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Design and Evaluation of Ion Induced in Situ Gel formulation For Levofloxacin Hemihydrateocular Delivery.

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ABSTRACT: *Eye drops are the conventional dosage forms which results in poor bioavailability due unique anatomy and physiology of eye[1]. 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. Ions activated in situ gel systems are capable of producing prolonged release of an active substance by crosslinking with cations present in tear fluid. The present work describes the formulation and evaluation of Levofloxacin Hemihydrate based on concept of ion activated in situ gelation. Sodium alginate was used as gelling agent in combination with HPMC K4M as viscofying agent. The rheological behaviour of all formulations was not affected by addition of Levofloxacin hemihydrate. In vitro studies indicated that sodium alginate – HPMC based in situ gel retained drug for long time.The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study in vitro drug release study and ocular irritation study on rabbits. 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: *Ophthalmic drug delivery system, Ion activated in situ gel, Levofloxacin hemihydrate.*

I. INTRODUCTION:

Ophthalmic drug delivery is challenging for drug delivery because of its unique anatomy which restricts drug absorption into deeper tissues. Poor bioavailability of drugs from conventional ocular dosage forms is mainly due to tear production, nasolacrimal drainage, and transient residence time, drainage of the instilled solution, tear turnover and limited corneal area[2]. 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 surface[3]. Several Novel drug delivery systems 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. Successful results have been obtained with inserts and collagen shields.However, these preparations have disadvantages such as poor compliance, especially by old age people and many patients sometimes lose the device without noticing it.

The fluoroquinolones represent an expanding class of broad-spectrum antibacterial which cover a host of Gram-negative and anaerobic species responsible for ocular infections. These antibacterial have gained popularity in the ophthalmology field since they have been shown to be equivalent to combination therapy in the treatment of many ocular infections. Fluoroquinolones are also effective against a variety of Gram-positive organisms, including Streptococcal and Staphylococcal species; however, resistance is emerging among some of these organisms. Fluoroquinolones act by inhibiting two enzymes involved in bacterial DNA synthesis, both of which are DNA topoisomerases that human cells lack and that are essential for bacterial DNA replication, thereby enabling these agents to be both specific and bactericide. DNA topoisomerases are responsible for separating the strands of duplex bacterial DNA, inserting another strand of DNA through the break, and then resealing the originally separated strands. DNA gyrase introduces negative superhelical twists in the bacterial DNA doublehelix ahead of the replication fork, thereby catalyzing the separation of daughter chromosomes. Various fluoroquinolones like Levofloxacin, Ofloxacin and Ciprofloxacin are available in the various dosage forms.

Polymeric eye formulations can be subdivided into three groups as follows;

[1] Viscosity enhancing polymers, which simply increase the formulation's viscosity, resulting in decreased lacrimal drainage and enhanced bioavailability.
[2] Muco-adhesive polymers, which interact with ocular mucin, therefore increasing the contact time with ocular tissues.

[3] 3.In situ gelling polymers, which undergo sol-to-gel phase transition upon exposure to the physiological stimuli.
[4] There are four broadly defined mechanisms used for triggering the in situ gel formation of

biomaterials:
[1] Physiological stimuli (e.g., Temperature and pH),
[2] physical changes in biomaterials (e.g., solvent exchange and swelling),
[3] Chemical reactions (e.g. Ion exchange, Enzymatic, chemical)
[4] Photo-initiated polymerization.

The preformed gels don't allow for précised administration of a drug. After administration of eye drops they 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 gelling[4]. Keeping the physiology of the ocular surface in mind, three parameters (temperature, pH and ionic strength) can be generally exploited. Here we will be discussing on in situ gel formation based on chemical reactions chemical reactions that results in situ gelation may involve precipitation of inorganic solids from supersaturated ionic solutions, enzymatic processes, and photo-initiated processes.
Ionic Crosslinking Polymers may undergo phase transition in presence of various ions. Some of the polysaccharides fall into the class of ion-sensitive ones. While k-carrageenan forms rigid, brittle gels in reply of small amount of K+, i-carrageenan forms elastic gels mainly in the presence of Ca2+. Gellan gum commercially available as Gelrite® is an anionic polysaccharide that undergoes in situ gelling in the presence of mono and divalent cations, including Ca2+, Mg2+, K+ and Na+. Gelation of the low-methoxypectins can be caused by divalent cations, especially Ca2+. Likewise, alginic acid undergoes gelation in presence of divalent/polyvalent cations example Ca2+ due to the interaction with guluronic acid blockThis type of formulation was prepared in deionized water as the gelling agent forms stiff gel when interact with the ions in the buffer system.

A number of studies have already been performed on thermosetting gels based on pluronic[5, 6] and pH sensitive formulations of chitosan and Carbopol[7, 8] 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 resulting in formation of 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 they are less irritating to the ocular tissues than in situ gelling systems depending on a change in pH ortemperature

II. MATERIALS AND METHOD:

Materials: Levofloxacin hemihydrate was kindly supplied as a gift sample from Neuland Laboratories Limted, Hyderabad, Andhra Pradesh. Sodium alginate was obtained as a gift sample from signet chemicals Ltd. Hydroxyl propyl methyl cellulose (HPMC K4M) was gift sample from Colorcon Asia Pvt. Ltd. All other chemicals were used of analytical grade.

Selection of vehicle: The solubility of Levofloxacin was tested in various buffers such as acetate buffer I.P. (pH 6.0 & 6.5), citrophosphate buffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 7.2 and 7.4) in order to select a suitable vehicle. Solutions of levofloxacin in the above buffers were prepared to test its solubility at the dosage level desired (0.5%, w/v).

Method for Preparation of the formulations: This type of formulation was prepared in deionized water as the gelling agent forms stiff gel when interact with the ions in the buffer system. Take 0.9% w/v of sodium chloride and add this under constant stirring, then add benzalkonium chloride (0.02%w/v) to above the solution as preservative[9]. After that add levofloxacin solution (0.5% w/v) in polymer solution under stirring to form uniform solution and lastly make up the volume up to 100ml.Table 1 shows the composition of all formulation. Formulations were tested for ocular irritation study (Protocol approval no. : MCP/IAEC/130/2014)

Table I: Different compositions of Levofloxacin hemihydrate in situ gel formulation.

Sr. No	Ingredients	Concentration in %w/v			
		F1	F2	F3	F4
1	Levofloxacin hemihydrate	0.5	0.5	0.5	0.5
2	Sodium alginate	0.4	0.7	1.0	1.3
3	HPMC K4M	0.5	0.5	0.5	0.5
4	Benzalkonium Chloride	0.02	0.02	0.02	0.02
5	Sodium chloride	0.9	0.9	0.9	0.9
6	Deionized water	100 ml	100 ml	100 ml	100 ml

III. RESULT AND DISCUSSION

[1] Determination of visual appearance, clarity and pH:

The appearance and clarity of the formulation was determined visually and the pH of the formulation was determined by the pH meter. Table 2 shows the observation of for the prepared formulations.

[2] Gelling capacity:

It was determined by placing drop of formulation in test tube containing simulated tear fluid which was freshly prepared and equilibratedat 37^0C. Gelling capacity was evaluated based on time required for gelation as well as time required to dissolve the gel was also noted. The observation was raked as "+" which indicates slow phase transition from liquid to gel and it dissolved rapidly. "++" indicates that vehicle is in liquid-gel like form and flow less readily, and also showed that gelation immediate and remains for few hours. "+++" indicates that vehicle is in gel form and very difficult to flow and also showed immediate gelation which remains for extended period of time. Table 3 gives information about the gelling capacity.

[3] % Drug content:

The drug content was determined by diluting 1-ml of formulation in 100 ml of simulated tear fluid pH 7.4. Aliquot of 5 ml of solution was withdrawn and further diluted with 25 ml of STF and the concentration was determined by UV method.

Table II: Result of Evaluation Parameter

Formulation	Appearance	pH	Gelling capacity	% Drug content
F1	Transparent	7.12	++	99.30
F2	Transparent	7.20	+++	100
F3	Transparent	6.98	+++	98.56
F4	Transparent	7.12	+++	100.10

[4] **Rheological study:**

Viscosity of the instilled formulation is an important in order to determine the residence time of the formulation in eye. It was determined by using Brookfield viscometer. Viscosity was measured at different angular velocities. Details are given in Table 3 and Figure 1.

Table III: Rheological study of in situ gel formulation.

Angular Velocity (rpm)	F1	F2	F3	F4
10	400	565	750	980
20	397	521	701	935
30	350	480	659	865
40	320	439	623	780
50	275	398	553	720
60	200	341	490	687
70	175	310	430	580
80	138	296	365	490
90	110	238	290	380
100	100	178	200	250

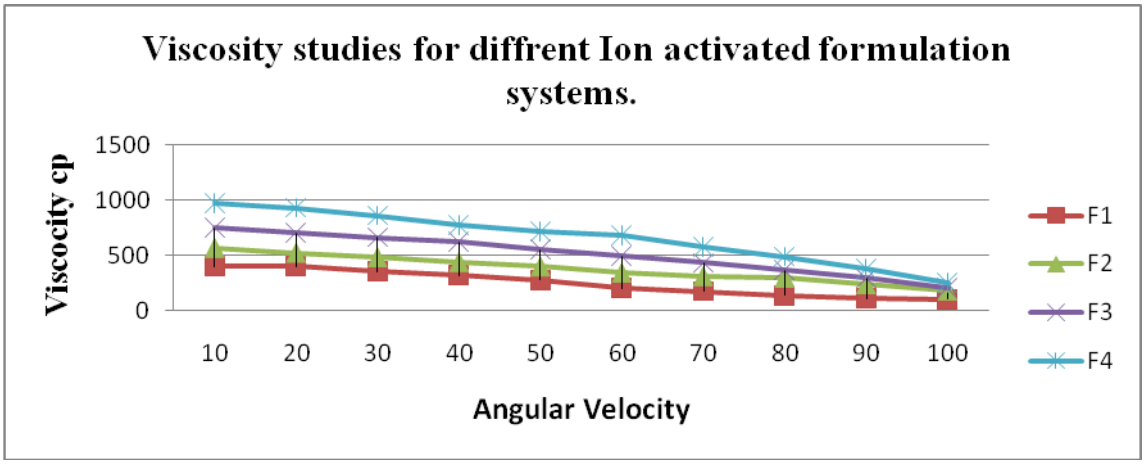

Figure 1: Rheological study of prepared in situ gel formulation.

In-vitro drug release study: The in vitro release from the formulations was studied using cellophane membrane. Dissolution medium used was freshly prepared (pH 7.4) artificial tear fluid. Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driven shaft and suspended in 50 ml of dissolution medium maintained at 37^0C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analysed by UV-vis. spectrophotometer at 288 nm. Table 4 and Figure 2 give information about the release profile of the formulation.

Table IV: % Cumulative drug release of in situ gel formulation.

Time in hours	F1	F2	F3	F4
	0	0	0	0
1	28.3	23.47	15.1	10.34
2	45.89	41.30	25.66	17.76
3	53.65	48.69	38.45	26.09
4	68.98	62.60	49.52	34.23
5	77.32	74.34	60.73	49.34
6	89.9	84.52	74.09	60.88
7	98.55	92.34	89.39	74.12
8	---	97.30	99.78	84.09

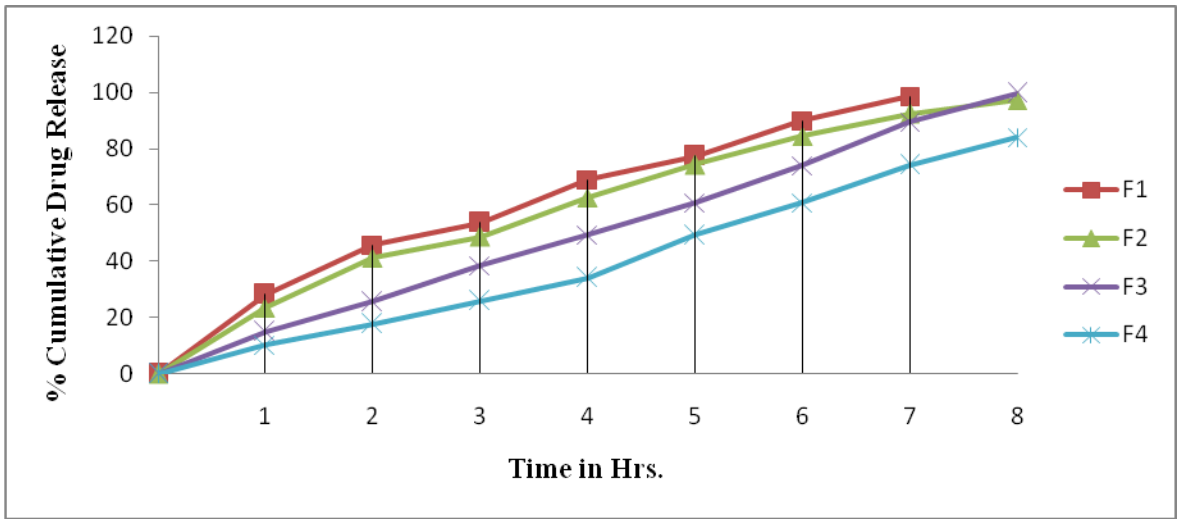

Figure 2: % Cumulative drug release from different formulation.

[5] Sterility test:
All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycolate medium and 20°C to 25°C in the case of soyabean-casein digest medium.

[6] Ocular irritation studies[10]:
Ocular irritation studies were performed on male albino rabbits weighing 1-2kg.The modified Draize technique was designed for the ocular irritation potential of the ophthalmic product. According to Draize test, the eye drops (100µl) was normally placed in the lower cul-de-sac and irritancy was tested at the time interval of 1hr, 24hrs, 48hrs, 72hrs, and 1 week after administration. The rabbits were observed 18 periodically for redness, swelling and watering of the eye.

[7] Stability studies[11]:
Stability is defined as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e. its shelf life), the same properties and characteristics that it possessed at the time of

its manufacture. Stability testing is performed to ensure that drug products retain their fitness for use until the end of their expiration dates. All the formulations were subjected to stability studies at accelerated condition i.e. 40^0C for a period of one month. The samples were withdrawn after 30 days and were evaluated for drug content, visual appearance and clarity.

Table V: Comparative data of % drug content and pH of the formulation over stability at accelerated condition.

Formulation	*Visual appearance*		*Clarity*		*pH*		*Drug Content (In %)*	
F3	**Initial**	**30 days**	**Initial**	**30 days**	**Initial**	**After 30 days**	**Initial**	**After 30 days <<**
	Transparent		Clear		7.1	7.2	98.65	98.23

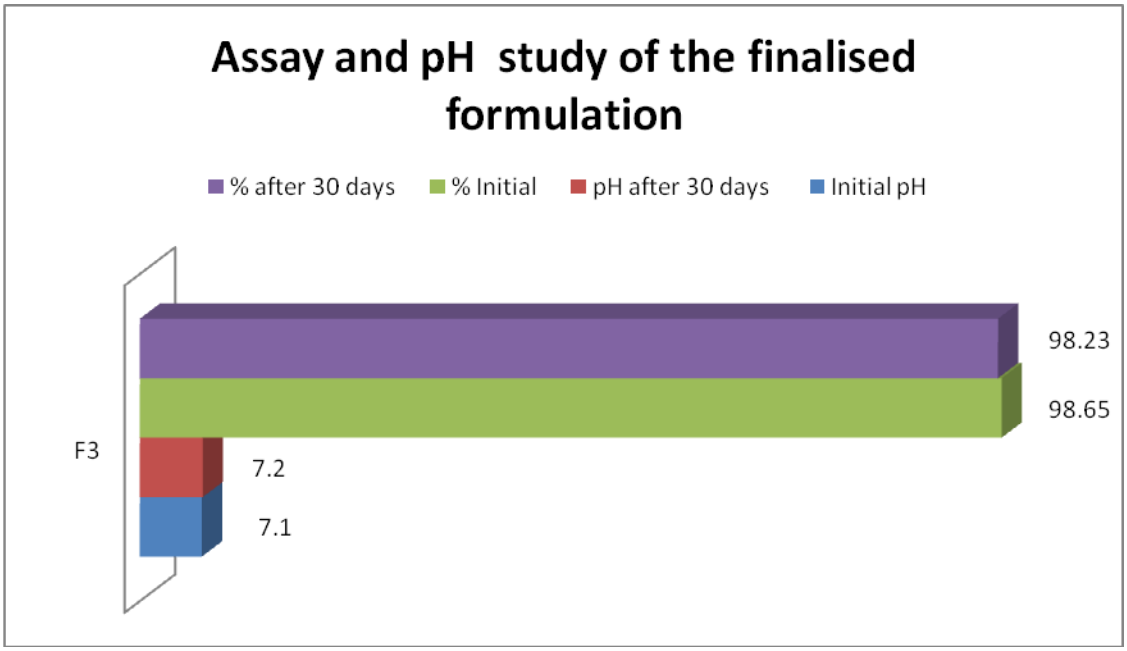

Figure 3: Assay and pH of Stability Samples at Accelerated condition.

IV. CONCLUSION:

Levofloxacin is a third generation antibiotic used for treatment of ocular conjunctivitis was successfully formulated using sodium alginate ion triggered and HPMC K4M as viscosity enhancing agent. In the present work F3 formulation found to produce sustained the drug release for 8 hours and it was compatible with eye and it is non irritating. The stability data of the formulation also suggest that it is stable and maintains its efficacy after one month. It also ensures the reduced dosing frequency by retaining the drug content for 8.0 hours their by improving patient compliance. As the process involved is not critical and availability of excipients is also possible, the present work canbe explored for its use in humans which will help to commercialize the same.

REFERENCES:

[1] Manish K and Kulkarni GT: Recent advances in ophthalmic drug delivery system. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(1): 387-94.
[2] Basavaraj KN, Manvi FV and Manjappa AS: In situ forming hydrogels for sustained ophthalmic drug delivery, Journal of Controlled Release 2007; 122: 119-134.
[3] Jarvinen K, Jarvinen T, Urtti A: Ocular absorption following topical delivery. Advanced Drug Delivery Reviews 1995; 16:3-19
[4] Lee VHL, Li VHK, Pro-drugs for improved ocular drug delivery. Advanced Drug Delivery Reviews 1989; 3:1-38.

[5] Kaur IP, Garg A, Singla AK and Aggarwal D: Vesicular system in ocular drug delivery an overview. International Journal of Pharmaceutics 2004; 269; 1-14.
[6] Krauland AH, Leitner VM and Bernkop SA: Improvement in the in situ gelling properties of deacetyllatedgellan gum by immobilization of thiol groups. Journal of Pharmaceutical Sciences 2003; 1234-41.
[7] El-Kamel AH:In vitro and in vivo evaluation of pluronic F-127 based ocular delivery system for timolol maleate. International Journal of Pharmaceutics 2002; 241: 47-55.
[8] Qi H, Chen W, Huang C, Li L, Development of poloxameranalogs/ carbapol based in situ gelling and mucoadhesive ophthalmic delivery system for puerarin. International Journal of Pharmaceutics 2007; 337: 178-87.
[9] Mahesh NS, Manjula BP, Study of an alginate/hpmc based in situ gelling ophthalmic delivery system for levofloxacin hydrochloride, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 4, Issue 3, 655-658
[10] John W Shell, PhD. Ocular Drug Delivery System-A review. Toxicol and Ocular Toxicol. 1982; 1(1): 49-63.
[11] Mohan EC, Jagan Mohan k, Venkatesham A. Preparation and evaluation of in situ for ocular drug delivery. Journal of Pharmacy Research. 2009; 2(6):1089-94