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Mucoadhesive, Thermosensitive, Prolonged-Release Vaginal Gel for Clotrimazole:β-Cyclodextrin Complex

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ABSTRACT

The purpose of this study was to achieve a better therapeutic efficacy and patient compliance in the treatment for vaginitis. Clotrimazole (1%) has been formulated in a vaginal gel using the thermosensitive polymer Pluronic F127 (20%) together with mucoadhesive polymers such as Carbopol 934 and hydroxypropylmethylcellulose (0.2% for both). To increase its aqueous solubility, clotrimazole was incorporated as its inclusion complex with 1:1 molar ratio with β-cyclodextrin. The inclusion complex was thoroughly characterized using various techniques, including ^{1}H NMR spectroscopy, FT IR spectrophotometry, differential scanning calorimetry, scanning electron microscopy, phase solubility studies, and determination of stability constant ($k_{1:1}$). The gelation temperature and rheological behavior of different formulations at varying temperatures were measured. In vitro release profiles of the gels were determined in pH 5.5 citrate buffer. It was observed that complexation with cyclodextrin slowed down the release of clotrimazole considerably. Carbopol 934, on the other hand, was found to interact with β-cyclodextrin, inducing precipitation. As far as rheological properties are concerned, thermosensitive in situ gelling was obtained with formulations containing drug: cyclodextrin complex rather than with free drug. Thus, the optimum formulation for a controlled-release thermosensitive and mucoadhesive vaginal gel was determined to be clotrimazole:β-cyclodextrin 1% with 0.2% hydroxypropylmethylcellulose in Pluronic F127 gel (20%) providing continuous and prolonged release of active material above MIC values.

KEYWORDS: clotrimazole, thermosensitive, mucoadhesive, prolonged release, cyclodextrin, vaginal.

INTRODUCTION

Genital tract infections, which are among the most frequent gynecological diseases, are treated commonly with imidazole-derivative antifungal agents such as clotrimazole since these drugs are locally active with no major side effects.[1,2] Current vaginal delivery systems include creams, foams, gels, tablets, pessaries, and irrigations, which are limited because of residence time at the genitourinary tract: they are removed rather rapidly by the self-cleansing action of the vaginal tract.[3,4] Moreover, the physiological conditions imposed by the protective mechanism of the genital tract, limiting the residence time and thus impairing the therapeutic efficacy of the drug, make multiple and frequent administration necessary for treatment.

Patient compliance when administering the dosage forms and following the repeated-dose therapeutic regimen is an important challenge in vaginal drug delivery. Patients are generally reported to tolerate gels better than other dosage forms,[5] but it is believed that vaginal therapy can be significantly improved if a delivery system can keep the drug at the site of administration for longer than the conventional dosage forms can.

Mucoadhesive vaginal gels containing polycarbophil, Carbopol, hydroxypropylcellulose, and polyvinylpyrrolidone as polymers enhancing the adhesion of the administered dosage form to the mucosal tissue have been added to in situ–gelling thermosensitive gels prepared with Pluronic F127.[6-10] Different techniques are adopted in drug delivery with thermosensitive gels, including dispersing the drug in the gel with a concentration higher than its solubility value and dispersing drug-loaded nanoparticles, liposomes, and drug:cyclodextrin complexes.[11-14]

Cyclodextrins are used in the pharmaceutical field to form inclusion complexes with drug molecules to increase their aqueous solubility, to enhance their aqueous stability or photostability, to mask unwanted characteristics, or to reduce side effects.[15,16] Cyclodextrins are also reported to convey controlled-release properties to certain active ingredients.[17]

The objective of this study was to design a vaginal gel formulation with thermosensitive and mucoadhesive properties to ensure longer residence at the infection site, providing a favorable release profile for the antifungal drug clotrimazole with the help of β-cyclodextrin complexation. The effect of β-cyclodextrin on drug release was believed to arise from its solubility-enhancing properties.

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Materials and Methods

Materials

Clotrimazole (molecular weight: 344 g/mol, aqueous solubility: 5.5 μmol/L), depicted in Figure 1, was purchased from Sigma Chemicals (Steinheim, Germany), and β-cyclodextrin (Kleptose) was supplied by Roquette Frères (Lestrem, France). Pluronic F127 was purchased from Sigma-Aldrich (St Louis, MO), Carbopol 934PH was of pharmaceutical grade and purchased from BF Goodrich (Cleveland, OH), and hydroxypropylmethylcellulose (HPMC) USP was purchased as Metolose 90SH 15000 from Seppic (Paris, France). Polyethylene glycol 400 (PEG400), used to solubilize the drug in the gel formulation, was purchased from Fluka BioChemika (Buchs, Germany). All other reagents were of analytical grade and used without further purification.

Methods

Preparation of Clotrimazole:β-cyclodextrin Complex

The inclusion complex of clotrimazole with β-cyclodextrin in a 1:1 molar ratio was prepared according to the colyophilization technique.[18] According to this technique, a fixed amount of clotrimazole was weighed and transferred to a round-bottomed flask. β-cyclodextrin solution in distilled water was added onto clotrimazole, and the powder was dispersed evenly. After ensuring that all clotrimazole powder was wetted by the β-cyclodextrin aqueous solution, the dispersion was left to equilibrate for 7 days at room temperature under constant stirring. At the end of equilibrium time, the dispersion was filtered and filtrate containing soluble clotrimazole:β-cyclodextrin complex (1:1) was lyophilized using a Heto-Holten A/S Lyolab Freeze Dryer (Allerod, Denmark) to obtain the complex in dry powder form.

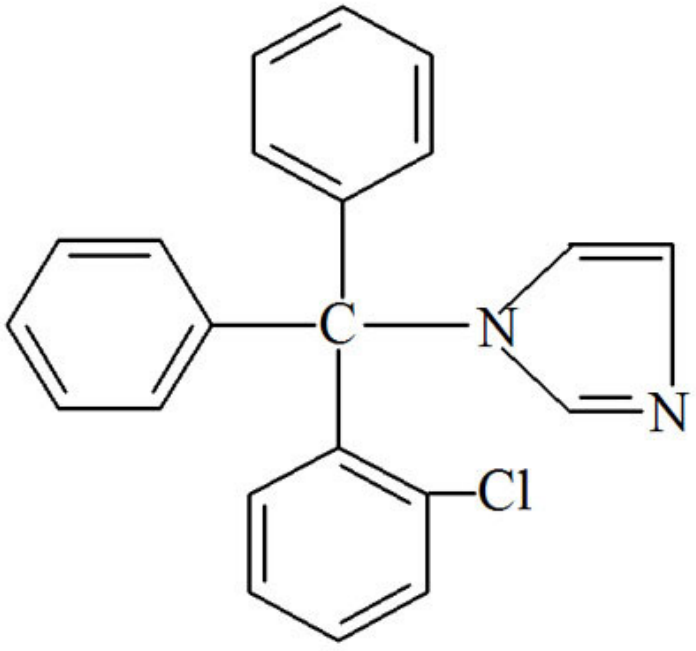

Figure 1. Structure of antifungal drug clotrimazole.

Characterization of Clotrimazole:β-cyclodextrin Complex

Protein nuclear magnetic resonance (^{1}H NMR) spectra of clotrimazole, β-cyclodextrin, and clotrimazole:β-cyclodextrin complex were taken by a Bruker DPX 400 digital Fourier transform-nuclear magnetic resonance (FT-NMR) spectrophotometer (Rheinstetten, Germany) at 400 MHz. Chemical shifts were given to external tetramethylsilane at 0 ppm with calibration using solvent signals (dimethylsulfoxide at 2.5 ppm, deuterated water at 4.75 ppm, and chloroform $CHCL_3$ at 7.25 ppm).

Fourier transform infrared (FT IR) spectra of clotrimazole, β-cyclodextrin, and clotrimazole:β-cyclodextrin complex were taken with a Nicolet 520 FT IR spectrophotometer (Thermo Electron Corp, Waltham, MA) using discs of each sample and previously prepared potassium bromide containing 0.01 g of sample in 0.1 g of potassium bromide between the wavelengths of 400 and 4000 cm^{-1}.

Differential scanning calorimetry (DSC) analyses were performed on lyophilized clotrimazole, lyophilized β-cyclodextrin, and clotrimazole:β-cyclodextrin complex samples with a DuPont 910 differential scanning calorimeter (Wilmington, DE). Each sample weighing ~3 mg was heated in hermetically sealed aluminum pans at a rate of 10ºC/min up to 200ºC in a dynamic nitrogen atmosphere.

Scanning electron microscopy (SEM) was performed to elucidate the crystal structure of lyophilized clotrimazole, β-cyclodextrin, and clotrimazole:β-cyclodextrin complex. The samples were mounted on metal stubs with a double-sided adhesive band and then sputtered with a 100 Å thick layer of gold. They were examined with a JEOL scanning electron microscope (SEM-ASID-10, Tokyo, Japan) at an acceleration voltage of 80 kV.

Phase solubility studies were performed as follows: 10 mg of clotrimazole was mixed with 10 mL of aqueous solutions containing varying amounts of β-cyclodextrin within the range of 0 to 16 mM. The suspensions were shaken until equilibrium was reached (7 days). The suspension was filtered through a 0.22-μm membrane filter. The filtrate was analyzed spectrophotometrically for the total amount of solubilized clotrimazole.

Preparation of Clotrimazole Gel Formulations

Thermosensitive and mucoadhesive clotrimazole gels were prepared according to the cold method.[19] Briefly, mucoadhesive polymer (Carbopol 934 or HPMC) (0.2% wt/vol) was slowly added to citrate phosphate buffer (0.1M, pH

Table 1. ^{1}H NMR Chemical Shifts, δ (ppm), of C-H Protons of β-cyclodextrin Alone and the Clotrimazole:β-cyclodextrin Complex Taken in Dimethylsulfoxide

	β-Cyclodextrin	Complex
H-1	4.82	4.80
H-2	3.30	3.30
H-3*	3.70	3.65
H-4	3.20	3.20
H-5*	3.65	3.40
H-6a,b	3.75	3.75

*Internal protons of cyclodextrin molecule that are expected to be affected by a potential inclusion complex formation.

4.0) at 4ºC with gentle mixing. Pluronic F127 (20% wt/vol) was then added to Carbopol or HPMC solution and allowed to dissolve overnight at 4ºC. Clotrimazole in free form (1%) was initially dissolved in a mixture of PEG400 and ethanol (5:3) and added to cold Pluronic F127 solution containing 0.2% Carbopol 934 or HPMC. On the other hand, for formulations containing drug:cyclodextrin complex, clotrimazole:β-cyclodextrin was dissolved directly in the cold Pluronic F127 solution containing 0.2% Carbopol 934 or HPMC with gentle mixing.

Characterization of Gels

The rheological behavior of gel formulations was determined. The flow curves of thermosensitive and mucoadhesive gels containing either free clotrimazole or clotrimazole:β-cyclodextrin complex were determined using a Brookfield Model DV-II Viscometer (Essex, UK) at controlled-rate mode. Measurements were made in a cone-and-plate geometry with a diameter of 40 mm (cone angle 2º). The shear rates ranged from 0.5 to 100 seconds^{-1}. Samples were applied to the lower plate using a spatula to ensure that formulation shearing and air bubbles did not occur. Measurements were made at 22ºC. Each point is the mean of at least triplicate analysis. Error bars have been omitted to retain clarity; however, in all cases, the coefficient of variation of replicate analyses was less than 5%.

The gelation temperature of the formulations was determined as follows: a 20-mL transparent vial containing a magnetic bar in 5 mL of Pluronic F127 gel was placed in a water bath. A thermometer connected to a thermistor was immersed in the gel, which was heated at a rate of 2ºC/min with constant (150 rpm) stirring. When the magnetic bar stopped moving because of gelation, the temperature displayed on the thermistor was recorded as the gelation temperature.[19,20]

The in vitro release of clotrimazole in free form and in complex with β-cyclodextrin was determined from different vaginal gel formulations using a dialysis bag placed in a sealed glass vial under constant magnetic stirring. The gel formulations (2.5 g) were packed into the dialysis bags (Spectra/Por Cellulose Ester Membrane MWCO: 100 000 Da, Spectrum Labs, Rancho Dominguez, CA) sealed with closures of 50 mm (Spectrum Labs). The release medium was 100 mL 0.1M citrate-phosphate buffer (pH 5.5) containing 1% Tween 80, providing sink conditions for clotrimazole. The medium was maintained at 37ºC and stirred at 100 rpm. At various time intervals, 5 mL of dissolution fluid was collected. Levels of clotrimazole in the samples were analyzed with a Shimadzu UV-VIS 160A spectrophotometer at λ = 210 nm. The exact amount of clotrimazole released from the formulation was calculated with a calibration curve with an analytically validated method (r^2 = 0.9994, repeatability coefficient of variation (CV) = 0.18%, reproducibility CV = 2.7%).

Results and Discussion

Characterization of Inclusion Complex

First, formation of a genuine inclusion complex of 1:1 molar ratio was evaluated using different techniques, including ^{1}H NMR spectroscopy, FT IR spectrophotometry, DSC, and SEM analysis. NMR spectroscopy has been used to elucidate the structure of drug:cyclodextrin complexes. NMR allows a clear distinction between inclusion and any other possible external interaction, with large effects observed on the protons located inside the hydrophobic cavity (H-3 and

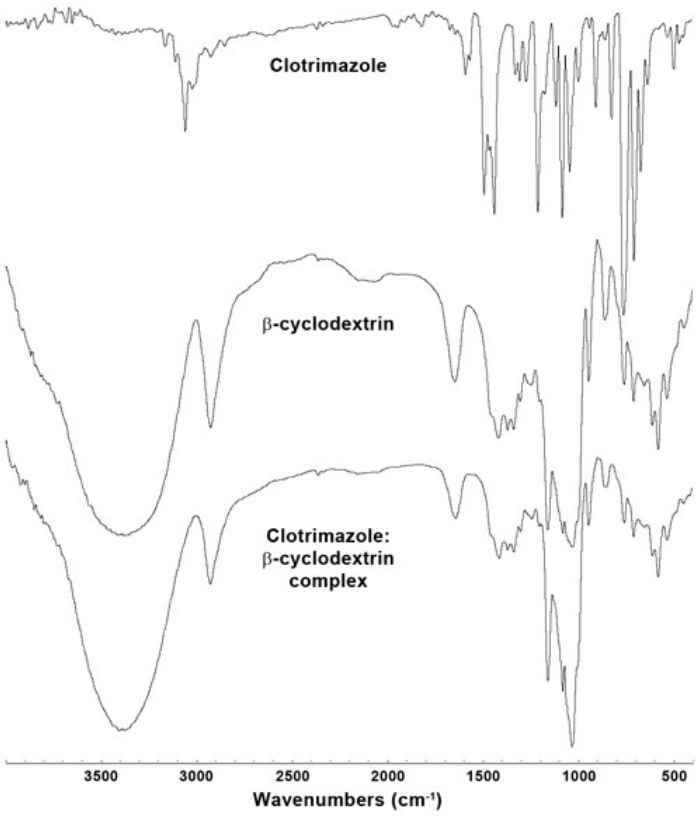

Figure 2. FT IR spectra of clotrimazole, β-cyclodextrin, and clotrimazole:β-cyclodextrin 1:1 complex.

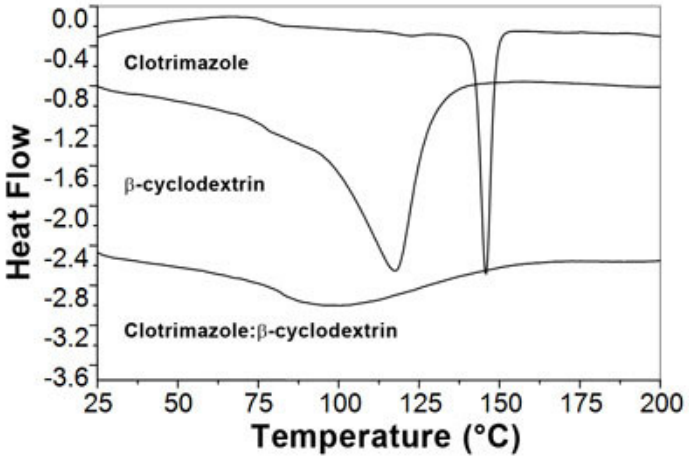

Figure 3. Differential scanning calorimetry data of lyophilized clotrimazole, β-cyclodextrin, and clotrimazole:β-cyclodextrin (1:1) complex.

H-5, with H-5 more pronouncedly affected) clearly proving inclusion. The chemical environment of the internal protons changes, resulting in changes in the chemical shifts of the protons because of shielding or deshielding effects. On the other hand, protons located on the outside experience very little or no effect.[21-24] Table 1 details the shifts observed for the internal proton H-5, suggesting that the drug has entered the cyclodextrin cavity to form an inclusion complex. There are no shifts for external protons, indicating that the complex formed can be classified as an inclusion complex.

Another indicator of the formation of a drug:cyclodextrin complex is the changes observed for the FT IR spectra of the drug in complex form. Figure 2 displays the FT IR spectra for clotrimazole, β-cyclodextrin, and the complex. It can be clearly observed that the FT IR spectrum for the complex is almost identical to that of β-cyclodextrin alone. On the other hand, clotrimazole's aromatic region peaks at around 600 to 800 cm^{-1} have disappeared, which suggests that these groups of the drug are included in the cavity.

For the demonstration of a genuine inclusion complex, results should be confirmed with different and independent techniques. The DSC thermogram in Figure 3 clearly indicates that clotrimazole's sharp melting endotherm at 148ºC and β-cyclodextrin's dehydration observed between 50 and 120ºC have completely disappeared for the thermogram of the complex, with a very slight water loss around 100ºC. Clotrimazole's DSC thermogram was taken after freeze drying in order not to overlook the effects of the lyophilization process on the crystalline structure of the drug. This might have affected the thermogram of the drug if a transformation from the crystalline state to the amorphous state had taken place, but this did not happen in this study.

SEM photomicrographs of clotrimazole, β-cyclodextrin, and the complex were taken with ×2500 magnification, as seen in Figures 4A to 4C. Figure 4A represents the crystalline structure of clotrimazole, which is close to cubic after lyophilization. On the other hand, β-cyclodextrin is more of a planar structure with large crystals, as seen in Figure 4B. The complex exhibits a totally different crystalline structure than the drug or the β-cyclodextrin, with needle- and flake-type crystals suggesting the formation of a complex.

The phase solubility diagram of clotrimazole with β-cyclodextrin can be observed in Figure 5. As can be seen, this diagram is an AL-type curve with a linear relationship between solubilized clotrimazole and molar β-cyclodextrin with a negative curvature. The initial linear ascending part of the solubility diagram in Figure 5 is generally ascribed to the formation of a 1:1 complex. The apparent stability constant ($K_{1:1}$) can be calculated from the solubility data using this formula:

$$K_{1:1} = \frac{Slope}{S_o(1-slope)} \qquad (1)$$

where S_0 is the intrinsic solubility of clotrimazole.

The apparent stability constant ($K_{1:1}$) for the clotrimazole:β-cyclodextrin complex was calculated from the solubility data and found to be 701M^{-1}, which confirms previously reported values.[25]

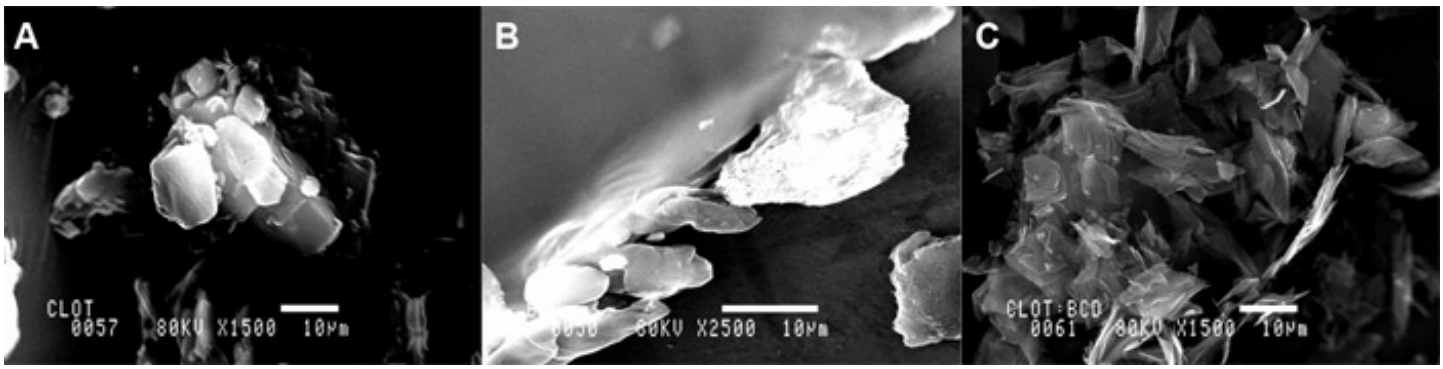

Figure 4. Scanning electron microscopy photomicrographs of clotrimazole (A), β-cyclodextrin (B), and complex (C) (magnification ×2500).

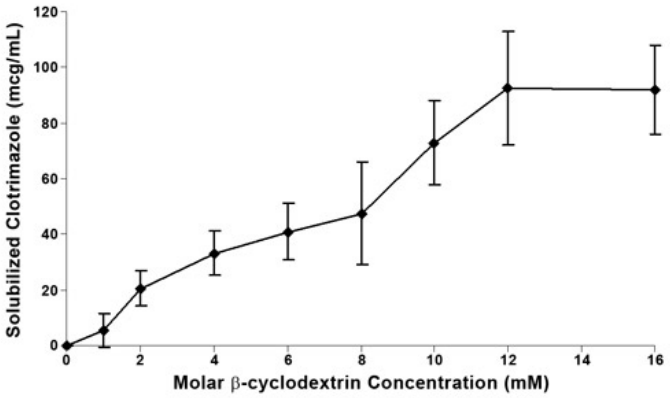

Figure 5. Phase solubility diagram of clotrimazole with β-cyclodextrin in pure water (n = 3).

Characterization of Thermosensitive Gels

Different formulations of Pluronic F127 gel with or without the mucoadhesive polymers Carbopol 934 and HPMC using free clotrimazole and clotrimazole:β-cyclodextrin complex were prepared using the cold method. Formulation components influenced gelling properties significantly. Carbopol 934, a mucoadhesive polymer known to interact strongly with low-molecular-weight molecules, polymers, and drugs,[26] displayed the same phenomenon with both free drug and the complex, causing precipitation within a few hours for gels containing Carbopol 934. It is believed that Carbopol interacts with the free drug, which has a considerably lower molecular weight, causing the drug's precipitation. Carbopol-containing formulations were not used for further studies, including viscosity measurements and in vitro release studies, because of this precipitation effect.

Another important factor influencing gel characteristics was whether the drug was employed in free form or in complex. Table 2 and Figure 6 display the significant differences in gelation temperature and in vitro release profiles of different formulations. Gelation temperature is dependent largely on polymer content; when clotrimazole is administered in free form, additional use of PEG400 as a solubilizing agent is required. Together with the high percentage of Pluronic (20%), known to lower gelation temperature,[14] an excess amount of polymer content with the presence of PEG causes the formation of gels at even 4ºC. On the other hand, as seen in Table 2, mucoadhesive polymer type seems to have no significant effect on gelation temperature. The presence of cyclodextrins such as HP-β-cyclodextrin was previously reported to lower gel viscosity.[14] This effect was attributed to the fact that the binding force (hydrogen bonding) of cross-linked Pluronic gel became weaker when HP-β-cyclodextrin was replaced in the gel matrix. The phenomenon was reported to be similar to the decrease in gel strength of sodium salicylate.

Flow plots of gels containing free and complexed clotrimazole with and without the mucoadhesive polymer HPMC are shown in Figure 6. Steady shear properties of Pluronic formulations were affected by temperature and overall polymer content.[7,27] Rheological properties of gels formulated with free clotrimazole are very much different from that of clotrimazole:β-cyclodextrin complex. As seen in Figure 6, gels containing free clotrimazole show significantly higher viscosity that was not affected by temperature. Gels containing clotrimazole complexed to β-cyclodextrin, however, show a temperature-sensitive rheological behavior.

Table 2. Gelation Temperatures (ºC) of Different Formulations of 20% Pluronic F127 Vaginal Gel (n = 3)*

Formulation	Gelation Temperature (ºC) ±SD
CLOT/PEG400-EtOH/PF127	10 ± 2
CLOT/PEG400-EtOH/HPMC/PF127	12 ± 1.5
CLOT:β-CD/PF127	32 ± 0.3
CLOT:β-CD/HPMC/PF127	33 ± 2

*CLOT indicates clotrimazole; PEG, polyethylene glycol; EtOH, ethanol; PF127, Pluronic F127; HPMC, hydroxypropylmethylcellulose; β-CD, β-cyclodextrin.

In vitro release experiments were realized on formulations that did not display precipitation. Thus, gels formulated with or without HPMC containing either free clotrimazole or clotrimazole:β-cyclodextrin complex were used. Figure 7 represents the in vitro release profiles of clotrimazole thermosensitive gel formulations. Gels containing free drug liberated most of the drug within 4 days. However, formulations containing complexed clotrimazole released only ~40% of the drug during this period. This fact could be attributed to 2 different phenomena. Complexation to β-cyclodextrin was performed because of the poor aqueous solubility of clotrimazole. Complexation with cyclodextrins is known to

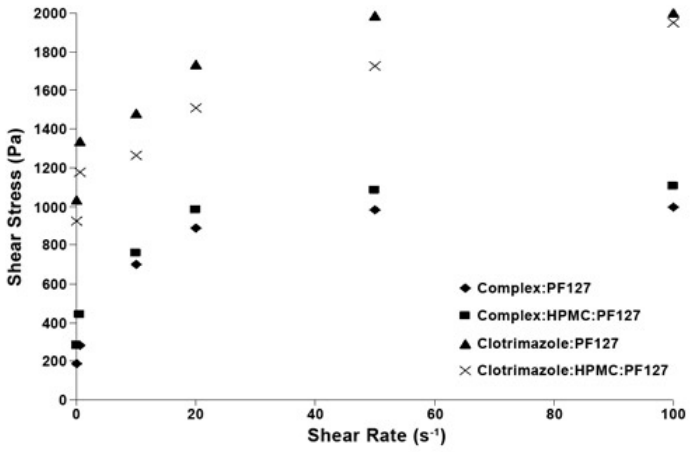

Figure 6. Flow curves of different clotrimazole vaginal gel formulations at room temperature (22ºC) (n = 3). The shear rate started at 0.5 seconds^{-1} and gradually increased up to a maximum of 100 seconds^{-1}. HPMC indicates hydroxypropylmethylcellulose; PF127, Pluronic F127.

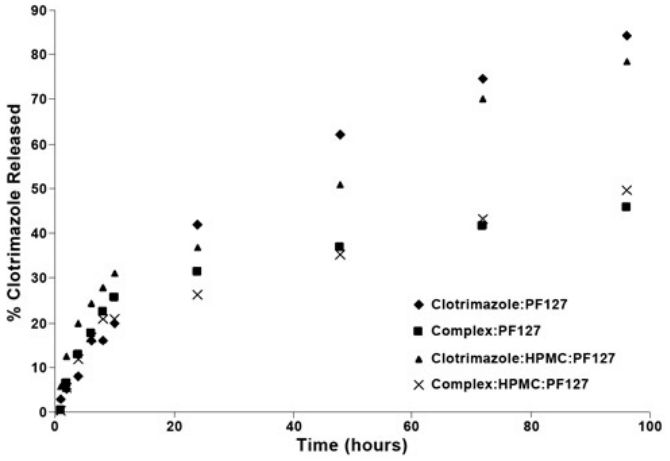

Figure 7. In vitro release profiles of clotrimazole vaginal gels at 37ºC in pH 5.5 citrate buffer. HPMC indicates hydroxypropylmethylcellulose; PF127, Pluronic F127.

enhance clotrimazole aqueous solubility considerably.[27] On the other hand, this solubilization effect has a different impact on release behavior of the gels, as seen in Figure 7. In extensive studies performed on the release behavior of thermosensitive hydrogels, it was reported that although the rate of dissolution of Pluronic gel is actually the controlling factor in drug release, it is not the only parameter affecting drug release. Because the interface is eroding at a constant rate, the surface concentration of the drug is held relatively constant and the drug diffuses out at a constant rate,[28] which was the case in our study, as seen in Figure 7. More concentrated gels were reported to dissolve at a slower rate than less concentrated ones because of the decreased water diffusion coefficient for the rate of water diffusing into the gel.[28] Release from the complex is, on the other hand, a competitive process controlled by diffusion of the drug upon dilution and competitive displacement of the drug by components in the dissolution media.[29] These 2 factors may have contributed to the prolonged release of clotrimazole from the thermosensitive gel in the complexed form. During the 92-hour release study, released amounts were significantly above the minimum inhibitory concentration (MIC) value for clotrimazole determined previously.[30]

This release period could be helpful in predicting the gel's behavior in the vagina. Most probably, a faster release of clotrimazole would be achieved in vivo because of the presence of salts and proteins. Even if the vaginal formulation's residence time was much less than 96 hours, this could be sufficient to obtain a high local dose of the drug for therapeutic efficacy.

Conclusion

The data in this study support the potential effectiveness of a vaginal gel with mucoadhesive properties to ensure longer residence time in the application site, avoiding surgery for implantation because of prolonged release properties when the drug is incorporated in an inclusion complex with β-cyclodextrin. Controlled release of the incorporated drug is achieved, ensuring an antimycotic efficacy within a long period, suggesting better patient compliance and higher therapeutic efficacy.

References

1. Ritter W, Patzschke K, Krause U, Stettendorf S. Pharmacokinetic fundamentals of vaginal treatment with clotrimazole. *Chemotherapy.* 1982;28:37–42.

2. Sawyer PR, Brogden RN, Pinder RM, Speight TM, Avery GS. Clotrimazole: a review of its antifungal activity and therapeutic efficacy. *Drugs.* 1975;9:424–447.

3. Deshpande AA, Rhodes CT, Danish M. Intravaginal drug delivery. *Drug Dev Ind Pharm.* 1992;18:1225–1279.

4. Wolfsson AD. Intravaginal drug delivery technologies. In: Rathbone M, ed. *Modified-Release Drug Delivery Technology.* New York, NY: Marcel Dekker; 2002:759–774.

5. Hardy E, Jimenes AL, de Padua KS, Zanewald LJD. Women's preferences for vaginal antimicrobial contraceptives, III: choice of a formulation, applicator and packaging. *Contraception.* 1998; 58:245–249.

6. Ceschel GC, Maffei P, Lombardi Borgia S, Ronchi C, Rossi S. Development of a mucoadhesive dosage form for vaginal administration. *Drug Dev Ind Pharm.* 2001;27:541–547.

7. Chang JY, Oh YK, Choi H, Kim YB, Kim CK. Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. *Int J Pharm.* 2002;241:155–163.

8. Chang JY, Oh YK, Kong HS, et al. Prolonged antifungal effects of clotrimazole-containing mucoadhesive thermosensitive gels in vaginitis. *J Control Release.* 2002;82:39–50.

9. Chu JS, Amidon GL, Weiner ND, Goldberg AH. Mixture experimental design in the development of a mucoadhesive gel formulation. *Pharm Res.* 1991;8:1401–1407.

10. Park H, Robinson JR. Physicochemical properties of water insoluble polymers important to mucin/epithelial adhesion. *J Control Release.* 1985;2:47–57.

11. Veyries ML, Couarrazze G, Geiger S, et al. Controlled release of vancomycin from poloxamer 407 gels. *Int J Pharm.* 1999;192:183–193.

12. Desai SD, Blanchard J. Pluronic F127-based ocular delivery system containing biodegradable polyisobutylcyanoacrylate nanocapsules of pilocarpine. *Drug Deliv.* 2000;7:201–207.

13. Bochot A, Fattal E, Grossiord JL, Puisieux F, Couvreur P. Characterization of a new ocular delivery system based on a dispersion of liposomes in a thermosensitive gel. *Int J Pharm.* 1998;162:119–127.

14. Kim EY, Gao ZG, Park JS, Li H, Han K. rhEGF/HP-β-CD complex in poloxamer gel for ophthalmic delivery. *Int J Pharm.* 2002; 233:159–167.

15. Thompson DO. Cyclodextrins—enabling excipients: their present and future use in pharmaceuticals. *Crit Rev Ther Drug Carrier Syst.* 1997;14:1–104.

16. Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins, I: drug solubilization and stabilization. *J Pharm Sci.* 1996;85:1017–1025.

17. Challa R, Alka A, Javed A, Khar RK. Cyclodextrins in drug delivery: an updated review. *AAPS PharmSciTech.* 2005;6:E329–E357.

18. Hedges AR. Industrial applications of cyclodextrins. *Chem Rev.* 1998;98:2035–2044.

19. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK. Development of in situ gelling and mucoadhesive acetaminophen liquid suppository. *Int J Pharm.* 1998;165:33–44.

20. Miyazaki S, Nakamura T, Takada M. Thermo-sensitive sol-gel transition of Pluronic F-127. *Yakuzaigaku.* 1991;51:36–43.

21. Schneider HJ, Hacket F, Rüdiger V, Ikeda H. NMR studies of cyclodextrins and cyclodextrin complexes. *Chem Rev.* 1998; 98:1755–1785.

22. Loukas YL. Measurement of molecular association in drug: cyclodextrin inclusion complexes with improved ^{1}H NMR studies. *J Pharm Pharmacol.* 1997;49:944–948.

23. Djedaini F, Perly B. Anthraquinone sulphonate as a general-purpose shift reagent for the NMR analysis of cyclodextrins. *Magnet Res Chem.* 1990;28:372–374.

24. Djedaini F, Perly B. Nuclear magnetic resonance investigation of the stoichiometries in β-cyclodextrin:steroid inclusion complexes. *J Pharm Sci.* 1991;80:1157–1161.

25. Van Doorne H, Bosch EH, Lerk CF. Formation and antimicrobial activity of complexes of beta-cyclodextrin and some antimycotic imidazole derivatives. *Pharm Weekbl Sci.* 1988;10:80–85.

26. Blanco-Fuente H, Esteban-Fernandez B, Blanco-Mendez J, Otero-Espinar FJ. Use of β-cyclodextrins to prevent modifications of the properties of carbopol hydrogels due to carbopol-drug interactions. *Chem Pharm Bull (Tokyo).* 2002;50:40–46.

27. Boulmedarat L, Grossiord JL, Fattal E, Bochot A. Influence of methyl β-cyclodextrin and liposomes on rheological properties of Carbopol®974 NF gels. *Int J Pharm.* 2003;254:59–64.

28. Anderson BC, Pandit NK, Mallapragada S. Understanding drug release from poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) gels. *J Control Release.* 2001;70:157–167.

29. Stella VJ, Rao VM, Zannou EE, Zia V. Mechanisms of drug release from cyclodextrin complexes. *Adv Drug Deliv Rev.* 1999;36:3–16.

30. Memisoglu E, Bochot A, Ozalp M, Sen M, Duchene D, Hıncal AA. Direct formation of nanospheres from amphiphilic β-cyclodextrin inclusion complexes. *Pharm Res.* 2003;20:117–125.