ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 12, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

# FORMULATION AND EVALUATION OF SOLUBILITY ENHANCED *IN SITU* GELLING EYE DROPS OF LOTEPREDNOL ETABONATE

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*Received: 20 March 2019, Revised and Accepted: 30 September 2019*

**ABSTRACT**

**Objective:** The aim of the present study is to improve the aqueous solubility and develop *in situ* gelling eye drops of loteprednol etabonate. Beta-cyclodextrin (β-CD)-assisted solubility enhancement was attempted and phase solubility studies were carried out.

**Methods:** The kneading technique was used to formulate drug/β-CD inclusion complexes with a ratio of 1:1. *In situ* gelling ophthalmic sol–gel systems were then developed. Ion-sensitive and pH-dependent trigger mechanisms were targeted. The former type was based on increasing gellan gum concentration, to formulate three systems F1, F2, and F3. The latter pH-dependent systems were formulated using a constant concentration of Carbopol 971P NF and varying concentrations of hydroxypropyl methylcellulose (HPMC) K4 M and HPMC K15M grades giving formulations F4, F5, and F6. All six formulations were subjected to physicochemical evaluation for clarity and appearance, texture analysis, pH, viscosity, isotonicity, *in vitro* gelation, drug content determination, and microbiological tests (sterility testing and effectiveness of preservative) which were also conducted.

**Results:** All the six formulations passed the analytical tests, with F2 and F4 emerging as the optimized formulations. Eight hour *in-vitro* drug release carried out in a fabricated diffusion cell revealed the release to a concentration dependent controlled one. One month stability studies at 40°C and 75% RH of the optimized formulations proved their robustness.

**Conclusion:** Extensive studies carried out revealed the optimized formulation for each category of sol-gel systems. Formulations F2 and F4 were found to be these optimized formulations.

**Keywords:** Loteprednol etabonate, Beta-cyclodextrin, Solubility, *In situ* gelling.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i12.33160

## INTRODUCTION

*In situ* forming gels are formulations that are applied as solutions or suspensions and undergo gelation due to physicochemical changes in the eye and are triggered by increased temperature, pH, or ionic strength.

Loteprednol etabonate is a topical corticoid anti-inflammatory. It is used in ophthalmic solution for the treatment of steroid-responsive inflammatory conditions of the eye such as allergic conjunctivitis, uveitis, acne rosacea, superficial punctate keratitis, herpes zoster keratitis, iritis, cystitis, and selected infective conjunctivitis [1].

Beta-cyclodextrin (β-CD) complexation with loteprednol etabonate enhances its solubility. Various methods can be applied to prepare drug/cyclodextrin complexes, including the solution method, the coprecipitation method, neutralization method, the slurry method, the kneading method, and the grinding method. In most cases, the presence of at least some water is essential for successful complex formation. In solution, cyclodextrin complexes are usually prepared by the addition of excess amount of drug to an aqueous cyclodextrin solution. The suspension form is equilibrated at the desired temperature (which may require periods of up to 1 week and then filtered or centrifuged to form clear drug/cyclodextrin complex solution). For the preparation of solid complexes, the water is removed from the aqueous drug/cyclodextrin solution by evaporation (e.g., spray drying) or sublimation (e.g., lyophilization). Topical corticosteroids have been used to treat ocular inflammatory conditions and have several drawbacks. *In situ* release products are an alternative [1,2].

## MATERIALS AND METHODS

### Materials

Loteprednol etabonate was obtained as a free gift sample from Cipla Ltd., Verna, Goa. Gellan gum was obtained from Oxford Laboratories, Mumbai, India. Carbopol® 971P NF was obtained from Lubrizol Advanced Materials India Pvt. Ltd., Mumbai, India. Hydroxypropyl methylcellulose (HPMC) K4 M and HPMC K15 M were gifted by Colorcon Asia Ltd., Goa. All the other chemicals used were of analytical grade.

### Methodology

*Preformulation studies*

Melting point determination

Melting point of drug was done by open capillary method. Drug was taken in a glass capillary sealed at one end in a flam and dipped in liquid paraffin inside the melting point apparatus [1,2].

Partition coefficient

The partition coefficient of loteprednol etabonate was determined using n-octanol and phosphate buffer pH 7.4 as the aqueous phase. The two phases were mixed in equal quantities (1:1) saturated with each other on a mechanical bath shaker at 32°C for 24 h. To it, 100 mg of drug was added. The flask was shaken at 32°C for 6 h to achieve a complete partitioning. The two phases were then separated by centrifugation at 100 rpm for 5 min. The solutions obtained were passed through a membrane filter and analyzed spectrophotometrically. The partition coefficient of the drug was calculated using the following expression [1-3]:

$$K_{o/w} = \frac{\text{Drug concentration in n – Octanol}}{\text{Drug concentration in phosphate buffer pH 7.4}}$$

### Solubility analysis

The solubility profile of loteprednol etabonate was determined using modified shake flask method in select suitable solvents. An excess of the drug was added to each of the 10 ml of the solvents taken in tubes maintained at room temperature. The tubes were shaken (vortex mixed).

Aliquot portions of the supernatants were taken, filtered, and suitably diluted for quantification of loteprednol etabonate [4].

**Calibration curve of loteprednol etabonate in simulated tear fluid (STF) phosphate buffer pH 7.4**

A stock solution of 1000 µg/ml of loteprednol etabonate was prepared in methanol by dissolving 25 mg of loteprednol etabonate in 25 ml of methanol. A working standard solution of 100 µg/ml of loteprednol etabonate was prepared by diluting 10 ml of the stock solution up to 100 ml in a volumetric flask using methanol. Using the working standard solution, a series of standard solutions of concentration ranging from 2 to 20 µg/ml were prepared by diluting with STF phosphate buffer pH 7.4. The absorbance of the solution was measured at the wavelength of maximum absorption against the reference blank and was plotted against concentration to obtain the standard calibration curve [3,4].

**Compatibility studies**

The compatibility studies were done by Fourier-transform infrared (FT-IR) spectroscopy and differential scanning calorimetry (DSC) studies [5].

**FT-IR spectroscopy**

The infrared (IR) absorption spectrum was obtained by preparing a simple physical mixture of the drug and the excipient. The mixture was then placed on the stage of the instrument and scanned by the passage of an IR beam through it. The spectra were scanned over a frequency range of 4000–400 $cm^{-1}$ [5].

**DSC**

DSC was used as a screening technique for assessing the compatibility of the pure drug with the individual solid-state excipients and also when in combination. To investigate the possible interactions between the components, the DSC curves of pure drug and each individual excipient were compared with those of their 1:1 w/w physical mixtures [5].

**Preliminary studies**

*Phase solubility studies*

A series of β-CD solutions in concentration ranging from 5 to 50 mM were prepared using phosphate buffer pH 7.4 each placed in 10 ml volumetric flasks. An excess amount of loteprednol etabonate (20 mg) was added and sonicated for 1 h and then vortex mixed at room temperature for further 24 h to achieve equilibration.

The suspensions were then filtered through 0.45 µ Millipore filter and analyzed spectrophotometrically. The phase-solubility profile of loteprednol etabonate in aqueous β-CD solutions was then plotted. It consisted of concentration of the drug in the aqueous solution in mM on the Y-axis plotted against the increasing concentration of β-CD on the X-axis. The association constant ($K_c$) for the complex formed was calculated from the slope of the phase-solubility profile and the aqueous solubility of loteprednol ($S_o$), using the equation.

$$Kc = \frac{Slope}{S_0(1 - Slope)}$$

The phase solubility studies were carried out in duplicate and the averages of both the trials calculated. The complexation efficiency (CE) was calculated from the slope of the phase-solubility diagram using the formula.

$$CE = S_0 . Kc$$

**Preparation of loteprednol etabonate β-CD (11) complex**

Loteprednol etabonate-β-CD (1:1) molecular inclusion complex was prepared by mixing drug and beta-cyclodextrin in mortar. A 1:1 hydroalcoholic solution of distilled water and methanol was used to knead the physical mixture into a paste and dried in a vacuum oven for 24 h at a temperature not exceeding 40°C. The complex was then evaluated for its solubility in distilled water and STF phosphate buffer pH 7.4 [6,7].

**Development of optimized placebo *in situ* gelling systems**

The concentrations of polymer to be employed for the development of the formulations were deduced by carrying out placebo gelling studies. Two types of polymers were evaluated for *in situ* gelation, namely, ion-sensitive gellan gum and pH-dependent Carbopol 971P NF. Placebo solutions of the polymers in varying concentrations were prepared. While gellan gum was formulated as an aqueous solution alone, Carbopol 971P NF was solubilized with varying concentrations of HPMC grades in phosphate buffer pH 6.8.

The gelling capacity was determined by placing 1 drop of the formulation in a vial containing 3 ml of freshly prepared STF equilibrated at 37°C. Gel formation was visually assessed and the time taken for gelation and the gel formed to dissolve were noted. In both the cases, the minimum concentrations at which the respective polymers showed gelling were noted [8].

**Formulation and development of optimized ion-triggered and pH-dependent *in situ* gelling ophthalmic solutions**

Three formulations each of ion-sensitive Gelrite™ gellan gum systems and pH-dependent Carbopol 971P NF in combination with HPMC K4 M and K15 M were formulated (Table 1). The drug-cyclodextrin complex equivalent to 0.5% w/v of loteprednol etabonate was dissolved in three-fourth of the vehicle. The solution was then filtered using a 0.45 µ membrane filter and vacuum filtration assembly. The polymer and viscosity-enhancing agents were added to the solution and hydrated overnight. The next day, the solution was slightly agitated using magnetic stirrer to achieve uniformity. All the other excipients were added and the volume made up using the vehicle. The formulation was then filled in 10 ml capacity amber-colored glass vials, stoppered with rubber closures, and ultimately sealed with aluminum caps. These were then subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min.

**Evaluation of the *in situ* gelling ophthalmic systems**

*Clarity and appearance*

The clarity of the developed formulations was visually evaluated against a black and white background. The sols were further converted to gels using STF and the clarity and physical appearance of the gel were taken note of Comstock and DeCory [9].

*Texture analysis*

The firmness, consistency, and cohesiveness of the formulations were assessed based on the flow properties of the sol. The pourability of the sol was evaluated so as to determine if the formulation could be easily administered *in vivo* [9].

*pH*

The pH of the *in situ* sol ophthalmic formulations was determined using calibrated pH meter. The measurements were conducted in triplicates [9].

**Table 1: Compositions of formulations F1-F6 (%w/v)**

| Formula | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Drug/ beta-cyclodextrin complex | ≈ 0.5 | ≈ 0.5 | ≈ 0.5 | ≈ 0.5 | ≈ 0.5 | ≈ 0.5 |
| HPMC K4 M | - | - | - | 0.2 | - | 0.1 |
| HPMC K15 M | - | - | - | - | 0.2 | 0.1 |
| Gellan gum | 0.5 | 0.75 | 1.0 | - | - | - |
| Carbopol 971P NF | | | | 0.3 | 0.3 | 0.3 |
| Benzalkonium chloride | - | - | - | 0.01 | 0.01 | 0.01 |
| Propyl paraben | 0.02 | 0.02 | 0.02 | - | - | - |
| Distilled water/ phosphate buffer pH 6.8 | q. s | q. s | q. s | q. s | q. s | q. s |

HPMC: Hydroxypropyl methylcellulose

*Viscosity*

The viscosity of the formulations was determined using a Brookfield viscometer (LVDV-I) fitted with spindle no. 2. The sample was placed in sample holder of the sample adapter assembly and the angular velocity was gradually increased from 0 to 60 rpm. Then, the hierarchy of angular velocity was reversed and the average of the two dial reading was considered to calculate the viscosity. The formulations were then converted from sol to gel by the addition of STF. The viscosity of samples was recorded before and after gelation [9].

*In vitro gelation*

The time taken for the first detection of gelation and for the time taken for the gels so formed to dissolve was noted [9].

*Isotonicity*

A drop of blood was suitably diluted with the formulation under test using red blood corpuscles (RBCs) pipette and observed under an optical microscope at ×45. The structure of the RBCs was compared with standard marketed preparation containing 0.5% loteprednol etabonate to assess if any changes occurred in it [10].

*Drug content*

The vials containing the formulation were shaken for 2–3 min and 1 ml was transferred to a 100 ml volumetric flask and volume was made up with STF phosphate buffer pH 7.4. The concentration of loteprednol etabonate was determined using ultraviolet–visible spectrophotometer after suitable dilution with STF phosphate buffer pH 7.4. The measurements were carried out in triplicates [11-13].

*In vitro release studies*

*In vitro* drug release studies of the *in situ* gelling systems were carried out using a fabricated diffusion cell. The diffusion cell consisted of a donor compartment and a receptor compartment with a cellophane membrane mounted between them. The receptor compartment was filled with 50 ml of STF phosphate buffer pH 7.4 and stirred at 20–30 rpm throughout the experiment. The temperature was maintained at 37±0.5°C. 1 ml of the formulation was placed in the donor compartment and 5 ml of the fluid from the receptor compartment was withdrawn at definite time intervals and was replaced by an equal volume of receptor fluid. The concentration of the drug in the receptor compartment was determined spectrophotometrically [13-16].

**Microbial tests**

*Sterility test*

The sterilized sample was aseptically transferred in Soybean Casein Digest Medium for bacteria and fluid thioglycollate medium for fungi. After inoculation, the media were incubated for not <14 days, at 30–35°C for bacteria and 20–25°C for fungi. The media were checked for growth at the end of the specified period of time [17,18].

**Effectiveness of preservative**

The test sample was inoculated with microbial strains and the effectiveness of the preservative is determined by its ability to check microbial growth. This is done by performing viable count at suggested intervals of time. Three strains of microorganism were used for the purpose of this study, which were Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus,* and fungi *Candida albicans*. The medium used was nutrient agar with incubation conditions 30–35°C for bacteria and 20–25°C for fungi. The time intervals for viable counting were on the $0^{th}$, $7^{th}$, $14^{th}$, and $21^{st}$ days [19,20].

**Stability studies**

The formulated ophthalmic sol–gel systems were subjected to repeated cycles at temperature 40°C and humidity 75% RH for 30 days and checked for any signs of instability, polymeric phase separation, gelling capacity, and drug content [21-23].

## RESULTS

**Preformulation studies**

*Identification tests (Table 2)*

**Table 2: Results of identification tests**

| S. No. | Test | Standard | Observation |
|---|---|---|---|
| 1. | Melting point | 222–224°C | Complies |
| 2. | Partition coefficient ($K_{o/w}$) | 3.4 | Complies |

**Solubility analysis (Table 3)**

**Table 3: Solubility analysis of loteprednol etabonate**

| S. No. | Solvent | Observation |
|---|---|---|
| 1. | Distilled water | Practically insoluble |
| 2. | Simulated tear fluid phosphate buffer pH 7.4 | Practically insoluble |
| 3. | Methanol | Freely soluble |
| 4. | Ethanol | Soluble |
| 5. | Dichloromethane | Soluble |

**Standardization of loteprednol etabonate (Fig. 1)**

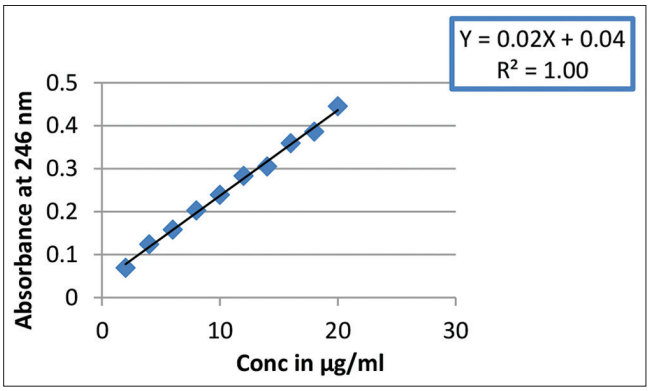


**Fig. 1: Calibration curve of loteprednol etabonate**

**Compatibility studies**

*FT-IR spectroscopy (Fig. 2)*

(Figure 2 is cited on next page)

**DSC (Fig. 3)**

(Figure 3 is cited on next page)

**Preliminary studies**

*Phase solubility studies (Fig. 4)*

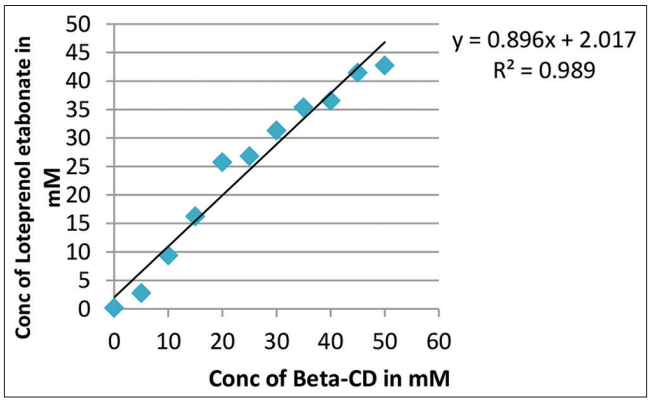


**Fig. 4: Phase-solubility profile of loteprednol etabonate in aqueous medium**

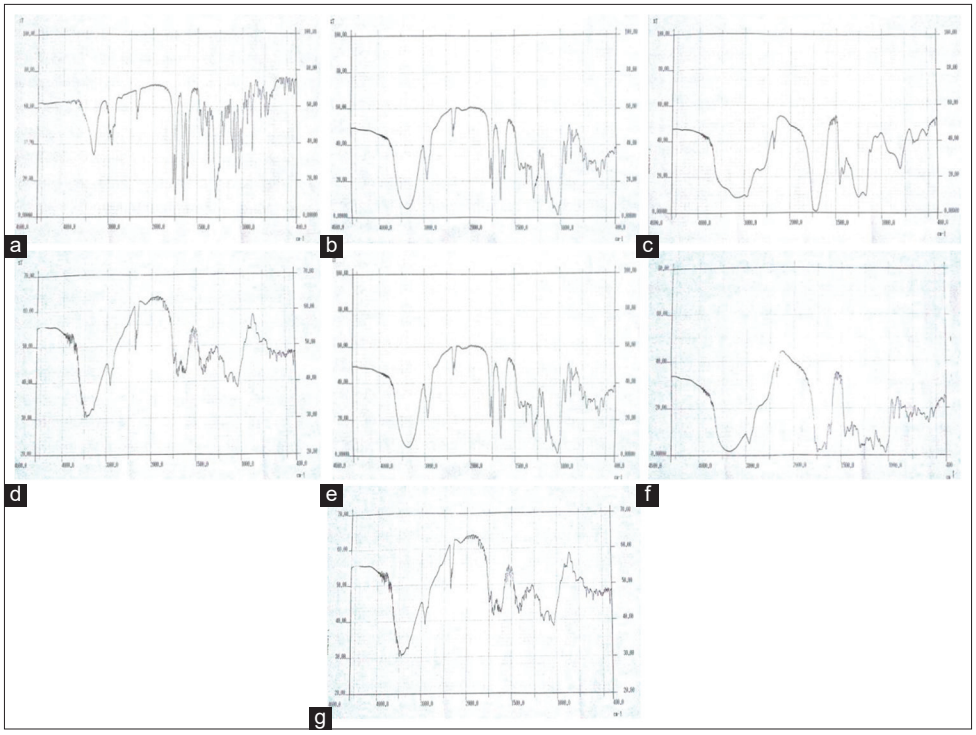

**Fig. 2: Fourier transform infrared spectra of (a) loteprednol etabonate, (b) beta-cyclodextrin (β-CD), (c) Carbopol 971P NF, (d) gellan gum, (e) loteprednol etabonate: β-CD (1:1) complex, (f) Drug-B-CD (1:1) complex+Carbopol 971P NF, (g) Drug-B-CD (1:1) complex+gellan gum**

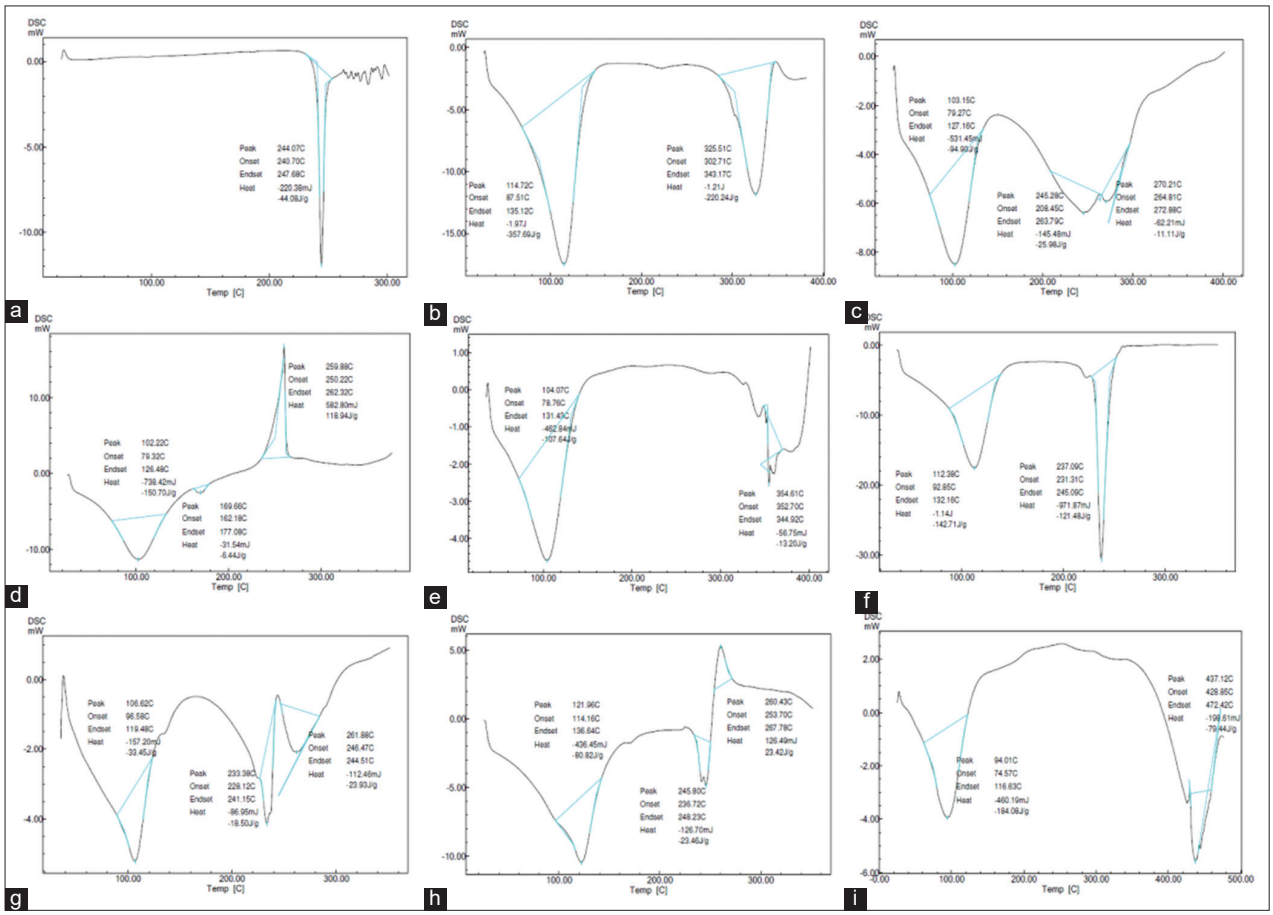

**Fig. 3 Differential scanning calorimetry scan of (a) loteprednol etabonate, (b) beta-cyclodextrin (β-CD), (c) Carbopol 971P NF, (d) gellan gum, (e) hydroxypropyl methylcellulose (HPMC), (f) loteprednol etabonate: β-CD (1:1) complex, (g) Drug-B-CD (11) Complex+Carbopol 971P NF, (h) Drug-B-CD (1:1) complex+gellan gum, (i) Drug-B-CD (1:1) complex + HPMC**

**Formulation and development of optimized ion-triggered and pH-dependent *in situ* gelling ophthalmic solutions (Fig. 5)**


**Fig. 5: Formulations F1-F6**

**Evaluation**

*Viscosity studies (Fig. 6)*

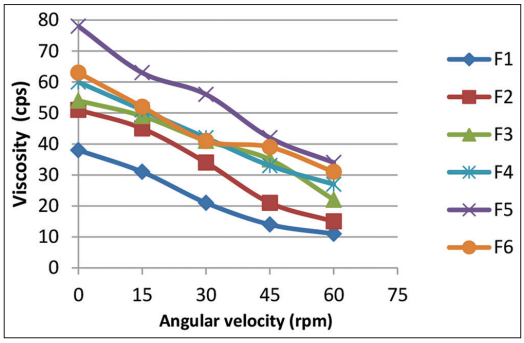


**Fig. 6: Viscosity studies of formulation F1-F6 as sols**

***In vitro* gelation and isotonicity (Fig. 7)**

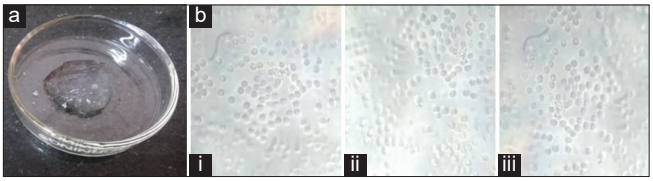


**Fig. 7: (a) *In vitro* gelation of formulation F2 (b) isotonicity evaluation (i) F2, (ii) F5, (iii) marketed product**

**Drug content (Table 4)**

**Table 4: Drug content of formulations F1-F6**

| S. No. | Formulation | Drug content (%) |
|---|---|---|
| 1. | F1 | 97.2 |
| 2. | F2 | 96.4 |
| 3. | F3 | 99.21 |
| 4. | F4 | 98.07 |
| 5. | F5 | 95.6 |
| 6. | F6 | 95.26 |

***In vitro* release studies (Fig. 8)**

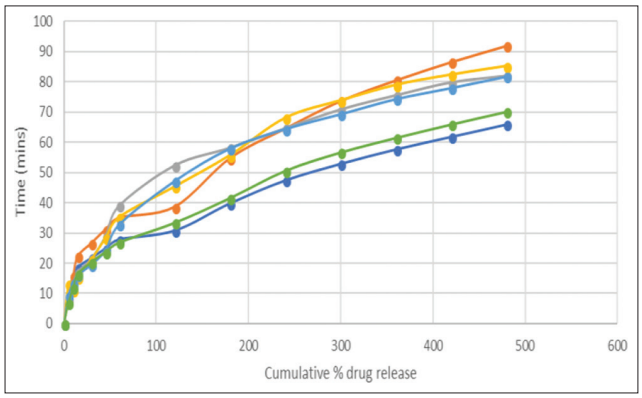


**Fig. 8: *In vitro* drug release profile**

**Sterility testing (Fig. 9)**

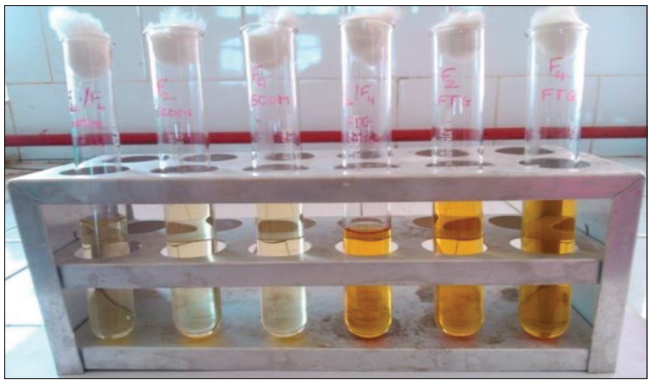

**Fig. 9: Photograph showing results of sterility tests on formulations F2 and F4**

**Stability studies (Table 5)**

**Table 5: Stability results**

| S. No. | Formulation | Phase separation | Gelling capacity | Drug content |
|---|---|---|---|---|
| 1. | F2 | None | Retained | 96.02% |
| 2. | F4 | None | Retained | 98.7% |

## DISCUSSION

### Compatibility studies

*FT-IR spectroscopy*

The IR spectra of loteprednol etabonate did not show any significant difference from those obtained for their physical mixtures. The results indicate that there was no positive evidence of interaction between drug and the polymers, more than if any hydrogen bonding, which may have occurred between the donating and accepting groups of both the drug and the polymers.

*DSC*

On comparison, the scans displayed that the physical mixtures of the active and various excipients did not show any peaks before the main peak in the thermal scan of loteprednol etabonate. Any peaks seen before were inherent in the individual thermal scans of the excipients.

### Preliminary studies

*Phase solubility studies*

The phase-solubility profile showed a type of solubility profile, indicating that the aqueous solubility of the substrate, i.e., the drug increases with the increase in the concentration of the ligand, namely, β-CD. The complex here is the first order with respect to ligand and first or higher order with respect to substrate. The apparent solubility of loteprednol etabonate increased linearly on addition of β-CD up to a concentration of 45 mM giving the $A_L$-type phase-solubility profile having an average slope of 0.896. Taking the aqueous solubility ($S_0$) of the drug 0.0171 mM (0.008 mg/ml), the stability constant $K_C$ was calculated using the formula.

The stability constant $K_C$ was found to be 501.0 $M^{-1}$ which is very close to the mean value of $K_C$ for β-CD. It also showed that the complex was formed in the ratio 1:1. From the value of the stability constant $K_C$, the CE was calculated using the formula. The CE was calculated to be 8.567.

### Preparation of loteprednol etabonate-β-CD (1:1) complex

The drug-β-CD 1:1 complex was prepared using the kneading technique. Hydroalcoholic solution of distilled water and methanol in the ratio of 1:1 was used as the binding solvent. Following kneading, the mass was oven dried for 24 h in a vacuum oven at a temperature not exceeding 40°C. Following the drying, the complex was evaluated to check its solubility. For this purpose, an amount of the complex equivalent

to the proposed dose of the drug was weighed and dissolved in the appropriate amount of vehicle, i.e., distilled water. The test showed that almost all the complex completely solubilized in the aqueous media with no or negligible residue left behind.

**Development of optimized placebo *in situ* gelling systems**

To deduce the concentrations of the polymers to be employed in the actual formulation aspect, placebo *in situ* gelling systems were developed and tested. These *in situ* gels were prepared in varying permutation and combinations to determine the best candidates for the formulation. While gellan gum was used alone, Carbopol 971P NF was evaluated in combination with various concentrations of HPMC grades. This was due to the acidic nature of polyacrylic acid that produced strongly acidic solutions when used in higher concentrations to achieve desired rheology characteristics. Hence, it had to be used in lower concentrations and the viscosity compensated by means of HPMC. The vehicle used for gellan gum was distilled water while that used for Carbopol 971P NF was phosphate buffer pH 6.8. In case of each of the test placebo system, the parameters evaluated were the gelling strength (gelling time), gel structure and cohesiveness, the clarity, and the gelling capacity.

It was observed that, increase in the polymer concentration in the liquid form, showed increase in viscosity and upon gelation it showed increase in cohesiveness of structure and consistency. The effects were similar with both the polymers i.e gellan gum and Carbopol 971P NF. While gellan gum produced clear gels, the ones made with Carbopol 971P NF were milky translucent in appearance. The minimum concentration of the gellan gum at which gelation was seen was 0.3% w/v. However, the gel produced was loose and watery in consistency and dissolved rapidly. The concentrations above 0.3% w/v showed promising results and were thus carried forward into the actual formulation.

In case of the polymer Carbopol 971P NF, the concentration of 0.3% w/v showed first signs of gelation; however, the gel formed was loose and inconsistent. Hence, concentrations above 0.3% w/v in combination of 0.2%w/v HPMC grades were chosen for further development.

**Formulation and development of optimized ion-triggered and pH-dependent *in situ* gelling ophthalmic solutions**

Formulations F1, F2, and F3 were based on gellan gum with distilled water as the vehicle and aimed at bringing about ion-sensitive gelation triggered by the calcium ions in the tear fluid. Formulations F4, F5, and F6 were based on combinations of Carbopol 971P NF and HPMC grades with phosphate buffer pH 6.8 as the vehicle. The nature of polyacrylic acid is such that higher concentrations yield desired rheological characteristics and form strongly acidic solutions that can be irritating to the eye. To circumvent this, Carbopol 971P NF was used in combination with two HPMC grades to enhance the viscosity of the formulations without the substantial lowering of the pH. The reasoning behind the latter choice was to buffer the formulations into the pH range of 6.0–6.5 which would have otherwise been strongly acidic due to polyacrylic acid.

Benzalkonium chloride was the preservative of choice for formulations F4, F5, and F6. The sensitivity and incompatibility or gellan gum to it, however, lead to the use of an alternative preservative in the formulations F1, F2, and F3. Upon terminal sterilization, thermoreversible gelling was noticed in case of formulations F4, F5, and F6. This was attributed to the use of HPMC that undergoes thermoreversible gelation at 40–45°C.

**Evaluation of the *in situ* gelling ophthalmic systems**

*Clarity and appearance*

All the formulations (F1-F6) were found to be free from any solid foreign particles. The formulations based on gellan gum were clear and transparent in appearance. However, formulations F4, F5, and F6 composed of polyacrylic acid and HPMC grades were milky in appearance. Upon gelation, the formulations F1, F2, and F3 formed clear gels with no precipitation or phase separation. Formulation F4, F5, and F6 formed translucent gels when triggered with no precipitation or phase separation.

*Texture analysis*

The firmness, consistency, and cohesiveness of formulation were assessed based on the flow properties of the sol. Upon gelation, the structure and cohesiveness of the *in situ* gels were also evaluated.

The pourability of the sol was quite satisfactory exhibiting a smooth flow ensuring easy administration of the product through the dropper. It was seen that upon standing for long periods of time, formulations with the highest concentration of polymer and containing the higher grade of viscolizer (HPMC K15 M), namely, F3 and F5 tended to settle. However, the application of shear stress brought them back to their original consistency. Formulation F6 with equal concentrations of both HPMC K4 M and K15 M showed intermediate behavior. In terms of pourability, formulations F2 and F4 showed optimum behavior.

Upon gelation, it was seen that the formulation F1 formed a loose watery matrix, whereas F2 and F4 with intermediate polymer concentrations formed soft but firm gels. Formulation F3, with highest gellan gum concentration, formulation F5 composed of maximum concentration of the high-grade HPMC K15 M, and formulation F6 containing intermediate and equal concentrations of both HPMC grades formed rigid hard gels that could discomfort the eye.

*pH*

The normal ocular pH of the eye ranges from 6 to 8. The formulations F1, F2, and F3 containing gellan gum showed a slightly basic pH so the final pH was adjusted using 0.1 N HCl. The pH of these formulations fell in the range of 7.0–8.0. Due to the strong acidic nature of polyacrylic acid, formulation F4, F5, and F6 containing Carbopol 971P NF showed pH values in the range of 6.0–6.5.

*Viscosity*

At non-physiological conditions, the formulations were in a liquid state and exhibited low viscosity. This viscosity increased with the increase in the polymer concentrations in the formulation. Formulation F1, F2, and F3 showed increase in viscosity on account of the increasing gellan concentration and so did F4, F5, and F6 due to the combinations of polymers used, in the order of F4-F5. Formulation F6 remained intermediate between F4 and F5 for it contained half the concentration of the higher grade HPMC as compared to F5 and the other half was HPMC K4 M. Formulation F5 showed the maximum viscosity due to the high concentration of HPMC K15 M.

An increase in the pH to 7.4 caused the solutions to transform into gels with high viscosity. The formulations exhibited pseudoplastic rheology both in the sol form and on gelation.

*In vitro gelation*

Formulations F1-F6 retained their gelling time within 60 s or less. The gel formed by formulation F1 was found to be loose and watery due to the low polymer concentration. Formulations F3, F5, and F6 formed rigid hard gels that showed the probability of causing discomfort to the eye. Formulations F2 (0.75% w/v gellan gum) and F4 (0.3% w/v Carbopol 971P NF and 0.2% w/v HPMC K4 M) produced soft but firm and flexible gels malleable in accordance of curvature of the eye. In all cases, the gelling was instantaneous and the gels so formed lasted for 9–10 h and then dissolved.

*Isotonicity*

All the formulations F1-F6 were evaluated for their tonicity. The reference standard used for comparison was a marketed product containing 0.5% w/v loteprednol etabonate. It was seen that all the formulations passed the isotonicity test causing neither shrinking nor bursting of the red blood cells when mixed with a drop of the individual formulations and observed under the microscope.

*Drug content*

All the formulations F1-F6 were assessed to determine the drug content and its uniformity. The formulations showed assay values within the

confidence limits of 95–105% with values being closer to the lower limit.

The assay values concluded that both the polymers irrespective of their trigger mechanisms were capable of forming suitable matrices for the entrapment of the drug. Furthermore, it was noted that soaking and hydration of the polymer in the drug solution as opposed to soaking the polymer in the pure solvent followed by solvation of the drug in the polymer solution significantly improved the formulation procedure and resulted in better drug content.

*In vitro release studies*

Eight-hour diffusion studies were carried out on all formulations. A fabricated diffusion cell consisting of a donor compartment and receptor compartment was used for the study. Samples were withdrawn in quantities of 5 ml from the receptor compartment and it was subsequently replenished with fresh diffusion medium. The samples were analyzed for drug release spectrophotometrically.

The release mechanism was understood to be slow controlled release type with an initial immediate burst. It was postulated that upon coming in contact with its trigger be it the presence of calcium ions or the pH change, each formulation undergoes rigidization and forms a tight matrix that entraps the solubilized drug and thus controls its release. The initial burst release is explained by the prehydration of the polymer during the formulation. It is eminent here to remember that the vehicles employed in both kinds of formulations were aqueous in nature. Furthermore, the polymers were hydrated in the vehicles for a good 24 h. With both the polymers, i.e., gellan and Carbopol having hydrophilic properties, the matrices when come in contact with the medium, due to the prehydration, the water permeation in them is not controlled resulting in the burst release. Over the course of time, the release follows a concentration-dependent controlled pattern. The immediate burst release was more prominent in formulations F1, F2, and F3 due to the highly hydrophilic nature of gellan gum. The release studies of each formulation were done in duplicates and triplicates to assess the reproducibility and reliability of the data.

In case of gellan gum-based *in situ* gels, the minimum cumulative percentage drug release was shown by formulation F1, i.e., 65.98%, whereas in the case of Carbopol 971P NF and HPMC-based formulations, F6 showed the minimum release of 70.16%. The overall maximum release was shown by formulation F2 composed of 0.75% w/v gellan gum.

*Microbial tests*

Based on the assessment of the results of parameters tested, one formulation each, i.e. ion triggered and pH dependent was chosen from all formulations and was subjected to microbial tests.

*Sterility testing*

A total of six tubes were incubated, three of each medium (Soybean Casein Digest Medium and Fluid Thioglycollate Medium). While two test tubes contained the test sample (formulation) each, the third test tube as acted as control. After the completion of the incubation period, upon observation, it was seen that the two tubes containing the test samples showed no viable growth just as in the case of control. Thus, in conclusion, both the test samples passed the sterility testing.

*Effectiveness of preservative*

The test samples were inoculated with specific cultures, namely, Gram-positive, Gram-negative, and fungi. Viable counts were taken by pour plate method on days 0, 7, 14, and 21. A control of each test sample was kept which was subjected to the viable count as in the case of test samples. At the end of 21 day period, it was seen that the viable count drastically decreased every 7 days and thus the preservatives passed as per I.P. limits.

*Optimized formulation*

Based on the results of the parameters evaluated and thorough interpretation of data of *in vitro* release studies and kinetic modeling, two formulations, one based on each *in situ* gelling polymer, were chosen to be optimized formulations.

Formulation F2 based on gellan gum (0.75% w/v) and F4 based on polyacrylic acid (0.3% w/v Carbopol 971P NF and 0.2% w/v HPMC K4 M) successfully passed all the evaluator tests, suggesting their superiority from the counterparts and catapulting their use in further carrying stability studies. They were thus optimized formulations.

*Stability studies*

It was seen that formulation F4 slightly thickened on account of the thermoreversible gelling of HPMC. However, the viscosity was retained subsequently with no signs of phase separation. Formulation F2 showed increase in viscosity on standing independent of the temperature and humidity conditions which was restored on application of shear stress. This proved the pseudoplastic rheology of the formulation. Both the formulations retained their gelling capacity. The assay of the formulations F2 and F4 revealed the values to be in the limits 95–105%. The stability studies indicated that the formulation was physically and chemically stable with no significant change in any of the parameters evaluated.

## CONCLUSION

To overcome reduced corneal permeability of steroid cyclodextrin, inclusion complexes was developed. Aqueous solubility and corneal perfusion are increased. β-CD, one of the natural unsubstituted cyclodextrins, was employed for solubility enhancement of the drug. Two different polymers with different triggering mechanisms were chosen. One polymer was gellan gum gelled in the presence of divalent and monovalent cations, namely, calcium and sodium present in the tear fluid. The second choice was polyacrylic acid (Carbopol 971P NF) in combination with two HPMC grades, namely, HPMC K4 M and K15 M. Eight hour *in vitro* drug release diffusion studies showed controlled release affected by the drug concentration gradient. Formulation F2 containing 0.75% w/v gellan gum and F4 containing 0.3% and 0.2% w/v Carbopol 971P NF and HPMC K4 M, respectively, were found to be these optimized formulations.

## ACKNOWLEDGMENTS

The authors are thankful to Oxford Laboratories, Mumbai, India, Lubrizol Advanced Materials India Pvt. Ltd., Mumbai, Colorcon Asia Ltd., Goa, for gift sample of drug and excipients.

## AUTHORS' CONTRIBUTIONS

All authors have contributed equally.

## CONFLICTS OF INTEREST

Declared none.

## REFERENCES

1. Harish NM, Prabhu P, Charyulu RN, Gulzar MA, Subrahmanyam EV. Formulation and evaluation of *in situ* gels containing clotrimazole for oral candidiasis. Indian J Pharm Sci 2009;71:421-7.
2. Merkli A, Tabatabay C, Gurny R. Biodegradable polymers for the controlled release of ocular drugs. Prog Polym Sci 1998;23:563-80.
3. Swarbrick J. Complexation: Cyclodextrins. In: Encyclopedia of Pharmaceutical Technology. 3rd ed., Vol. 1. New York: Informa Healthcare Inc.; 2007. p. 171-97.
4. Loftsson T, Jarho P, Másson M, Järvinen T. Cyclodextrins in drug delivery. Expert Opin Drug Deliv 2005;2:335-51.
5. Patil JS, Kadam DV, Marapur SC, Kamalapur MV. Inclusion complex system; a novel technique to improve the solubility and bioavailability of poorly soluble drugs: A review. Int J Pharm Sci Rev Res 2010;2:29-34.
6. Sweetman SC. Corticosteroids. Martindale: The Complete Drug

Reference. 36th ed. London: RPS Publishing; 2009. p. 1490-537.
7. Bajaj IB, Survase SA, Saudagar PS, Singhal RS. Gellangum: Fermentative production, downstream processing and applications. Food Technol Biotechnol 2007;45:341-54.
8. Carter SJ. Ophthalmic products. In: Cooper and Gunn's dispensing for pharmaceutical students. 12th ed. New Delhi: CBS Publishers; 2013. p. 635-8.
9. Comstock T, DeCory H. Advances in corticosteroid therapy for ocular inflammation: Loteprednoletabonate. Int J Inflam 2012;789623:1-11.
10. Loftsson T, Hreinsdóttir D, Másson M. Evaluation of cyclodextrin solubilization of drugs. Int J Pharm 2005;302:18-28.
11. Paulsson M, Hägerström H, Edsman K. Rheological studies of the gelation of deacetylated gellan gum (Gelrite) in physiological conditions. Eur J Pharm Sci 1999;9:99-105.
12. Bodor N, Drustrup J, Wu W. Effect of cyclodextrins on the solubility and stability of a novel soft corticosteroid, loteprednol etabonate. Pharmazie 2000;55:206-9.
13. Davies NM, Wang G, Tucker IG. Evaluation of hydrocortisone/hydroxypropyl-β-cyclodextrin solution for ocular drug delivery. Intl J Pharm 1997;156:201-9.
14. Sanz Taberner T, Martín-Villodre A, Pla-Delfina JM, Herráez JV. Consistency of carbopol 971-P NF gels and influence of soluble and cross-linked PVP. Int J Pharm 2002;233:43-50.
15. Deulker A, Gude R, Sancoalcar A, Vaidya S. Formulation development and evaluation of long acting ophthalmic *in situ* gelling system of dorzolamide hydrochloride. Int J Drug Dev Res 2013;5:156-63.
16. Mohan EC, Kandukuri JM, Allenki V. Preparation and evaluation of *in situ* gels for ocular drug delivery. J Pharm Res 2009;2;1089-94.
17. Liu Y, Liu J, Zhang X, Zhang R, Huang Y, Wu C, *et al. In situ* gelling gelrite/alginate formulations as vehicles for ophthalmic drug delivery. AAPS PharmSciTech 2010;11:610-20.
18. Kumar JR, Muralidharan S. Formulation and *in vitro* evaluation of gellan gum/carbopol and sodium alginate based solution to gel depot of ketotifenfumarate system. J Pharm Sci Res 2012;4:1973-7.
19. Lena MT. Formulation and evaluation of floating oral *in situ* gel of metronidazole. Int J Pharm Pharm Sci 2014;6:265-9.
20. Doijad RC, Manvi FV, Malleswara VS, Alase P. Sustained ophthalmic delivery of gatifloxacin from *in situ* gelling system. Indian J Pharm Sci 2006;68:809-14.
21. Rathore KS. *In situ* gelling ophthalmic drug delivery system: An overview. Int J Pharm Pharm Sci 2010;2:30-4.
22. Kumar M, Kulkarni GT. Recent advances in opthlmic drug delivery system. Int J Pharm Pharm Sci 2012;4:387-94.
23. Shakta MS, Shalini A, Bharti C, Laxminarayan K, Rajadurai P, Rajdip LV. Effect of fucithalmic and sofinox eye drops on experimental allergic conjunctivitu in rats. Int J Pharm Pharm Sci 2014;6:458-60.