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Physically Cross-Linked Polyvinyl Alcohol for the Topical Delivery of Fluconazole

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The aim of this study was to develop fluconazole in an ultra-pure polyvinyl alcohol (PVA) hydrogel able to deliver the drug in a sustained release pattern for local treatment of skin fungal infections. The topical fluconazole hydrogels were prepared using PVA hydrogels physically cross-linked by freeze–thaw technique. Polyethylene glycol (PEG) was added as a hydrophilic excipient as a release enhancer of fluconazole. The effects of PVA molecular weight, PEG molecular weight, and PEG concentration were studied using a 2 × 4 × 2 factorially designed experiment. The selected fluconazole hydrogel proved to be physically stable over a period of 6 months and to be effective in the topical treatment of cutaneous candidiasis. Therefore, it could be concluded that the formula composed of 10% PVA 205000 and 1.5% PEG 4000 and 2% fluconazole and prepared by three cycles of freezing, and thawing is very promising in the local treatment of skin fungal infection as an alternative to the systemic use of fluconazole.

Keywords fluconazole; PVA; hydrogels; physical crosslinking; factorial design

INTRODUCTION

Hydrogels are three-dimensional, water-swollen structure composed mainly of hydrophilic homopolymers or copolymers (Peppas, 1987). They are rendered insoluble because of the presence of physical or chemical cross-links. The physical cross-links can be entanglements, crystallites, or weak associations such as van der Waals forces or hydrogen bonds. These cross-links provide the network structure and physical integrity. Hydrogels have been considered for use in a wide range of biomedical and pharmaceutical applications. Based on their high water content and rubbery nature, hydrogel materials resemble natural living tissue more than any other class of synthetic biomaterials (Peppas, 1987; Ranter, Hoffman, & Andare, 1976).

Polyvinyl alcohol (PVA) is a polymer that has been studied extensively because of its numerous desirable characteristics, specifically its good film-forming, gel-forming, and physical properties; high hydrophilicity, processability, biocompatibility, and good chemical resistance. These properties have led to its broad use in industrial, pharmaceutical, and biomedical applications (Hernandez, Sarafian, Lopez, & Mijangos, 2004). Also other properties of PVA such as high tensile strength and tack make PVA useful as a water-resistant adhesive (Peppas & Peppas, 1993). This will be of particular importance for topically applied dosage forms which require long contact time with the skin to achieve better efficacy as well as better patient compliance. PVA can be cross-linked through the use of difunctional cross-linking agents or by gamma irradiation (Peppas, 1987). Some of the common cross-linking agents that have been used include glutaraldehyde, acetaldehyde, formaldehyde, and other monoaldehydes. As with any cross-linking agent, however, residual amounts are present in the ensuing PVA gel, rendering the gel not acceptable for biomedical and pharmaceutical applications. A third mechanism of hydrogel preparation involves physical cross-linking due to crystallite formation (Stauffer & Peppas, 1992). This method addresses toxicity and carcinogenicity issues because it does not require the presence of a cross-linking agent.

Aqueous PVA solutions have the unusual character of crystallite formation upon repeated freezing and thawing cycles. Although the most recent researchers suggested that the hydrogel formation is attributed to hydrogen bonding formed between PVA molecular chains (Hatakeyema, Uno, Yamada, Kishi, & Hatakeyama, 2005). However, the reason of this phenomenon is a subject of significant discussion. Three basic models have been proposed in an effort to explain the mechanisms inducing the gel formation: (a) hydrogen bonding, (b) polymer crystallite formation, and (c) liquid–liquid phase separation

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process. Hydrogen bonding is thought to be the directional interaction that causes a physical cross-link (tie point) to form in the gel. The polymer crystallite formation theory hypothesizes that gelation is due to the organization of network chains into crystallites. Liquid–liquid separation explains the phenomenon as a phase separation into polymer-rich and polymer-poor regions by spinodal decomposition (Peppas & Stauffer, 1991). Another theory suggests that these processes are not completely separated from each other and each may play an interdependent role in the formation of the gel.

The properties of the gel generally depend on the molecular weight of the polymer, the concentration of the aqueous PVA solutions, the temperature and time of freezing and thawing, and the number of freezing/thawing cycles (Hernandez, et al., 2004).

Fluconazole is a triazole fungistatic agent used for superficial mucosal (oropharyngeal, oesophageal, or vaginal) candidiasis and for fungal skin infections as well as serious systemic fungal infections. Fluconazole is given by mouth or intravenous infusion in similar doses. Fluconazole is available commercially as tablets and injections only despite its well-known adverse effects including GIT and taste disturbances. Other adverse effects include headache, dizziness, leucopenia, thrombocytopenia, hyperlipidemias, and raised liver enzyme values. Serious hepatotoxicity has been reported in patients with severe underlying disease such as AIDS or malignancy. Anaphylaxis and angioedema have been reported rarely. As the fungal infections require long duration of therapy, systemic administration of fluconazole leads to higher incidence of adverse effects. This embraces the development of topical fluconazole preparation to avoid them. Most of the topical delivery systems in the market have a residence time insufficient to provide adequate therapeutic effects. Hence, the development of an effective drug delivery system with prolonged contact time with the skin surface and providing sustained drug release would be beneficial.

The primary objective of this study was to develop a hydrogel formulation for the topical delivery of fluconazole using PVA physically cross-linked by repeated freezing and thawing. As this cross-linking usually results in delaying the drug release from such hydrogels, enhancement of the fluconazole release was done using the hydrophilic excipient polyethylene glycol (PEG). The effect of number of freezing and thawing cycles and the concentration of PVA was studied. A full factorial design experiment was conducted to study the effects of PVA molecular weight, PEG molecular weight, and PEG concentration on the different properties of the prepared fluconazole hydrogels. In addition, in vivo studies were conducted on the selected formula.

MATERIALS AND METHODS

Materials

Fluconazole was kindly provided by SEDICO Company (Cairo, Egypt). PVA of average molecular weight 130,000, degree of polymerization 2,700, ester number 130–150, degree of hydrolysis 86.7–88.7 mol%, viscosity of 4% solution in water at 20°C 16–20 mPa.s and PVA of average molecular weight 205,000, degree of polymerization 4,200, ester number 130–150, degree of hydrolysis 86.7–88.7 mol%, viscosity of 4% solution in water at 20°C 38–42 mPa·s were purchased from Fluka GmbH. PEG, molecular weight 400, 1,500, 4,000, 6,000, and semipermeable cellulose membrane, molecular weight cutoff 12,000–14,000, were purchased from Sigma Chemical Co. (USA). Citric acid monohydrate and disodium hydrogen phosphate were obtained from El-Nasr Pharmaceutical Chemical Co. (Abu Zaabal, Egypt). Sabouraud dextrose agar (SDA) was purchased from Oxoid Limited (Hampshire, England). Clinical isolate of *Candida albicans* was obtained from Specialized Ain Shams Hospital (Cairo, Egypt). All other chemicals were of analytical grade or equivalent quality.

Preparation of Fluconazole Hydrogels

Aqueous solutions of PVA of molecular weight 130,000 and 205,000 of three concentrations (8, 10, and 12%) were prepared by dissolving the required amount of the polymer in distilled water at 90°C. After complete polymer dissolution, the solutions were cooled to room temperature. Specified amount of the fluconazole, equivalent to 2% (with or without PEG) was then added to the cooled PVA solutions.

The mixtures were frozen at −20°C for 8 h and then allowed to thaw at room temperature for 12 h. This procedure consisted one full cycle. All the prepared mixtures were subjected to two, three, and four cycles.

For the full factorial design experiment, all the hydrogels were prepared using 10% PVA and three cycles of freezing and thawing. The factors studied were the PVA molecular weight at two levels (130,000 and 205,000), the PEG molecular weight at four levels (PEG 400, PEG 1500, PEG 4000, and PEG 6000), and the concentration of PEG at two levels (0.5 and 1.5%) giving 2 × 4 × 2 = 16 formulae.

The assigned codes and exact composition of PVA hydrogels containing PEG used in the factorial design are shown in Table 1.

TABLE 1
The Assigned Codes and Composition of Polyvinyl Alcohol (PVA) Hydrogels Containing Polyethylene Glycol (PEG) Used in the Factorial Design

	Concentration of PEG (%, wt/wt)	Molecular Weight of PEG			
		400	1,500	4,000	6,000
PVA (M wt 130,000)	0.5	1	3	5	7
	1.5	2	4	6	8
PVA (M wt 205,000)	0.5	9	11	13	15
	1.5	10	12	14	16

Physical Evaluation of the Prepared Hydrogels

Visual Inspection

The prepared formulae were examined for their physical characteristics: color, homogeneity, and phase separation.

Spreadability

A sample of 0.1 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 min where no more spreading was expected (Contreras & Sanchez, 2002; De Martin & Cussler, 1975; Lucero, Vigo, & Leon, 1994; Vennat, Gross, & Pourrat, 1994). Diameters of spreaded circles were measured in centimeter and were taken as comparative values for spreadability. The results obtained are average of three determinations.

In Vitro Release Study of Fluconazole from PVA Hydrogels

The study was carried out using the modified USP dissolution apparatus I. Samples, each of 2 g of the preparation, were spreaded on a cellophane 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 tubes were tied up with a rubber to prevent leakage and attached to the apparatus shaft (Chang, Oh, Choi, Kim, & Kim, 2002). After fixation to the apparatus, the tubes were immersed in the dissolution vessel which contained 100 mL of the release medium (McIlvain buffer pH 5.5) (El-Laithy & El-Shaboury, 2002) and maintained at 32 ± 0.5°C (Siewert, Dressman, Brown, & Shah, 2003). The tubes were rotated at 50 rpm, and aliquots each of 3 mL were withdrawn from the release medium at time intervals of 10, 20, 30, 60, 90, 120, 180, 240, 300, and 360 min. Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed spectrophotometrically at λmax 261 nm for fluconazole content, and the concentration of the drug was determined from the previously constructed calibration curve. The equation for % drug release was % fluconazole released = $A \cdot k \cdot 100$, where A is the UV absorbance of the collected sample at 261 nm, k is the procedural constant obtained from the slope of the calibration curve then multiplied by 100 to obtain the amount released in 100 mL.

The presence of PVA and PEG did not interfere with the analysis as confirmed from blank experiments conducted using plain bases. Experiments were carried out in triplicates and results were averaged.

Analysis of the Factorial Design Data

The diameter of the spreaded circle and the percent of fluconazole released at 6 h (T6 h) were the chosen responses for analysis in the full factorial design experiment.

The analysis of these values was performed by using the statistical software (MINITAB® Release 14.20, 2005). The analysis of variance including sum of squares, subsequent significance tests, and the calculation of the average values were also obtained using this software.

Kinetic Analysis of the Drug Release Data

The data obtained from the in vitro release experiments were analyzed using linear regression method according to zero order ($Ct = C0 - Kt$), first order ($\ln Ct = \ln C0 - k1t$), and Higuchi diffusion model ($Mt/M\infty = kt^{1/2}$), where Ct is the concentration of the drug in the gel at time t, $C0$ is the initial concentration of drug in the gel, K is the zero order release constant, $k1$ is the first-order release constant, $Mt/M\infty$ is the fraction of the released drug at time t, and k is the Higuchi dissolution constant.

Stability Studies on the Selected Fluconazole Hydrogel

Based on the results obtained from the previous studies, stability studies as well as the in vivo evaluation were performed on the selected formula; the one showing suitable spreadability and appropriate release characteristics. This hydrogel was stored in well stoppered glass container for 6 months at room temperature in the dark. Physical evaluation of the sample's stability was performed by visual inspection and spreadability testing. The effect of storage on the in vitro drug release was evaluated as well (Tas, Ozkan, Savaser, & Baykara, 2003). The results obtained from the freshly prepared samples and after storage were compared using student t test and the software utilized was Gragh Pad Instat V2.04 with 5% level of significance.

In Vivo Evaluation of the Selected Fluconazole Hydrogel

Preparation of the Animals

The method utilized was that of Ben-Josef, Cutright, Manavathu, and Sobel (2003). A total of 24 male guinea pigs each weighing 400–450 g (Animal house, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt) were used in this study. Animals were housed at room temperature and had access to food and water ad libitum. To achieve a heavy cutaneous infection, animals were immunosuppressed with cyclophosphamide (100 mg/kg body weight) by intraperitoneal injection on days –5, –3, and –1, where day 0 was the day of infection.

This study was performed in accordance with the guidelines of the experimental protocol approved by the Ethics Committee of EAPRU (Experimental and Advanced Pharmaceutical Research Unit, Faculty of pharmacy, Ain Shams University).

Preparation of the Microorganisms

Clinical isolate of *C. albicans* was used to infect the animals. A working culture of the candida was grown for 48 h at 30°C on Sabouraud dextrose agar (SDA). The cells were then collected, washed, and then resuspended to a final concentration of 1×10^7 blastospores/mL in sterile phosphate-buffered saline (PBS) for infection.

Cutaneous Infection

The animal's back was shaved with electric clippers. Approximately 2.5 cm^2 area was marked on each animal's back. The marked area was infected with a 0.025 mL suspension containing 1×10^2 blastospores/mL.

The suspensions were gently rubbed onto the skin with a sterile cotton-tipped swab until no more visible fluid was observed. Infection was produced under an occlusive dressing. The infected area was covered with a sterile adhesive bandage and was held in place with extra adherent tape for 48 h, (Lyoyama, Uchida, & Yamaguchi, 1997) before treatment began. Negative control animals were infected in the same manner and did not receive any treatment.

Treatment of the Infection

Treatment began 2 days after the infection was induced and was applied once daily for 7 days. The experimental animals were divided into four groups each of six animals:

- Group I: Negative control group where the animals didn't receive any treatment.
- Group II: Oral control group where the animals received single dose oral fluconazole 10 mg/kg, (Nagino, Shimohira, Ogawa, Uchida, & Yamaguchi, 2000).
- Group III: The animals received treatment by the fluconazole hydrogel.
- Group IV: The animals received treatment by the nonmedicated hydrogel.

All animals were killed by ether anaesthesia 48 h after the last treatment, and 2.5 cm^2 skin from the infected sites was excised and cut into 10 equal blocks. Each block was implanted onto SDA plates containing 100 mg/L of cyclohexamide plus 50 mg/L gentamicin and incubated at 30°C for 48 h. All 10 blocks from the same animal were implanted onto one plate. Each skin block was examined visually for *C. albicans* growth, and skin blocks yielding fungal growth were regarded as culture positive. The fungal growth often occurred surrounding the skin block. The negative skin blocks had no growth around them or at any side of the skin block.

Statistical Analysis

The number of positive skin blocks in each group was counted. Differences between groups were tested for significance using a two-sided Chi square test. p values of $< .05$ were considered significant.

RESULTS AND DISCUSSION

Inspection of the prepared hydrogels indicated that they were white, smooth, and of semisolid consistency. It was observed that PVA solutions remained transparent (clear) after the first freezing and thawing cycle indicating that the gel was not fully developed after only one cycle. This observation was also seen by Lozinsky, Zubov, Kulakova, Titova, and Rogozhin (1992). Upon further freezing, the PVA gels experienced an obvious increase in turbidity which increased by increasing the number of freeze–thaw cycles, and it was more prominent in the gels prepared with the higher PVA molecular weight (205,000). Similar results were obtained by Peppas and Stauffer (1991) and Peppas and Scott (1992) who referred this turbidity to particles densification or crystallization leading to an increase in their diameter. This theory was supported by the fact that the larger particles absorbed more light and so transmit less. No phase separation or precipitation was reported for all formulae.

According to a preliminary study done on the hydrogels prepared without PEG (data are not shown), it was found that increasing the number of cycles from two to three was accompanied by a decrease in both spreadability and in vitro release of fluconazole. However, these two parameters did not change upon increasing the number of freeze–thaw cycles from three to four. So three cycles were maintained constant for the rest of the work. In addition, the 8% concentration of PVA 130,000 yielded unacceptable soft gel in contrast to its 10% concentration, while both 8 and 10% of the PVA 205,000 gave gels with acceptable consistency, but to keep all the conditions of the following study constant, 10% PVA of each molecular weight was selected for further study. After 6 h, the percentage of the drug released from the hydrogels prepared with 10% of the PVA molecular weights 130,000 and 205,000 and subjected to three cycles of freezing and thawing were 63.4 and 53.72%, respectively (Table 2). Attempts were made to enhance the drug release from these hydrogels by the incorporation of PEG (hydrophilic excipient). To investigate the effect of both the concentration and the molecular weight of PEG used on the physical properties of the hydrogels as well as fluconazole release, a full factorial design study was established.

Analysis of the Factorial Design Data

The spreadability and the percent of fluconazole released at 6 h (T6 h) from different fluconazole hydrogels prepared according to the factorial design are shown in Table 3. The release profiles of these hydrogels are shown in Figures 1 and 2.

Effect of PVA Molecular Weight, PEG Concentration, and Molecular Weight on the Spreadability of the Hydrogels

The ANOVA test (Table 4) shows that all the main factors under study—namely, PVA molecular weight, PEG molecular weight, and PEG concentration—had significant effects ($p = 0.000$) on the spreadability of the hydrogels. The highest effect was that of the PVA molecular weight (Figure 3) and the

TABLE 2
Percent Fluconazole Released from Different Polyvinyl Alcohol (PVA) Hydrogels After 6 h

PVA (M wt)	PVA Concentration (%)	Number of F/T Cycles	% Fluconazole Released After 6 h ± *SD*
130,000	8	2	Not done as the gels were too soft to be used
		3	
		4	
	10	2	82.93 ± 0.31
		3	63.4 ± 0.78
		4	62.81 ± 1.12
	12	2	63.61 ± 0.61
		3	61.85 ± 1.54
		4	59.37 ± 0.21
205,000	8	2	73.25 ± 0.24
		3	67.71 ± 1.14
		4	66.96 ± 1.35
	10	2	72.44 ± 1.24
		3	53.72 ± 1.93
		4	52.13 ± 0.82
	12	2	59.12 ± 0.35
		3	51.41 ± 0.15
		4	51.82 ± 1.41

lowest one was that of the PEG concentration, their respective *F*-values were 4967.13 and 29.39.

The average value for spreadability of the PVA molecular weight 130,000 was 5.263 cm compared to 3.845 cm in case of PVA 205,000. This can be simply explained by the longer chain length and consequently the higher viscosity value of the higher molecular weight of PVA which in turn led to the lower spreadability.

It was found that the main effects of PEG molecular weight and concentration were significant with respective *F*-values 70.03 and 29.39. The spreadability of the different hydrogels containing different PEGs increased up to an optimum molecular weight (PEG 4000), after that it decreased. Average spreadability values ranged between 4.325 and 4.708 cm and can be arranged in the following sequence, PEG 4000 > 1500 > 6000 > 400, with respective values of 4.708 > 4.642 > 4.558 > 4.325 cm. Concerning the effect of PEG concentration, the hydrogels prepared with 0.5 and 1.5% had mean spreadability values of 4.613 and 4.504 cm, respectively. Although the main effects of the PEG concentration and molecular weight and the interaction effects of the different factors on the spreadability of the hydrogels were statistically significant, yet the spreadability values exhibited a narrow range from 4.325 to 4.708 cm, hence these effects could be considered of limited value from the practical point of view.

TABLE 3
Spreadability and *T*6 h of Fluconazole Hydrogels Prepared According to the Full Factorial Design

		Responses (Mean of Three Readings ± *SD*)	
Formula Code	Composition	Diameter of Spreaded Circle (cm)	*T*6 h
1	PVA M wt 130,000 + 0.5% PEG 400	5.3 ± 0.0	62.24 ± 0.57
2	PVA M wt 130,000 + 1.5% PEG 400	5.1 ± 0.08	74.7 ± 2.8
3	PVA M wt 130,000 + 0.5% PEG 1500	5.6 ± 0.0	65.05 ± 1.5
4	PVA M wt 130,000 + 1.5% PEG 1500	5.2 ± 0.05	70.3 ± 0.49
5	PVA M wt 130,000 + 0.5% PEG 4000	4.6 ± 0.0	66.15 ± 1.9
6	PVA M wt 130,000 + 1.5% PEG 4000	5.1 ± 0.05	70.1 ± 1.33
7	PVA M wt 130,000 + 0.5% PEG 6000	5.7 ± 0.15	62.8 ± 0.12
8	PVA M wt 130,000 + 1.5% PEG 6000	5.3 ± 0.0	56.8 ± 0.5
9	PVA M wt 205,000 + 0.5% PEG 400	3.6 ± 0.0	52.18 ± 1.66
10	PVA M wt 205,000 + 1.5% PEG 400	3.2 ± 0.05	56.8 ± 0.12
11	PVA M wt 205,000 + 0.5% PEG 1500	3.7 ± 0.1	52.9 ± 2.5
12	PVA M wt 205,000 + 1.5% PEG 1500	3.9 ± 0.1	62.5 ± 0.14
13	PVA M wt 205,000 + 0.5% PEG 4000	4.3 ± 0.0	61.7 ± 0.58
14	PVA M wt 205,000 + 1.5% PEG 4000	4.9 ± 0.05	78.4 ± 1.97
15	PVA M wt 205,000 + 0.5% PEG 6000	3.9 ± 0.0	68.5 ± 1.57
16	PVA M wt 205,000 + 1.5% PEG 6000	3.2 ± 0.0	55.3 ± 2.86
17	PVA M wt 130,000	5.2 ± 0.0	63.4 ± 0.78
18	PVA M wt 205,000	3.8 ± 0.0	53.72 ± 1.93

*T*6 h, percent of fluconazole released after 6 h.

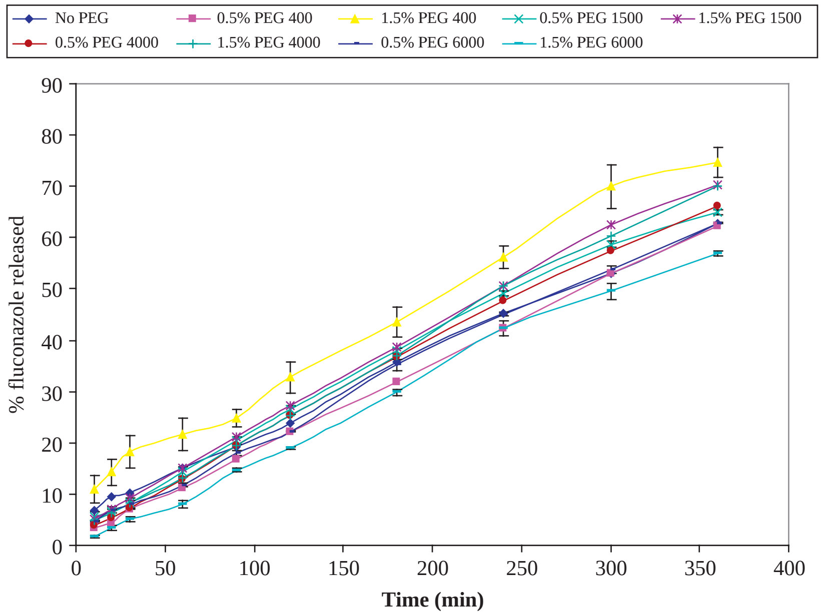

FIGURE 1. In vitro release profiles of different fluconazole hydrogels (PVA M wt 130,000) containing polyethylene glycol (PEG).

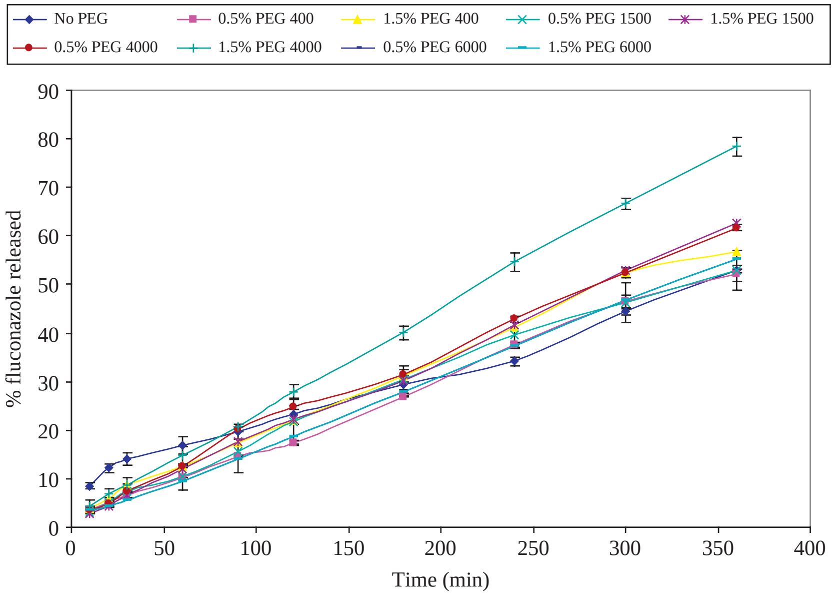

FIGURE 2. In vitro release profiles of different fluconazole hydrogels (PVA M wt 205,000) containing polyethylene glycol (PEG).

Effect of PVA Molecular Weight, PEG Concentration, and Molecular Weight on the In Vitro Fluconazole Release from the Hydrogels

The ANOVA test (Table 5) shows that all the factors studied—namely, the molecular weight of PVA, the molecular weight of PEG, and the concentration of PEG—have significant effects on $T6$ h ($p = 0.000$).

Figure 4 shows in detail the main effect of each of the three variables. The mean percent fluconazole released after 6 h from the two different PVA molecular weights 130,000 and 205,000 were 66.03 and 61.03%, respectively. Increasing the molecular weight of PVA caused a reduction in the fluconazole release from the hydrogels. This effect as previously reported is due to the increase in the crystallinity of the system and its mechanical strength upon increasing the molecular weight of PVA (Peppas & Scott, 1992).

The effect of increasing the PEG molecular weight as shown in Figure 4 seemed to increase the release of fluconazole

TABLE 4
The Analysis of Variance (ANOVA) for Spreadability of Fluconazole Hydrogels
Prepared According to the Factorial Design

Source of Variation	*df*	Sum of Squares	Mean Square	*F*-value
PVA molecular weight	1	23.8008	23.8008	4,967.13*
PEG molecular weight	3	1.0067	0.3356	70.03*
PEG concentration	1	0.1408	0.1408	29.39*
PVA M wt – PEG M wt	3	5.6958	1.8986	396.23*
PVA M wt – PEG concentration	1	0.0133	0.0133	2.78
PEG M wt – PEG concentration	3	2.0092	0.6697	139.77*
PVA M wt – PEG M wt – PEG concentration	3	0.2967	0.0989	20.64*
Error	32	0.1533	0.0048	—
Total	47	33.1167	—	—

df, degrees of freedom.
*Significant at $p = 0.000$.

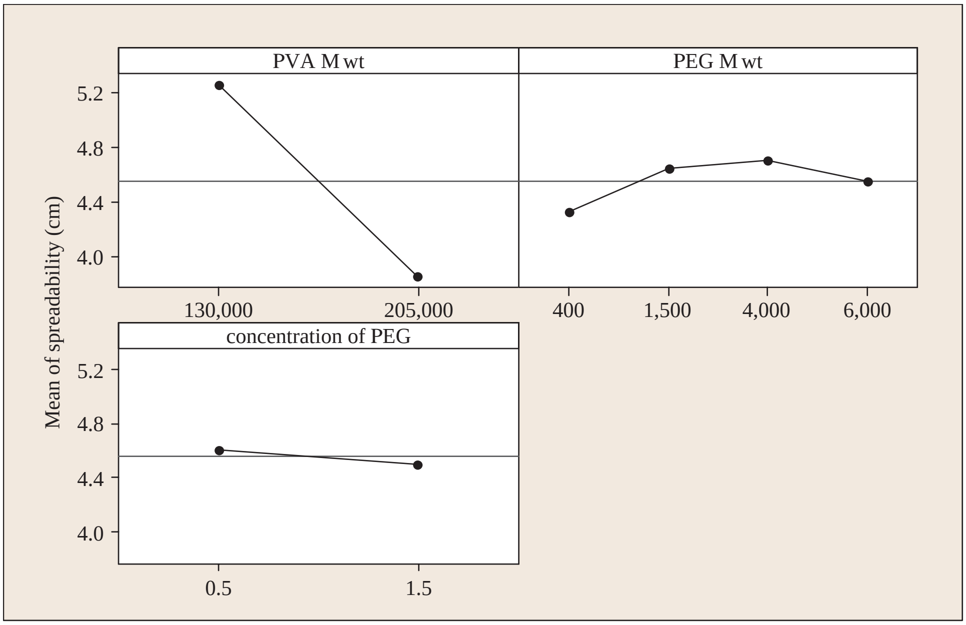

FIGURE 3. Main effect plots of different factors on the spreadability of fluconazole hydrogels.

reaching its maximum at the level of PEG 4000 and then decreased again with PEG 6000. This finding was in agreement with the spreadability results confirming that there is an optimum molecular weight of PEG at which the best spreadability and drug release were obtained. The mean percent fluconazole released from hydrogels containing different PEG molecular weights can be arranged in the following descending order 69.08% > 63.21% > 60.99% > 60.86% corresponding to the PEG molecular weights 4,000, 1,500, 400, and 6,000, respectively. PEG is a hydrophilic excipient that is commonly used as a release enhancer due to its high water solubility, (Desai & Blanchard, 1998). The effect of changing the molecular weight of PEG on the fluconazole release could be explained by the fact that higher molecular weight PEG occupied a larger volume in solution and appeared to be much more effective in disrupting the crystalline structure of the PVA hydrogels (Pandit & McGowan, 1998). This fact was in accordance with our results, but it could not explain the lower release enhancing effect of PEG 6000 compared to other PEGs. It was reported that the number of hydroxyl groups per unit mass of the low-molecular weight PEG is higher than that of the high-molecular weight PEGs, indicating the higher hydrophilicity of the former polymer. So the less hydrophilic character of PEG 6000 seemed to hinder the expected release enhancing effect due to its high molecular weight (Kim, Choi, & Park, 2000).

TABLE 5
The Analysis of Variance (ANOVA) for the Percent Fluconazole Released After 6 h from Hydrogels Prepared According to the Factorial Design

Source of variation	*df*	Sum of Squares	Mean Square	*F*-value
PVA molecular weight	1	300.10	300.10	110.82*
PEG molecular weight	3	533.44	177.81	65.66*
PEG concentration	1	206.26	206.26	76.16*
PVA M wt – PEG M wt	3	601.78	200.59	74.07*
PVA M wt – PEG concentration	1	1.06	1.06	0.39
PEG M wt – PEG concentration	3	782.24	260.75	96.26*
PVA M wt – PEG M wt – PEG concentration	3	263.46	87.82	32.43
Error	32	86.66	2.71	—
Total	47	2,775.00	—	—

df, degrees of freedom.
*Significant at $p = 0.000$.

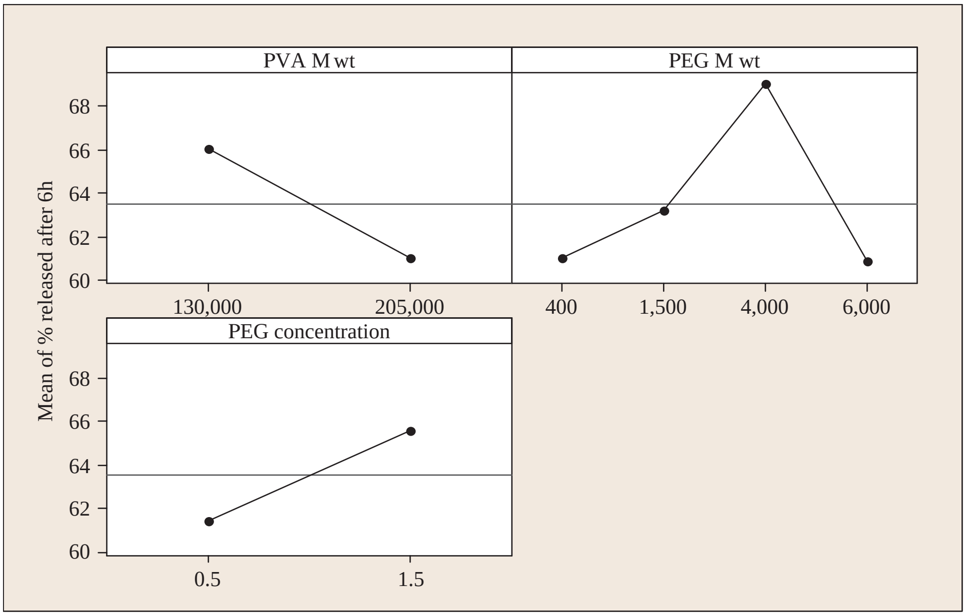

FIGURE 4. Main effect plots of different factors on the percent fluconazole released after 6 h.

Further inspection of Figure 4 reveals that increasing the concentration of PEG resulted in an increase in the fluconazole release. The two concentrations used in this design were 0.5 and 1.5%, yielding mean percent fluconazole release of 61.46 and 65.61%, respectively. This of course can be attributed to the increase in the hydrophilicity of the hydrogel upon increasing the PEG concentration.

According to the results of ANOVA test (Table 5), there were two significant interactions. The first is between the PVA molecular weight and PEG molecular weight and the second is between PEG molecular weight and PEG concentration ($p = 0.000$). Figure 5 shows in detail the two-way interactions between the different variables.

Concerning the interaction between the PVA and the PEG molecular weights, it can be seen that the highest release enhancing effect was achieved upon the addition of the PEG 4000 to the PVA of the high molecular weight 205,000. This can be explained according to West and Hubbell (1995) that the higher the molecular weight of the polymer, the larger will be the pore size in the hydrogel formed by adding another substance interfering with the crystallization process. This in turn allowed diffusion of larger amount of the drug**.**

Another significant interaction was found between the PEG molecular weight and its concentration, where it was found that increasing the concentration of PEG with increasing the PEG molecular weight always enhanced the release of fluconazole

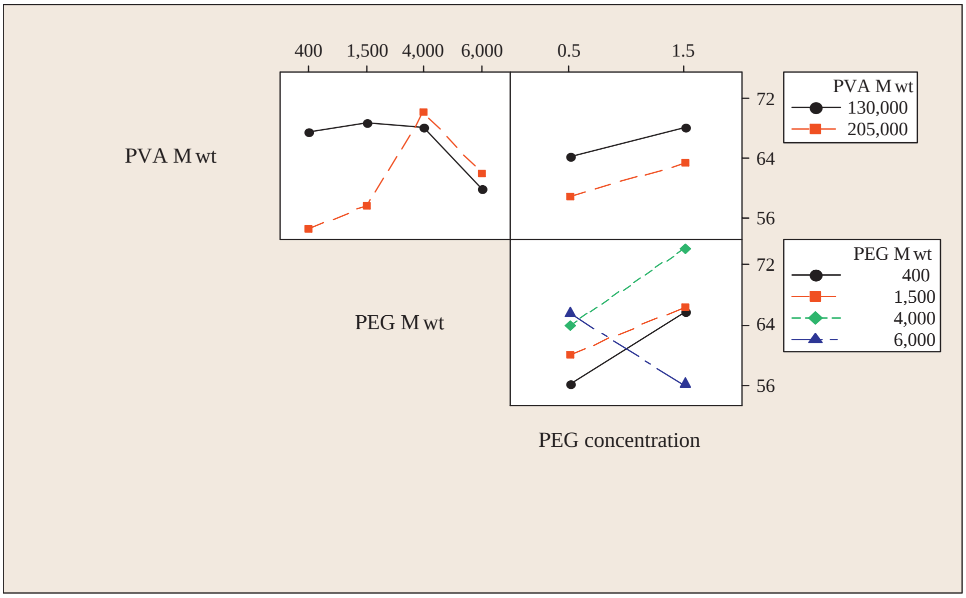

FIGURE 5. Interaction effect plots of different factors on the percent fluconazole released after 6 h.

except for PEG 6000 which showed a reverse pattern. As previously mentioned, the less hydrophilic character of PEG 6000 led to release retarding effect, and this effect was of course increased with increasing its concentration. This result was explained in another situation by Hashizaki et al. (2005), who found that the addition of PEG had two opposing effects on the drug leakage from liposomes: an enhancing effect resulting from the increase in mobility of the lipid molecules and phase separation enhanced by the PEG, and a shielding effect where the exposed PEG chains on the liposomal surface shield drug release from the liposomes. This shielding effect increased with increasing PEG concentration. In the lower molecular weight PEGs, the enhancing effect was superior to the shielding effect even at the high concentrations while in case of the high-molecular weight PEGs the shielding effect dominated.

From the previous results and Table 3, it could be concluded that the highest fluconazole release (78.4%) after 6 h was obtained from the hydrogel prepared with the PVA molecular weight 205,000 in the concentration of 10% with the addition of 1.5% PEG 4000 and subjected to three cycles of freezing and thawing. Furthermore, this formula showed an acceptable consistency (the diameter of the spreaded circle was 4.9 cm). Therefore, this formula was selected for stability studies and the in vivo evaluation.

Kinetic Analysis of the Drug Release Data

The in vitro release data of different fluconazole hydrogels were analyzed according to zero-order, first-order, and diffusion-controlled release mechanisms using linear regression. By trying the three different models, the highest coefficient of determination was obtained with zero-order mechanism. This means that none of the studied factors in the factorial design had an effect on the release order. This result was in accordance with what was obtained by Takamura, Ishii, and Hidaka (1992), where the release of both hydrophilic and hydrophobic drugs was found to be of zero order.

Stability Studies on the Selected Fluconazole Hydrogel

The selected hydrogel presented good stability. Upon visual inspection, no macroscopical physical changes were observed during storage. Spreadability and in vitro drug release testing were repeated after 6 months of storage and showed no significant difference when compared to the fresh ones (using Student's *t* test the $p > .05$).

In Vivo Evaluation of the Selected Fluconazole Hydrogel

From the results shown in Figure 6, it can be seen that the negative control group showed severe infection and did not show any improvement throughout the 7 days of treatment where 58 skin blocks out of 60 showed positive growth of candida. The group of guinea pigs receiving plain base (placebo preparations), although having a lower number of positive skin blocks than the negative control animals, showed large populations of candida (50 blocks out of 60).

Statistical analysis of the results obtained from the fluconazole hydrogel and its comparison with the plain base revealed that there was significant improvement after treatment with the medicated formulation ($p < .0001$) where only 10 positive skin blocks were obtained out of 60. Comparing the results of the topical treatment with the systemic (oral) treatment of the infection showed that the hydrogel was significantly better

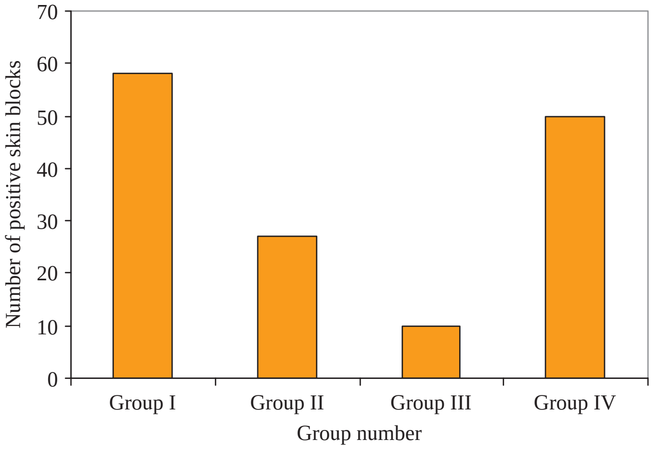

FIGURE 6. The number of positive skin blocks of different groups.

than the oral use of fluconazole (p = .001) with 27 blocks showing positive candida growth out of 60.

CONCLUSION

From this study, it can be concluded that fluconazole hydrogel prepared with 10% PVA molecular weight 205,000 containing 1.5% PEG 4000 and subjected to three cycles of freezing and thawing was more efficient in treating cutaneous candidal infection than the orally administered fluconazole. Furthermore, this preparation would be expected to have a better patient compliance as local application would substantially minimize systemic side effects. In addition, the prepared hydrogel was only removed by either peeling or washing of the skin; this can be an important advantage when used in humans, especially in covered areas.

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