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MUCOADHESIVE, THERMORESPONSIVE, PROLONGED-RELEASE VAGINAL GEL FOR MICROBICIDE

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ABSTRACT

A novel mucoadhesive, thermoresponsive vaginal gel for microbicide was developed with gelation temperature 24-35 °C. Poloxamer 407 (P407) or: and poloxamer 188 (P188) were used to confer the temperature-sensitive gelation property. The mixtures of P407 (15%) and P188 (15–20%) existed as a liquid at room temperature, but gelled at 30–36°C. To modulate the gel strength and the bioadhesive force of Ciclopirox olamine gel, mucoadhesive polymer such as polyox WSR N-60K was used. Among bioadhesive polymers, polyox polymer enhanced gel strength most efficiently. These polymers reinforced the bioadhesive forces 4-7 fold compared to P407/P188 (15:15) alone and 3-6 fold compared to P407/P188 (15:20) alone. Differential scanning calorimetry (DSC) was employed to investigate the effect of poloxamer gel on the conformational changes of rat vaginal membrane. The in-situ gelling liquid with polyox polymer inserted into the vagina of women without difficulty and leakage and retained in the vagina at least 6-8 h. These results suggest that in situ-gelling and mucoadhesive vaginal microbicide gel for women can be further developed as a more convenient and effective vaginal dosage form for treating sexually transmitted disease.

Key-words: Mucoadhesion, Vaginal drug delivery, Polyox polymers, Microbicide, Sexually transmitted disease, Conformational change

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INTRODUCTION

Heterosexual transmission of human immune deficiency virus (HIV-1) accounts for nearly 90% of all HIV-1 infections in women.[1] Currently, an estimated 19.2 million women worldwide are infected with HIV-1, accounting for ~50% of the 40 million adults living with HIV/AIDS.[2] In the United States, the proportion of HIV acquisition attributable to heterosexual transmission in women has increased from 21% in 1986 to 42% in 2002.[3,4] Women are 4 to 16 times more likely to contact HIV from infected males than vice versa, and young women are especially vulnerable.[5] In addition to HIV, an estimated 45 million Americans older than 14 years of age suffer from genital herpes (1 in 5), and 20 million from human papilloma virus (HPV) (1 in 10), and more than half a million each have sexually transmitted hepatitis B virus infection (HBV).[6]

The emergence of HIV/AIDS as a disease spread through sexual intercourse, combined with growing public awareness about the problems associated with other viral sexually transmitted infections (STIs), has prompted the search for new, cost-effective, and safe vaginal microbicides for curbing transmucosal viral transmission.[7, 8] Microbicides would provide protection by inactivating viruses or preventing viruses from replicating either in semen or the infected host cells that line the vaginal wall. Microbicides that are currently being investigated are directed mainly at preventing pregnancy as well as protection against STIs.[9, 10]

To accelerate the development of microbicides in India, where HIV and other sexually transmitted infections (STIs) are a serious problem, more than 2,200 topical products marketed in that country were reviewed for potential anti-HIV and cytotoxic activity, and 60 pharmaceutical ingredients or formulations were tested more extensively. [11] Those with significant HIV-inhibitory activity were evaluated for activity against gonococcus, chlamydia, lactobacillus, and sperm. The products with the best results were evaluated for safety in the rabbit vaginal irritation assay and condom compatibility test. Based on these tests, one of the most promising compounds is Ciclopirox olamine (CO). Ciclopirox olamine (6-cyclohexyl-1-hydroxy-4-methyl-2-[1H]-pyridone ethanolamine salt) is marketed worldwide, including in the United States and Europe, as a topical antifungal agent. This active ingredient is found in at least 14 vaginal antifungal products marketed in India, Italy, Spain, Switzerland, Turkey, and other countries. The dosage forms include creams, ovules, foams, powders, solutions, and washes which usually contain 1% CO. Ciclopirox olamine is effective against yeasts, dermatophytes and fungi *Candida albicans, Candida glabrata* and other *Candida species*.[12-15] In addition, CO is an excellent anti-inflammatory agent [16,17] and is absorbed into the skin and vaginal wall.[18]

In vaginal drug delivery, the physiological conditions imposed by the protective mechanisms of the vagina often lead to the limited contact time of administered drugs with vaginal mucosa and a short duration of therapeutic efficacy, making a frequent dosing regimen necessary. Moreover, conventional vaginal dosage forms such as inserts and ointments give discomfort to the patients. Although the patients are known to tolerate gels better than inserts or ointments, the direct application of gels into the disease sites of vagina might be difficult as well as inconvenient. Vaginal therapy would be thus significantly improved if an intravaginally administered drug can retain at the site of administration for prolonged period after more convenient dosing.[19, 20]

Recently, in situ-gelling liquids have been investigated as a more convenient dosage form of topical applications. The liquids applied to the topical areas such as eyes can make transition to gels as a result of a chemical/physical change induced by the physiological environments. Poloxamer, a block copolymer made of polyoxyethylene and polyoxypropylene is known for its excellent compatibility with other chemicals, high solubility capacity for different drugs, and good drug release characteristics.[21] Mucoadhesion can be used as a means to improve intimacy of contact, as well as a way to increase dosage form residence time to various administration routes (Park and Robinson, 1985; Robinson et al., 1987; Lee et al., 2000).[22] Hence the present work was envisaged to develop a stable, novel, and aesthetic bioadhesive vaginal microbicide drug delivery system with improved efficacy.

MATERIALS AND METHODS

Poloxamer (P407, P188) were supplied from Signet chemicals (Mumbai, India). Ciclopirox olamine was kindly provided by Cipla research and development (Mumbai, India). Polyox polymers were obtained as a gift sample from The Dow Chemical Company (USA). All other chemicals were of laboratory grade.

Fourier Transformed Infrared Spectroscopy

The drug, polymer and other formulation ingredients were characterized by IR Spectroscopy using a FTIR 8400S, Shimadzu, Toyota, Japan. The spectrum was taken in KBr discs in the range of 4000- 500 cm^{-}.

Preparation of *In Situ* gels

Thermo sensitive and mucoadhesive gels were prepared by using the cold method (Choi et al., 1998).[23] In brief, Polyox WSR N 60-K (0-2 w/ v%) was slowly added to citrate phosphate buffer (0.1 M, pH 4.0) at 4 °C with gentle mixing. P407 (15 w/v %) and P188 (15 or 20 w/v %) were then added to polyox WSR N 60-K solution and allowed to dissolve overnight at 4°C. Ciclopirox

olamine (0.77 %) was initially dissolved in the mixture of ethanol and polyethylene glycol 400 (3:5), and added to the cold P 407/P188 solution containing various content of polyox WSR N 60-K (1–2%) with gentle mixing. The composition of thermoreversible gels is shown in Table-1.

Table-1: Composition of thermoreversible gel

Batch code	P 407:P 188	Polyox WSR N-60 K (%)
G1	15:15%	0
G2		1
G3		1.5
G4		2
G5	15:18%	0
G6		1
G7		1.5
G8		2
G9	15:20%	0
G10		1
G11		1.5
G12		2

Preparation of Vaginal Fluid Simulant

Simulated vaginal fluid was prepared as described (Owen and Katz, 1999).[24] To 1 L of distilled water, NaCl (3.51 g), KOH (1.4 g), Ca(OH)2 (0.22 g), bovine serum albumin (0.018 g), lactic acid (2.00 g), acetic acid (1.00 g), glycerol (0.16 g), urea (0.4 g), and glucose (5.00 g) were added and dissolved. The pH of the mixture was then adjusted to 4.2 using HCl.

Drug Content Evaluation

Drug content was determined by dissolving accurately weighed quantity of gels in methanol (AR grade). After suitable dilution absorbance was recorded by using Systronic 2201 UV/Vis double beam Spectrophotometer at 299 nm. Drug content was determined using slope of standard curve, previously plotted.

pH

2.5gm of gel was accurately weighed and dispersed in 25ml of purified water. The pH of dispersions was measured using pH meter (Systronics digital-DI-707).

Viscosity

Viscosities of gels were determined using Brookfield Viscometer (Japan). Gels were tested for their rheological characteristics at 25 °C using Brookfield Viscometer (DV-III programmable Rheometer). The measurement was made over the whole range of speed settings from 10 rpm to 100 rpm with 30 seconds between 2 successive speeds and then in a descending order.

Measurement of Gelation Temperature

A 20-ml transparent vial containing a magnetic bar and 10 g of poloxamer solution was placed in a low-temperature thermostat water bath. A digital thermosensor connected to a thermistor was immersed in the poloxamer solution. Poloxamer solution was heated at a constant rate with constant stirring. When the magnetic bar stopped moving due to gelation, the temperature displayed on the thermistor was determined as a gelation temperature (Miyazaki et al., 1991).[25]

Measurement of Gel Strength *In Vitro-In Vivo*

Poloxamer solution (50 g) was put in a 100 ml-mass cylinder and gelled in a thermostat at 36.5°C. The apparatus for measuring gel strength (weight: 35 g) was then placed onto the gelled poloxamer. The gel strength was determined by the time(s) it took to move the apparatus 5 cm down through the poloxamer gel (Schmolka, 1972).[26, 27] In cases that took more than 10 min to drop the apparatus into the gel, various weights were placed on top of the apparatus and gel strength was described by the minimal weights that pushed the apparatus 5 cm down through the gel. The gel strength suitable for poloxamer gel was also investigated by inserting poloxamer gels into the vagina of a healthy women volunteers and observing any leakage after insertion. The protocol for this work was approved by Institutional ethics committee of S.K.Patel College of Pharmaceutical Education and Research, Ganpat University and the protocol no. was SKPCPER-IEC/2008/02.

Determination of Bioadhesive Force

A section vaginal skin of the rabbit and secured with mucosal side out on to each glass vial using a rubber band and an aluminum cap. The vials with the rectal tissues were stored at 36.5°C for 10 min. Next, one vial was connected to the balance and the other vial was placed on a height-adjustable pan. Poloxamer gels were added onto the rectal tissue on the other vial. Then, the height of the other vial was adjusted so that the gel could be placed between the mucosal skin of both vials. The weights of the apparatus were kept raised until two vials became separated. Bioadhesive force, the detachment stress (dyne: cm^2), was determined from the minimal weights that detached two vials (Ch'ng et al., 1985; Lehr et al., 1990). [28, 29] The rectal tissue pieces were changed for each tensile measurement.

***Ex Vivo* Permeation Studies**

Kiesary Chien diffusion cell mounted with hairless rabbit vaginal skin was used for drug ex vivo permeation study. 1 gm of gel was taken into the cell (donor compartment) and vaginal fluid stimulant pH 4.2 in receptor compartment which is agitated using magnetic stirrer (100rpm) and temperature maintained to 37±10 °C was maintained. The sample was withdrawn at

predetermined time intervals and same volume replaced with fresh vaginal fluid stimulant. Absorbance was measured after suitable dilution at 299nm to estimate Ciclopirox olamine.

DSC Study of the Rat Vaginal Membrane

Vaginal membrane was freshly excised from female hairless rats euthanized with a lethal dose of sodium pentobarbital (50 mg/kg, i.p.). Rat vaginal membrane was then treated with 15% poloxamer gel for 6 h. The poloxamer-treated membrane was rinsed with distilled water to wash away the remaining poloxamer gel on the surface of the membrane. The membrane treated with distilled water for 6 h was used as the control. Rat vaginal membrane was analyzed using a differential scanning calorimeter (DSC – 60 Simadzu–Japan).[30] Vaginal membrane samples (both poloxamer-treated and the untreated control), each weighing 10–15 mg, were placed into aluminum crucibles and hermetically sealed to avoid water evaporation. During the analysis, the sealed pan was placed in the sample cell of the DSC-60 and scanned from 50 to 300°C, with a heating rate of 10 degree/min. The protocol for this work was approved by Institutional Animal Ethics Committee of S.K.Patel College of Pharmaceutical Education and Research, Ganpat University and the protocol no. was SKPCPER-IAEC/2008/10.

Analysis of Release Mechanism

Curve fitting was performed using Microsoft Excel 2007 version. The dissolution data were fitted to the following equations.[31] Release exponent "*n*" was calculated

$$Mt/M = kt^n \quad (1)$$

$$\log (Mt/M) = \log k + n \log (t) \quad (2)$$

Where *Mt*/*M* is the fraction of released drug at time *t*, *k* is a characteristic constant of the thermoreversible gel and *n* is an indicative of release mechanism.

RESULTS AND DISCUSSION

FTIR Study

Figure-1. Demonstrates the FTIR Transmittance spectra of Ciclopirox olamine, polymer and other formulation ingredients. The FTIR spectrum of Ciclopirox olamine showed two characteristic peaks at C-NH_2 stretching at (790.84 cm^-, 1633.76 cm^-); C-N stretching (1134.18 cm^-); one characteristic peaks for C-NO bending (1511.28 cm^-). The same characteristic peaks are also observed for drug – excipients mixture. As shown in Figure-1, we can predict that the characteristics of the peaks of Ciclopirox olamine were not altered after mixing with the other formulation ingredients, indicating no chemical reaction or interaction between drug and excipients.

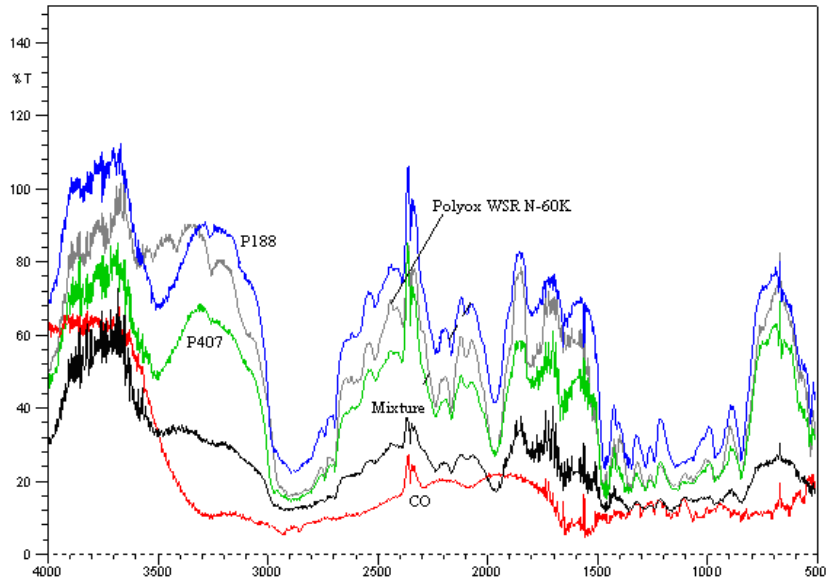

Figure-1: FTIR spectra of Ciclopirox olamine and formulation excipients

Characterization of various Ciclopirox Olamine gel formulation

In the present study efforts were made to prepare thermoreversible gels of Ciclopirox olamine using poloxamer 407 (P407) and/or poloxamer 188 (P188) and polymer polyox WSR N-60K.Drug content of the formulations was well within the range between 96.01 ± 0.1 to 99.1 ± 0.3 and pH 4.1 to 5.0,suitable for vagina (Table-2). The viscosity of various formulated Ciclopirox olamine gels was measured using a Brookfield viscometer. In gel systems, consistency depends on the ratio of solid fraction, which produces the structure, to liquid fraction. Differences in concentration and kind of gelling agents result in changes in the occurring structure consistency.

Table-2: Characteristics of various gel formulations

Batch code	pH	Viscosity (m.pas)	Drug content(%w/v)	Drug release after 8 hrs.
G1	4.5	2000	97.01 ± 0.1	99.33*
G2	4.1	2500	98.0 ±0.5	97.85
G3	4.4	3400	99.1 ± 0.3	100.03
G4	4.5	4000	97.2 ± 0.3	92.29
G5	4.5	2700	97.0 ±0.1	97.51*
G6	4.9	3200	98.4 ± 0.3	96.8
G7	5.0	3500	98.67 ±0.3	93.08
G8	4.2	4300	96.01 ± 0.1	91.72
G9	4.5	3300	96.99 ± 0.4	94.77*
G10	4.3	3900	97.8 ±0.2	94.04
G11	4.2	4220	98.4 ±0.1	87.54
G12	4.2	4500	97.45 ± 0.4	85.31

***** Formulation G1, G5 and G9 released 95% of the drug within 5-6 hrs.

Gelation temperatures of poloxamer solutions

Gelation temperature is the temperature at which the liquid phase makes a transition to gel. A gelation temperature range suitable for thermoreversible gel would be 30–36°C. If the gelation temperature of thermoreversible gel is lower than 30°C, gelation occurs at room temperature leading to difficulty in manufacturing, handling and administering. If the gelation temperature is higher than 36°C, the gel still stays as a liquid at body temperature, resulting in leakage from the anus. Therefore, thermoreversible gel must have the suitable gelation temperature, 30–36°C, to be a liquid form at room temperature and to form a gel phase instantly in the vagina. All formulations existed as a liquid form at the room temperature and might gel at the human vaginal temperature, known to be 37.2 °C (Rashad et al., 1992).[32] As bases of thermoreversible gel with the suitable gelation temperatures (30–36°C), poloxamer 407 (P407) and/or poloxamer 188 (P188) were selected due to their thermo-sensitive gelling properties. In addition, P407 and P188 are known to have low toxicity, less skin irritation, excellent water-solubility, and high Solubilizing capacity for sparingly soluble drugs, good drug release characteristics and compatibility with other chemicals.[33]

Various mixtures of poloxamer P407 and P188 gelled at the suitable gelation temperatures, while solutions of each poloxamer alone did not gel at the desirable range (Table-3). Solutions of single poloxamer containing less than 16% of P407 or less than 25% of P188 did not form a gel over the temperature ranges tested. The gelation temperatures of poloxamer solutions containing 18–25% of P407 alone or 30% of P188 were 13–25°C and 48°C, respectively. This indicates that P407 or P188 alone could not provide the suitable gelation temperature. In cases of P407 and P188 mixtures, several formulations gelled at the body temperature. As the concentration of P407 increased, the mixtures needed smaller amounts of P188 to gel at the desirable gelation temperature.[34] The w: w percentage ratios of P407:P188 with a gelation temperature in the range of 30 36°C were 9:25, 12:20 and 15:15–15:20. Among these compositions, three formulations of P407:P188 mixtures (15:15–15: 20) were selected as the systems of choice for the thermoreversible gel since they might give flexibility in formulation with other components.

Gelation time

All formulations showed gel-like rheological properties at 36 °C, but showed different rate of gelation depending on the content of P188. Gelation time was defined as the time when the elasticity modulus became higher than the viscosity modulus. The gelation of P407/P188/Polyox (15/20/1) was observed at 35 s, but it took longer time for P407 / P188 /Polyox (15/15/1) which

began to show the viscoelastic property of a gel at 72 s. At 37 °C, the formulation with 20% of P188 gelled within 34 s. The higher gelation rate of the formulation with 20% of P188 might have resulted from the stronger association of P188 with other components via hydrogen-bonding and ionic interaction. After intravaginal application, the shorter gelation time observed in the formulation P407/P188/Polyox (15/20/3) would be advantageous in that the rapidly gelled formulation might face the less change of drainage from the site of application, leading to a prolonged retention of Ciclopirox olamine in the vaginal cavity.

Table -3: Gelation temperatures of poloxamer solutions

Poloxamer	Concentration (%, w:w)	Gelation temperature (°C)a
P407	10	>50
	20	22 ± 0.2
	25	14 ± 0.5
P188	20	>50
	25	>50
	30	48 ± 0.2
P407/P188	9/10	>50
	9/20	44 ± 0.2
	9/25	34 ± 0.1
	12/5	>50
	12/10	47 ± 0.5
	12/20	36 ± 0.5
	15/10	42 ± 0.3
	15/15	35 ± 0.5
	15/20	29 ±0.5
	15/25	24 ± 0.5

Gel strength in vivo

In the development of thermoreversible gel, gel strength is important in finding the condition which allows the easy insertion of the gel and no leakage from the applicator. Thus, the ranges of gel strength suitable for poloxamer gel were investigated by inserting poloxamer gels into the vagina of a healthy woman volunteers and observing any leakage after insertion. We observed two thresholds in gel strength; the upper and the lower limit. Above the upper threshold of the gel strength, it was difficult to insert the gel. Under the lower limits, the gel leaked out from the applicator. The threshold ranges differed between gels containing various amount of bioadhesive polymers. In the gels with Polyox WSR N-60 K (1-2%), the threshold range was (15–40 s) (Table-4), while in the gels with no Polyox WSR N-60 K, the range was 10–20 s. The broader threshold range of liquid gel containing bioadhesive polymer Polyox WSR N-60 K (15–40 s)

appears to be due to the stronger bioadhesive force of polyox polymers (Figure-2).As the concentration of Polyox WSR- N-60 K increased the gel strength of the formulations also increased (Figure-3).

Table-4: Gelation temperatures of prepared formulations and its bioadhesive strength

Batch code	P 47:P 188	Polyox WSR N-60 K (%)	Gelation temperature (°C)a	Gel strength (S)	Bioadhesive force (dyn/cm^2)
G1	15:15%	0	35 ± 0.5	10	3560
G2		1	34.5 ± 0.2	15	3700
G3		1.5	34± 0.2	21	4200
G4		2	32 ±0.1	26	4500
G5	15:18%	0	30 ± 0.2	15	3325
G6		1	29 ±0.3	24	4460
G7		1.5	27± 0.4	31	4780
G8		2	27± 0.4	37	5390
G9	15:20%	0	27 ±0.5	20	3610
G10		1	26 ±0.5	26	4900
G11		1.5	25.5 ±0.5	33	5200
G12		2	24 ± 0.5	40	5500

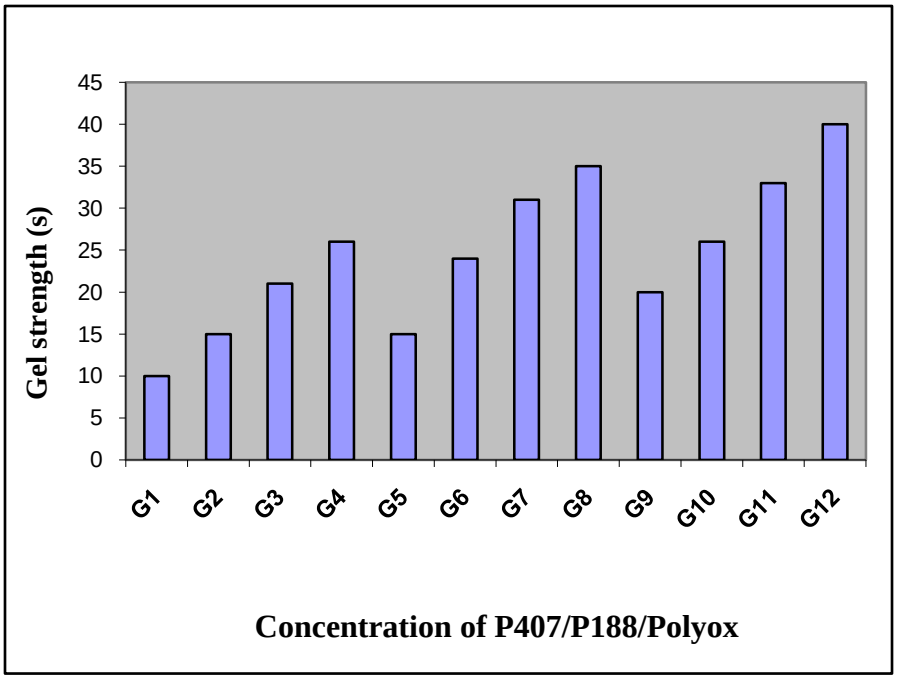

Figure-2: Effect of concentration of P407/P188/Polyox on gel strength (s) in formulations G1 to G12

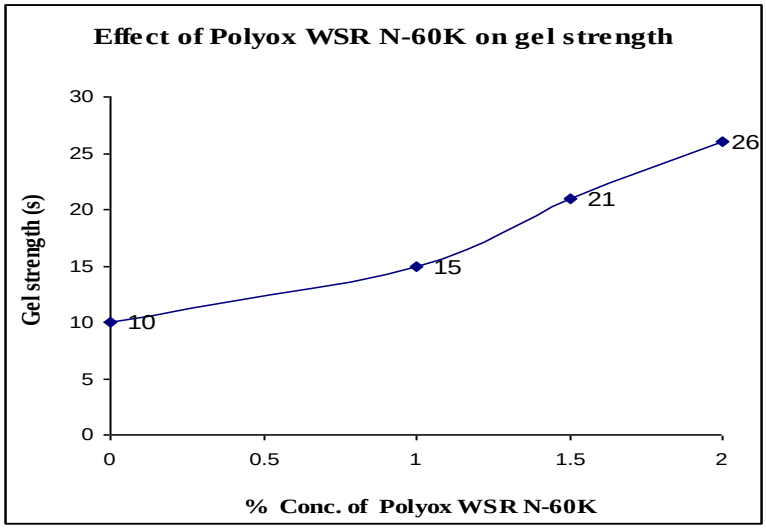

Figure-3: effect of concentration of polyox WSR N-60K on gel strength Bioadhesive force

Bioadhesive force means the force with which thermoreversible gel bind to vaginal membrane at 36.5°C ±0.5°C. The bioadhesive force is known to be dependent on the nature and the concentration of bioadhesive polymers. The stronger the bioadhesive force is, the more it can prevent the gel leaching from the vaginal tract. But if the bioadhesive force is too excessive, the gel can damage the vaginal mucous membrane (Robert et al., 1988; Leung and Robinson, 1990).[35, 36] Therefore, thermoreversible gel must have the suitable bioadhesive force. Among bioadhesive polymers, polyox polymers enhanced gel strength most efficiently. These polymers reinforced the bioadhesive forces 4-7 fold compared to P407/P188 (15:15) alone and 3-6 fold compared to P407/P188 (15:20) alone. All the formulations having bioadhesive force in the range of 3325 to 5500 dyn/cm^2 (Figure-4).

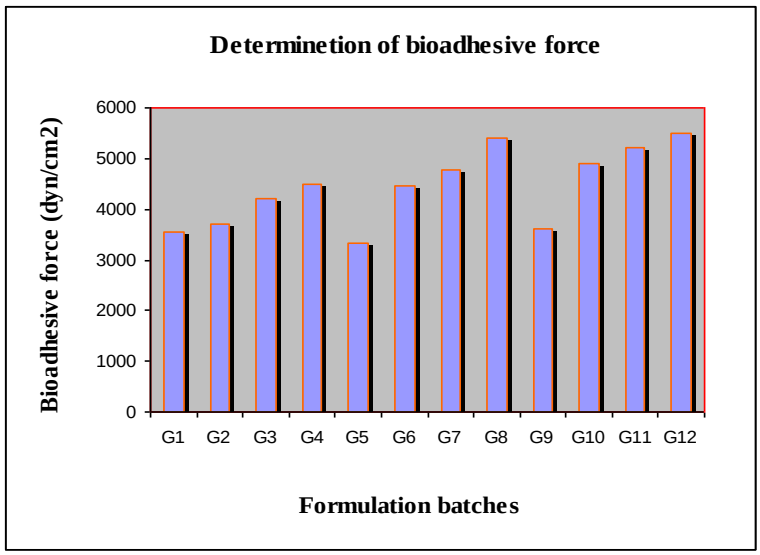

Figure-4: Determination of bioadhesive force

Ex vivo permeation (In vitro release) of Ciclopirox olamine from liquid gel

To test whether P 188 or Polyox WSR N-60 K affects the release rates of ciclopirox olamine from the liquid gel, the release test was performed with the formulations composed of constant amount of P 407 (15%) and variable amounts of P 188 (15:15%–15:20%). The release of ciclopirox olamine was variously affected by the components of liquid gel such as P 188 and the bioadhesive polymer, polyox WSR N-60 K. Poloxamer P 188 showed little effect on the release rates of Ciclopirox olamine from liquid gel. Regardless of the contents of P 188, ~ 40- 50% of Ciclopirox olamine was released out within 3-4 h and about 99 % of the drug was released at 6 h in all formulations (Figure-5) Polyox WSR N-60 K delayed the release rates of ciclopirox olamine from a certain concentration.

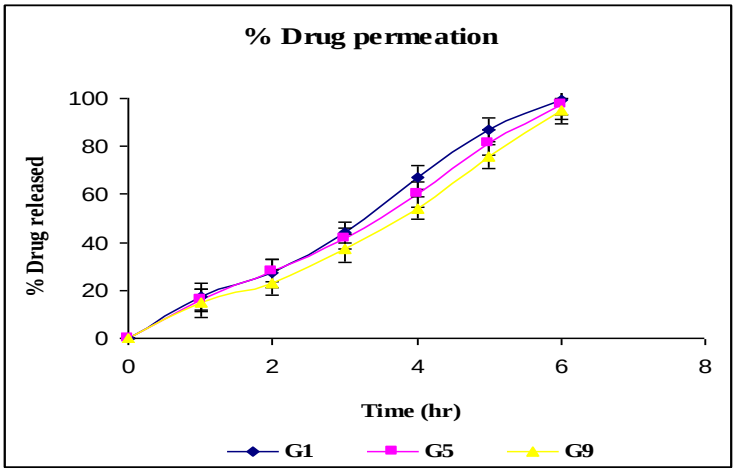

Figure-5: Release of Ciclopirox Olamine from gel formulations without polyox polymer

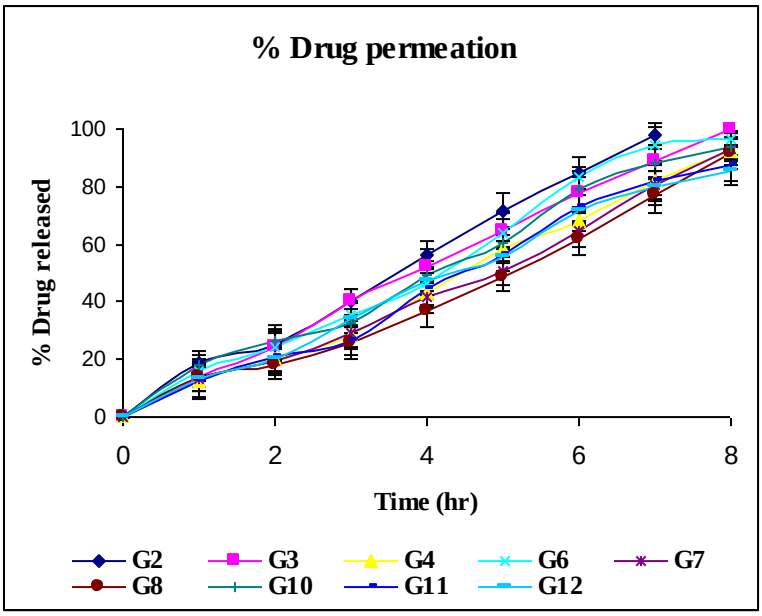

Figure-6: Percentage drug release from gel formulations with polyox polymer

Although the release rates of ciclopirox olamine did not significantly differ between no Polyox WSR N-60 K and 1 % Polyox WSR N-60 K -loaded gel. The release rates of Ciclopirox olamine tended to decrease as the concentrations of Polyox WSR N-60 K increased than 1 %. Polyox WSR N-60 K consistently decreased the release of Ciclopirox olamine in various liquid gels with different contents of poloxamer P 188. Among various gels containing different amounts of P 188 and Polyox WSR N-60 K, the slowest release of Ciclopirox olamine was shown in the gel with 2 % Polyox WSR N-60 K and 20% P 188 (Figure-6). Such a slow release of ciclopirox olamine appears to be contributed by the higher gel strength of the formulation with 20% P 188 and 3 % Polyox WSR N-60 K than other formulations.

DSC Study of the Rat Vaginal Membrane

In this study, DSC is used as a qualitative tool to assess the differences in the thermal stability of poloxamer-treated rat vaginal membrane and the untreated control. In the DSC profile of poloxamer-treated and the untreated control, of special interest is the peak at around 64.45°C, which is the thermal denaturation temperature (Td) of the vaginal membrane (Figure-7). The thermal denaturation temperatures of the poloxamer -treated membrane and the untreated control were 64.45°C and 63.19°C, respectively, which were not significantly different from each other (Table-6). The results of DSC analysis of rat vaginal membrane showed that the profiles of thermal stability for both poloxamer-treated and the untreated control at temperatures ranging from 50 to 300°C were almost identical, indicating that poloxamer does not affect thermal stability of vaginal membrane.

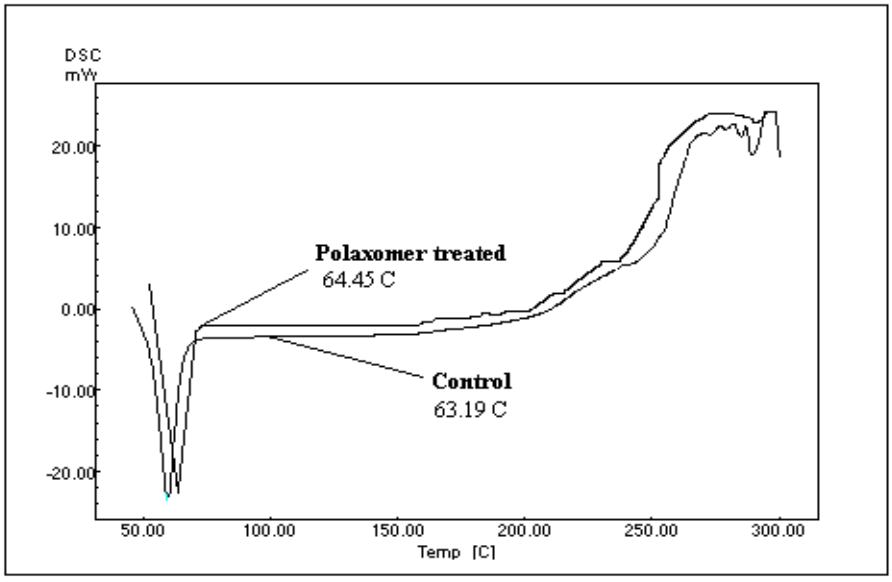

Figure-7: Thermal denaturation of the rat vaginal membrane (carbopol-treated vs. control)

Table-6: Thermal denaturation of the rat vaginal membrane

Condition	Thermal denaturation temperature(°C) ±SD
Poloxamer-treated membrane	64.45 ± 1.01*
Untreated control	63.19 ± 1.11*

* No statistically significant difference.

DSC has been used to elucidate a direct relationship between membrane stability and the degree of disorder of the lipid layers upon exposure to polymer-based delivery system by demonstrating any changes in the lipid conformation with the cervical mucosa and the viscoelastic property of membrane. The conformational change of the mucosa membrane before and after treatment of intravaginal formulations made of various combinations of polymer including microbicidal agents was investigated using DSC. The results of this study add to evidence that this system can be efficiently and safely used as female drug delivery system.

Analysis of release mechanism

To investigate the mechanism of drug releases the above mentioned equations (1) and (2) were used. Where, *Mt*/*M* is the fraction of released drug at time *t*, *k* is a characteristic constant of the thermoreversible gel and *n* is an indicative of release mechanism. The *n* value of 1 corresponds to zero-order release kinetics, 0.5<*n*<1 means a non-Fickian release model and *n*=0.5 indicates Fickian diffusion (Higuchi model) (Peppas, 1985). From the plot of log (*Mt/M*) versus log (*t*) (Figure-8), kinetic parameters, *n* and *k*, were calculated. Table-5 shows that most of *n* values are close to 0.5<*n*<1, suggesting that Ciclopirox olamine might be released from the gel by non-Fickian diffusion or anomalous transport. However, the *k* values indicate that Ciclopirox olamine

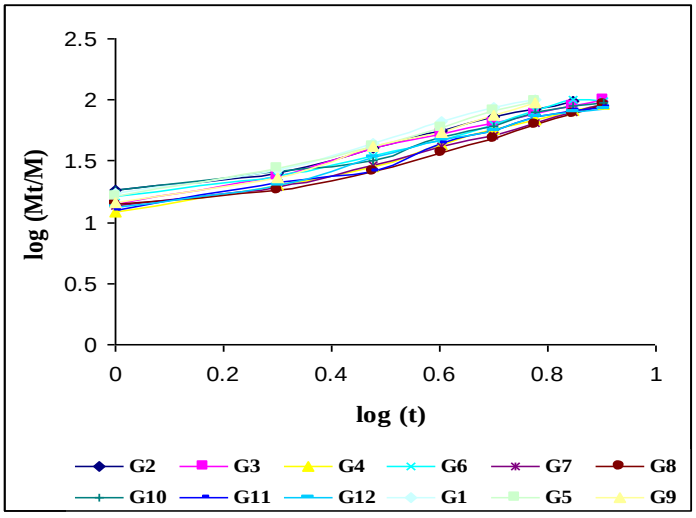

Figure-8: Release kinetics of Ciclopirox olamine (Logarithm of released fractions of Ciclopirox olamine was plotted against logarithm of time)

Table-5: Release kinetic parameters

Batch code	Polyox WSR N-60 K (%)	Release exponent (*n*)	Kinetic constant (*k*, %:h*n*)	Correlation coefficient (*r*)
G1	0	1.0342	1.1862	0.9801
G2	1	0.9145	1.2003	0.9759
G3	1.5	0.9638	1.1335	0.9963
G4	2	1.0124	1.0378	0.9857
G5	0	1.0204	1.1733	0.9886
G6	1	0.9419	1.147	0.9745
G7	1.5	0.9644	1.0567	0.9757
G8	2	0.9382	1.0505	0.9479
G9	0	1.0655	1.1162	0.9803
G10	1	0.8521	1.1944	0.9665
G11	1.5	1.0577	1.0394	0.9776
G12	2	0.9541	1.0847	0.9881

released more slowly from liquid gel with higher concentration of P 188 and polyox WSR N-60K. P 188 and polyox WSR N-60K seems to affect the release rates by influencing the physicochemical property of gel matrix.

CONCLUSION

Taken together, it is concluded that the mixtures of P407:P188: Polyox (15:15:1.5) is the optimal systems which have the gelation temperature suitable for Ciclopirox olamine gel, which remained at the administered sites due to strong gel strength and mucoadhesive force. Furthermore, the desirable physicochemical properties such as in *situ* gelling property, suitable gel strength and bioadhesive force of the thermoreversible microbicide gel, could alleviate the patients a feeling of alienation, discomfort and refusal during application, increasing patient compliance. DSC study demonstrated that female control drug delivery system based on poloxamer in combination with polyox polymer showed good biocompatibility and did not cause any conformational changes in rat vaginal membrane. This system might be applicable for the development of thermoreversible and mucoadhesive microbicide gel for woman as a more convenient and effective vaginal dosage form.

REFERENCES

1. Piot P, Bartos M, Ghys PD, Walker N, Schwartlander B. The global impact of HIV/AIDS. Nature 2001; 410: 968-973.
2. United Nations Program on HIV/AIDS. AIDS epidemic update. Available at: www.unaids.org. December, 2003.

3. Centers for Disease Control and Prevention. HIV AIDS, Surveill Rep., 2002, 14, 1-48.
4. Samuel MC, Hessol N, Shiboski S, Engel RR, Speed TP, Winkelstein WJ. Factors associated with human immunodeficiency virus sero-conversion in homosexual men in three San Francisco cohort studies 1984—1989. J Acquir Immune Defic Syndr1993; 6:303-312.
5. Rivers K, Aggleton P. Women & HIV. Adolescent sexuality, gender and the HIV epidemic. BETA 2001; 14: 35-40.
6. American Social Health Association. Sexually Transmitted Diseases in America: How Many Cases, and at What Cost? Research Triangle Park, NC: ASHA, 1998.
7. Uckun FM, D Cruz OJ. Prophylactic contraceptives for HIV/AIDS. Hum Reprod Update 1999; 5: 506-514.
8. D'Cruz OJ, Uckun FM. Clinical development of microbicides for the prevention of HIV infection. Curr Pharm Des 2004; 10: 315-336.
9. Turpin JA. Considerations and development of topical microbicides to inhibit the sexual transmission of HIV. Expert Opin Investig Drugs 2002; 11:1077-1097.
10. D'Cruz OJ, Uckun FM. Pre-clinical safety evaluation of novel nucleoside analogue-based dual-function microbicides (WHI-05 and WHI-07). J Antimicrobial Chemother 2002: 793-803.
11. Zaneveld LJ, Waller P. Ciclopirox olamine: A vaginal product with microbicide potential (Program for the Topical Prevention of Conception and Disease, TOPCAD), J. Sellors (PATH), F. Camus-Bablon (PATH). Available at www.path.org
12. Dittmar WA. Microbiologist Laboruntersuchungen mit Ciclopirox olamine. Arzneimittel-Forschung/Drug Research 1981; 31: 1317-1322.
13. Dittmar WA. A new antimycotic compound with a broad antimicrobial spectrum: laboratory results. Arzneimittel-Forschung/Drug Res 1973; 23: 670-674.
14. Abrams BB. Ciclopirox olamine: A hydroxyl pyridon antifungal agent. Clinics in Dermatology 1992; 9: 471-477.
15. Jue SG. Ciclopirox olamine 1% cream: a preliminary review of its antimicrobial activity and therapeutic use. Drugs 1985; 29: 330-341.
16. Bagatell FK. Evaluation of a new antifungal cream, Ciclopirox olamine 1% in the treatment of cutaneous candidosis. Clin Ter. 1985; 8: 4-48.

17. Kligman AM. An in vitro human skin model for assaying topical drugs against dermatophytic fungi. Acta Derm Venereol 1987; 67: 243-248.
18. Coppi G. Pharmacokinetics of Ciclopirox olamine after vaginal application to rabbits and patients. J Chemother 1993; 5: 302-306.
19. Mandal TK. Swelling-controlled release system for the vaginal delivery of miconazole. Eur J Pharm Biopharm 2000; 50: 337–343.
20. Jung Yun Chang, Yu-Kyoung Oh, Han-gon Choi, Yang Bae Kim, Chong-Kook Kim. Rheological evaluation of Thermosensitive and Mucoadhesive vaginal gels in physiological conditions. Int J Pharm 2002; 241: 155–163
21. Morishita M, Barichello JM, Takayama K, Chiba Y, Tokiwa S, Nagai T. Pluronic® F-127 gels incorporating highly purified unsaturated fatty acids for buccal delivery of insulin. Int J Pharm 2001; 212: 289–293.
22. Park H, Robinson JR. Physicochemical properties of water insoluble polymers important to mucin/epithelial adhesion. J Control Release 1985; 2: 47–57.
23. 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.
24. Owen DH, Katz DF. A vaginal fluid simulant. Contraception 1999; 59: 91–95.
25. Sanjay Bidkar, Devendra Jain, Amol Padsalg, Krishna Patel, Vinod Mokale. Formulation development and evaluation of Fluconazole gel in various polymer base formulation: Development and evaluation of Fluconazole gel in various polymer bases. Asian J Pharm 2007; 1: 63-68.
26. Miyazaki S, Nak amura T, Takada M. Thermo-sensitive sol-gel transition of Pluronic F-127. Yakuzaigaku 1991; 51: 36–43.
27. Schmolka IR. Artificial skin I. Preparation and properties of Pluronic F-127 gels for treatment of burns. J Biomed Mater Res 1972; 6: 571–582.
28. Ch'ng, HS, Park H, Kelly P, Robinson JR. Bioadhesive polymers as platforms for oral controlled drug delivery II: synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. J Pharm Sci 1985; 74: 399–405.
29. Lehr CM, Bouwstra JA, Tukker JJ, Junginger HE. Intestinal transit of bioadhesive microspheres in an in situ loop in the rat: a comparative study with copolymers and blends based on poly (acrylic acid). J Control Release 1990; 13: 51–62.

30. Corbo DC, Liu J , Chien YW. Characterization of the barrier properties of mucosal membranes. J Pharm Sci 1990; 79: 202–206.
31. Peppas NA. Analysis of Fickian and non-Fickian drug release polymers. Pharm Acta Helv. 1985; 60:110–111.
32. Rashad AL, Toffler WL, Wolf N, Thoraburg K, Kirk EP, Ellis G, Whitehead WE Vaginal P02 in healthy women and in women infected with Trichomonas vaginalis: Potential implications for metronidazole therapy. Ame J Obstet Gynecol 1992; 166: 620–624.
33. Jung Yun Chang, Yu-Kyoung Oh, Han-gon Choi, Yang Bae Kim, Chong-Kook Kim. Rheological evaluation of thermo sensitive and mucoadhesive vaginal gels in physiological conditions, Int J Pharm. 2002; 241:155–163.
34. Han-Gon Choi, Jae-Hee Jung, Jei-Man Ryu, Sung-June Yoon, Yu-Kyoung OH, Chong-Kook Kim. Development of in situ-gelling and Mucoadhesive acetaminophen liquid suppository. Int J Pharm 1998: 165: 33–44.
35. Robert CR, Buri PA, Peppas NA. Experimental method for bioadhesive testing of various polymers. Acta Pharm Technol 1988; 34: 95–98.
36. Leung SS, Robinson JR Bioadhesives in drug delivery. Polymer News 1990; 15: 333–342.