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### A Review on Design and Development of Thermosensitive Ocular In Situ Gel Ophthalmic Formulation

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The design of the ophthalmic delivery system that enhances the bioavailability and to increase the residence time with improved mechanical properties and mucoadhesive properties has to be prepared in order to overcome the problems associated with the conventional ophthalmic solutions i.e., these conventional formulations eliminate rapidly after administration and cannot provide and maintain an adequate concentration of the drug in the pre corneal area and in order to avoid the rapid dilution, formulations with an increased viscosity have been evaluated. Among them, the thermo sensitive in situ gel-forming formulations, which undergo phase transition from liquid to semisolid gel upon exposure to physiological environments, seem to be a promising tool. Thermo sensitive in situ gelling and mucoadhesive ophthalmic drug delivery system is designed by employing poloxamer407 as thermo gelling agent under physiological conditions in the ocular environment it undergoes sol to gel phase transition. The mucoadhesive material includes carbopol, chitosan, hydroxypropylcellulose (HPC), polycarbophil, polyvinylpyrrolidone (PVP) in various ratios in order to obtain optimum mucoadhesive which plays a vital role in enhancing bioavailability.

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**Keyword:** In situ gel, Ocular route, Ophthalmic Drug Delivery, Thermo sensitive.

#### 1. Introduction

The principle treatment of ocular diseases, especially the drug must exhibit a localized action, when topical administered as eye drop solution. But in several cases, topical action of ophthalmic solutions are not effective due to protective mechanisms of the human eye. The protective mechanism of eye that is lacrimal secretion and the blinking reflex cause rapid drainage of the formulation. The short pre-corneal contact time combined with corneal impermeability results in low bioavailability, and as a result, frequent dosing is usually needed. In order to avoid the rapid dilution, the ophthalmic product with an increased viscosity have been to

designed. The in situ gel-forming formulations undergo phase transition from sol to semisolid gel upon exposure to physiological environments, which appears to be a promising tool. These thermo sensitive in situ gels should be a free-flowing polymeric solution of thermo gelling and mucoadhesive materials at room temperature to allow easily reproducible administration into the eye as a drop<sup>[1]</sup>.

These thermo sensitive in situ gels must undergo phase transition in such a way that the gel that is formed at the physiological environment must be capable in withstanding the mechanical and shear forces in the cul-de-sac. The poloxamer

407 or different grades of poloxamer under thermo gelification .i.e., the poloxamer is block copolymer which under goes aggregation and converts in to the micelles. These micelles are spherical in shape spherical with a dehydrated polyoxypropylene (POP) core with an outer shell of hydrated swollen polyoxyethylene (PEO) chains. An increase in the temperature leads to dehydration and conformational changes at the hydrophobic chains regions, increasing chain friction and entanglement of the polymeric network. . At this point, gelation has occurred, and the micelles remain apparently intact and orderly packed, which has been described as “hard-sphere crystallization”<sup>[1,2]</sup>.

### 1.1 An Ideal Ophthalmic Formulation Should Be One That

1. Can be delivered in a drop form without causing blurred vision or irritation;
2. Has a suitable strength to endure the lacrimal fluid dilution without rapid pre corneal elimination after administration;
3. Has a suitable mucoadhesive force to improve the retention of the drug in the pre corneal area, thereby facilitating the reservoir effect of the cornea for the drug and increasing the bioavailability.

## 2. Materials and Methods<sup>[3,4,5]</sup>

### 2.1 Poloxamer

Poloxamer (trade name Pluronic), a block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature .At a concentration of 18% (w/w) or higher in aqueous solution, poloxamer 407 (P407), in which the ratio of PEO and PPO is 7:3, is transformed from a low viscosity solution to a gel under the ambient temperature. But this lower concentration solution will lose the gelation ability after diluted by lacrimal fluid. So 25% (w/w) P407 should be used to ensure the completion of the phase transition under physiological condition. However, in this case, the gelation temperature (GT) is lower than room

temperature and the solution must be stored in refrigerator, which causes great inconvenience for the preparation and the use. Therefore, poloxamer 188 (P188), which is an analog of P407, was added to P407 solution as a regulatory substance. It exhibits a good perspective to increase the GT of P407.

Concentrations from 14% to 20% and 0.5% to 1.5% w/w of poloxamer and chitosan, respectively, were evaluated by oscillatory rheology, with the purpose of obtaining an optimal gelation temperature. The rheological and mechanical properties, as well as the mucoadhesive ability of the poloxamer gels as a function of chitosan concentration, were evaluated.

### 2.2 Mucoadhesive Materials<sup>[5,6]</sup>

**2.2.1 Carbopol resins:** Acrylic-acid based polymer which are available in a range of molecular weights and may be linear, branched or cross-linked have been investigated very frequently for the development of ocular drug delivery systems owing to their excellent mucoadhesive property. Carbopol is the gelling agent but as its concentration increases in the vehicle, its acidic nature may cause stimulation to the eye tissue.

Eg: Carbopol  
Chitosan  
Hydroxy propylmethyl cellulose (HPMC)  
hydroxypropylcellulose (HPC),  
polycarbophil  
polyvinylpyrrolidone (PVP)

**2.2.2 Chitosan:** for the ocular delivery of timolol maleate were already studied . It was shown that these polymers can be used in combination to produce clear, sterile and non-irritating ophthalmic formulations.

### 2.3 Preparation of Gels<sup>[7,8]</sup>

All poloxamer solutions used in this study (14–20% w/w) were prepared by weighing the polymer in cold ultrapure water. The solutions were kept in a refrigerator for at least 24 h to ensure complete dissolution. In cases where chitosan (0.5–1.5% w/w) was used, it was

initially dissolved in a solution of acetic acid 0.5% v/w. The chitosan solution was then refrigerated and used as a solvent for the poloxamer dispersion. Benzalkonium chloride solution was added 0.006% as preservative in all solutions. All the sample solutions were adjusted to pH  $4.0 \pm 0.1$  or  $7.4 \pm 0.1$  by 0.5 M sodium hydroxide solution, sterilized at 121°C and 15 psi for 20 min and then stored in the refrigerator prior to the evaluation of their rheological properties.

## 2.4 Evaluation Of In Situ Gels [8,9,10] Gelling Capacity

### Method 1: (Formulation And Evaluation of Ph-Triggered in Situ Gelling System of Prulifloxacin).

The prepared in situ gelling system was evaluated for gelling capacity in order to identify the composition suitable for use as in situ gelling system. The in situ gelling system was mixed with simulated tear fluid (in the proportion of 25:7 i.e. application volume 25(ii and normal volume of tear fluid in the eye is 7ul) to find out the gelling capacity of the ophthalmic product. The gelation was then assessed visually by noting the time for the gelation and the time taken for dissolution of the formed gel.

### Method 2: (Design and evaluation of baicalin-containing in situ pH-triggered gelling system for sustained ophthalmic drug delivery).

Gelling systems of various concentrations of Carbopol and HPMC were prepared and evaluated for gelling capacity in order to identify the compositions suitable for use as an in situ gelling system. The gelling capacity was determined by placing 100µL of in situ gelling system in a vial containing 2mL of phosphate buffer at pH 6.8 and equilibrated at  $(35 \pm 1)$  °C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve.

## 2.5 Measurement of Gt (Development of A Poloxamer Analogs/Carbopol-Based in Situ Gelling and Mucoadhesive Ophthalmic Delivery System For Puerarin).

Ten milliliters of sample solution and a magnetic bar were put into a transparent vial that was placed in a low temperature water bath. A thermometer with accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at the rate of 1 °C/1–2 min with the continuous stirring of 100 rpm. The temperature was determined as GT, at which the magnetic bar stopped moving due to gelation. Each sample was measured at least in triplicate.

## 2.6 In Vitro Drug Release [9,10]

### Method 1: (Enhancing and Sustaining the Topical Ocular Delivery of Fluconazole Using Chitosan Solution and Poloxamer/Chitosan in Situ Forming Gel).

Drug release rates from chitosan solutions (0.5, 1.0 or 1.5% w/w) and from in situ forming gels comprised of poloxamer (16% w/w) and chitosan (0.5, 1.0 or 1.5% w/w) were measured through a cellulose membrane in a Franz-type diffusion cell with a diffusion area of 0.64 cm<sup>2</sup>. Comparisons were made with the poloxamer (16% w/w) gel alone, and with aqueous solution, all of them containing 2 mg/ml drug. Aqueous solution containing drug 2 mg/ml was used as a control. The donor compartment was filled with 1 ml of studied formulation while the receptor compartment contained 35 ml of pH 7.4 HEPES buffer solution. The system was maintained under magnetic stirring (600 rpm) and at 35°C with an outer bath. Samples (1 ml) were withdrawn from the receiving solution each hour for 6 h and replaced with fresh receiving fluid. The amount of drug that permeated across the membrane, i.e., the amount of the drug in the receiving solution, was analyzed by HPLC. The diffusion coefficients (D) of drug from each vehicle were calculated using the following equation.

$$Q=2C_0(Dt/\Pi)^{1/2}$$

where Q is the cumulative amount of drug released per unit area, C<sub>0</sub> is the initial drug concentration in the vehicle, and D is the diffusion coefficient and t is time.

The drug release rate (K) was also determined by the slope of the linear portion of the plots of the drug cumulative amount versus the square root of time.

**Method 2:** The drug release from the prepared formulation was studied by placing the test solution in a circular Teflon cup of 2.5cm internal diameter and 1.2cm depth. This was in turn placed on an inverted USP basket kept inside a 250-ml beaker containing 200 ml of simulated tear fluid (pH7.4) as a dissolution medium and it was stirred by a magnetic stirrer (50 rpm) maintained at a temperature of  $37 \pm 0.1$  °C. Samples (1ml) were withdrawn at regular intervals and replaced with an equal volume of fresh medium. The absorbance of the diluted samples was measured at max (288nm) by UV-spectrophotometer using simulated tear fluid as a blank to calculate amount of drug release from in situ gel. The percentage of drug release was plotted against time to find the drug release pattern of all in situ gel preparations. Then, the release of selected in situ gelling system was compared with that of marketed eye drops.

### 2.7 Rheological Studies<sup>[10,11]</sup>

#### **Method 1: (Development of a poloxamer analogs/carbopol-based in situ gelling and mucoadhesive ophthalmic delivery system for puerarin).**

The rheological properties were determined using a cone and- plate geometry rheometer with a diameter of 40mm (cone angle  $4^\circ$ ). The shear stress of the sample solutions was measured at different shear rates at  $25 \pm 0.1$  and  $35 \pm 0.1$  °C, respectively. A typical run comprised of changing the shear rate from 0 to 200  $s^{-1}$  at a controlled ramp speed (keeping a period of 6 s at each shear rate). Then, the hierarchy of shear rate was reversed ( $200-0 s^{-1}$ ) for a similar period of 6 s. The average of two readings was used to calculate the shear stress. Evaluations were conducted in triplicate. Error bars have been omitted to retain clarity, however, in all cases the standard deviations of replicate analyses were less than 5%. In order to simulate the physiological disposition of gels more literally,

the polymer solutions were diluted by STF in a ratio of 40:7 and then adjusted to physiological pH value ( $7.4 \pm 0.1$ ) by adding the required amount of sodium hydroxide before the rheological studies were conducted at  $35 \pm 0.1$  °C.

**Method 2:** The relationship between contact time and the rheology was easily understood for viscosity enhanced ophthalmic solutions. It was noted from various literature that the formulations before gelling should have a viscosity of 5 to 1000 mpa and after gelling in the eye will have a viscosity from about 50-50,000 mpa. Rheological studies of the prepared formulations were carried out by Brookfield synchroelectric viscometer (LVOV Pro II) using spindle S18 (small sample adaptor). The viscosity of the formulations were determined at different speed conditions (10,20,50,75 to 100 rpm).

### 2.8 *In Vitro* Mucoadhesive Strength: (A Poloxamer/Chitosan In Situ Forming Gel With Prolonged Retention Time For Ocular Delivery)<sup>[11,12]</sup>

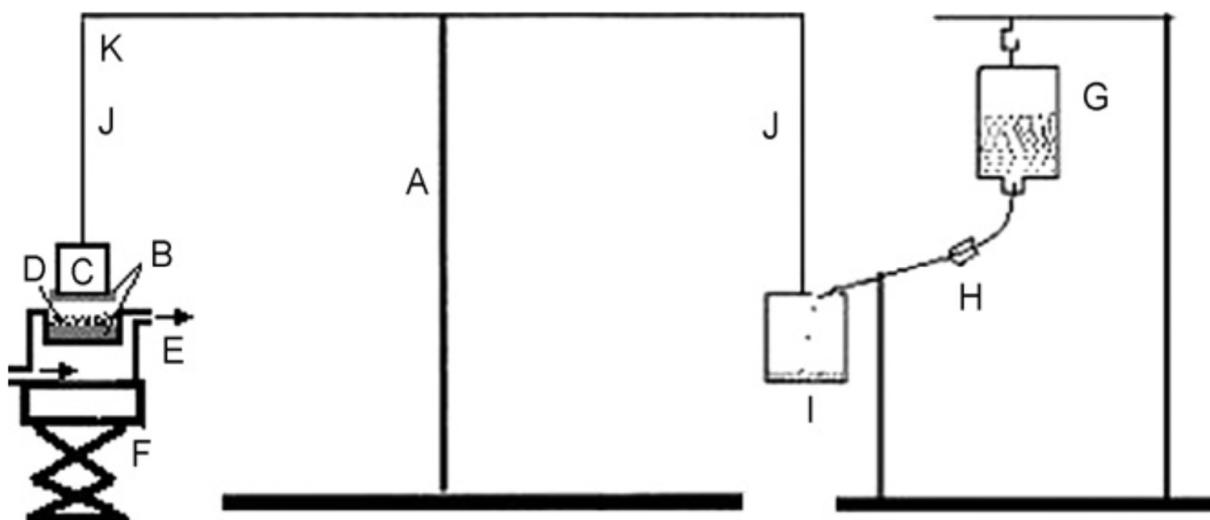
The mucoadhesive strength of the formulations under investigation was evaluated in vitro by measuring the force required to detach the formulation from a mucin disc using an Instron universal testing machine. Mucin discs were prepared by compression of a known weight of crude porcine mucin (250 mg), using a ring-press with a 9 mm diameter. These discs were then horizontally attached to the lower end of the cylindrical probe (1 cm diameter) by using double-sided adhesive tape. Prior to mucoadhesion testing, the mucin disc was hydrated by submersion in a 5% solution of mucin for 30 s. Excess surface liquid was removed by gentle blotting. The analytical probe was then lowered until the mucin disc was in contact with the surface of the sample, which had been packed into shallow cylindrical vessels. The probe and the disc remained in contact for 30 s. The probe was then moved upwards at a constant speed of 1.0 mm/s, and the force required to detach the mucin disc from the surface of each formulation was determined from the resulting force-time plot. All measurements were

performed at 35°C, and at least three replicates were carried out.

### 2.9 Measurement of Mucoadhesive Force: (Development of A Poloxamer Analogs/Carbopol-Based In Situ Gelling and Mucoadhesive Ophthalmic Delivery System for Puerarin).

The experimental technique used for determining the bioadhesive force has been derived from a previously published method. The experimental setup is presented in Figure below. Briefly, a section of tissue was cut from the cornea of the rabbit and washed with physiological saline, then immersed in newly prepared Glutathione Bicarbonate Ringer's solution at 35 °C for 10 min, which was prepared according to. The corneal tissues were attached to the undersurfaces (0.785 cm<sup>2</sup>) of the Teflon cylinder (C) and the sample cell of the thermostat (E) using a cyanoacrylate adhesive, respectively. The Teflon cylinder (C) was suspended by means of a thin steel wire (J) to the left of the balance (A). The balance (A) was made balanced. Forty microliters of polymer solution was added onto the sample cell of the thermostat (E) at 35 °C, which was placed on a height-adjustable pan (F). The height

of the pan (F) was adjusted quickly to make the polymer solution just come into contact with the corneal tissue before the polymer solution shifted into gel. Then, the balance of the balance (A) was destroyed with a weight (5.0 g) put onto the left end of the balance bar (K), so that the contact was made with the force of the Teflon cylinder (C) (5.0 g). After 10 min contact, the weight was removed, so that the balance (A) could regain balance. Then, the switch (H) of the infusion apparatus was opened to make the water drop into the glass vial (I) with a constant flow rate of 5 mL/min. The weight of the water in the glass vial (I) kept increasing until the gel and the corneal tissue were detached. Bioadhesive force, the detachment stress (dyne/cm<sup>2</sup>), was determined from the minimal weights that detached the gel and the corneal tissue. The corneal tissue pieces were changed for each measurement. To evaluate the bio adhesive force change after instillation and mixing with the tear fluid, the bioadhesive force measurements were taken at 35 °C, pH 7.4 and after diluting the formulations with STF (tear fluid).



**Fig: 1: Mucoadhesive force-measuring device:** (A) modified balance; (B) corneal tissue; (C) Teflon cylinder; (D) gel; (E) thermostat; (F) height-adjustable pan (G) dropping bottle; (H) infusion apparatus; (I) glass vial; (J) thin steel wire; (K) balance bar.

### 2.10 Determination of Bioadhesive Force<sup>[13,14]</sup>

The bioadhesive force of the liquid suppository was determined by using the measuring device shown in Figure. In brief, a section of tissue was cut from the fundus of rabbit rectum and secured with mucosal side out onto each glass vial (C) using a rubber band and an aluminum cap. The vials with the rectal tissues were stored at 36.5°C for 10 min. Next, one vial with a section of tissue (E) was connected to the balance (A) and the other vial was placed on a height-adjustable pan (F). Liquid suppository (D) was added onto the

rectal tissue on the other vial. Then, the height of the vial was adjusted so that the liquid suppository could be placed between the mucosal tissues of both vials. The weights (B) were kept raised until the two vials were attached. Bioadhesive force, the detachment stress ( $\text{dyncm}^2$ ), was determined from the minimal weights that detached the two vials. The rectal tissue pieces were changed for each measurement.

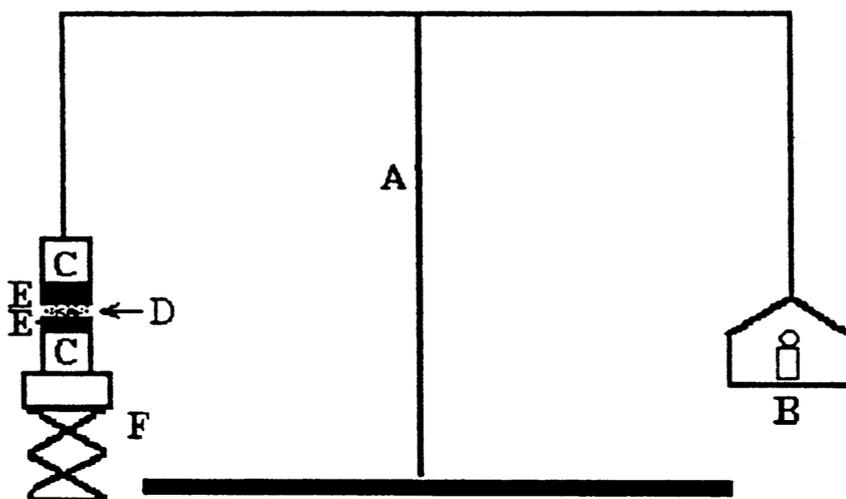


Fig: 2: Bioadhesive force-measuring device: (A) modified balance; (B) weights; (C) glass vial; (D) liquid suppository; (E) rectal tissue; (F) height-adjustable pan.

### 2.11 Texture Profile (A poloxamer/chitosan in situ forming gel with prolonged retention time for ocular delivery).

Texture profile analysis (TPA) was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, England) in TPA mode. Formulations (35 g) were transferred into 50-ml bottles, taking care to avoid the introduction of air into the samples. A cylindrical analytical probe (35 mm diameter) was forced down into each sample at a defined rate (1 mm/s) and to a defined depth (10 mm). At least five replicate analyses of each sample were performed at temperatures of 25°C and 35°C. From the resulting force–time plots, the hardness (the force required to attain a given deformation), compressibility (the work required to deform the

product during the first pass of the probe) and adhesiveness (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived.

### 2.12 Antimicrobial Activity (Formulation and Evaluation of Ph-Triggered in Situ Gelling System of Prulifloxacin)<sup>[14,15]</sup>

Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This was determined in the agar diffusion medium employing “cup plate technique”. Sterile solution of marketed drug eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken

into separate cups bored into sterile Muller Hinton Agar (MHA) previously seeded with organisms (Staphylococcus aureus and Pseudomonas aeruginosa). After allowing diffusion of solutions for two hours, the plates were incubated for 24 h at 37 ° C. The zone of inhibition (ZOI) was compared with that of the standard .

### **2.13 Sterility Testing ( Formulation and Evaluation of Ph-Triggered in Situ Gelling System of Prulifloxacin).**

IP method (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating formulations for not less than 14 days at 30 to 35 °C in the fluid thioglycolate medium to find the growth of bacteria and at 20 to 25° C in the soyabean-casein digest medium to find the growth of fungi in the formulations<sup>[15,16]</sup>.

### **2.14 Ft-Ir Studies ( Formulation and Evaluation of Ph-Triggered in Situ Gelling System of Prulifloxacin).**

The possibility of drug-excipients interactions were investigated by FTIR studies. The FTIR graph of pure drug and combination of drug with excipients were recorded using KBR pellets<sup>[17]</sup>.

### **2.15 Ocular Irritancy Studies (Formulation and Evaluation of Ph-Triggered in Situ Gelling System of Prulifloxacin).**

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 (100 ml) was normally placed in the lower cul-de-sac and irritancy was tested at the time interval of 1 hr, 24hrs, 48hrs, 72hrs, and 1 week after administration. The rabbits were observed periodically for redness, swelling and watering of the eye<sup>[18,19]</sup>.

### **2.16 Appearance, Clarity, Ph and Drug Content ( Formulation and Evaluation of Ph-Triggered In Situ Gelling System of Prulifloxacin).**

The appearances of all formulations were light yellow in colour and were clear except the formulation. Terminal sterilization by autoclaving had no effect on the formulations. The haziness observed during autoclaving due to precipitation at elevated temperature was found to disappear and the clarity was regained after overnight standing. The pH of all the formulations was found to be within the range of 6.0 to 6.4, which is desirable for the ophthalmic formulations. The drug content of all the formulations was within the range of 98.35% to 100.13%, showed the uniform distribution of drug in the ophthalmic formulations and the results<sup>[20]</sup>.

### **3. Conclusion**

Ophthalmic drug delivery system is burgeoning field in which most of the researchers are taking challenges to combat various problems related to this delivery. Steady advancement in the understanding of principles and processes governing ocular drug absorption and disposition and continuing technological advances have surely brought some improvements in the efficacy of ophthalmic delivery systems. The primary requirement of a successful controlled release Product focuses on increasing patient compliance which the in-situ gels offer. Exploitation of polymeric in-situ gels for controlled release of various drugs provides a number of advantages over conventional dosage forms.

Sustained and prolonged release of the drug, good stability and biocompatibility characteristics make the in situ gel dosage forms very reliable. Use of biodegradable and water soluble polymers for the in-situ gel formulations can make them more acceptable and excellent drug delivery systems. In-situ activated gel-forming systems seem to be favoured as they can be administered in drop form and produce appreciably less inconvenience with vision. Moreover, they provide better sustained release properties than drops. This type of dosage forms are used now a day in combat glaucoma, dry eye syndrome, Sjogren's syndrome, ARM D, trachoma etc.

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