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DEVELOPMENT OF pH BASED PHASE CHANGE SOLUTIONS FOR OPHTHALMIC DRUG DELIVERY OF GATIFLOXACIN

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ABSTRACT

Conventional dosage forms like gels and eye drops show relatively low bioavailability due to poor precorneal contact time. *In situ* gelling systems are of great importance in providing prolonged ocular drug delivery due to their elastic properties. It resists ocular drainage leading to longer contact time. In the present study an *in situ* gelling solution was formulated for gatifloxacin an antibacterial agent, using different ratios of hydroxypropyl cellulose and Sodium alginate with a view to increase gelling strength and bio-adhesion force in order to increase pre-corneal contact time, thereby increasing ocular bioavailability of the drug. The prepared formulations were evaluated for various physical parameters such as, clarity, pH, viscosity, drug content, sterility, and *in vitro* drug release. The formulated gels were transparent, uniform in consistency and had sufficient spreadability with a pH range of 6.8 to 7.1. It was found that with an increase in the concentration of the bioadhesive polymer sodium alginate the drug release was prolonged for longer period of time. Amongst the different polymer combinations studied the G-7 showed satisfactorily higher gel strength, and sustained drug release for more than twelve hours and was stable throughout the stability studies. Thus it can be concluded that the G-7 was more suitable combination for the formulation of ophthalmic *in s*itu gelling system for sustained ocular drug delivery of gatifloxacin, which improves the bioavailability of the drugs on the precorneal area.

Keywords: Ophthalmic *In situ* gel, Gatifloxacin, Bioadhesive polymers, Sodium Alginate.

INTRODUCTION

Topical administration of drugs to the eye is the most common method for the treatment of various ocular diseases. This route shows low bioavailability of drugs because of low residence volume (7-10 µl), loss of administered dose due to rapid elimination of drug by nasolachrymal duct, non-productive absorption through conjunctiva leading to some undesirable side effects. To overcome these problems various ophthalmic dosage forms, such as viscous solutions, ointments, gels or polymeric inserts have been prepared to improve the ocular residence time of medications for topical application to the eye [1,2,3]. The rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect which gives multiple dosing regimen necessary. Ocular administration of drug would be significantly improved if the precorneal residence time of drugs could be increased. Several novel formulations have been developed for ophthalmic use, not only to prolong the contact time to the ocular surface, but also to slow down drug elimination from ocular surface [4]. Many reports have been published about ocular inserts and collagen shields, however, these formulations have poor patient compliance. These problems can be overcome by the use of polymeric solutions, which can be formulated as a liquid dosage form, to be administered into the eye, upon exposure to physiological conditions, such as

pH, temperature and ion mediated sol-gel systems, which leads to changes into the gel phase thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability [5]. Alginate was chosen as a vehicle for ophthalmic formulations since it exhibits several favourable 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 [6]. 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-guluronic acid (G). The polymer forms three-dimensional hydrogel matrices and the high α-L-guluronic acid (G) content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lacrimal fluid [7].

The objective of the present study was to develop an pH activated *in situ* gelling system for gatifloxacin, a fluoroquinolone derivative used to treat external infections of the eye such as acute and subacute bacterial conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers which can prevent frequent drug administration and improve patient compliance. The preparation is Sodium Alginate with the combination of hydroxy propyl cellulose (HPC) as the viscosity enhancer for the Gatifloxacin solution (0.3% w/v),which undergo gelation when instilled into the cul-de-sac of the eye and provide prolonged release of the drug[8,9,10].

MATERIALS AND METHODS

Materials: Gatifloxacin was obtained as a gift sample from Cadila Health Care Ltd., Ahmedabad, India. Sodium alginate with high Glucuronic acid content was supplied by Colorcon India., And HPC was purchased from Anilax Enterprises Inc. Columbia Turnpike, Florham Park USA. Cellulose membrane was purchased from Sigma-Aldrich chemicals pvt. Ltd., New Delhi, All other reagents and solvents were of analytical grade and used as received.

Formulation of *in situ* gelling system: The solutions were prepared aseptically and composition of all the prepared formulation is given in the **Table-1**. The alginate and alginate/HPC solutions were prepared by dispersing the required amount in distilled water with continuous stirring. Gatifloxacin (0.3% w/v) was dissolved separately in dilute acetic acid and the pH was adjusted to 6.5 by 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 up the volume up to 100ml.

Evaluation of *insitu* gelling system of gatifloxacin

Test for appearance/ clarity: All the prepared formulations were checked for general appearance i.e. color, odour, any suspended particulate matter etc. The clarity of the formulation was checked by using wooden board with black and white background. The glass vials were held horizontally and gently rotated immediately under the lamp and then inverted once or twice to detect foreign particles.

Determination of pH: The pH of all formulations was measured using a digital pH meter. The pH meter was calibrated with buffer solutions of pH 4 and pH 7 before use. The pH of all formulations was measured immediately after preparation as well as next day after 24 hours of storage at room temperature.

Gelling capacity: The formulations were evaluated for gelling property in order to identify the formulations compatible for use in eye as in situ gelling systems. The gelling capacity was determined by placing 100µl of prepared system in a vial containing 2 ml of freshly prepared artificial tear fluid and equilibrated at 35°C.

The gel formation was visually evaluated; time for gelation and the time taken for the gel to dissolve were noted. 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) [12]. The lowest scores (+) were given to those formulations in which the phase transition occurred only after 1-3 min. and the formed gels dissolved within 1-3 hrs. The highest scores (+++) were assigned to those prepared formulations for which the phase transition commenced within 1-2min, and the formed gels were stable for about 7-8 hrs. The moderate scores (++) were assigned to those formulations, which could form the gel in 1-2min., but failed to maintain gel structure for more than 3hours.

Viscosity: The viscosity of the instilled formulation is an important factor in determining residence time of drug into the eye. The prepared gatifloxacin solutions were allowed to gel in the simulated tear fluid and then the viscosity of the formulation was determined by using Brookefield DV-II+ Rheometer

with spindle LV-3 with angular velocity run from 10 to 100rpm [11, 12].

Content uniformity: The formulations were tested for drug content uniformity. Vials (n = 3) containing the formulation were properly shaken for 2–3 min. 1 ml of the formulation was transferred into a 100 ml volumetric flask. 50 ml of simulated tear fluid (pH 7.4) was added to the flask. The formed gel was completely suspended with the help of a glass rod, followed by vigorous shaking until it gets completely dispersed to give a clear solution[13]. The volume was adjusted to 100 ml with simulated tear fluid. The solution was filtered through a 0.45-mm filter membrane and the drug concentration was determined with a UV-Visible spectrophotometer at 293 nm.

***In vitro* drug release:** *In vitro* release rate of the gatifloxacin from the *in situ* gelling system was determined by the diffusion process. 1 ml of the formulation was kept in the donor compartment over a cellophane membrane which was rinsed and soaked for the 24 hours in the diffusion medium. The donor compartment was immersed in the receptor compartment containing 50ml of the phosphate buffer of pH 7.4, the beaker containing diffusion medium (receptor compartment) was maintained at 37^0 C with the constant stirring at 22 rpm [14] using the magnetic stirrer. 1 ml aliquots were withdrawn from the diffusion medium every hour for the 12 hours and same quantity of fresh, pre-warmed diffusion medium was replaced for the amount withdrawn. The samples withdrawn were analyzed at 293 nm for the gatifloxacin using Shimazdu Double beam UV-Visible spectrophotometer.

Sterility testing: All ophthalmic solutions should be sterile therefore the test for sterility is very important evaluation parameter. The test for sterility was performed according to Indian Pharmacopoeia. 2 ml of liquid from test container was taken with a sterile pipette. The test solution was aseptically transferred separately to fluid thioglycollate medium (20 ml) and soybean-casein digest medium (20 ml). The liquid was mixed with the media and the inoculated media were incubated for not less than 14 days at 30°C to 35°C in the case of fluid thioglycollate medium and for 20°C to 25°C in the case of soybean-casein digest medium[15].

In vitro antibacterial activity: The *in vitro* antibacterial studies were performed to ascertain the biological activity of the optimized formulation and marketed eye drops against microorganisms. *S. aureus* and *P. aeruginosa* were used as the test microorganisms. A layer of nutrient agar (20 ml) seeded with the test organism (0.2 ml) was allowed to solidify in the Petri plate. Cups were made on the solidified agar layer with the help of sterile borer of 4 mm diameter. Then volume of the final formulations (optimized formulation and marketed eye drops) containing equivalent amounts of drug was poured into the cups. After keeping Petri plates at room temperature for 4 h, the plates were incubated at 37 °C for 24 h. The diameter of zone of inhibition was measured by using an antibiotic zone finder. The selected formulations were stored at ambient humidity conditions between 2-8^{o}C, ambient temperature and at 40^{o}C for a period of one month. The samples were withdrawn at frequent intervals and evaluated for the parameters viz. pH change, appearance, gelation studies, drug content and *in vitro* drug release [16].

RESULTS AND DISCUSSION

The use of polymers like Sodium Alginate, HPC-50 *in situ* gel-forming systems is substantiated by the property of its aqueous solutions to transform into stiff gels when the pH is raised. However, the concentration of Sodium Alginate required forming stiff gels results in acidic solutions which are easily neutralized by the buffering action of the tear fluid. The formulations were light yellow in colour and the clarity was found to be satisfactory. The pH of all the formulations **Table 2** was within the acceptable range and hence would not cause any ocular irritation upon instillation into the eye.

The **Table 1** shows gatifloxacin concentration in formulations, it was determined at 293 nm by using UV-Visible spectrophotometer. The absorbance of solution at 293 nm was used to calculate percentage drug content. Drug content of gatifloxacin in all 7 formulations was between 98.30% - 100.9%. The two main basics of gelling system are viscosity and gelling capacity. Except for the formulations G-1and G-2, all the formulations gelled immediately 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. The viscosities of all the prepared formulations were in the range of 10-50cps which significantly improves the residence time in the eye. The viscosity of the formulations was in the range of 12-50cps due to 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 as shown in **Fig.1**. The administration of ophthalmic formulations should have as little effect as possible

on the pseudo-plastic character of the pre-corneal film. The ocular shear rate is very high, ranging from $0.03S^{-1}$ during inter-blinking periods to 4250-28, 500 S^{-1} during blinking, viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often preferred. Comparative study of *in vitro* release profile of all *in situ* gel formulation is shown in **Fig. 2.**

The formulations, G-7 showed sustained drug release for 12 hours, which may be due to the higher concentration of sodium alginate along with HPC. It show that the alginate (at low conc.) alone does not give prolong release. The comparative *in vitro* drug release profile shown in **Fig. 3** between the marketed conventional ophthalmic drops and the formulation G-7 showed 40% and 16% after initial 30min. At the end of 90 min the drug release was found to be 99% and 22% from the marketed product and G-7 indicating that the drug release was significantly prolonged by using the *in sit*u gelling systems. The higher regression coefficient values for each formulation suggested that, all the formulations G-1 to G-7 behaved matrix type of drug release, kinetic data of all the formulation was further given in **Table 3**.

Sterilization of the selected formulation was carried by using the medium described above. The formulation G-7 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 soybean casein digest medium. The optimized gel forming solution showed antimicrobial activity when tested microbiologically by cup plate technique. Clear zones of inhibition were obtained in case of formulation G-7 and eye drops in the market. The diameter of zone of inhibition produced by formulation G-7 against both test organisms were greater than those produced by marketed eye drops of the market **Table 4.** The antimicrobial effect of the GS *in situ* gelling formulation is probably due to a fairly constant release of drug from the cross-linked hydrogel drug reservoir which permits drug to be released to the target site relatively slowly. The stability studies indicated that the formulation G-7 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 gatifloxacin was stable at selected storage conditions with most suitable storage conditions at the refrigeration temperature. Final conclusion is that the present work was carried out to develop a novel *in situ* gum based ophthalmic drug delivery system of gatifloxacin. The methodology adopted for preparation of *in-situ* gel solution was very simple and cost effective. It is novel approach to improve easy eye instillation, increased residence time and bioavailability and prolong drug release. From the study conducted, the following conclusions were drawn, by varying the concentration of HPC with different ratios of gelling agent results in increased residence time and sustained drug release was found to be best viscosity enhancer in combination with polymers to increase duration of action and drug release. The study revealed that an appropriate ratio of Sodium Alginate to polymers is an important factor in achieving increased duration of action and also drug release from the dosage form to achieve sustained effect. The *in situ* gel formed afforded sustained drug release over 12 hrs. The formulations exhibited therapeutic efficacy. The developed formulation is a viable alternative to conventional eye solutions by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release.

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TABLE 1: COMPOSITION OF *IN SITU* GELLING SYSTEMS OF GATIFLOXACIN

Ingredients	G-1	G-2	G-3	G-4	G-5	G-6	G-7
Gatifloxacin (%w/v)	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium Alginate(%w/v)	0.5	1	0.5	0.75	1	1.25	1.5
HPC-50 cps(%w/v)	---	---	0.75	0.75	0.75	0.75	0.75
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled Water(ml) q.s	100	100	100	100	100	100	100

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *IN SITU* GELLING SYSTEMS OF GATIFLOXACIN

Parameter	G-1	G-2	G-3	G-4	G-5	G-6	G-7
pH	6.5	6.7	6.4	6.5	6.7	6.6	6.8
Drug content	100.3	99	98.3	100.7	99.1	99.5	100.9
Viscosity (cps)	12	20	21	25	32	36	50
Gelling capacity	+	++	+	++	+++	+++	+++

(+) Gelation after a few minutes and dissolves rapidly; (++) Gelation immediate and remains for few hours; (+++) Gelation immediate and remains for extended period.

TABLE 3: REGRESSION CO-EFFICIENT ANALYSIS AND BEST MODEL FIT ANALYSIS FOR ALL THE PREPARED FORMULATIONS

Formulations	First order	Zero order	Peppas	Matrix	Hixon crowell
G-1	0.9884	0.9146	0.9791	0.9891	0.9454
G-2	0.9776	0.912	0.9838	0.9892	0.9876
G-3	0.9705	0.9008	0.9819	0.9981	0.9634
G-4	0.8818	0.9558	0.9569	0.9639	0.9556
G-5	0.9391	0.9391	0.9541	0.9571	0.9554
G-6	0.9847	0.9777	0.9639	0.9893	0.9871
G-7	0.9821	0.9866	0.9867	0.9835	0.9833

TABLE 4: COMPARATIVE ZONE OF INHIBITION PRODUCED BY THE OPTIMIZED FORMULATION G-7

Microorganism	Area of the zone of inhibition(mm^2) after 24 h of incubation	
	Formulation G-7	Marketed Eye Drop
E .Coli	640± 1.8	582± 1.5
S. Aureus	605± 2.9	527± 2.1

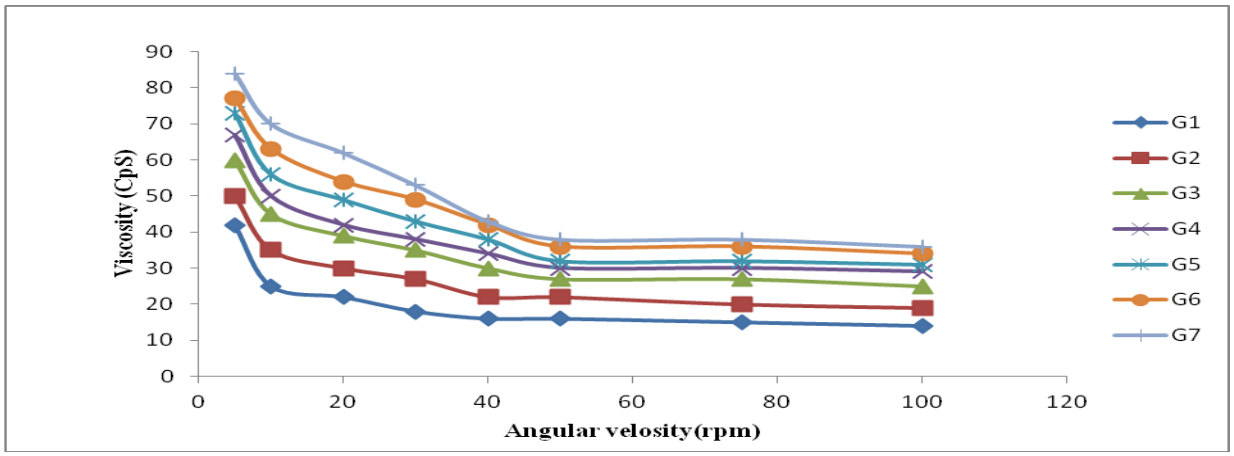

Fig. 1: Rheological profiles of *in situ* gelling systems of gatifloxacin from formulations G1-G7

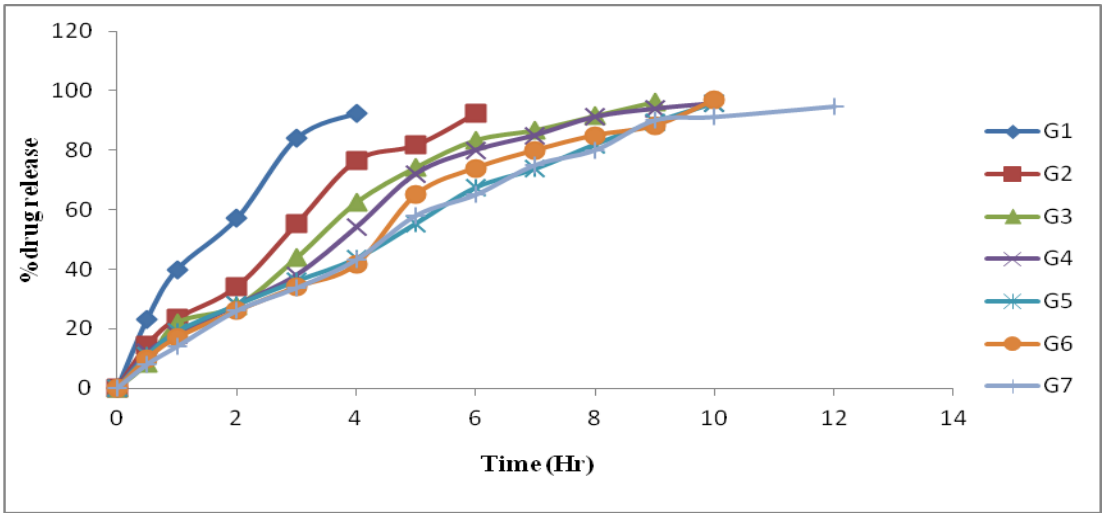

Fig. 2: Plot of *in vitro* drug release of gatifloxacin *in situ* gelling systems for formulations G1, G2, G3, G4, G5, G6 and G7

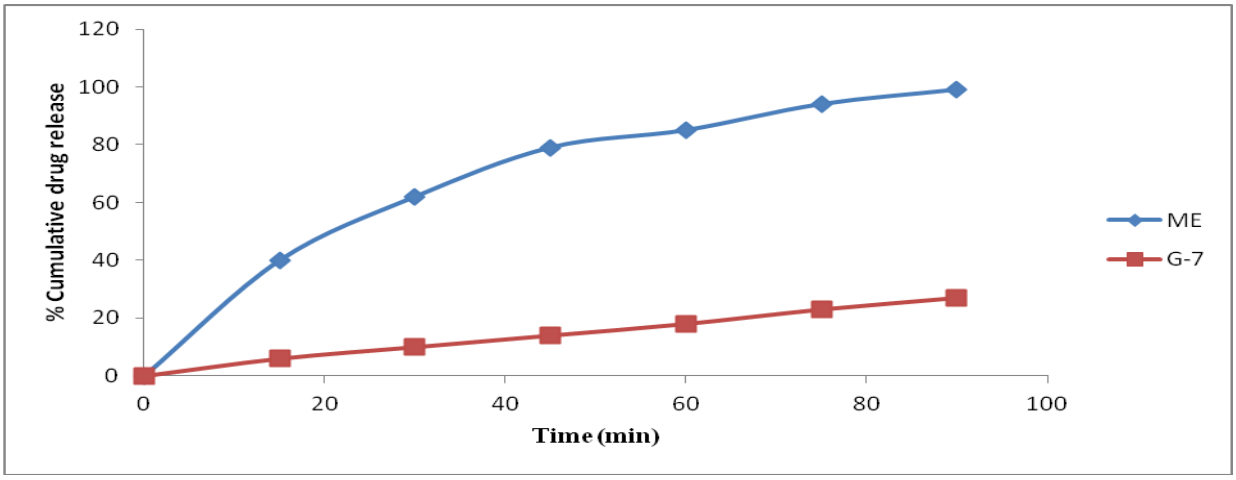

Fig.3: Comparative study of drug release profile of marketed conventional eye drop with formulation G-7. [-♦- ME] Marketed conventional eye drop, formulation code G-7 [-■-]

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