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Development and characterization of bioadhesive vaginal films of clotrimazole for vaginal candidiasis

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Abstract

The purpose of this work was to design and optimize bioadhesive vaginal films of clotrimazole that could be retained in the vagina for prolonged period for more effective treatment against vaginal candidiasis. Bioadhesive films were formulated by solvent casting technique using bioadhesive polymers such as chitosan, HPMC and sodium CMC. Glycerine and PEG-400 were used as plasticizer. The films were characterized for various physical, mechanical, and aesthetic properties. Bioadhesive strength, antifungal activity and *in vitro* release studies suggested that the prolonged release bioadhesive vaginal film formulation of clotrimazole is useful and effective dosage form for treating vaginal candidiasis.

Keywords: vaginal candidiasis, clotrimazole, bioadhesive vaginal film, chitosan, HPMC, Sodium CMC.

Introduction

Vaginal candidiasis (VC) is the most common reason why women seek the help of a gynecologist. It is estimated that nearly 75% of all adult women have had at least one genital yeast infection in their lifetime; at least 50% of these women will experience one or more recurrent episodes of VC. Of the 150 members of the yeast-like genus Candida, only 10 members are pathogenic in humans. *Candida albicans* is responsible for 90% of vaginal fungal infection cases; however, the noxious role in the genesis of VC has recently been stressed also for other Candida species, for example *C. glabrata* and *C. parapsilosis* (Redondo-Lopez et al. 1990, Horowitz et al. 1992, Sobel et al. 1993). For the affected women, the symptoms of the disease are disquieting, sometimes to the point where they interfere with their usual activities and life functions.

Conventional vaginal dosage forms are associated with limitations of poor retention, leakage and messiness causing inconvenience to users, leading to poor subject/patient compliance and loss of therapeutic efficacy. These limitations can be overcome by novel bioadhesive vaginal drug delivery systems (Robinson and Bologna 1994, Vermani and Garg 2000). There is a need to advance vaginal formulation technology to fulfill certain functions such as product dispersion throughout the vagina, retention for intended intervals, physicochemical interaction with vaginal

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milieu, release of active ingredients and effects on target organisms (Garg et al. 2005). Aesthetic qualities of vaginal products are also important to ensure proper compliance and regular use. In many parts of the world, vaginal films are preferred over gels due to their aesthetic appeal (Coggins et al. 1998). In addition, the films have several advantages of portability, ease of application (without applicator), easy storage and handling, feasibility of large scale production, and improved stability of drugs at extremes of temperature and humidity. Hence films may be more suitable than gels for tropical climates.

The present study describes a novel approach to vaginal delivery of Clotrimazole (CTZ) for local treatment of VC. A bioadhesive film has been developed using polymers like chitosan and combinations of HPMC and Sodium CMC in different concentrations. The present study describes a novel approach to vaginal delivery of Clotrimazole (CTZ) for local treatment of VC. A bioadhesive film has been developed using polymers like chitosan, HPMC and Sodium CMC using different concentrations. This system has been designed to ensure longer residence at the infection site, providing a favorable release profile for the antifungal drug clotrimazole. The film was evaluated in respect of its formulation, drug release and Bioadhesion characteristics.

Materials and Methods

Materials

Clotrimazole was obtained as gift sample from Zyg Pharma Mumbai, India. Hydroxypropyl methylcellulose (HPMC) and Sodium carboxymethylcellulose (SCMC), PEG-400, Tween 80 were obtained from Loba Chemie, Mumbai, India. Chitosan was obtained from Sigma-Aldrich Corp, St. Louis, USA. Glycerine, Potassium di-hydrogen ortho phosphate and Sodium hydroxide were obtained from Ranbaxy Lab, India. Vaginal fluid simulant (VFS) was prepared from 3.51 g/L NaCl, 1.40 g/L KOH, 0.222 g/L $Ca(OH)_2$, 0.018 g/L bovine serum albumin, 2 g/L lactic acid, 1 g/L acetic acid, 0.16 g/L glycerol, 0.4 g/L urea, 5 g/L glucose. The pH of the mixture was adjusted to 4.5 using 0.1M HCl (Owen and Katz 1999). All other materials used in the current study were of analytical grade.

Formulation of polymeric films

Different bioadhesive vaginal film formulations of CTZ were formulated using varying amounts of polymers along with Glycerine/ polyethylene glycol (PEG) and Tween-80 as plasticizer and dispersing agent respectively. The formulations (F1-F4) contained chitosan and lactic acid as solvent. Required quantity of low molecular weight chitosan was transferred to the beaker. 10 mL of 1% lactic acid solution was added to it. It was heated to 30°C, with continuous stirring. Drug was dispersed into this polymeric solution using tween 80 with stirring. Glycerin was added to it and made up the volume using 1% lactic acid. The formulations (F5-F8) contained HPMC and SCMC and water as solvent. Required quantity of HPMC was added to 10 mL of water in the beaker and required quantity of SCMC was added to 10 mL of distilled water in another beaker. Drug was dispersed into this polymeric solution using Tween 80 with continuous stirring. PEG was added to it and made up the volume using distilled water. These solutions were transferred to the Petri dish and it was kept for drying for 24 h. The films formed were peeled off and utilized for further studies. An optimized amount of Clotrimazole was added to get a constant amount of drug (10 mg/cm^2) Table 1 lists the various compositions employed during the study.

Table 1. Formulation chart of the bioadhesive films

Ingredients	Formulation Code							
	F1	F2	F3	F4	F5	F6	F7	F8
Chitosan (%)	1	1.5	2	2.5	-	-	-	-
HPMC (%)	-	-	-	-	0.8	0.8	1	1.2
SCMC (%)	-	-	-	-	0.8	1	0.8	0.8
Glycerine (mL)	0.5	0.5	0.5	0.5	-	-	-	-
Poly Ethylene Glycol (mL)	-	-	-	-	0.5	0.5	0.5	0.5
Tween-80 (%)	1	1	1	1	1	1	1	1
Lactic acid q.s (mL)	20	20	20	20				
Distilled water q. s.(mL)	-	-	-	-	30	30	30	30

Drug excipient compatibility studies

The FTIR spectra of the samples were obtained using FT– infrared spectrophotometer (Shimadzu-8400 S, Japan) by KBr pellet method in the wave number range 600 - 4000 cm^{-1}. The position of peak in FT-IR spectra of pure CTZ is compared with those in FT-IR spectra of CTZ plus excipients.

Drug content

The prepared films were cut in to 3 x 3 cm^2 and taken into a 100 mL volumetric flask and dissolved in VFS. Suitable dilution was made and absorbance was checked at 261 nm, using UV spectrophotometer (Shimadzu -1601, Japan).

Pharmaceutical characterization

Films were evaluated for various aesthetic, physical, mechanical, and performance parameters including appearance, color, odor, transparency, thickness uniformity, weight variation, flexibility, mechanical properties (tensile strength and elongation at break), assay, release profile, bioadhesion, adhesion time, retention in simulated vaginal environment, antifungal activity, Surface pH and Stability studies.

Thicknesses of whole patch were measured at different points using a micrometer screw gauge taking five patches together. The average thickness of single patch was calculated. Weight variation of patches was carried out by cutting the patch size of 1x1 cm^2 and measuring the weight of each patch individually.

Tensile strength of patches

It was determined using Hounse field universal testing machine. It consisted of two loaded grips. The upper one was movable and the lower one was fixed. The test patch of specific size (5x1 cm^2) was fixed between these load grips and force was gradually applied till the patch broke. The tensile strength of the patch was taken directly from the dial reading in Newtons.

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}} \quad (1)$$

Percentage elongation of the patches

It is carried out by Hounse field universal testing machine. The percentage elongation at break point is measured on the scale of the machine using the following equation.

$$\text{Percentage elongation} = \frac{\text{Maximum length recorded at break} - \text{original length}}{\text{Original length}} \times 100 \quad (2)$$

Bioadhesion in simulated vaginal environment

Bioadhesive strength of films in simulated vaginal environment was measured using isolated sheep vaginal tissue with modified two-armed balance method (Yamsani et al. 2007). A circular piece of sheep vaginal mucosa was fixed to the tissue holder and was immersed in SVF and temperature was maintained at 37±0.5°C. Then the film was placed on the mucosa by using a preload of 50 g and kept aside for 3 min. After preloading time, the preload was removed and the weights were added on the other pan until film detached from the sheep vaginal mucosa. The weight required to detach film from sheep vaginal mucosa was noted.

In vitro drug release studies

A patch of the film measuring 3x3 cm^2 was kept in basket (mesh).This assembly was then made to dip into beaker containing 30mL of SVF. The contents in the assembly were maintained at 37±5°C and stirred on a magnetic stirrer. Samples were withdrawn after every 1 hour and assessed for drug content. Drug release data obtained from *in vitro* dissolution were analyzed and were fitted into the Korsmeyer-Peppas model (Korsmeyer et al. 1983)

$$\frac{M_t}{M_\infty} = k_1 t^n + k_2 t^{2n} \quad (3)$$

Where M_t is the amount of drug released at time t, M_∞ is the amount of drug released at an infinite time, k_1 and k_2 are the magnitudinal contributions of diffusion and polymer relaxation mechanism, and n is the Fickian diffusion coefficient. Based on the phenomenological analysis, the type of release, *i.e.*, whether Fickian, non-Fickian (anomalous) or zero-order, was predicted.

Determination of Surface pH

The Surface pH of the prepared CTZ vaginal film was determined to evaluate the possible irritation effects on the mucosa. The patch was left to swell in 10 mL of SVF in small beakers and the pH was measured at time intervals of 1, 2, 3, 4 up to 8 h by placing the electrode in contact with the surface of the film.

Accelerated Stability Studies

Accelerated stability studies of films were performed at 40°C and 75% RH for 6 months. CTZ films were sealed in sachets prepared by heat sealing of aluminum foil placed in cartons, and stored at accelerated stability conditions (40°C and 75% RH). Samples were evaluated periodically at the end of 1, 2, 3 and 6 months for color, odor, softening time, dissolution time, surface pH, mechanical properties and drug content characteristics of CTZ in film.

Results and Discussion

Selection of polymers

The polymers, *viz.* Chitosan, sodium CMC and HPMC, were selected owing to their excellent bioadhesive strength, release rate controlling ability, non-toxicity, non-irritancy and stability at vaginal pH (Yamsani et al. 2007). Chitosan, a partially deacetylated chitin, which is a biologically safe biopolymer, prolongs the adhesion time of oral formulation and drug release from them. It is a promising bioadhesive material at physiological pHs. Chitosan provides a prolonged adhesion since it does not become inactivated after the first contact and shows no drop in mucoadhesion (Lehr et al. 1992). Chitosan also possesses antimicrobial property (Kast and Bernkop-Schnurch 2001). Successful use of the polymer combination of SCMC and HPMC

is known to provide the formulation with controlled drug release along with desired mucoadhesive properties (El-Kamel et al. 2002).

Drug-excipient compatibility

The IR spectra of CTZ and drug-loaded films (formulation F3 & F6) were found to be identical and presented (Fig. 1). The peak at 3450 nm indicating the -NH stretching, 2961 nm for the C-H stretching, 1564 for the aromatic N-O stretching and 1266 nm for the c-o stretching are the major peaks of the drug. The FTIR spectra of the pure drug and formulation F3 & F7 indicated that characteristics peaks of CTZ were not altered in their position after successful entrapment in the film, indicating no chemical interactions between the drug and carriers used.

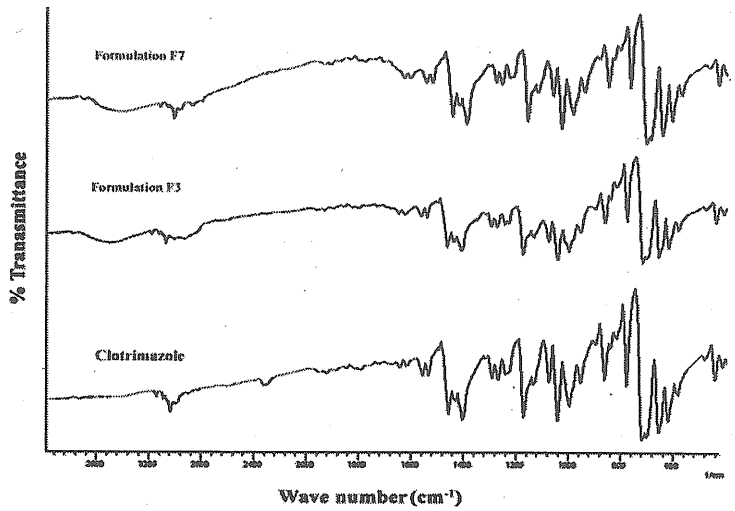

Figure 1. FTIR spectra of pure drug and formulations

Drug content and physical evaluation

Table 2. Results of drug content and physical evaluation. Data are depicted as the mean ± SD of three experiments.

Formulation	Drug Content (%)	Thickness (mm)	Average Weight (mg)
F1	98.61±0.015	0.32±0.0023	11.2±0.18
F2	99.15±0.045	0.31±0.0058	10.8±0.16
F3	99.23±0.021	0.33±0.0037	11.0±0.21
F4	99.10±0.005	0.34±0.0041	11.2±0.21
F5	99.14±0.042	0.36±0.0032	11.7±0.14
F6	99.21±0.030	0.38±0.0047	11.9±0.24
F7	99.30±0.134	0.41±0.0045	12.2±0.18
F8	99.31±0.143	0.43±0.0032	12.5±0.23

The experimental results of the film evaluation parameters are summarized in Table 2. Homogeneous films were translucent, colorless and soft, and no spot or stain was found on the films. The average thickness of the patches ranged from 0.32 and 0.43 mm. No batch varied more than 5% of the average mass and thickness indicating consistency in the preparation of the

films with minimal batch to batch variation. Average drug content was 98.61 and 99.31% and the results of the drug content uniformity test indicates homogenous drug distribution.

Tensile strength of patches

The films that are intended as a dosage form for intravaginal drug delivery should possess sufficient strength to withstand mechanical damage during production, handling and application. The tensile strength is defined as the maximum stress sustained by the material. As shown in Fig. 2, tensile strength varied according to the compositions of the formulation. As concentration of polymer increased the tensile strength also increased. The tensile strength of films made with chitosan was higher compared to that of HPMC and SCMC films.

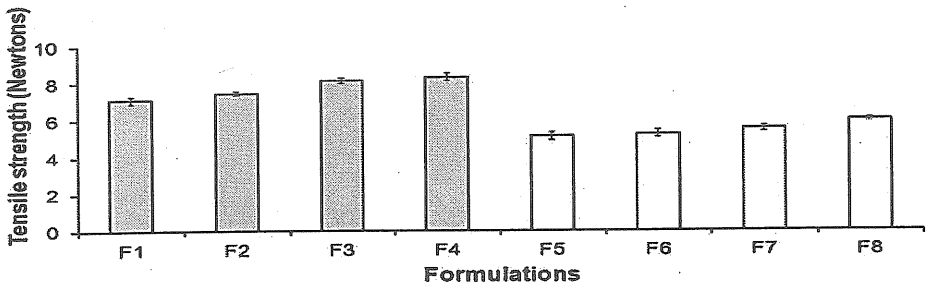

Figure 2. Comparison of tensile strength of polymeric films. Each data point represents the mean±S.D. of three replicates.

Percentage elongation of the patches

Patches exhibited decreased percentage elongation with increase in concentration of polymer. The result of percentage elongation studies is shown in Fig. 3. This study is useful in studying the flexibility of the prepared film. The films must possess both flexibility and strength for desired effectiveness. Increased concentration of polymer results in increased extent of cross linking and decreases flexibility of the film and as a result the percentage elongation decreases. The chitosan films made using glycerine as plasticizer exhibited more percentage elongation than that of HPMC and SCMC films plasticized with PEG.

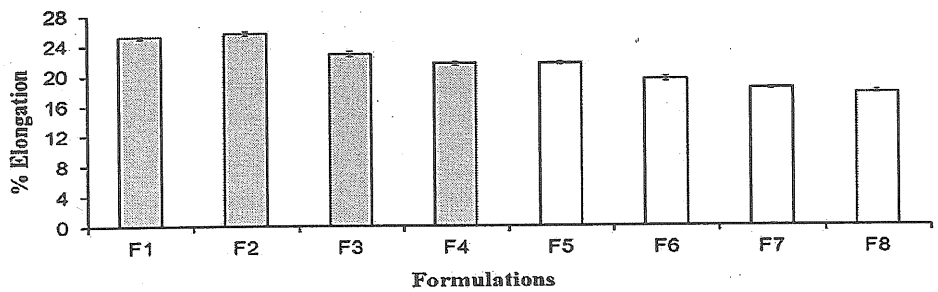

Figure 3. Comparison of percentage elongation of polymeric films. Each data point represents the mean ± S.D. of three replicates.

Bioadhesion in Simulated Vaginal Environment

There is a strong prophylactic and clinical need to develop vaginal products with desired characteristics such as product dispersion throughout the vagina and retention for intended intervals. Retention of a dosage form in vaginal cavity for prolonged intervals is desirable for therapeutic efficacy and minimizes the need of frequent dosing intervals. As shown in Figure 4, the maximum force of detachment increased with increasing polymer concentration. The chitosan films showed higher bioadhesion strength compared to films HPMC and SCMC films. The initial interaction between the polymer and the biological surface is through electrostatic interaction followed by mechanical interlocking of the polymer chains and therefore, the surface charge density of polymers is important for electrostatic behavior during the adhesion process (Tur and Ch'ng 1998). Chitosan possesses OH and NH_2 groups that can give rise to hydrogen bonding. Its linear molecules express sufficient chain flexibility, and their conformation is highly dependent on ionic strength. These properties are considered essential for mucoadhesion (Robinson and Mlynek 1995). SCMC can increase surface charge density and the carboxylic group can form hydrogen bonds with tissue (Peh and Wong 1999). HPMC is the long chained, non-ionic polymer and the mucoadhesive property could be due to the formation of physical or hydrogen bonding with the mucus components. HPMC can relieve the dryness and irritation even in the case of reduced mucus secretions (Yoo et al. 2006). SCMC and HPMC show faster hydration rate and thereby swelling which helps in the interpenetration of mucus and polymer resulting in bioadhesion. The developed CTZ films are bioadhesive in nature, would be retained in vagina for prolonged intervals, hence are expected to reduce the leakage and messiness, avoid user inconvenience, prolong the contact time in vaginal cavity, and thereby increase the effectiveness of CTZ.

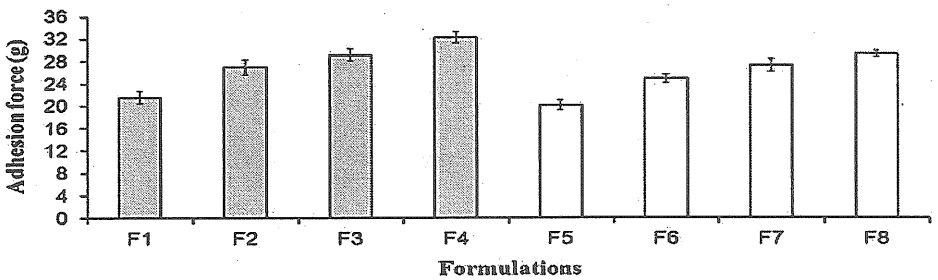

Figure 4. Comparison of percentage elongation of polymeric films. Each data point represents the mean ± S.D. of three replicates.

In vitro release studies

The release rate of CTZ from the films is shown in Fig. 5a and 5b. The simulated dynamic vaginal system used in this study mimics the physic dynamic conditions of the vagina. As evident from the diverse nature of dissolution profiles the influence of polymer seems to be vital in regulating the drug release. A comparison of the release rates of clotrimazole from films made with combinations of HPMC and SCMC showed a slower release than the films made with chitosan. Films formulated with 1% chitosan (formulation F1) released 85%, whereas films

prepared with 0.8% HPMC and 0.8% SCMC (formulation F5) released 59%. An increase in the thickness of the diffusional path length due to higher relaxation of the cellulosic chains in films containing HPMC and SCMC seems to be responsible for slowing down the release of CTZ. Drug release rate curves of the formulations shows that with increase in the Polymer (chitosan, HPMC, Sodium CMC) concentration drug release decreases. The films containing 1.2% HPMC and 0.8 % SCMC showed only 49 % drug release in 12 h. As the ratio of HPMC to SCMC concentration in the formulation increased, the CTZ release rate from the film decreased, indicating the drug release rate from the film can be controllable by the ratio of HPMC to SCMC concentration. HPMC decreases the release of the drug as it builds up an excessively viscous gel, which is more resistant to water penetration and erosion. All the film formulations exhibited a good sustained release effect.

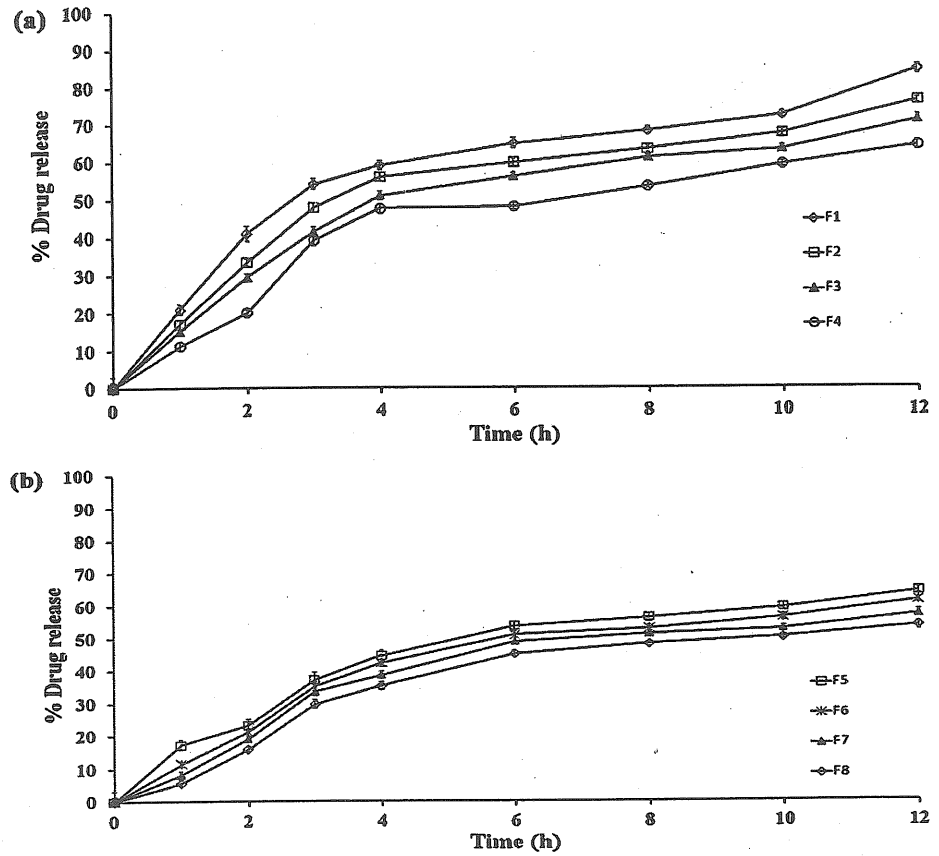

Figure 5. Dissolution of clotrimazole from prepared bioadhesive films: effect of polymer type and ratio (a) Chitosan films and (b) HPMC & SCMC films. (n = 3, mean ± standard deviation).

The release kinetic studies indicated that the "*n*" values were between 0.586-0.868, exhibiting a non-Fickian release behavior controlled by a combination of diffusion and chain relaxation mechanism. Values of the kinetic constant, *k,* showed a declining trend with an increase in the level of each polymer, construing an appreciable change in the polymer matrix with a change in the polymer composition.

Surface pH

The prepared bioadhesive films showed pH near to the neutral pH, which indicates that none of the formulation will cause irritation in vaginal mucosa. The surface pH of all formulations ranged from pH 6.3 to 6.6. The chitosan containing formulations shows the pH away from the neutral pH. The formulations containing HPMC and Sodium CMC showed pH nearer to neutral pH.

Accelerated Stability Studies

During stability studies, no significant change in color, odor, dissolution time, surface pH, mechanical properties and content of clotrimazole in films was observed at the end of 6 months of storage at accelerated conditions (40°C and 75% RH).

Conclusions

To summarize, novel bioadhesive vaginal films of CTZ (3×2 cm^2, containing 90 mg drug) with acceptable aesthetic, physical, and mechanical properties were formulated. Films were prepared using Chitosan and combinations of HPMC and SCMC. The clotrimazole vaginal bioadhesive films formed with mixture of SCMC and HPMC slowly released the drug compared to Chitosan films. All the film formulations exhibited a good sustained release effect. The formed films have good bioadhesive property that would us enhance the retention & sustained drug release in the vagina. CTZ vaginal films are stable under accelerated conditions. These results indicate the potential use of the proposed formulation as mocoadhesive vaginal dosage form with increased patient compliance.

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References

Coggins, C., Elias, C.J., Atisook, R., Bassett, M.T., Ettiegnene-Traore, V., Ghys, L. Jenkins-Woelk, P.D., Thongkrajai, E. and Van Devanter, N.L. (1998). Women's preferences regarding the formulation of over-the-counter vaginal spermicides. *AIDS* 12: 1389–1391.

El-Kamel, A.H., Sokar, M.S., Naggar, V.F. and Al-Gamal, S.S. (2002). Bioadhesive controlled release metronidazole vaginal tablets. *Acta Pharm.* 52: 171–179.

Garg, S., Vermani, K., Garg, A., Anderson, R.A., Rencher, W.B. and Zaneveld, L.J.D. (2005). Development and characterization of bioadhesive vaginal films of sodium polystyrene sulfonate (pss), a novel contraceptive antimicrobial agent. *Pharm. Res.* 22: 584-595.

Horowitz, B.J., Giaquinta, D. and Ito, S. (1992). Evolving pathogens in vulvovaginal candidiasis: implications for patient care. *J. Clin. Pharmacol.* 32: 248-255.

Kast, C.E. and Bernkop-Schnurch, A. (2001). Thiolated polymers-thiomers: development and *in vitro* evaluation of chitosan-thioglycolic acid conjugates. *Biomaterials* 22: 2345–2352.

Korsemeyer, R.W., Gurny, R., Doelker, E., Buri, P. and Peppas, N. (1983). Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.* 15: 23–35.

Lehr, C.M., Bouwstra, J.A., Schacht, E.H. and Junginger, H.E. (1992). *In vitro* evaluation of mucoadhesive properties of chitosan and some other natural polymers. *Int. J. Pharm.*78: 43-48.

Owen, D.H. and Katz, D.F. (1999). A vaginal fluid simulant. *Contraception* 59: 91–95.

Peh, K.K. and Wong, C.F. (1999). Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. *J. Pharm. Pharm. Sci.* 2: 53–61.

Redondo-Lopez, V., Lynch, M. and Schmitt, C. (1990). *Torulopsis glabrata* vaginitis: clinical aspects and susceptibility to antifungal agents. *Obstet. Gynecol.* 76: 651-5.

Robinson, J.R. and Mlynek, G.M. (1995). Bioadhesive and phase-change polymers for ocular drug delivery. *Adv. Drug Del. Rev.* 64: 45-50.

Robinson, J. and Bologna, W.J. (1994). Vaginal and reproductive system treatments using a bioadhesive polymer. *J. Control. Release* 28: 87–94.

Sobel, J.D., Vasquez, J. and Lynch, M. (1993). Vaginitis due to *Saccharomyces cerevisiae*: epidemiology, clinical aspects and therapy. *Clin. Infect. Dis.* 16: 93-9.

Tur, K.M. and Ch'ng, H.S. (1998). Evaluation of possible mechanism(s) of bioadhesion. *Int. J. Pharm.*160: 61-74.

Vermani, K. and Garg, S. (2000). The scope and potential of vaginal drug delivery. *Pharm. Sci. Technol. Today* 3: 359–364.

Yamsani, V.V., Gannu, R., Kolli, C., Rao, M.E. and Yamsani, M.R. (2007). Development and *in vitro* evaluation of buccoadhesive carvedilol tablets. *Acta. Pharm.* 57: 185–197.

Yoo, J.W., Dharmala, K, and Lee, C.H. (2006) The physicodynamic properties of mucoadhesive polymeric films developed as female controlled drug delivery system. *Int. J. Pharm.* 309: 139-45.

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