

FORMULATION AND EVALUATION OF CROCONAZOL HYDROCHLORIDE TOPICAL GELS

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تناولت الدراسة استعمال بعض البوليمرات لدراسة مدى ملاءمتها لتحضير هيدروكلوريد الكروكونازول كهلاميات موضعية ومن المعروف أن هذا العقار من مجموعة الأزولات التي لها تأثير على الفطريات و كان تركيز العقار في الهلاميات واحد في المائة بالوزن. وقد اختيرت بعض البوليمرات في تركيبات مناسبة لتحضير الهلاميات مثل ميثيل السليلوز والتيلوز وكحول عديد الفينيل والبلورونك ف الجليكول عديد الاثيلين والكريبول بي والاديسبرت. وتناول البحث دراسة انطلاق العقار من الهلاميات المختلفة عبر غشاء السلوفان واستعمل محلول الفوسفات المنظم ذو الأس الهيدروجيني كوسط مستقبل للعقار المنطلق من الهلاميات ووجد أن انطلاق العقار من الهلاميات يترتب تبعاً لهذا الترتيب المثل سليلوز، التيلوز، البلورونك ف، كحول عديد الفينيل ثم الهلاميات الأخرى. كما وجد أن آلية انطلاق العقار من الهلاميات تتبع نظام الانتشار وقد تم حساب بعض معاملات هذه الآلية. كما تم دراسة تأثير تركيز العقار وتركيز البوليمر على انطلاق العقار من هلاميات البلورونك ف واستعملت عدة تركيبات للعقار هي نصف وواحد واثنين في المائة وعدة تركيبات للبوليمرات وهي عشرون وخمس وعشرون وثلاثون. وأثبتت النتائج وجود علاقة طردية بين زيادة تركيز العقار ومعدل الانطلاق وكذلك وجود علاقة عكسية بين معدل انطلاق العقار وزيادة تركيز البوليمر. وأخيراً تم دراسة فعالية هلاميات العقار ضد بعض الفطريات المزروعة على الأجار اجار ووجد أن الهلاميات لها تأثير جيد ضد الفطريات المستعملة.

This study was designed to evaluate different polymers for their suitability as vehicles for topical drug delivery systems. Croconazol hydrochloride is an azol derivative used as antimycotic agent. It was incorporated in this vehicles as a gel forms in a concentration of 1.0% w/w. Polymers used in this study are methylcellulose (MC), Tylose (Ty), Polyvinyl alcohol (PVA), Pluronic F-127 (pl. F-127), Polyethylene glycol (PEG), Carbopol 974P (Carb.) and Eudispert mv. (Eud). They were used in a suitable concentration for gel formulation.

In-vitro release characteristics of the drug from different gels were carried out using dialysis membrane in phosphate buffer pH 5.2. The release data were treated with various kinetic principles to assess the relevant parameters. The general rank order of Croconazol hydrochloride release from the prepared gel were MC > Ty > pl. F-127 > PVA > Carb > Eud > PEG. The results showed that, the release of drug from the prepared gels obeyed the diffusion model (Higuchi's equation). Some kinetic parameters were calculated such as diffusion coefficient, permeability coefficient and the partition coefficient. The results indicated a direct dependence of the release rate on the diffusion coefficient. The influence of initial drug concentration (0.5, 1.0 and 2.0% w/w), and pl F-127 concentration (20, 25, 30% w/v) on the release patterns was studied. The obtained data revealed an inverse correlation between the drug release rate and the pluronic F-127 concentration and a direct correlation between the drug release rate and the initial drug concentration. The anti-fungal activity of the different gel formulations was evaluated by agar-cup plate technique using five fungal species. The results obtained indicated that, all gel formulations have good anti-fungal activities.

INTRODUCTION

Topical products are important class of drug delivery systems and their uses in the therapy become more widespread. The purpose of topical dosage form is to conveniently deliver drugs to localized area of the skin.¹

Superficial fungal infections such as dermatophytosis or candidiasis are increasing and represent common dermatological problems. The therapeutic efficacy of a topically applied drug used for dermatological diseases is not always as complete as desirable because it depends on the ability of the drug to penetrate the skin and accumulate in the deeper layers of the skin.² Dermatophytes are a group of closely related fungi that have capacity to invade keratinized tissues (skin, hair, and nails) of human and animals to produce a harmful infection. Many anti-fungal peptides produced by some organisms or synthesized compound are active against the growth of yeast and filamentous fungi.³⁻⁵

Croconazol hydrochloride is a synthetic imidazole derivative (phenyl vinyl derivative) It is a potent topical anti-fungal agent. It is active against dermatophytes (the causative organisms of tinea infections) and yeasts (*Candida albicans*).⁶ Croconazol hydrochloride is water insoluble drug, so hydrogel may be an excellent vehicle for the topical delivery of this drug. Many authors used hydrogel bases such as Carbopol,⁷ Poloxamers,⁸ Cellulose derivatives⁹ and Eudispert¹⁰ as topical drug delivery systems.

The purpose of the present study is to formulate Croconazol hydrochloride in different gel forms, to determine the *in-vitro* release of the drug from these formulations, to study the effect of initial drug concentration and polymer concentration on drug release profile and lastly to study the anti-fungal activity of these formulations.

EXPERIMENTAL

Materials

Croconazol hydrochloride (Shionogi, Japan), Methylcellulose (Dow Chemical Co. USA), Carbopol 974P (BF. Goodrich Chem. Co. USA), Eudispert mv (Rhom Pharma, GmbH, Darmstadt, Germany), Pluronic F-127

(BASF, Ludwigshafen, Germany), Tylose (methylhydroxyethylcellulose) (Clariant GmbH, Wiesbaden, Germany), Polyethylene glycol, MW 600, 1000 (Merck-Schuchardt, Munchen, Germany), polyvinyl alcohol (BDH, England). Triethanolamine, sodium dihydrogen orthophosphate (EL-Naser Chemical Co. Egypt). Semipermeable Fisher cellophane membrane No. 30/32 (Fisher Sci. Co. London, England). Sabouraud-dextrose agar (Oxoid, England). Miconazol cream (MUP, Egypt containing miconazol nitrate 2% w/w) was used as control in the microbiological study. All other chemicals are of pharmaceutical grades.

Apparatus

Electronic balance (Percisa 205A, Oerlikon AG, Zurich, Switzerland), Magnetic stirrer with hotplate (Gallenkamp, England), pH-meter (Jenway, Ltd. UK), UV-spectrophotometer (Schimadzu, Japan).

Methods

Preparation of gels

Croconazol hydrochloride gels (1.0% w/w) were prepared by different methods according to the polymer used as the following.

Preparation of pluronic F-127 gel

Pluronic F-127 gel (25% w/v) was prepared by the cold method described by Schmolka.¹¹ The weighed amount of the polymer was slowly added to water with gentle mixing. The mixture was left in refrigerator (4°) overnight to complete dissolution of the polymer. After the formation of a clear viscous solution, a solution of the drug was added to the polymer solution and mixed very gently with a glass rod. The solution was left at room temperature until a clear gel is formed.

Preparation of Carbopol 974P gel

Carbopol 974P gel (2.0% w/v) was prepared adopting the method of French *et. al.*¹² Carbopol was added to the water portion wise with gentle mixing. After complete addition of the polymer and mixing, the drug solution was added. The gel was spontaneously formed by addition of two millilitres of triethanolamine.

Preparation of Eudispert mv gel

Eudispert mv (5.0% w/v) was sprinkled gradually into water with gentle mixing, the drug solution was added. Two millilitres of triethanolamine were added until a gel is obtained.

Preparation of methylcellulose and Tylose gels

The weighed amount of the powder (methylcellulose 5.0% w/v, Tylose 2.0% w/v) was added gently to hot water. Stirring was continued to prevent the formation of lumps. The drug solution was added, then left overnight in a refrigerator (4°) to complete solubility and gel formation.

Preparation of polyvinyl alcohol gel

The weighed amount of polyvinyl alcohol (15% w/v) was added to water and mixed very gently till complete solubilization of the powder and gel formation, then the drug solution was added.

Preparation of polyethylene glycol gel

The polyethylene glycol gel was prepared using the heat and mechanical incorporation method. Polyethylene glycol of MW 1000 and 600 at a ratio of 1:2 w/w were placed in a beaker and heated on water bath till complete mixing and stirred until congealed. The drug solution was mixed gently to produce the final gel.

In-vitro release of Croconazol hydrochloride from different gel formulations

In-vitro release of the drug from different gel formulations was evaluated using standard cellophane membrane stretched at the end of the dialysis cell (2.5 cm in diameter and 10 cm in length) that contain 1.0 gm of gel (donor compartment). The acceptor compartment composed of glass beaker (250 ml) containing 100 ml of phosphate buffer pH 5.2 as release medium, and kept at 37° ± 0.5. The content of the beaker was stirred using magnetic stirrer with hotplate (50 rpm).

Aliquots (5.0 ml) were withdrawn at time intervals and measured spectrophotometrically at max 284 nm.¹³ against blank similarly treated. The release medium kept constant by adding a fresh buffer to the acceptor compartment.

All experiments were carried out in triplicate and the average values were calculated.

Mathematical treatment of the data

The *in-vitro* release data of Croconazol hydrochloride from the investigated gel formulations was linearized according to zero-order, first-order and diffusion models kinetics. The permeation parameters of the drug (permeability coefficient p_m , diffusion coefficient, D_{app} , and partition coefficient k_p) were calculated according the following equations:

$$Q = 2 C_o \sqrt{Dt} \quad \text{Eq. 1}$$

$$D = (\text{slope} / 2 C_o)^2 \quad \text{Eq.2}$$

$$P = (k.D) / L \quad \text{Eq. 3}$$

Where:

Q: the amount of drug released per unit area (mg/cm²).

C_o: the initial drug concentration (mg).

t: time interval (hr.).

D: Diffusion coefficient.

P: permeability coefficient.

K: partition coefficient.

L: thickness of membrane (cm).

The release profile was constructed by plotting the cumulative amount of the released drug per unit area (mg/cm²) versus $t^{1/2}$. The diffusion coefficient was calculated from the slope obtained, C_o was the initial concentration of the drug in the vehicle. The partition coefficient (k) was calculated from P and D and the thickness of membrane (equation 3).

Anti-fungal activities

Agar-cup diffusion technique was adopted.¹⁴ Five fungal species were used, some of them are pathogenic for human and animals, namely, *Aspergillus flauus*, *Candida albicans*, *Chrysosporium tropicum* (keratinophyte), *Penicillium chrysogenum* and *Trichophyton rubrum* (dermatophyte) were cultivated on Sabouraud-dextrose agar. After solidification, 1 cm holes were made and filled with accurately weighed 0.25 gm of gel formulations or control (miconazol cream). The plates were incubated at 28° for 3 days. The inhibition zones around the perforations were measured in mm.

RESULTS AND DISCUSSION

In-vitro release of Croconazol hydrochloride from different gel formulations

The results of the percentage release of Croconazol hydrochloride from various gel formulations, evaluated through four hours period, are exhibited in Figure 1. The general rank order of the release of the drug was observed to be MC gel > Ty > pl. F-127 > PVA > Carb. > Eud and finally PEG gel (Fig. 1).

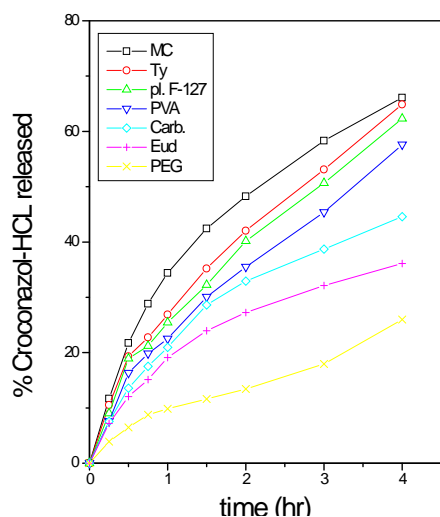


Fig. 1: *In-vitro* release profile of Croconazol hydrochloride (1% w/w) from different topical gel formulations.

Effect of vehicle on drug release

It is well known that, the nature of the vehicle has a great influence on drug release. In the present study, different hydrogel bases with different polymer structure were used to compare the release profile of the drug from these vehicles. The diffusion of the drug from any base depends on the nature and composition of the individual base.¹⁵ The cellulose derivative and pl. F-127 exhibited a superior drug release which might be related to vehicle nature. Croconazol hydrochloride release from these gel formulations (after four hours) was 1.5, 2, 3-folds higher than Carb, Eud and PEG gel respectively, that may be reflect the affinity of the drug to the gel base.

Kinetic of the *in-vitro* release studies

Various kinetic models, namely zero-order, first-order fashions and Higuchi diffusion mechanism were applied to obtain the best fit of the data. The confirmation between the different mechanisms was dependant upon the correlation coefficient (r) Table 1. The results revealed that, the release pattern of the drug from the tested formulations followed the Higuchi diffusion model ($r = 0.974-0.999$).

Table 2 represents the kinetic parameters of diffusion mechanism of Croconazol hydrochloride released from different gel formulations. From Table 2, it was shown that, cellulose derivative gels and pl. F-127 gel have higher diffusion coefficient (D_{app}) than other

Table 1: Correlation coefficient (r) after mathematical treatments of the release data of Croconazol hydrochloride from different gel formulations according to zero-order, first-order and diffusion mechanisms.

Polymer used	Zero-order *(r)	First-order *(r)	Diffusion *(r)
Methylcellulose	0.943	0.841	0.986
Tylose	0.990	0.943	0.997
Pluronic F-127	0.986	0.896	0.999
Polyvinyl alcohol	0.968	0.895	0.994
Carbopol 974P	0.977	0.935	0.995
Eduspirt mv	0.962	0.882	0.994
Polyethylene glycol	0.918	0.939	0.974

*r : Correlation coefficient.

Table 2: The kinetic parameters of diffusion mechanism of Croconazol hydrochloride released from different topical gel formulations.

Polymer used	$D_{app} \times 10^{-3}$ (cm^2/sec)	$P_m \times 10^{-2}$ (cm/sec)	$K_p \times 10^{-3}$
Methylcellulose	8.082×10^{-3}	3.08×10^{-2}	3.810×10^{-3}
Tylose	7.655×10^{-3}	3.47×10^{-2}	4.533×10^{-3}
Pluronic F-127	7.558×10^{-3}	3.26×10^{-2}	4.323×10^{-3}
Polyvinyl alcohol	6.829×10^{-3}	3.43×10^{-2}	5.022×10^{-3}
Carbopol 974P	6.430×10^{-3}	4.48×10^{-2}	6.980×10^{-3}
Eudispert mv	5.864×10^{-3}	5.54×10^{-2}	9.440×10^{-3}
Polyethylene glycol	4.842×10^{-3}	7.71×10^{-2}	15.90×10^{-3}

D_{app} : Diffusion coefficient (cm^2/sec).
 K_p : Partition coefficient.

P_m : Permeability coefficient (cm/sec).

gel formulations. Therefore a greater release of the drug expected when there is less affinity of the drug for the base. As in case of methylcellulose gel which gave the highest diffusion coefficient value ($8.082 \times 10^{-3} cm^2/sec$). Also, it was observed that, the gel form with highest release of the drug and high diffusion coefficient yielded the low partition coefficient value (k) 3.81×10^{-3} . PEG gel with the lowest drug release gave the highest partition coefficient value of $15.9 \times 10^{-3} cm/sec$. These results were in agreement of the finding by Babar *et al.*¹⁵

Effect of drug concentration on the release profile of the drug

The release profile of Croconazol hydrochloride from the pl. F-127 gel formulation (25% w/v) containing different concentration of the drug (0.5, 1.0, 2.0% w/w) was plotted according to Higuchi model (Figure 2). It showed that, the release of the drug increased with increase the loaded drug in the vehicle, also the diffusion coefficient increased with increase the initial concentration of the drug Table 3. Similar results were reported by EL-Gendy *et al.*¹⁶ for release of flurbiprofen from different gel bases. Also, Hassan *et al.*¹⁷ reported that, the release of ciprofloxacin hydrochloride and norfloxacin from different gel bases was dependant on the concentration of initial drug concentration.

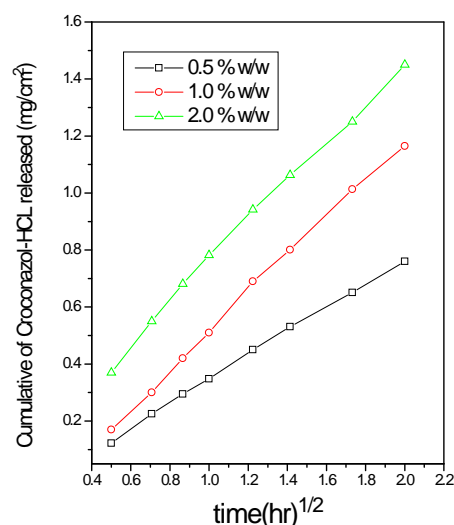


Fig. 2: *In-vitro* release profiles of Croconazol hydrochloride from Pluronic F-127 gel (25% w/v) as a function of initial drug concentration.

Effect of polymer concentration of the gel on the release of the drug

The release profile of Croconazol hydrochloride (1.0% w/w) from the gel bases composed of 20%, 25% and 30% w/v pl. F-127 presented in Figure 3. In general, it can be seen as the concentration of pl. F-127 increased the diffusion coefficient of the drug decreased Table 3, (7.65×10^{-3} , 7.558×10^{-3} and $6.86 \times 10^{-3} cm^2/sec$ for 20%, 25%, and 30% w/v) polymer concentration respectively. EL-Gibaly *et al.*¹⁸ reported similar results on their study on clotrimazole release from different gel formulation.

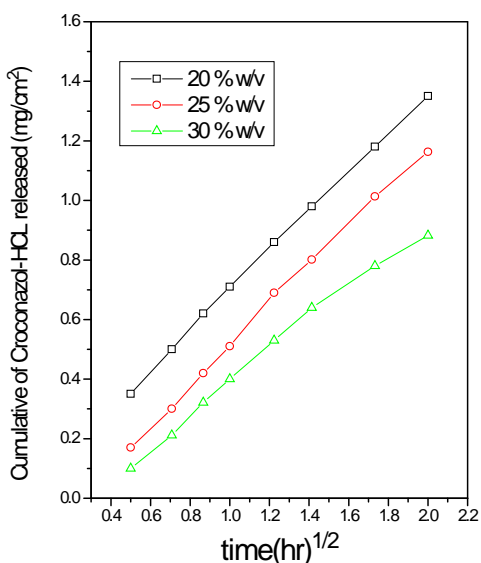


Fig. 3: *In-vitro* release profile of Croconazol hydrochloride (1% w/w) from pluronic F-127 gel as a function of polymer concentration.

Anti-fungal activity of Croconazol hydrochloride gel

The anti-fungal activity of the gel formulations of Croconazol hydrochloride was carried out by measuring the inhibition zone in (mm) and compared by the effect of miconazol gel (MUP, Egypt) as control. Table 4 displayed the inhibition zone of Croconazol hydrochloride gel formulations. All the tested formulations gave a good inhibition zone. From the obtained results, it appeared that, all gel formulations had a high inhibitory effect on the most tested fungal species, but had a moderate inhibitory effect against *Candida albicans*. Also, It is observed that, the inhibition zone (mm) of Croconazol hydrochloride gels was larger than the control gel. In addition, the control gel was inactive (no inhibition zone) against *Penicillium chrysogenum*, but all Croconazol hydrochloride gel had highly inhibition activity. Lastly, from the obtained results, it is seemed that, there was a slight difference in inhibition activity of the different gel formulations, but with the same rank order of the *in-vitro* release of the drug from the gel forms, with an exception, PEG gel.

Table 3: Effect of drug concentration and polymer concentration on the Kinetic parameters of diffusion of Croconazol hydrochloride from pl. F-127 gel formulations.

	D_{app} (cm ² /sec)	P_m (cm/sec)	K_p
- drug concentration			
0.5% w/w	6.110×10^{-3}	5.26×10^{-2}	8.610×10^{-3}
1.0% w/w	7.558×10^{-3}	3.26×10^{-2}	4.323×10^{-3}
2.0% w/w	8.000×10^{-3}	3.432×10^{-2}	4.290×10^{-3}
Polymer concentration			
20 % w/v	7.654×10^{-3}	2.96×10^{-2}	3.867×10^{-3}
25 % w/v	7.558×10^{-3}	3.26×10^{-2}	4.323×10^{-3}
30 % w/v	6.861×10^{-3}	4.53×10^{-2}	6.610×10^{-3}

D_{app} : Diffusion coefficient (cm²/sec).

P_m : Permeability coefficient (cm/sec).

K_p : Partition coefficient.

Table 4: Anti-fungal activity of different Croconazol hydrochloride gel formulations.

Gel type	Inhibition zone (mm) using the following different fungal speices				
	<i>Aspergillus Flauus</i>	<i>Candida albicans</i>	<i>Chryso. tropicum</i>	<i>Pencillium chryso</i>	<i>Trichophyton rubrum</i>
Methylcellulose	45	26	70	50	52
Tylose	37	19	60	50	46
Pluronic F-127	40	16	62	48	45
PVA	32	16	47	37	38
Carbopol 974P	40	24	64	58	51
Eudispert mv	23	16	46	36	30
PEG	70	35	72	60	64
Control (miconazol)	30	16	15	0	22

0 : no inhibition zone

Conclusion

- The *in-vitro* study is considered a useful methodology for screening Croconazol hydrochloride topical gel formulations.
- The release mechanism of the drug from the different gel formulations obeys diffusion mechanism.
- The amount of drug release of the drug depends on the nature of the polymer used for preparation of gel.
- There is a direct relationship between the amount of drug released from the pl. F-127 gel and the initial drug concentration and an inverse relationship with the concentration of the polymer.
- The Croconazol hydrochloride gel formulations show an excellent activity against different species of fungi.

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