AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Mucoadhesive Microemulsion Based Prolonged Release Vaginal Gel for Anti-Fungal Drug

Anita Patel[1*], Jayvadan Patel [2]

1. Research Scholar, Bhagwant University, Ajmer, Rajsthan, India

2. Departments of Pharmaceutics, Nootan Pharmacy College, Visnagar, Gujarat, India

ABSTRACT

The objective of the present investigation was to develop and evaluate microemulsion based gel for the vaginal delivery of Sertaconazole. The solubility of Sertaconazole in various oils, surfactants and co-surfactants were checked to identify components of the microemulsion. The ternary diagrams were plotted to identify the area of microemulsion existence. Various gelling agents were evaluated for their potential to gel the Sertaconazole microemulsion without affecting its structure. Carbopol 940 was selected for the formulation of microemulsion based gel. The prepared formulations of Sertaconazole Microemulsion based gel was evaluated by checking its pH, spreadability, rheological studies, mucoadhesive strength, *in-vitro* drug release studies and *ex-vivo* retention studies. The Sertaconazole Microemulsion based gel showed good *in vitro* bioadhesion and anti-fungal activity. The Sertaconazole Microemulsion based gel has potential be successfully used for the topical treatment of vaginal candidiasis.

Keywords: Phase diagram, Microemulsion based gel, Mucoadhesion**,** Vaginal candidiasis

*Corresponding Author Email: patelanita21@gmail.com

Received 26 June 2012, Accepted 2 July 2012

Please cite this article in press as: Patel A *et al.*, Mucoadhesive Microemulsion Based Prolonged Release Vaginal Gel for Anti-Fungal Drug. American Journal of PharmTech Research 2012.

INTRODUCTION

Invasive fungal infections are an increasing threat to human health. In the developed world, these infections predominantly occur in the context of increasingly aggressive immunosuppressive therapies. Recently, there have been an increasing number of profound fungal infections caused by fungi such as those belonging to the genus Candida, the genus aspergillus and the genus Cryptococcus. This is a particular complication encountered in transplant patients, those administered a large quantity of antibiotics, anticancer drugs (carcinostatic) or steroidal agents over a long period.[1] Most of females have at least suffer with vulvovaginal candidiasis once during their lifetime. The episodes of vulvovaginal candidiasis are often painful and very uncomfortable and can include itching, irritation, continuous vaginal discharge and dysuria.[2,3,4]

The most commonly prescribed treatment for vulvovaginal candidiasis in recent years has been the imidazole antifungal. Imidazole antifungal agents which are available in various dosage forms such as vaginal creams and pessaries and oral tablets. Sertaconazole is an antifungal agent very effective for treatment of *Candida albicans* infections. Sertaconazole is an imidazole derivative antifungal agent developed for treatment of human mycotic infections and plays an essential role in antifungal chemotherapy.[5] It is lipophilic with limited water solubility except at low pH and has poor topical permeation posses certain challenge in for vaginal drug delivery.[6] For treatment of vulvovaginal candidiasis local antifungal has been favored due to numerous side effects, toxicity and teratogenic potential of systemically applied drug. Hence, for solubilization of sertaconazole microemulsion appeared to be a suitable approach. Microemulsion is a clear, thermodynamically stable, isotropic mixture of oil, water, surfactant and co-surfactant. The short to medium chain alcohols are generally considered as co-surfactants in the microemulsion system. The presence of surfactant and co-surfactant in the system makes the interfacial tension very low. Therefore microemulsions form spontaneously, with an average droplet diameter of 10-140 nm or less.[7] Microemulsion system has several advantages including enhanced drug solubility, ease of manufacturing, good thermodynamic stability and enhancement effect on transdermal ability over conventional formulation.[8, 9]

Microemulsion offers a significant advantage of increasing solubility of drugs through skin or mucous membrane and also can reduce their barrier due to its powerful permeation enhancing effect.[10] The solubilization of sertaconazole in microemulsions would improve its vaginal availability. However, it is also essential to have a dosage form which adheres to the vaginal mucosa and increases the residence time of sertaconazole in vagina. This functionality can be

imparted by gelling of the sertaconazole using bioadhesive agent. A gel can be described as the cross-linked material that retains a large amount of solvent inside its medium and if the solvent retained in organic one, such material is known as organogels.

Thus, in the present investigation, the potential of microemulsion based mucoadhesive gel of sertaconazole was investigated for vaginal delivery. The developed microemulsion based mucoadhesive gel of sertaconazole was evaluated for *in vitro* antifungal activity and *ex vivo* retention study.

MATERIALS AND METHODS

Sertaconazole was kindly gifted by Cipla Ltd. (Mumbai, India), Carbopol 940P and Acrysol K140 were kindly gifted by Corel pharmaceutical Ltd. (Ahmedabad, India). Captex-100, Capmul MCM C8, Cremophore-EL and Transcutol were kindly gifted by Abitec Corporation, U.S.A. Isopropyl myristate, Oleic acid, Olive oil, Tween-80, Span-80, Span-20 and Propylene glycol (All AR grade) were procured from SD fine chemical Ltd, Mumbai, India and all other chemicals were of analytical reagent grade. Candid®-V gel was purchased from local market.

Solubility study

The solubility of Sertaconazole was carried out to select appropriate oil (Acrysol K140, Oleic acid, Olive oil, Capmul MCM C8, Isopropyl myristate, and Captex-100), surfactant and co-surfactant (Cremophore-EL, Transcutol, Tween-80, Span-80, Span-20, Propylene glycol). An excess amount of Sertaconazole was added to 5 ml of oil or surfactant or co-surfactant and the resulting mixture was shaken reciprocally at 37°C for 72 hour followed by centrifugation for 10 min at 5000 rpm. The supernatant was filtered through a membrane filter paper (0.45µm) and the filtrate was analyzed in UV-Visible spectrophotometer with suitable dilution with methanol at 260nm.[11]

Construction of pseudo-ternary phase diagrams

In order to find out the concentration range of components for the existing range of micro emulsions, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature (25°C).[12] Oleic acid was screened as the oil phase. Tween 80 and Propylene glycol were selected as surfactant and co surfactant, respectively. Distilled water was used as an aqueous phase. Four phase diagrams were prepared with the 1:1, 1:2, 1:3 and 2:1 weight ratios of Tween 80 to propylene glycol, respectively. For each phase diagram at a specific surfactant/co surfactant weight ratio, the ratios of oil to the mixture of surfactant and co surfactant were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Water was added drop wise to each oily mixture with

continuous stirring at 37±0.5ºC until the mixture became clear at a certain point. The pseudoternary phase diagrams were prepared by considering three components (A, oil; B, the mixture of surfactant and co-surfactant; C, water). The amount of components required for formulation of microemulsion was chosen from phase diagram.

Formulation of microemulsions

From the phase diagrams, Sertaconazole-loaded microemulsions were selected at different component ratios. Microemulsion systems were prepared by mixing oil with the mixture of surfactant and co-surfactant and, water was added precisely drop by drop into oily phases with magnetic stirring at 37±0.5ºC (Table 1) The systems were equilibrated with gently magnetic stirring for 30 min followed by dissolving of appropriate amount of Sertaconazole under ultrasonication.[13] The final concentration of Sertaconazole in microemulsion formulation was 2% w/w.

Characterization of microemulsion

The pH values of microemulsion were determined at 25°C. The viscosity of various microemulsions was measured by using Brookfield Digital viscometer. The globule size and size distribution of microemulsion were determined by Malvern Zetasizer version 6.20 based on laser light scattering principle. The physical stability of microemulsion was tested by centrifuging the microemulsion at 3000 rpm for 30 min.[13,14]

Formulation of microemulsion based gel of sertaconazole

Various gelling agents namely, xanthan gum, sodium alginate, hydroxypropyl methylcellulose and Carbopol 940 were evaluated for their ability to gel sertaconazole microemulsion. Gelling agent was dispersed or dissolved slowly in 10ml of the sertaconazole microemulsion with the help of stirrer at 1,000 rpm. The suitable gelling agent was selected on the basis of compatibility with microemulsion structure, feel, and ease of spreadability. The optimized composition of sertaconazole microemulsion based gel is shown in Table-1.

Table 1: Different formulation of drug loaded microemulsion based vaginal gels

Ingredients in (% w/w)	F1	F2	F3	F4
Sertaconazole	2	2	2	2
Oleic Acid	3	5	5	5
Tween 80	21	27	20	50
Propylene glycol	21	54	60	25
S : CoS Ratio	1:1	1:2	1:3	2:1
Gelling agent (Carbopol 940)	1.2	1.4	1.2	1.4
Water	q.s	q.s	q.s	q.s

Characterization of sertaconazole microemulsion based gel

Appearance and pH

Appearance of gel was evaluated on the basis of visual evaluation. The pH of the gel was determined using digital pH meter calibrated using suitable buffer solutions.

Drug content

For determination of drug content, about 1gm of the gel was weighed in a 100 ml volumetric flask and dissolved in methanol the samples were appropropriately diluted and the absorbance was measured at 260 nm using UV-VIS spectrophotometer.[14]

Spreadability

Spreadability of microemulsion based gel was measured in terms of diameter of gel circle produced when placed between two glass plates of definite weight. A weighed quantity 0.5gm gel was placed within a circle of 1cm diameter premarked on a glass plate over which a second glass plate was placed. A weight of 500 gm was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading of the gels was noted.[15, 16]

Determination of Mucoadhesive Strength

The mucoadhesive forces for microemulsion based gels were determined by means of Mucoadhesive force measuring apparatus using tissue specimen obtained from mucosal side of goat vagina (procured from a slaughter house). At the time of testing section of tissue secured to upper side of glass vial using cyanoacrylate adhesive. One vial with the section of tissue connected to balance and other vial fixed on height adjustable pan. Simulated vaginal fluid was evenly spread on membrane and gel was applied on vaginal tissue. The height of vial adjusted so that the gel could adhere to the mucosal surface of both vials. The weights of the apparatus were kept raised until two vials became separated.[17] After the adhesive bond has formed, the force (weight) required to detach the tissue from surface of each formulation was measured and calculated as mucoadhesive strength.

Rheological Characterization

The rheological studies of samples were carried out with Brookfield Digital viscometer (LV DV-E model) using S-64 spindle number. The developed formulations were poured into the small sample adaptor of the Brookfield viscometer and the angular velocity increased gradually from 0.5 to 100 rpm. The average of the two readings was used to calculate the viscosity.

***In vitro* drug release study**

The in vitro drug release was, performed in sink conditions, by means of a Franz diffusion cell, diameter 20 mm, with water jacketed receptor chamber (15 ml) and a donor chamber

thermostated at 37±0.5ºC. The receptor solution was constantly stirred by magnetic stirrer. The two chambers were separated by a cellulose membrane and each formulation was spread on a circular portion of the membrane. A quantity of 1 ml samples were withdrawn and analyzed by Shimadzu UV-visible spectrophotometer at 260 nm. [14, 18]

Ex- Vivo Retention Measurement

An intact tubular piece of goat vagina was procured from a slaughter house. Vagina was suspended vertically with the help of a loop of wire and a stand.[19] The tissue was surrounded with a cotton pad moistened with normal saline, further surrounded by aluminum foil in order to keep the tissue moist for the duration of the experiment. An electronic balance was placed below the suspended tissue to measure the weight of gel falling down. The room temperature was maintained at 37±0.5ºC. A test sample (5gm) was introduced into the isolated vaginal tube with the help of a 10mL syringe (without needle), taking care to avoid spillage. The expulsion of gel from the lower end under the influence of gravity was then recorded as a function of time for 2 hrs as a measure of retention.

***In Vitro* Antifungal Activity**

The microbiological studies were performed against *Candida albicans* ATCC 10231 in Sabouraud's agar medium by the cup plate method. The microbiological studies were carried out on the optimized formulation, Candid®-V and 2% w/v of plain drug solution of sertaconazole for comparison against micro-organism. The diameter of zone of inhibition was measured.

RESULT & DISCUSSION

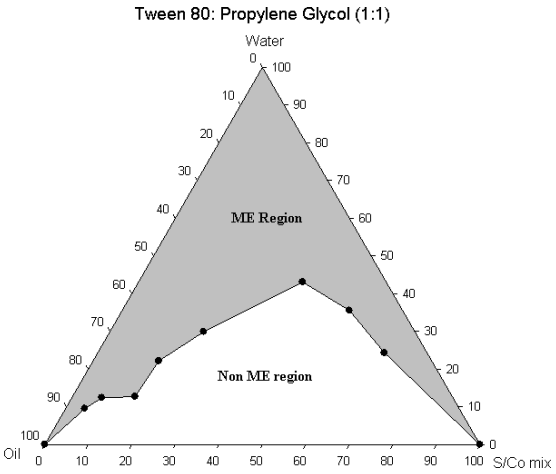
Pseudo ternary phase diagrams of microemulsions

The microemulsion existence region was determined by constructing phase diagrams. Figure-1 **(a) to (d)** describes the pseudo ternary phase diagram with various weight ratios of Tween 80 to Propylene glycol. The translucent region presented in phase diagram reveals the microemulsion existence region. No distinct conversion from water-in-oil (w/o) to oil-in-water (o/w) microemulsion was observed. The rest of region on the phase diagram represents the turbid and conventional emulsions based on visual inspection. After preparing the pseudo ternary phase diagram the medicated microemulsions were formulated.[20]

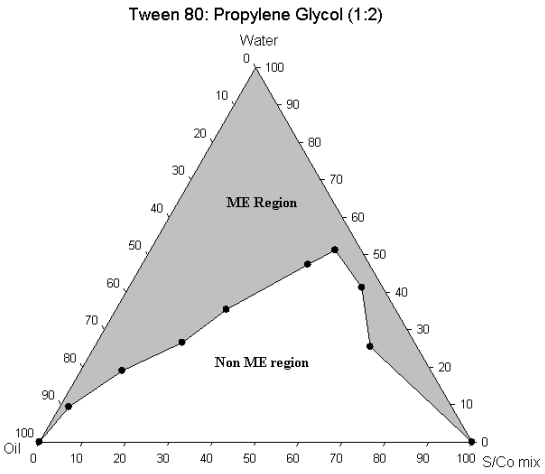
Characterization of microemulsion

All microemulsion formulation appeared uniform in color and transparency. The microemulsion formulations had appropriate observed pH values for vaginal application. Viscosities of all microemulsion were in the range of 2020 ± 115.33cps to 2720 ± 329.70cps. In general the

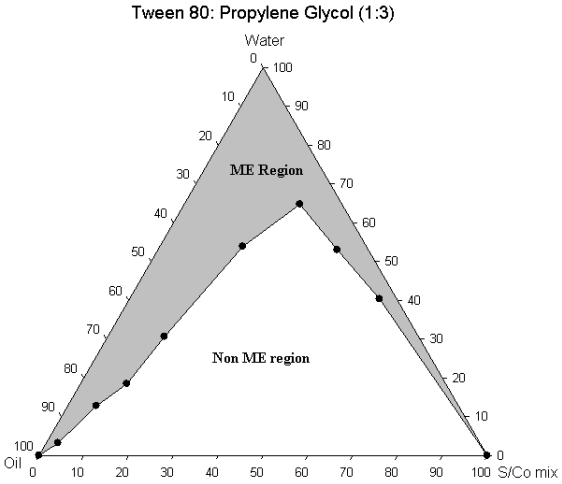
viscosity of the microemulsions was found to be increasing with increasing surfactant: co-surfactant concentrations. None of the microemulsion systems showed signs of phase separation on centrifugation at 3000 rpm for 30 minutes. This result provided a rapid and full proof identification of the system as microemulsion. The results of pH, viscosity, and globule size and zeta potential measurement of all the formulations are shown in Table-2.



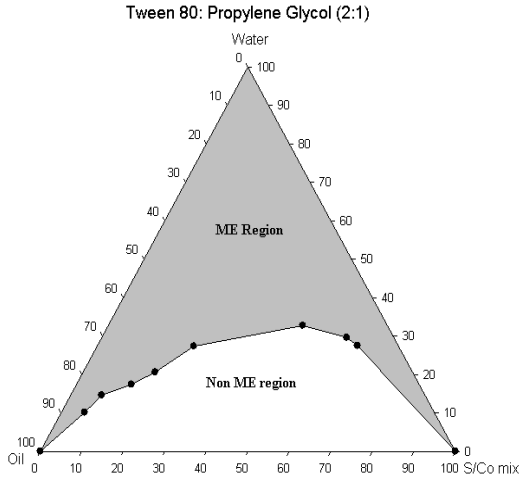
(a)



(b)



(c)



(d)

Figure 1 (a) to (d): Ternary phase diagram of surfactant /Co-surfactant (Tween 80/Propylne Glycol) 1:1, 1:2, 1:3 and 2:1 ratio respectively

Table 2: Globule size and zeta potential of microemulsion

Formula	pH	Viscosity (cps)	Globule size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
F1	4.24 ± 0.06	2020 ± 115.33	26.18	0.549	-0.256
F2	4.20 ± 0.20	2330 ± 170.88	162.4	0.668	-0.195
F3	4.25 ± 0.10	2500 ± 208.81	125.2	1.000	0.0536
F4	4.28 ± 0.32	2720 ± 329.70	7.208	0.907	-0.252

Formulation Development of Microemulsion Based Gel

Microemulsions have lower viscosity and are difficult to retain in vagina so for the ease of application they are tried to be gelled with suitable gelling agent. Various gelling agents such as xanthan gum, sodium alginate, hydroxypropyl methylcellulose and Carbopol 940 were evaluated for the gelling of sertaconazole microemulsion. It was observed that sodium alginate affected the structure of the microemulsion and resulted in separation of oily phase. This observation could be attributed to that fact that salts like sodium alginate can affect the structure of the microemulsion.[21, 22, 23] Xanthan gum and hydroxypropyl methylcellulose was unable to yield viscosity desirable for the gel formulation of acceptable consistency. Only Carbopol 940 at a concentration of 1 % w/w was able to thicken the microemulsion, could yield gel consistency without disturbing the microstructure of the sertaconazole microemulsion. Hence, Carbopol 940 was selected for the formulation of microemulsion based gel.[24, 25, 26]

Characterization of various sertaconazole microemulsion based gel formulation

All prepared gels were clear and transparent in appearance. Sertaconazole content in the microemulsion based vaginal gel was found to be in the range of 97.94±2.22 to 99.62±1.47 of the theoretical value (2.0 %w/w). The pH of microemulsion based gel systems was found to be in the range of 4.18±0.16 to 4.28±0.15 and acceptable to pH of vagina summarized in Table-3.

Table 3: Different evaluation parameters of sertaconazole microemulsion based vaginal gel formulations (mean ± SD, n=3)

Formula	Drug content	pH	Spreadability (cm)	Mucoadhesive Force (N)	*Ex-vivo* retention Time (min)
F1	98.92 ± 2.46	4.21±0.17	3.85 ± 0.17	0.220 ± 0.016	88 ± 2.3
F2	99.62 ± 1.47	4.18±0.16	3.53 ± 0.16	0.238 ± 0.010	97 ± 3.6
F3	99.31 ± 0.71	4.28±0.15	3.92 ± 0.26	0.242 ± 0.023	93 ± 1.8
F4	97.94 ± 2.22	4.26±0.10	3.58 ± 0.25	0.250 ± 0.012	102 ± 3.2

Spreadability

It is important parameter for uniform and ease of application of topical preparation from patient compliance point of view. High spreadability value of sertaconazole microemulsion based gel indicates better spreading ability at the site of application. The diameter of circle for sertaconazole microemulsion based gels was depicted in Table-3.

Mucoadhesive Force

Mucoadhesive force means the force with which microemulsion based gel bind to vaginal membrane at 37°C±0.5°C. The bioadhesive force is known to be dependent on the nature and the concentration of mucoadhesive polymers. The stronger the mucoadhesive force is, the more it can prevent the gel leaching from the vaginal tract. But if the mucoadhesive force is too

excessive, the gel can damage the vaginal mucous membrane.[27, 28] Therefore, microemulsion based gel must have the suitable mucoadhesive force. Mucoadhesive polymers, carbopol 940 enhanced gel strength most efficiently. Detachment stress of different formulations of sertaconazole microemulsion based vaginal gel was determined (Table-3). The formulations show adequate mucoadhesion.

Rheological Characterization

Viscosity of the gel was measured at different shear rates and rheological profile was generated. Rheological behavior of the microemulsion based gel systems indicated that the systems were non-newtonian in nature showing shear thinning and decrease in viscosity with increasing shear rates. This pseudo plastic rheology of formulation is useful for topical application of gels. The viscosity data and rheological profiles are shown in Figure-2.

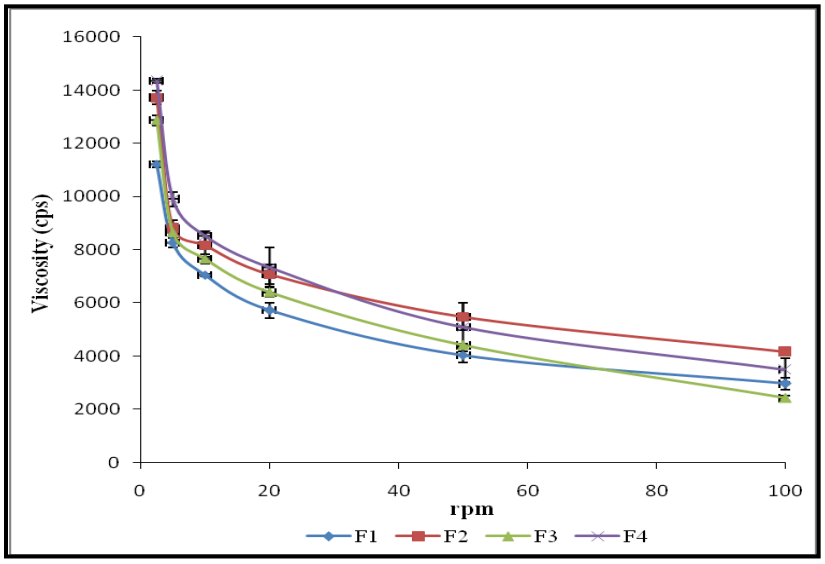


Figure 2: Rheological profile of F1, F2, F3 and F4 sertaconazole microemulsion based vaginal gel formulations

***In vitro* drug release study**

In vitro drug release kinetics was carried out by the use of Franz diffusion cells in order to evaluate sertaconazole microemulsion based vaginal gel release profile. The results of in vitro drug release study are shown in Figure-3. This pattern of the drug release confirms the controlled release behavior of the formulation. The release data of various formulations showed that; the concentration of polymer and the surfactant mixture content in the formulation significantly affected the percentage drug release. With increase in surfactant: co-surfactant ratio and polymer concentration the drug diffusion was decreased accordingly. The gel containing 1.2 % of carbopol 940 showed releases up to 97.87% at the end of 6 hrs while the gel containing 1.4 % of

carbopol showed release up to 99.48 % at the end of 8 hrs which indicates that the drug release from gels can be controlled by polymer concentration. Curve fitting of in vitro release data of all the formulation was compared with different release model to select best fitting model. The examination of correlation coefficients (R^2) indicated that drug release followed diffusion controlled mechanism from microemulsion based vaginal gels as the values for the correlation coefficient higher in case of zero order equation. This indicates that drug release depends on swelling, relaxation and erosion of polymer with zero order release kinetics.

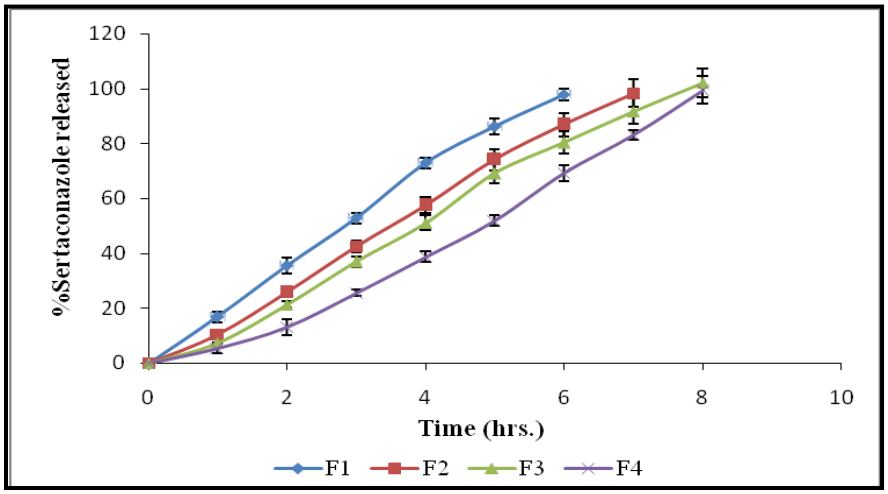


Figure 3: Release of Sertaconazole from microemulsion based vaginal gel formulations

Ex- Vivo Retention Measurement

Bioadhesion and long retention are desirable characteristics of a vaginal formulation. Carbopol 940 acts as mucoadhesive polymer. Performed adhesion force studies and retention studies proves the mucoadhesive nature of Carbopol 940. All the microemulsion based vaginal gel formulation exhibits good retention time. It was possible to note that Carbopol 940 possess good bioadhesive and retention properties. Carbopol 940 presence improved gel performances and made them able to be employed in vaginal therapy (Table-3).

***In Vitro* Antifungal Activity**

The sertaconazole microemulsion based gel formulation showed antimicrobial activity when tested microbiologically by cup plate technique. Clear zone of inhibition were obtained. The values of zone of inhibition produced by sertaconazole microemulsion based vaginal gel sertaconazole standard, and Candid-V gel were shown in Figure-4. It is clearly indicate that sertaconazole microemulsion based vaginal gel showed higher anti-fungal activity as compared to the marketed Candid-V gel ($P < 0.05$) and sertaconazole standard. The enhanced *in vitro* antifungal activity of sertaconazole microemulsion based vaginal gel may be attributed to

enhanced penetration of oil globules containing sertaconazole through fungal cell walls to inhibit ergosterol synthesis.

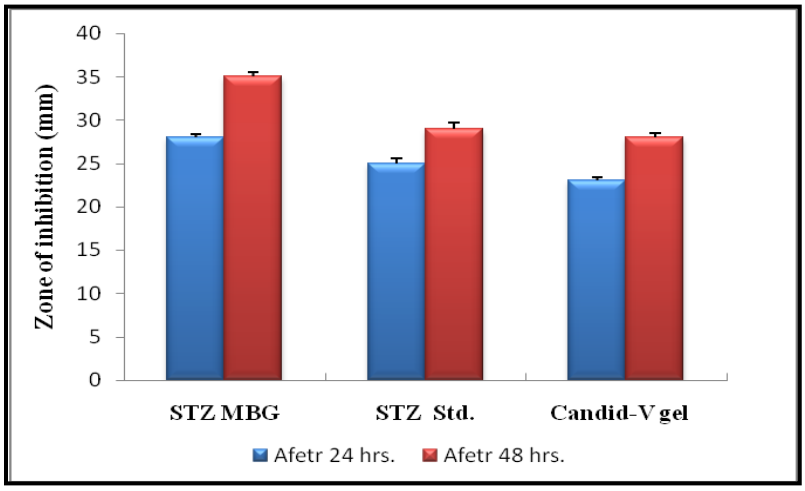


Figure 4: Zone of inhibition (mm) for sertaconazole microemulsion based vagina gel (STZ MBG), sertaconazole standard (STZ Std.) and Candid- V gel

CONCLUSION

Gel formulation of sertaconazole microemulsion with mucoadhesive properties is promising for prolonging the vaginal residence time and thereby better therapeutics effects. In addition they provide intimate contact between dosage form and vaginal mucosa which may result in high drug concentration in local area. The sertaconazole microemulsion based vaginal gel could be successfully formulated for the topical treatment of vaginal candidiasis. The developed sertaconazole microemulsion based vaginal gel showed good *in vitro* antifungal activity against *Candida albicans* when compared with standard and capable of loading therapeutics dose of sertaconazole, to control its release for 8 hrs. The studies indicated that sertaconazole microemulsion based vaginal gel could be a viable alternative to the current topical formulations available for the treatment of vaginal candidiasis.

ACKNOWLEDGEMENT

We are thankful to Cipla Pharmaceuticals, Abitec Corporation, U.S.A., Corel pharmaceutical Ltd for the gift samples of the drug and excipients, Department of Pharmaceutics, Nootan Pharmacy College, Visnagar for providing facilities to carry out entire research work and Institute of Research and Development Gujarat Forensic Science University, Gandhinagar for globule size analysis.

REFERENCES

1. Bodey GP. Azole antifungal agent. Clin Infect Dis 1992; 14(Suppl.): 161-169.

2. Sobel JD. Candidal vulvovaginitis. Clin Obstet Gynecol 1993 Mar; 36(1): 153–165.
3. Horowitz BJ, Giaquinta D, Ito S. Pathogens in vulvovaginal candidiasis: Implications for patient care. J Clin Pharmacol 1992; 32(3): 248–255.
4. Nencioni T, Pamparana F, Jacobellis M. Mycotic vulvovaginitis and topical cyclopiroxolamine. Minerva Ginecol 1988; 40(12): 719-722.
5. Tawfique K, Danesment TK, Warnock DW. Clinical pharmacokinetics of ketoconazole. Clin. Pharmacol 1988; 14: 13–34.
6. Daneshmend TK, Warnock DW, Turner A, Robert CJ. Pharmacokinetics of ketoconazole in normal subjects. Journal of Antimicrobial Chemotherapy 1981; 8: 299-304.
7. Attwood D, Kreuter J. Colloidal Drug Delivery Systems. New York: Marcel Dekker; 1994: 31–71.
8. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Deliv Rev 2000; 45: 89–121.
9. Gasco MR. Microemulsions in the Pharmaceutical Field: Perspectives and Applications. Industrial Applications of Microemulsions. New York: Marcel Dekker Inc; 1997: 97–122.
10. Baroli B, Lo´pez-Quintela MA, Delgado-Charro MB, Fadda AM, Blanco-Me´ndez J. Microemulsions for topical delivery of 8-methoxsalen. J Control Release 2000; 69: 209–218.
11. Hilton J, Woollen BH, Scott RC, Auton TR, Trbilcock KL, Wilks MF. Vehicles effect on *in-vitro* percutaneous absorption through rat and human skin. Pharm Res 1994; 11: 1396–1400.
12. Corswant CV, Engstrom SS, Söderman O. Langmuir.1997; 13: 5061–5070.
13. Rhee YS, Choi JG, Park ES, Chi SC. Transdermal delivery of ketoprofen using microemulsions. Int J Pharm 2001; 228: 161– 170.
14. Kavita A, Sivaramkrishna M, Nelinic CN, Nappinnai H. Formulation and evaluation of topical drug delivery system of fluconazole. Indian Drugs 2003 Dec; 40(2): 720-723.
15. Desai KG. Enhanced Skin Permeation of Rofecoxib Using Topical Microemulsion Gel. Drug Dev Res 2004; 63: 33–40.
16. Bachhav YG, Patravale VB. Microemulsion based vaginal gel of fluconazole: Formulation, *in vitro* and *in vivo* evaluation. Int J Pharm 2009; 365: 175–179.

17. Laithy HM, El-Shaboury K MF. The development of cutina lipogels and microemulsion gel for topical administration of fluconazole. AAPS PharmaSciTech 2002; 3(4): Article 35.
18. Puranajoti P, Patil RT, Sheth PD, Bommareddy G, Dondeti P, Egbaria K. Design and Development of Topical Microemulsion for Poorly Water-Soluble Antifungal Agents. The Journal Applied Res 2002; 2: 1-4.
19. Vermani K, Garg S, Zaneveld LJD. Assemblies for In Vitro Measurement of Bioadhesive Strength and Retention Characteristics in Simulated Vaginal Environment. Drug Deve Ind Pharm 2002; 28(9): 1133–1146.
20. Chen H, Chang X, Weng T, Zhao X, Gao Z, Yang Y, Xu H, Yang X. A study of microemulsion systems for transdermal delivery of triptolide. J Control Rel 2004; 98: 427–436.
21. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. Crit Rev Ther Drug Carrier Syst 1999; 16: 461–521.
22. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. Adv Drug Del Rev 2000; 45: 89–121.
23. Date AA, Patravale VB. Microemulsions: applications in transdermal and dermal delivery. Crit Rev Ther Drug Carrier Sys 2007; 24: 547–96.
24. Sharma G, Jain S, Tiwary K, Kaur G. Once daily bioadhesive vaginal clotrimazole tablets: design and evaluation. Acta Pharm 2006; 56: 337–45.
25. Knuth K, Amiji M, Robinson J. Hydrogel delivery systems for vaginal and oral applications. Adv DrugDeliv Rev 1993; 11: 137–67.
26. Baloglu E, Özyazıcı M, Hızarcıoglu SY, Karavana HA. An in vitro investigation for vaginal bioadhesive formulations: bioadhesive properties and swelling states of polymer mixtures. Il Farmaco. 2003; 58: 391–6.
27. Robert CR, Buri PA, Peppas NA. Experimental method for bioadhesive testing of various polymers. Acta Pharm Technol 1988; 34: 95–98.
28. Leung SS, Robinson JR. Bioadhesives in drug delivery. Polymer News 1990; 15: 333–342.