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Formulation and *In vitro* Evaluation of Fluconazole Topical Gels

Aml Mekkawy[1*], M. Fathy[1] and Sohair El-Shanawany[1]

[1]Faculty of Pharmacy, Assuit University, Assuit, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author AM managed the literature searches, carried out the experimental work, performed the statistical analysis, and wrote the first draft of the manuscript. Authors MF and SES designed the study, wrote the protocol and managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Topical drug delivery of fluconazole, an antifungal drug, in gel form was formulated to avoid the side effect of the oral route.
Study Design: In this study I prepare different formulation from different polymers and select the best formulation to undergo further antifungal and stability studies.
Place and Duration of Study: Faculty of Pharmacy, Department of Pharmaceutics, Assiut University, between May 2010 and July 2011.
Methodology: Different polymers; Sodium carboxymethyl cellulose, Sodium alginate, Carbopol 934P, Hydroxypropylmethyl cellulose, Pluronic F-127 and hydroxypropyl cellulose, were used. The compatibility of fluconazole and different gelling polymer was assessed through differential scanning calorimetry and infrared absorption spectroscopy. The influence of polymer type and concentration on fluconazole release from the prepared gels were studied. The prepared gel formulations were evaluated for pH, drug content, rheology, spreadability and in vitro drug release
Results: The rheological behavior of all the prepared gels showed a pseudoplastic flow (shear thinning) which is a good characteristic in the pharmaceutical gels. With the increase of the polymer concentration in the formulation, viscosity increased and in vitro release of fluconazole decreased. Among all the prepared formulations, 0.5% Carbopol 934P gel showed desired properties and exhibited the best fluconazole in vitro release

**Corresponding author: Email: dr_amola85@yahoo.com;*

that reaches 77% over a 3-hr period. This gel showed a good inhibition to the fungal growth against *Candida albicans* and *Trichophyton mentagrophyte* using cup plate method and also, showed good stability.
Conclusion: 0.5% Carbopol 934P / Fluconazole gel is a promising dosage form for the treatment of superficial fungal infections and could be used for further clinical studies.

Keywords: Fluconazole; solubility; hydrogels; polymers; calorimetry (DSC); rheology; in vitro release; kinetics; antifungal activity.

1. INTRODUCTION

Fungal infections are very common in human beings, especially in the tropical regions. Fungi produce a wide spectrum of human infections ranging from superficial skin infections affecting the outer layers of skin, hair, nails and mucous membranes to systemic infections (internal organ invasion) [1-3]. Fungal infections usually occur as a result of decrease in the natural human defenses due to either immunosuppressive diseases or immune suppressive agents and also in association with opportunistic heavy exposure to the fungus [4]. When fungi infect the skin surface, they invade the stratum corneum to avoid being shed from the skin surface by desquamation, so the management of the superficial fungal infection begins with topical agent that can penetrate the stratum corneum cells [5]. So the topical treatment is greatly valuable when there are no extensive lesions and is much favorable as it generates high local tissue levels [6]. Fluconazole (FLZ) as an azole derivative is a bis-triazole antifungal agent which inhibits the fungal lanosterol 14-α demethylase enzyme that converts lanosterol to ergosterol. According to adverse effects mentioned by Sweetman [7], fluconazole mainly affects the gastrointestinal tract including abdominal pain, diarrhea, flatulence, nausea and vomiting. Topical products for the treatment of dermatological diseases include a wide choice of vehicles ranging from solids to semisolids and liquid preparations including creams, gels, ointments, pastes, aerosols and solutions. Gel topical formulations offer better patient compliance [8]. Gel is easily spreadable, easily removable, emollient, non-staining and compatible with several excipients [9]. They are rheologically referred to as pseudo plastic (shear thinning) systems. A number of polymers are used to provide the structural network that is the essence of a gel system. Gel formulations show variation with the variability of polymer type and concentration which affect drug release and hence the formula quality which must be optimized. The present work aimed to formulate and evaluate topical fluconazole gel formulations using different polymers as sodium carboxymethyl cellulose (Na CMC), hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), sodium alginate (Na alginate), Carbopol 934P (Carb.934P) and Pluronic F-127 (Pl.F-127) in order to avoid the drawbacks of the oral administration of fluconazole and enhance the release of fluconazole from the prepared gels.

2. MATERIALS AND METHODS

2.1 Materials

Fluconazole (FLZ.) was kindly provided by SEDICO (Cairo, Egypt). Spectra/Por® dialysis membrane (12000 to 14000 molecular weight cut off) (Spectrum Laboratories Inc., USA). Sodium alginate (Na alginate) (Judex Laboratories Reagent, England). Sodium carboxymethyl cellulose (Na CMC) (30000 M.Wt.), potassium dihydrogen orthophosphate, triethanolamine (TEA), propylene glycol (PG), Calcium chloride ($CaCl_2$) (Adwic, EL-Nasr

Pharmaceutical Chemicals Co., Egypt). Hydroxypropylmethyl cellulose (HPMC), Pluronic® F-127 (Pl.F-127) (Sigma Chemical Co., USA). Carbopol 934-P (Carb.934P) (B.F.Goodrich Chemical Co., USA). Hydroxypropyl cellulose (HPC) (Kolmar Company, California, USA). *Candida albicans* No 11 & *Trichophyton mentagrophyte* No 5500 (supplied from Mycological center, Faculty of Science, Assiut University).

2.2 Solubility of Fluconazole

The solubility of fluconazole was performed to validate experimental conditions. It was determined in phosphate buffer pH 7.4 (release medium), water and mixture of water and propylene glycol (80:20% w/w) (vehicle used in gel preparation). An excess amount of the drug was added to a flask containing 10 ml of each solvent. Then the mixtures were agitated at 100 rpm in a thermostatically controlled shaking water bath (Gallenkamp, England) at 37± 0.5ºC for 24 hours, filtered through filter disk 0.8µ (Sartorious), diluted and measured spectrophotometrically at 261nm using an UV spectrophotometer (Jenway 6305, U.K) against a blank similarly treated.

2.3 Study of the Physicochemical Compatibility of Fluconazole and Gelling Polymers

Differential scanning calorimetry (DSC) and Infrared absorption spectroscopy (IR) measurements were performed for predicting any interaction between fluconazole and polymers used in preparing gel formulations.

2.3.1 Differential scanning calorimetry (DSC)

DSC measurements were done using differential scanning calorimeter (DSC-50; Shimadzu, Japan) calibrated with indium. DSC measurements were done for the powder of fluconazole, polymers (Na CMC, Na alginate, Carb. 934P, HPMC, Pl. F-127 and HPC) and physical mixture of drug and polymers (1:1). 3-5 mg sample was sealed in standard aluminum pan and heated over a temperature range of 20-200ºC. The thermograms were obtained at a constant increasing rate of 10ºC/min in a nitrogen flow rate 20 ml/min.

2.3.2 Infrared absorption spectroscopy (IR).

IR measurements were performed using Infrared Spectrophotometer (IR-470; Shimadzu, Japan) by the KBr disc method. The samples were ground, mixed thoroughly with KBr and compressed using IR compression machine and then scanned over the range of 4000 to 400 cm-1. Infrared spectroscopic analysis was done for the powder of fluconazole, polymers (Na CMC, Na alginate, Carb. 934P, HPMC, Pl. F-127 and HPC), physical mixture of drug and polymers 1:1.

2.4 Preparation of Fluconazole Gel Formulations

Fluconazole concentration was 1% w/w in all the prepared formulations. FLZ gels were prepared by dissolving an accurately weighed 1 g of FLZ powder in 20 g propylene glycol using magnetic stirring bar. Propylene glycol was added as a cosolvent, release enhancer of the drug from the formulation and also as penetration enhancer. The calculated amount of water required to prepare 100 g was added. The specified amounts of the gelling polymers;

sodium carboxymethyl cellulose (2 & 4% w/w), sodium alginate (6 & 8% w/w), hydroxypropylmethyl cellulose (2 & 4% w/w) and hydroxypropyl cellulose (16 & 18% w/w) were added slowly to the previously formed mixture and allowed to soak overnight for complete polymer solvation. The mixture was continuously stirred to get the required gels. To study the effect of addition of calcium chloride to sodium alginate gels, 0.3% w/w CaCl2 was added to Na alginate gels prepared as previously using 4% and 5% w/w sodium alginate. Carbopol 934P gels (0.5 & 1% w/w) were prepared by dispersing the specified amount of Carbopol 934P on drug, propylene glycol and water mixture prepared as previously mentioned using magnetic stirring bar. The dispersion was left overnight to ensure complete swelling of the polymer. Carbopol gels were spontaneously formed by the addition of triethanolamine dropwise till neutralization. Pluronic F-127 gels (20 & 25% w/w) were prepared by the cold method described by Schmolka [10]. The required amount of Pluronic F-127 was dissolved in drug, propylene glycol and cold water mixture prepared as previously mentioned using magnetic stirring bar. Then the solution was left in a refrigerator overnight, a clear transparent gel was obtained when the solution was left at room temperature.

2.5 Evaluation of the Prepared Fluconazole Gel Formulations

2.5.1 Physical appearance, pH and actual drug content

The prepared formulations were inspected visually for their color and homogeneity. The pH of the prepared medicated gel formulations was determined directly after preparation using a digital pH meter, (Jenway, U.K.). The drug content was determined by dissolving accurately weighed 1 g of the formulation in phosphate buffer pH 7.4 using magnetic stirrer for 3 hours in order to get complete solubility of the drug. Then, the mixture was quantitatively transferred into volumetric flask 25 ml and completed to mark with phosphate buffer then filtered through filter disk 0.8µ. The absorbance was recorded by using UV-spectrophotometer at 261 nm. The same procedure was adopted for the plain gel and used as blank.

2.5.2 Viscosity and rheological behavior

The viscosity of the prepared gel formulations was determined using BrookField DV-III ULTRA programmable rheometer, model RV, helipath spindle set (Brookfield Engineering laboratories, USA) using T-bar spindle numbers 94, 95 and 96. The viscosity was measured at temperature 25^{o}C and 37^{o}C to study the effect of temperature on the gel viscosity using 20g sample. In order to identify the flow behavior of each formulation, the measurement was made over the whole range of speed settings from 10 rpm to 100 rpm. One minute interval between 2 successive speeds was adopted to generate a complete flow curve. The rheogram was generated by plotting viscosity readings in centipoises (cps) versus spindle speed (rpm) using the same spindle for each polymer concentrations. This experiment was performed for both the plain and the medicated formulations.

2.5.3 In vitro release studies

The in vitro release of fluconazole from the prepared formulations was studied using dialysis method. A one gram sample of each formulation was accurately weighed and placed on a semi permeable cellophane membrane (previously immersed in phosphate buffer pH 7.4 for 24 hours) to occupy a circle of 2.5 cm diameter. The loaded membrane (donor compartment) was firmly stretched over the lower open end of a glass tube of 2.5 cm internal diameter and

made watertight by rubber band. The tube was then immersed in a beaker containing 25 ml of phosphate buffer pH 7.4 which is the release medium (receptor compartment). The system was maintained for 3 hours at 37 ± 0.5ºC in a thermostatic shaking water bath at 50 rpm. Samples of 5 ml were withdrawn at intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 hours. The volume of each sample was replaced by the same volume of fresh buffer (kept at the same temperature) to maintain constant volume. Samples were analyzed for fluconazole content spectrophotometrically at λmax 261 nm against blank similarly treated.

2.5.4 Analysis of the release data

The release mechanisms of fluconazole from the semisolid formulations were elucidated by fitting the release data to four kinetic models. Regression analysis was adopted to compute the constants and correlation of data (r^2).

Zero order kinetics

$$Q = k_o t \quad [11] \tag{1}$$

Where Q is the % of drug released at time t, ko is the zero order release constant and t is the time in hours.

First order kinetics

$$\ln (100-Q) = \ln 100 - k_1 t \quad [11] \tag{2}$$

Where k_1 is the first order release constant.

Higuchi kinetics

$$Q = k_H t^{1/2} \quad [12] \tag{3}$$

Where Q is the amount of drug released at time t per unit area & k_H is the Higuchi release rate constant.

$$KH = 2C_o (D/\pi)^{1/2} \tag{4}$$

Where C_o is the initial drug concentration & D is the diffusion coefficient.

Korsmeyer peppas equation

$$Mt/M\infty = k_t n \quad [13] \tag{5}$$

Where Mt/M∞ is the fraction of released drug at time t & n is the release exponent.

n value is indicative for the drug release mechanism, If n ≤ 0.5 it is a fickian diffusion mechanism, 0.5 < n < 1 it is a non-fickian mechanism (anomalous diffusion) and if n = 1, so release mechanism from the formulation follows a zero order mechanism (case-2 relaxation). In case of n > 1, it indicates a super case-2 transport. Anomalous diffusion or non-fickian diffusion refers to combination of both diffusion and erosion controlled release rate while case-2 relaxation and super case-2 transport refer to erosion of the polymeric chain.

2.5.5 Statistical analysis

All studies were performed in triplicate and the values were expressed as mean ± S.D. The data were analyzed by one way ANOVA and Post Hoc Turkey-Test at a significance level of

.05, homogeneity of variance was evident by Levene's test in most cases and assumed in few others since no transformations were valid. Student T-test was also considered in some cases at a significance level of .05. SPSS statistical package [14] was used in these analysis.

2.5.6 In vitro antifungal activity

Agar cup-plate method was adopted for this study. Different concentrations of fluconazole in DMSO (1%, 0.5%, 0.1%, .05% and 0.01%) were used to study the in vitro antifungal activity of fluconazole against *Candida albicans* (as a representative Yeast fungus) and (1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.1%, .05% and 0.01%) against *Trichophyton Mentagrophyte* (as a representative Dermatophyte fungus). Also the solvent used; DMSO was tested as positive growth control. A single isolate of each fungus was picked from the agar slab culture to prepare spores suspensions in sterile water and was adjusted to be 1×10^6 spores/ml. One ml of the spores' suspension was mixed with Sabouraud agar (15-20 ml) in sterile Petri dish (9 cm in diameter) and the agar plates were allowed to solidify. After solidification, a single well was made in each agar plate using a porer of size 5mm and filled with 50µl of the specified concentrations of fluconazole solutions using DMSO as control solution. The plates were incubated at 25± 1ºC for 3 days (for Candida isolates) and 8 days (for Trichophyton isolates) and then they were examined for the inhibition zone diameter which is an indicator for the antifungal activity.

The selected gel formulation (0.5 % Carbopol 934 P) that showed the best release was subjected to this study. The formulation was tested for its *in vitro* release of fluconazole to the agar and its antifungal activity against the same two fungi using the same method. Plain gel formulation (without drug) was also tested as a positive growth control result. The same methodology as mentioned previously was repeated with changing the diameter of the wells to be 1 cm and filling them with an accurately weighed 0.5 gm of each formula (either medicated or plain). The mean value of the inhibition zone diameter from three plates was calculated.

2.5.7 Stability studies

The selected formulation (0.5% Carbopol 934P / FLZ gel) was subjected to accelerated stability study. It was kept at 40ºC (incubator) for three months [15]. Physical appearance, pH, rheology and spreadability were studied before and after the period of storage. HPLC analysis was performed to evaluate the drug content before and after the stability study period to predict the stability of the drug in the storage temperature (40ºC).

2.5.7.1. Analytical condition

The HPLC analysis was performed on reversed phase high-performance liquid chromatographic system (Young lin. Autochro-3000). Fluconazole was separated on a Venusil XBP C18 (L) reversed phase analytical column (250mm × 4.6mm, 5µm) (Agela Technologies, USA). The mobile phase used was water: acetonitrile (76:24 v/v) with 1 ml/min flow rate at 210 nm using Young Lin UV/Vis Detector (Model UV730D), with dual wavelength (LTD., Korea). The sample was injected through Rheodyne 5020 injection valve, equipped with 20µl sample loop (Rheodyne, Berkeley, CA, USA). The retention time of fluconazole was 5.15 minutes that permits a rapid determination of the drug.

2.5.7.2. Preparation of the sample solutions

0.5 g of the gel sample was accurately weighed and dissolved in 20 ml double distilled water using magnetic stirrer for 3 hours in order to get complete solubility of the drug. Then, the mixture was quantitatively transferred into volumetric flask 25 ml and completed to mark with the double distilled water to give 200 µg/ml of fluconazole solution. The solution was filtered through 0.8 µ membrane filter and diluted to obtain 100 µg/ml.

3. RESULTS AND DISCUSSION

3.1 Solubility of Fluconazole

Solubility of fluconazole at 37ºC in phosphate buffer pH 7.4 (release medium) was 6.85± 0.11 mg/ml. Solubility of fluconazole in water (7.5± 0.13 mg/ml) is similar to the value reported (8 mg/ml) by Dash and Elmquist (16). For enhancing water solubility of fluconazole to ensure complete solubility of the desired drug concentration added to the prepared formulations (10 mg/ml), a mixture of water and PG (80:20% w/w) was used. Fluconazole solubility in this mixture was investigated and found to be equal to 15.87± 0.6 mg/ml.

3.2 Study of the Physicochemical Compatibility of Fluconazole and Gelling Polymers

3.2.1 Differential scanning calorimetry (DSC)

The physicochemical interaction between FLZ and the gelling polymers was evaluated by DSC. The DSC thermograms of pure FLZ, Na CMC, Na alginate, Carb. 934P, HPMC, Pl. F-127 and HPC and their physical mixtures at weight ratio of 1:1 are shown in Fig. 1. Fluconazole powder showed a single sharp endothermic peak at 140.6^{o}C (16) corresponding to its melting point and representing its crystalline nature. FLZ, in physical mixture, had retained its endothermic peak without significant changes. The DSC curves showed that there was no any incompatibility between FLZ and the gelling polymers.

3.2.2 Infrared absorption spectroscopy (IR)

Fig. 2 shows the IR spectra of fluconazole, the gelling polymers (Na CMC, Na alginate, Carb. 934P, HPMC, Pl.F-127 and HPC) and their corresponding physical mixtures. Fluconazole displayed a broad band at 3200 cm-1 due to hydrogen bonded O-H stretching vibrations, aromatic C-H stretching vibrations at 3120 cm-1, aromatic C=N stretching vibrations at 1620 cm-1 and aromatic C-F stretching vibrations at 1210, 1220 cm^{-1}.The principle peaks of fluconazole were observed in the spectra of the drug / polymer physical mixtures indicating no interactions had been occurred.

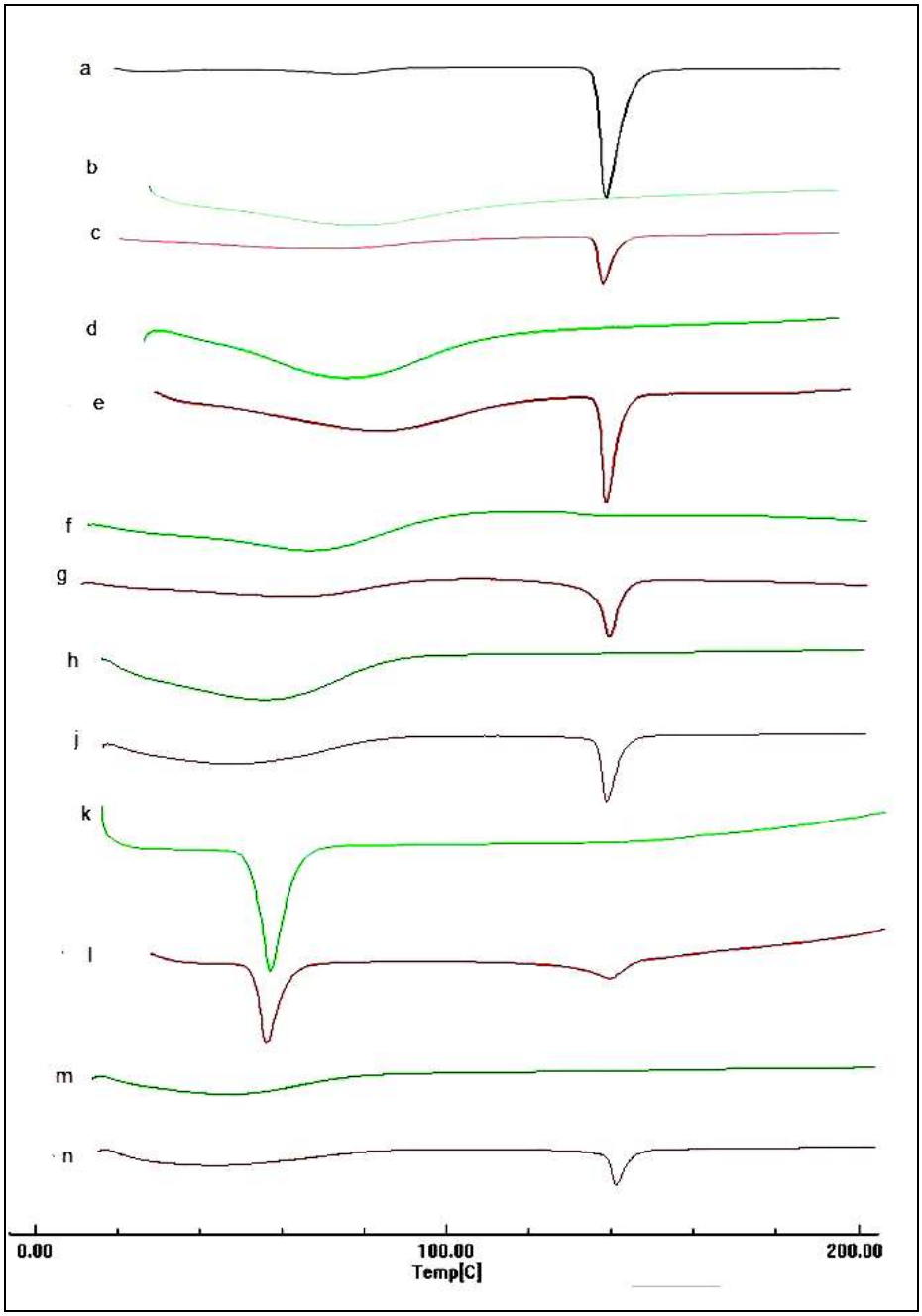

Fig. 1. DSC thermograms of (a) Fluconazole (FLZ) (b) Sodium carboxymethyl cellulose (NaCMC) (c) Physical mixture of FLZ: NaCMC (d) Sodium alginate (e) Physical mixture of FLZ: sodium alginate (f) Carbopol 934P (g) Physical mixture of FLZ: Carbopol 934P (h) Hydroxypropylmethyl cellulose (HPMC) (j) Physical mixture of FLZ: HPMC (k) Pluronic F-127 (l) Physical mixture of FLZ: Pluronic F-127 (m) Hydroxypropyl cellulose (HPC) (n) Physical mixture of FLZ:HPC. (Physical mixtures with ratio1:1)

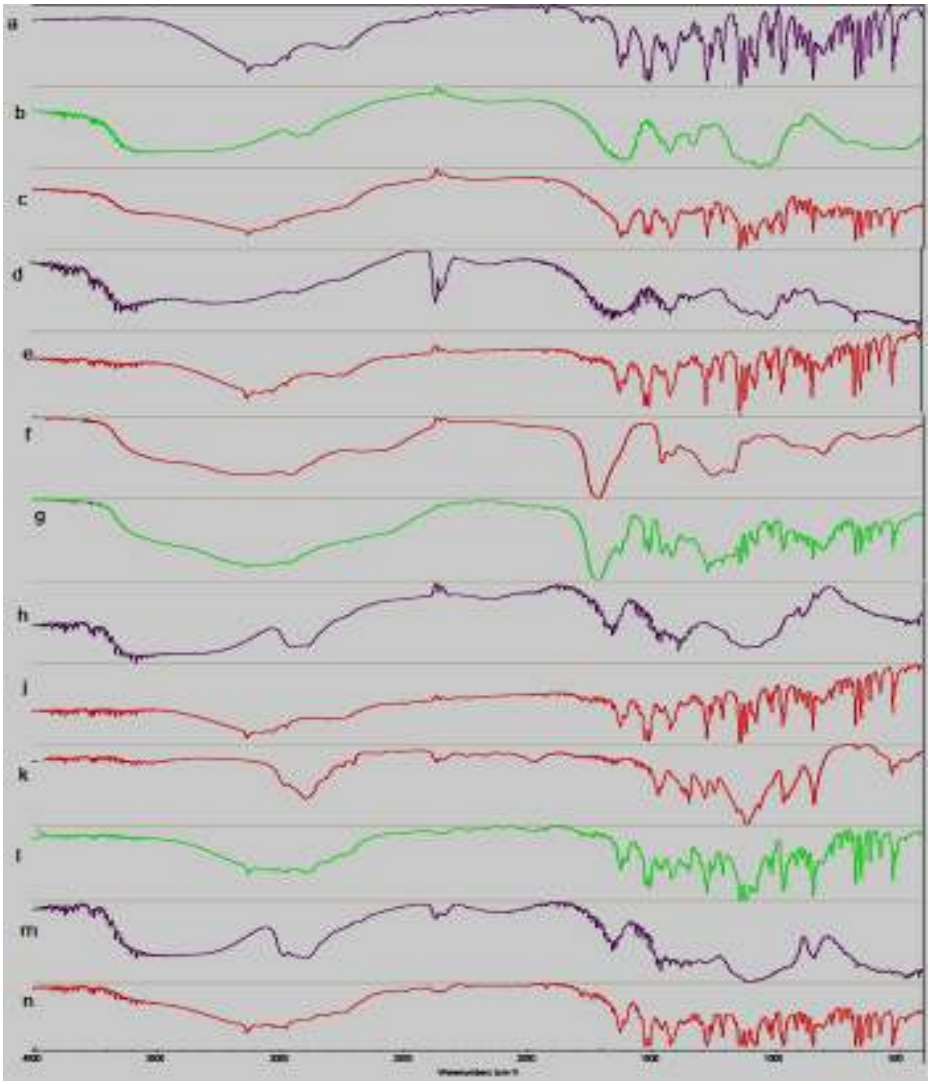

Fig. 2. IR spectra of (a) Fluconazole(FLZ) (b) Sodium carboxymethyl cellulose (NaCMC) (c) Physical mixture of FLZ: NaCMC (d) Sodium alginate (e) Physical mixture of FLZ: sodium alginate (f) Carbopol 934P (g) Physical mixture of FLZ: Carbopol 934P (h) Hydroxypropylmethyl cellulose (HPMC) (j) Physical mixture of FLZ: HPMC (k) Pluronic F-127 (l) Physical mixture of FLZ: Pluronic F-127 (m) Hydroxypropyl cellulose (HPC) (n) Physical mixture of FLZ:HPC. (Physical mixtures with ratio1:1)

3.3 Evaluation of the Prepared Fluconazole Gel Formulations

3.3.1 Physical appearance, pH and actual drug content

All the tested formulations were homogenous and transparent. The formulations showed a pH range between 5.5-7.3 and drug content range was 95–103%.

3.3.2 Viscosity and rheological behavior

The presence of the drug didn't affect the rheology of the formulations as there were no differences between plain and medicated gels. The changes in the viscosity of the prepared fluconazole gel formulations due to the differences in type of the gelling agents, concentration of the gelling agents and the temperature are illustrated in Figs. 3 (a-f). From these figures it is concluded that:-

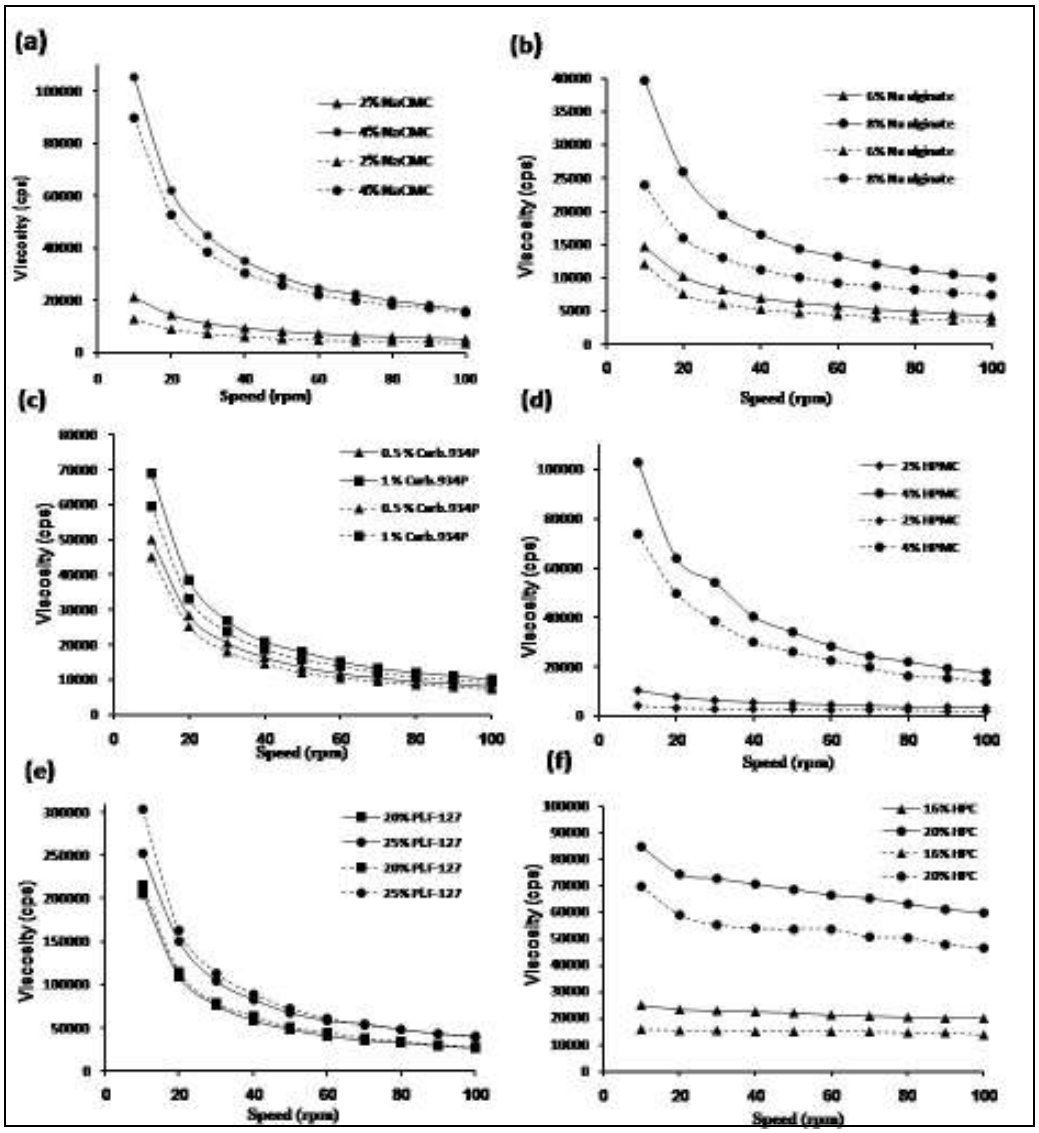

Fig.3. Rheological behavior of fluconazole gels at 25ºC and 37ºC prepared using the polymers; Sodium carboxymethy cellulose NaCMC (a), Sodium alginate (Na alginate) (b), Carbopol 934P (Carb. 934 P) (c), Hydroxypropylemethyl cellulose (HPMC) (d), Pluronic F-127 (Pl.F-127) (e) and Hydroxypropyl cellulose (HPC) (f) (solid line = 25ºC, dotted line = 37ºC)

Pluronic F-127 gels exhibit the highest viscosity while HPMC and Na alginate gels exhibit the lowest viscosity from all the prepared gel formulations.

The viscosity increases upon increasing the polymer concentration for all the used polymers. Meshali et al. [17] reported that the increase in gel-viscosity with increasing HPMC concentration was due to the formation of dense network by entanglement or attraction between HPMC molecules through hydrogen bonds or van-der Waal forces. These forces increase as increasing the polymer concentration leading to aggregation which is manifested by increasing viscosity.

The viscosity has an inverse relationship with the temperature in all the prepared gels except for Pluronic F-127 gels which exhibit a thermoreversible behavior and hence increase the viscosity upon increasing the temperature. Similar observations were obtained by Fetih [18].

The shape of the rheograms showed a pseudoplastic behavior (shear thinning behavior) which is a good characteristic in the pharmaceutical gels for filling and topical application requirements. At lower speed (rpm) the rate of decrease in the viscosity is much higher than that at higher speed. At higher speed, the curves seem to be straightened and so the gel reaches a limiting viscosity, this can be explained as the molecules in the three dimensional network structure of the gel are entangled together with enclosing the immobilized solvent and upon increasing the shear rate (speed), the network structure is disrupted and molecule became disentangled and align themselves in the direction of flow and so molecules offer less resistance to flow and hence decreasing the gel viscosity which also is due to release of some of the water that was entrapped in the destroyed network structure; similar interpretation was recorded by El laithy and El Shaboury [19] and Mahrous [20].

3.3.3 In vitro release studies

The percent of fluconazole released over a period of 3 hours from the prepared gel formulations containing the same initial drug concentration (1% w/w fluconazole) are discussed according to two variables; different polymer concentrations and different polymer types.

From Figs. 4(a-f), it is observed that the release of fluconazole from the prepared gel formulations decrease as an inverse function of polymer concentrations.

The percent of fluconazole released from the prepared Na CMC gels (Fig. 4a) decreases significantly (P = .05) as the polymer concentration increases from 2% to 4%. This result is similar to those reported by Mohammed [21] and Tas et al. [22]. It may be due to the increased viscosity of the gel due to increasing the polymer concentration.

The percent of fluconazole released from the prepared Na alginate gels (Fig. 4b) decreases significantly (P = .05) as the polymer concentration increase from 6% to 8%. An analogous situation has been reported by Ahuja et al. [23], Al-Kubati [24] and Fetih [18].

Release of fluconazole from Carbopol gels was inversely related with the polymer concentration. Carbopol gel (0.5% w/w) showed a significantly (P = .05) higher fluconazole release than 1% w/w Carbopol gels (Fig. 4c). The increase in polymer concentration will increase the crosslink density which increase the tortuosity of the gel from which the drug release occur within the hydrogel network. These findings are in agreement with the data obtained by Songkro et al. [25]. Different results were reported when Macedo et al. [26]

studied the effect of increasing Carbopol concentration from 1 % to 2 % w/w. They found that the increase in polymer concentration had no significant effect on tolmetin release from gel formulations.

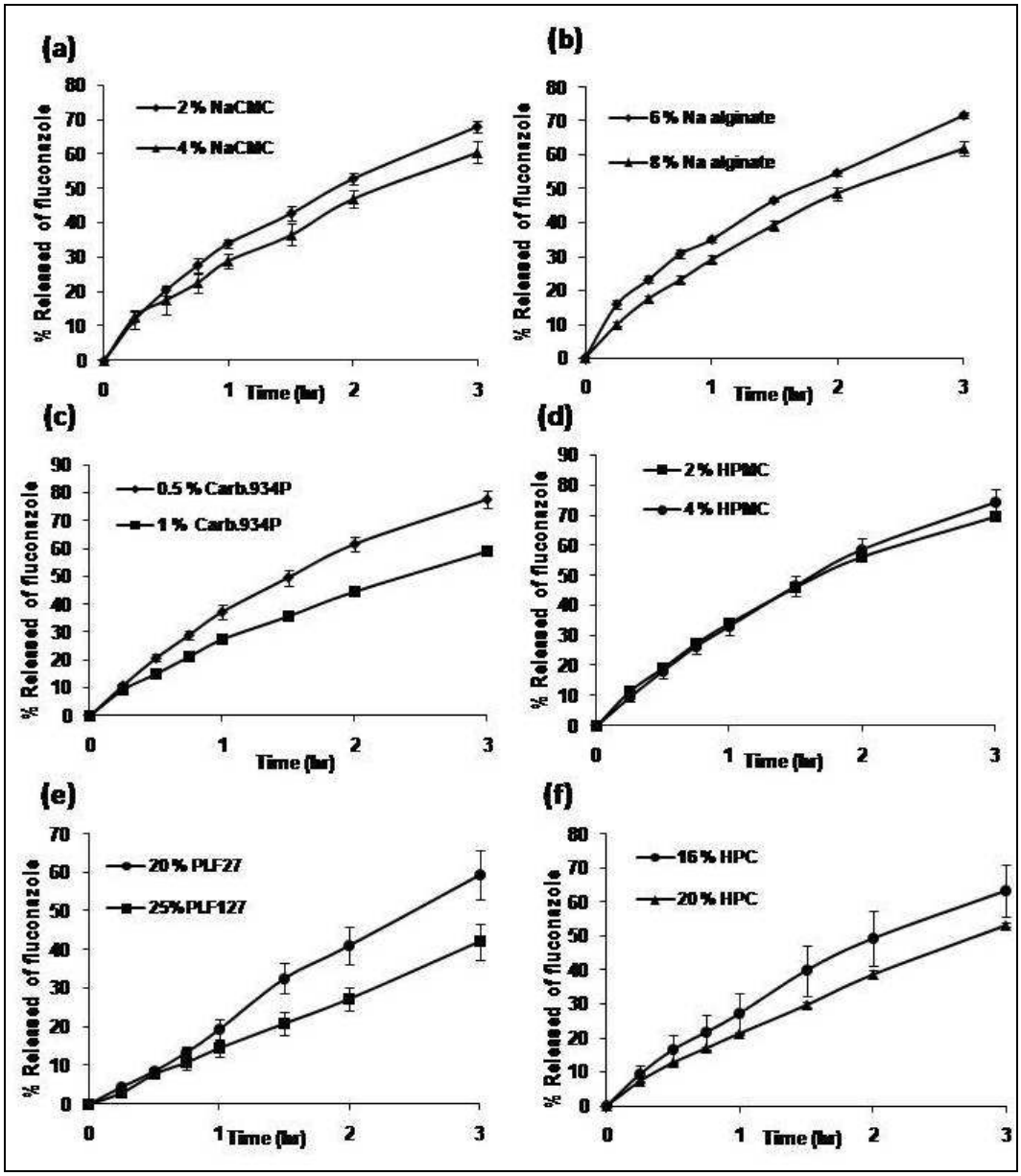

Fig. 4. Effect of different concentrations of Sodium carboxymethy cellulose NaCMC (a), Sodium alginate (Na alginate) (b), Carbopol 934P (Carb. 934 P) (c), Hydroxypropylemethyl cellulose (HPMC) (d), Pluronic F-127 (Pl.F-127) (e) and Hydroxypropyl cellulose (HPC) (f) on the release of fluconazole from the prepared gels

The percent of fluconazole released from HPMC gels didn't differ significantly ($p > .05$) upon changing the concentration of the polymer from 2 to 4 % w/w in spite of the increase in the viscosity (Fig. 4d). These results may be explained by the low percent modification in the polymer concentration. However, Songkro et al. [25] has found that increasing the HPMC concentration from 4% to 10% w/w decreased the percent of nicotinamide released from the prepared oral gel formulations.

The percent of fluconazole released from Pluronic F-127 gels is displayed in Fig. 4e. The release of fluconazole from the prepared gel was found to decrease significantly ($P = .05$) upon increasing the Pluronic concentration. This may be explained as follow; the drug release from Pluronic gels depends on the number and size of the micelles which affect the number and size of water channels so the change in Pluronic concentration affect the diffusional pathways and thus the drug release. Moore et al. [27] explained that behavior by that the increase in the number of micelles at higher Pluronic concentrations will result in a more entangled system and a more rigid gel as was discussed in the viscosity part. This inverse relationship between the Pluronic concentration and the drug release has been shown for all the drug studied and has been reported by several investigators [28-33].

The percent of fluconazole released from HPC gels was found to decrease insignificantly ($p>.05$) with increasing the polymer concentration from 16 % to 18 % (Fig. 4f). this result is in agreement with Andrews et al. [34] who reported that a decrease in release rate was observed upon increasing HPC concentration.

Fig. 5 shows the effect of addition of 0.3% w/w $CaCl_2$ to different concentrations of Na alginate on the release of fluconazole. It was found that the release of fluconazole from 6% w/w Na alginate gel alone was significantly ($P = .05$) higher than 4% w/w Na alginate gel + $CaCl_2$ and 5% w/w Na alginate gel + $CaCl_2$. This release profile may be explained as the sodium ions (Na+) of the polymer were exchanged with the calcium ions (Ca^{+2}) so the polymer became crosslinked as calcium ions can form two bonds unlike sodium ions which form only one bond [(35)]. So, the addition of a solution of calcium chloride to sodium alginate solution can form a gel at lower concentration than that gel formed by sodium alginate alone. Additionally, this cross linking imparts a resistance for drug molecules to be released.

In general, the inverse relation between polymer concentration and FLZ released is in agreement with lauffer's molecular diffusion theory of polymer gels [36]. The theory states that the diffusion of a solute is inversely proportional to the volume fraction occupied by the gel forming agent. Welin-Berger et al. [37] found that an increase in the macroviscosity may affect the release rate of the active compound inversely. Many investigators mentioned the same inverse relationship between the release and the polymer concentration in their studies on various drugs using different polymers [18,21,23,24].

It is obviously clear from the previous results that all the prepared fluconazole gel formulations with the different polymers showed an acceptable drug release. Noticing the release of fluconazole from the gel prepared from the lowest concentration of each polymer, it was found that the release from Pluronic gel was significantly ($P = .05$) lower than the release from other polymer gels nearly at all time points. Cellulosic derivative polymer (Na CMC, HPMC, and HPC) gels showed no significance difference in the fluconazole release over most of the time points. At three hours, Carbopol gel showed a significantly ($P = .05$) higher drug release over all the other polymers. These findings are in agreement with the data of El Gendy et al. [30]. They found a significant difference in flubiprofen release from Carbopol and Pluronic gels indicating that the drug release is influenced by the nature of

each individual polymer. This result is in agreement with Patel et al. [38] who stated that aceclofenac Carbopol gel showed superior drug release followed by Na CMC, HPMC and sodium alginate gels. The structure of Carbopol plays an important role in drug release, the main barrier for drug release from the aqueous Carbopol polymer gels is a mechanical layer formed by the random network of the polymer molecules which bind and entraps the surrounding water, and this aqueous phase may be the region for drug diffusion from the gel. In case of Pluronic gel, its aqueous solution is composed of micelles formed by the polymer and the aqueous phase. The aqueous phase form channels through which the drug is available for release. HPMC gels showed higher drug release than Na CMC and HPC gels. This result may be due to the low viscosity of HPMC gels and the greater hydrophilicity of HPMC. Cheong et al. [39] reported that the HPMC molecules are giant macromolecules compared to drug and water molecules. They are made up of hundreds of chain segments in random coils held tightly by hydrogen bonding. HPMC being a hydrophilic has a great affinity for water so when the polymer chain comes in contact with water, polymer-water interaction replaces the polymer-polymer attraction.

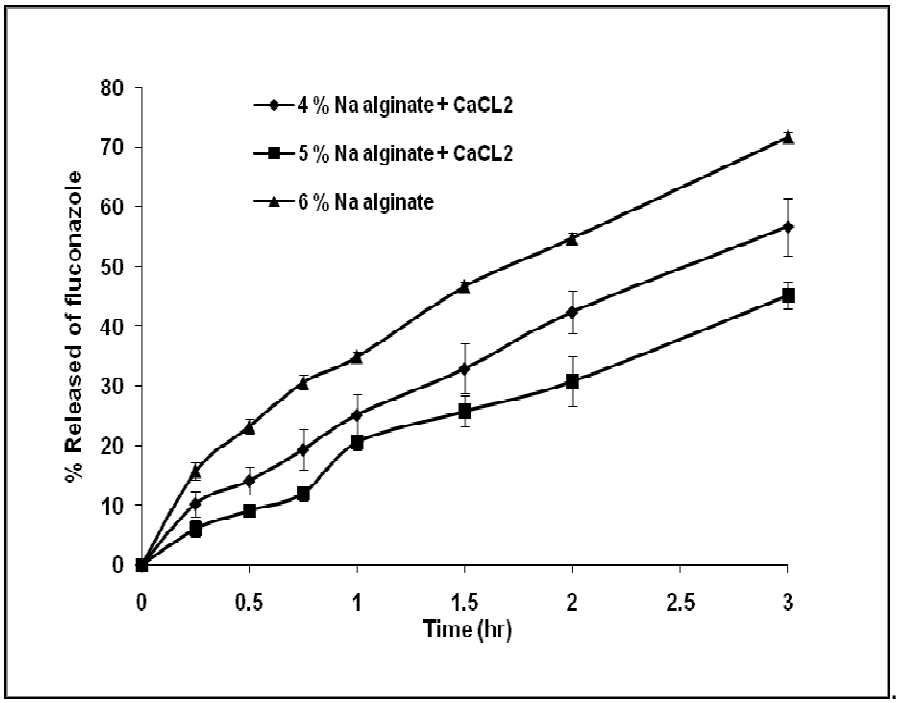

Fig.5. Effect of addition of 0.3% calcium chloride ($CaCl_2$) to different concentrations of sodium alginate (Na alginate) on release of fluconazole

The viscosity of the prepared gel formulations increased in the order of 2% HPMC < 6% Na alginate < 2 % Na CMC < 16% HPC < 8 % Na alginate < 0.5% Carb.934P = 1% Carb.934P = 20 % HPC <4 % HPMC < 4% Na CMC < 20 % Pl.F-127 < 25 % Pl.F-127.

However, drug release after a 3-hr period decreased in the order of 0.5% Carb.934P > 4% HPMC > 6% Na alginate > 2% HPMC > 2% Na CMC > 16% HPC > 8% Na alginate > 4% Na CMC >20% Pl.F-127 >1% Carb.934P > 20% HPC > 25% Pl.F-127. It is seemed that they do not follow the expected behavior in which - when increasing the viscosity of the gel

decreasing the drug release - so this lack of correlation indicates that the bulk viscosity is not the primary factor that affecting the release of the drug and release mostly depends also on the nature of the polymer.

These observations are confirmed by the relationship of log viscosity (at 10 rpm) and the percent of FLZ released after 3 hrs from the prepared gels (Fig. 6). It was appeared that there is a weak correlation between the log viscosity of the formulations and the percent of FLZ released from the gels (R^2 = 0.351). This result is not in agreement with those obtained by Songkro et al. [25] who stated that there is a high correlation between the log viscosity and the release rate of nicotinamide from the prepared gels (R^2=0.7289).

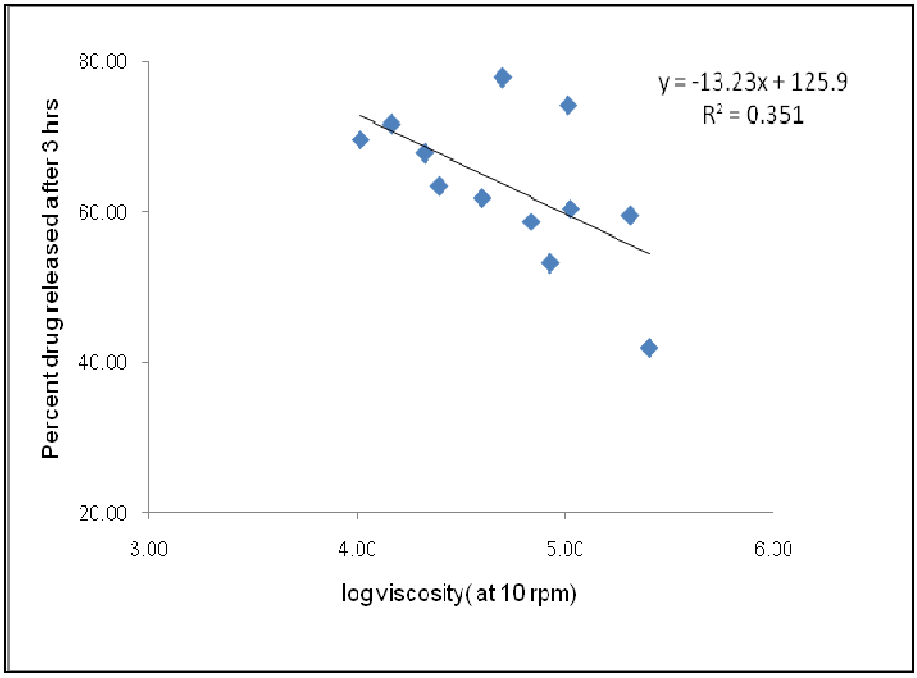

Fig.6. Relationship between log viscosity and percent of FLZ released after 3 hrs from the prepared gels

The previous results suggest that an appropriate choice of the polymer used in preparing the gel is very important for achieving the desired drug release profile. In conclusion, the diffusion of any drug through the different polymers depends on the nature and the composition of the polymers and the release rate can be altered by changing their nature and the composition.

3.3.4 Analysis of the release data

The preference between the release mechanisms depends on the coefficient of determination (R^2; squared correlation coefficient) and the release exponent (n) of korsmeyer-Peppas equation. The in-vitro release data of FLZ from the prepared gel formulations are in favor of first order kinetics except for Pluronic F-127 20% & 25% gels (Table 1). Also, their release exponent n of Peppas equation was found to be > 0.5 and < 1. This indicates that non-fickian diffusion controls the drug release from the gels. These results

are in a good agreement with Mahrous [20], who reported that the mechanism of release of fluconazole from the prepared buccal gels is first order. Also Habib et al. [40] reported that the release mechanism of ketorolac tromethamine from Na alginate and Na CMC gels is first order. While in case of Pluronic F-127 20 % & 25 % gels, R^2 and n values support a zero order release processes (n=1). Similar results were obtained by El-Houssieny and Hamouda [41], Moore et al. [27], Paavola et al. [42] and Wang et al. [43] . They found that the release of the studied drugs from Pluronic F-127 gels follows zero order process and the drug release is controlled by gel dissolution rather than by drug diffusion.

Table 1. Kinetic analysis of the release data of fluconazole from the prepared gel formulations

Polymer	Zero Order		First Order			Higuchi Diffusion model Q/A vs. $T^{1/2}$		Best fitted model
	R^2	$K_{o\ (\%\ h^{-1})}$	R^2	$K_{1\ (h^{-1})}$	T0.5 (h)	R^2	$D(cm^2/hr)$	
2 % NaCMC	0.955	21.691	*0.997*	0.367	1.890	0.847	4.19E-03	first order
4 % NaCMC	0.966	19.191	*0.995*	0.299	2.319	0.845	3.22E-03	first order
6 % Na alginate	0.947	22.241	*0.993*	0.399	1.739	0.852	4.78E-03	first order
8 % Na alginate	0.965	20.171	*0.999*	0.318	2.182	0.842	3.40E-03	first order
0.5 % Carbopol	0.964	25.777	*0.997*	0.498	1.392	0.839	5.36E-03	first order
1 % Carbopol	0.975	19.075	*0.999*	0.289	2.398	0.838	2.91E-03	first order
2 % HPMC	0.955	22.898	*0.999*	0.396	1.750	0.843	4.47E-03	first order
4 % HPMC	0.972	24.877	*0.998*	0.452	1.533	0.832	4.68E-03	first order
20 % Pl.F-127	*0.995*	20.443	0.985	0.304	2.279	0.782	2.32E-03	zero order
25 % Pl.F-127	*0.999*	13.908	0.990	0.179	3.879	0.791	1.13E-03	zero order
16 % HPC	0.975	20.984	*0.999*	0.334	2.074	0.834	3.41E-03	first order
20 % HPC	0.991	17.334	*0.997*	0.247	2.802	0.826	2.16E-03	first order

R^2: Coefficient of determination, K_o: Zero order release constant, K_1: First order release constant, $T_{0.5}$: Half-life of first-order reaction,D: Diffusion coefficient.

3.3.5 In-vitro antifungal activity

The in vitro antifungal activity of different concentrations of FLZ in DMSO (1%, 0.5%, 0.1%, .05% and 0.01% w/v) on *Candida albicans* and (1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.1%, .05% and 0.01% w/v) on *Trichophyton mentagrophyte* was evaluated by the measurement of the mean diameter of growth inhibition zones in millimeter (Table 2). It was found that *Candida albicans* is more susceptible than *Trichophyton mentagrophyte* to FLZ solution even at low concentrations down to 0.01% w/v (equivalent to 5 µg FLZ). Figs. 7(a) & (b) show the growth inhibition zone of 1 % FLZ solution in DMSO against these two fungi. It was also found that the diameter of the growth inhibition zone increases with increasing the fluconazole concentration. The solvent used (DMSO) showed no antifungal effect on the tested fungi. The results of the selected Carbopol gel formulation that subjected to antifungal activity are shown photographically in Figs. 7 (c) & (d). They exhibited a good inhibition zones; 56.7 ± 2.89 mm and 53.3 ± 2.89 mm against *Candida albicans* and *Trichophyton mentagrophyte* respectively. Plain gel showed no growth inhibition.

Table 2. Effect of different concentrations of fluconazole solution in dimethyl sulphoxide (DMSO) on Trichophyton mentagrophyte and Candida albicans

Candida albicans		*Trichophyton mentagrophyte*	
Conc.of FLZ solution in DMSO	**Average zone of inhibition (mm) ± SD**	**Conc.of FLZ solution in DMSO**	**Average zone of inhibition (mm) ± SD**
1%	50 ± 0.00	1%	21 ± 1.41
0.50%	40 ± 0.00	0.90%	19 ± 1.41
0.10%	37.7 ± 0.58	0.80%	19 ± 1.41
0.05%	33 ± 0.00	0.70%	18 ± 2.83
0.01%	24.7 ± 0.58	0.60%	15 ± 0.00
DMSO alone	0.00	0.50%	- ve
		0.10%	- ve
		0.05%	- ve
		0.01%	- ve
		DMSO alone	0.00

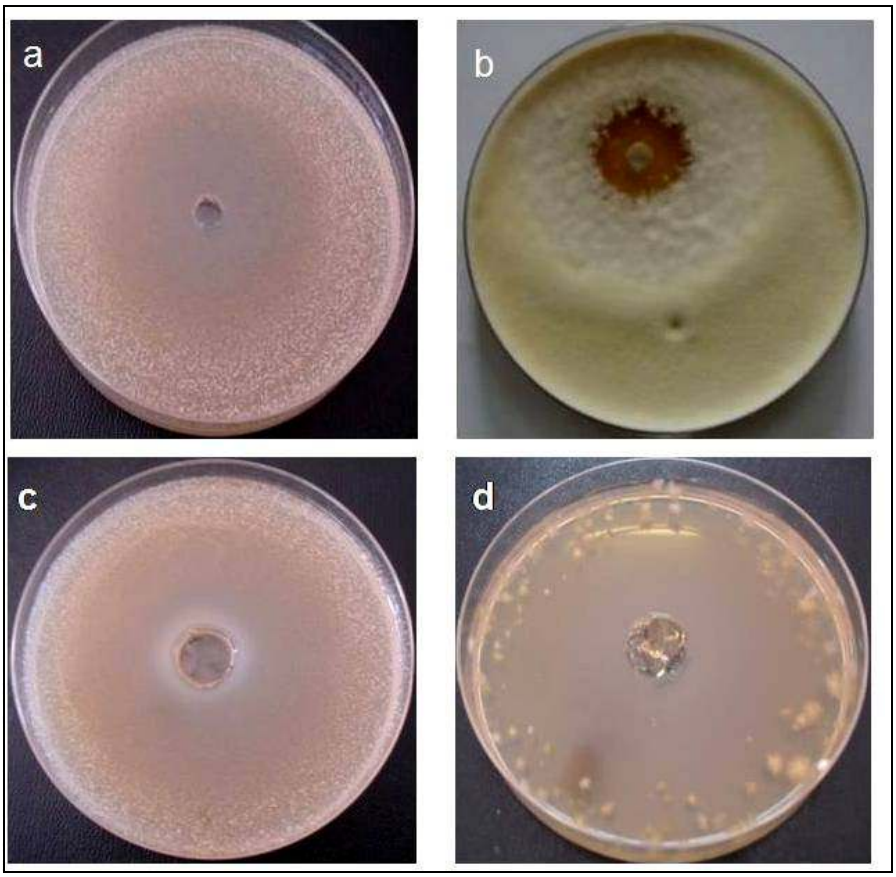

Fig.7. The growth inhibition zones of 1% fluconazole solution in DMSO (a) & (b) and 0.5% Carbopol 934P gel (c) & (d) against *Candida albicans* and *Trichophyton mentagrophyte* respectively

3.3.6 Stability studies

The tested gel sample in the studied temperature 40ºC (incubator) was inspected visually after the period of three months. There was no change in the color, homogeneity, no mold growth, no syneresis and no recrystallization. The pH of the fresh formulation was 6.2 and after 3 months of storage was found to be 6.05. There was a slight decrease in the viscosity of the gel with no change in the rheological behavior. Also the drug content was 99.3%. The calibration curve for fluconazole was constructed by plotting FLZ solution different concentrations versus peak height and it showed a good linearity in 25-100 µg/ml range. Fig. 8 shows the HPLC chromatograms of the tested gel sample at zero time and after three months at 40ºC and also the chromatogram of the plain Carbopol gel. The chromatogram of the gel sample after 3 months shows no new peaks and hence there is no degradation had occurred.

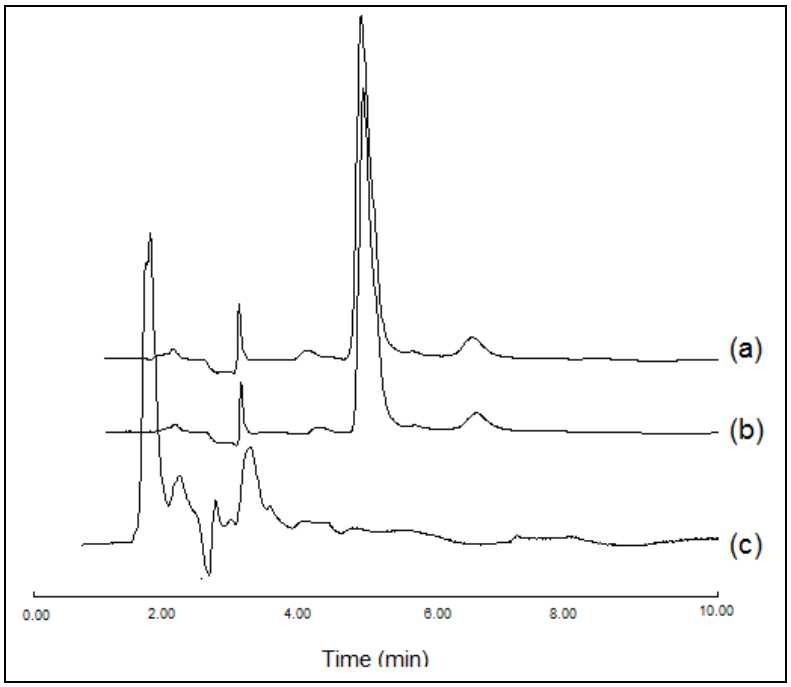

Fig.8. HPLC chromatograms of: (a) fluconazole / 0.5% Carbopol gel at zero time; (b) fluconazole / 0.5% Carbopol gel after three months at incubator temperature (40°C); (c) 0.5% Carbopol plain gel.

4. CONCLUSION

The results obtained showed that the formulated gels had good physical characteristics and an acceptable FLZ release.0.5% Carbopol gel showed the highest *In vitro* FLZ release after 3 hours, high *in vitro* fungal inhibition and good stability at accelerated temperature 40ºC after 3 months. So, it could be a promising topical alternative for the treatment of skin fungal infections and could be subjected for further clinical studies.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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