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Formulation and evaluation of ophthalmic delivery of fluconazole from ion activated *in situ* gelling system

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ABSTRACT

Fungal keratitis is a sight threating ocular infection that most frequently occur as a infection of candida species. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antifungal agent, fluconazole, based on the concept of ion-activated in situ gelation. ocular in situ gels can increase the drug residence time thus increasing the bioavailability. Gelrite was used as the gelling agent in combination with HPMC E-50(Hydroxy Propyl methyl Cellulose) that acted as a viscosity-enhancing agent. Formulations were evaluated for physical parameter like clarity, pH, drug content, rheological studies, sterility test, in vitro drug release studies. the formulations were therapeutically efficacious, stable and provide sustained release of drug over a period of 8 Hrs. These results demonstrate that developed system is a best alternative to conventional ophthalmic drops.

Keywords: Gelrite, fluconazole, in situ, Gelation.

INTRODUCTION

Ocular drug delivery is extremely interesting and highly challenging in field of pharmaceutical research. Eye drops that are conventional ophthalmic delivery system often result in poor bioavailability & therapeutic response, because of high tear fluid turn over & naso-lacrymal drainage which leads to side-effects hence frequent administration & use of concentrated solutions serve to provide desired therapeutic effect .ocular therapy would be significantly improved if the precorneal resindence time of drug could be increased. Various ophthalmic delivery system such as inserts, aqueous gels & ointments have been developed in order to lengthen resindence time but because of drawbacks like blurred vision and low patient compliance from inserts not much used.[1,2]

Various in situ gelling system have been developed by the use of polymeric solutions which exhibit sol to gel phase transition as a result of exposure to physiological temp. ph or ionic composition of the lachrymal fluid. Such systems upon administration undergo sol to gel transition and increase ocular bioavailability. In situ gelling systems are of three types PH triggered system eg. Carbopol, cellulose acetate phthalate latex, Temp. Dependant eg. Pluronic & tetronic, Ion-activated ex, gelrite,sodium alginate. Gelrite is an anionic deacylated exocellular polysaccharide secreted by pseudomonas elodea with a tetra saccharide repeating unit of one-L-rhamnose, one d-glucouronic acid and two-o-glucose residues. It has the capacity o form gels in presence of cations. HPMC used is an viscosity enhancing agent.
The purpose of present study was to develop an ion-activated in situ gelling system for fluconazole which is a fluorinated bis-triazole derivative that has been effective against Candida species. Gelrite was used in combination with HPMC E-50 as viscosity enhancer for formulation of fluconazole eye drops (0.3%w/v) which undergo sol to gel phase transition when instilled into cul-de –sac of eye.

MATERIALS AND METHODS

Materials

Fluconazole was obtained as a gift sample from FDC Pvt. Ltd. India. Gellan gum and HPMC were obtained from sisco labs pvt. Ltd. and H.D. Fine Chemicals, Mumbai, India respectively. All other reagents were of analytical grade.

Preparation of formulations

A 3^2 factorial design was used for formulation design, gellan gum and HPMC E-50 were chosen as a independent factor. There effect on dependant factors like viscosity and drug release was observed. Aqueous solution of varying concentrations of gellan gum and HPMC were prepared and evaluated for gelling capacity and viscosity in order to identify the compositions suitable for as in situ gelling system. The solution Polymer was prepared by dispersing gellan gum into de ionized water and heating up to 90^0c for 20 minutes followed by cooling to room temperature. Drug solution was prepared by dissolving fluconazole in water, to this solution add HPMC E-50 & Mannitol. Drug solution was mixed with polymer solution using a magnetic stirrer, Benzalkonium chloride was added which acts as preservative. The prepared in situ gels were filled in glass vials closed with rubber closures and sealed with aluminium caps and sterilized by autoclave at 121^0c for 20 minutes .[3,4]

Table1. Variables in Optimization Study

Variables	level used		
Independent	Lower(-1)	Middle(0)	Upper(+1)
Gelrite(X1)	0.3	0.4	0.5
HPMCE50(X2)	0.5	0.6	0.7

Table 2. Composition of fluconazole in situ gel formulation

Ingradients	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fluconazole	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
HPMC E-50	0.5	0.6	0.7	0.5	0.6	0.7	0.5	0.6	0.7
Gellan gum	0.3	0.3	0.3	0.4	0.4	0.4	0.5	0.5	0.5
Mannitol	5	5	5	5	5	5	5	5	5
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Water q.s.	100	100	100	100	100	100	100	100	100

Evaluation studies:-

a. Clarity

The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds.

b. FTIR Interaction Study:-

IR spectra were taken by using fourier transform infrared spectrophotometer (shimadzu, miracle-10). The technique used was attenuated total reflectance and spectra was scanned in range of 4000-400cm^{-1}. FTIR study was carried on pure drug, drug + physical mixture of polymers, formulations to confirm compatibility of drug with other Excipients used in the preparation of in situ gels**.**

c. Differential Scanning Calorimetry (DSC) study of physical mixture of drug and polymer

The differential calorimetric scanning of physical mixture of drug and individual polymer were carried out using Differential Scanning Calorimeter (mettler Toledo star system, switzer Land). Samples were placed in aluminium crucibles and DSC thermo grams were recorded at the heating rate of 10^0C/min in the range of 0^0C to 300^0C. Air was purged at the rate of 10 ml/min.

d. pH

The pH of each of prepared ophthalmic formulations was determined by using pH meter (equip-tronics). The pH meter was calibrated before each use with standard pH 4, 7 and 9.2 buffer solutions. [5]

e. Assay

The specified volume (1ml) of each of the ophthalmic formulations was taken and diluted with distilled water to make concentration 20µg/ml. The samples were analyzed spectrophotometrically at λmax of 261 nm. The concentration of fluconazole in samples was determined from a previously prepared calibration curve. The study was done in triplicate.

f. Test for gelling ability

Gelling capacity of formulations was evaluated in order to identify the formulations suitable for use as in situ gelling systems. The individual ophthalmic formulations (100µl) were added into 2 ml of simulated tear fluid contained in glass vials. The phase transition of solution to viscous gel was observed. Accordingly scores were assigned.[6]

Composition of simulated tear fluid:

Sodium chloride	: 0.670gm
Sodium bicarbonate	: 0.200gm
Calcium chloride dehydrate	: 0.08gm
De ionized water	: 100ml

g. *In vitro Diffusion studies*

In vitro release studies were carried out using bi chambered donor receiver compartment mode (Franz diffusion cell). *In vitro* release of fluconazole was carried out in formulations with different concentrations of gelrite using dialysis membrane. The diffusion medium 26ml of simulated tear fluid stirred at 50 rpm at 37^0 C $\pm 0.5^0$ C. One end of the diffusion tube was covered by a dialysis membrane. The1 ml formulation were spread on the dialysis membrane and membrane was placed such that it just touches the diffusion medium (STF) present in receptor compartment. The drug samples were withdrawn at the interval of one hour for the period of 8 hrs from diffusion medium and analyzed by a UV spectrophotometer at 261 nm using simulated tear fluid as blank.[7]

h. Determination of viscosity of ophthalmic formulations

The viscosity values were estimated for both the preparations i.e. ophthalmic solutions of fluconazole as well as the preformed gels.[8]

i. Determination of viscosity of ophthalmic solutions

The specified volume of prepared ophthalmic solution was transferred in sample cell which was placed carefully within the adaptor (Brookfield DV-II + PRO viscometer, Adapter spindle No-18).The water of 25^oC was circulated through jacket of the adaptor. The viscosity values were recorded.

ii. Determination of viscosity of preformed ophthalmic gels

The gellan gum gel formulations were prepared by adding CaCl2 solutions to formulations. The gels were formed due to the interaction between gelrite and Ca2+ ions. The viscosity values were recorded using Brookfield DV-II + PRO (spindle No. F with helipath attachment).

i. Sterility Testing

Sterility testing was performed for aerobic and anaerobic bacteria and fungi by using fluid thioglycolate and soybean casein digest medium respectively as per the Indian Pharmacopoeia.

The method used for sterility testing was direct inoculation method.10 ml culture was added to 100 ml of culture medium. Both media were kept for incubation at 32^0C for 7 days and observed for any microbial growth. The sterility test results were compared with positive and negative controls.[9]

j. Ocular irritancy studies

The optimized formulations was used for in vivo studies, the protocol was approved by Institutional Animal Ethics committee with approval no.004/2012. The Draize technique was designed for the ocular irritation potential of the ophthalmic products .According to the Draize test, the amount of substance applied to the eye is normally 100µl placed into the lower cul-de-sac with observation of the various criteria made at a designed required time interval of 1hr, 24hrs, 48hrs, 72hrs and 1 week after administration.[10,11] Three male rabbits weighing 1.5 to 2kg were used for the present study. The sterile formulation was instilled twice a day for a period of 7 days, and a cross-over study was carried out (with a 3 day washing period with saline was carried out before the cross-over study). Rabbits were observed periodically for redness, swelling, watering of the eye.

k. Accelerated stability studies

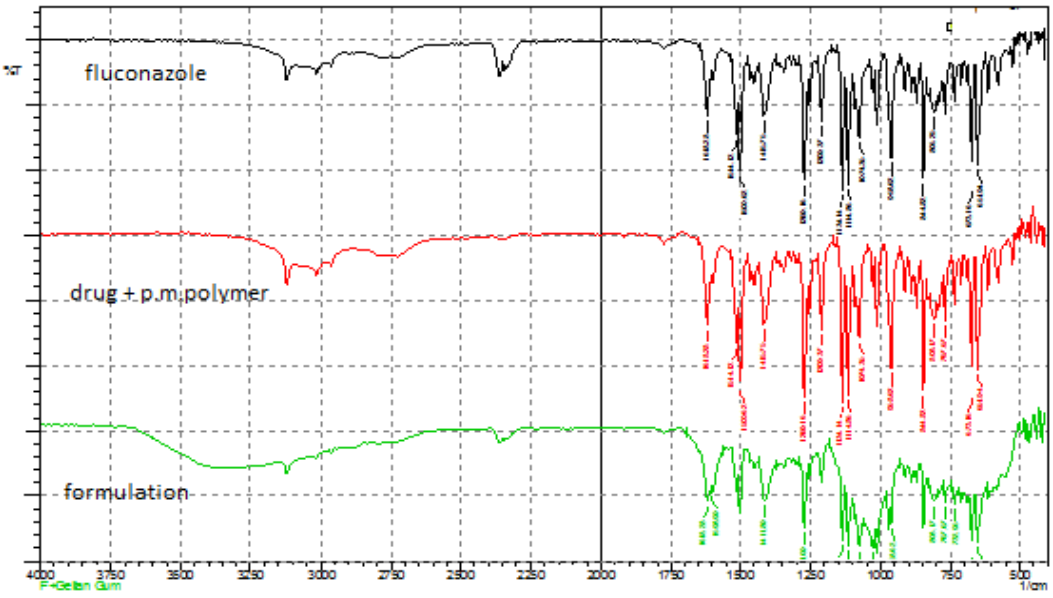
Selected sterilized formulations were stored at 4 ± 1^0c, room temperature (27 ± 1^0c), 45 ± 1^0c for a period of 1 month. The formulations were evaluated at periodic intervals for drug content, clarity, pH, gelling capacity, rheology, in vitro drug release and sterility.

RESULTS AND DISCUSSION

The use of gellan in in situ gel-forming is by the property of its aqueous solution to transform into stiff gels when it comes in contact with ions of tear fluid. The appearances of formulations were found to be clear. The two main pre requisites of an in situ gelling system are viscosity and gelling capacity. The formulation should have an optimum viscosity that will allow easy instillation into the eye as a liquid which undergo rapid sol to gel transition. Additionally, formed gels should preserve its integrity without dissolving for a prolonged period of time. The formulation showed good gelling ability and had phase transition within 60 seconds. A concentration of 0.3%-0.5%of gellan gum and 0.5%-0.7% of HPMCE50 was selected as it had satisfactory effect on viscosity and gelling capacity. The increase in viscosity is caused by cross linking of the negatively charged polysaccharide helices by monovalent and divalent cations. Less viscosity at solution stage facilitate easy installation of formulation in to eye and spread due to blinking of eye. In contact with simulated tear fluid at 37^{o}C the solutions instantly transformed to gel form. The PH of all the formulations was in the range of 6.8-7.2. Terminal sterilization by autoclaving had no effect on the appearance, pH, gelling capacity and viscosity of formulations. The haziness that was observed after autoclaving was found to disappear and original clarity was regained after standing for sometime.

Table 3 Evaluation parameters

Formuation code	PH	gelation	Drug content
F1	6.8	++	98.91
F2	6.8	++	97.78
F3	7.1	+++	95.34
F4	7.2	+++	104.01
F5	6.7	+++	99.03
F6	6.8	+++	98.78
F7	6.9	+++	103.09
F8	7.0	+++	99.83
F9	7.2	+++	101.44

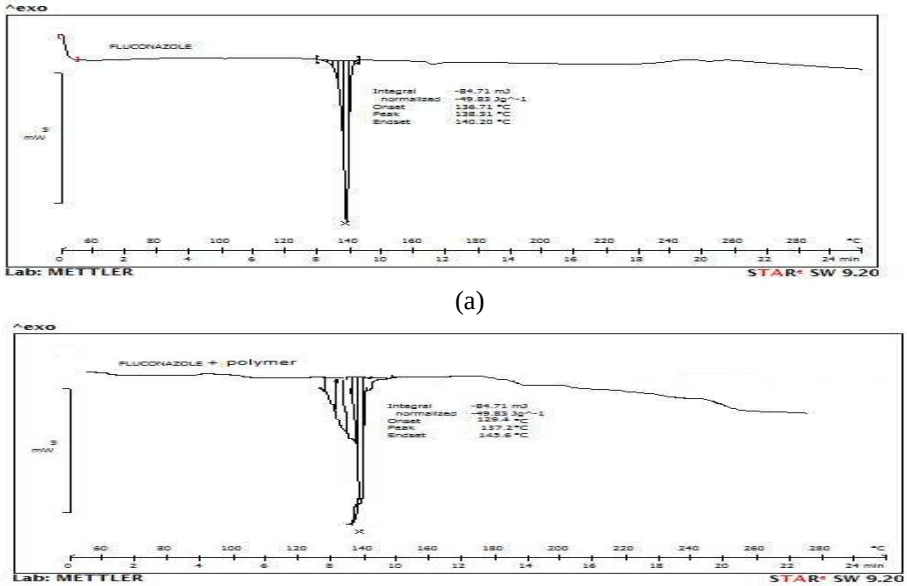


Note: ++ gelation immediate and remains for few hours; +++ shows gelation immediate which last for few hrs.

Fig1:- FTIR spectra of drug, drug+ polymer and prepared in situ gel formulation

The prepared in situ gelling system were evaluated for interaction studies to ensure that there is no interaction occurred between drug and polymer. For confirmation of stability of drug IR spectra of formulation was taken and compared with pure drug. The results of these studies reveal that there were no definite changes obtained in the bands of drug with that of pure drug.

The DSC thermograms of physical mixture showed characteristic endothermic peak corresponding to fluconazole around 136^{0}C. Therefore, there was no interaction between the drug and polymer.



(b)

Fig.2:- DSC thermogram of (a) fluconazole,(b)physical mixture of drug+ polymer

Rheological Evaluation:

It was observed that there was corresponding increase in viscosity at solution phase of each Formulation with increasing concentration of bio adhesive polymer i.e. HPMC E-50 from 0.5% w/v to 0.7% w/v and also the concentration of gellan gum from 0.3 to 0.5 %w/v. Viscosities increases from 35-79 cps at 20 rpm. The Formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity. As angular velocity increases from 20-100 rpm there was decrease in viscosity. The administration of ophthalmic preparations should influence as little as possible pseudo plastic character of the precorneal film. Since the ocular shear rate is high during blinking, viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rate are preferred.

To study effect of polymer conc. On viscosity eq.1 was generated, it shows that as the conc. Of polymers increased increase in viscosity as in fig. 2

Viscosity = 59.44+15.17* x1+6.33* x2-1.17 * $x1^2$-0.67*$x2^2$……(1)

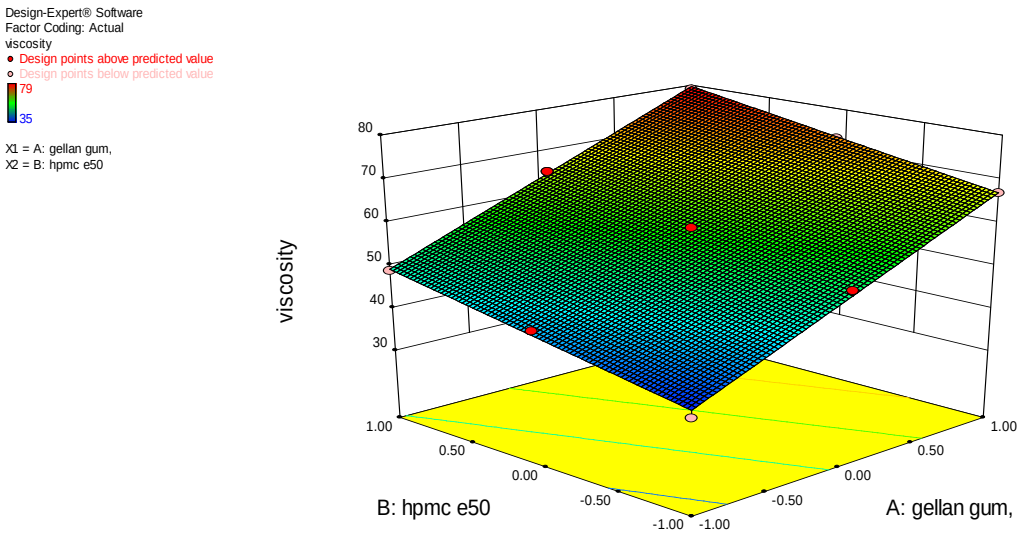


Fig.2:-Response surface plot for viscosity.

Estimation of fluconazole

Estimation of drug contents in ophthalmic solutions was carried out by spectrophotmetrically using UV spectrophotometer (UV 1800, Shimazdu). The contents of fluconazole in the selected *in situ* gelling solution systems were found to be 95%w/v-104%w/v.

In vitro diffusion study

Formulation f1 to f9 showed retardation of fluconazole release as concentration of gelrite increased from 0.3% to 0.5% and concentration of HPMC E-50 increased from 0.5% to 7%. Total release in 8 hrs was decreased from 93.96 % to 76.91 %. Hydroxy propyl methyl cellulose is hydrophilic and swellable polymer, hence it caused increase in viscosity & decrease in diffusion of the drug over the period of 8 hrs. Also formulations show retardation in drug release as compared to marketed formulation. The response surface plot shows the effect of polymer concentration on drug release.

The drug release conditions in vitro may be very different from those likely to be in the eye. However, the results clearly show that gels have the ability to retain fluconazole release and premature drug release will not occur. In cul-de-sac, the gels will probably undergo faster release of drug due to shearing action of eyelid.

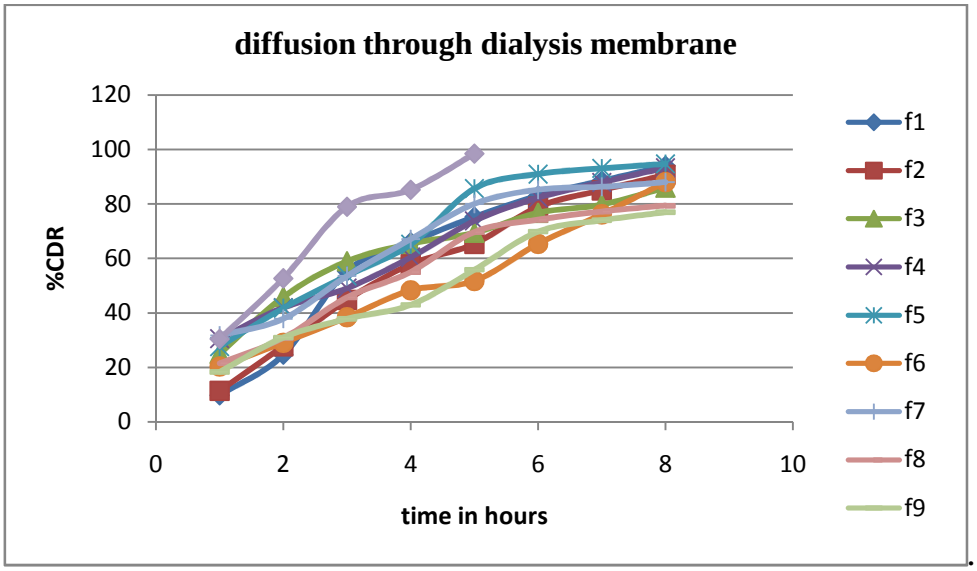


Fig 4. % Cumulative drug release of fluconazole in situ gel formulations compared with marketed formulation F- 10. (Zocon eye drop)

To understand the effect of polymer conc. On drug release, coefficient observed for release was fitted in eq. (2). The (-) coefficient of both variables shows that as polymer conc. Increases there was decreases in drug release fig.5

Drug release = 91.16- 4.40 * x1- 4.94* x2……(2)

Sterility testing

The formulations cleared the sterility test as there was no evidence of microbial growth when incubated for more than 7 days. Results mentioned in table 4.

Table 4 : Sterility test data of prepared In situ gels

Formulation code	Days of incubation						
	1	2	3	4	5	6	7
F1	-	-	-	-	-	-	-
F2	-	-	-	-	-	-	-
F3	-	-	-	-	-	-	-
F4	-	-	-		-	-	-
F5	-	-	-	-	-	-	-
F6	-	-	-	-	-	-	-
F7	-	-	-	-	-	-	-
F8	-	-	-	-	-	-	-
F9	-	-	-	-	-	-	-

Note:- (-)sign indicates no growth

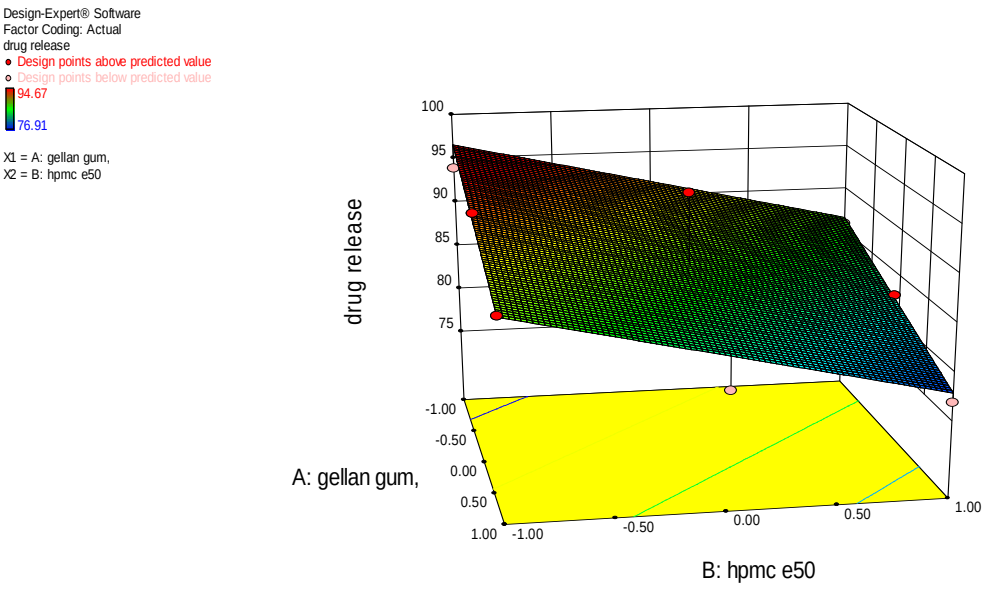


Fig.5:- Response surface plot for drug release.

Ocular irritancy studies

The results of ocular irritation studies indicate that an optimized formulation was non-irritant. No ocular damage or abnormal signs to the cornea, iris or conjunctivae were visible. Excellent ocular tolerance was noted.

Accelerated stability studies

Stability studies were carried out on optimised formulations. The formulations tested was found to be clear with no change in PH , drug content, viscosity, in vitro release and gelling capacity.

CONCLUSION

Fluconazole which is a anti fungal agent used in the treatment of fungal keratitis was successfully formulated as a in situ gel using gelrite as a polymer. Gelrite as a gelling agent used in combination with methocel E50 as a viscosity enhancing agent. The formulation was liquid and underwent rapid gelation upon coming in contact with ions of tear fluid. The gel formed in situ afforded sustained drug release over an 8-h period. The formulations were therapeutically efficacious. Stability data indicates that the formulations to be stable. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

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