Sustained Ophthalmic Delivery of Gatifloxacin from *In Situ* Gelling System

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The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of drug may be overcome by the use of *in situ* gel-forming systems that are instilled as drops into the eye and 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, gatifloxacin, based on the concept of ion-activated systems. Sodium alginate was used as the gelling agent in combination with hydroxy propyl methyl cellulose (Methocel E50LV), which acted as a viscosity enhancing agent. The developed formulations were therapeutically efficacious, stable, non-irritant and provided sustained release of the drug over an eight hour period. The developed system is thus a viable alternative to conventional eye drops.

The landscape of ophthalmic drug delivery is highly competitive and rapidly evolving. New classes of pharmaceuticals and biologics are fuelling the demand for novel drug delivery systems. Ophthalmic drug delivery is one of the most interesting and challenging endeavors. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. It is a common knowledge that, the ocular bioavailability of drugs applied

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topically as eye drops is very poor. The absorption of drugs in the eye is severely limited by some protective mechanisms that ensure the proper functioning of eye and by other concomitant factors[1].

Recently, controlled and sustained drug delivery has become the standard in modern pharmaceutical design and an intensive research has been undertaken in achieving much better drug product effectiveness, reliability and safety[2]. In this regard, many polymers are very useful which undergo reversible solution to gel phase transition in response to physiological stimuli[3]. *In situ* gels are conveniently dropped as a solution into the conjunctival sac, where they undergo a transition into a gel with its favorable residence time. The sol-gel transition occurs as a result of a chemical/ physical change induced by physiological environment. This type of gel combines the advantage of a solution being patient convenient with the favourable residence time of a gel for enhancing the ocular bioavailability. The sol-gel transition can be induced by a shift in the pH as for cellulose acetate phthalate, a shift in temperature as for the thermogelling Poloxamer 407 or by presence of cations as for deacetylated gellan gum and alginates[3].

Thus, with the use of these *in situ* gelling systems, residence time of the drug in the eye is increased. Continuous delivery of drugs in a controlled manner to the anterior chamber of the eye will eliminate the requirement for frequent drug administration, causing better patient compliance and resulting in extended duration of action. Hence, lower amount of total dose will be required, which will minimize the local and/or systemic side effects[4,5].

Gatifloxacin and HPMC E50 LV (hydroxypropyl methylcellulose) were obtained as gift samples from Ajanta Pharma Ltd., Mumbai and Colorcon Asia Pvt. Ltd., Goa respectively. All other chemicals were of laboratory grade.

The preparation of *in situ* gelling system was carried out following the manufacturing process as described previously[6]. Table 1 shows the composition of all the formulations. The formulations were subjected to different evaluation parameters.

The general appearance of the formulation was observed which included clarity and color of solution[7]. The pH of the prepared formulations was checked by using pH meter. The drug content was determined by taking 1 ml of the formulation and diluting it to 100 ml with distilled water. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with distilled water. Gatifloxacin concentration was determined at 290 nm by using UV-Vis spectrophotometer (UV-1201, Shimadzu Corporation, Japan). The *in situ* gelling system was mixed with STF (simulated tear fluid) in the proportion 25: 7 (application volume 25 µl, normal volume of tear fluid in the eye is 7 µl). Gelation was assessed by visual examination[8]. Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye.

TABLE 1: COMPOSITION, pH AND DRUG CONTENT OF *IN SITU* GELLING SYSTEMS OF GATIFLOXACIN

Ingredients	F1	F2	F3	F4
Gatifloxacin sesquihydrate equivalent to gatifloxacin (% w/v)	0.3	0.3	0.3	0.3
Sodium alginate (% w/v)	0.8	0.4	0.6	0.8
HPMC E50LV (% w/v)	–	0.2	0.2	0.2
Sodium chloride (% w/v)	0.9	0.9	0.9	0.9
Benzalkonium chloride (% w/v)	0.01	0.01	0.01	0.01
Phosphate buffer pH 6.8 (ml) qs to	100	100	100	100
pH	6.7	6.8	6.8	6.8
Drug content	98.33	103.3	101.6	102.5

The viscosity determination of prepared formulations were carried out using Brookfiled DV-111+ Rheometer with spindle LV-3. The prepared solution was allowed to gel in the STF and then viscosity was measured. Viscosity of samples was measured at different angular velocities. A typical run comprised changing angular velocity from 10 to 100 rpm with equal wait for each rpm. The angular velocity was reversed (100 to 10 rpm) with similar weight. The average of two readings was used to calculate the viscosity[9].

The *in vitro* release of gatifloxacin from the formulation was studied through cellophane membrane using modified apparatus. The dissolution medium used was STF, freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium was tied to one end of a specifically designed glass cylinder (open at both ends). One ml of formulation (equivalent to 3 mg of gatifloxacin) was accurately placed into this assembly. The cylinder was attached to a stand and suspended in 50 ml of dissolution medium maintained at 37±1°, the membrane just touching the receptor medium surface. The dissolution medium was stirred at low speed using magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Vis spectrophotometry at 285 nm[10]. The *in vitro* release

studies were carried out for the marketed product in order to compare the release profile with the prepared *in situ* gelling system of gatifloxacin. All ophthalmic preparations should be sterile; therefore, the test for sterility is a very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia[11].

Antimicrobial efficacy studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This was determined by agar diffusion test employing "cup plate technique". Sterile solution of gatifloxacin (Gate Eye Drops, Ajanta Pharma Ltd, Mumbai) as a standard was used and the developed formulations (test solutions) were poured into cups bored into sterile Muller Hinton Agar (MHA) previously seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). After allowing diffusion of solutions for two hours, the plates were incubated for 24h at 37°. The zone of inhibition (ZOI) measured around each cup was compared with that of the standard. Both positive and negative controls were maintained throughout the study[12].

The ethical committee of the University had permitted the *in vivo* study. Ocular irritation studies were performed on four male albino rabbits each weighing 1-2 kg. The sterile formulations were instilled twice a day for a period of 21 d and the rabbits were observed periodically for redness, swelling and watering of the eyes. All the four formulations were subjected to stability studies at ambient humidity conditions at 2° to 8°, ambient temperature at 40±1° for a period of one month. The samples were withdrawn after 7, 15 and 30 d. The samples were evaluated for parameters viz. pH, appearance, gelation studies, drug content and *in vitro* drug release[13].

Clarity of all the formulations was found to be satisfactory. The formulations were light yellow in color. Terminal sterilization with autoclaving had no effect on the physicochemical properties of the formulations. The haziness that was observed after autoclaving (due to precipitation of HPMC E50LV at elevated temperature) was found to disappear and the original clarity was regained after overnight standing. pH values for all the formulations are given in Table 1. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation. Table 1 also shows the result of percent drug content for all the formulations. The drug content was found to be in acceptable range for all the formulations. Percent drug content in all four formulations were in the range 98.3 - 103.3% indicating uniform distribution of drug.

The two main prerequisites of gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity, which will allow its easy instillation into the eye as a liquid (drops), which will then undergo rapid sol-to-gel transition due to ionic interaction. Moreover, to facilitate sustained release of drug to the ocular tissue, the *in situ* formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time. All the formulations gelled instantaneously (less than a minute) on contact with STF. By visual inspection, the formulations formed a translucent matrix on addition to the STF. The gelation may be due to ionic cross-linking of the alginate chains by the divalent cation.

The formulations exhibited pseudoplastic rheology, as evidenced by shear thinning on increase in shear stress with increased angular velocity. The viscosity was directly dependent on the polymeric content of the formulation. The viscosity increased with increasing concentration of sodium alginate. F4 showed the maximum viscosity of 45 cps at 20 rpm whereas the minimum viscosity at 20 rpm was shown by F2. This indicated that addition of HPMC E50LV led to increase in viscosity. The viscosities of formulations were in following order F4>F1>F3>F2.

Generally viscosity values in the range of 15-50 cps significantly improve the contact time in the eye[14]. Higher viscosity values offer no significant advantage and have a tendency to leave a noticeable residue on the lid margin. The administration of ophthalmic preparation should influence as little as possible the pseudoplastic character of the pre-corneal film. Since the ocular shear rate is very high, ranging from 0.03 s^{-1} during interblinking periods to 4250 - 28,500 s^{-1} during blinking[3], 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 rheological profile of prepared *in situ* gelling systems of gatifloxacin is shown in fig. 1.

The *in vitro* release studies were carried out for all formulations using STF as the dissolution medium. The data of these studies are presented in Table 2. Results indicated that F4 showed better sustaining effect amongst all formulations. This may be due to the higher concentration of sodium alginate along with HPMC E50LV in F4. The release profile is shown in fig. 2.

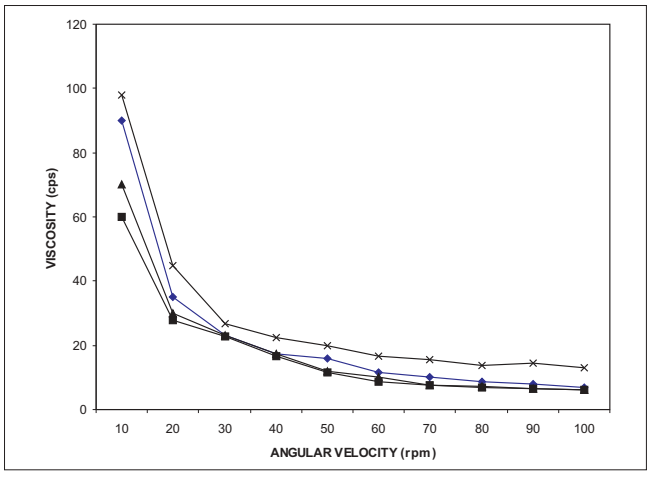

Fig. 1: Rheological profile of *in situ* gelling systems of gatifloxacin (F1-F4)
Formulation-1 [-◆-], formulation-2 [-■-], formulation-3 [-▲-] and formulation-4 [-x-]

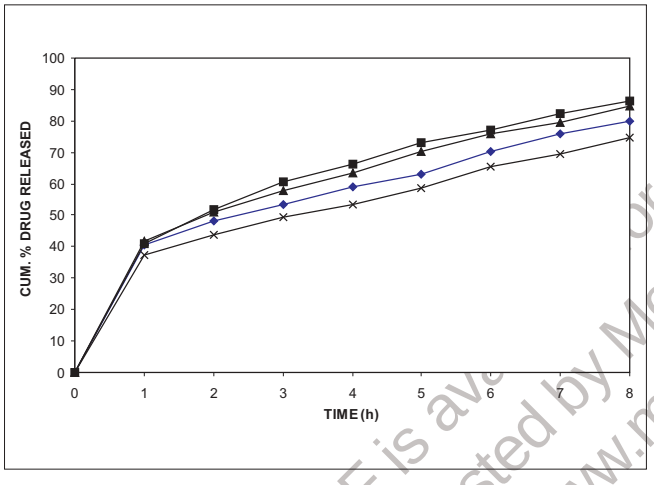

Fig. 2: Plot of cumulative % drug released vs. time for F1-F4 *in situ* gelling gatifloxacin formulations
Formulation F-1 [-◆-], formulation F-2 [-■-], formulation F-3 [-▲-] and formulation F-4 [-x-]

TABLE 2: RELEASE PROFILE OF *IN SITU* GELLING SYSTEMS OF GATIFLOXACIN FORMULATION

Time (T) (h)	Cumulative percent drug released			
	F-1	F-2	F-3	F-4
1	40.67	40.98	41.93	37.39
2	48.13	51.63	50.96	43.77
3	53.55	60.49	57.90	49.52
4	59.15	66.22	63.38	53.33
5	63.05	73.27	70.32	58.53
6	70.16	77.21	75.80	65.52
7	75.93	82.45	79.51	69.43
8	79.83	86.22	84.67	74.63

The drug release pattern obtained for the gelled samples is characteristic for hydrophilic matrices. The initial fast release of gatifloxacin can be explained by the fact that, alginate eye drops are formulated in water and hence the polymer was completely hydrated. When they come in contact with STF and gelation occurs, a prehydrated matrix is formed in which hydration and water penetration no longer limit drug release leading to an apparent diffusion-controlled release. The regression coefficients for the formulations F1-F4 of zero order plots were found to be 0.9979, 0.9859, 0.9937 and 0.9989 respectively. The regression values for formulations F1 to F4 of first order plots were found to be 0.9909, 0.9981, 0.9955 and 0.9923 respectively. These results indicated that formulations F1 and F4 followed zero order kinetics whereas F2 and F3 followed first order kinetics as shown in fig. 3. When the release data was subjected to Higuchi matrix plots, it was observed that formulations F1, F3 and F4 with regression coefficients of 0.9933, 0.9989 and 0.9911 followed Higuchi matrix suggesting diffusion controlled release. The 'n' values obtained from Peppas equation were less than 0.5, which indicated that all the formulations showed drug release by Fickian diffusion mechanism[15].

The *in vitro* release profile of F4 (Due to the constraint of time, only the formulation F-4 which showed viscosity of 45 cps at 20 rpm and 74.63% drug release was compared with marketed formulation) was compared with marketed formulation of gatifloxacin (Zigat Eye Drops). The drug release was found to be 40% and 13% for marketed product and F4, respectively after initial 15 min. At the end of two hours the drug release was 92.33% and 44.55% for marketed product and F4, respectively. The comparative plot is shown in fig. 4. Results indicated that, the drug release was significantly prolonged by using the *in situ* gelling system due to the addition of the polymers: sodium alginate and HPMC E50LV.

There was no appearance of turbidity and hence no evidence of microbial growth when all the formulations were incubated for not less than 14 d at 30° to 35° in case of fluid thioglycolate medium and at 20° to 25° in the case of soyabean-casein digest medium. The preparations being examined, therefore, passed the test for sterility. The *in vitro* efficacy study indicated that gatifloxacin retained its antimicrobial efficacy when formulated as an *in situ* gelling system and the drug was active against the selected strains of microorganisms. The prepared *in situ* gelling systems of gatifloxacin showed satisfactory ocular tolerance. No ocular damage or abnormal clinical signs were visible. Only a few signs of increased lacrimation were noted.

All the formulations did not show any change in

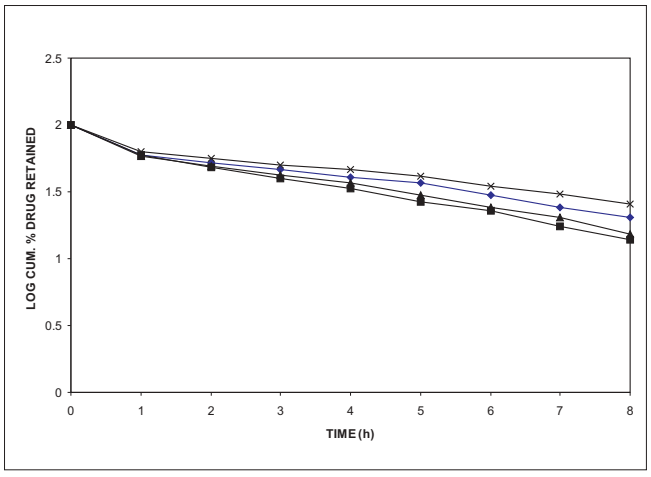

Fig. 3: Plot of log cumulative percent drug retained vs. time for F1-F4 *in situ* gelling gatifloxacin formulations
Formulation F-1 [-◆-], formulation F-2 [- ■ -], formulation F-3 [-▲-] and formulation F-4 [-x-]

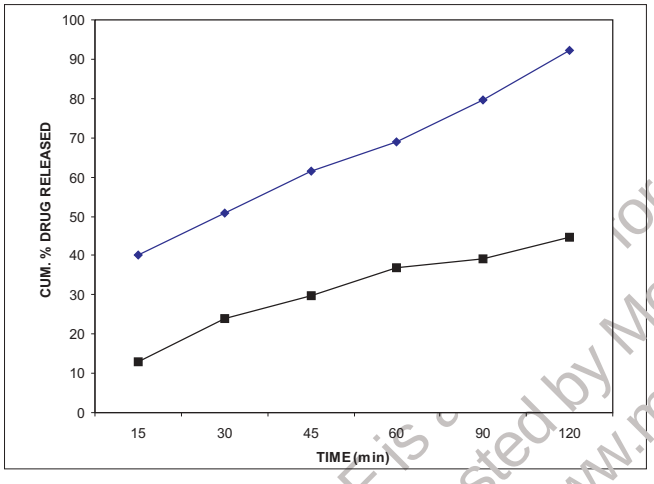

Fig. 4: Comparison of release profile of marketed product with formulation F4
Marketed product (Zigat eye drops) [-◆-] and formulation F4 [- ■ -]

appearance at any of the storage condition in regards to clarity and color of the preparation. It was also observed that there was no change in the gelation properties of the prepared *in situ* gelling systems at any of the storage conditions; however, the time of gelation was delayed for all the formulations stored at 40±1° after one month. Variations were observed in pH values at all the storage conditions. The pH of formulations was found to decrease slightly with time. The maximum change was observed for formulations stored at 40±1°, followed by ambient temperature, followed by 2°-8°. It was, revealed that, least changes in drug content were observed when the formulations were stored at 2° to 8°. It was also found that drug release was higher for formulations stored at 2°-8°. From stability studies it was observed that there was no significant change in any of the parameters evaluated as all the results obtained were within acceptable range. It was therefore observed that all four formulations were stable at selected storage conditions. It was found that 2° to 8° was the most suitable storage condition for the prepared *in situ* gelling system of gatifloxacin.

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