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Original Research Paper

Development of Ion triggered *In-situ* gel containing Levofloxacin hemihydrates for ocular delivery: *In-vitro* and *In-vivo* evaluation

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ABSTRACT

In-situ gelling systems are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in specific physico-chemical parameters like temperature, ionic strength, or pH. A major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to pre-corneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered can be altered by increasing the retention time of medication into the eye by using in situ gel forming systems, thereby preventing the tear drainage. The aim of the present investigation is to prepare and evaluate novel Ion-triggered ocular gelling systems of Levofloxacin Hemihydrates. These gelling systems involve the use of Sodium alginate as Ion-sensitive polymer and HPMC K4M as viscofying agent. Nine formulations (S1-S9) based on Ion activated In situ gelation using Sodium alginate were preparedwith HPMC K4M as a common viscolyzer. A 3^2 full factorial design has been applied with conc. of Sodium alginate and HPMC K4M as independent variables studied at three levels to study their influence on drug release and gelling capacity as dependent variables. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, in vitro drug release and in-vivo studies in rabbit eye. The developed formulations showed sustained release of drug for up to 8 hrs. The formulations were found to be non-irritating with no ocular damage.

KEYWORDS: Ion-sensitive In-situ gel, Sodium alginate, Levofloxacin hemihydrates

1-.INTRODUCTION

Topical administration is generally considered the preferred route for the administration of ocular drugs due to its convenience. Drugs applied in this manner can be packaged in multiple forms, including solutions, ointments, and suspensions. Drug absorption occurs through corneal and non-corneal pathways. Most non-corneal absorption occurs via the nasolacrimal duct and leads to non-productive systemic uptake, while most of the drugs transported through the cornea are taken up by the targeted intraocular tissue. Unfortunately, corneal absorption is limited by drainage of the instilled solutions, lacrimation, tear turnover, metabolism, tear evaporation, non-productive absorption/adsorption, and limited corneal area, poor corneal permeability, binding by the lachrymal proteins, enzymatic degradation, and the corneal epithelium itself. These limitations confine the absorption window to a few minutes after administration and reduce corneal absorption to < 5%[1].Consequently; multiple daily dosingis often required, leading to decreased patient compliance. To increase both retention time on the

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surface of the eye and corneal absorption, novel topical systems have been developed, including ones that utilize in situ gelling polymers, mucoadhesive polymers, inserts, and ocular penetration enhancers[2]. In situ-forming hydro gels are liquid upon instillation andundergo phase transition in the ocular cul-de-sac to form viscoelasticgel and this provides a response to environmentalchanges. Three methods have been employed to causephase transition on the surface: change in temperature, pH, and electrolyte composition[3].

Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, β-D-mannuronic acid (M) and α-L-glucuronic acid (G) which forms three-dimensional hydrogel matrices .Sodium Alginate was chosen as a vehicle for ophthalmic formulations since it exhibits 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. Alginate transforms into stable gel upon exposure to divalent cations, which is not easily eroded by tear fluid[4].

Levofloxacin hemihydrates are a fluoroquinolone derivative used to treat external infections of eye such as acute and sub-acute bacterial conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers[5, 6]. It is available in market as 0.5%w/v eye drops.The objective of present study was to develop and evaluate an Ion activated In-situ ophthalmic gel system for Levofloxacin hemihydrates by using Sodium alginate as gelling agents.

EXPERIMENTAL

Materials and methods:

Levofloxacin hemihydrates were kindly supplied as a gift sample from Orgochem Ltd, Gujarat. Sodium alginate supplied by Signet chemicals. Hydroxyl propyl methyl cellulose (HPMC K4M) was obtained from Colorcon Asia Pvt. Ltd. All chemicals were used of analytical grade.

Preparation of the formulations

Table (1) shows the composition of In-situ gelling system based on Sodium alginates gelling agent. Ion activated in-situ solutions were prepared by dispersing the required amount of Sodium alginate and HPMC K4M in distilled water with continuous stirring. Levofloxacin hemihydrates (0.5% w/v) were dissolved in distilled water. Solution of benzalkonium chloride and mannitol were then added to the above solution. The resultant solution was then added to alginate solution under constant stirring to obtain a uniform solution. Distilled water was then added to make the volume up to 100 ml[7, 8].

Evaluation of formulations

Test for appearance/ clarity

All the formulations were checked for general appearance i.e. colour, odour, any suspended particulate matter etc. The clarity was checked using wooden board with black and white background. The vials were held horizontally and gently rotated immediately under the lamp and then inverted once or twice to detect foreign particles[9].

Determination of pH

The pH of each formulation was recorded using a calibrated pH meter. The pH of all formulations was recorded immediately after preparation as well as after 24 hours of storage at room temperature.

Gelation Studies

The gelling capacity was determined by placing 2 drops of prepared system in a vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37^0C.The gel formation was visually evaluated; time for gelation and the time taken for the gel to dissolve were noted[10]. The composition of artificial tear fluid was sodium chloride (0.670 g), sodium bicarbonate (0.200 g), calcium chloride·2H2O (0.008 g), and purified water q.s. (100 g). To note gelation observations the lowest scores (+) were assigned to those products in which the phase transition occurred only after 60-90 sec. and the formed gels collapsed within 1-2 hrs. The highest scores (+++) were assigned to those products for which the phase transition commenced within 60-90 sec. and the gels so formed were stable for about 7-8 hrs. The moderate scores (++) were assigned to the products, which could form the gel in 60-90 sec.but failed to maintain gel structure for more than 3hrs.

Drug content

The drug content was determined by suitably diluting solution with phosphate buffer pH 7.4. Aliquot of 1ml was withdrawn and further diluted to 10 ml with phosphate buffer pH 7.4. Levofloxacin concentration was determined at 288 nm by using UV-Visible spectrophotometer.

Rheological studies

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 Brookfield viscometer with angular velocity run from 10 to 100 rpm.

In-vitro release study:

The in vitro release of Levofloxacin from the formulations was studied through cellophane membraneusing a modified Franz diffusion cell. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). Cellophanemembrane, previously soaked overnight in the dissolution medium, was tied to one end of a specificallydesigned glass cylinder. A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37^0C so

that the membrane just touched thereceptor medium surface. The shaft was rotated at 100 rpm. Aliquots, each of 1-ml volume, were withdrawnat hourly intervals and replaced by an equal volumeof the receptor medium. The aliquots were dilutedwith the receptor medium and analyzed by UVspectrophotometer at 288 n

In vivo drug release studies

In vivo release studies of Levofloxacin hemihydrates from the prepared *in situ* gelling formulations were carried out using six male New Zealand albino rabbits each weighing 2.5–3.0 kg and with no signs of ocular inflammation or gross abnormalities.

Method:

50-µl of Levofloxacin hemihydrates optimized *in situ* gelling formulations (0.5%w/v) and marketed eye drop (0.5% w/v; marketed preparation) were instilled in the lower cul-de-sac of each eye, and the upper and lower eyelids were gently held closed for 2 min to maximize drug-cornea contact. At 0.5, 1, 2, 4, 6, and 8 h post dosing, the eyes were anesthetized using 4% topical xylocaine solution and the aqueous humor was sampled from 6 eyes for each formulation using a 28-gauge needle. Aqueous humor samples (100 µl) were mixed with acetonitrile and kept in a refrigerator for 1 h. The mixture was then centrifuged at 3000 r/min for 15 min and 20 µl of the supernatant obtained was used to determine the Levofloxacin hemihydrates content by HPLC.

HPLC analysis:

Quantitative estimation of Levofloxacinwas done by HPLC. A filtered and degassed mixtureof phosphate buffer pH 3.0 and Acetonitrile (80:20) wasused as the mobile phase. The mobilephase was delivered at a flow rate of 1.0 ml/min, theinjection volume was 20 µl and the effluent was monitoredat 235 nm using Gatifloxacin as internal standard.

Pharmacokinetic analysis

The area under the curve, $AUC_{(0-8)}$(µg.h/mL) for Levofloxacin concentration in the aqueous humor was calculated using the trapezoidal rule. The maximum Levofloxacin concentration Cmax (µg/ml) in the aqueous humor and the time at which Cmax is achieved i.e. Tmax (hr) were determined from actual data point.

Sterility testing:

IP method (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubatingformulations for not less than 14 days at 30 to 35^0 C in the fluid thioglycolate medium to find the growth of anaerobic bacteria and at 20 to 25^0 C in the soybean-casein digest medium to find the growth of aerobic bacteria and fungi in the formulation.

Antimicrobial efficacy study

Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms (Staphylococcus aureus and Pseudomonas aeruginosa) this was determined in the agar diffusion medium employing "cup plate technique". Sterile solution of marketed Levofloxacin hemihydrates eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile Muller Hinton Agar (MHA) previously seeded with organisms (*Staphylococcus aureus and Pseudomonas aeruginosa*). After allowing diffusion of solutions for twohours, the plates were incubated for 24 h at 37^0C. The zone of inhibition (ZOI) was compared with that of the standard. The activity of optimized formulations were also checked in infectious condition of eye i.e. STF pH 6.0 and compared with that of normal condition of eye i.e. STF pH 7.4

Selection of vehicle

Buffers contribute significantly to chemical stability and clinical response and also influence the comfort and safety of the product, hence it is important to select a suitable buffer which ensures product stability and desired drug solubility. The studies in various buffer solutions indicated that the drug was soluble in acetate buffer IP of pH 4.6, 4.8 and 5.0 and in citrophosphate buffer of pH 6.0 at the dosage level of 0.5%, w/v. The solutions were stable to elevated temperatures and autoclaving. However, their instability to light as evidenced by discoloration of the exposed solutions necessitated their packing in amber vials. It has been reported that the ocular penetration of Levofloxacin, is maximum at pH of about 6.5. Citrophosphate buffer, pH 6.0, was therefore selected as a vehicle for the pH activated in-situ gel preparation. Sodium alginate was found to be more stable in deionised water as compared to buffer that's why it was selected as vehicle for Ion activated in-situ gel preparation.

Selection of excipients:

Sodium alginates were selected as gelling agent because of its property to transform into gel in contact with tear fluid. These agents alone could not be used for ocular therapeutics due to its low mucoadhesive property; hence HPMC K4M was used along with these gelling agents to impart mucoadhesive properties. Benzalkonium chloride is mostly used in ophthalmic as preservative and also it is stable over wide temperature and pH conditions. Sodium chloride and Mannitol were used as tonicity adjusting agents to maintain preparation isotonic.

The composition, drug content, gelling capacity of the various Ion activated formulations are shown in Table (1). The drug content was found to be satisfactory.

Table (1): Characterization of Sodium alginate In-situ gelling system of Levofloxacin

Code	Sodium	Hpmc	Gelling	pH	Drug	Clarity	Appearance

	algi nate	k4 m	capa city		cont ent		
S1	1.0	0.4	+++	6. 0	99.9 8	Cle ar	Light yellow
S2	0.5	0.3	+	6. 0	102. 02	Cle ar	Light yellow
S3	1.5	0.5	+++	6. 4	99.7 6	Cle ar	Light yellow
S4	0.5	0.5	+	6. 2	98.7 5	Cle ar	Light yellow
S5	0.5	0.4	++	6. 2	100. 03	Cle ar	Light yellow
S6	1.5	0.3	+++	6. 3	100. 48	Cle ar	Light yellow
S7	1.5	0.4	+++	6. 0	101. 20	Cle ar	Light yellow
S8	1.0	0.5	+++	6. 2	99.3 4	Cle ar	Light yellow
S9	1.0	0.3	++	6. 3	100. 36	Cle ar	Light yellow

Appearance, clarity, pH and drug content:

The appearances of all formulations were light yellow in colour and were clear. Terminal sterilization by autoclaving had no effect on the formulations. The haziness observed during autoclaving due to precipitation of HPMC at elevated temperature was found to disappear and the clarity was regained after overnight standing. The pH of all the formulations was found to be within the range of 6.0 to 6.5, which is desirable for absorption of Levofloxacin hemihydrates. The drug content of ion activated in-situ formulations was within the range of 98.75% to102.02%, showed the uniform distribution of drug in the ophthalmic formulations and the results are shown in Table (1).

Gelling capacity:

The viscosity and gelling capacity plays important role for In-situ gelling system. The formulation should have an optimum viscosity for easy instillation into the eye as a liquid which undergo sol-to-gel transition. Formulations S1, S3, S6, S7, and S8 showed better gelling capacity as shown in Table (1).

Rheological studies:

The formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity (pseudo plastic rheology). The administration of ophthalmic preparations should influence as little as possible the pseudo plastic character of the precorneal tear film. Since the ocular shear rate is very large ranging from 0.03 s^{-1} during interblinking periods to 4250–28,500 s^{-1} during blinking, viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear are preferred.An increase in pH to 7.4 (the pH of the tear fluid) caused the solutions to transform into gels with high viscosity as shown in case of Ion activated in-situ gelling system shown in Table(3).

Table (3): Viscosity of ophthalmic formulations of Sodium Alginate with HPMC K4M at 10, 50 & 100 rpm.

Code	Viscosity of formulations at 10, 50 and 100 rpm both at 25°C and 37°C					
	25^0c	**37^0c**	**25^0c**	**37^0c**	**25^0c**	**37^0c**
	10	**10**	**50**	**50**	**100**	**100**
S1	72	38242	63	11220	58	9729
S2	60	18400	56	7840	52	6289
S3	78	42212	72	12200	69	9810
S4	62	21432	58	7920	55	6123
S5	67	20121	53	6850	49	4024
S6	71	39273	61	12121	57	9932
S7	76	40280	64	12010	54	9813
S8	79	38276	63	11389	53	9623
S9	68	36209	48	11254	41	9523

In-vitro release:

Figure1.Show the percentage of Levofloxacin hemihydrates released as a function of time for S1-S9 respectively. As per figures, S3 showed better release of 86.2 up to 8 hrs. The *in vitro* drug release conditions may be very different from those likely to be encountered in the eye. However, the results clearly showed that the gels have the ability to retain Levofloxacin hemihydrates for a prolonged period of time (8 hr) and premature drug release does not occur. From the results it is concluded that the high viscosity plays important role in controlling the release of drug from the formulations, when the polymer concentration increases drug release decreases, and when polymer concentration decreases drug release from the formulation increases[11]. Comparison of in-vitro release of formulations S3 with marketed eye drop is shown in Figure 2.

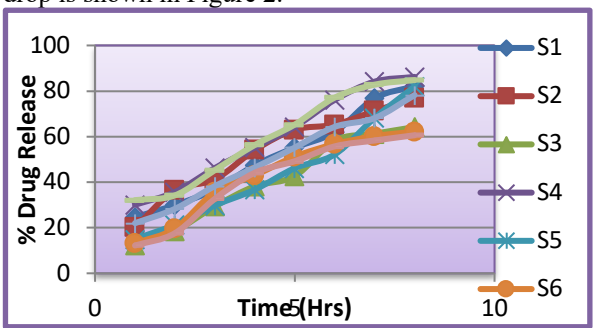


Figure 1: In-vitro release kinetics of S1-S9 batches

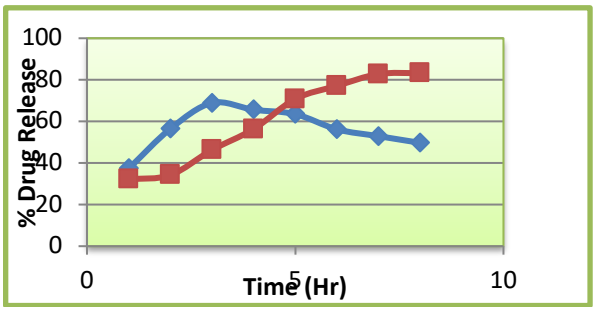


Figure 2: comparison of In-vitro release kinetics of S3 with marketed formulation (MF)

In-vivo release:

The *in vitro* release studies were completely free from any complications due to variability in precorneal factors, such as blinking, lachrymation, tear turnover, and drug washout[12]. The *in vitro* studies provided the relative permeation characteristics of Levofloxacin from different formulations, but they could not simulate real *in vivo* conditions. It is, therefore, necessary to study the *in vivo* ocular absorption of the drug from the formulations. The results of the *in vivo* studies are shown in Figure 3.

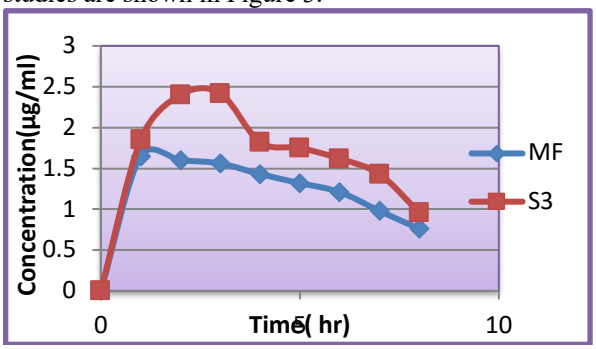


Figure 3:Tear fluid concentration profile of Marketed eye drop & Ion activated in-situ gel (S3)

Table (3): Pharmacokinetic parameters of Levofloxacin in aqueous humor after instillation of S3

Ocular delivery system	Cmax (µg/ml)	Tmax (h)	$AUC_{(0-8)}$ (µg.h/ml)*
MF	1.65	1	12.69
S3	2.40	3	18.30

The level of Levofloxacin in aqueous humor after topical instillation of S3 was shown in (Figure.2). The aqueous humor content of Levofloxacin was significantly higher at all time points after administration of S3 than other formulations. It was interesting to note that the aqueous humor level showed a maximum at 3 hr post administration which decreased gradually afterwards since the higher concentration of the antibiotic is always desirable at early time of infection. More specifically, the intraocular Levofloxacin level attained in the aqueous humor following administration of S3 was fairly high for up to 8 hr in contrast to other formulations.

Sterility test

The optimized formulation S3 passed the test for sterility as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 14 days a 30-35 ^{0}C in case of fluid thioglycolate medium and at 20-25^{0}C in the case of soya bean casein digest medium.

Antimicrobial efficacy study:

The study indicated that the Levofloxacin retained its antimicrobial efficacy even after formulated as an *in situ gelling system*, this was indicated by same zone of inhibition (3.5 cm) observed in case of both marketed formulation and in-situ gel system against staphylococcus aureus as shown in figure4. In infectedcondition of eye, pH of eye becomes acidic i.e. pH 5-6. Antimicrobial efficacy of optimized in-situ formulations were tested in acidic condition i.e. STF(pH 6) and compared its zone of inhibition in STF (pH 7.4) against Staphylococcus aureus (2024) and Pseudomonas aeruginosa (NCIM-5029) which was same as shown in figures 5.This indicates that Levofloxacin shows antimicrobial activity even in acidic condition of eye (Infectious condition of eye).

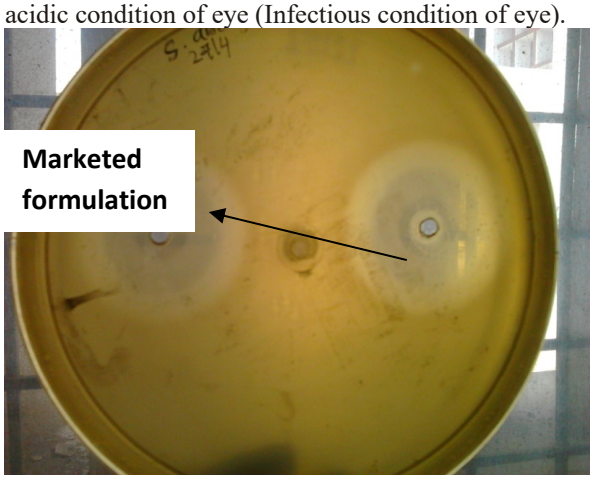


Figure 4: Zone of inhibition shown by marketed formulation with In-situ gelling system against staphylococcus aureus (NCIM-2024)

(a) Staphylococcus aureus (b) Pseudomonas aeruginosa

Figure 5: Zone of inhibition shown by S3 against (a) Staphylococcus aureus (NCIM-2024) (b) Pseudomonas aeruginosa(NCIM-5029)

CONCLUSION:

Levofloxacin hemihydrates which is a broad spectrum Anti-bacterial agent used in the treatment of various ocular infections was successfully formulated as Ion activated In-situ gel using HPMC K4M as polymer. The formulated systems provided sustained release of the drug for 8 hr period. The developed formulation is a viable alternative to conventional eye drop due to its ability to enhance bioavailability through its longer

precorneal residence time and ability to sustain release of the drug. Also important is its ease of administration and decreased frequency of administration resulting in better patient compliance.

REFERENCES:

1. Bourlais CAL, Acar LT, Rhodes CT, Sado PA, Leverge R. New Ophthalmic Drug Delivery Systems. Industrial Pharmacy 1995; 21:19-59.
2. Bourlais CAL, Acar LT, Zia H, Sado PA, Leverge R. Ophthalmic drug delivery systems recent advance. Industrial Pharmacy 1998; 17:33-58.
3. Rathore KS, Nema RK, Sisodia SS. Timolol maleate a gold standard drug in glaucoma used as ocular films and inserts: an overview. IJPSRR 2010; Volume 3, Issue 1, Article 005: p23-29.
4. Odile Sechoy, Gerard Tissie, Chantal Sebastian, Florence Maurin, Jean-Yves Driot and Claude trinquand (2000). A new long acting ophthalmic formulation of Carteolol containing alginic acid. Int. J. Pharmaceutics, 207: 109-116.
5. Nanjawade, B.K., Manvi, F.V., Manjappa, A.S., Journal of Controlled Release. 2007, 122, 119–134.
6. Lin, H.-R., Sung, K.C., Journal of Controlled Release. 2000, 69, 379–388
7. Sindhu abraham, sharonfurtado, bharath s, basavarajbv, Deveswaran r and madhavan v,Sustained ophthalmic delivery of ofloxacin from an ion-activated in situ gelling systempak. J. Pharm. Sci., vol.22, no.2, april 2009, pp.175-179
8. *B*. *Srividya et al*., Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system,p-207
9. Madan M, Bajaj A, Lewis S et al. In-situ forming polymeric drug delivery systems. Ind J Pharm Sci 2009; 71; 242-51
10. Subimol S, AniShree GS, Radhakrishnan M. Fabrication of ophthalmic in-situ gel of Diclofenac Potassium and its evaluation. Scholars academic J Pharm 2013; 2:101-106.
11. Rathore KS. In-situ gelling ophthalmic drug delivery system: an overview. Int J Pharm Sci Res 2010; 2:30 34.
12. Amaliyar PR, Chaudhary S. In-situ gel forming a novel approach for sustained ophthalmic drug delivery. Int J Pharm Sci Bio Sci 2014; 3(2): 502-524.