Preparation and in-Vitro Evaluation of Mucoadhesive Clotrimazole Vaginal Hydrogel

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Abstract

Clotrimazole (CLO) is an antimycotic imidazole derivative applied locally for the treatment of vaginal yeast infections. In this study, CLO was formulated as vaginal mucoadhesive hydrogel, using different types of mucoadhesive polymers to ensure prolonged contact between active ingredient and vaginal mucosa.

Physicochemical properties of the prepared formulas were evaluated as a visual inspection, pH, swelling index, spreadability, and mucoadhesive characteristics, in addition to an *in-vitro* drug release. The influence of type and concentration of polymers as CMC-Na (1.5, 2.5, and 3.5%w/w), carbopol 940(0.25, 0.5, and 1 %w/w) and poloxamer 407 (15, 25, 30%w/w) on CLO release from the prepared gels were also investigated. All the different concentrations of each polymer gave a percent of drug release profile inversely proportional with the polymer concentration. The lowest concentration showed faster release within the first half an hour of 99± 2% of drug release , while CMC-Na at 3.5% (F2) demonstrated the least release of 40 % after three hours.

On the other hand, poloxamer 407 of 25% gel (F7) produced the highest release of 98%, while carbopol 1% (F5) and CMC-Na (F1) produced 85% and 80% ,respectively. All the prepared formulas were following Higuchi kinetic model. Based on overall result, CLO can be formulated as mucoadhesive vaginal hydrogel using 25%poloxamer as the best prepared formula.

Key words: Hydrogel, Clotrimazole, Mucoadhesive.

تحضير و تقييم خارج الجسم الحي لمادة كلوتريمازول بشكل هلام مائي باستخدام قواعد مخاطية الالتصاق للاستخدام المهبلي

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الخلاصة

يعتبر عقار الكلوتريمازول واحدا من مشتقات الايميدازول يستخدم موضعيا في علاج فطريات المهبل . في هذه الدراسة تم تحضير الكلوتريمازول بصيغة هلام مائي له القابليةعلى الالتصاق بالجدران المخاطية .

تم تقيم الصيغ من حيث الخواص الفيزياوية-الكيميائية مثل(الوصف الخارجي ,الاس الهيدروجيني، نسبة الانتفاخ,قابلية الانتشار وقابلية الالتصاق)بالاضافة الى تقيم تحرر الدواء خارج الجسم .تمت دراسة العوامل المؤثرة في تحرر الدواء كنوع وتركيز القواعد الجلاتينية (١,٥- ٢,٥ - ٣,٥)% من الكاربوكسي مثيل سيليلوزصوديوم و(٠,٥- ٠,٢٥ - ١)% من الكاربابول ٩٤٠ و (١٥-٢٥ - ٣٠)% من البولوكسامير ٤٠٧.

وقد ظهرت ان كمية تحرر كلوتريمازول تتناسب بصورة عامة عكسيا مع زيادة تركيز البوليمرفي جميع الصيغ المحضرة باختلاف تراكيزها, حيث ان تحرر الدواء كان بمعدل٩٩±٢% خلال نصف الساعة الاولى للضيغ ذوات التراكيز القليلة لكافة البوليمرات ,بينما ابطئ تحرر في تركيبة كاربوكسي مثيل سليلوز صوديوم ٣,٥% بمقدار ٤٠% خلال ثلاث ساعات . من الناحية الاخرى , ان التركيبة الحاوية على البولوكسامير ٤٠٧ (٢٥%) اظهرت اعلى تحررللدواء(٩٨%) بعد مرور ساعتين ومن ثم الكاربابول٩٤٠ (٨٥%) واخيرا الكاربوكسي مثيل سيليلوز صوديوم(٨٠%).ان تحررالدواءمن الهلام المائي لكافة الصيغ المحضرة تخضع لقانون العالم هيجوشي .

بناءا على النتائج المستحصلة اثبت بان دواء الكلوتريمازول يمكن تشكيله بصيغة هلام مائي مهبلي باستخدام ٢٥% البولوكسامير ٤٠٧.

الكلمات المفتاحية : هلام مائي ، كلوتريمازول ، الالتصاق المخاطي

Introduction

Vaginal delivery as a route of drug administration is currently of a great interest to scientists and pharmaceutical industry [(1)]. Many different approaches have been tested to develop vaginal drug delivery system (DDS) that can meet clinical and patient requirements [(2)]. Considerable attention has been focused on the development of vagina l (DDS) that providing a gradual release of drug over time. One interesting group of auxiliary agent is the mucoadhesive polymers, which are the basis of newly designed system. The most widely used mucoadhesive vaginal (DDS) is (hydrogel) [(3, 4)].Hydrogel is one of the

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upcoming classes of polymer-based controlled release (DDS) due to several factors among them are their hydrophilicity, biocompatibility mucoadhesive characteristics [5]. Also gels are easy to manufacture, comfortable, and have ability to spread onto the surface of mucosa to achieve an intimate contact with vaginal mucosa. Moreover, because of their high water content and their rheological properties, they provide hydration and lubrication action [6].

Clotrimazole is an imidazole derivative with a broad spectrum of antimycotic activity, the drug is a weak base described as a white to pale yellow crystalline powder, practically insoluble in water, freely soluble in chloroform and methanol [7, 8].The objective of this study is to formulate 1% Clotrimazole vaginal mucoadhesive hydrogel using three different types of polymers with different concentration of each.The physicochemical characteristics of gel and mucoadhesive property of selected formulas were also evaluated.

Materials and Method

Materials

Clotrimazole and methyl paraben were supplied by (Samara Drug Industry), Sodium carboxy-methylcellulose (BDH chemicals Ltd, Pool, England), Cabopol 940(Hi Media lab.,Ltd, Mumdai, India), Poloxamer 407. (Sigma, Germany), monobasic potassium phosphate (Fluka , Switzerland), tween 80 (Merk-Schuncherdt, Germany).

Method

Preparation of Clotrimazole vaginal gels:

Nine formulas of CLO hydrogel were prepared by cold mechanical method[9]as shown in (table 1). Pre weighed amount of polymers was dispersed in sufficient quantity of distilled water (containing 1% tween 80), the dispersion was homogenized using magnetic stirrer for 1 hour and then left to for 24 hours for complete swelling and equilibration of polymer. Weighted amount of CLO and methyl paraben were added with continuous stirring. In case of carbopol 940 containing gel, [specific amount of triethanolamine (TEA) drop by drop was added with continuous mixing] and the final weight was completed to 100g with the aqueous solution. The formulas were placed in refrigerator to complete the formation of hydrogel[10].

Table (1) Composition of clotrimazole vaginal hydrogels formulas (%w/w).

* Sodium carboxymethyl cellulose

Formula No.	Clotrimazole	CMC-Na*	Carbopol 940	Poloxamer 407	TEA**	Tween 80	Methyl-paraben	Distilled Water
F1	1	3.5	---	---	---	1	0.4	Up to 100
F2	1	2.5	---	---	---	1	0.4	Up to 100
F3	1	1.5	---	---	---	1	0.4	Up to 100
F4	1	---	1.0	---	2.0	1	0.4	Up to 100
F5	1	---	0.5	---	1.0	1	0.4	Up to 100
F6	1	---	0.25	---	0.5	1	0.4	Up to 100
F7	1	---	---	30	---	---	0.4	Up to 100
F8	1	--	--	25	---	---	0.4	Up to 100
F9	1	--	---	15	---	---	0.4	Up to 100

** Triethanol-amine

Evaluation of the prepared hydrogel formulas

Visual examination

The examination considered a series of visual characteristics (consistency, homogeneity) [11].

pH determination

The pH of the prepared gel was measured using pH – meter by putting the tip of the electrode into the gel and after 2 minutes the result was recorded [12].

Swelling study

One gram sample from each formula was soaked into 5ml of phosphate buffer pH 4.0 and left for a specific time, and then the excess buffer was removed and reweights the samples again. This test done for time intervals after one and three hours. The following formula will be used to calculate the swelling ratio.

Swelling ratio= $Ws\text{-}W_0 / W_0 \times 100$

Where *Ws* is the weight of the swollen hydrogel at time t and $W_\circ$ is the initial weight[13].

Spreadability measurement

Spreadability was measured on the basis of"Slip" and "Drag" characteristics of gels. 1.0gm of gel was placed on the center of ground slid plate and spread over an area of 1 cm diameter. The gel was then sandwiched between two slides by the application of 200 gm weight. The spread diameter was recorded after 5 minutes, and measured in cm. This measured area was taken as comparative values for spreadability, (diameter of the spread circle – initial diameter)[12] .

Estimation of drug content by HPLC analysis

The content of CLO in prepared formulas was determined using HPLC analysis method stated in the United State Pharmacopeia (USP) for CLO assay [8]. HPLC from Knauer ,Germany was utilized.

A specified quantity of the gel equivalent to 50 mg was stirred using ultrasound with about 30 ml of the diluents [a mixture of 25% phosphate buffer (4.35mg/ml of K2HPO4) and 75% methanol] and then the volume was made up to 50 ml with same diluents. The above solution was further diluted to obtain solution having concentration of 0.2mg/ml. The chromatographic system was carried out using the following specifications:-

-Column: 4.6-mm x25 –cm; 5µm packing L1.

-Mobile phase: Acetonitrile and Buffer (3: 1), filtered and degassed.

(Buffer: - 4.35mg/ml of dibasic potassium phosphate)

-Detector: UV 254nm.

-Flow rate: 1.5 ml/min.

-injection size: 20 µl.

Mucoadhesive strength measurement

The mucoadhesive strength of selected formula was determined by measuring the weight required to detach the gel from the sheep vaginal mucosal tissue by using a modified chemical balance. A section of vaginal mucosa was cut from the sheep's vaginal cavity and instantly fixed with mucosal side out onto each glass vial using a rubber band.

The vials with vaginal mucosa were stored at 37°C for five minutes; the vial was attached to the balance instead of one of the pans by a height-adjustable hook. Next, glass plate was placed under the mucosal tissue .The gel was placed on the glass plate; then, the height of the vial was adjusted so that the gel could touch the mucosal tissue which was allowed to adhere for three minutes to the gel (preload time)[14] .

On the other side of the balance, a plastic cup was placed to collect water, and balanced with the other side. Water was added drop by drop to the plastic cup until the weight of water in the cup detached the glass slide from the mucosal tissue.

The detachment stress (dyne/cm^2), was determined from the minimal weights that detached two surfaces from each other, was calculated using the following equation

$F = 980\ m/\pi\ r^2$

Where F is the detachment force (dyne/cm^2) per unit area of mucosa (cm^2) ($\pi\ r^2$ in which r is the radius of the vial), (r =1.2 cm) by the balance weight m (g) and 980 is acceleration due to gravity (cm/sec^2).

In-vitro dissolution test

The *in vitro* release of CLO from all hydrogel formulas was performed by using dissolution apparatus type 2 (paddle type).A weighing quantity of a gel (10 gm that contain 100 mg CLO) was uniformly spread on petri dish 4.5cm in diameter, and this was immersed in dissolution jar filled with 900ml dissolution media (phosphate buffer pH 4.0 B.P. containing 2% tween 80) at 37˚C[15]. The paddle was placed about 2cm above the petri dish and rotated at speed of 50 rpm. Samples of 5ml were withdrawn at intervals of 30, 60, 120, and 180 minutes and were replaced with equal volume of the fresh buffer solution each time to maintain constant volume. Samples were analyzed for CLO by HPLC [16].

Variable affecting release profile

Effect of different polymer concentration on the release profile of clotrimazole

The effect of different concentrations of the used polymer on the release profile of the drug was studied; all formulas were subjected to this study.

Effect of different types of polymers on the release profile of clotrimazole

One formula was selected from each polymer CMC-Na, carbapol 940 and poloxamer 407 (the highest release profile). Comparion between these formulas were achieved to show the effect of the polymer on the release.

Release kinetics

To analyze the mechanism and rate of drug release from prepared gel, the dissolution data obtained were fitted to kinetic equations including Zero order, First order, Higuchi and Korsmeyer- peppas. R^2 value was used to show the best fit model to be selected [17].

Results and Discussion

Macroscopic feature:

Visual inspection of prepared gel indicated the homogeneity of all formulas, no phase separation, with semi-rigid texture and white to off white smooth gel. This result is similar to that defined in references [7, 18].

pH

The pH of all formulas were almost neutral as show in table (2).

Table (2) pH of prepared formulas

Formula no.	pH
F1	6.4 ± 0.10
F2	6.4 ± 0.11
F3	6.2 ± 0.08
F4	7.3 ± 0.06
F5	7.4 ± 0.05
F6	7.1 ± 0.08
F7	7.3 ± 0.07
F8	7.0 ± 0.10
F9	7.1 ± 0.085

Swelling index

Swelling of the polymer depends on the concentration of the polymer, degree of cross-linking, ionic strength and presence of water [18]. Increasing in cross- linker concentration shows a direct negative effect due to tighter structure [19] .

Tablet (3) shows that F1 and F2 started swelling rapidly and continued for three hours due to high amount of hydroxyl group in each cellulose unit which contributing in presences of hydrogen bound[20].

F4 shows less swelling index at the beginning but polymer when fully hydrated swelled in phosphate buffer and surface disintegration of the gel occurred at the end of three hours [21].

The swelling ratios of ionic hydrogels were often an order of magnitude higher than those of neutral gels because of the presence of intermolecular interactions.

(F7-F9) being contain neutral gel have no swelling appearance may be due to full hydration of the polymer [20, 22].

Table (3) Swelling ratio of prepared formulas

Formula no.	Swelling ratio w/w after 1 hour	Swelling ratio w/w after 3 hours ± S.D.
F1	50% ± 1%	80% ± 1.8%
F2	45% ± 1. 2%	150% ± 0. 23%
F3	0%	50% ± 0.5%
F4	8% ± 0.3%	30% ± 0.65%
F5	--	--
F6	--	--
F7	--	--
F8	--	--
F9	--	--

-- : swelling ratio is negligible because the polymer used is water soluble [20].

Spreadability measurement

All prepared gels using different concentrations of different polymers were spreadable on the tissue surface as show in table (4). Spreadibilty of prepared gels was decreased as the polymer concentration increased as in formula (F1,F5,F7). It should be mentioned that the addition of polysorbate 80 improved the physical characteristics including spreadability, consistency and skin feel duo to an increase in the diameter of the spread circle of the gel [23].

Table (4) Spreadability values of prepared gels.

Formula No.	F1	F2	F3	F4	F5	F6	F7	F8	F9
Spread-ability cm ± S.D	1.5 ±0.1	2 ±0.08	3 ±0.14	1.5 ±0.08	2.2 ±0.1	2.8 ±0.12	0.4 ±0.02	0.6 ± 0.04	3 ±0.15

Mucoadhesive strength measurement

The mucoadhesive strength of the prepared gels was affected by polymer type and it was found in the following order: poloxamer 407 >carbopol 934 > CMC Na (F8, F4, and F2) respectively as illustrated in table (5). This is due to the fact that mucoadhesive strength depends on the polymer structure, molecular weight, and other physicochemical properties which are varied according to the polymer type [24, 25].

Table (5) Result of force required for detachment

Formula No.	F2	F4	F8
Detachment stress ($dyne/cm^2$) ±S.D.	4009.6 ± 2.7%	5310.1 ± 3.5%	6783.8 ± 4.3%

Content uniformity

A validated HPLC method was used for determination of CLO content in the gels. Table 6 indicated that the results obtained were within the limit of USP monograph (90-110) % of the theoretical content of the prepared gel [8].

Table (6) Drug content uniformity

Formula No.	F1	F2	F3	F4	F5	F6	F7	F8	F9
% of original amount od drug ± S.D.	103 ± 0.20	104 ± 0.30	99 ± 0.80	95.2 ± 0.23	97.5 ± 1.00	101 ± 1.20	98.8 ± 0.43	97.6 ± 0.66	99 ± 0.50

Effect of polymer concentration on the in vitro release

It was found in formulas F1, F2 and F3 that the amount of drug release from CMC-Na hydrogel was decreased with increasing polymer concentration as in figure (1). This result could be attributed to an increase in the density which leads to increase in diffusional path way of drug from more sticking cellulose matrix [26].

Figure (2) illustrates the difference in percent of drug release which was attributed to the mesh size of the network. The mesh size depends on the grade of crosslinking and the chemical structure of the monomers, so the diffusivity of the incorporated drug was effected [27]. The hydrogel with low degree of crosslinking (F6) was fast released. On the other hand, gradual release of drug occurred in F4 due to the tighter network [28].

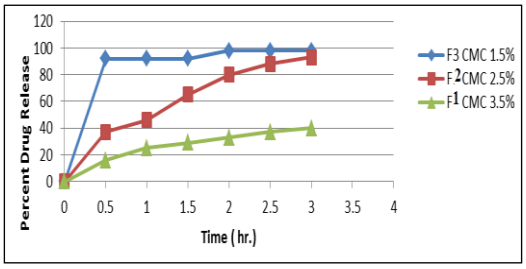

Figure 1: Effect of CMC-Na concentration on the release of clotrimazole from three different formulas.

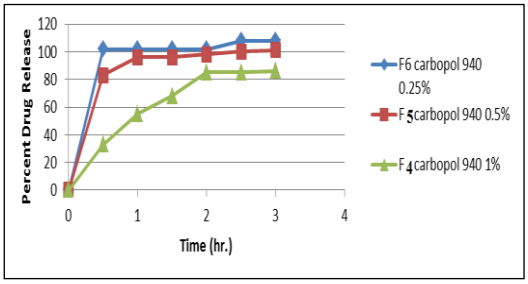

Figure 2: Effect of different carbopol 940 concentration on the release of clotrimazole from three different formulas.

Figure (3) showed that the increase in polyxamer 407 concentration led to decrease the release rate (F7- F9). It is hypothesized that the drug is released by diffusion through the extra micellar water channels of the hydrogels matrix, and higher concentration of polyxamer 407 causes smaller size of water channels [29].

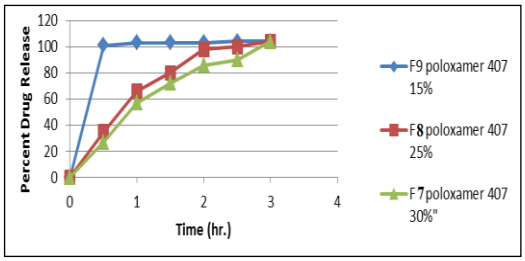

Figure 3: Effect of different poloxamer 407 concentration on the release of clotrimazole from three different formulas.

Effect of polymer types on in vitro release

Three formulas of different types of polymers which show best release profile were chosen for this study and illustrated in figure (4). Poloxamer 407 (F8) performed higher release with control rate when compared with other formulas. This due to the mechanism of drug delivery via poloxamer which occurred by diffusion and dissolution of the gel at administered site. Poloxamer presented as complete dissolution in three hours, and this feature can be used to prepare pharmaceutical formulation. The amount of CLO released, was found in the following order: poloxamer >carbopol and CMC, this may be due to higher viscosity which was responsible for hindering drug release from gel matrix [30]. Figure 4: Effect of different polymers on the clotrimazole release from different formulas.

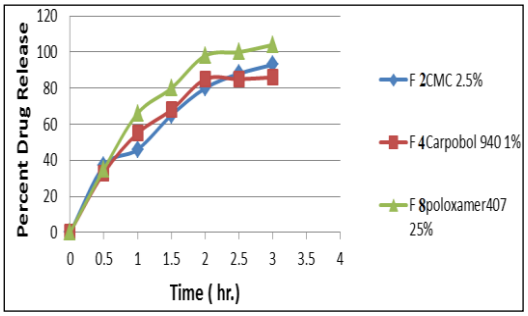

Figure 4: Effect of different polymers on the clotrimazole release from different formulas.

Mathematical modelling of drug release profile

According to the results obtained and shown in table (7), the release of CLO from polymer network forming hydrogel for formulas F1-F3, F4, F6 and F9 obeys higuchi release kinetic as there (R^2)values gave high results, while F4, F7, and F8 follow zero-order kinetics. The mechanism of release depending on the composition of hydrogel, geometry, preparation technique and environmental conditions during drug release [17].

Table (7) Mathematical finding of clotrimazole release from repared gels.

Formula No.	R^2			Krossmeyr-peppas n
	Zero order	First order	Higuchi	
F1	0.979	0.861	0.997	0.22
F2	0.982	0.845	0.990	0.221
F3	**0.730**	0.733	0.961	
F4	**0.999**	0.861	0.973	0.1
F5	0.831	0.754	0.992	
F6	0.730	0.733	0.962	
F7	0.997	0.891	0.986	0.216
F8	0.999	0.876	0.974	0.167
F9	0.734	0.734	0.970	

Conclusion

The present study demonstrates that the CLO confirm a promising vaginal mucoadhesive hydrogel since it provided highest release profile with maximum adhesion property especially for poloxamer 407 of 25% (F8), in addition to the acceptable physiochemical parameter such as pH, swelling index, drug content, and spreadability.

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