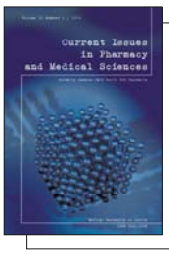

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Formulation and stability evaluation of hydrogels with ketoconazole

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ABSTRACT

This study is focused on designing hydrogels with ketoconazole (KET) based on two polymers - Carbopol 980 or hydroxyethyl cellulose (HEC). Hydrogels were stored for 6 months at different temperature and relative humidity conditions (4°C ± 2°C, 25°C ± 2°C and 60% ± 5% RH, 40°C ± 2°C and 75% ± 5% RH). It was found that hydrogels made of Carbopol 980 exhibited more favourable physicochemical properties and better stability . In HEC hydrogels stored at 40°C ± 2°C and 75% ± 5% RH a decrease of pH, differences in the content and sedimentation of KET were observed. *In vitro* release study showed that amount of released KET from designed hydrogels was definitely higher than from registered product.

Keywords: ketoconazole, hydrogel, Carbopol 980, HEC, stability

INTRODUCTION

Topical formulations are important class of drug delivery systems and their use in therapy is becoming more widespread. These formulations range in physicochemical nature from solid through semisolid to liquid [6, 11]. Currently, a great attention is devoted to the hydrogels, which are three-dimensional and hydrophilic polymer networks capable of swelling in water or biological fluids and retaining a large amount of fluids in the swollen state. They can be prepared from a wide variety of materials of natural origin, as well as from materials obtaineded by the modification of the natural structures and from the synthetic polymeric materials [14]. The most common gelling agents are: hydroxyethyl cellulose, hydroxypropyl methylcellulose, methylcellulose, sodium carboxymethylcellulose and different types of Carbopol. Hydrogels for dermatological use have several favourable features such as mucoadhesion, tixotropy, spreadability or ease of removal. Furthermore, hydrogels are characterized by ease of application and better percutaneous absorption than other semisolid preparations. They provide faster and more complete release of the drug from the vehicle to the skin and therefore, in the consequence - higher efficacy than creams or ointments [1, 7].

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Ketoconazole (KET), an imidazole derivative, is a broad spectrum antifungal agent active against a wide variety of fungi and yeasts [2]. The main effect of imidazoles is the inhibition of the sterol-14α-desmetilase, an enzymatic system dependent upon cytochrome P 450, with a consequent inhibition of fungal development. KET is used in the treatment of topical or systematic fungal infections [3, 4] and is available in different topical preparations (cream, shampoo) and orally as tablets. KET is practically insoluble in water (0.0866-17.0 μg/ml) [5, 16] and in aqueous solutions it might undergo chemical degradation (oxidation and hydrolysis) [12].

As there is no registered hydrogel with KET on Polish pharmaceutical market, the aim of this study was to formulate and evaluate stability and physicochemical properties of hydrogels containing KET, obtained with different types of polymers (hydroxyethyl cellulose or Carbopol 980).

MATERIALS AND METHODS

Reagents. Ketoconazole was received as a gift sample from Polfarmex S.A. (Kutno, Poland), Carbopol 980 (Lubrizol, Cleveland, USA), hydroxyethyl cellulose – HEC (A.C.E.F., Piacenza, Italy), propylene glycol, sodium hydroxide potassium, dihydrogen phosphate, disodium hydrogen phosphate, anhydrous sodium acetate anhydrous, acetic acid 80% anhydrous (Chempur, Piekary Śląskie, Poland), 2-bromo-2 nitropropane-1,3-diol-bro-

nopol – (Sigma Aldrich, Buchs, Switzerland), Tween 80 (Sigma Aldrich, Madrid, Spain), ethanol 99.9% (J.T. Baker Deventer, Holland), methanol HPLC grade (Witko, Łódź, Poland), acetonitryle HPLC grade, sodium dodecyl sulphate – SDS (POCH, Gliwice, Poland), water HPLC grade (Milli-Q Reagent Water System, Billerica, USA). All used reagents were of analytical grade. Commercially available product – Nizoral® cream 20 mg/g (Janssen-Cilag, Beerese, Belgium); composition: propylene glycol, stearyl alcohol, cetyl alcohol, sorbitan stearate, Polysorbate 60, isopropyl mirystate, anhydrous sodium sulphite, Polysorbate 80, purified water.

Preparation of hydrogels. The hydrogels were prepared by dissolving bronopol in purified water and then polymers (Carbopol 980 or HEC) were gradually added into the solution and stirred for 60 minutes using mechanical stirrer (IKA-Werke, Staufen, Germany) until homogenous mixture appeared. In the case of Carbopol 980, mixture was neutralized (to pH 6.0) by dropwise addition of 20% solution of sodium hydroxide to allow gel formation. Mixing was continued until a transparent gel was received and afterwards KET (suspension in propylene glycol) and Tween 80 were added (Table 1). The blending was continued to get uniform dispersion of KET in the hydrogel.

Table 1. Composition of various hydrogels with ketoconazole

Ingredient (g)	Formulation code					
	H1	H2	H3	C1	C2	C3
Ketoconazole	2.0	2.0	2.0	2.0	2.0	2.0
HEC	2.75	2.75	2.75	–	–	–
Carbopol 980	--	–	–	0.4	0.4	0.4
20% NaOH	–	–	–	q.s	q.s.	q.s
Propylene glycol	10.0	10.0	10.0	10.0	10.0	10.0
Tween 80	–	1.0	3.0	–	1.0	3.0
Bronopol	0.01	0.01	0.01	0.01	0.01	0.01
Purified water (up to)	100.0	100.0	100.0	100.0	100.0	100.0

Stability studies. The prepared hydrogels were stored for six months in sealed polyethylene containers at three different conditions (4°C±2°C, 25°C±2°C and 60%±5% RH, 40°C±2°C and 75%±5% RH) in climatic test chambers (CTC 256, Memmert, Schwabach, Germany; KBF 115, Binder, Tuttlingen, Germany) and evaluated periodically for viscosity, pH, particles size, KET content and inspected visually for their colour, homogeneity and consistency.

Viscosity measurement. The viscosity was determined using Brookfield viscometer (Model RVDV-III Ultra, Brookfield Engineering Laboratories, Middlebro, USA) at 25°C±1°C and shear rate 11,52 s^{-1}.

pH determination. The pH was measured by a glass electrode of the pH-meter Orion 3 Star (Thermo Scientific, Waltham, USA).

Particle size analysis. Samples from the hydrogels were observed (under magnification x 100) using optical microscope Motic BA 400 equipped with a camera (Moticon, Wetzlar, Germany). The particles size was additionally evaluated to see if none exceeds the 90 µm size limit [9].

HPLC analysis. KET content was determined after extraction of samples of hydrogels in ethanol 99.9% and analysed by HPLC method in the following conditions: Zorbax Eclipse XDB-C18, 4.6 X 150 mm, 5 µm column (Agilent, Waldbronn, Germany); mobile phase: methanol – acetonitrile – phosphate buffer pH 6.8 (35:40:25, v/v); flow rate 1.0 ml/min; detection at 231 nm; retention time 4.0 min [15].

***In vitro* release of KET.** The release of KET was measured through natural cellulose membrane (Cuprophan®, Medicell, London, UK) using an enhancer cell with surface area of 3.80 cm^2. The enhancer cell consisted of a Teflon load ring, a cap, a membrane and a drug reservoir. This study was performed using the USP dissolution apparatus 2 (Erweka DT 600, Heusenstamm, Germany) with mini vessels (250 ml) and mini paddles. Samples, each of about 3 g, were placed in the enhancer cell which was then immersed in the dissolution vessel containing 100 ml of the release medium (acetic buffer pH 5.5 with 1% SDS), to provide the *sink* conditions, previously warmed and maintained at 32°C ± 0.5°C. Agitation was affected by mini paddle at 75 rpm and aliquots each of 2 ml were withdrawn from the release medium at different time intervals (0.5, 1, 2, 3, 4, 5 and 6 h). Withdrawn samples were replaced by equal volumes of fresh release medium. The samples were assayed by HPLC method as described earlier and the concentration of KET was determined from the previously constructed calibration curve. Standard calibration curve in dissolution medium was linear over the range of 1-100 µg/ml (R^2 = 0.9991). All the experiments were carried out in triplicate.

RESULTS AND DISCUSSION

During designing topical formulations, the choice of the base as well as the addition of excipients plays an important role, because they determine the quality of the final dosage form, its stability and efficacy. In this study, six hydrogels with KET and two different polymers (Carbopol 980 or HEC) were prepared (Table 1) and their physicochemical properties and stability were evaluated.

Carbopol 980 is polyacrylic acid polymer with highly ionized carboxyl groups after neutralization that leads to gel formation due to the electrostatic repulsion among the charged polymer chains. The hydrogels of Carbopol 980 are transparent, have an attractive appearance, good mucoadhesive properties and pleasant cool feeling. HEC is a non-ionic water soluble cellulose derivative, it does not interfere with the pH of final formulation and is widely used in pharmaceutical products.

Hydrogels may tend to dehydratation and lose their original texture, therefore propylene glycol as a humectant was added. Propylene glycol and Tween 80 were also used to improve solubility of KET and as permeation enhancers. Initially, as preservatives, nipagines M and P were chosen, but because of their interaction with KET (appearance of pink colour after 7 days of storage of hydrogels at 40°C±2°C and 75%±5% RH), bronopol as compatible excipient was used (Table 1). Bronopol is soluble in water, it shows antibacterial activity at pH 5.0-8.0 and it is generally regarded as a non-irritant and nonsensitizing material at concentrations up to 0.1% w/v [10].

It was noted that all obtained hydrogels had uniform appearance and appropriate consistency. They were easily spreadable with acceptable adhesion and good mechanical properties. Over time it appeared that there were no significant changes in organoleptic properties of prepared hydrogels (data not shown). A topical dosage form needs to have a semisolid consistency, to be easily spreadable on the skin and able to remain in the application site. Therefore, viscosity of prepared hydrogels during 6 months storage at different temperature and relative humidity conditions was monitored. In hydrogels stored 6 months at 4°C±2°C, 25°C±2°C and 60% ± 5% RH no significant changes in Carbopol 980 and HEC hydrogels viscosity were observed (viscosity was about 13000 mPa·s and 12700 mPa·s, respectively). However, hydrogels stored for 6 months at 40°C±2°C and 75%±5% RH have become slightly more fluid (from 13008 mPa·s to 12833 mPa·s in Carbopol 980 hydrogels and from 12700 mPa·s to 10959 mPa·s in HEC hydrogels) and in HEC hydrogels stored at this conditions sedimentation of KET was additionally observed.

The pH value of all prepared hydrogels was set in the range 6-7 not to affect degradation of KET, which is stable at pH 6-8 [12]. However, in HEC hydrogels stored 14 days at 40°C±2°C and 75%±5% RH, decline of pH was reported. Consequently it might cause degradation of KET and decrease of its activity. In Carbopol 980 hydrogels, stored for 6 months at all analyzed conditions of temperature and relative humidity, no pH changes were noted.

During 6 months of storage particle size in any of the hydrogels was not higher than acceptable 90 µm [9] and ranged 31-36 µm.

KET content, determined by HPLC method, in Carbopol 980 hydrogels was within acceptable limit 90 - 110% [13], but in HEC hydrogels stored at 40°C±2°C and 75%±5% RH significant changes in KET concentration was observed (Table 2), what might be the consequence of the sedimentation of KET particles. It should also be noted that no additional peaks in the chromatograms of analyzed samples were observed, what indicates stability of KET.

Table 2. Content of ketoconazole in examined hydrogels stored for 6 months at different conditions of temperature and relative humidity

Formulation code	Ketoconazole content (%)*				
	Days of storage				
	0	14	30	90	180
4°C±2°C and 60%±5% RH					
H1	97.09±2.86	93.53±0.92	92.08±2.19	95.05±2.62	92.91±0.50
H2	91.78±1.09	94.23±1.53	98.10±.01	92.80±2.27	105.36±2.36
H3	93.90±1.03	93.45±1.14	98.83±1.44	101.90±1.57	99.28±1.81
C1	96.82±0.51	101.85±0.87	96.15±4.78	104.00±0.91	104.55±1.14
C2	94.68±1.55	98.70±0.87	97.70±0.26	97.50±0.07	96.30±0.58
C3	97.81±0.64	99.70±0.36	101.50±0.81	98.95±1.37	98.30±0.28
25°C±2°C and 60%±5% RH					
H1	97.09±2.86	98.10±4.29	93.14±1.06	97.85±0.77	95.05±0.24
H2	91.78±1.09	96.02±1.37	105.70±1.03	101.45±0.70	103.20±2.61
H3	93.90±1.03	94.90±0.77	95.41±2.31	102.45±2.82	94.70±2.77
C1	96.82±0.51	98.55±0.45	98.10±0.04	98.20±0.23	103.85±1.47
C2	94.68±1.55	99.60±0.72	95.85±0.54	97.20±1.17	98.30±0.54
C3	97.81±0.64	100.35±0.25	99.75±0.08	100.85±0.53	99.00±1.14
40°C±2°C and 75%±5% RH					
H1	97.09±2.86	76.73±0.09	88.81±0.83	99.35±0.23	115.75±1.50
H2	91.78±1.09	97.60±2.28	94.71±1.52	105.00±0.06	98.35±1.18
H3	93.90±1.03	97.90±1.25	89.62±1.56	109.65±0.13	107.15±1.82
C1	96.82±0.51	97.90±0.35	93.35±0.95	93.35±0.89	102.95±0.43
C2	94.68±1.55	95.65±0.61	98.05±0.76	107.10±3.22	100.65±1.92
C3	97.81±0.64	98.40±0.51	96.85±0.42	99.70±3.23	102.70±0.59

*The mean values ± S.D. from three independent experiments are presented

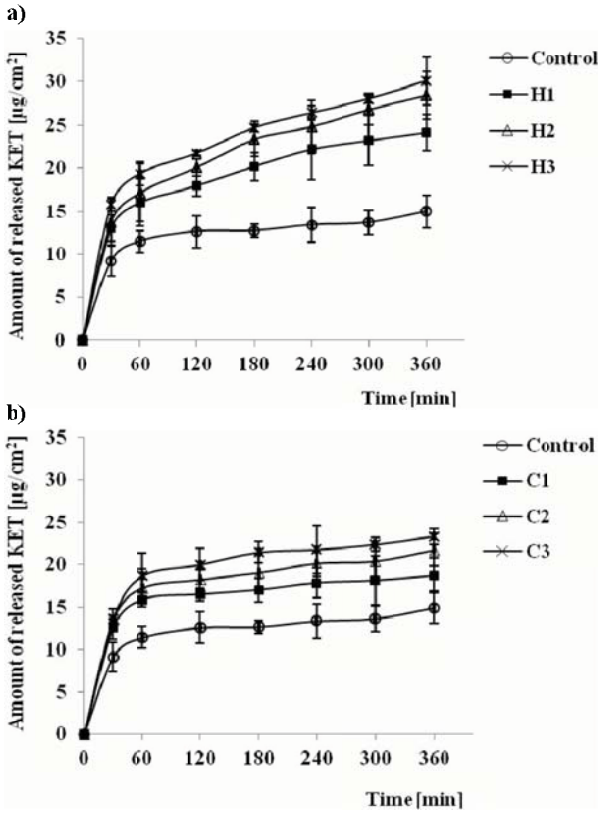

Fig. 1. Amount of ketoconazole per unit area (µg/cm^2) released from HEC hydrogel formulations (a), Carbopol 980 formulations (b) and commercially available product (control)

Results of the drug release evaluation followed for 6 h are presented in Figure 1. As it is shown, KET was significantly faster released from HEC hydrogels. After 6 h, the

cumulative amount of KET released from HEC hydrogels was in the range 24.16-30.07 μg/cm^2, whereas at the same time the amount of KET released from Carbopol 980 hydrogels ranged 18.70-23.37 μg/cm^2. This might be probably caused by differences in polymers properties. Carbopol 980 is an acidic polymer and the compounds, which are weak bases like KET, are slower released from hydrogels based on this polymer [8]. It was also found that the amount of released KET significantly increased with increasing concentration of Tween 80, which improved KET solubility. The greatest amount of KET was released from hydrogels H3 and C3 with 3% addition of Tween 80 (30.07 μg/cm^2 and 23.37 μg/cm^2, respectively). For comparison, the amount of KET released from commercially available product was definitely lower (14.96 μg/cm^2).

CONCLUSIONS

In this study, it was demonstrated that hydrogels with KET based on Carbopol 980 possess the most favourable physical and chemical properties. They were more stable at different temperature conditions and relative humidity than HEC hydrogels and no significant pH, viscosity or KET content changes were noticed during 6 months of storage. *In vitro* release study showed that KET was definitely faster released from all prepared hydrogels than from commercially available product. The obtained results suggest that designed Carbopol 980 hydrogels can be successfully used as vehicle of KET to topical dosage forms.

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