole in pluronic F_{127}.

3.2.2. *Test for spreadability*

Results for spreadability testing are shown in Table 3. All prepared gels using different concentrations of different release enhancers were spreadable on the skin surface. It should be mentioned that the addition of propylene glycol, polyethylene glycol 400, and polysorbate 80 improved the physical characteristics including spreadability, consistency and skin feel (*37*). Also, its addition helped the dissolution of the drug and prevented precipitation upon storage. With each type of release enhancer used, its addition led to a significant increase ($p < 0.05$) in the diameter of the spread circle of the gel.

3.2.3. *pH and particle size of the prepared formulae*

pH values of the prepared clotrimazole formulae ranged from 5.28-6.05 which is decreased from the pH of the plain base to some extent (Table 3). This pH range allows maximum stability for all release enhancers used and is also suitable for skin.

Relevant to determination of particle size measurement, the mean particle size diameter value ranged from 0.985-6.319 μm where formula F_7 showed the lowest value and F_1 showed the highest value (Table 3).

3.2.4. *Rheological measurements and data analysis*

The rheological parameters of the prepared formulations, namely, the flow index (*n*) and the consistency index (η) are shown in Table 4, while their rheograms are shown in Figure 3. Based on the

Table 3. Physical parameters of clotrimazole gels

Formulae	Drug content (%)	Spreadability (cm)	pH	Particle size (μm)
F_1	98.9	2.3	6.05	1.755 ± 0.87
F_2	99.1	3.3	5.89	4.232 ± 1.548
F_3	99.6	3.1	5.55	1.637 ± 0.669
F_4	99.3	3.0	6	2.577 ± 0.896
F_5	99.0	3.2	6	1.581 ± 0.939
F_6	100.5	3.1	6.02	5.843 ± 2.453
F_7	100.4	4.8	6	1.441 ± 0.514
F_8	100.2	3.9	5.28	2.104 ± 1.233
F_9	101.3	4.3	5.97	1.291 ± 0.753
F_{10}	100.9	3.8	5.92	6.319 ± 2.506
F_{11}	101.1	3.4	5.91	0.985 ± 0.357
F_{12}	99.7	2.9	5.67	1.465 ± 0.684
F_{13}	99.4	2.6	5.91	0.241 ± 0.139
F_{14}	99.8	3.0	5.64	1.289 ± 0.641

values of *n*, calculated after the power law equation and which were all < 1 as shown in Table 4, it could be confirmed that all the formulations exhibited shear thinning behavior. This is a desirable property in a topical semisolid preparation, since it should thin during application (*38*). The cause of pseudoplastic flow revealed by gels may be due to progressive rupture of the internal structure of the formulations and its later reconstruction by means of Brownian movement (*38,39*).

It is obvious that increasing all the penetration enhancer concentrations led to a decrease in the flow index values. This result was explained by the formation of full structured three dimensional polymer lattices due to increased enhancer concentration (*40*). Also, this effect may be attributed to the plasticizing effect of these compounds. The plasticizer would have the ability to weaken polymeric intermolecular attractions, thus allowing polymer chains to move more readily, improving the flexibility of the polymer. Consequently, the higher relaxation and mobility of the chains provoke greater entanglement and increase gel viscosity (*41*).

An important rheological parameter is the apparent viscosity of the gels. It is related to mechanical and physical properties such as spreadability, consistency and hardness of the preparation which in turn are related to ease of product removal from container, ease of application on the skin surface and product feel on the application site.

3.2.5. *In vitro release and effect of some penetration enhancers on drug release*

The percent of clotrimazole released after 6 h, apparent diffusion coefficient, permeability coefficient, and partition coefficient were represented in Table 5 and Figures 4-6. It is clear that 20% pluronic F_{127} gave a very low percent of clotrimazole release after 6 h (34.41%). This may be due to the fact that pluronic F_{127} gels consist of a large population of micelles and the drug is released by diffusion through extra micellar aqueous channels of gel matrix (*12,42-44*).

The addition of hydrophilic or lipophilic additives into pharmaceutical formulations in order to modulate

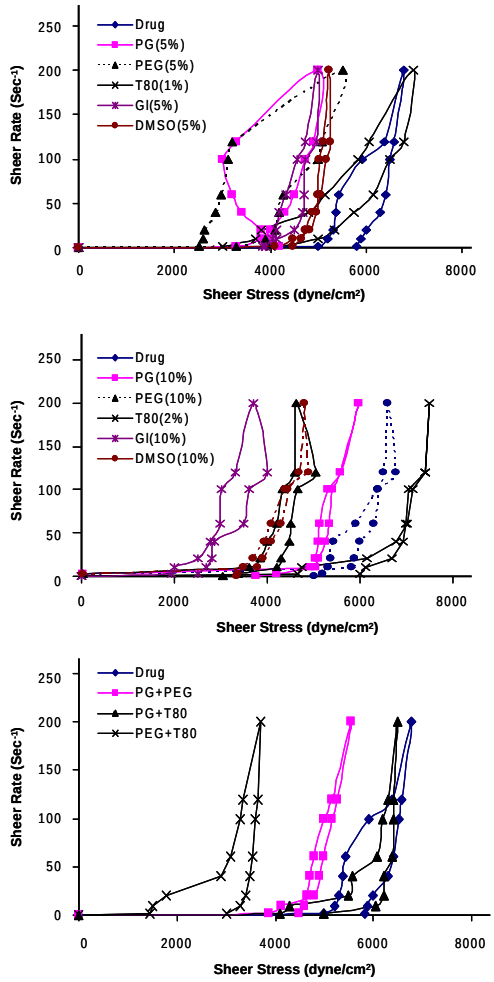

Figure 3. Rheogram of clotrimazole from 20% pluronic F_{127} gels containing different release enhancers. PG, propylene glycol; PEG, polyethylene glycol 400; T80, polysorbate 80; Gl, glycerin; DMSO, dimethyl sulphoxide.

Table 4. Rheological parameters of clotrimazole gels

Formulae	Consistency index (η)	Flow index (*n*) at shear rate 120
F_1	4,500	0.066
F_2	3,800	0.048
F_3	3,200	0.087
F_4	4,000	0.100
F_5	3,800	0.050
F_6	4,220	0.036
F_7	4,600	0.030
F_8	3,814	0.038
F_9	5,963	0.041
F_{10}	2,555	0.048
F_{11}	3,722	0.031
F_{12}	4,350	0.037
F_{13}	4,730	0.054
F_{14}	1,390	0.166

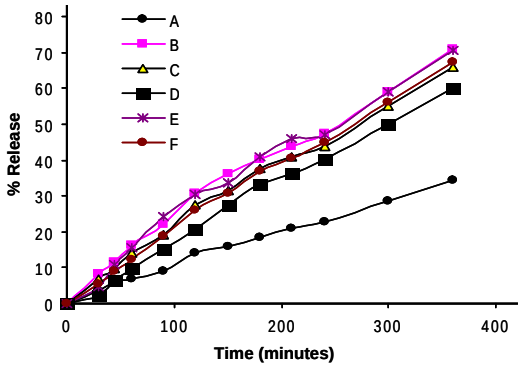

Figure 4. Release profiles of clotrimazole from 20% pluronic F_{127} gels containing different release enhancers. A, drug; B, propylene glycol (5%); C, polyethylene glycol 400 (5%); D, polysorbate 80 (1%); E, glycerin (5%); F, dimethyl sulphoxide (5%).

the release of drugs is most common. Polarity produced by the additives is an important factor that affects drug release. The higher the water solubility of the additives the greater the enhancement of dissolution of the formulation and thereby, the higher rate of drug release and *vice versa* (*45*). In this study, all the additives used, namely, propylene glycol, polyethylene glycol, glycerin, and dimethyl sulfoxide in concentrations of 5 and 10% (w/w) and also polysorbate 80 in concentrations of 1 and 2% (w/w), showed significant enhancement of the release of clotrimazol in comparison with the additives free base, but to a variable extent. The higher release was obtained from formula (F_7) containing 10% propylene glycol (86.94%) after 6 h in comparison with other additives or their combination, followed by formula (F_9) containing 2% polysorbate 80 (80.415%) and then formula (F_8) containing 10% polyethylene glycol (73.53%).

It has been reported that the addition of polyethylene glycol 400, propylene glycol, and glycerin (in concentrations ranging from 2.5% to 10%) into different polymer bases including pluronic F_{127} significantly enhanced drug release rate. This was related to the high water solubility of glycols where they readily absorb water and therefore speed up the hydration rate and so the dissolution rate of the polymer into which these hydrophilic agents are incorporated (*46-49*). The high solubility of these polyhydroxy compounds resulted also in a decrease of the resistance to drug diffusion by increasing the formulation porosity following their dissolution. These results were in accordance with Korsmeyer *et al.* and Jones *et al.* (*50,51*).

A further advantage of these polyhydroxy compounds is that they act as humectants, to prevent drying and crusting of the vehicles (*52*).

From the results, it is clear that addition of 10% propylene glycol gave the highest release pattern. This is because propylene glycol may change the microviscosity of the base (*47*).

Addition of the non-ionic surfactant polysorbate 80 to pluronic F_{127} gels has also enhanced the release rate which could be attributed to increasing the number and dimensions of the aqueous channels available for drug diffusion, thus increasing the effective porosity

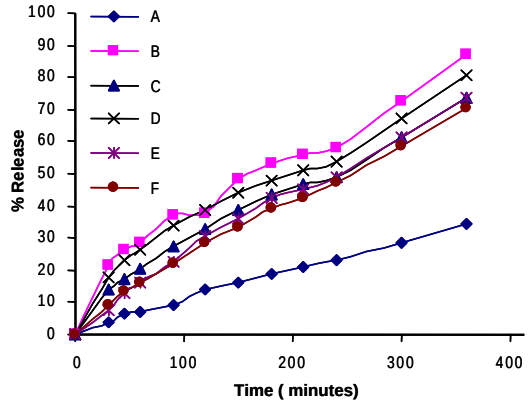

Figure 5. Release profiles of clotrimazole from 20% pluronic F_{127} gels containing different release enhancers. A, drug; B, propylene glycol (10%); C, polyethylene glycol 400 (10%); D, polysorbate 80 (2%); E, glycerin (10%); F, dimethyl sulphoxid (10%).

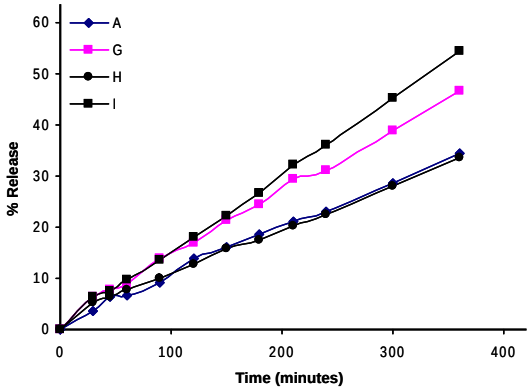

Figure 6. Release profiles of clotrimazole from 20% pluronic F_{127} gels containing different release enhancers. A, drug; G, propylene glycol:polyethylene glycol (5% + 5%); H, propylene glycol:polysorbate 80 (5% + 1%); I, polyethylene glycol:polysorbate 80 (5% + 1%).

Table 5. Effect of different penetration enhancers on the *in vitro* release characteristics of clotrimazole from pluronic F_{127} gels

Formulae	Order of reaction	% clotrimazole released after 6 h	Dapp ($\times 10^{-3}$ µg•cm^2•h^{-1})	P_m ($\times 10^{-3}$ cm•h^{-1})	K_p
F_1	zero	34.41	14.91	10.59	0.1419
F_2	zero	71	9.45	20.90	0.4425
F_3	zero	65.93	8.63	19.20	0.4450
F_4	zero	60.133	13.24	18.30	0.2764
F_5	zero	70.65	6.55	19.80	0.6047
F_6	zero	67.38	14.33	20.65	0.2882
F_7	zero	86.94	4.92	23.11	0.9391
F_8	zero	73.53	6.35	20.48	0.6449
F_9	zero	80.415	5.97	22.16	0.74198
F_{10}	zero	73.53	8.59	21.37	0.4979
F_{11}	zero	70.64	12.78	21.43	0.3354
F_{12}	zero	46.73	15.99	14.48	0.1811
F_{13}	zero	33.69	26.56	10.73	0.0808
F_{14}	zero	54.34	19.28	18.25	0.0189

Dapp, apparent diffusion coefficient; P_m, permeability coefficient; K_m, partition coefficient; H, membrane thickness; K_p, Pm × H/Dapp.

of the gel matrix. Also, polysorbate 80 has emulsifying properties due to increasing formation of intrapolymeric micelles which decrease interpolymeric connections allowing enhanced drug release. This was in accordance with what was reported by Hansson and Lindman (*53*).

Addition of dimethyl sulfoxide at concentrations of 5 and 10% to pluronic F_{127} gels also enhanced the release rate. The most enhanced effect was found to be dependent on the additive concentration (*54*).

Kinetic analysis of release data have shown that by trying the three different models, the highest regression coefficient was always obtained with a zero order release model. This result was previously found by Wang *et al.* and Paavola *et al.* (*55,56*).

3.3. *Stability study on selected clotrimazole gels*

No significant change ($p > 0.05$) in the measured parameters (spreadability, rheological behavior, and *in vitro* clotrimazole release) were noticed after 6 months of storage, indicating high physical stability of all clotrimazole gels under study.

3.4. *Evaluation of antimicrobial activity*

Clotrimazole, which is an imidazole derivative antifungal agent, was widely used for treatment of mycotic infections of the genitourinary tract (*57*).

The results presented here confirm the earlier reports of Plempel *et al.* (*3,4*) regarding the *in vitro* activity of clotrimazole against pathogenic fungi. This compound is, indeed, a "broad spectrum" antifungal agent; its inhibitory action is seen against systemic and opportunistic pathogenic fungi (Figure 7).

Antifungal activities of plain gels and standing solutions were determined using the pour diffusion method described by CLSI 2006 (*30*). In this process, the clear zone of inhibition indicated the sensitivity of the organism to the chemical agents (*58*). *In vitro* antifungal activity of clotrimazole was carried out by the agar diffusion method. Table 6 shows the antifungal activity of clotrimazole in propylene glycol (10%, w/w), polyethylene glycol 400 (10%, w/w) and polysorbate 80 (2%, w/w) as well as in drug form.

The effect of gel against the tested organisms was estimated by the width of the inhibition zone. The clotrimazole plain form exhibited the highest activity against *Aspergillus flaves* with an inhibition zone of 46 mm. On the other hand, clotrimazole gels with release enhancer propylene glycol (10%, w/w) showed antifungal activity against all the tested microorganisms with a zone inhibition of nearly 30 mm. In the case of clotrimazole gels with release enhancer polyethylene glycol 400 (10%, w/w), it was found to be effective against *A. niger*, *A. flaves*, *C. albicans*, and *S. cerevisiae* with inhibition zones of 39, 39, 35, and 32 mm, respectively.

Table 6. Antifugal activity of coltrimazole gels

Organisms tested	Inhibition zones for fungi (mm)[a]				
	PD	PG	PEG	P	SC
Aspergillus flaves	46	44	39	37	40
Aspergillus niger	37	38	39	35	32
Candida albicans	30	35	35	31	42
Saccharomyces cerevisiae	32	30	32	28	32

[a]Diameter of inhibition zones from triple readings. Abbreviations: PD, plain drug; PG, propylene glycol (10%, w/w); PEG, polyethylene glycol 400 (10%, w/w); P, polysprbate 80 (2%, w/w); SC, standard clotrimazole.

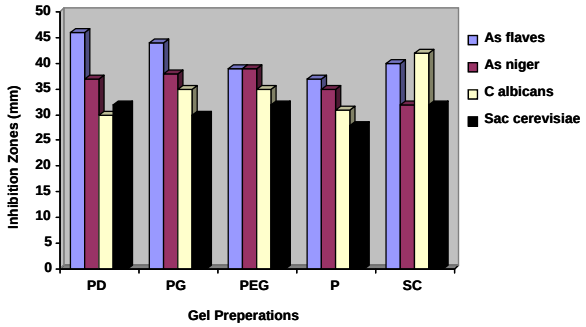

Figure 7. Antifungal activity of tested preparations. PD, plain drug; PG, propylene glycol (10%, w/w); PEG, polyethylene glycol 400 (10%, w/w); P, polysprbate 80 (2%, w/w); SC, standard clotrimazole.

Clotrimazole inhibits biosynthesis of the sterol ergosterol, an important component of fungal cell membranes. Its action leads to increased membrane permeability and apparent disruption of enzyme systems bound to the membrane, resulting in leakage and loss of essential intracellular compounds, and eventually cell lysis.

4. Conclusion

The solubility of clotrimazole was significantly increased in the presence of PF_{127}. The micellar solubilization was an energetically favored, spontaneous process resulting in a lower thermodynamic orderliness. The best release enhancers were propylene glycol and polyethylene glycol 400 at a concentration of 10% and polysorbate 80 at a concentration of 2%. The formulae containing it were physically stable and proved to be effective against *A. niger*, *A. flaves*, *C. albicans*, and *S. cerevisiae* with inhibition zones of 39, 39, 35, and 32 mm, respectively. Therefore, it was concluded that these formulae could be a very promising topical alternative for the treatment of skin fungal infections.

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