Design, formulation, and evaluation of in situ gelling ophthalmic drug delivery system comprising anionic and nonionic polymers

Fatima Sanjeri Dasankoppa, Pooja Solankiy, Hasanpasha N. Sholapur¹, Vilas G. Jamakandi, Vinuta M. Sajjanar, Pooja M. Walvekar

Abstract:

BACKGROUND: The significant problem in the ocular drug delivery is the attainment of optimal drug concentration at the site of action. Development of therapeutic agents that require repeated long-term administration is a driver for the sustained release drug delivery systems, to result in less frequent dosing, and less invasive techniques. Therefore, to overcome the anatomical barriers and ocular bioavailability constrains, a novel drug delivery system in situ gels have been developed.

MATERIALS AND METHODS: The in situ gelling system comprises gellan gum, an anionic polymer responsible for the ionic gelation. Methylcellulose a nonionic polymer contributes for the viscosity and gels at the body temperature. The formulation was characterized for clarity, appearance, pH, gelation time, drug content estimation, rheological evaluation, effect of sterilization on the viscosity, in vitro diffusion study, isotonicity testing, and ocular irritation testing.

RESULTS AND DISCUSSION: The developed formulations exhibited sustained release of drug over 8 h thereby increasing residence time of the drug. Sterilization caused no effect on viscosity of the formulation. Optimized formulation was selected on the bases of ability to form instant gel and with increased viscosity of gel with a slow and prolonged in vitro drug release pattern. The optimized formulation was found to be nonirritating with no ocular damage or abnormal clinical signs to the cornea, iris, and conjunctiva.

CONCLUSION: Hence, the developed ophthalmic in situ gel by virtue of its prolonged corneal residence time and sustained drug release could be considered a viable alternative to the conventional eye drops in achieving enhanced bioavailability.

Keywords: Draize test, gellan gum, in situ gel, methylcellulose, power-law model, rheology

Introduction

The modern text on design and evaluation of therapeutic products must place into unique perspective, the nature of the eye, and requirements of ophthalmic dosage forms. The eye is perhaps better than any other bodily organ and serves as a model structure for the evaluation for the drug activity.[1] A major problem being faced in ocular therapeutics is the attainment of an optimal drug concentration at the site of action. Tear production, transient residence time, and nonpermeability of corneal epithelium create major problem resulting in poor bioavailability and absorption of ocular dosage form.[2]

New modes of delivering a drug into the eye range from solid to hydrophobic or
hydrophilic devices that are inserted into the ophthalmic cul-de-sac, increase the drug residence time, thereby providing drug for absorption for prolonged periods, and reducing the frequency that a given drug product must be administered.[3]

The volume of tears in the eye is 7 µL, most of which resides in the conjunctival sacs with 1 µL covering the cornea. Commercial eye drop dispenses 50 µL. Due to anatomical constraints; the eye often eliminates rapidly within 5–6 min after administration, and only a small amount (1%–3%) of an eye-drop actually reaches the intraocular tissue.[4]

To overcome the ocular drug delivery barriers and bioavailability constraints, novel drug delivery systems have been developed; emulsion, ointments, suspensions, aqueous gels, nanoparticles, nanosuspensions, and in situ gels.[5]

In situ gels are viscous liquids, undergo sol-to-gel transitions under the influence of pH, temperature, and the presence of electrolytes.[6] These in situ gels are non-Newtonian formulations, displaying pseudoplastic properties, that is, viscosity decrease with increasing shear rate, caused by ocular movement and blinking. Pseudoplasticity offers significantly less resistance to blinking and shows much greater acceptance than viscous Newtonian formulations.[7]

Materials and Methods

Materials

Ofloxacin was obtained as a gift sample from Elegant Drugs Pvt Ltd., Hubballi. Gellan gum (Life Expressions, Bengaluru, Karnataka, India), benzalkonium chloride (AstraZeneca Ltd., Bengaluru, Karnataka, India) methylcellulose (MC), polyethylene glycol 400, sodium chloride, sodium bicarbonate, potassium chloride (S D Fine-chem Limited., Bengaluru, Karnataka, India), dialysis membrane (HiMedia, Mumbai, Maharashtra, India). All the reagents used were of analytical grade.

Methods

Preparation of standard calibration curve of ofloxacin

A composition of 25 mg of the drug was accurately weighed in 25 ml volumetric flask containing 0.1N HCl to obtain stock solution-I (SS-I) with 1000 µg/ml concentration. From the SS-I, 10 ml was pipetted out and volume was made up to 100 ml with 0.1N HCl to obtain SS-II with 100 µg/ml concentration. Further aliquots were taken, and the solutions were made up to the mark 10 ml with 0.1N HCl to get a final concentration in the range of 2–10 µg/ml. All the working standards were measured in the ultraviolet (UV) range 200–400 nm and the absorbances were noted at λmax 293 nm against 0.1N HCl as blank.[8]

Drug–polymer compatibility studies

The term “incompatibility” refers to interactions of active pharmaceutical ingredients (APIs) with excipients that lead to change in physical, chemical, and therapeutic properties of a pharmaceutical dosage forms. Potential physical and chemical interactions between APIs and excipients can affect the chemical nature, stability, and bioavailability of APIs and in consequence, their therapeutic efficacy and safety.[9]

Fourier transform infrared spectroscopy

Compatibility studies were carried out using Fourier transform infrared (FTIR) spectrophotometer and employing KBr disc method. Infrared (IR) spectroscopic analysis of the pure drug and the drug–polymer physical mixture in appropriate ratio (1:1) were carried out. The spectra were recorded over a range of 4000–400 cm⁻¹ wave number. These IR spectra were employed as compatibility screening tool. Functional peaks of ofloxacin were compared with that of the mixture samples for possible interactions.[10]

Preparation of ophthalmic in situ gelling system

All the formulations were designed by Design-Expert (Design-Expert 10). A 2²-factorial design was employed for statistical optimization of formulations. The amounts of gellan gum and MC were considered as the independent factors, and percentage complementarity-determining region (CDR) and viscosity as the dependent variables (responses). The three-dimensional response surface graph represented the main and interaction effects of the independent variables, whereas two-dimensional contour plot gives a visual representation of values of the response.

Polymers were dispersed in deionized water and kept overnight for complete hydration. MC was kept in refrigerator to obtain transparent polymeric solution. The polymeric solutions were homogenized by stirring on a magnetic stirrer. Ofloxacin 0.3% w/v was solubilized and added to the polymeric solution. Finally, the volume was made up with distilled water and the pH was adjusted in the range 6.8–7.4.[11]

An ideal in situ gelling delivery system should be free-flowing liquid with low viscosity at nonphysiological condition (pH 4.0; 25°C) to allow reproducible instillation into the eye as drops and should undergo phase transition to form strong gel and sustain the drug at physiological conditions (pH 7.4; 37°C).[12]

Characterization of ofloxacin in situ gel

Physical appearance

Formulations were examined for color and clarity under fluorescent light against white and black background for the presence of particulate matter if any present.[13]
**pH determination**

The pH of ophthalmic formulation indicates its stability and at the same time, there would be no irritation to the patient on administration of the formulation. Ophthalmic formulations should have a pH range between 5 and 7.4.[14]

**Rheological study**

Viscosity of instilled formulation is an important factor in determining residence time of drug in the eye. From the literature, it is evident that the formulations in the sol form need to have viscosity ranging 5–1500 cps and gels with viscosity ranging about 50–50,000 cps. Viscosity of the sols was measured at 25°C (room temperature) and of the gels (after addition of artificial tear fluid [ATF]) at 37°C ± 0.5°C (body temperature) with the thermostatic water bath connected to the small sample adapter. The viscosity of gels was measured with 1:3 ratio of formulation and ATF, respectively. The angular velocity of the spindle was increased to 0.3, 0.5, 0.6, 1, 1.5, 2, 2.5, 3, 3.4, 5, 6, 10, and vice versa; and the viscosity of the formulation was recorded.[13]

**In vitro gelation study**

Gelling strength of formulations were evaluated by placing a drop (100 μl) of polymeric solution in vials containing 1 ml of freshly prepared ATF, equilibrated at 37°C. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and period for which the formed gel remains as such.

(+)– Gels after few minutes and dissolves quickly

(++)– Gels immediately and remains for <4–5 h

(+++)– Gels immediately and remains for >8 h period.

The composition of ATF is Sodium chloride 0.670 g Sodium bicarbonate 0.200 g Calcium chloride 0.008 g Purified water (q. s)-100 ml.[18]

**Effect of sterilization on viscosity of in situ gelling formulations**

The formulations were subjected to sterilization by means of an autoclave at 121°C with a sterilization cycle of 15–20 min to check the rigors of sterilization on formulations. After gelation in ATF, the samples were subjected to viscosity measurement at an angular velocity of 1.5 rpm. Appropriate spindle (TL6) to be used to measure the viscosity before sterilization and after sterilization.[17]

Viscosity of non-Newtonian fluids is characterized by more than one parameter and is better expressed in the form of a power-law model.

\[
\mu_a = K \left( \frac{N}{N_0} \right)^n (4 \pi N)^{n-1}
\]

Where,

\(\mu_a\)–the apparent viscosity (cps)

N–the spindle speed (rpm)

K–the consistency index and the dimensionless index of flow behavior.

The values of ln (μa) and ln (4 πN) were fitted into the equation for a straight line. From the slope and intercept values of the line of best fit, the flow behavior index “n” and consistency coefficient “K” were determined.[18]

**In vitro drug release of in situ gel forming system**

*In vitro* drug release studies were carried out in Franz-diffusion cell using dialysis membrane (previously soaked overnight in the ATF, HiMedia) and ATF (pH: 7.4) as diffusion membrane and receptor medium, respectively. Formulation and ATF in the 1:3 ratio, respectively, was placed in the donor compartment, which was in contact with receptor medium. The receptor medium (ATF) was stirred continuously at 50 rpm to simulate blinking action of eyelids. The whole assembly was adjusted on magnetic stirrer and maintained at 37°C ± 0.5°C to mimic physiological condition at which gelation occurs. Aliquots of medium were withdrawn at 1 h time interval for an extended period of 8 h. Equal volumes of fresh media were added to replace the withdrawn samples. Finally, the withdrawn samples were diluted and estimated by UV-spectrophotometer (UV-1800) at 293 nm using the blank.[19]

**Drug content estimation**

Accurately measured 1.0 ml of the sol form of the formulation was pipetted out and diluted to 100 ml with distilled water to give 30 μg/ml stock. 3 ml from the above stock was pipetted, diluted to 10 ml with distilled water, and analyzed by UV-spectrophotometer (UV-1800). Ofloxacin obeys Beer’s range of 2–10 μg/ml. Drug content was estimated by utilizing the linear equation obtained from the standard calibration curve.[20]

**Isotonicity evaluation**

Isotonicity is an important characteristic of the ophthalmic formulation. Isotonicity need to be maintained to prevent tissue damage or irritation of eye. Formulations were subjected to isotonicity testing to evaluate their isotonic (osmotic pressure same as body fluids), hypotonic (osmotic pressure greater than body fluids), and hypertonic (osmotic pressure less than body fluids) nature. The formulations were mixed with one drop of blood and observed under microscope at ×45 magnification and compared with standard ciprofloxacin eye drops which depicts the isotonic nature.[21]

**Ocular irritancy test**

The Draize test: It is a rabbit eye toxicity and irritation test. This test is officially accepted in the OECD countries for regulatory purposes in the classification of slightly and moderately irritating chemicals. It is based on the subjective scoring of three tissues of the eye: the cornea, conjunctiva, and iris. In the study, Albino rabbit (New Zealand White rabbit) is used as
test species. Approval from the Institutional Animal Ethics Committee was obtained prior to the commencing of the study. This study group had 2 rabbits of either sex weighing 1.5–2 kg. One hundred microliter of the formulation was placed in the lower cul-de-sac of one eye and the other eye served as control. The evaluation of ocular lesions was made at 1, 4, 24, 48, 72 h, and 1 week after administration. A 3-day washing period with saline or distilled water was carried out. The sterile formulation was instilled twice a day for 7 d, and a crossover study was carried out. The rabbit eye was periodically observed for redness, swelling, and secretions from the eye.[22–24]

**Ex vivo corneal permeation study**
Goat’s cornea was used to study the permeation across the corneal membrane. Whole eyeballs of goat were procured from a slaughter house. This study was carried out using Franz-diffusion cell by placing the corneal side in continuous contact with formulation in the donor compartment. The formulation to be tested was added to the donor chamber with the help of a micropipette. The receptor compartment was filled with ATF at 37°C ± 0.5°C. The receptor medium was stirred on a magnetic stirrer at 50 rpm.

The samples were withdrawn at 1 h time interval for 8 h and analyzed for drug content. Receptor medium was replaced with an equal volume of ATF at each time interval to maintain sink conditions. The samples were appropriately diluted and analyzed.[25]

**Accelerated stability studies**
The purpose of stability testing is to provide evidence on how the quality of an API or medicinal product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a retest period for the API or a shelf life for the medicinal product and recommended storage conditions.

Stability studies were carried out on the optimized formulation according to the International Conference on Harmonization (ICH) guidelines. The optimized formulation was packed and sealed in amber-colored ampules and placed in the stability chamber for 30 days with 40°C ± 2°C, 75% RH ± 5% RH. Samples were withdrawn at 0, 7, 14, 21, and 28 d intervals. The formulation was evaluated periodically for drug content, pH, gelling capacity, viscosity, and in vitro drug release.[26,27]

**Results**

**Standard calibration curve of ofloxacin**
From the standard calibration curve of API, it was observed that the drug obeys beer’s law in concentration range from 2 to 10 µg/ml in 0.1 N HCl. The linear equation (y = 0.0903x + 0.003) generated was used for the calculation of amount of drug release.

**Drug–polymer compatibility studies**
Physical mixture of the model drug and polymer was characterized by FTIR spectra for any physical as well as chemical alterations of the drug characteristics [Figure 1].

**Preparation of ophthalmic in situ gelling system**
Ofloxacin ophthalmic in situ gels were formulated using various ratios of anionic polymer and nonionic polymer gellan gum and MC, respectively. Benzalkonium chloride served as a preservative. The formulations were adjusted to pH and tonicity as shown in [Table 1].

**Characterization and evaluation of in situ gels**
The in situ gels were formulated and accessed for their physical appearance, clarity, pH measurement, gelling capacity, drug content estimation, rheological evaluation [Table 2 and Figures 2, 3], effect of sterilization on viscosity [Table 3], in vitro drug diffusion study [Figure 4], ocular irritancy study, ex vivo corneal permeation study, isotonicity testing [Figure 5], and accelerated stability studies [Table 4 and Figure 6].

**Discussion**
The present study involves design and formulation of ophthalmic in situ gelling system for the treatment of bacterial infection comprising different ratios of gellan gum and MC polymers and the drug ofloxacin using Design-Expert software. Four formulations were formulated using 2<sup>3</sup> level-factorial design. The formulations were characterized, evaluated, and stability accessed. Physical mixture of the model drug and polymer was characterized by FTIR spectra for any physical as well as chemical alterations of the drug characteristics. IR spectrums exhibited no interference in the functional groups, as the principle peaks of the model drug were found to be unaltered in the spectra of the drug–polymer physical mixture. All the formulations were subjected to postformulation evaluation parameters. All the formulations were found to be clear and light yellow in color. The pH of all the formulations was within the acceptable range of 6.94 ± 0.02–7.30 ± 0.01 giving satisfactory results.

| Table 1: Formulation design using design expert |
|-----------------------------------------------|
| Formulation code (%w/v) | df1 | df2 | df3 | df4 |
|-------------------------|-----|-----|-----|-----|
| Ofloxacin              | 0.3 | 0.3 | 0.3 | 0.3 |
| Gellan gum              | 0.2 | 0.65 | 0.25 | 0.65 |
| Methylcellulose        | 0.5 | 0.5 | 0.8 | 0.8 |
| PEG 400                 | 1   | 1   | 1   | 1   |
| Benzalkonium chloride  | 0.01| 0.01| 0.01| 0.01|
| Water quantity sufficient | 100 | 100 | 100 | 100 |
| PEG: Polyethylene glycol |     |     |     |     |
Dasankoppa, et al.: Formulation development of ophthalmic in situ gelling drug delivery system

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hence causing no irritation to the eye on instillation. The \textit{in situ} formed gels should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs. The gelling capacity is characterized by their gel forming time (gels after few 2 min ++, immediate gelation >30s ++++, and immediate gels within seconds (instantly) ++++), and gel erosion time (dissolves rapidly >3 h ++, remains for 4–6 h ++++, and remains for extended period <8 h +). The percentage drug content was in the range of 75.42 ± 0.12–96.35 ± 0.12. Viscosity of the \textit{in situ} gel determines the residence time of the drug in the eye. An optimum viscosity is desired to administer the formulation as solution, which later under goes phase transition from sol to gel. Sol-gel transitions are characterized by pseudoplastic nature. For the pseudoplastic nature power-law model specifies $n < 1$. The viscosity of all the formulations decreased as the shear rate increased, indicating the character of pseudoplastic fluid. All the formulations were subjected to sterilization by means of autoclaving at 121°C with a sterilization cycle of 15–20 min to check the rigors of sterilization on formulations. All the formulations retained their texture and viscosity without any effect of sterilization. Design of experiments (DOE) optimized formulation sustained the drug for 8 h with percent cumulative drug release 79.89%. Increased gellan gum concentration increased the percentage CDR, whereas MC contributed for the increase in viscosity as shown in the 3D response plot [Figures 7 and 8]. Ideally, ophthalmic formulations should be isotonic with blood cells to prevent any irritation or damage to the eye. Microscopic observations demonstrate that integrity of RBC’s was maintained with no lysis or crenation of RBC’s [Figure 5]. The formulation was found to be nonirritating with no ocular damage or any abnormal signs to the conjunctiva, iris,

Figure 1: Compatibility studies of ofloxacin (a) and polymer combinations (b) used in the formulation at accelerated temperature (40°C) and humidity conditions (75% RH) for 28 days

Figure 2: Pseudoplastic flow behavior of design of experiments formulations DF1, DF2, DF3, and DF4

Figure 3: Power-law model plot of design of experiments formulations DF1, DF2, DF3, and DF4

![Figure 1](image1.png)

![Figure 2](image2.png)

![Figure 3](image3.png)
and cornea. There was no significant change of viscosity, gel forming ability, pH, and drug content. Hence, the specified conditions were considered to be suitable for the storage of formulation [Table 4]. “n” value signifying flow behavior at 25°C and 40°C for 28 days remains constant [Figure 6].

**Conclusion**

Ophthalmic *in situ* gels decrease the tear turnover rate and nasolacrimal drainage, to overcome the drawbacks of conventional eye drops. Polymer concentrations play a vital role in influencing the viscosity, drug residence time, and the drug release from the gelling system. Increased residence time of the formulation in the eye contributes for the increased bioavailability and decrease the dosing frequency gives better patient compliance. The cross-linking between negatively charged helices and the ions of the tear fluid cause the gellan gum gelation and the increased temperature cause viscosity increment contributed by the MC. The formulations had competent pH values corresponding to the tear fluid pH (7.4). Hence, making them nonirritating on instillation. There was instant gelation and lasted for longer duration. In *situ* gels showed satisfactory viscosity, with pseudoplastic flow behavior index. The flowing nature of the sol decreased with the increased ionic concentrations and temperature leading to form gels. The optimized formulation exhibited a 4–5-fold (approximately) increase in viscosity corresponding to the sol form. Drug release was achieved through the diffusion of the drug from the polymer matrix for a sustained period of 8 h. All the formulations retained the viscosity and the integrity of the polymers after sterilization effects. Ocular toxicity studies in rabbit’s eye showed no abnormal signs to the cornea, conjunctiva, and iris, resulting in the nonirritational and safety for ocular administration. Stability studies

### Table 2: Evaluation of the physiochemical parameters of design of experiments formulations

| Formulation code | Visual appearance                  | pH (at 25°C) | Gel forming time | Gel erosion time | Drug content (%) | Spindle TL6 |
|------------------|-----------------------------------|--------------|------------------|------------------|-----------------|-------------|
| df1              | Clear, light yellow solution      | 7.25±0.01    | ++++             | ++++             | 96.35±0.12      | 1710        |
| df2              | Clear, light yellow solution      | 7.25±0.01    | ++++             | ++++             | 96.35±0.12      | 2210        |
| df3              | Clear, light yellow solution      | 7.25±0.01    | ++++             | ++++             | 96.35±0.12      | 3490        |
| df4              | Clear, light yellow solution      | 7.25±0.01    | ++++             | ++++             | 96.35±0.12      | 3170        |

++++: Gelation is immediate within seconds

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**Figure 4:** Comparative *in vitro* diffusion study profile of *in situ* formulations

**Figure 5:** Isotonicity testing for optimized *in situ* gelling formulations

**Figure 6:** Comparison of flow behavior (n) values, at 25°C and 40°C for 28 days

**Figure 7:** Three-dimensional response plot for percentage complementarity determining region
Dasankoppa, et al.: Formulation development of ophthalmic in situ gelling drug delivery system

Table 3: Viscosity values for in situ formed gels before and after sterilization and percentage viscosity variation at 1.5 rpm

| Formulation code | Viscosity value for in situ formed gels (cps) Before sterilization | Viscosity value for in situ formed gels (cps) After sterilization | Percentage viscosity variation (mean values±SD, n=3) |
|------------------|---------------------------------------------------------------|---------------------------------------------------------------|--------------------------------------------------|
| GMCP 1           | 910.53±0.85                                                   | 1118.33±2.08                                                 | −22.82±0.124                                     |
| GMCP 2           | 142.90±1.05                                                  | 182.33±2.08                                                  | −27.59±1.450                                     |
| GMCP 3           | 118.00±2.00                                                  | 123.66±3.21                                                  | −4.90±2.720                                     |
| GMCP 4           | 962.00±2.64                                                  | 1167.70±1.40                                                 | −21.52±0.198                                     |
| GMCP 5           | 133.20±2.46                                                  | 142.00±3.60                                                  | −4.00±2.160                                     |
| GMCP 6           | 831.00±5.29                                                  | 712.30±10.78                                                 | 14.27±1.740                                     |
| GMCP 7           | 8162.86±2.79                                                | 9722.83±2.31                                                 | −19.11±0.012                                     |
| GMCP 8           | 2793.66±1.66                                                | 3526.96±4.81                                                 | −26.26±0.178                                     |

SD: Standard deviation

Table 4: Results for accelerated stability studies for optimized formulation df1 stored at 40°C/75% relative humidity

| Parameters                  | 0 day | 7 days | 14 days | 21 days | 28 days |
|-----------------------------|-------|--------|---------|---------|---------|
| pH                          | 7.25±0.01 | 7.25±0.01 | 7.24±0.01 | 7.24±0.01 | 7.23±0.02 |
| Physical appearance         | Clear, light yellow solution | Clear, light yellow solution | Clear, light yellow solution | Clear, light yellow solution | Clear, light yellow solution |
| Percentage of drug content  | 96.35±0.01 | 96.35±0.01 | 96.34±0.01 | 95.35±0.1 | 95.36±1.02 |
| Gel forming time (h)        | Instantly | Instantly | Instantly | Instantly | Instantly |
| Gel erosion time (h)        | 7-8    | 7-8    | 7-8     | 7-8     | 7-8     |
| Viscosity (sol) cps at 1.5 rpm, spindle TL5 | 600 | 601.4 | 595.9 | 360.9 | 360.0 |
| Viscosity (gel) cps at 1.5 rpm, spindle TL5 | 2130 | 2129.1 | 2120.5 | 2119.6 | 2119.4 |
| Percentage viscosity variation (mean±SD, n=3) | 0 | +0.045±0.001 | +0.446±0.005 | +0.488±0.008 | +0.497±0.008 |

SD: Standard deviation

Figure 8: Three-dimensional response for viscosity

as per the ICH guidelines, 40 ± 2°C/75 ± 5% RH storing conditions for 1 month shows no significant change in the polymers viscosity, drug release, gelling ability, and drug content. Hence, the formulation was found to be stable.

On the concluding remarks, the designed ophthalmic in situ gelling system has the ability to form stiff gel on contact with the tear fluid at 37°C ± 0.5°C. The developed formulation could be a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal retention time and its ability to sustain the drug release.

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Conflicts of interest

There are no conflicts of interest.

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Dasankoppa, et al.: Formulation development of ophthalmic in situ gelling drug delivery system

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