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In situ gelling system of ofloxacin- *In vivo* precorneal drainage study

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ABSTRACT

Topical application is the preferred route of administration for bacterial conjunctivitis and keratitis because the drops provide therapeutically effective concentrations; the drops wash away bacteria and bacterial antigens; adverse systemic effects of the drugs are decreased or eliminated. Our present work describes the formulation and evaluation of an ocular delivery system of ofloxacin based on the concept of an ion activated in situ gelling system. Gellan alone was investigated as vehicle for the formulation of eye drop of ofloxacin which undergoes gelation when instilled into the cul-de-sac of the eye. The developed formulation was characterized for pH, clarity, in vitro drug release profile, transcorneal permeation profile, ocular irritation and in vivo precorneal drainage study. In situ gel forming ability of the developed system significantly controls precorneal drainage as studied by gamma scintigraphy. The formulation was also found to be nonirritant and well tolerable. The developed in situ gelling system form good clear gel over the corneal surface immediately after administration. In situ gel forming ability of the developed system significantly controls precorneal drainage; thus increased residence time in the eye that would help to increase ocular bioavailability.

Keywords: ofloxacin, gellan gum, topical formulation, gamma scintigraphy

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INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. The goal of pharmacotherapeutics is to treat a disease in a consistent and predictable fashion. An assumption is made that a correlation exists between the concentration of a drug at its intended site of action and the resulting pharmacological effect [1].Topical application is the preferred route of administration for bacterial conjunctivitis and keratitis because the drops provide therapeutically effective concentrations; the drops wash away bacteria and bacterial antigens; adverse systemic effects of the drugs are decreased or eliminated. The factors that contribute to achieving effective therapeutic concentrations of the drug in the cornea include the frequency of administration, the concentration of the drug, the lipophilic nature of the drug where the epithelium is intact, the length of contact time of the drug with the cornea, and the lack of an intact corneal epithelium [2]. Whenever an ophthalmic drug is applied topically to the anterior segment of the eye, only a small amount (5%) actually penetrates the cornea and reaches the internal anterior tissue of the eyes. Rapid and efficient drainage by the nasolacrimal apparatus, noncorneal absorption and the relative impermeability of the cornea to both hydrophilic and hydrophobic molecules, all account for such poor ocular bioavailability [3, 4]. A significant challenge to the formulator is to circumvent (bypass) the protective barriers of the eye without causing permanent tissue damage. Thus to increase the ocular bioavailability of drug, we need to increase the ocular residence time of the drug. Several *in situ* gelling systems have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and

consequently enhance ocular bioavailability [5]. *In situ* forming gels are formulations, conveniently dropped in the eye as a solution, where undergo transition into a gel. These systems exhibit sol-to-gel phase transitions due to a change in a specific physicochemical parameter (e.g.: pH, temperature and ions) in the cul-de-sac [6] and thus prolong the precorneal residence time. Gamma scintigraphy is a non-invasive technique that allows monitoring of the corneal residence without disturbing normal physiological functions [7]. It was first described by Rossomond [8] and has since been widely used, i.e. to assess the precorneal drainage of, artificial tear products [9], ophthalmic ointments [10], liposomal formulation of tropicamide [11], w/o microemulsions [12], thermosetting gels [13], alginate and HMPC based an ion activated *in situ* gelling system [14] and an ion and pH activated *in situ* gelling system based on gellan gum and chitosan [15].

The objective of the present work was to develop ion activated *in situ* gelling systems of ofloxacin. To perform *in vivo* precorneal drainage study of optimized system. Gellan gum alone was used in ion activated *in situ* gelling system. Of the currently available fluoroquinolones, ofloxacin has the highest intrinsic solubility [16]; is well tolerated because of its near-neutral pH (6.4), and has the highest rate of penetration into ocular tissues [17, 18].This high rate of tissue penetration may also be significant.

MATERIALS AND METHODS

Ofloxacin (NuLife Pharmaceuticals Pimpri Pune), Gellan gum (Applied Biosciences Mumbai), all other solvents used were of analytical grade unless mention. *In vivo* precorneal drainage study was conducted in SPECT Lab. Pune, under the guidance of Dr. Solav. The ocular irritation was performed according to Draize technique on New Zealand white albino rabbits, each weighing 2–3 kg. All the experiments were approved and conducted as per guidelines of Institutional Animal Committee (Reg.No.1036/A/07/CPCSEA).

Preparation of the ion activated *in situ* gelling system

Different combinations of placebo formulations were developed and evaluated for clarity and gelling capacity to identify the composition suitable for use as *in situ* gelling system (Table 1). Gellan gum was dispersed in deionized water. Further dispersions were heated to 90^{o}C for 20 min while stirring. Ofloxacin solution was prepared in water with the aid of 0.1M NaOH to get clear solution. This solution is added to gellan dispersion to obtain drug concentration of 0.3%w/v. The pH was adjusted to 7.0 ± 0.1 using drops of 0.5M NaOH, and the dispersion was equilibrated at 4^{o}C overnight. Mannitol and methyl paraben were added as isotonicity agent and preservative, respectively. The formulations were sterilized by terminal autoclaving at 121^{o}C for 20 min at 15 psi. All glassware used during the preparation of the *in situ* forming gels was sterilized by autoclaving and the entire procedure was carried out in a laminar flow hood.

Table 1: Physico-chemical properties of ion activated *in situ* gelling placebo systems

Sr. No.	Gellan gum (% w/v)	pH	Clarity	Gelling capacity
1	0.1	7.0±0.2	Transparent	+
2	0.2	7.0±0.2	Transparent	++
3	0.3	7.1±0.2	Transparent	+++
4	0.4	6.90±0.2	Transparent	+++
5	0.5	6.90±0.2	Transparent	+++

Note: (+) Phase transition within 60 sec, collapse of gel structure within 1-2 hr, (++); Phase transition within 60 sec, collapse of gel structure within 3-4 hr, (+++) Phase transition within 60 sec and gel structure stable for more than 6 hr.

Table 2 : Developed medicated *in situ* gelling systems

Formulation code	Ofloxacin (% w/v)	Gellan gum (% w/v)
G1	0.3	0.3
G2	0.3	0.4
G3	0.3	0.5

Drug polymer interaction studies

The IR and ultraviolet spectrum of pure drug solution and optimized formulation was taken before and after autoclaving. Thin layer chromatogram of pure drug and optimized formulation was obtained using a solvent system consists of n-butanol, methanol and ammonia in the ratio of 5:1:1.5. Interaction studies investigated any interaction between the drug and excipients and studied the effect of method of sterilization.

In vitro drug release studies

In vitro release of ofloxacin in medicated formulations (Table 2) was studied using a modified USP dissolution testing apparatus. The dissolution medium used was freshly prepared simulated tear fluid pH 7.4 maintained at

temperature of 37±1°C. Cellulose membrane (Spectra/Por dialysis membrane, 12,000–14,000 MW cut off), previously soaked overnight in the dissolution medium, was tied to one end of specifically designed glass cylinder (open at both ends and of 2.0 cm diameter), allowed to rotate at 50 rpm. The drug content in the withdrawn samples was determined at 293 nm using UV-visible double beam spectrophotometer. The results were the means of three runs (Figure 1).

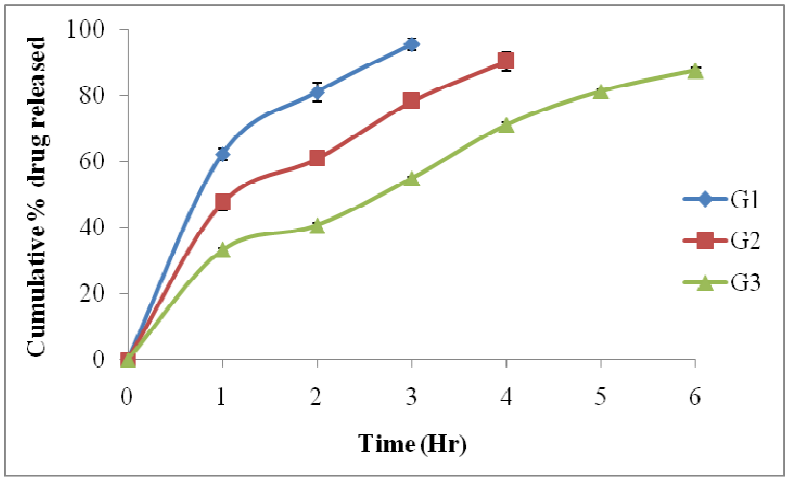

Figure 1: *In Vitro* drug release profile of *in situ* gelling system (mean±SD; n=3)

In vitro transcorneal permeation study

In vitro transcorneal permeation study of optimized formulation was performed using modified Franz diffusion chamber [19]. Simulated tear fluid was used as a diffusion medium. Fresh goat corneal membrane was separated, soaked in simulated tear fluid, and mounted on by sandwiching between the clamped donor and receptor compartment. Prior to application of formulations, the membrane was allowed to equilibrate for 30 minutes. 1 ml of sample was withdrawn and replaced with fresh simulated tear fluid in order to maintain sink conditions. The samples were appropriately diluted and the absorbance was measured at 293 nm using a Shimadzu UV-VIS spectrophotometer. The results were the means of three runs (Figure 2).

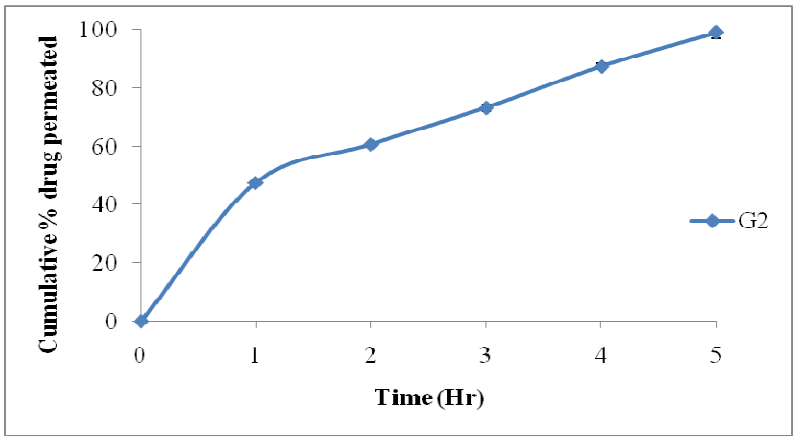

Figure 2: Transcorneal permeation profile from optimized *in situ* gelling system
(mean±SD; n=3)

Ocular irritation studies

The ocular irritation was performed according to Draize technique on New Zealand white albino rabbits, each weighing 2–3 kg. 50 ul of optimized formulation was instilled into the lower cul-de-sac of the left eye of the rabbit. The right eye, which remained untreated, served as a control. To prevent loss of test material, the lower eye lid was gently held together for app. 5 sec. The sterile formulations were instilled twice a day and the rabbits were observed after 1hr, 24 hr, 48hr, and 72 hr for redness, excessive tearing, and inflammation of the eye (Table 3).

Table 3: Ocular irritation test of optimized *in situ* gelling systems

Parameter	Duration			
	G2			
	1hr	24hr	48hr	72hr
Redness	0	0	0	0
Excessive Tearing	0	0	0	0
Inflammation	0	0	0	0

(0 - No redness, no inflammation or excessive tearing, 1 - Mild redness with inflammation & slight tearing, 2 - Moderate redness with moderate inflammation and excessive tearing, 3 - Severe redness with severe inflammation and excessive tearing).

Gamma scintigraphy

In vivo precorneal drainage of radionuclide was studied using single photon emission computing tomography autotuned to detect the 140 KeV 99m radiation of Tc. Optimized formulation G2 was assessed on a group of four rabbits with a minimum washout period of 3 days. Solution of ofloxacin was placed in an amber colored bottle and radiolabeled with Tc-99m by direct labeling method using stannous chloride as reducing agent. This radiolabeled drug solution was then mixed with other ingredients in such a way that the final solution would contain 0.3% w/v ofloxacin and required concentration of polymers. Rabbits were anaesthetized using ketamine HCl injection given intramuscularly in a dose of 15 mg/kg body weight. The rabbits were positioned 5 cm in front of the probe and 25 µL of the radio labeled formulation (equivalent to ~100 µ ci) was instilled onto the left corneal surface of the rabbits. Recording was started immediately after instillation and continued for 10 min using 128 × 128 pixel matrix. Individual 60 frames were captured by dynamic imaging process. Region of interest (ROI) was selected on the one frame of the image and time activity curve was plotted to calculate the rate of drainage from eye (Figure 3). A single whole body static image also was taken after 2 hr of instillation of formulation (Figure 4).

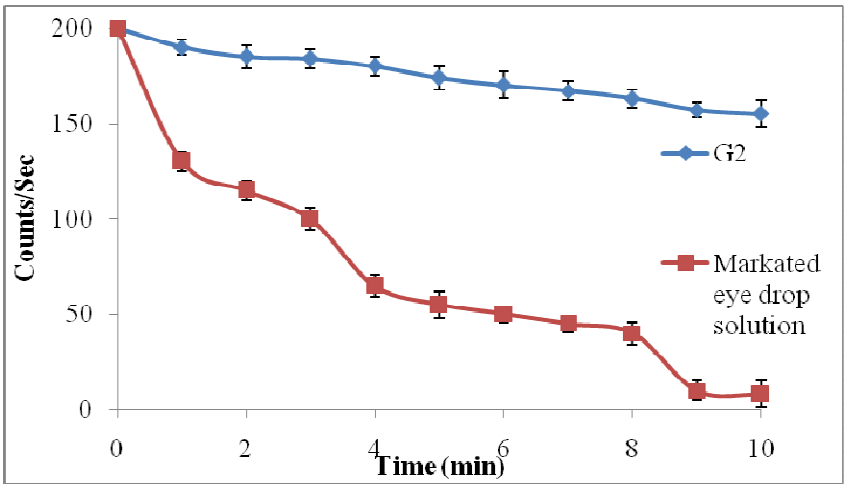

Figure 3. Time activity curve shows precorneal drainage of optimized formulation G2 and marketed eye drop solution

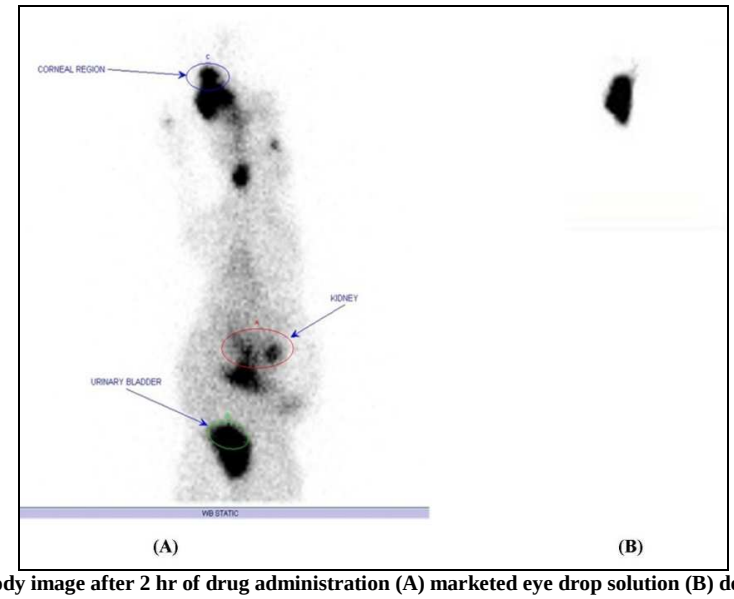

Figure 4. Static whole body image after 2 hr of drug administration (A) marketed eye drop solution (B) developed *in situ* gel system

RESULTS

The main prerequisites of ocular gelling system are clarity and gelling capacity (speed and extent of gelation). All the formulations containing different concentrations of gellan gum (0.1%-0.5 % w/v) showed the gelling ability in the presence of simulated tear fluid (Table 1). The nature of the gel formed depended on the polymer concentration. Based on this study, *in vitro* drug release of ofloxacin in medicated formulation (Table 2) was studied. Release of drug from gel matrix is inversely proportional to concentration of gelling agent (Figure 1). Transcorneal permeation profile was comparable to that of *in vitro* drug release profile. Good transcorneal permeation was observed because of two factors, as drug is formulated in aqueous base, drug diffuses from the formed gel matrix upon addition to the dissolution medium and highest rate of penetration of ofloxacin into ocular tissue (Figure 2).

Gel forming ability of gellan gum produces a dense matrix in simulated tear fluid and release of drug was possibly influenced by diffusion and/or erosion of the matrix. The combination of these processes seemed to result in the overall diffusion controlled release kinetics, as indicated by the *n* values. The results of the ocular irritation studies (Table 3) indicate that optimized formulation was non-irritant. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible. For scintigraphic studies, during prelabeling efficiency, labeling parameters like $SnCl_2$ concentration and pH were optimized. 50 µg $SnCl_2$ at pH 3.0 was found to give the maximum labeling efficiency (95.4%). *In vitro* stability of the Tc-99m labeled complex was also tested and the complex was found to be stable for up to 3 hr. The observation of the acquired gamma camera images showed that, developed *in situ* gelling systems form good clear gel over the corneal surface immediately after administration as compared with marketed eye drop solution.

DISCUSSION

Gellan, was investigated as vehicle for the formulation of eye drops of ofloxacin (0.3% w/v), which undergoes gelation when instilled into the cul-de-sac of the eye and improves contact time of drug. It is attributed to formation of a three dimensional network of gellan gum by its complex formation with Ca^{++} ions and hydrogen bonding with water. Interaction studies revealed that the ingredients were compatible to each other and no physicochemical reactions took place. It also demonstrated that the formulation ingredients were stable to heat and final formulation could be terminally sterilized by autoclaving. Developed system allows its easy instillation into the eye as a liquid (drops), forms transparent gels and spread over the corneal surface. The release of ofloxacin was inversely proportional to gellan gum concentration (Figure 1), as might be due to more and denser gel structure. A similar release pattern was reported for carteolol, wherein the initial fast release (burst effect) decreased with an increase in polymer concentration [20]. Transcorneal permeation profile was comparable to that of *in vitro* release profile. Good transcorneal permeation attributed to gel matrix formation and intrinsic lipophilicity of ofloxacin. For scintigraphic studies, the observation of the acquired gamma camera images showed that, developed *in situ* gelling systems form good clear gel over the corneal surface immediately after administration. Marketed eye drop solution cleared very rapidly from the corneal region whereas, *in situ* gelling systems were cleared at slow rate and retained at corneal surface for longer duration (Figure 3). *In situ* gel forming ability of the developed system significantly controls precorneal drainage (Figure 4). Thus, increased residence time in eye would help to increase ocular bioavailability. The period of drug absorption is short because the activity gradient decreases rapidly owing to precorneal solution drainage and conjunctival systemic absorption. A minimum of 5-10 min of ocular contact time was determined to be necessary for significantly reducing systemic drug absorption [21].

CONCLUSION

The developed gellan gum based ophthalmic *in situ* gel formulation containing ofloxacin (0.3% w/v) was successfully prepared. The formulation was found to be nonirritant, showed enhanced transcorneal drug permeation and prolonged retention at corneal site. It was also found suitable for delivery to eyes and can prove as better alternative to conventional eye drops for the better drug therapy.

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