IJPSR (2014), Vol. 5, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 31 October, 2013; received in revised form, 12 December, 2013; accepted, 24 March, 2014; published 01 April, 2014

FORMULATION AND EVALUATION OF STIMULI SENSITIVE pH TRIGGERED *IN-SITU* GELLING SYSTEM OF FLUCONAZOLE IN OCULAR DRUG DELIVERY

S. Nagalakshmi*, Seshank, Radhika Ramaswamy and S. Shanmuganathan

Department of Pharmaceutics, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai-116, Tamil Nadu, India

Keywords:

Fluconazole, Hydrogels, Carbopol, HPMC, pH triggered, *In situ* gel

Correspondence to Author:

S. Nagalakshmi

Lecturer, Department of Pharmaceutics, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai-116, Tamil Nadu, India

E-mail: nagalakshmimpharm@gmail.com

ABSTRACT: The present research finding focuses with the formulation and development of *in situ* gelling system of fluconazole using stimuli sensitive hydrogels. The quick elimination of the drug in the pre-corneal region thereby reducing the bioavailability of conventional ophthalmic solutions may overcome by means of *in situ* gel forming systems. These are instilled as drops into the eye and undergo a sol-gel transformation in the cul-de-sac. Fluconazole is an effective antifungal agent against superficial fungal infections in the eye. Hence an effort was made to develop *in situ* gelling system that can be triggered by pH and for providing sustained release of fluconazole. The drug that has been loaded with polymeric carrier undergoes transition from solution to gel upon triggered by pH. The main purpose of the study was to formulate pH triggered *in situ* gelling system of fluconazole using carbopol and HPMC polymers in order to attain a better bioavailability, considerable increase in ocular residence time, and reduce frequent instillation, thereby improving patient compliance. Fluconazole *in situ* gelling system was formulated by using polymers of pH sensitive grade like polyacrylic acid (carbopol940) along with hydroxy propyl methyl cellulose (HPMC). Carbopol solutions are less viscous and transform into firm gels upon increase in pH of the eye as it is acidic in nature and when it combines with HPMC, a well-known ocular viscosity enhancing agent. The developed formulation provided sustained release over a period of 8 hours and it was proved that the formulation was stable and safe which can be considered an alternate to the conventional eye drops.

INTRODUCTION: The most important and essential organs of our body are the eyes. Eye drops are one of the conventional ophthalmic drug delivery systems, ultimately lead to very less bioavailability in ocular area. This is attributed owing to its ocular anatomical and physiological constraints like relative impermeability of epithelial membrane of cornea, tear dynamics and drainage due to nasolacrimal fluid [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(4).1339-44

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(4).1339-44

The intention of pharmacotherapeutics is to seek the achievement of an adequate drug concentration at the specified site of action for an ample period of time to show a pharmacological response [2]. This has been a major challenge to develop a formulation in ocular therapeutics. Recently, *in situ* gel formulations have extensively been studied to improve ocular bioavailability and period of drug therapy [3].

The reduced bioavailability and therapeutic response elicited by commercial ophthalmic solutions due to quick pre-corneal elimination of the drug can be overcome by the means of *in situ* gels [4]. An increase in the pre-corneal residence time of drug and improved bioavailability could be

achieved by the usage of ocular delivery systems based on the concept of *in situ* gel formation. These *in situ* gelling systems exhibit sol to gel transition in the *cul de sac* of the eye. The transition is due to alterations in the pH, temperature, and ionic strength and various physicochemical parameters [5].

The challenge of this research finding is to develop an *in situ* gelling system of fluconazole using carbopol940 and HPMC LV 50 that is activated by pH. To formulate fluconazole (0.3%w/v) eye drops, pH sensitive polymers were used as the vehicle. The challenge is to transform eye drops in to gel and to afford sustained release of the drug, in turn to improve the patient comfort by reducing the dose and frequency of administration.[4,5]

MATERIALS AND METHODS:

Materials: Fluconazole, Carbopol940 (HIMEDIA LABORATORIES, LOBACHEMIE), Hydroxyl propyl methyl cellulose (LOBA CHEMIE), EDTA, Sodium Chloride, Benzalkonium chloride and pH 4 buffer. All chemicals either of analytical or pharmaceutical grade were used without further purification.

Method: The novel formulation of *insitu* gel was developed as per the method reported by Kumar and Himmestein, et al.[6]Different concentrations of pH sensitive polymers were used to develop eye drops of fluconazole and the composition is shown in table 1. Combination of polymers of required quantity was allowed to hydrate in pH 4 buffer. Calculated quantities of EDTA, sodium chloride, benzalkonium chloride were mixed to the buffered polymeric solution. The resulting solution was mixed with the drug solution and was stirred continuously to get a uniform solution. The final volume was adjusted by using pH 4 buffer. The developed formulations were stored in aseptic conditions. Sterilization procedure was carried out by autoclaving (121ºC and 15 psi) for 20 minutes and characterization studies were performed further.

TABLE 1: COMPOSITION OF VARIOUS INSITU GELS

Ingredients	F1(g)	F2(g)	F3(g)	F4(g)	F5(g)	F6(g)
Fluconazole %w/v	0.3	0.3	0.3	0.3	0.3	0.3
Carbpol 940 %w/v	0.2	0.2	0.4	0.4	0.3	0.2
HPMC LV50 %w/v	0.2	0.4	0.2	0.4	0.2	0.3
EDTA	0.1	0.1	0.1	0.1	0.1	0.1
NaCl %w/v	0.9	0.9	0.9	0.9	0.9	0.9
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
pH4	100	100	100	100	100	100

EVALUATION:

Visual Appearance, Clarity and pH: The prepared formulations were checked for clarity, color and transparency visually. The pH was measured by using a pH meter [4, 5, 6].

Gelling capacity: The developed *in situ* gel was assessed for its gelling capacity. The dilution of formulation was done by mixing with simulated tear fluid (25:7). The time taken for gelation and dissolution by the developed gel was noted [5, 7].

Drug content estimation: The content of drug in the *in situ* gel was calculated by taking a sample (2ml) of the *in situ* gel and mixed with simulated tear fluid of pH 7.4 in 100 ml flask to get the concentration of 10µg/ml in 100 ml. The absorbance values were read by using UV spectrophotometer at 260 nm in order to calculate the percentage of drug content [8].

***In vitro* Release Studies:** Dialysis method was used to study the release of drug. Open ended glass cylinders of dimensions of about 10 cm height, 3.7 cm outer diameter were used as a permeation cell.

A cellophane membrane (0.8µm pore size previously soaked in phosphate buffer of pH 7.4) was attached to one end of the cylinder by adhesive tape. 2 ml of the prepared formulation (eye drops) solution was placed inside the cell and the cell is submerged to a depth of 1 cm in 100 ml phosphate buffer pH 7.4. The cell was maintained at 37±1˚C throughout the experiment.

Aliquots were withdrawn from the receptor compartment respectively (0.5, 1, 1.5, 2, 2.5hrs). After each withdrawal, the volume of liquid in the receptor compartment was replaced by fresh phosphate buffer of pH 7.4.The drug concentration was determined spectrophotometrically at 260nm.The release pattern of the *in situ* gel preparation was calculated by plotting the drug release with time. The *in-vitro* drug release for all the developed formulation was calculated and amongst them the formulation showing good release profile was compared with the commercially available eye drops [8, 9].

***In vitro* Release Studies using goat's cornea:** Goat's cornea was used in the permeation study of the drug across the corneal membrane. Eye ball of the goat was procured from the slaughter house and was transferred while in cold condition submerged in normal saline at 4^{o}C to the laboratory. Cornea was removed along with 5-6mm of the surrounding scleral tissue and was washed with cold saline. The drug release from the prepared formulation was studied by using dialysis method using goat's cornea as semi permeable membrane [10].

Sterility testing: IP method (1996) was followed for the sterility testing of the eye drops. Sterility testing was performed by incubating the formulations for not less than 14 days at 30-35˚C in fluid thioglycollate medium to check the growth of bacteria and at 20-25^{o}C in the soya bean casein digest medium to check the growth of fungi in the formulations [1, 11].

Antifungal assay: The antifungal activity of the selected formulation was carried out on *Candida albicans* species by antifungal susceptibility test.

The nutrient agar medium was prepared by dissolving potato dextrose in hot distilled water and media was autoclaved at 121˚C for 15 minutes. By using diffusion method, test organisms were previously seeded in the nutrient agar medium. The aliquot test samples were poured in to petri plates containing nutrient agar medium using a micropipette.

The plates were left undisturbed for 20min and then incubated at 25˚C for 24hr.The diameters of zone of inhibition for *Candida albicans* were measured up to 48-72hrs respectively [12].

FTIR studies: The FTIR study reveals the possibility of interaction between drug and the polymer. Potassium bromated (KBr) pellets were used to record the FTIR graph for pure drug and the polymer [13].

Accelerated stability testing: The developed formulations were stored in amber colored vials which were then subjected to accelerated stability studies by storing at 40±2˚C and 75±5% RH as per ICH guidelines. During the stability studies, samples were periodically analyzed for any change in their appearance, pH, gelling capacity and drug content [14].

RESULTS:

Visual Appearance, Clarity and pH: The nature and the color of prepared eye drops were seem to be light yellow in color, except F2 and F6. All the formulations were found to be clear without any haziness. The developed ophthalmic formulations were found to have pH within the range of 6-6.4 (refer **table 2**).

Table 2: EVALUATION OF INSITU GELS

Formulation	Appearance	Clarity	pH	%Drug content	Gelling capacity
F1	Light yellow	clear	6.0	99.72±0.770	+:
F2	Light yellow	cloudy	6.1	98.35±0.551	++:
F3	Light yellow	clear	6.2	98.46±0.255	+++:
F4	Light yellow	clear	6.2	99.86±0.636	+++:
F5	Light yellow	clear	6.1	99.35±0.525	++:
F6	Light yellow	cloudy	6	98.88±0.702	+:

+: Gels slowly and dissolves,

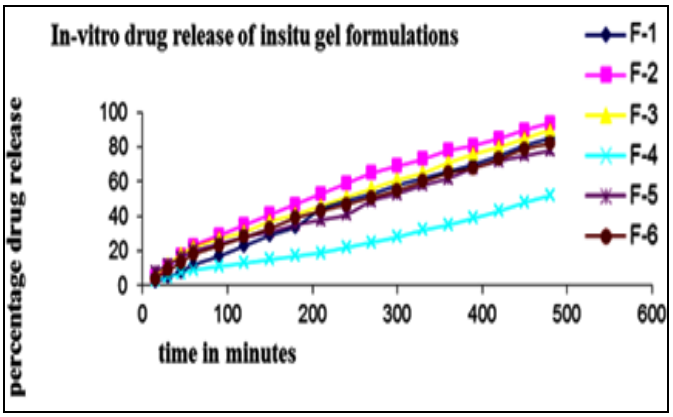
++: Gelation immediate and remains for few hours,

+++: Gelation immediate and remain for extended period.

Gelling capacity: Formulations F1 and F6 gels slowly and dissolves. F2 and F5 showed immediate gelation and remained for few hours. Formulations F3 and F4 also showed immediate gelation but remained for an extended period (refer table 2).

Drug content estimation: The drug content was within the range of 98.35% to 99.88% and the results are shown in table 2.

***In vitro* Release Studies:** The best formulation was found to be F4 and the release pattern was compared with the marketed formulation. The release pattern of all the gels are shown in **figure 1** and the comparative release is shown in **figure 2**.



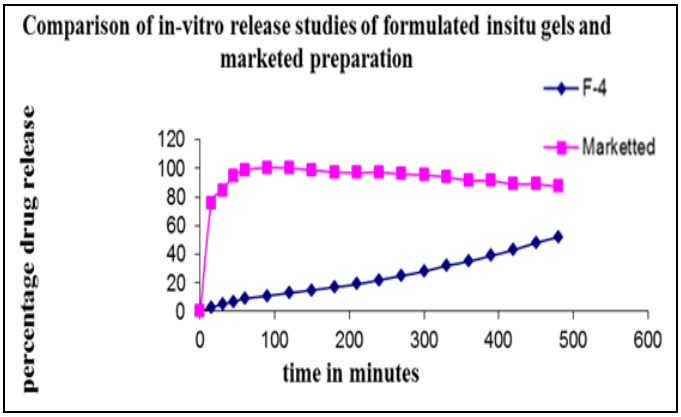


FIGURE 1 FIGURE 2

Sterility testing: The formulation F4 passed the test for sterility testing.

Antifungal assay: Test sample (formulated product) representeda maximum zone of inhibition and the results for antifungal assay are shown in table 3 and refer figure 3.

TABLE 3: EVALUATION ANTI FUNGAL ASSAY

S. No	Samples	72hrs (ZOI)mm
1.	***Candida albicans***	
	Test sample	7
	Sample control	4
	Marketed formulation	5
	Amphotericin B	6

No of organism: 1No (*Candida albicans*); Amphotericin B (Conc. 100 mg/ml -100µl for 100 ml media)

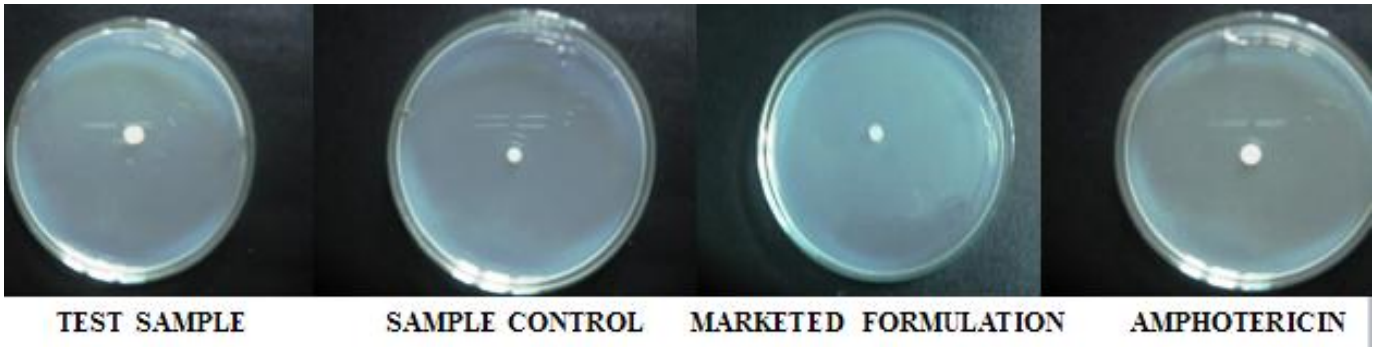


FIGURE 3: ZONE OF INHIBITIONS FOR THE TEST FORMULATION, SAMPLE CONTROL, MARKETED PREPARATION AND AMPHOTERICIN B

FTIR studies: The FTIR graph representing pure drug and mixture of drug and polymers were studied. From the observed peaks for pure drug and mixture of drug and polymers, it was confirmed that there were no significant interaction between the drug and polymers.

Accelerated stability testing: There were no significant variations in the drug content, consistency, pH and physical appearance of the formulations after storing at the prescribed temperatures as mentioned earlier.

DISCUSSIONS:

Visual Appearance, Clarity and pH: Sterilization had no specific effect on the formulations. The lack of clarity which was noted during autoclaving procedure mainly occurred by the precipitation of HPMC at elevated temperatures. The haziness disappeared and the solution attained clarity on overnight standing. The pH was found to be suitable for eye drops.

Gelling capacity: The rheology and gelling capacity are significant parameters for the development of insitu gel formulation. The formulations should provide favorable viscosity for easy instillation into the eye as a drop which then transforms in to gel form.

Drug content estimation: The percentage drug content tells that the drug has been uniformly distributed in all the developed formulations.

***In-vitro* Release Studies:** It was evident from in-vitro dissolution data that F4 showed better sustaining effect amongst all formulations. The in-vitro release profile of F4 was then compared with marketed formulation of fluconazole and was found that the drug release was about 52% at the end of 8hrs study.

Sterility testing: The formulation F4 passed the sterility tests, since no turbidity appeared in the formulation and hence there was no proof of microbial growth when incubated in not less than 14 days at 30-35˚C in case of fluid thioglycollate medium and at 20-25˚C in the case of soyabean casein digest medium.

Antifungal assay: The test sample showed a maximum zone of inhibition when compared to the sample control, marketed product and amphotericin, which proves that the test formulation has better efficacy and can provide an excellent antifungal property.

FTIR studies and Accelerated stability testing: No interaction with the drug and polymers were to be found which states that the formulation was stable. And the results from accelerated stability tests confirmed that the formulated *in situ* gel did not show any variations and could withstand the various temperatures and humidity.

CONCLUSION: The novel pH triggered ocular *insitu* gelling drug delivery was successfully developed and evaluated for its appearance, drug content, clarity, pH, gelling capacity, in vitro release and stability studies etc. The formulation was in the form of a liquid at pH 6 and undergoes immediate gelation when the pH was raised to 7.4. The gel exhibited release over a period of 8 hours in a sustained manner. Hence this developed formulation is a viable alternate choice over the commercial eye drops by the virtue of its improved bioavailability through its increased corneal residence time and ability to sustain drug release. Thus patient compliance could be achieved by decreasing the frequency of drug administration.

ABBREVIATIONS:

EDTA-Ethylene diamine tetra acetic acid

IP- Indian Pharmacopoeia

FTIR- Fourier Transform Infrared Spectroscopy

REFERENCES:

1. Abdul HasanSathali A, Mohanambal E, Arun K. Formulation and Evaluation of pH trigerred in situ gelling system of Levofloxacin, Indian Journal of Pharmaceutical Education and Research, 2011;45: 58-64.
2. Rathore KS, Nema RK. Formulation and evaluation of ophthalmic films for timolol maleate. Planta Indica 2008; Vol.no.4: p 49-50.
3. SaettoneMF, and Salminen L. Ocular inserts for topical delivery, Advanced drug delivery reviews 1995; 16(1): 95
4. J.Padma Preetha, K. Karthika, Rekha. NR and Khalid Elshafie. Formulation and evaluation of insitu ophthalmic gels of Diclofenac sodium, J. Chem. Pharm. Res., 2010, 2(3):528-535.
5. Lokhande Umesh Ramchandra et al . Design and development of pH triggered insitu gelling system of Ciprofloxacin, IRJP 2012,3(5).
6. Kumar SR, Himmestein, KJ. Modification of in situ gelling behavior of carbopol solutions by hydroxyl propyl methyl cellulose. J Pharm Sci: 1995: 84:344-348.
7. Nagesh C, Manish Patil, S Chandrashekhara and Rahul Sutar. A novel insitu gel for sustained ophthalmic delivery of Ciprofloxacin hydrochloride and Dexamethasone-design and characterization, Der Pharmacia Lettre, 2012, 4 (3):821-827
8. Wei G, Xu H, Ding PT, Li SM. Thermosetting gels with modulated gelation temperature for ophthalmic use: Rheological and gamma scintigraphic studies, J Contr Rel2002; 83:65-74.
9. Eaga Chandra Mohan, Jagan MohanKandukuri, Venkateshan Allenki. Preparation and Evaluation of in situ gels for ocular drug delivery. J pharm Res. 2009: 2(6): 1089-1094.
10. Sirish Vodithalai *et al*. Formulation and Evaluation of ion activated Ocular Gels of Ketorolac Tromethamine, Intl. Journal of Current Pharmaceutical Research, Vol 2, issue 3, 2010.33-37.

11. Sindhu Abraham, Sharon Furtoda, Bharath S, Basavaraj BV, Deveswaran R and Madhavan N. Sustained Ophthalmic delivery of ofloxacin from an ion-activated insitu gelling system. Pak J Pharm Scvi.2009: 22 (2): 175-179.
12. Pravin Pawar, Heena Kashyap, Sakshi Malhotra, and Rakesh Sindhu.Hp-*β*-CD-VoriconazoleIn Situ Gelling Systemfor Ocular Drug Delivery: In Vitro, Stability, and Antifungal Activities Assessment, Bio Med Research International, Volume 2013, 1-9.
13. Amal El- Kamal, Heba Al-Dosari, fahad Al-Jenoobi. Environmentally Responsive Opthalmic gel Formulation of Carteolol Hydrochloride. Drug Del, 2006:13:55-59.
14. MuniahAhuja et al. Stability Studies on aqueous and oily ophthalmic solutions of Diclofenac, Pharmaceutical Society of Japan, Vol 129(4) 495-502 (2009).

How to cite this article:
Nagalakshmi S, Seshank, Ramaswamy R and Shanmuganathan S: Formulation and evaluation of stimuli sensitive pH triggered *in-situ* gelling system of Fluconazole in Ocular Drug Delivery. *Int J Pharm Sci Res* 2014; 5(4): 1339-44.doi: 10.13040/IJPSR.0975-8232.5(4).1339-44