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Design and Evaluation of *In Situ* Gelling System for Ocular Drug Delivery of Baloflaxacin

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ABSTRACT

The objective of the present study was to develop ophthalmic delivery systems based on polymeric carriers that undergo sol-to-gel transition upon change in temperature or in the presence of cations so as to prolong the effect of HP-*β*-CD baloflaxacin *in situ* gelling formulations. The *in situ* gelling formulations of baloflaxacin were prepared by using pluronic F-127 (PF-127) or with combination of pluronic F-68 (PF-68) and Metolose SR by cold method technique. The prepared formulations were evaluated for their physical appearance, drug content, gelation temperature (*T*gel), *in vitro* permeation studies, and stability studies. All batches of *in situ* formulations had satisfactory pH ranging from 6.8 to 7.4, drug content within range of 90.5 % to 98.19 %, show uniform distribution of drug. As the concentration of each polymeric component was increased, that is, PF-68 and Metolose SR, there was a decrease in *T*gel with increase in viscosity. The *in vitro* drug release decreased with increase in polymeric concentrations. The designed formulation has prolonged effect and retained its properties against various bacterial infections of eye such as conjunctivitis.

INTRODUCTION

In the ophthalmic drug delivery systems, protective barriers of eye lead to low absorption of drug and it leads to poor bioavailability of therapeutic drugs. The cul-de-sac normally holds 7–9 L of tears but can retain up to approximately 20–30 L without overflowing. The normal tear flow rate and film thickness are 1 L/min and 4–9 m. The normal pH of the tears is 6.5–7.6. The drainage of instilled solutions (25–50 L) away from the front of the eye is essentially completed at around 90 sec. Under normal conditions, the eye can accommodate only a very small volume without overflowing. Commercial eye drops have a volume of 30 L, which is about the volume of the conjuctival sac in humans; however, after a single blink, only an estimated 10 L remains [1]. The poor bioavailability and less therapeutic response of conventional eye drops occurs mainly due to the gravity induced lacrimal flow and normal tear turnover of the eye. Frequent dosing is usually associated with patient noncompliance and tear drainage of the administered dose passes via the nasolacrimal duct into the gastrointestinal tract, leading to side effects. Due to this drug loss in front of the eye, very small drug is available to enter the cornea and inner tissue of eye. It leads to very small corneal contact time (about 1-2 mins) in humans for instilled solution usually less than 10 %. Therefore, only small amount of drug actually penetrates the cornea and reaches intraocular surface [2, 3].

An ideal ophthalmic drug delivery must be able to release the drug in sustained manner and remain in the front of eye for prolong period of the time. As a result, various attempts have been made to prolong the contact time of drug on the ocular surface and also to slow down the drug elimination [4], that is, development of viscous gel to prolong the precorneal drug retention [5, 6], microparticle suspension [7], or polymeric solution [8], inserts [9], and collagen shields [10]. However, these dosage forms also comprise some disadvantages such as discomfort especially in elderly patients, loss of device during sleep, or rubbing eye and poor compliance, as well as blurred vision.

The ophthalmic drug delivery based on in situ gel can overcome these problems. As in situ activated gel forming systems can be administered in drop form and create considerably fewer problems with vision and also provide better sustained properties than drops these *in situ* gelling systems consist of polymers that exhibit sol-to-gel phase transitions due to change in specific

physicochemical parameters (pH, temperature, and ionic strength) in the environment, cul-de-sac in the case of eye [11]. There are different approaches used for triggering the *in situ* gel formation: physiological stimuli (e.g., temperature induced and pH induced), physical changes in biomaterials (e.g., diffusion of solvent and swelling), and chemical reactions (e.g., enzymatic, chemical, and photoinitiated polymerization).

Polymers such as pluronics (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPOPEO) Triblock), polymer networks of poly(acrylic acid) (PAA), and polyacrylamide (PAAm) or poly(acrylamide-co-butyl methacrylate) are temperature-induced polymers which are liquid at room temperature (20°C–25°C) and undergo gelation when arrive in contact with body fluids (35°C–37°C), due to an increase in temperature [12, 13]. Certain polymers such as PAA (Carbopol, carbomer) or its derivatives, mixtures of poly(methacrylic acid) (PMA) and poly(ethylene glycol) (PEG), show change from sol to gel with change of pH [14, 15]. In presence of various ion such as k+, Ca+2, Mg+2, and Na+, certain ion sensitive polysaccharides such as carrageenan, gellan gum (Gelrite), pectin, and Metolose SR undergo phase transition [16, 17]. Poloxamer 407 (PF-127) is a nonionic surfactant composed of poly(ethylene oxide)-b(poly(propylene oxide)-b-poly(ethylene oxide) (PEO-PPO-PEO) showing amphiphilic behavior due to hydrophobic propylene oxide domains and hydrophilic ethylene oxide domains. Pluronic F127 exhibits sol to gel transition at 37°C when used at a higher concentration of (25%–30%) (w/v). By using different series of poloxamers, cross-linking agents, by changing pH and ionic strength gelation, temperature can be adjusted within the range of 33–36°C [18–20].

Balofloxacin (BLFX), chemically named 1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methylamino-piperidin-1-yl)-4-oxoquinoline-3-carboxylic acid, is the fourth generation of a new class of synthetic antibacterial fluoroquinolone agents. It has broad antibacterial spectrum, ranging from gram-positive bacteria to gram-negative bacteria. BLFX exhibited excellent antibacterial activity against gram-positive bacteria such as multiple-drug-resistant staphylococci and pneuropiummococci, is prescribed for the treatment of various bacterial infections including uncomplicated urinary tract infections, infective ophthalmitis, community-acquired pneumonia, acute exacerbation, sinusitis, skin infections and chronic bronchitis.

Balofloxacin is a lipophilic drug. Due to low solubility of Balofloxacin, it is encapsulated in *β*-cyclodextrin derivative in order to increase the solubility and stability of Balofloxacin in aqueous solutions, while maintaining its lipophilicity and high corneal permeability. Hydroxypropyl-*β*-cyclodextrin (HP-*β*-CD), a cyclic oligosaccharide with outer hydrophilic surface and a lipophilic cavity, is capable of forming inclusion complexes with many lipophilic drugs.

Treatment of bacterial conjunctivitis requires frequent administration of antibiotics as eye drops. This is associated with transient peaks of high drug concentration in the eye, which in turn results in undesirable side effects. These short comings may be overcome by the development of "*In situ* gelling system", which have the potential to enhance bioavailability and to reduce the side effects of potent new drugs. In this study, *in situ* gelling system of balofloxacin were prepared using polymers pluronic F127 (12 w/v), Metolose SR (0.5 % to 1.5 % w/v) and pluronic F-68(10 %-14 %).

MATERIALS AND METHODS

Balofloxacin was obtained from Spectrum Pharma Labs (Mumbai, India). Pluronic F127, HPMC E50LV and Metolose SR. All other chemicals and solvents were of analytical grade.

FORMULATION

In situ gelling liquids were prepared using different concentrations of pluronic F-68 and Metolose SR with fixed concentration of pluronic F-127. Baloflaxacin (0.1 w/v) was weighed separately and dissolved in the distilled water with (1.0 % w/v) HP-β-CD. Metolose SR solutions of different concentrations (0.5 %, 1 %, and 1.5 %) were prepared by dispersing the required amount in distilled water with continuous stirring until completely dissolved. The Baloflaxacin solution was added to the alginate solution under constant stirring until uniform, clear solution was obtained. Further, to this mixture pluronic F-127 (12 % w/v) and different concentrations of pluronic F-68 (10 %, 12 %, and 14 %) were added. Benzalkonium chloride (0.01 % w/v) was added as a preservative to the previous solutions. Sufficient amount of sodium chloride was added to the mixture to maintain the isotonicity. Finally, the volume was adjusted with distilled water up to 100 mL. Partially the dissolved pluronic solutions were stored overnight in a refrigerator at 4°C for hydration and stirred periodically until clear homogenous solutions were

obtained. Nine batches of formulation were prepared by using different concentrations of Metolose SR and PF-68.

Table 1. Formulations of Baloflaxacin of in situ gelling system for ocular drug delivery

Ingredients	f1	f2	f3	f4	f5	f6	f7	f8	f9
Baloflaxacin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
pluronic F127	12	12	12	12	12	12	12	12	12
Metolose SR	-	-	-	0.5	1	1.5	-	-	-
pluronic F68	-	-	-	-	-	-	10	12	14
benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
water	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml	100ml

EVALUATION OF FORMULATION

Visual Appearance and Clarity

Visual appearance and Clarity was done under fluorescent light against white and black back ground for presence of any particulate matter.

pH

The pH of the prepared *in situ* gelling system after addition of all the ingredients was measured using pH meter.

Drug Content Analysis

Drug content analysis of prepared *in situ* gelling systems was carried out using Spectrophotometric method. The assay of these formulations was carried out by pipetting 0.1 ml of all four optimized formulations, and it was diluted up to 100 ml of Simulated Tear Fluid (pH 7.4). The absorbance was measured at 293 nm using UV-Visible spectrophotometer.

In Vitro Gelation

The Gelling capacity of the formulations containing different ratio of Pluronic F127 and HPMC (E50LV) Metolose SR was evaluated. It was performed by placing a drop of polymeric solution in vials containing 1 ml of Simulated Tear Fluid, freshly prepared and equilibrated at 34^{O}C, and visually assessed the gel formed and time for gelation as well as time taken for the gel formed to dissolve.

Measurement of Gelation Temperature

At room temperature, ten milliliters of cold sample solution (pluronic containing formula) were put into a beaker (25mL) and placed in a low temperature water bath. A thermometer was immersed into the sample solution for constant monitoring. The solution was heated with stirring at 200rpm using a magnetic bar (9 × 25mm). The temperature at which the magnetic bar stopped moving due to gelation was reported as the gelation temperature (*T*gel). Each sample was measured in triplicate.

Rheological Studies

It is the important factor to determine the residence time of drug in the eye by considering the viscosity of the instilled formulation. The prepared solutions were allowed to gel at physiological temperature and then the viscosity determination was carried out by using Brookfield viscometer (Brookfield DV+Pro, Brookfield Engineering Laboratories, Middleboro, MA, USA). By plotting graph of shear rate versus shear stress, the flow pattern was checked.

Interaction studies

Prepared *in situ* gel formulations were tested for the intactness of drug in the various formulations by comparing with pure drug. These were done to ensure that, the therapeutically active drug has not undergone any change after it has been subjected to processing steps during preparation of *in situ* gelling systems. These studies were performed by taking IR spectra using KBr method.

***In Vitro* Release Studies**

In vitro drug permeation studies were carried out by putting them *in situ* gelling formulation on Millipore membrane filter (0.15mm) between the donor and receptor compartments of an all glass modified Franz diffusion cell. To simulate the corneal epithelial barrier, the Millipore membrane filter was used, as isolated cornea will not remain viable beyond 4hrs. The receptor compartment of an all glass modified Franz diffusion cell was filled with 10 mL freshly prepared simulated tear fluid (pH 7.0), and all air bubbles were expelled from the compartment. An aliquot (1mL) of test solution was placed on the Millipore membrane filter, and the opening of the donor cell was sealed with a glass cover slip. The receptor fluid was kept at 37 ± 0.5°C with constant stirring using a Teflon coated magnetic stir bead. Permeation study was continued for

10hrs, and samples were withdrawn from receptor and analyzed for baloflaxacin content by measuring absorbance at 293 nm in a spectrophotometer. Drug permeation experiments were also carried out using freshly excised goat cornea. Whole goat eyeballs were transported from the local butcher shop to the laboratory in cold (4°C) normal saline within 1hr of slaughtering of the animal. The cornea was carefully excised along with 2 to 4 mm of surrounding scleral tissue and was washed with cold normal saline till the washing was free from proteins. Freshly excised cornea was fixed between clamped donor and receptor compartments in such a way that its epithelial surface faced the donor compartment. For the analysis of baloflaxacin withdrawn from receptor compartment, the same procedure was adopted as mentioned earlier. Results were expressed as cumulative percentage of drug released versus time.

RESULTS AND DISCUSSION

Pluronic F127 became one of the most extensively investigated temperature responsive materials due to its unique thermoreversible gelation properties, but the phase transition temperature strongly depended on pluronic F127 concentration [21]. Pluronic F68 is incorporated into pluronic F127 in order to modulate the phase transition temperature for ophthalmic drug delivery system [22]. Pluronic 127 (12 %, w/v) was selected as the basis of formulation because below this concentration it loses its sol-gel transition properties and it is used in combination with pluronics F68 and metalose SR in different concentrations. Metalose SR was combined in formulations.

Drug Content: All the prepared *in situ* gelling systems were evaluated for preliminary tests such as Visual appearance, Clarity, pH, and Drug content. These formulations were transparent and clear. The pH of the formulations were found to be 6.5±0.5, and drug content was in between 90.5 % to 98.19 % shown in Table 2.

***In Vitro* Release Studies**: Results (shown in Table 3) reveal that all formulations exhibited sustained release of the drug (above 50 %) from the Metolose SR and Pluronic F-127/F-68 network over 10 hours. Cellulose derivatives like Metolose SR dissolve in water and yield much more viscous solution. Thus, the increase in viscosity might have contributed to the decrease in rate of drug release from these formulations.

Ocular Irritancy Studies: All the formulations were found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris, and conjunctiva observed.

Interaction Studies: The results of these studies reveal that there were no definite changes obtained in the bands of drug with respect to pure drug.

Table: 2 Evaluation of pH, Drug Content Analysis, *In Vitro* Gelation

formulations	Metolose SR (%)	PF 68(%)	gelation temperature	PH	Drug content
f1	0.5	10	36.92±0.15	6.3	95.14±1.22
f2	1	12	36.52±0.45	6.8	95.27±1.56
f3	1.5	14	35.95±0.22	6.9	92.39±2.01
f4	0.5	10	32.9±0.50	7.1	94.78±1.86
f5	1	12	31.8±0.33	6.9	93.65±0.532
f6	1.5	14	30.9±0.48	7.2	98.19±0.478
f7	0.5	10	28.36±0.12	6.8	96.45±1.89
f8	1	12	27.68±0.28	6.6	92.36±2.46
f9	1.5	14	25.43±0.72	7.2	90.5±0.66

Table: 3 *In Vitro* Release Studies

TIME	f1	f2	f3	f4	f5	f6	f7	f8	f9
0	0	0	0	0	0	0	0	0	0
1	17.52	15.16	11.02	10.35	9.12	9.38	9.89	10.22	8.69
2	21.45	18.83	16.14	14.12	10.89	12.06	11.65	14.98	11.02
3	29.06	26.36	22.12	21.83	12.36	17.21	15.65	17.65	14.8
4	32.52	38.04	35.57	33.05	19.36	20.09	17.35	20.63	17.25
5	38.36	42.42	38.44	39.56	26.38	27.54	22.29	24.66	21.12
6	40.22	45.36	43.12	41.63	32.49	31.22	36.53	28.36	26.36
7	47	48	46.23	45.89	38.57	37.52	43.22	32.88	33.75
8	56.11	50.95	52.13	49.25	42.86	41.42	49.57	38	39.69
9	64.53	58.21	59.12	57.91	45.4	47.25	52.13	40.26	43.12
10	80.69	76.54	71.99	63.01	52.13	50.26	59.62	47.96	46.23

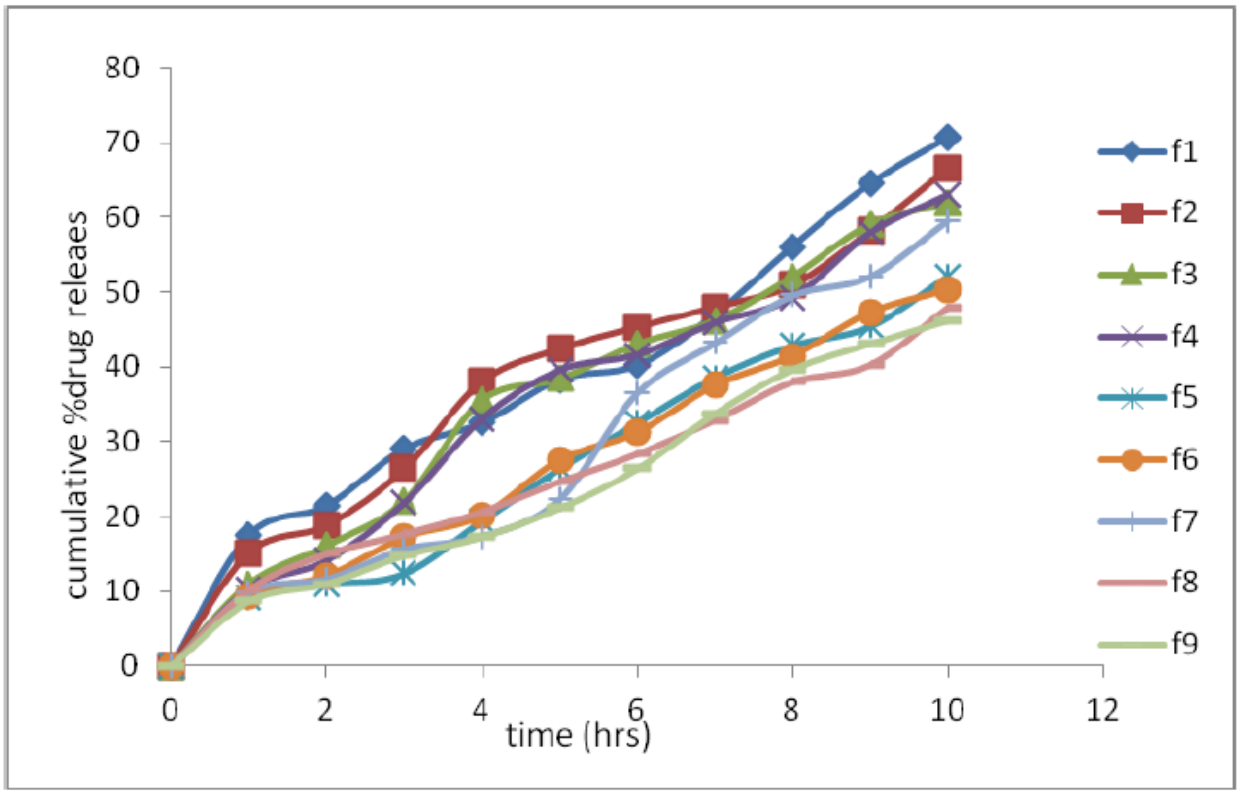

Fig.1. *In vitro* drug release studies

CONCLUSION

Baloflaxacin prescribed for infective opthalmitis, sinusitis, chronic bronchitis, pneumonia, skin infections. It was successfully formulated as a *in situ* gel using Pluronic as a polymer. Pluronic as a gelling agent used in combination with methocel SR/ Metolose SR as a viscosity enhancing agent. The formulation was liquid and underwent rapid gelation upon coming in contact with tear fluid. The gel formed *in situ* afforded sustained drug release over an 10hrs period. The formulations were therapeutically efficacious. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability of sustained drug release. Also important factor is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

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