Brief Report

Formulation and evaluation of *in situ* gelling thermoreversible mucoadhesive gel of fluconazole

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ABSTRACT: The purpose of the present study was to develop ophthalmic gel formulations of fluconazole. Intraocular delivery of topically applied drugs such as fluconazole is hampered by elimination of the solution due to tear turnover, so an *in situ* gelling thermoreversible mucoadhesive gel was formulated. Thermoreversible mucoadhesive gels were prepared using the cold method along with poloxamer 407 and different mucoadhesive polymers such as hydroxy ethyl cellulose (HEC), hydroxy propyl methyl cellulose (HPMC) K4M, and polyvinyl pyrrolidone (PVP) K30. Gels were evaluated for physical parameters like appearance, gelation temperature, pH, spreadability, drug content, gel strength, bioadhesion, and *in vitro* permeation. A modified device (modified K-C diffusion cell with a sheep's eye corneal membrane as a diffusion membrane) was used for evaluation of drug permeation through a sheep's corneal membrane. The formulated gels were transparent, uniform in consistency, and had spreadability with a pH range of 6.8 to 7.3. Satisfactory bioadhesion on the sheep's corneal surface and good gel strength were also observed. Diffusion studies have shown that a matrix is the best-fit model. As the concentration of mucoadhesive agent increases, the rate of permeation decreases. The order of drug permeation through the membrane was HEC > PVP K30 > HPMC K4M. This study found that a thermoreversible polymer and mucoadhesive polymers can be effectively used to prolong residence time.

Keywords: Ophthalmic gels, Poloxamer 407, Mucoadhesive polymers, *In situ* gelling

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1. Introduction

In the field of pharmaceutical research, ophthalmic delivery is extremely interesting and highly challenging (*1*,*2*). Topical ocular infections and especially fungal infections can be effectively treated with ocular delivery itself rather than using an oral delivery of drugs. Popular treatments such as eye drops and suspensions are available for topical administration of ophthalmicaly active drugs to tissues around the ocular cavity. These suffer from a drawback in that the active constituent becomes diluted in tear film as the preparation is instilled into the *cul-de-sac* and is rapidly drained away from pre-corneal cavity by constant tear flow and lacrimo-nasal drainage. As a result, only a small fraction of the dose is absorbed by ocular tissues. Hence, frequent administration and use of concentrated solutions serve to provide the desired therapeutic effect (*3*). Candidal endophthalmitis is a sight-threatening ocular infection that most frequently occurs as a complication of candidemia. Fluconazole is a fluorinated bis-triazole derivative that has been reported to be effective against *Candida albicans* in various experimental animal models and clinical settings (*4*).

Thermoreversible gels made of poloxamer 407 have *in situ* gelling behavior (*5*). *In situ* gels formulated using poloxamer 407 can be conveniently applied to the conjunctival sac, where they undergo transition from a sol to a gel. Prolongation of residence time due to these *in situ* gelling systems will help to deliver a drug continuously in a controlled manner to the anterior chamber of eye, eliminating the need for frequent administration of drug and thus resulting in better patient compliance and prolonged action. This will result in a dose reduction, helping to minimize local and systemic side effects (*6*). In the present study, mucoadhesive polymers such as hydroxy propyl methyl cellulose (HPMC) K4M, hydroxy ethyl cellulose (HEC), and polyvinyl pyrrolidone (PVP) K30 were used to prolong the residence time of the gels and may

have helped to provide an additional advantage to gels containing poloxamer 407 alone.

2. Materials and Methods

2.1. *Materials*

Fluconazole was donated by Glenmark Pharmaceuticals (Mumbai, India), poloxamer 407 was donated by BASF India (Mumbai, India), and HEC, HPMC K4M, and PVP K30 were supplied by Cipla (Mumbai, India). All other chemicals used were of analytical grade.

2.2. *Methods*

Thermoreversible mucoadhesive gels of fluconazole were prepared using the cold method (*7*). Accurately weighed quantities of fluconazole and mucoadhesive polymer (Table 1) were dissolved in sterile water. To these solutions the required amount of poloxamer 407 was added. Weight was adjusted with distilled water to reach a final concentration of fluconazole of 0.2% (w/w). The solution was mixed well and stored at 4°C for 12 h. All solutions were adjusted to a pH in the range of 6.8 to 7.3 with the help of 2 M NaOH. All gel formulations were evaluated for physical appearance, consistency and spreadability.

2.3. *Measurement of gelation temperature*

Gelation temperatures of the gels were measured according to the method described by Gilbert *et al.* (*8*). Two mL aliquots of the gel were transferred in a test tube sealed with a parafilm and immersed in a water bath at 4°C. The temperature of the bath was increased in increments of 1°C and left to equilibrate for 15 min at each new setting. The samples were examined for gelation, which is considered to have occurred when the meniscus would no longer move when tilted more than 90°C. All measurements were performed in triplicate (n = 3).

2.4. *Content uniformity*

All prepared gel formulations were tested for content uniformity.

2.5. *In vitro bioadhesion evaluations*

The bioadhesive force of all of the batches was determined by the method described by Choi *et al.* (*9*). A sheep's corneal membrane was cut from the eye of a sheep and instantly fixed with the mucosal side outwards onto a glass vial using a rubber band. Vials with the corneal membrane were stored at 37°C for 5 min. Then, the next vial with a section of membrane was connected to a balance in an inverted position while the first vial was placed on a height adjustable pan. The gel was placed onto the corneal membrane of the first vial. Then, the height of the second vial was adjusted so that the membrane surfaces of both vials came in close contact. A ten minute contact time was chosen. Then, the weight was allowed to increase in the pan until the vials had detached. The bioadhesive force was the minimum weight required to detach two vials. The corneal membrane was replaced for each measurement (n = 3).

2.6. *Measurement of gel strength*

A sample of 50 g of gel was placed in a 100 mL graduated cylinder and gelled in a thermostat at 37°C. The apparatus for measuring gel strength as described by Choi *et al.* (*9*) was allowed to penetrate the gel. Gel strength, *i.e.* the viscosity of the gels at physiological temperature, was determined by the time (in seconds) taken by the apparatus to sink down 5 cm through the prepared gel. All measurements were performed in triplicate (n = 3).

2.7. *Permeation studies across a sheep's corneal membrane*

A modified device (modified K-C diffusion cell with a sheep's eye corneal membrane as a diffusion membrane) was used for evaluation of drug permeation through a sheep's corneal membrane. This membrane was tied to a specifically designed glass cylinder (open at both ends). Simulated tear fluid ($NaHCO_3$ 0.218 g, NaCl 0.678 g, $CaCl_2 \cdot 2H_2O$ 0.0084 g, and KCl 0.138 g in 100 mL of water) was used as the diffusion medium. The formulation to be tested was added to the donor chamber with the help of a micropipette.

Table 1. Formulation design of thermoreversible mucoadhesive gels of fluconazole

	Formulation contents (% w/w)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fluconazole	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Poloxamer 407	18	18	18	18	18	18	18	18	18
HEC	0.5	1	1.5						
HPMC K4M				0.5	1	1.5			
PVP							0.5	1	1.5
2M NaOH	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Sterile water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2. Results of various evaluation parameters

Formulation	Gelation temperature (°C)	Content uniformity (%)	Bioadhesion potential (dynes/cm^2)	Gel strength (sec)
F1	36.66 ± 0.32	100.5 ± 0.15	3,438.00 ± 6.04	105.33 ± 0.57
F2	35.16 ± 0.40	100.2 ± 0.05	4,039.5 ± 11.78	111.66 ± 0.57
F3	33.93 ± 0.65	99.90 ± 0.20	4,288.8 ± 16.18	117.66 ± 1.52
F4	35.36 ± 0.55	99.8 ± 0.15	4,464.06 ± 8.28	123.33 ± 0.55
F5	33.10 ± 0.55	100.2 ± 0.10	5,055.66 ± 10.1	129.3 ± 1.52
F6	31.86 ± 0.45	100.16 ± 0.05	4,953.03 ± 20.99	131.66 ± 0.57
F7	31.56 ± 0.20	101.1 ± 0.08	5,134.41 ± 11.22	135.33 ± 0.57
F8	29.10 ± 0.69	100.2 ± 0.06	5,376.41 ± 6.69	137.33 ± 0.57
F9	27.63 ± 0.30	100.1 ± 0.18	5,423.07 ± 9.44	137.66 ± 1.52

Table 3. Permeation kinetics of various formulation batches

Formulation	Zero order kinetics (R^2)	First order kinetics (R^2)	Matrix model (R^2)	Peppas model (R^2)	Hixon-Crowell model (R^2)	Korsmeyer-Peppas kinetics release exponent (n)
F1	0.8293	0.8310	0.9902	0.9806	0.8304	0.4361
F2	0.7909	0.7929	0.9840	0.9801	0.7923	0.3890
F3	0.8141	0.8159	0.9866	0.9734	0.8153	0.4292
F4	0.8068	0.8087	0.9864	0.9775	0.8081	0.4158
F5	0.7828	0.7847	0.9789	0.9593	0.7841	0.4129
F6	0.7929	0.7998	0.9832	0.9692	0.7992	0.4229
F7	0.8489	0.8504	0.9887	0.9674	0.8499	0.5003
F8	0.8607	0.8623	0.9908	0.9717	0.8618	0.4820
F9	0.8459	0.8474	0.9778	0.9342	0.8469	0.5117

The donor surface of the membrane was constantly in contact with the simulated tear fluid. A temperature of 37 ± 0.5°C was maintained throughout the study. A magnetic stirrer to the cell provided continuous agitation. At regular time intervals, 1 mL of sample was withdrawn and replaced by fresh simulated tear fluid in order to maintain sinking conditions. The samples were appropriately diluted and the absorbance was measured at 261.5 nm using a Shimadzu 1700UV-VIS spectrophotometer.

3. Results and Discussion

The prepared ophthalmic gel formulations exhibited optimum physical properties. Gels were transparent, uniform in consistency, and had spreadability with a pH range of 6.8 to 7.3.

3.1. *Measurement of gelation temperature*

As the concentration of mucoadhesive polymers increased, the gelation temperature of gel decreased (Figure 1). This ability of mucoadhesive polymers to lower gelation temperature may be due to increased viscosity after dissolution of polymers. The ability of mucoadhesive polymers to lower gelation temperature could be explained by their ability to bind to the polyoxyethylene chains present in the poloxamer 407 molecules. This would promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding, thus leading to gelation at lower temperature (*10*). Increasing the concentration of any

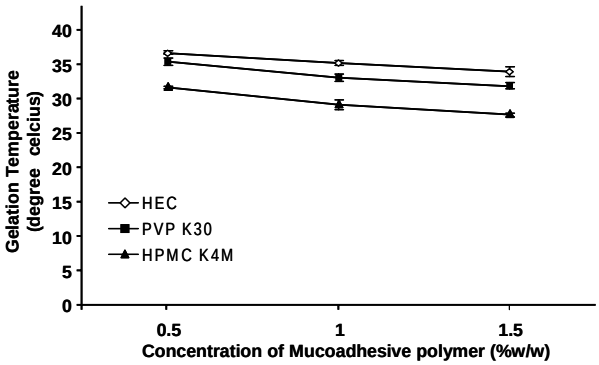

Figure 1. Effect of concentration of mucoadhesive polymer on gelation temperature.

of the mucoadhesive polymers used from 0.5 to 1.5% produced a gradual decrease in the gelation temperature of the corresponding solutions. Action to lower gelation temperature for the gels prepared using mucoadhesive polymers was, in order, HEC > PVP K30 > HPMC K4M. The results of gelation temperatures of different formulations are indicated in Table 2.

3.2. *In vitro permeation studies*

In vitro permeation across the sheep's corneal membrane was fit to various kinetic models of release (Figure 2). All batches indicated that a matrix model of permeation kinetics was the best-fit model. A faster release initially indicates that the drug in the solution in the space outside the gel matrix initially diffuses quickly. The release of drug within the gel is controlled by both the nature and concentration of polymer

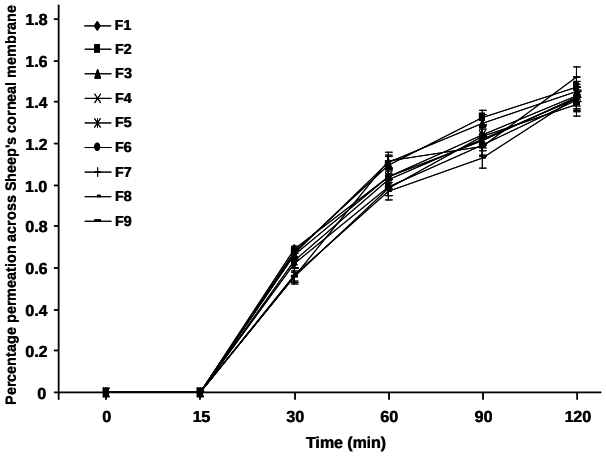

Figure 2. Permeation profile of *in situ* gelling thermoreversible gels of fluconazole.

used. Drug permeation through the cornea with a thermoreversible gel containing various mucoadhesive polymers occurred in the order of HEC > PVP K30 > HPMC K4M. '*n*' values (Table 3) greater than 0.5 indicate non- Fickian release whereas those less than 0.5 indicate Fickian release.

4. Conclusion

In this study, an *in situ* gelling thermoreversible ophthalmic gel of fluconazole was developed using poloxamer 407 and various mucoadhesive polymers. These *in situ* gelling formulations were free-flowing, transparent, had uniform consistency, and had spreadability at room temperature. A satisfactory gelation temperature and bioadhesion were observed in these gels. *In vitro* permeation studies across corneal mucosa showed both Fickian and non-Fickian release. Therefore, these gels represent a viable alternative to conventional eye drops. This study demonstrated that a thermoreversible polymer and mucoadhesive polymer can effectively be used to prolong residence time.

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