Jaya raja kumar et al /J. Pharm. Sci. & Res. Vol.4(2), 2012, 1716--1723

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

Highly Effective Ciclopriox Olamine Temperature-Induced In Situ Gel for Oral Thrush

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Abstract:
The endeavor of this study was the formulation and assessment of a novel temperature-induced and mucoadhesive *in situ* gel delivery system, which can be used in the treatment of oral thrush. A mucoadhesive polymer was used as a thermo responsive material, because pluronic 188 and carbomer 934 has thermal gelation properties at certain temperature. The thermal gelation temperature 15% w/w pluronic 188 and carbomer 934 1% w/w solutions is above body temperature, but by using different ratio of pluronic and carbomer combination of system, it can be shifted near to body temperature. The optimized formulations were evaluated for gelling capacity, viscosity, gel strength, mucoadhesive force, drug release, anti fungal activity, FTIR, DSC and XRD.
Keyword: Prolong Release, Ciclopriox olamine, pluronic 188, Carbomer 934, temperature induced, Oral Thrush

INTRODUCTION

Oral candidiasis (also known as "thrush"[1]) is an infection of yeast fungi of the genus *Candida* on the mucous membranes of the mouth. It is frequently caused by *Candida albicans*, or less generally by *Candida glabrata* or *Candida tropicalis*.

Candida species are everywhere, human fungal pathogens capable of initiating a variety of recurring superficial diseases especially in the oral and vaginal mucosa [2,3]. In the late 1950s there was a progressively 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 broad use of steroids, immunosuppressive agents in organ transplant recipients[6,7] myeloablative radiation therapy[8,9,10], and antineoplastics in patients with hematologic malignancies[11,12,13] contributed to the increasing morbidity associated with *Candida*. More lately, mucosal *Candida* infections have received profuse attention due to the advent of the human immunodeficiency virus (HIV) infection. For instance, it is now known that up to 90% of HIV-infected individuals suffer from oropharyngeal candidiasis[14]

Even though the clinical efficacy of systemic antifungal treatment is well established, the potency is decreased by thousand fold when reaches the target site, and also large dose and/or prolonged administration is often necessary to maintain an effective drug concentration. The long-term use of systemic antifungal drugs is associated with potential adverse effects and patient non-compliance and also less drug availability at the site of infection, limits its use. In such condition a safe and effective local route of drug delivery device, which will reduce the dose and increase the concentration of drug in the oral cavity with low systemic concentration is highly desirable.

Hence, attention has been focused on extended released topical antifungal formulations aimed at prolonging active drug concentrations in the oral cavity. Using appropriate carriers, which can effectively administer the drug for an extended period of time will reduce the systemic side effect but also improve the therapeutic efficacy and patient compliance.

MATERIAL AND METHOD:

Material: pluronic 188 were kindly gifted by pharmaceutical Pvt. Ltd, (Bombay). Ciclopriox olamine was used as a standard drug from Glenmark, India. All other chemical used were of analytical grade.

Method: Thermo reversible gels were prepared using cold technique. The method involved slow addition of polymers in required quantity of cold distilled water further it was kept overnight for swelling. The polymer solution was taken in a beaker stirred continuously using a magnetic stirrer until a uniform solution was obtained and it was kept at ambient temperature for 24 hrs. A small amount of triethanolamine was added to adjust the pH 7. An appropriate amount of selected drugs solubilized in physiologically compatible solvents with continuous stirring until a uniform drug solution was obtained. The detailed composition of prepared formulation is depicted in Table- 1

Table1. Composition of Temperature induced formulation code RR

Ingredients (w/w) %	RR 1	RR 6	RR 7
Ciclioprioxolamine	1	1	1
Pluronic 188	15	15	15
Carbomer 934	---	0.03	0.04
Alcohol	q.s	q.s	q.s
Citric Acid	0.18	0.18	0.18
Triethanolamine	q.s	q.s	q.s
Cold. Distilled Water	q.s	q.s	q.s

EVALUATION OF *IN SITU* GEL

Determination of pH:

The pH of the gel was determined using a calibrated pH meter. The readings were taken for average of 3 samples[15]

Gelling capacity:

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated saliva fluid (7.4) freshly prepared and equilibrated at 37^0 C and visually assessing the gel Formation and noting the time for gelation and the time taken for the gel formed to dissolve. Different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time[15].

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^o 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[16].

Viscosity Studies:

The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosity of *in situ* gel and the solution were determined at different angular velocities (0, 10, 20, 30, 40….to 100 rpm) and average of two reading were used to calculate the viscosity.

Spreadability:

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability[17] (S)

S= ML/T

Where, M = weight tide to upper slide (g)

L = length moved on the glass slide (cm)

T = time taken (sec)

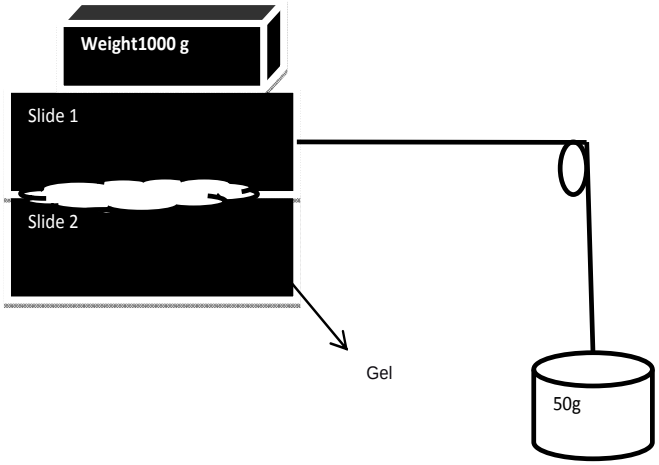
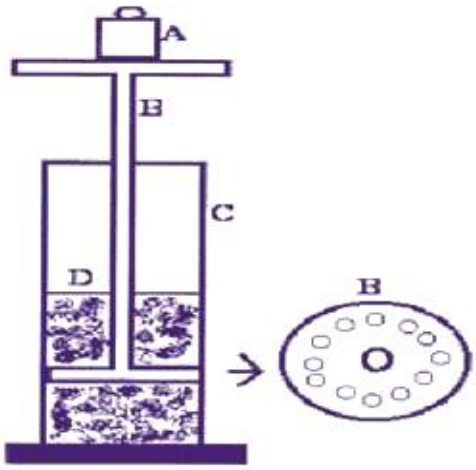


Figure -1. Diagramatic representation of spreadability measuring device.

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 37^0 C. The apparatus for measuring gel strength (apparatus as shown in figure 23 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[18]



(A)Weights (B) device (C) measuring cylinder (D) gel

Figure - 2.Diagramatic representation of gel strength measuring device.

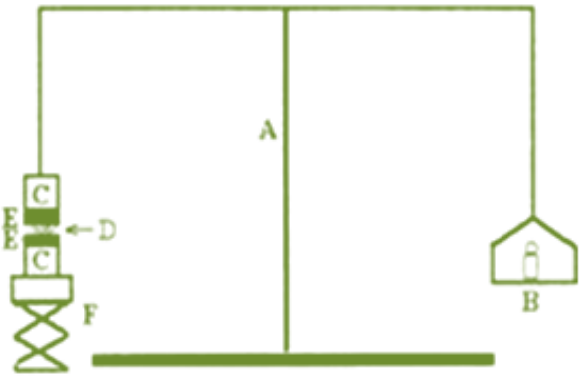
Determination of Mucoadhesive Force:

The mucoadhesive force of all the optimized batches was determined as follows, a section of mucosa was cut from the chicken cheek portion and instantly fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken cheek mucosa was connected to the balance in inverted

position while first vial was placed on a height adjustable pan. Oral gel was added onto the buccal mucosa of first vial. Before applying the gel, 150µL of simulated saliva solution (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) was evenly spread on the surface of the test membrane. 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 weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The cheek mucosa was changed for each measurement[17].

Detachment stress (dynes/cm^2) = m g/A

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2; and A is the area of tissue exposed.



(A) Modified Balance,(B) weight (C) Glass vial
(D) Gel (E) mucous membrane (f) Height adjustable pan

Figure -3.Diagramatic representation of bioadhesive force measuring device

Diffusion across the chicken cheek mucosa:

Chicken cheek mucosa 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[15].

Assembly of diffusion cell-for *in vitro* diffusion studies:

The diffusion medium used was phosphate buffer (2.38 gm Na_2HPO_4, 0.19 gm KH_2PO_4 and 8 gm 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^{0}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.

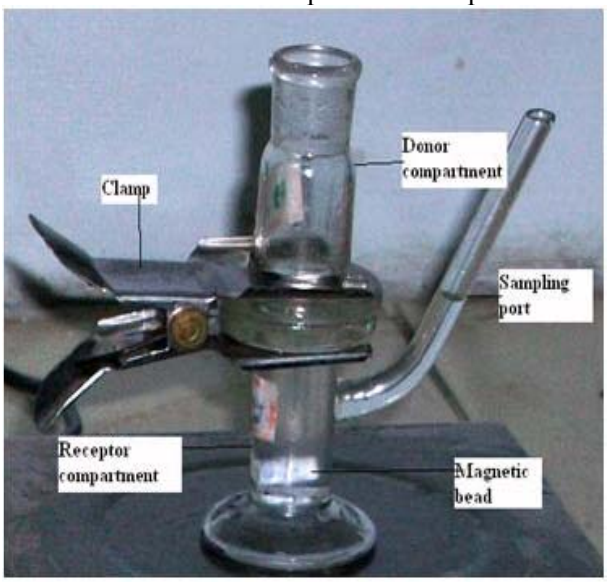


Figure - 4.diagramatic representation of apparatus used for finding diffusion study

Content Uniformity

Tests for content uniformity were carried out for all the prepared gel formulations. The vials (n =3) containing formulation were properly shaken for 2-3 min[19]. One ml of the formulation was transferred into 100 ml volumetric flask with 1 ml calibrated graduated Pipette. 50 ml of simulated saliva pH 7.4 was added. The formed gel was completely crushed with the help of glass road followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution. 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. (Shimadzu UV1700, Japan)

RESULT AND DISSCUSSION

Physicochemical properties of ciclopiroxolamine *in situ* gels

Physicochemical properties such as viscosity, pH, appearance and clarity were performed and the results are recorded in Table -2.

Table- 2: Physicochemical Evaluation of optimized ciclopriox Solution:

Tests	RR1	RR6	RR7
visual appearance	***	***	***
clarity	***	***	***
pH	6.8	6.9	7.0
Viscosity (Cps)	43.11	49.34	50.05

Gelation temperature

GT of temperature induced *in situ* gels decreased with increase in concentration of Pluronic188 in RR1, RR2, RR3 and RR4 at 35.5°C, 34.2°C, 25.1°C and 23.4°C respectively. By adding with Pluronic188 in increasing ratio (15:0.03), (15:0.04), (15:0.05) an increase in gelation temperature has occurred in the formulations RR5, RR6 and RR7 to 28.3°C, 35.6°C and 36.5°C respectively. The Pluronic188 concentration was optimized at 15%. The buccal gel prepared with Pluronic188 (15%) with in RR8, RR9, RR10 and RR11 (0.1% to 0.4%) increased the GT (42.2°C to 60.2°C). Since RR1, RR6 and RR7 showed gelation at a temperature near to body temperature, they have been selected as optimized formulae for further studies (Fig-5).

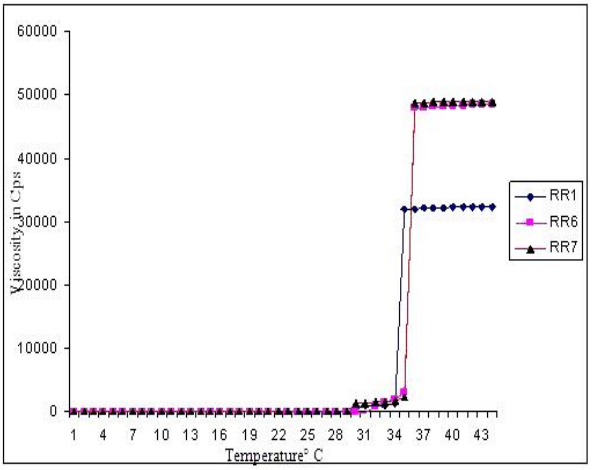


Figure -5. Showing the Viscosity versus temperature curves of ciclopriоxolamine In situ gel

Viscosity Study

The optimized formulations RR1, RR6 and RR7 exhibited an increase in viscosities (31943, 47830, 48750 Cps) at body temperature. But the other formulations (RR8, RR9, RR10 and RR11) have not exhibited an increase in viscosity at body temperature which may be due to the increasing concentration of polymers (0.1 to 0.4) (Fig- 5)

Gelation time

The gelation time of ciclopirox olamine formulations RR1(15%), RR6 (15%:0.04%) and RR7(15%:0.05%) demonstrated gel-like rheologic properties at 35.5°C, 34.6^{0}C and 36.5^{0}C depending on the percentage of P188/CP 934. whereas the formulation RR1 with 15% of P188 showed gelation within 43 sec .The gelation time of solutions containing P188/ CP 934 K6 (15/0.02), K7(15/0.03) was observed at 35 sec. and 34 sec,. This effect may be due to increased concentration of in the formulation. Every addition of (0.1% w/w CP 934) into the formulations leads to 5^{0}C increase in gelation temperature (Table- 3).

Table -3. Characteristics of optimized ciclopriox*in situ* Gel FormulationsRR code

Formulation	Viscosity (Cps)	% drug content (w/w)	Mucoadhesive force (dynes/cm^2)	Gel strength(sec.)	Gelation Capacity	Gelation Time (sec.)
RR1	31943	97.5	3673.12	108	***	43
RR6	47830	98.7	4801.14	112	***	35
RR7	48750	98.7	4992.06	116	***	34

Content Uniformity

All the optimized formulation of ciclopirox olamine (RR1, RR6, and RR7) was checked for their content uniformity. The content was determined at 298 nm, which is of drug using Shimadzu 1700UV-VIS spectrophotometer**.**

Gel Strength

At 37°C, the gel strength of formulation RR6 (112 sec) was found to be more as compared to formulation RR1 (108 sec) and gel strength of formulation RR7 (116 sec) which was found to be more as compared to the other formulations This may be due to reversible gelation properties of Pluronic at 37°C (Table- 3)

Determination of mucoadhesive force

Adhesive properties of Pluronic188 formulations increased with the increase in concentration of in formulations RR6 (P188 15% w/w: CP 934 0.02% w/w) (4801.14 dynes/cm^2) and RR7 (P188 15% w/w: CP 934 0.03% w/w).(4992.06 dynes/cm^2).. Whereas the formulation RR1 which contains only Pluronic (P188 10%) showed less viscosity (3673.12 dynes/cm^2) (Table-3)

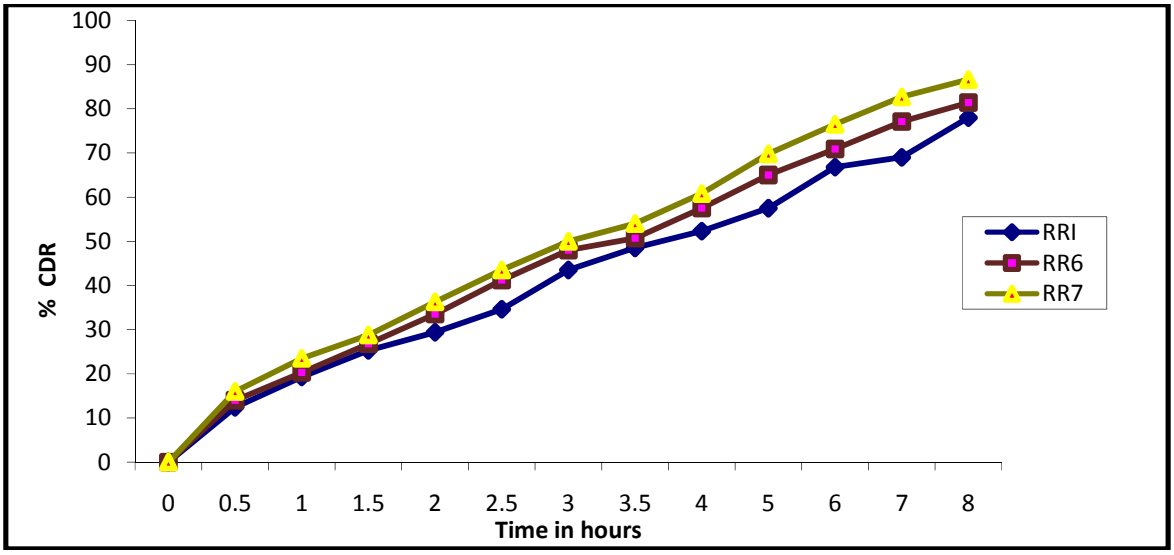


Figure -5. Showing the Diffusion of RR Code- optimized formulation

***In vitro* Release Studies:**

The percentage ofCiclopiroxolamine drug diffused through chicken cheek mucous membrane over a period of 8 hrs from formulation RR1, RR6 and RR7 was found to be 78.5%, 81.4% and 86.6 % respectively. The diffusion of drug from formulation RR1 was less which may be due to presence of Pluronic188 in the gel which retards the drug release rate owing to reduction in dimension of water channel. While diffusion of drug from formulations RR6, RR7 was found to be more which may be due to presence of , which undergoes rapid swelling properties (Fig-5)

Drug Release Kinetics

The correlation coefficient 'r' indicated that the drug release followed diffusion controlled zero order mechanism from the *in situ* gel of Ciclopirox olamine, as the value of 'r' for zero order kinetics ranged from 0.991 to 0.993 and also found to be more than that of first order which ranged from 0.990 to 0.991. The value of 'r' for Higuchi kinetics ranged from 0.990 to 0.991. It was clearly indicated that RR code formulation were following predominantly zero order release

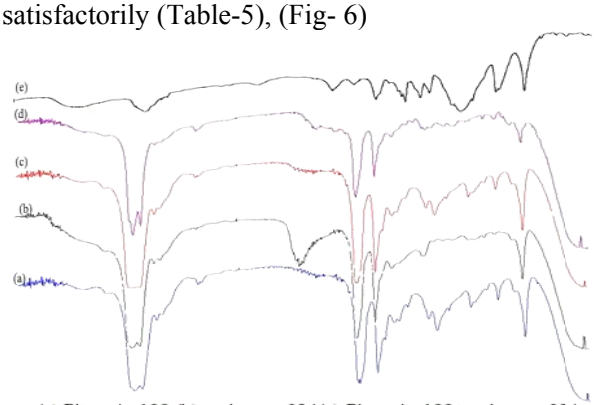
The formulations RR1, RR6 and RR7 exhibited good *in vitro* release kinetics with fickian type of diffusion mechanism. More over to comprehend the drug release mechanism the data were fitted in to korsmeyer-peppas exponential model where the 'n' values were in the range of 0.055 to 0.057. It was understood that RR code formulations were following predominantly zero order and fickian diffusion mechanism of drug release (Table- 4)

Table -4. Release kinetics of RR Code formulation

Order of process	RR1	RR6	RR7
Zero order	R^2 0.991	0.992	0.993
	M -0.046	-0.053	-0.066
	C 2.053	2.066	2.111
First order	R^2 0.991	0.991	0.99
	M 6.029	6.474	6.854
	C -0.926	-0.215	0.361
Higuchi	R^2 0.991	0.991	0.99
	M 6.029	6.474	6.854
	C -0.926	-0.216	0.361
korsmeyer	R^2 0.966	0.949	0.965
	M 0.057	0.057	0.055
	C 1.293	1.336	1.376

FTIR Spectra

The ciclopirox has greater intensity in absorption where as it is made formulation with Pluronic the said compound absorption decrease, still it retains its activity after making form without getting disturbed in the structure. Ciclopriox olamine can be formulated with Pluronic188 and RR formulation satisfactorily (Table-5), (Fig- 6)



(a) Pluronic 188 (b) carbomer 934(c) Pluronic 188+carbomer 934 combination (d) ciclopirox olamine (e) Pluronic 188+carbomer 934+ ciclopirox olamine

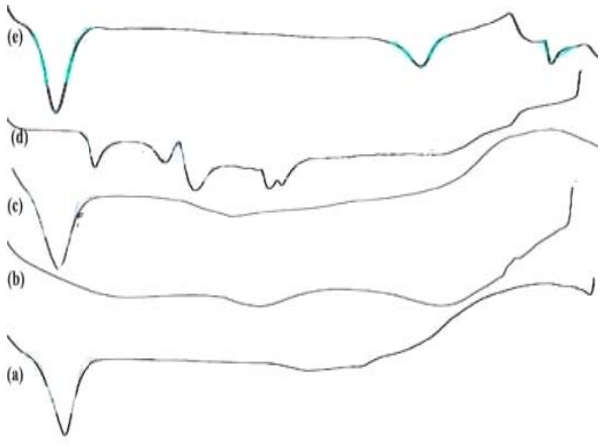
Figure -6. FTIR spectra

Table -5. FTIR spectra

Compound cm^{-1}	CH stretching cm^{-1}	Unsaturated nitrogen compound cm^{-1}	Poloxamar/1120 cm^{-1}		cm^{-1}		C-C multiple bond stretching cm^{-1}	Corboxlate anion cm^{-1}	Ar-N-tertiary vibration cm^{-1}	CN vibration cm^{-1}	OH bending cm^{-1}	free OH cm^{-1}
			C-O polymeric associated	CH_2 - CH_3 CO	OH	COOH						
Ciclopriox olamine	2960	1653 C-C Multiple Bond Streaching						1460	1380	LESS	720	3650
Pluronic188			1380	1320								
Pluronic188+	2960		1380	1320		1140-1720 cluster						
Pluronic188+ Ciclopriox Olamine	2960	1653 C-C multiple bond streaching						1467	1280	1280	720	3458

DSC Spectra:

Ciclopiroxolamine showed a long and sharp characteristic endothermic peak at 110.67 ºC and 124.15°C and 157.83ºC. The DSC thermogram of showed endothermic peak between 160°C to 240˚C.The DSC thermograms of physical mixtures of ciclopiroxolamine with and grades of P188 showed peaks at 57.63 ºC, 193.46 ºC and 242.05 ºC. The DSC thermograms of physical mixtures of polymer combination showed endothermic peaks at 57.63 ºC. The Pluronic188 peaks at 62.96°C, Therefore, Pluronicand may be used as excipients in the formulation of ciclopiroxolamine*in situ* gels. (Fig- 7)

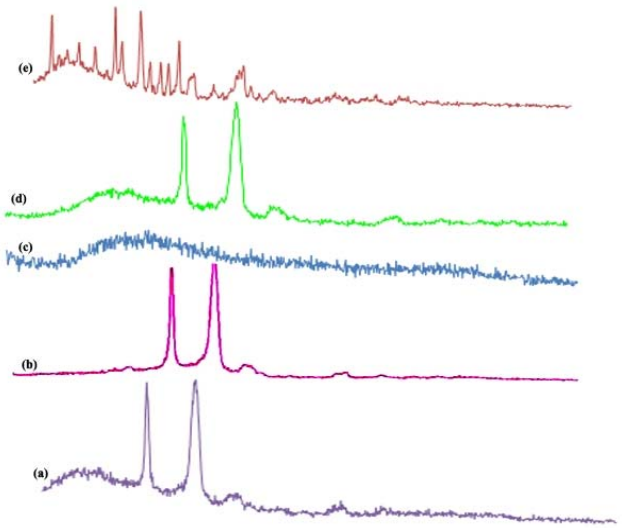


(a) Pluronic 188 (b) carbomer 934 (c) ciclopirox olamine (c) Pluronic 188+carbomer 934 combination (e) Pluronic 188+carbomer 934+ ciclopirox - olamine

Figure -7. DSC spectra

XRD Spectra:

The X-ray diffractograms of pure ciclopiroxolamine showed the distinct peaks at between 11.65 θ, 17.5 θ, 19.95 θ and other peak at 23.5 θ, Pluronic188 showed a peak at between 22 θ to 23 θ and showed peak at 15.95 θ, the peak of polymer combination was observed at 19.3 θ and 23.35 θ, The polymer combination with drug was peak at 19.15 θ and 23.35 θ, There was a slight reduction in peak level which may be due the conversion of drug from crystalline form to amorphous form in the formulations (Fig-8)



(a) ciclopirox olamine (b) pluronic 188 (c) carbomer 934 (d) pluronic 188 + carbomer 934 combination (e) pluronic 188 + carbomer 934 +ciclopirox olamine combination

Figure - 8.XRD spectra

Antifungal activity:

The antifungal activity of ciclopiroxolamine was determined by agar diffusion method by taking various concentrations of standard solutions and *in situ* gels (MIC: 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg).It was observed from the results the zone of inhibition for standard solutions were in the range of 0.9 cm- 2.7 cm, whereas it was in the range of 1.7 cm- 3.1 cm for the *in situ* gels (Table- 6), (Fig. 9-10).

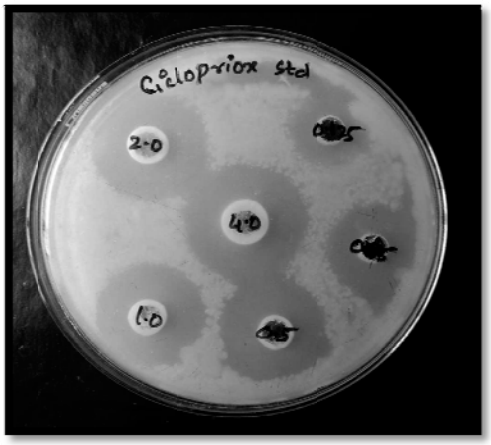


Figure -9.Photography showing the zone of inhibition of cicloprioxolamine standard

Figure -10. Photography showing the zone of inhibition of cicloprioxolamine*in situ* system

Table 6: Antimicrobial activity of *in situ* gel in comparison to reference standard using *Candida Albican*

	0.125 mg	**0.25 mg**	**0.5 mg**	**1.0 mg**	**2.0 mg**	**4.0 mg**
In situ gel	1.7	2.0	2.4	2.6	2.8	3.1
standard	0.9	1.5	1.9	2.1	2.3	2.7

SUMMARY AND CONCLUSION

Gelation temperature of temperature induced *in situ* gels of ciclopiroxolamine decreased with increase in concentration of Pluronic 188 from 35.5^{o}C to 23.4^{o}C for a concentration of 10% to 15% (RR1 to RR4).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 of formulation RR6 and RR7 (112, 116 sec) exhibited good gel strength among all optimized RR code formulation which may due to increase in concentration of Pluronic and its reversible gelation character at 37ºC.The mucoadhesive force is an important physicochemical parameter of topical application in buccal cavity. The mucoadhesive force was significantly increased from 3673.12 dynes/cm^2 (RR1) to 4992.06 dynes/cm^2 for the formula RR7 which consists of 0.03% 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 RR1, RR6 and RR7 released 78.5%, 81.4% and 86.6% respectively at the end of 8thhour.The diffusion of drug from formulation RR1 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 RR6, RR7 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 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.

The preparation RR7 was the best formula among the reversible thermo sensitive ciclopiroxolamine*in situ* gels with all the necessary characters of the *in situ* gels for mucoadhesion to effectively treat the oral thrush.

REFERENCE

[1] James, William D.; Berger, Timothy G.; et al. (2006). *Andrews' Diseases of the Skin: Clinical Dermatology*. Saunders Elsevier.

[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, corticosteroids' 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. Bacterial. 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. Microbial. 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] Samaranayake, L. P. 1992. Oral mycoses in HIV infection. Oral Surg. Oral Med. Oral Pathol. 73:171-180.

[15] HarishMatapady,Narayana et al . Development of a gellan gum based mucoadhesive in situ gels for buccal local delivery system of fluconazole,int.j.chhem.sci:7(1),2009,315-326

[16] 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

[17] Mitra Jelvehgaria, Mohammad-Reza Rashidib, Hedayte Samadi a, b Mucoadhesive and Drug Release Properties of Benzocaine Gel Iranian Journal of Pharmaceutical Sciences Autumn 2006: 2(4): 185-19

[18] 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.

[19] Jagdish Balasubramaniam., In vitro and in vivo evaluation of the Gelrite® gellan gum-based ocular delivery system for indomethacin, Acta Pharm. 53 (2003) 251–261