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Research Article

FORMULATION AND EVALUATION OF FLUCONAZOLE TOPICAL GEL

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ABSTRACT

Fluconazole is an imidazole derivative used for the treatment of local and systemic fungal infection. The oral use of fluconazole is not recommended as it has many side effects. The present study was designed to formulate and evaluate different formulae of topical gel containing fluconazole for treatment of fungal infection of skin. The gel was formulated by using different polymers with different concentration as Carbopol 940, Hydroxypropyl methylcellulose E4M, Methyl cellulose, Pectin and Pluronic P407. Ten different formulae were prepared and characterized physically in term of color, syneresis, spreadability, pH, drug content and rheological properties. Drug-excipients compatibility studies were confirmed by carrying out DSC and FT-IR. In-vitro drug release in phosphate buffer pH 5.5 and permeation study through cellulose membrane, using a modified Franz diffusion cell, were performed. Candida albicans was used as a model fungus to evaluate the antifungal activity of the prepared formulae achieved using Nizoral® cream as control. The results of in vitro drug release and its permeation studies showed that the highest values was from F3(91.3% of drug released after 2 hr). Also F3 shows the highest antifungal activity. The rheological behavior of the prepared formulae showed shear-thinning flow indicating structural breakdown of the existing intermolecular interactions between polymeric chains. Moreover, the stability study revealed no significant difference between before and after storage for selected formula.

INTRODUCTION

Topical preparations are formulae which are applied directly to an external body surface by spreading, rubbing, spraying or instillation[1].The topical route of administration has been utilized either to produce local effect for treating skin disorder or to produce systemic drug effects. Within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations[2].

Gels often provide a faster release of drug substance, independent of the water solubility of the drug, as compared to creams and ointments[3]. They are highly biocompatible with a lower risk of inflammation or adverse reactions, easily applied and do not need to be removed[4].

Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removed, emollient, non-staining, compatible with several excipients and water soluble or miscile[5-6].

Fungal infections traditionally have been divided into two distinct classes: systemic and superficial. Consequently, the major antifungal agents are classified into systemic and topical drugs[7]. Antifungal drugs are classified according to their chemical structure as: polyene antifungals, azole antifungals, allylamine antifungals, echinocandin antifungals and others[8].

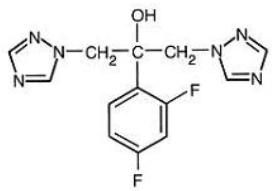

Fig. 1: Shows the chemical structure of fluconazole

Fluconazole is a synthetic antifungal agent belonging to the group of triazole.it is one of the commonly used antifungal agents for most kinds of fungal infections including superficial and invasive fungal infections[9] Fluconazole differs markedly from other imidazole in its pharmacokinetic properties. The presence of two triazole rings (bis-triazole) makes this compound less lipophilic and more hydrophilic when compared with other azoles antifungal agents. The presence of halogenated phenyl ring increases its antifungal activity[10]. Fluconazole is available commercially as tablets and injections only in spite of its well known adverse effects including nausea, vomiting, bloating and abdominal discomfort. In order to bypass these disadvantages, the gel formulations have been proposed as topical application.

The goal of our research to formulate and evaluate various polymers with varying concentrations for the preparation of a safe, effective and stable gel containing Fluconazole and evaluate the in-vitro performance, stability and also evaluate the in-vitro antifungal activity for prepared formulae.

MATERIALS AND METHODS

Materials

Fluconazole was kindly gifted from Egyptian International pharmaceutical industries company, Cairo, Egypt. carbopol 940, pluronic F-127 were obtained from Goodrich (USA). methyl cellulose(MC), hydroxypropylmethyl cellulose (HPMC), pectin, methyl and propyl paraben were from El-Nile pharmaceutical company,Cairo, Egypt. glycerin, propylene glycol, triethanolamine, disodium hydrogen phosphate and potassium dihydrogen phosphate were purchased from EL-Nasr chemical company, Cairo, Egypt.

Methods

Preparation of Fluconazole topical gels

The composition of fluconazole topical gel formulae are shown in table 1. Fluconazole(1% w/w) was dissolved in a hot mixture containing propylene glycol (20% w/w)and glycerin (10% w/w) as moistening agent [11].

Polyacrylic acid polymer (carbopol 940), cellulose polymers (HPMC, MC), polysaccharide polymer (Pectin) gel were prepared by dispersing the calculated amount of polymer in calculated amount of warm water with constant stirring using magnetic stirrer at a moderate speed. Then add the previous mixture containing the drug. The pH of carbopol gel was adjusted using TEA. While polymer undergoing transition (Pluronic)[12-14] was dispersed slowly in cold water 4ºC with constant stirring according to cold technique[15-16]. Finally methyl and propyl paraben as preservatives were added slowly with continuous stirring until gel formation. The prepared gels were packed in wide mouth glass jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in dark and cool place [17].

Table 1: Shows composition of Fluconazole topical Gel (% w/w)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Fluconazole	1	1	1	1	1	1	1	1	1	1
Carbopol 940	**0.5**	**1**	—	—	—	—	—	—	—	—
HPMC	—	—	**1.5**	**2**	—	—	—	—	—	—
Methyl Cellulose	—	—	—	—	**2**	**4**	—	—	—	—
Pectin	—	—	—	—	—	—	**3**	**4**	—	—
Pluronic F127	—	—	—	—	—	—	—	—	**15**	**18**
Glycerin	10	10	10	10	10	10	10	10	10	10
Propylene Glycol	20	20	20	20	20	20	20	20	20	20
Methyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified water to	100	100	100	100	100	100	100	100	100	100

Physicochemical Evaluation of Prepared Fluconazole Gels

Drug-Excipients Compatibility Studies

A-Differential scanning calorimetry (DSC)

The DSC studies were performed for the drug, the polymers and the drug-polymer physical mixtures in the ratio 1:1. The samples (3-4 mg) were inserted in aluminum pan and heated in the rate of 10ºC /min, to a temperature of 200ºC using a differential scanning calorimeter (TA-501; shimadzu corporation, Japan).Results are shown in figures(2-6).

B- Fourier Transfer Infrared spectrophotometer (FTIR)

The FTIR studies were carried for the drug, the polymers and the drug-polymer physical mixture in the ratio 1:1 were mixed separately with IR grade KBr in the ratio of (100:1) and corresponding discs were prepared by applying 5.5 metric ton of pressure in a hydraulic press using FTIR Spectrophotometer (Genesis II, Mattson, England). The disks were scanned over a wave number range (4000 - 400cm).

Visual examination

All developed gel formulae were inspected for their homogeneity[18], color; syneresis and presence of lumps by visual inspection after the gels have been set in the container.

Spreadability test

A sample of 0.5 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected[19]. Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations. Results are shown in table 2.

pH determination

The pH of the gels was determined using digital pH meter[20] (3310, Jenway, UK).The readings were taken for average of 3 times. Results are shown in table 2.

Drug Content determination

A specific quantity of developed gel was taken and dissolved in 100ml of phosphate buffer of pH 5.5. The volumetric flask containing gel solution was shaken for 2hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered using Millipore filter (0.45µm). After suitable dilution drug absorbance was recorded by using UV- visible spectrophotometer (UV – 1700, Shimadzu, Japan) at λ_{max} 260 nm using phosphate buffer (pH 5.5) as blank .Results are shown in table 2.

Rheological Studies

The viscosity of the different gel formulae was determined at 25°C using rotational Brookfield viscometer of cone and plate structure with spindle CPE-41 and CP-52[21]. The apparent viscosity was determined at shear rate 40 sec^{-1}. The flow index was determined by linear regression of the logarithmic form of the following equation:

$\tau = k \gamma n$……Equation (1)

Where "τ" is the shear stress, "γ" is the shear rate, k is the consistency index, and n is the flow index. When the flow is Newtonian n=1, if n>1 or n<1, shear thickening or shear thinning is indicated, respectively. Evaluation was conducted in triplicate. The entire rheograms are shown in Figures (7-11).The flow behavior is shown in table 3.

In Vitro Release Studies

The study was carried out using (Varien dissolution tester, model VK 7010,with an auto sampler unit VK 8000, USA).One gram of fluconazole was placed in the watch glass covered with aluminum mesh. The watch glass was then immersed in the vessel containing 500 ml of the release medium, phosphate buffer pH 5.5 at 37ºC ± 0.5 ºC with a paddle speed of 50 rpm. Aliquots (5ml) were withdrawn at specified time intervals every 10 minute over 2 hours and immediately replaced with fresh dissolution medium. The samples were assayed spectrophotometrically at λmax 260 nm and the concentration of the drug was determined from the previously constructed calibration curve. Experiments were carried out in triplicates, the results were averaged and blank experiments were carried using plain bases. Results are shown in Fig. 12.

Drug Release Kinetic Study

The data obtained from the *in vitro* release experiments were analyzed using linear regression method according to the following equations:

a- Zero – order equation:

$Q = k_o t$

Where Q is the amount of drug released at time t, and k_0 is the zero – order release rate.

b- First – order equation:

$In (100 – Q) = In\ 100 – k_1 t$

Where Q is the percent of drug release at time t, and k_1 is the first – order release rate constant.

c- Higuchi's equation:

$$Q = k\ t^{1/2}$$

Where Q is the percent of drug release at time t, and k is the diffusion rate constant[22]

Results were tabulated in table 4**.**

In-vitro Drug Diffusion Study

Cellulose membrane (0.45µm, obtained from sigma chemicals) was used for this study. A sample of 1g of the preparation was spreaded on a cellulose membrane previously soaked overnight in the release medium. The loaded membrane was firmly stretched over the edge of a glass tube of 2 cm diameter; the membrane was tied up with a rubber to prevent leakage [23,24] .Tubes were then immersed in the dissolution vessel which contained 50 ml of the release medium, phosphate buffer pH 5.5, and maintained at 37ºC ± 0.5ºC[25]. The shafts were rotated at 50 rpm and aliquots each of 3 ml were withdrawn from the release medium at specified time intervals. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed spectrophotometrically at λmax

260 nm and the concentration of the drug was determined from the previously constructed calibration curve. Each data point represented the average of three determinations. *In vitro* release studies were recorded for a four hour period. Previous solubility tests were made so as to ensure sink conditions for drug dissolution in the donor medium. The flux, lag time and permeability coefficients of fluconazole through synthetic membrane are shown in table 5.

Antifungal study

The prepared formulae were tested in a triplicate manner using agar cup method against Candida albican strain. Cup of 10 mm in diameter were made aseptically in Sabouraud dextrose agar after being inoculated with tested fungal suspension strain by spreading on the agar surface. The cups were filled with each prepared formula by sterile syringe. Then the zone of inhibition of each cup was observed and calculated the radius of the zone of inhibition and compared to the control formula Nizoral ®cream. Results are shown in table 6**;** Fig. 13**.**

Stability studies

The stability study was carried out for the most satisfactory formulation. The most satisfactory formulations were packed in a collapse tubes and stored at room temperature for 6 months. At the end of each month the samples were analyzed for their physical properties, spreadability, pH, the drug content, rheological properties, drug release and antifungal activity by procedure stated earlier.

RESULTS AND DISCUSION

Topical and transdermal drug delivery systems offer several advantages over oral delivery systems. However it has been found so many side-effects were proved by the oral delivery system of fluconazole and here to over the side-effects of oral dosage form. The oral dosage form has been changed by formulation and evaluation of fluconazole topical gel.

Drug-Excipients Compatibility Studies

Any formulation development work has to be proceeded by preformulation studies. This preformulation study includes drug-excipients compatibility deliberate by DSC and FT-IR analysis. FT-IR study showed that there was no major change in the position of peak obtained in the drug alone and in a mixture of drug with excipients, which shows that there was no interaction between drug and excipients.

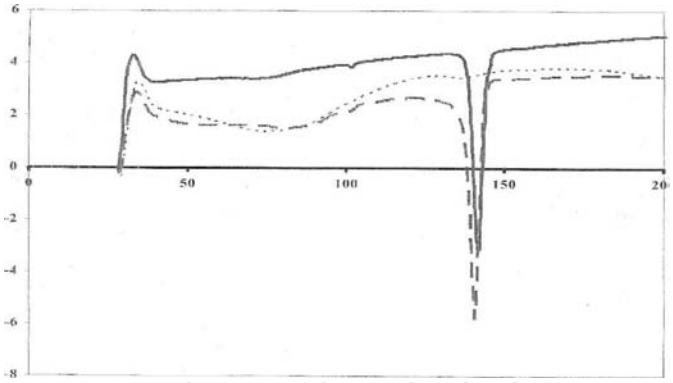

Fig. 2: Shows DSC of Fluconazole,carbopol 940

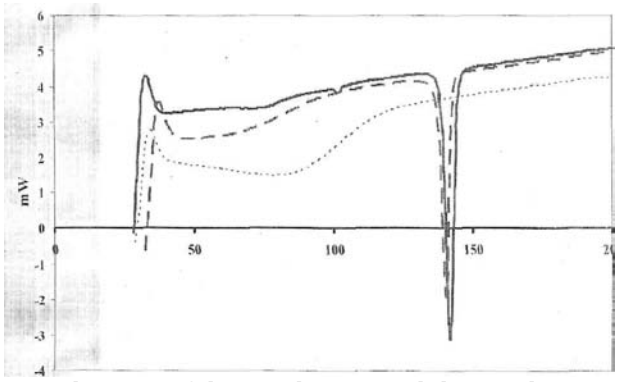

Fig. 3: Shows DSC of Fluconazole, HPMC and Fluconazole-cp 940 Fluconazole-HPMC

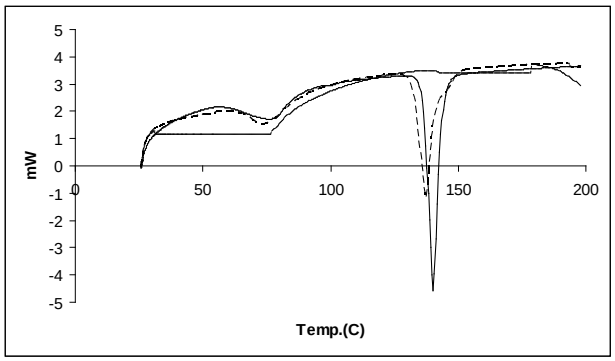

Fig. 4: Shows DSC of Fluconazole, MC

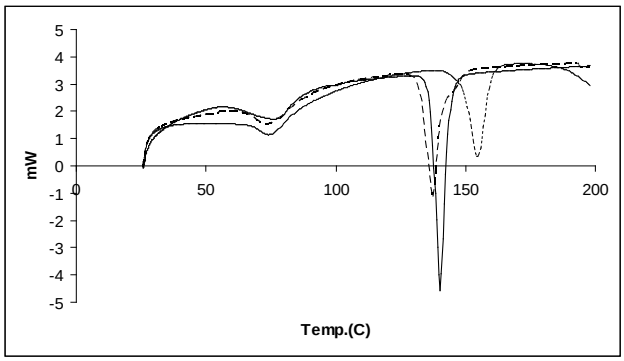

Fig. 5: Shows DSC of Fluconazole, Pectin and Fluconazole-MC Fluconazole-pectin

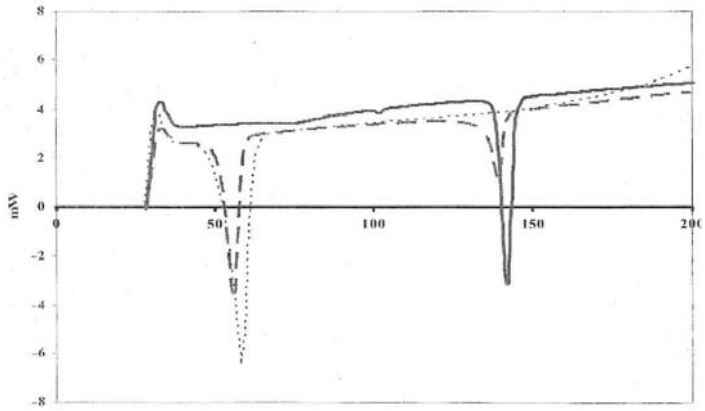

Fig. 6: Shows DSC of Fluconazole,Pluronic P-407 andFluconazole-p luronic

In addition, it is remarked that the DSC thermogram of fluconazole is characterized by one sharp endothermic peak at about 139.08 ºC which correspond to the melting point of the drug[26]. It is clear that there is no change in the position of the characteristic peak of the drug in all the physical mixtures with polymers. That indicates that there was no interaction between fluconazole and all polymers used in the preparation of gels. Results are shown in figures (2-6).

Evaluation of Fluconazole Topical Gel

Visual examination

The prepared gel formulae were inspected visually for their color and syneresis. The developed preparations were much clear and transparent except pectin gel is buff, opaque. All developed gel formulae showed good homogeneity with absence of lumps and syneresis. Results are shown in table (2)

Spreadability

The spreadability is very much important as show the behavior of gel comes out from the tube. The values of spreadability shown in table (2) indicate that all the polymers used gave gels spread by small amount of shear. The diameters of the spreaded circles ranged from 3 cm seen with the Pluronic F127gel and 5 cm seen with carbopol and HPMC gel. Data in table (2) revealed that increasing the concentration of any of the gelling agents was always associated with a decrease in the spreadability as expressed by the lower diameter of the spreaded circle.

pH Determination

The pH values of all developed formulae was in range 5-6 which is considered acceptable to avoid the risk of irritation upon application to the skin.[27,28] with the exception of pectin gel;pH was about 3.5; results are tabulated in table (2).

Drug Content determination

Results of drug content are shown in table (2). After various formulation of fluconazole gel the drug content of the formulated gel was estimated and the results were in the official limits with range of 9.5 to 9.99 mg/gm gel**.** The drug content determination also showed that the drug was uniformly distributed throughout the gel.

Table 2: Shows the Physical Properties of Fluconazole Topical Gels

Topical Gels	Color	Synerisis	Spredablity (cm)	pH	Drug content (mg/gm gel)
F 1	Shiny transparent	-ve	4.5	6.1	9.55
F 2	Shiny transparent	-ve	4	5.99	9.7
F 3	transparent	-ve	5	5.60	9.99
F 4	transparent	-ve	5	5.67	9.78
F 5	Translucent yellowish	-ve	4.5	6.1	9.99
F 6	Translucent yellowish	-ve	3.6	6.13	9.7
F 7	Opaque ,buff	-ve	5	3.6	9.7
F 8	Opaque ,buff	-ve	3.5	3.7	9.98
F 9	transparent	-ve	3.6	6.22	9.93
F 10	transparent	-ve	3	6.3	9.89

Table 3: Shows the rheological properties of Fluconazole Topical Gels

Formula №	Coefficient of determination (R^2)	Flow Index (n)	Viscosity* (centipoise) (η)	Flow Behavior
F 1	0.916	0.2384	1709	shear thinning
F 2	0.9291	0.2350	1918	shear thinning
F 3	0.9976	0.2251	1012	shear thinning
F 4	0.9823	0.21031	1036	shear thinning
F 5	0.908	0.2307	1449	shear thinning
F 6	0.908	0.2307	2083	shear thinning
F 7	0.9304	0.1214	1247	shear thinning
F 8	0.9819	0.1436	2289	shear thinning
F 9	0.9375	0.1390	2441	shear thinning
F 10	0.9459	0.1428	3261	shear thinning

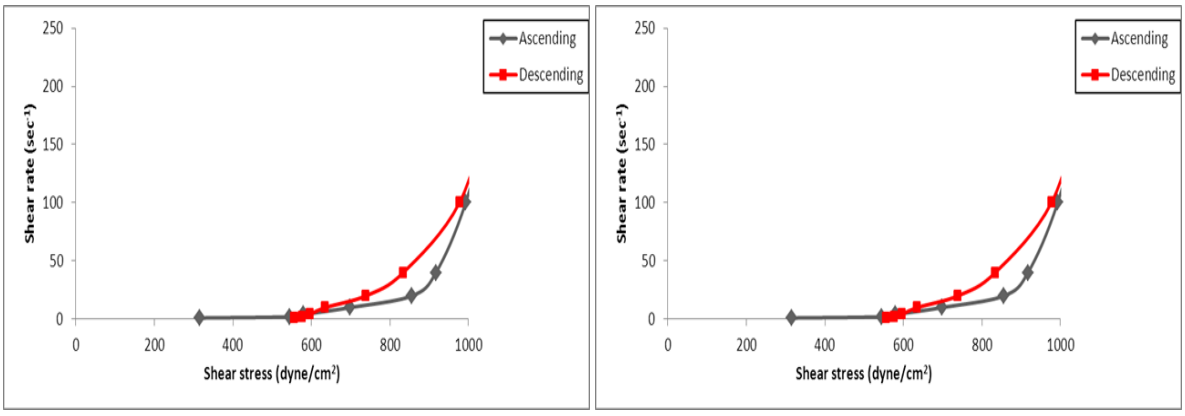

Fig. 7: Show rheograms of Carbopol 940 topical gel (A): F-1, (B): F-2.

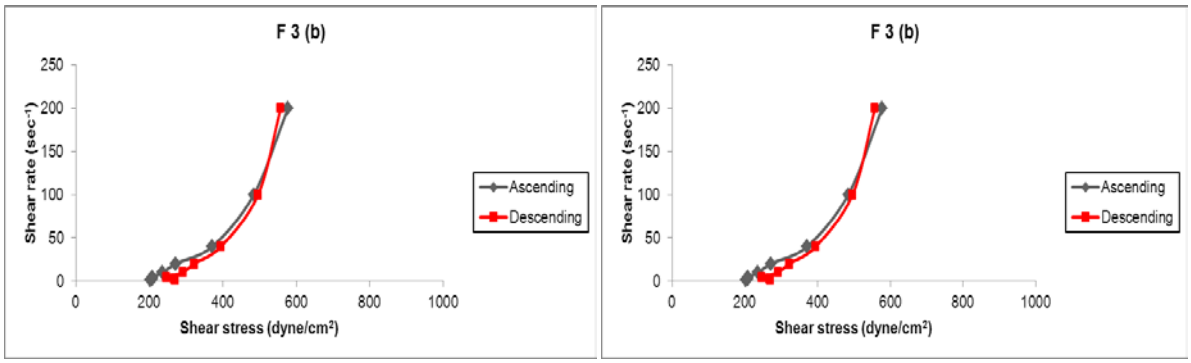

Fig. 8: Show rheograms of HPMC topical gel (A): F-3, (B): F-4.

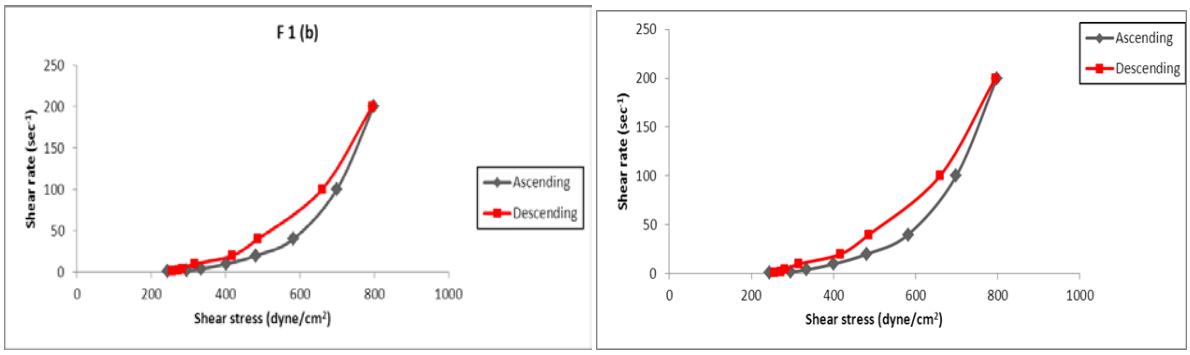

Fig. 9: Show rheograms of MC topical gel (A): F-5, (B): F-6.

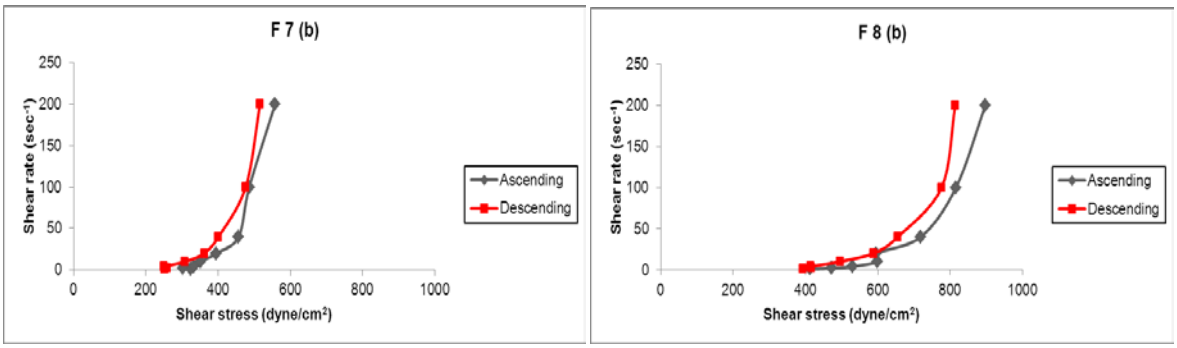

Fig. 10: Show rheograms of Pectin topical gel (A): F-7, (B): F-8.

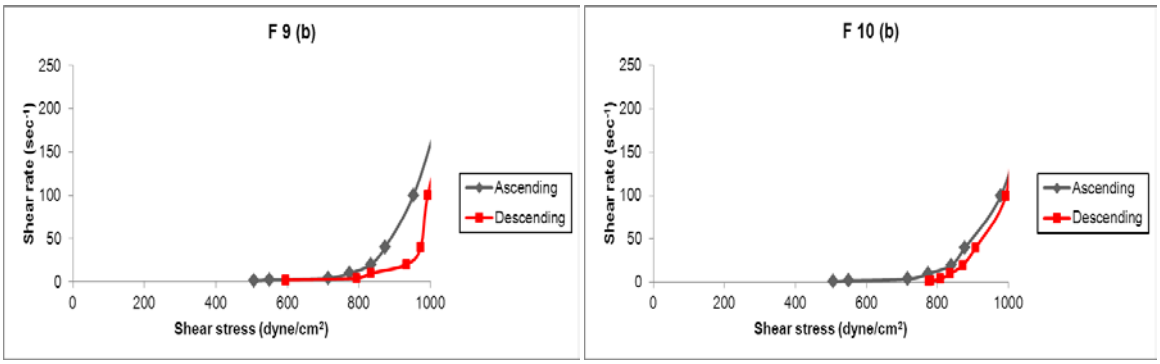

Fig. 11: Show rheograms of Pluronic topical gel (A): F-9, (B): F-10.

In-Vitro Release Studies

The In-Vitro release profile of fluconazole topical gel formulae was represented in Fig. (12). It was observed that the release of the drug from its different formulae can be ranked in the following descending order: F 3>F 4>F 5>F 7>F 9>F 8>F 1>F 10>F 2>F 6; where the amounts of the drug released after 2 hours were 91.3%, 89.6%, 84.5%, 82.3%, 79.8%, 77.6%, 73.6%, 73%, 69.5% and 58.6% respectively. It was observed that the most influenced factor in the drug release is polymer type followed by the concentration of the polymer.

Drug Release Kinetic Study

The release data analysis was carried out using the various kinetic models i.e using cumulative % drug release vs. time (zero order

kinetic model); log cumulative % drug remaining vs. time (first order kinetic model) and cumulative % drug release vs. square root of time (Higuchi model) [32-34]. The R^2 values are tabulated in table 4. All formulae showed best fitting to Higuchi model kinetics.

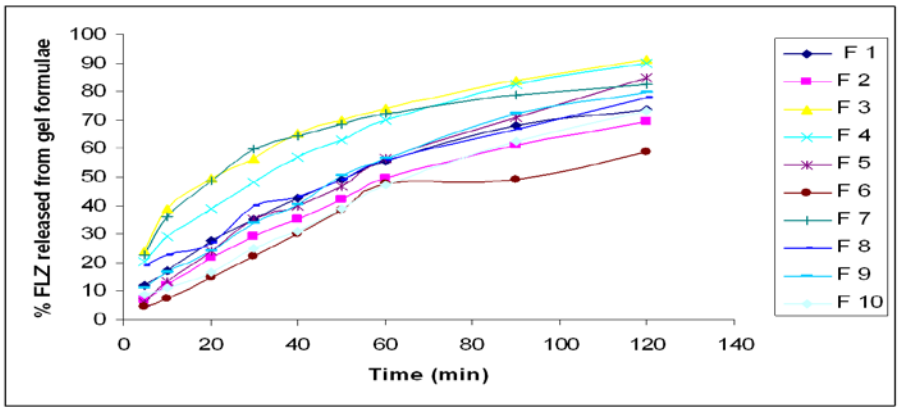

Fig. 12: Shows Release profile of Fluconazole from its gel formulae.

Table 4: Shows the Kinetic study of the In vitro release data of fluconazole from its different formulae.

Formula №	Correlation Coefficient(R^2)		
	Zero order	First order	Diffusion
F1	0.9339	0.9896	0.9929
F2	0.954	0.9958	0.9960
F3	0.8727	0.9722	0.9938
F4	0.9268	0.9928	0.9994
F5	0.9651	0.9893	0.9975
F6	0.903	0.9438	0.9649
F7	0.7823	0.9255	0.9255
F8	0.9666	0.9876	0.9950
F9	0.9593	0.9905	0.9964
F10	0.9833	0.9774	0.9935

In-vitro Drug Diffusion Study

The results of *in vitro* permeation studies of topical gel formulae across cellulose membrane are shown in table 5. The amount of fluconazole released from all gel formulae show a linear relationship with the square root of time (r >0.9). The cumulative amounts permeated at 4 hrs were 220.63, 246.2, 174.23, 243.8, and 144.44µg/cm^2/h for F1, F3, F5, F7, and F9, respectively. The vehicle composition can affect drug release and skin permeability properties[35, 36]. These results suggest that F 3 is effective for topical application as highest percentage of the applied drug permeated through the human epidermis after 4 hours.

Antifungal study

The antifungal activity of fluconazole from its different gel formulae compared with Nizoral® cream as control revealed in table (6) and Fig. (13). The antifungal activity was determined by measuring the inhibition zone. The results of all formulae were satisfactory as the greatest activity was observed with F3 where the inhibition zone reaches 37mm, while the lowest activity was found with F2 where the inhibition was 19 mm. These results are in agreement with the results obtained from the in vitro release study. This indicates good correlation between the chosen dissolution model and the in vitro antimicrobial susceptibility testing.

Table 5: Show the In vitro Drug Diffusion Study of the Selected Topical Gels

Topical gel	Js(ug cm^{-2} hr^{-1})	P(cm hr^{-1})	K	r
F 1	220.63	0.022	36.94	0.9475
F 3	246.37	0.024	146.53	0.9999
F 5	174.23	0.017	6.76	0.99999
F 7	243.83	0.024	61.66	0.9865
F 9	144.44	0.004	-428.71	0.9945

Table 6: Shows the inhibition zone of the prepared topical gel formulae

Formula №	Inhibition zone(mm)
Nizoral® cream (CONTROL)	22
F 1	22
F 2	19
F 3	37
F 4	36
F 5	36
F 6	27.5
F 7	35
F 8	32
F 9	32.5
F 10	30

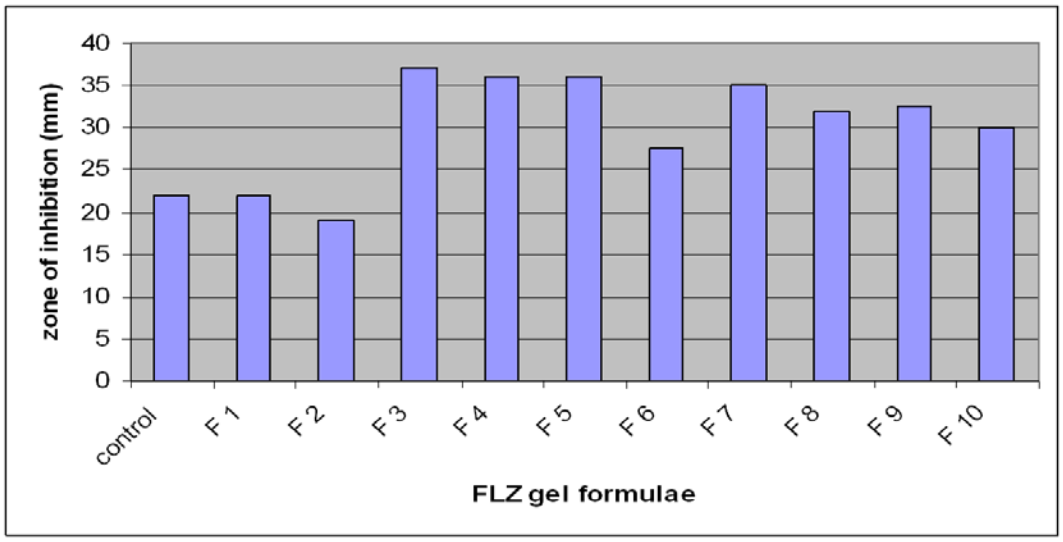

Fig. 13: Shows zone of inhibition of Fluconazole topical gel formulae

Stability Study

Entirely the prepared fluconazole gel formulae were found to be stable upon storage for 6 months at room temperature, where no significant change was observed in the parameters evaluated like physical appearance as color, syneresis, pH, rheological properties, drug release and antifungal activity.

CONCLUSION

On the basis of the previous findings we can concluded that Fluconazole was successfully incorporated into the different topical gel preparations. From among all the developed formulation the formula F 3 shows good spreadability, viscosity, drug release and antifungal effect. Therefore, it was concluded that our formulae could be very promising topical alternative for the treatment of skin fungal infections. However, further preclinical and clinical studies are required.

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