

STUDY OF AN ALGINATE/HPMC BASED *IN SITU* GELLING OPHTHALMIC DELIVERY SYSTEM FOR LEVOFLOXACIN HYDROCHLORIDE

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ABSTRACT

Conventional eye drops can result in poor drug bioavailability due to unique ocular anatomy and physiology¹. Ion-activated *in situ* gelling systems are able to crosslink with cations present in the tear fluid, therefore forming a gel on the ocular surface, which results prolonged corneal contact time.

The present work describes the formulation and evaluation of an anti-bacterial agent -levofloxacin hydrochloride, based on the concept of ion-activated in-situ gelation. Sodium alginate was used as the gelling agent in combination with HPMC-E-15 (Hydroxy Propyl Methyl Cellulose) that acted as a viscosity enhancing agent. The rheological behaviour of all the formulations were not affected by the incorporation of levofloxacin hydrochloride, both in vitro and in vivo pre-corneal retention studies indicated that the alginate or HPMC-E-15 solutions retained the drug better than the alginate or HPMC-E-15 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 economical, patient compliant and an alternative to conventional ophthalmic drops, patient compliance, industrially oriented and economical.

Keywords: Ophthalmic delivery systems, Sodium alginate, Levofloxacin.

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging areas facing the pharmaceutical scientist. The unique anatomy and physiology of the eye make it difficult to achieve an effective drug concentration at the target site. The challenge remains to circumvent the protective barriers of the eye without causing significant tissue damage or increasing the risk of systemic side effects. Various delivery systems have been investigated during past decades, pursuing two main strategies: to increase the corneal permeability and to prolong the contact time on the ocular surface². While former is mainly achieved by the use of prodrugs³, penetration enhancers^{4, 5} and colloidal delivery systems such as nanoparticles^{6, 7, 8}, liposomes⁹ and niosomes¹⁰, latter relies on rheological and mucoadhesive properties of the ophthalmic formulation.

Polymeric eye formulations can be subdivided into three groups: viscosity enhancing polymers, which simply increase the formulation's viscosity, resulting in decreased lacrimal drainage and enhanced bioavailability, muco adhesive polymers, which interact with ocular mucin, therefore increasing the contact time with ocular tissues, and *in situ* gelling polymers, which undergo sol-to-gel phase transition upon exposure to the physiological conditions present in the eye. The latter are highly advantageous over the preformed gels, which do not allow for accurate and reproducible administration of a drug and after administration, often produce blurred vision, crusting of eye lids and lacrimation. *In situ* gelling systems, on the other hand, can be easily and accurately instilled in liquid form, and are capable of prolonging the formulation's residence time on the surface of the eye due to gelling¹¹.

Keeping the physiology of the ocular surface in mind, three parameters (temperature, pH and ionic strength) can be generally exploited. A number of studies have already been performed on thermosetting gels based on pluronic^{12, 13} and pH sensitive formulations of chitosan and Carbopol^{14, 15}, but besides a few carbomer based artificial tear products none of these formulations has made it into market. The majority of studies have been conducted on ion-activated *in situ* gelling systems which are able to cross link with the cations present in the tear fluid, forming a gel on the ocular surface. They can be formulated at optimal pH for ocular delivery using buffers, can be easily and accurately instilled at room temperature and may therefore be less irritating to the ocular tissues than *in situ* gelling systems depending on a change in pH or

temperature. Gellan gum (gelrite) based systems have been evaluated for many drugs such as Timolol maleate¹⁶, ciprofloxacin hydrochloride¹⁷, indomethacin¹⁸ and gatifloxacin¹⁹ and have shown prolonged corneal residence time and increased ocular bioavailability of the drug. However, only a limited number of studies have been performed on xanthan gum and carrageenan systems²⁰ with a substantial lack of studies comparing the various *in situ* gelling polymer formulations. The objective of the present study was to develop an ion activated *in situ* gelling for Levofloxacin, a fluoroquinolone derivative used to treat external infections of the eye such as acute and sub-acute bacterial conjunctivitis, keratitis, keratoconjunctivitis²² 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 methyl cellulose-E-15 (HPMC-E-15) as the viscosity enhancer for the formulation of Levofloxacin eye drops (0.5% w/v), which undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug.

MATERIALS AND METHODS

Materials

Levofloxacin hydrochloride was obtained as gift sample from Cadila health care Ltd., Ahmedabad, India. Sodium alginate (Hi-Media labs India) and HPMC-E-15 from Apotex pharma, all other reagents were of analytical grade.

Preparation of formulations

Table I shows the composition of all the formulations. The alginate/HPMC-E-15 hydrogel was prepared by dispersing the required amount in 0.9% W/V sodium chloride with constant stirring using benzalkonium chloride (0.02% W/V) as preservative. Levofloxacin hydrochloride (0.5% W/V) was dissolved in water and added to polymer 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 & 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 system.

Gelling was assessed by mixing the formulation with simulated tear fluid in the proportion 25:7 and the gelation was assessed by visual examination²¹. 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 phosphate buffer pH 6.8. Aliquot of 1ml was withdrawn and further diluted to 25ml with phosphate buffer pH 6.8. Levofloxacin concentration was

determined at 288.5nm by using UV-Visible spectrophotometer (Elico SL-159). 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 was carried out by using Brookfield DV-II+ pro extra viscometer with spindle LV-3 with angular velocity run from 10 to 100rpm¹⁷.

Table 1: Formulation Design of Levofloxacin Hydrochloride containing in situ hydro gels.

| S. No. | Ingredients | Concentration In %W/V | | | |
|--------|-----------------------|-----------------------|-------|-------|-------|
| | | F-1 | F-2 | F-3 | F-4 |
| 1 | Levofloxacin HCl | 0.5 | 0.5 | 0.5 | 0.5 |
| 2 | Sodium alginate | 0.25 | 0.5 | 0.75 | 1.0 |
| 3 | HPMC-E-15 | 1 | 1 | 1 | 1 |
| 4 | Sodioum chloride | 0.9 | 0.9 | 0.9 | 0.9 |
| 5 | Benzalkonium chloride | 0.02 | 0.02 | 0.02 | 0.02 |
| 6 | Distilled water | 100ml | 100ml | 100ml | 100ml |

In vitro release studies of levofloxacin from formulations was studied through a fabricated apparatus consisting of 1ml of formulation placed in donor compartment over a cellophane membrane which was rinsed and soaked for 24hours in diffusion medium. The donor compartment was immersed in the receptor compartment containing 50ml of STF (Simulated Tear Fluid) maintained at 37±1°C with constant stirring at 22 rpm using magnetic stirrer. At specified intervals of time, 1ml 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 levofloxacin hydrochloride (0.5% 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 using autoclave at 121°C, 15lbs pressure for 20mins and sterility testing was performed²⁶. 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 5°C, 25°C±2°C/65% RH±5% and 40°C±2°C /75% RH±5% 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 release²³.

RESULTS AND DISCUSSION

The formulations were light yellow in color 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 II. The two main prerequisites of gelling system are viscosity and gelling capacity. Except for the formulations F1 and F2, 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.

Table 2: Evaluation results of pH, Drug Content and Gelling Capacity.

| Parameters | F-1 | F-2 | F-3 | F-4 |
|------------------|-------|-------|------|-------|
| pH | 7.1 | 7.1 | 7.1 | 7.1 |
| Drug Content (%) | 103.5 | 101.6 | 98.0 | 103.0 |
| Gelling Capacity | + | + | ++ | +++ |

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

Generally viscosity values in the range of 15-50cps significantly improve the contact time in the eye. The viscosity of the formulations F1 to F4 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 I. The administration

of ophthalmic preparations should have as little effect as possible on the pseudo-plastic character of the pre-corneal film²⁴. Since the ocular shear rate is very high, ranging from 0.03 s⁻¹ during inter-blinking periods to 4250-28500 s⁻¹ during blinking²⁵, visco-elastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often preferred.

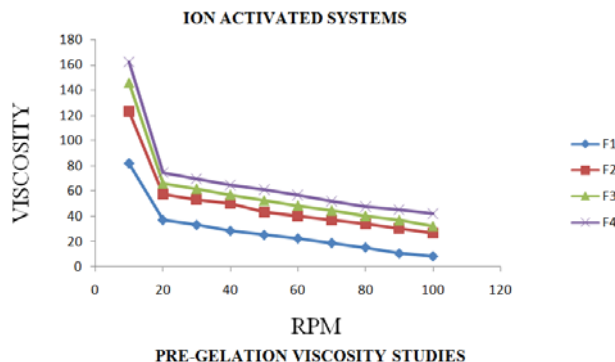
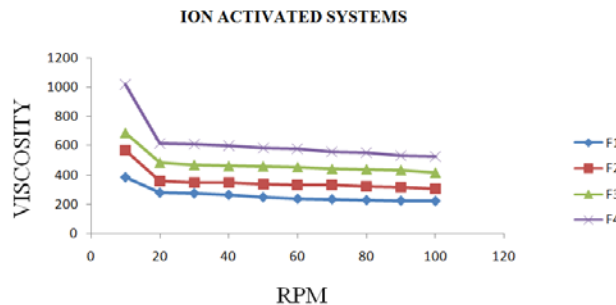


Fig. I: (SPINDLE NO LV-03)



POST- GELATION VISCOSITY STUDIES

The In vitro release studies fig II indicated that amongst all the formulations, F-4 sustained drug release for 8 hours, which may be due to the higher concentration of sodium alginate along with HPMC.

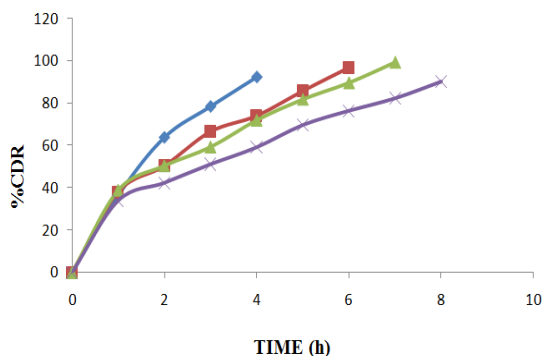


Fig. 2: Plot of *in vitro* release of levofloxacin as a function of time for formulations F1-F4 from *in situ* gelling systems

Note: %CDR = Cumulative % Drug Released

The higher regression coefficient values tableIII for each formulation suggested that the formulations F-1 to F-4 behaved matrix type of drug release.

Table 3: Regression co-efficient analysis and best fit analysis for the formulations

| Formulations | Zero order | First order | Matrix | Peppas | Hixon Crowell |
|--------------|------------|-------------|--------|--------|---------------|
| F-1 | 0.7935 | 0.7941 | 0.9957 | 0.9885 | 0.9420 |
| F-2 | 0.8608 | 0.8609 | 0.9965 | 0.9960 | 0.8609 |
| F-3 | 0.8082 | 0.8083 | 0.9961 | 0.9916 | 0.8083 |
| F-4 | 0.7663 | 0.7664 | 0.9952 | 0.7664 | 0.7664 |

The comparative *in vitro* drug release profile fig III between the marketed conventional ophthalmic drops and the formulation F-4 showed 41.69 % and 33.98 % after initial 60min. At the end of 180

min. the drug release was found to be 99.35% and 51.16% from the marketed product and F-4 indicating that the drug release was significantly prolonged by using the *in situ* gelling systems.

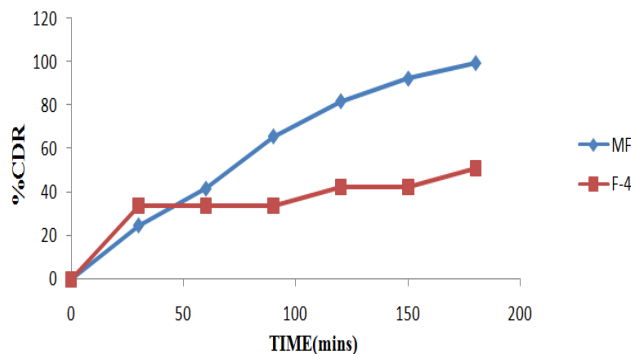


Fig. 3: Comparison of drug release profile of marketed conventional eye drop with formulation F-4

The formulation F-4 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°C in case of fluid

thioglycollate medium and at 20-25°C in the case of soyabean casein digest medium. The results of the ocular irritation studies indicated that the formulation F-4 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-4 was physically and chemically stable with no significant change in any of the parameters evaluated when stored at the ambient humidity conditions between 5°C, 25°C±2°C/65% RH±5% and 40°C±2°C /75% RH±5%. From stability studies it was observed that the *in situ* gelling system of levofloxacin was stable at selected storage conditions.

CONCLUSION

Levofloxacin, 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 HPMC-E-15 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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