K. Jaya raja kumar et al /J. Pharm. Sci. & Res. Vol.4(8), 2012, 1885 - 1889

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Physiological Stimuli of Solution to Gel Depot Systems Containing Clotrimazole for Oral Thrush

K. Jaya raja kumar*, Selvadurai Muralidharan and Sokkalingam Arumugam Dhanaraj
Faculty of Pharmacy, AIMST University, Semeling, Bedong, Malaysia

Abstract
The scope of the present study is to develop a prolonged the delivery of the active drug in the oral cavity using a suitable carrier such as *in situ* gels which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance. Local drug delivery systems are better suitable for antifungal drugs particularly for oral thrush. The viscosity of in situ system was found to be in the range (42.8 to 49.55 cps) for the sol, whereas for the gels it was up to (47820 cps). The maximum gel strength and mucoadhesion was found to be up to (120 seconds) and (4211 dynes/cm^2) respectively. Moreover they sustained the drug release for more than eight hours with a fickian diffusion mechanism and zero order release kinetics; however the *in vitro* anti fungal effect was appreciable in the developed formulation. Different techniques, X-ray diffraction (XRD), FTIR spectroscopy and differential scanning calorimetry (DSC) were used to estimate the crystallinity degree and incompatibility.
Keywords: Oral thrush, Clotrimazole, Mice model, Pluronic 188, Carbomer 934, Temperature induced gel depot.

INTRODUCTION

Oral thrush is caused by candida albicans, the predominant causative agent of all forms of mucocutaneous candidiasis. The importance of oral thrush, especially in more severe cases, is that the persofinds it difficult to swallow. This further compromises the individual's nutritional status and ability to swallow medication. The symptoms of oral thrush include a burning pain, altered taste sensation, and difficulty swallowing liquids and solids[1].*Candida* species are ubiquitous, human fungal pathogens capable of initiating a variety of recurring superficial diseases especially in the oral and vaginal mucosae [2-3]. In the late 1950s there was a steadily increasing number of reports on superficial *Candida* infections associated with the administration of broad-spectrum antibiotics such as tetracycline [4-5]. In subsequent years, the extensive use of steroids, immunosuppressive agents in organ transplant recipients [6-7] myeloablative radiation therapy [8-10], and antineoplastics in patients with hematologic malignancies [11-13] contributed to the increasing morbidity associated with *Candida*. More recently, mucosal *Candida* infections have received profuse attention due to the advent of the human immunodeficiency virus (HIV) infection. Digits of antifungal agents are available for the treatment of candidal infections [14]. The most important agents that are currently used for oropharyngeal candidiasis belong to the polyenes group like nystatin and amphotericin B, the imidazoles group like clotrimazole, econazole, ketoconazole and miconazole or the triazoles groups like fluconazole, itraconazole [15]. Amphotericin B is less broadly used for this purpose due to its treatment-limiting adverse effects such as nephrotoxicity[16]. Clotrimazole (1-o-chloro-α, α-diphenylbenzyl) imidazole is a synthetic imidazole, having a broad spectrum of fungicidal activity, being effective against both dermatophytes and yeast-like fungi. Several options are available for the treatment of oropharyngeal thrush includes the systemic administration and topical solution. Topical agents, such as nystatin and clotrimazole troches, are generally used as first-line therapy, but they are inconvenient for patients to administer, multiple daily doses are usually required, less concentration of drug at the site of infection, dilution of drug in oral cavity, rapid elimination of topically applied drugs due to the flushing action of saliva, less effective than systemic therapies [17-19] and systemic adverse effects. Objective of the present study is to prolong the delivery of the active drug in the oral cavity using a suitable carrier such as *in situ* gels which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance. The *in situ* systems are made of biodegradable products, which can be injected via a syringe into the infected area and once injected; solidify to form a semisolid depot [20].

MATERIALS AND METHODS

Material

Pluronic 188 was supplied from BASF (Ludwigshafen, Germany). Carbomer 934 obtained from Merk, Mumbai. Clotrimazole was kindly provided by Fourt's India, Chennai. All other chemical used were of analytical grade (Merk , Mumbai).

Method

Thermosensitive and solution to gel depot systems were prepared by using the cold method [21]. Four monopolymeric systems were prepared by dissolving the required amount of Pluronic 188 (P 188; 10, 11, 13 and 15 %, w/w) in 10 ml of cold distilled water using a magnetic stirrer (100 rpm) and overnight at 4°C. An appropriate amount of clotrimazole was solubilized in physiologically compatible solvents (ethanol and polyethylene glycol) and the drug solution was added to above monopolymeric system with continuous stirring until a uniform drug solution was obtained. A small amount of triethanolamine was added to adjust the pH 7.

Seven binary polymer systems were manufactured containing P188 (15 % w/w) and Carbomer 934 (0.02, 0.03, 0.04, 0.1, 0.2, 0.3 and 0.4 % w/w). The required amount of Carbomer 934 was initially dissolved in cold distilled water (at 4 ºC)

using a mechanical stirrer, following which the required amount of pluronic was added to this solution and stirred to ensure complete mixing. Gel depot systems prepared according to the procedure described above.

EVALUATION OF *IN SITU* GEL

Gelation temperature

The different formulations of *in situ* system combinations were evaluated for gelation temperature. The gelation temperature was determined by heating the solution (1-2° C) min in a test tube with gentle stirring until gel was formed. The gel was said to have formed when there was no flow after container was overturned [22].

Determination of Mucoadhesive Force

The experimental technique used for determining the bioadhesive force has been derived from a previously published method [23-24]. The experimental setup is presented in figure 1. The mucoadhesive force of the formulations was determined as follows; a section of membrane was cut from the chicken and instantly fixed with mucosal side out onto each glass vial (E) using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan (A). Clotrimazole was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then, the switch (C) of the infusion apparatus was opened to make the water drop into the glass vial (B) with a constant flow rate of 5 ml/min. The weight of the water in the glass vial (B) kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dyne/cm^2), was determined from the minimal weights that detached the gel. The chicken membrane pieces were changed for each measurement. All measurements were performed in triplicate (*n* = 3).

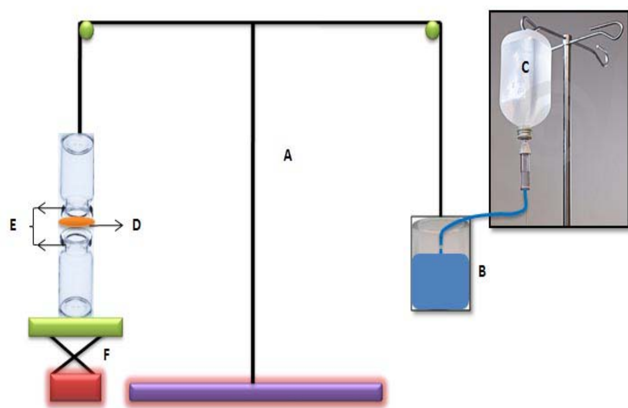


Figure 1. Mucoadhesive Force measuring device

Measurement of Gel Strength

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37 $^{\circ}$C. The apparatus for measuring gel strength (apparatus as shown in figure 2 weighing 27 gm) was allowed to penetrate in gel. The gel strength, which means the viscosity of the gels at physiological stimuli was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel [23].

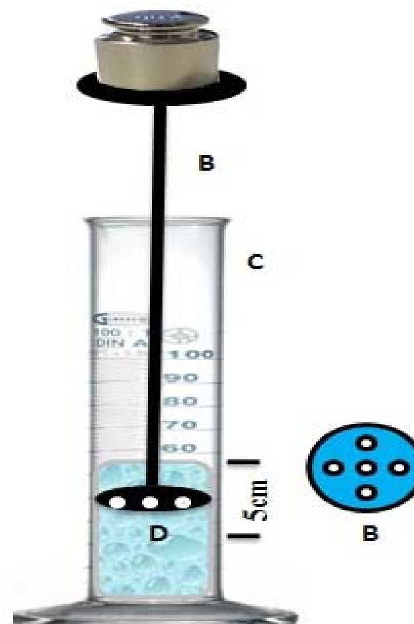


Figure 2. Gel strength measuring device

Viscosity Studies

The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosities of the solution were determined at different temperature and averages of two reading were used to calculate the viscosity.

Diffusion across the chicken cheek mucosa

Chicken cheek mucosa [25] was isolated from a healthy chicken which was obtained from the local slaughter house and was cleaned to remove blood cells. It was stored in normal saline with few drops of gentamycin sulphate injection, to avoid bacterial growth. The diffusion medium used was phosphate buffer (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl in 1000 ml of distilled water adjusted to pH 7.4).The oral diffusion cell was designed as per the dimension given. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir maintained at 37 ± 0.5°C and was connected to water jacket of diffusion cell using rubber latex tubes. The receptor compartment was filled with fluid. Then the prepared chicken cheek mucosa was mounted on the cell carefully so as to avoid the entrapment of air bubble under the mucosa. Intimate contact of mucosa was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment with the help of micropipette. Aliquots of samples were withdrawn at time intervals of one hour from sampling port of receptor compartment and same volume was replaced with receptor fluid solution in order to maintain sink condition. The samples were withdrawn and drug content was determined as per the above procedure.

Content Uniformity

The formed gel (1g) was completely crushed with the help of glass road followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution[26]. Final volume was adjusted to 100 ml with simulated saliva pH 7.4.Obtained solution was filtered through 0.45 micron filter membrane and the drug concentration was determined by UV Visible spectrophotometer at 260 nm. (Shimadzu UV1700, Japan)

Anti Fungal Activity
Methodology

Media Used: Potato Dextrose Agar (PDA)
Name of the analysis method: Agar diffusion method
Fungi analyzed: *Candida albicans*
Concentrations screened: 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg
The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl 10^{-4} CFU) and spread evenly on the plate. After 20 min, the wells were filled with of compound at different concentrations. The control plates with standard antibiotics were also prepared. All the plates were incubated at 27 °C ±1 for 48 hrs and the diameter of inhibition zone were noted [27, 28].

RESULTS AND DISCUSSION

Four monopolymeric systems and seven binary polymer systems were prepared (D1 to D7). The optimized formulations D1, D6 and D7 were taken for further studies.
Pluronics polymers as they are ABA type of copolymers, show characteristic property of thermoreversible gelation. The reversible thermal behavior of poloxamer (pluronic) in case of dilute as well as concentrated solutions was studied extensively [29]. Generally, this type of behaviour was observed in aqueous solutions of concentration range 15 to 20 % w/w. They are liquid when refrigerated (4 to 5 °C) but turn into gel form when at room temperature. The gel thus formed is reversible on again cooling [30]. Gelation temperature of temperature induced *in situ* gels of clotrimazole (Fig 3) decreased with increase in concentration of pluronic 188 from 35°C to 23°C for a concentration of 10 % to 15 % (D1 to D4). Gelation temperature of the D1 formula which contains 10% of pluronic was found to be 35°C and to have a viscosity of 30810 cps was taken for further studies. However the optimized concentration of pluronic (15%) showed a viscosity of 47640 cps and a gelation temperature of 23°C which is less than body temperature which is not suitable for maintaining a 'Sol' form at room temperature. So, carbomer was added an increasing concentration (0.02% to 0.4%) which led to the increase in gelation temperature from 34.2°C (D6), 36.5°C (D7) and a viscosity of 45500 cps, 47820 cps respectively (Fig 4). The optimized concentration of carbomer was found to be 0.03% and 0.04% for D6 and D7 and further addition led to an increase of gelation temperature more than body temperature. So, the formulae D6 and D7 were considered to be optimized formulae.

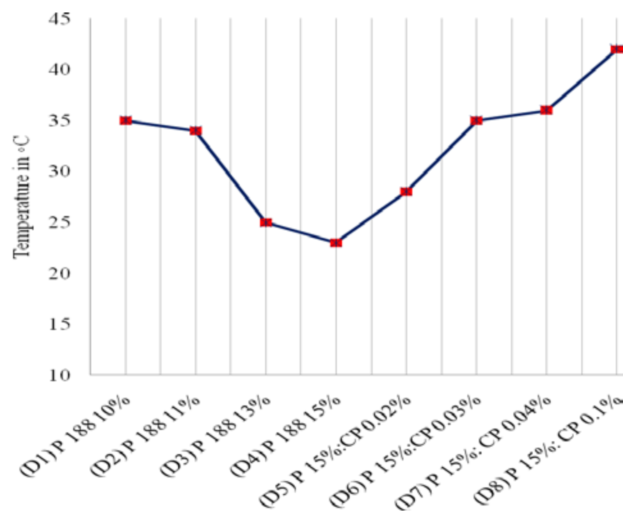


Figure 3. Effect of temperature induced systems on thermal gelation temperature

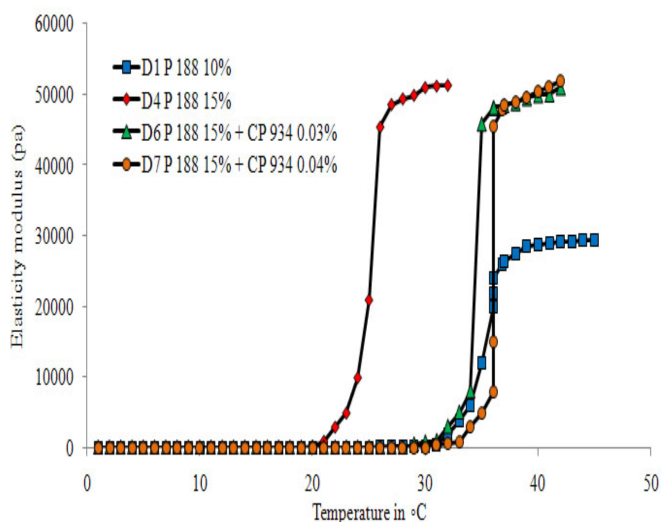


Figure 4. Temperature-dependent changes of the elasticity modulus.

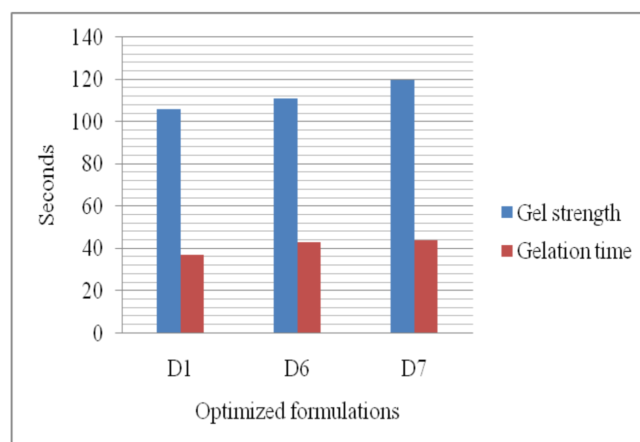


Figure.5 showing the gel strength and gelation time

The gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out from the site of administration. The gel strength (Fig. 5) of formulation D6 and D7 (111, 120 sec) exhibited good gel strength among all optimized D code formulation which may due to increase in concentration of pluronic and its reversible gelation character at 37 ºC.

Table.1 Characteristics of optimized clotrimazole *in situ* Gel

Formulation	% drug content (w/w)	Mucoadhesive force (dynes/cm^2)	Gelation Capacity
D1	98.4	3655	***
D6	98.6	4211	***
D7	98.6	4912	***

*** Good

The mucoadhesive force (Table 1) is an important physicochemical parameter of topical application in buccal cavity. The mucoadhesive force was significantly increased from 3655 dynes/cm^2 (D1) to 4912 dynes/cm^2 for the formula D7 which consists of 0.04% of carbomer and 15% of pluronic, as the concentration of mucoadhesive polymer (carbomer) increased. This also proved that carbomer has better mucoadhesive property than pluronic.

The *in vitro* diffusion studies conducted through the chicken cheek membrane from the formulae D1, D6 and D7 released 78.2%, 83.3% and 86.6% respectively at the end of 8th hour. The diffusion of drug from formulation D1 was less may be due to presence of pluronic 188 in the gel which retards the drug release rate owing to reduction in dimension of water channel. While diffusion of drug through formulation D6, D7 was found to be more which may be due to presence of carbomer 934, which undergoes rapid swelling and helps in faster diffusion**.** The value of release kinetics (Table 2) showed that the optimized formulae of thermo sensitive *in situ* gels followed zero order release mechanism and more over the 'n' value of korsemeyer equation confirmed that the release mechanism was fickan.

Table .2 Release kinetics of optimized formulation

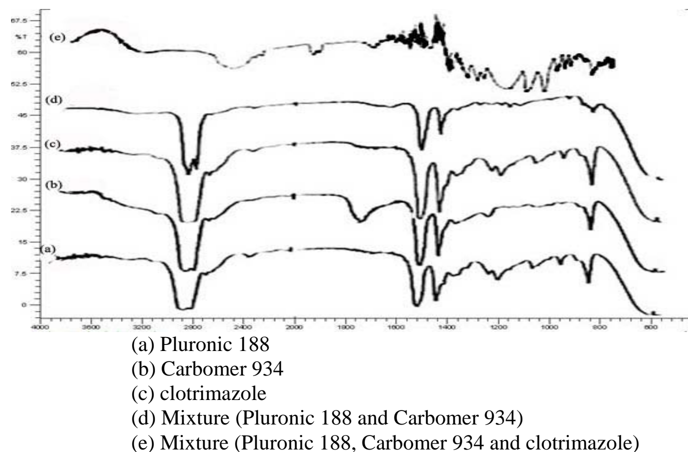
Order of process	**D1**	**D6**	**D7**
Zero order	R² 0.990	0.992	0.993
	M 6.155	6.602	6.795
	C -0.480	-0.8	0.476
First order	R² 0.991	0.99	0.991
	M -0.048	-0.061	-0.071
	C 2.051	2.116	2.191
Higuchi	R² 0.990	0.992	0.989
	M 6.155	6.602	6.795
	C -0.480	-0.8	0.476
korsmeyer	R² 0.968	0.955	0.96
	M 0.056	0.058	0.055
	C 1.318	1.33	1.373

Clotrimazole compound formed the polymer active with no disturbance the functional group. Therefore polymerized active constituent has no change of effect after polymerization (Table 3), (Fig 6).

Table .3 FTIR Spectra

compound cm^{-1}	cH stretching	C-Cl stretching	poloxamar112 0 cm^{-1}		carbopol 934 cm^{-1}		C-H Bending cm^{-1}	Ar-N-tertiary	CN vibration cm^{-1}
			C-O polymeric associated	CH_2 - CH_3 CO	OH	COOH			
Clotrimazole	2920	720					1460		1360
P			1380	1320					
CP 934									
P+CP 934	2960		1380	1320		1140-1720 cluster			
P188+CP 934+ Clotrimazole	2910	720	1380	1320			1460		1360

Pluronic 188–P, Carbomer 934-CP



(a) Pluronic 188
(b) Carbomer 934
(c) clotrimazole
(d) Mixture (Pluronic 188 and Carbomer 934)
(e) Mixture (Pluronic 188, Carbomer 934 and clotrimazole)

Figure.6 Showing the FTIR Spectra

Clotrimazole showed a narrow and sharp endothermic peak at 122.31˚C and151.40°C. The DSC thermograms of physical mixtures of clotrimazole with Carbomer 934 and grades of P188 showed shift peaks at 193.46 and 242.05. The DSC thermograms of physical mixtures of polymer combination showed endothermic peaks at 57.63. The pluronic 188 peaks at 62.96°C, The DSC thermogram of Carbomer 934 showed endothermic peak between 160°C to 240°C. These findings indicate there was no interaction (Fig 7) occurs between clotrimazole, polaxamer 188 and Carbomer 934 combination. Therefore, pluronic and Carbomer 934 can be used as excipients in the formulation of clotrimazole *in situ* gels

The X-ray diffractograms of pure clotrimazole showed the distinct peaks at between 12.55 θ and other peak at 19.6 θ, Pluronic 188 was peak at between 22 θ to 23 θ and Carbomer 934 showed peak at 15.95 θ, the polymer combination peak observed at 19.3 θ and 23.35 θ, the polymer combination with drug was peak at 19.4 θ and 23.34 θ, There is no appreciable change in the position of the peak with respect to θ values of physical mixture (Fig 8).

Table 4. Zones of inhibition (in cm)

	0.125 mg	0.25 mg	0.5 mg	1.0 mg	2.0 mg	4.0 mg	MIC mg
Clotrimazole (standard)	0.9	1.5	1.9	2.1	2.3	2.7	> 0.125
Clotrimazole (*in situ* gel)	1.7	2.0	2.4	2.6	2.8	3.1	> 0.125

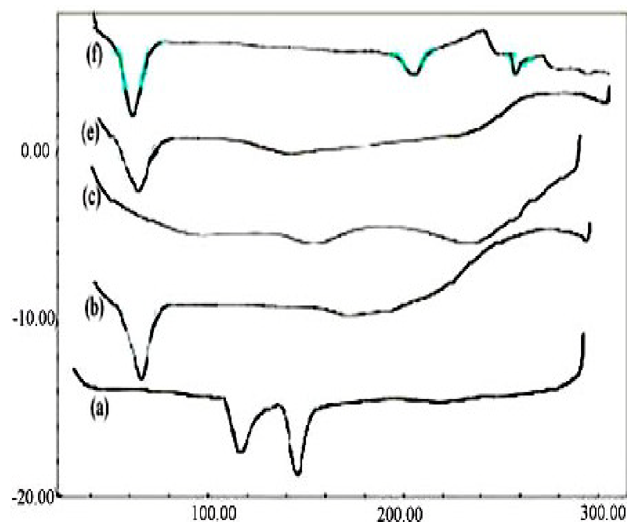


Figure. 7 Showing the DSC Spectra

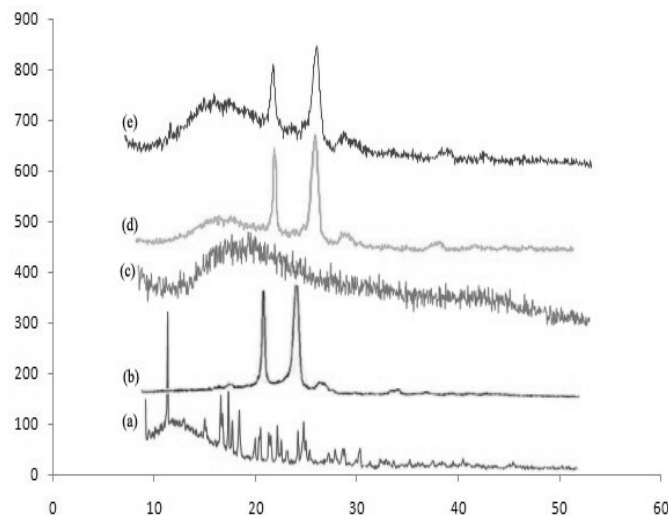


Figure.8 Showing the XRD Spectra

(a) Pluronic 188 (b) Carbomer 934 (c) clotrimazole (d) Mixture (Pluronic 188 and Carbomer 934)
(e) Mixture (Pluronic 188, Carbomer 934 and clotrimazole)

The antifungal activity the zone of inhibition for standard solutions was in the range of 0.9 cm to 2.7 cm (p > 0.125), whereas it was in the range of 1.7 cm- 3.1 cm (p > 0.125) for the *in situ* gels. It is clearly understood that the formulations showed better antifungal activity against *candida albicans* in comparison to standard solution of the drug used in the study (table 4).

CONCLUSION

Thermo sensitive *in situ* gels of clotrimazole were prepared with pluronic 188 and Carbomer 934 by taking 15% w/w pluronic 188 and 0.01 to 0.04% w/w Carbomer 934. These hydrogels are liquid at room temperature (25 ºC) and undergo gelation when in contact with body temperature (37 ºC). All the formulae found to have less gelation time and more mucoadhesive force with compatible viscosity at physiological temperature. Moreover they sustained the drug release for more than eight hours with a fickian diffusion mechanism and zero order release kinetics, moreover the *in vitro* anti fungal effect was appreciable in the formulation.

REFERENCES

[1] Fichtenbaum, C. J., Koletar, S., Yiannoutsos, C., Holland, F.,Pottage, J., Cohn, S.E., Walawander, A., Frame, P.,Feinberg, J., Saag, M., van der Horst, C., Powderly,W.G., 2000. Refractory mucosal candidiasis in advanced human immunodeficiency virus infection. Clin. Infect. Dis.30, 749–756

[2] Odds, F. C. 1988. *Candida* and candidosis: a review and bibliography. Bailliere Tindale, London, United Kingdom

[3] Samaranayake, L. P., and T. W. MacFarlane**.** 1990. Oral candidosis: an old disease in new guises. AIDS Commentary, Dental update January/February 1990

[4] Knight, L., and J. Fletcher. 1971. Growth of *Candida albicans* in saliva: stimulation by glucose associated with antibiotics, corticorsteroids and diabetes mellitus. J. Infect. Dis. 123:371-377.

[5] Seelig, M. S. 1966. Mechanisms by which antibiotics increase the incidence and severity of candidiasis and alter the immunological defenses. Bacteriol. Rev. 30:442-459.

[6] Saltarelli, C. G., K. A. Gentile, and S. C. Mancuso. 1975. Lethality of *Candida* strains as influenced by the host. Can. J. Microbiol. 21:648-654.

[7] Taschdjian, C. L., M. S. Seelig, and P. J Kozinn. 1973. Serological diagnosis of candidiasis. Crit. Rev. Clin. Lab. Sci. 4:19-60.

[8] Heimdahl, A., and C. E. Nord. 1990. Oral yeast infections in immunocompromised and seriously diseased patients. Acta Odontol. Scand. 48:77-84.

[9] Horn, R., B. Wong, and T. E. Kiehn. 1985. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. Rev. Infect. Dis. 7:646-655.

[10] Wingard, J. R., W. G. Merz, M. G. Rinaldi, T. R. Johnson, J. E. Karp, and R. Saral. 1991. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N. Engl. J. Med. 325:1274-1277.

[11] Bodey, G. P. 1986. Fungal infection and fever of unknown origin in neutropenic patients. Am. J. Med. 80:112-119.

[12] Gold, J. W. M. 1984. Opportunistic fungal infections in patients with neoplastic disease. Am. J. Med. 76:458-463.

[13] Maksymuik, A. W., S. Thongprosert, and R. Hopfer. 1984. Systemic candidiasis in cancer patients. Am. J. Med. 77:20-27.

[14] Ginnis mc MR, Rinaldi MG. Antifungal drugs: mechanisms of action, drug resistance, susceptibility testing and assays of activity in biological fluids. In V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore, Md. 1996; 176-211.

[15] Ellepolla ANB, Samaranayake LP. Oral candidal infections and antimycotics. Crit. Rev. Oral Biol. Med. 2000; 11: 172-198.
[16] Kauffman, CA. Role of azoles in antifungal therapy. Clin. Infect. Dis. 1996; 22:148-153.
[17] Koletar SL, Russell JA, Fass RJ, Plouffe JF. Comparison of oral fluconazole and clotrimazole troches as treatment for oral candidiasis in patients with human immunodeficiency virus. *Antimicrob Agents Chemothe*: 1990; 34:2267-2268.
[18] Pons V, Greenspan D, Debruin M, and the Multicenter Study Group. Therapy for oropharyngeal candidiasis in HIV-infected patients: A randomized, prospective multicenter study of oral fluconazole versus clotrimazole troches. *J Acquired Immune Dejk Syndr.* 1993 ; 6:1311-1316.
[19] Sangeorzan JA, Bradley SF, He X, et al. Epidemiology of oral candid & is in HIVinfected patients: Colonization, infection,treatment, and emergence of fluconazoleresistance. *Am J Med.* 1994; 97:339-346.
[20] Hatefi A, Amsden B. Biodegradable injectable in situ forming drug delivery systems. Journal of Controlled Release 2002; 80:9-29
[21] Jones, D.S., Brown, A.F., Woolfson, A.D., 2003a. Solute and solvent effects on the thermorheological properties of poly (oxyethylene)-poly (oxypropylene) block copolymers: implications for pharmaceutical dosage form design. J. Appl. Polym. Sci. 87, 1016–1026.
[22] Pandit NK, Kisaka J. Loss of gelation ability of Pluronic 127 in the presence of some salts [J]. Int J Pharm, 1996; 145,129-136
[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] Koffi FAA, Agnely G, Ponchel JL, Grossiord,Modulation of the rheological and mucoadhesive properties of thermosensitive pluronic-based hydrogels intended for the rectal administration of quinine. European Journal of Pharmaceutical Sciences.2006; 328–335-27
[25] Harish Matapady,Narayana. Development of a gellan gum based mucoadhesive in situ gels for buccal local delivery system of fluconazole, int.j.chhem.sci: 2009; 7, 315-326.
[26] Jagdish Balasubramaniam., In vitro and in vivo evaluation of the Gelrite gellan gum-based ocular delivery system for indomethacin, Acta Pharm. 2003; 53 251–261.
[27] Threlfall E.J., Fisher I.S.T., Ward L., Tschape H. & Gerner-Smidt P. (1999). Harmonization of antibiotic susceptibility testing for *Salmonella:* Results of a study by 18 national reference laboratories within the European Union-funded Enter-Net group. Microb. Drug Resist. **5**, 195–199.
[28] Walker R.D. (2000). Antimicrobial susceptibility testing and interpretation of results. *In:* Antimicrobial Therapy in Veterinary Medicine, Prescott J.F., Baggot J.D., Walker R.D., eds. Ames, IA, Iowa State University Press, 12–26.
[29] Henry RL, Schmolka IR: Burn wound coverings and the use of pluronics preparations. Crit. Rev. Biocompatibility. 5:207-220, 1989.
[30] Wanka G, Hoffmann H, Ulbricht W: Phase diagrams and aggregation behavior of poly (oxyethylene)-poly (oxypropylene)-poly (oxyethylene) triblock coplymers in aqueous solutions. Macromolecules 27:4115-4159, 1990.