SUSTAINED OPHTHALMIC DELIVERY OF OFLOXACIN FROM AN ION-ACTIVATED *IN SITU* GELLING SYSTEM

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ABSTRACT

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of *in situ* gel forming systems that are instilled as drops into the eye and then undergo a sol-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent ofloxacin, based on the concept of ion-activated *in situ* gelation. Sodium alginate was used as the gelling agent in combination with HPC (Hydroxy Propyl Cellulose) that acted as a viscosity-enhancing agent. *In vitro* release studies indicated that the alginate/HPC solution retained the drug better than the alginate or HPC solutions alone. The formulations were therapeutically efficacious, sterile, stable and provided sustained release of the drug over a period of time. These results demonstrate that the developed system is an alternative to conventional ophthalmic drops, patient compliance, industrially oriented and economical.

Keywords: Ophthalmic delivery systems, sodium alginate, *in situ* gelling, ofloxacin, sustained.

INTRODUCTION

In ocular delivery, the physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, resulting in a short duration of the therapeutic effect. When a drug solution is instilled into the eye, the effective tear drainage and blinking action of the eye results in a drastic reduction in the drug concentration (Maurice, 1987) and the limited permeability of the cornea contributes to the low absorption of ocular drugs. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination (Bourlais *et al*., 1998). Successful results have been obtained with inserts and collagen shields. However, these preparations have some disadvantages such as poor compliance, especially by elderly people and many patients sometimes lose the device without noticing it.

From the point of view of patient acceptability, a liquid dosage form is preferable. This problem can be overcome by the use of polymeric solutions, which change to a gel as a result of exposure to the physiological temperature, pH or ionic composition of the lacrimal fluid. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye, which upon exposure to physiological conditions, changes to the gel phase thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability (Zhidong Liu *et al*., 2006).

Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, b-D-mannuronic acid (M) and a-L-guluronic acid (G). The polymer forms three-dimensional hydrogel matrices and the high G content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lacrimal fluid. Sodium Alginate was chosen as a vehicle for ophthalmic formulations since it exhibits several favorable biological properties such as biodegradability and non-toxicity. A prolonged precorneal residence of formulations containing alginic acid was looked for, not only based on its ability to gel in the eye but also because of its mucoadhesive properties (Odile Sechoy *et al*., 2000).

The objective of the present study was to develop an ion activated *in situ* gelling for Ofloxacin, a fluoroquinolone derivative used to treat external infections of the eye such as acute and subacute bacterial conjunctivitis, conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers which can prevent frequent drug administration and enhance patient compliance. Sodium alginate was used as the gelling agent in combination with hydroxy propyl cellulose (HPC) as the viscosity enhancer for the formulation of Ofloxacin eye drops (0.3% w/v), which undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug.

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Table 1: Composition, pH, drug content, viscosity and gelling capacity of *in situ* gelling systems of ofloxacin

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6
Ofloxacin (%w/v)	0.3	0.3	0.3	0.3	0.3	0.3
Sodium alginate (%w/v)	0.5	1.0	0.5	0.75	1.0	1.5
HPC-50cps (%w/v)	---	---	0.5	0.5	0.5	0.5
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml) q.s	100	100	100	100	100	100
Evaluation						
pH	6.7	6.6	6.4	6.6	6.6	6.8
Drug content (%)	101.3	99.6	99.3	100.6	99.2	100.8
Viscosity (cps)	14	21	18	28	36	48
Gelling capacity	+	++	+	++	+++	+++

Note: + gels slowly and dissolves; ++ gelation immediate and remains for a few hours; +++ gelation immediate and remains for an extended period.

Table 2: Regression co-efficient analysis and best model fit analysis for the formulations

Formulations	Zero	First order	Matrix	Peppas	Hixon Crowell
F-1	0.9125	0.9894	0.9982	0.9848	0.9874
F-2	0.9120	0.9672	0.9872	0.9853	0.9706
F-3	0.9008	0.9105	0.9701	0.9579	0.9392
F-4	0.9585	0.8718	0.9814	0.9535	0.9440
F-5	0.9391	0.9869	0.9888	0.9753	0.9808
F-6	0.9947	0.7909	0.9555	0.9939	0.9239

MATERIALS AND METHODS

Materials

Ofloxacin was obtained as a gift sample from Cadila Health Care Ltd., Ahmedabad, India. Sodium alginate and HPC were purchased from Anilax Enterprises Inc. Columbia Turnpike, Florham Park USA. All other reagents were of analytical grade.

Preparation of formulations

The table 1 shows the composition of all the formulations. The alginate and alginate/HPC solutions were prepared by dispersing the required amount in distilled water with continuous stirring. Ofloxacin (0.3% w/v) was dissolved in dilute acetic acid and the pH was adjusted to 6.5 using 0.1N NaOH. Benzalkonium chloride (0.02% v/v) solution was then added to the above solution. The drug solution was then added to the alginate or alginate/HPC solution under constant stirring to obtain a uniform solution. Distilled water was then added to make the volume up to 100ml.

Evaluation studies

The general appearance of the formulations was observed which included colour and clarity of solution. The pH of the prepared formulations was checked using a pH meter. The Gelling capacity of the formulations was evaluated for gelling property in order to identify the formulations suitable for use as in situ gelling systems. Gelling was determined by mixing the formulation with simulated tear fluid in the proportion 25:7 and the gelation was assessed by visual examination (Carlfors *et al*., 1998). The time taken for gel to form and the time taken for it to dissolve was noted. The drug content was determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. Aliquot of 5ml was withdrawn and further diluted to 25ml with distilled water. Ofloxacin concentration was determined at 288.5nm by using UV-Visible spectrophotometer (UV-1201, Shimadzu Corporation, Japan). Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. The prepared solutions were allowed to gel in the simulated tear fluid and then the viscosity determination were carried out by using Brookefield DV-II+ Rheometer with spindle LV-3 with angular velocity run from 10 to 100rpm (Balasubramaniam *et al*., 2003).

In vitro release studies of ofloxacin from the formulations was studied through a fabricated "Through Flow Cell" which simulates the eye (Bharath S. and Shoba Rani Hiremath; 1999). 1ml of the formulation was placed in the through flow cell and 100ml of simulated tear fluid (STF) was taken into a receptor compartment. Necessary arrangements and connections were done such that the solution from the receptor compartment is continuously circulated to "through flow cell" and back with the flow

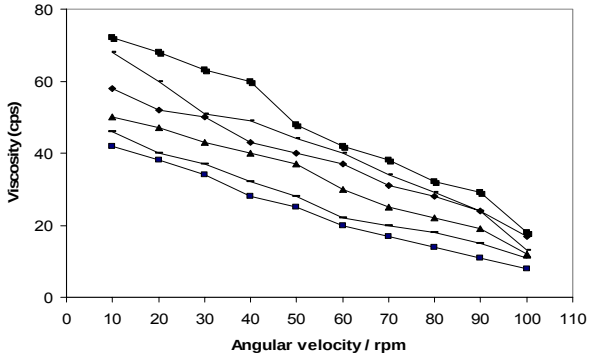


Fig. 1: Rheological profiles of *in situ* gelling systems of ofloxacin from formulations F1-F6

Formulation F-1[-▪-], Formulation F-2[-▪-], Formulation F-3[-Δ-], Formulation F-4 [-◊-], Formulation F-5 [-*-] and Formulation F-6 [-▪-]

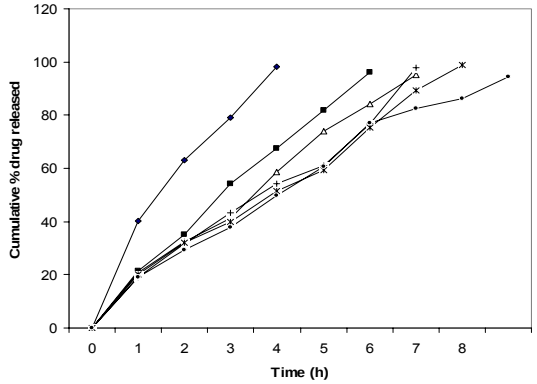


Fig. 2: Plot of *in vitro* release of ofloxacin as a function of time for formulations F1-F6 from *in situ* gelling systems

Formulation F-1[-◊-], formulation F-2[-▪-], Formulation F-3 [-Δ-], Formulation F-4 [-+-], Formulation F-5 [-*-] and Formulation F-6 [-·-]

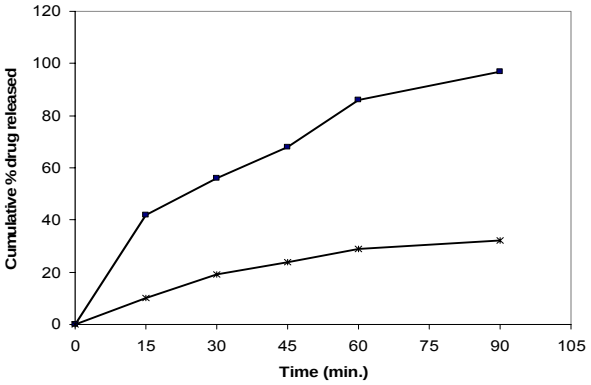


Fig. 3: Comparison of drug release profile of marketed conventional eye drop with formulation F-6.

Marketed conventional eye drop [-▪-] and formulation F-6 [-*-]

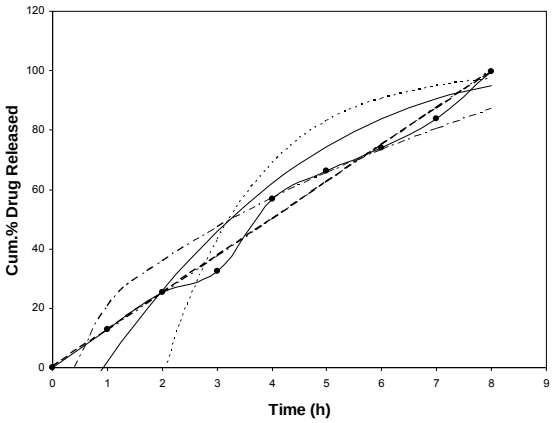
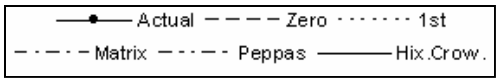


Fig. 4*: In-vitro* drug release kinetics and best fit model plot for the formulation F-6.



rate of 11 drops/minutes at 37±1°C temperature. At specified intervals of time, 2ml of the sample solution was withdrawn from the receptor compartment and replaced with the fresh STF. The samples were analyzed after necessary dilutions by UV-Visible spectrophotometer at 288.5nm. The *in vitro* release studies were also carried out for the marketed conventional ophthalmic drops of ofloxacin (0.3% w/v) in order to compare the release profile of the drug with the prepared *in situ* gelling system.

Sterilization of the selected formulations was carried out by gamma radiations at the dose of 1.0 M rad and the sterility testing was performed (IP, 1996). The ethical committee of the institution had permitted the ocular irritation studies. Four male albino rabbits of each weighing 1.5-2kg were instilled with selected sterile formulations twice a day for a period of 21 days and the rabbits were observed periodically for redness, swelling and watering of the eyes.

The selected formulations were stored at ambient humidity conditions between 2-8°C, ambient temperature and at 40°C for a period of one month. The samples were withdrawn at frequent intervals and evaluated for the parameters viz. pH, appearance, gelation studies, drug content and in vitro drug release (Kulkarni *et al*., 2004).

RESULTS AND DISCUSSION

The formulations were light yellow in colour and the clarity was found to be satisfactory. The pH of all the formulations was within the acceptable range and hence would not cause any irritation upon administration. The

drug content of all the formulations was in range. The evaluation results are also mentioned in table 1.

The two main prerequisites of gelling system are viscosity and gelling capacity. Except for the formulations F1 and F3, all the formulations gelled instantaneously with a translucent matrix on addition to the STF, which may due to ionic cross linking of the alginate chains by the divalent cation and extended for few hours.

Generally viscosity values in the range of 15-50cps significantly improve the contact time in the eye (Katrizky, 1997). The viscosity of the formulations F1 to F6 ranged from 14-48cps with increase in the alginate concentration within the system. All the formulations exhibited pseudo-plastic rheology, as shown by shear thinning and a decrease in the viscosity with increased angular velocity fig. 1. The administration of ophthalmic preparations should have as little effect as possible on the pseudo-plastic character of the pre-corneal film (Bothner *et al*., 1990). Since the ocular shear rate is very high, ranging from 0.03 s^{-1} during inter-blinking periods to 4250-28, 500 s^{-1} during blinking (Kumar and Himmestein, 1995), viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often preferred.

The *in vitro* release studies fig. 2 indicated that amongst all the formulations, F-6 sustained drug release for 8 hours, which may be due to the higher concentration of sodium alginate along with HPMC. The higher regression coefficient values table 2 for each formulation suggested that the formulations F-1 to F-5 behaved matrix type of drug release whereas formulation F-6 showed zero order drug release kinetics which was further proved fig. 3 by the best fit zero order model.

The comparative *in vitro* drug release profile fig. 4 between the marketed conventional ophthalmic drops and the formulation F-6 showed 45% and 19% after initial 30min. At the end of 90 min. the drug release was found to be 97.6% and 38% from the marketed product and F-6 indicating that the drug release was significantly prolonged by using the *in sit*u gelling systems.

The formulation F-6 passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 14 days at 30-35^{o}C in case of fluid thioglycollate medium and at 20-25^{o}C in the case of soyabean casein digest medium. The results of the ocular irritation studies indicated that the formulation F-6 is non-irritant with excellent ocular tolerance. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible.

The stability studies indicated that the formulation F-6 was physically and chemically stable with no significant change in any of the parameters evaluated when stored at the ambient humidity conditions between 2-8^{o}C, ambient temperature and 40^{o}C except for a slight decrease in the pH with time at 40^{o}C. From stability studies it was observed that the *in situ* gelling system of ofloxacin was stable at selected storage conditions with most suitable storage condition at the refrigeration temperature.

CONCLUSION

Ofloxacin, a broad spectrum antibacterial agent used in the treatment of ocular infections was successfully formulated as an ion- activated *in situ* gel forming ophthalmic solution using sodium alginate in combination with HPC as a viscosity enhancer which sustained the drug release over a period of 8 hours. The polymers used are inexpensive and easily available. The formulation also promises to reduce the frequency of drug administration, thus improving patient compliance. As the concept involved is novel and the methodology used for the preparation is simple as that of conventional ophthalmic liquid dosage form, it is industrially oriented and economical.

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