THERMOSENSITIVE IN SITU GEL OF BRINZOLAMIDE FOR SUSTAINED OCULAR DRUG DELIVERY

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Abstract: The objective of this research was to formulate and evaluate sustained release in situ gels of Brinzolamide, an anti-glaucoma agent. Thermo reversible in situ gels were prepared using polymers like Poloxamer 338, HPMC K4M and Carbopol 974 as viscofying agent by cold process. The in situ gels were evaluated for physical characteristics, pH, gelling capacity, sol-gel transition temperature, rheological behavior before and after gelation and uniformity in drug content. Infrared spectroscopy and differential scanning calorimetry were performed to confirm the interaction of drug and polymers in formulation. The optimized formula were further evaluated for ex-vivo permeation studies using goat cornea, scanning electron microscopy, stability studies, sterilization and ocular irritancy studies. The in situ gelling formulations were free-flowing, transparent, had uniform consistency, and had spreadability at room temperature. Among the formulations, formula containing Poloxamer 338 (22%) and HPMC K4M (1%) was clear transparent solution, having pH of 7.1, gelation was within 60 seconds and remained stable for 6 hours, sol-gel transition temperature was 37°C, viscosity before gelation was 37.8 ± 0.48 mPas and after gelation was 155 ± 0.65 mPas. Optimized formulation showed controlled release with 94.64 ± 0.554 at the end of 24 hrs. Ex-vivo permeation was found to be 89.18%± 0.748 after 24 hrs. The SEM of in situ gel suggested that the drug was well dispersed in the polymer matrix. No interaction was found in drug-polymer mixture. The results obtained from the developed controlled release systems are an alternative to conventional ophthalmic delivery systems, are patient compliance, industrially oriented and economical.

Keywords: In situ gels, HPMC K4M, Poloxamer 338, Ocular drug delivery, Sustained delivery

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INTRODUCTION

Glaucoma is a slowly progressive pathology that can lead to vision loss or complete blindness [1]. Globally, 60.5 million had glaucoma in 2010, so glaucoma is considered as the leading cause of world’s blindness. With the current therapeutic systems in the field of ophthalmic drug delivery and with the aging of the world’s population, the number of glaucoma blindness may increase to almost 80 million by 2020 [2].

The main cause of patients experiencing undiagnosed loss of vision until the advanced stages of the disease has occurred is because of the asymptomatic nature of the disease in the early phases. Therefore this disease is also called as the “silent thief of sight” [3].

Early diagnosis & treatment becomes very important for open-angle glaucoma as it can delay progression of the disease [4]. The currently available five main classes of topical medications for the treatment of glaucoma which are used to either decrease aqueous production with beta blockers (BB), alpha agonists (AA), and carbonic anhydrase inhibitors (CAIs) or improve aqueous outflow with cholinergics and PGAs. These medications are commonly available as eye drops [5]. Conventional eye drops face rapid tear turnover, blinking, lacrimation, lachrymal nasal drainage and various barriers of the eye. Therefore the bioavailability of ophthalmic drugs is very poor due to efficient protective mechanisms of the eye. So to overcome these problems current innovation in glaucoma treatment is focused on the improvement of drug delivery methods. The aim is to develop intra ocular pressure lowering sustained release therapies for the treatment of glaucoma [6].

The novel ocular drug delivery system includes nanotechnology based delivery systems like nano-particles, liposomes, nano-suspensions, etc, in situ gelling system, contact lens, ocular inserts, implants and microneedles as shown in the figure [7]. The present study deals with the development of ocular in situ gels for sustained release of Brinzolamide.

![Fig. 1: Novel ocular drug delivery systems](image-url)
Brinzolamide is a potent inhibitor of carbonic anhydrases ($IC_{50}$s = 3.2 and 45.3 nM for Carbonic anhydrase II and IV, respectively) that lowers intraocular pressure by locally inhibiting carbonic anhydrase in the ciliary processes and suppressing aqueous humor secretion. Brinzolamide is available as 1% w/v suspension & administered as one drop in the affected eye(s) 3 times daily for Ocular Hypertension and Glaucoma [8]. In clinical trials, ophthalmic preparations of brinzolamide were similar in efficacy to dorzolamide for the management of primary open-angle glaucoma and ocular hypertension. Topical carbonic anhydrase inhibitors such as brinzolamide generally possess a more favourable adverse event profile than their oral predecessor (acetazolamide). In comparative studies, brinzolamide produced ocular discomfort less frequently than dorzolamide [9].

In situ gels which have been developed are liquid dosage forms based on an in situ gelling solution, which consists of some polymers undergoing sol–gel phase transitions as a result of a special physical/chemical change (for example, pH, temperature or a specific ion) induced by the physiological environment have been investigated as more convenient dosage form of topical ocular application. Such systems upon administration undergo sol to gel transition and increase ocular bioavailability of the drug [10]. In situ gelling systems are of three types viz pH triggered systems (Carbopol, cellulose acetate phthalate latex), temperature dependant (Pluronic&tetronic), Ion-activated (gelrite, sodium alginate). Poloxamer (trade name Pluronic®), is known for exhibiting the phenomenon of reversible thermal gelation under a certain concentration and temperature [11].

Poloxamers are non-ionic poly (ethylene oxide) (PEO) – poly (propylene oxide) (PPO) copolymers. All poloxamers have similar chemical structures but with different molecular weights and composition of the hydrophilic PEO block (a) and hydrophobic PPO block (b) [12]. Poloxamer is available in different grade based on the physical parameters like Molecular Weight, Weight % of oxyethylene etc. The common available grades are poloxamer (68, 88, 98, 108, 124, 188, 237, 338, and 407). Three polymers from this class, poloxamer 188, poloxamer 338 and poloxamer 407, show inverse thermosensitivity; therefore, they are soluble in aqueous solutions at low temperature, but will gel at higher temperature [13]. Poloxamer 338 is used primarily as thickening agent and gel formers, but also as co-emulsifiers and consistency enhancers in creams and liquid emulsions. Owing to its ability to affect viscosity, Poloxamer 338 is suitable as stabilizer for topically administered suspensions [14]. Thermoreversible gelation is observed in aqueous solutions of concentration range 16-30%w/w. they are liquid when refrigerated (4-5°C) or heated to temperatures exceeding 70°C but turn into gel form when at room temperature. They exhibit maximum viscosity at 30-60°C. The gels thus formed are reversible again on cooling or heating [15].

MATERIALS AND METHODS
Materials

Brinzolamide was provided by Cipla pharmaceuticals Ltd. Hydroxy Propyl Methyl Cellulose (HPMC K4M) was a gift sample from Colorcon, Mumbai. Carbopol 974 was a gift sample from Lubrizol, Mumbai. Poloxamer 338(Lutrol F108) was provided by BASF, Mumbai. PEG 400, Benzalkonium chloride, Sodium chloride were procured from S.D. Fine Chem. Ltd Mumbai India. All other reagents used were of analytical grade.

ANALYTICAL METHOD DEVELOPMENT OF BRINZOLAMIDE

Analytical method development and validation play an important role in the formulation and manufacture of pharmaceuticals. For routine analysis of drug in respective formulations, assessment of drug content in the developed formulations and investigation of release of drug from the developed formulations, suitable analytical methods need to be developed and validated. The method was validated for Linearity, Accuracy, Precision, robustness, limit of detection, limit of quantification.

UV Method Development in simulated tear fluid pH 7.4

Preparation of simulated tear fluid (STF) pH 7.4:

Dissolve 6.7 g of sodium chloride, 2 g of sodium bicarbonate, and 0.08 g of calcium chloride dehydrate in 1000ml of distilled water. The pH of the solution was adjusted to 7.4 using pH meter [16].

Preparation of Brinzolamide standard stock solution:

Brinzolamide standard stock solution was prepared by dissolving accurately weighed 10 mg of Brinzolamide in 100 ml volumetric flask. The volume was then made up to 100 ml by using STF pH 7.4 to obtain the solution of 100µg/ml.

Scanning of Brinzolamide by UV-Visible spectrophotometer in STF pH 7.4:

From the standard stock solution, 1 ml was transferred to 10 ml volumetric flask. The Volume was made up to 10 ml with STF pH 7.4. The resulting solution containing 10µg/ml was scanned between 200 and 400 nm and the spectrum was drawn. The λ max was found to be 254 nm and was used as analytical wavelength throughout the study.

Calibration curve of Brinzolamide:

From the Brinzolamide standard stock solution (100µg/ml), appropriate aliquots of 0.5, 1, 1.5, 2, 2.5, 3 ml were taken into different volumetric flask and made up to 10 ml with STF pH 7.4, so as to get drug concentrations from 0 to 30 ppm. The absorbance of these drug solutions were
estimated at $\lambda_{\text{max}}$ 254 nm. This procedure was performed in triplicate to validate the calibration curve. A calibration curve was constructed using the data.

**PREPARATION OF THERMOREVERSIBLE IN SITU GELS OF BRINZOLAMIDE:**

Thermoreversible *in situ* gels of Brinzolamide were formulated using different ratios of Poloxamer 338 as gelling agent in combination with HPMC or Carbopol 940, as viscosity enhancing agents.

**Preparation of ocular *in situ* gel:**

Aqueous dispersions of selected concentrations of HPMC K4M, Carbopol 974 and Poloxamer 338 were prepared. The Poloxamer/HPMC combination and the Poloxamer/Carbopol combination were prepared by dispersing the Pluronic in the desired concentration of respective polymer solutions. Then the partially dissolved solutions were refrigerated until thoroughly mixed. An appropriate amount of Brinzolamide was dissolved in PEG 400, benzalkonium chloride and sodium chloride was added with continuous stirring until uniform solution was obtained. The drug solution was finally added to polymer solution with continuous stirring. The developed formulations were filled in ophthalmic squeeze dispenser (OSD) provided by Aptar Pharma [17].

**Table 1: Composition of in situ gels of Brinzolamide**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formulation code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
</tr>
<tr>
<td>Drug</td>
<td>1%</td>
</tr>
<tr>
<td>Poloxamer 338</td>
<td>22%</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>1.50%</td>
</tr>
<tr>
<td>Carbopol 974</td>
<td>-</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>0.02%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.90%</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

**Evaluation of ocular *in situ* gels:**

a) **Physical parameters**

The formulated *in situ* gel solution was tested for clarity & transparency.

b) **Gelling capacity**
The gelling capacity was determined by placing a drop of the formulation in a watch glass containing 2ml of freshly prepared simulated tear fluid and visually observed. The time taken for gelation and to dissolve was noted visually.

c) Rheological studies

The viscosity measurements were made using Brookfield viscometer (DV III ULTRA programmable Rheometer). The in situ gel formulations were placed in the sample tube. Viscosity was checked before and after gelling in simulated tear fluid respectively. The angular velocity of the spindle was increased to 5, 10, 20, 30, 50, 100 rpm and the rheological behaviour of the formulation was measured.

d) pH

The pH of ophthalmic formulations was determined using pH meter (EUTECH instrument).

e) Drug content

Drug content of in situ gels was determined by dissolving 0.2ml of the formulation in 10 ml of methanol in a volumetric flask and filtering. The absorbance of each of these solutions was measured on UV-visible spectrophotometer at 254 nm.

f) Measurement of the Sol-Gel transition temperature

An aliquot of 2ml of formulation was transferred to a test tube and sealed with paraffilm. The tube was maintained in a thermostatically controlled water bath at 4°C. The temperature of the water bath was increased gradually in increments of 3°C in the beginning of the experiment and then 1°C increment in the region of sol-gel transition temperature. The gelation was said to occur when the meniscus would no longer move upon tilting through angle 90°.

g) In Vitro diffusion Study of ocular in situ gels of Brinzolamide:

The in vitro diffusion of drug from the ophthalmic inserts was explored using Franz diffusion cell. Dialysis membrane No.150 (HiMedia Laboratories, Mumbai) was tied to one end of open cylinder, which acts as a donor compartment. The dialysis membrane was considered as corneal epithelium. 1 ml of the ocular in situ gel was accurately pipette on the dialysis membrane of the donor compartment. An ophthalmic insert was placed in the donor compartment. The content of the receptor compartment was stirred continuously using a magnetic stirrer at 200 rpm and temperature was maintained at 37± 2°C. Aliquots (2ml) were withdrawn at 10, 20, 30, 45, 60 minutes, & 2, 3, 4, 5, 6, 8, & 24 hours and replaced by equal volume of fresh solution each time. The samples were analyzed spectrophotometrically at 254
nm against reference standard using STF as blank. Cumulative % drug diffused was calculated. A graph of time (in mins) Vs cumulative % drug diffused was plotted.

h) **Ex Vivo Permeation Study of ocular in situ gel of Brinzolamide:**

The ex vivo permeation studies were carried out for optimized formulations using Franz diffusion cell. Goat cornea was mounted onto a Franz-diffusion cell in such a way that corneum side continuously remained in an intimate contact with in situ gel in the donor compartment.

*Corneal membrane preparation:* Fresh whole eye ball of goat was transported from the local butcher shop to the laboratory in cold normal saline within 1 hour of slaughtering of the animal. The cornea was carefully excised along with 2 to 4mm of surrounding sclera tissue and was washed with cold normal saline till the washing was free from proteins.

*Permeation Experiment:* Isolated cornea was mounted by sandwiching surrounding sclera tissue between clamped donor and receptor compartments of Franz diffusion cell in such a way that its epithelial surface faced the donor compartment. The receptor compartment was filled with 22 ml of freshly prepared simulated tear fluid (pH 7.4). An aliquot (1 ml) of test formulation was placed on the cornea and opening of the donor compartment was sealed with a glass cover slip, while the receptor fluid was maintained at 37± 2ºC with constant stirring at 200 rpm, using a Teflon-coated magnetic stir bead. 2 ml samples were withdrawn from the receptor compartment at 10, 20, 30, 45, 60 minutes, & 2, 3, 4, 5, 6, 8, & 24 hours. The samples were analyzed for Brinzolamide content spectrophotometrically at 254 nm. Each sample withdrawn was replaced with equal volume diffusion medium (simulated tear fluid pH 7.4) [18].

i) **Retention study:** After completion of 24 hrs of diffusion study, the mounted cornea was removed and scrapped-off gently to remove the formulation overlying on the cornea. The corneal tissue was smashed with required amount of methanol and was evaluated for brinzolamide content. Percent brinzolamide retained on the corneal membrane was calculated.

![Figure 2: Photograph of a) Goat eye  b) Goat cornea](image_url)
j) **Surface morphology-SEM analysis:**

The morphology of ocular in situ gel was studied using a Quanta 200 ESEM scanning electron microscope (SEM). The surfaces of the ocuserts & *in situ* gel were analyzed. Quanta 200 ESEM scanning electron microscope has the following features: resolution-3nm, magnification-300,000 x, Secondary & Backscattered Imaging, Boron-Uranium Elemental Analysis (Microanalysis). The devices were analyzed at 0.2 – 30 kV acceleration voltages using varying magnification like 500x to 2000x for each sample. Representative micrographs were taken.

k) **Drug-excipient compatibility studies**

Drug-excipients compatibility studies were investigated using Fourier transform infrared spectroscopy (FTIR) and Differential scanning calorimetry.

**Fourier Transform Infrared Spectroscopy (FTIR):** IR spectra of Brinzolamide & blends of drug and poloxamer 338, HPMC K4m, physical mixture of formulation were recorded on a FTIR spectrophotometer (Perkin Elmer RX-1) in the range of 4000–400 cm\(^{-1}\) using potassium bromide discs. Individual samples as well as the mixture of drug and excipients were ground, mixed thoroughly with potassium bromide for 3-5mins in a mortar and compressed into disc by applying a pressure in hydraulic press. The concentration of sample in potassium bromide should be in the range of 0.2% to 1%. The pellets were placed in light path and spectrum was obtained and reviewed for evidence of any interactions.

**Differential Scanning Calorimetry:** A drug-excipients compatibility study of API and formulation was investigated using Differential scanning calorimetry. In Differential Scanning Calorimetry (METTLER-TOLEDEO DSC1) any drastic changes in the formulation with the thermal behavior of either the drug or the excipients are visualized. It is a thermodynamic technique where the sample and reference material are subjected to a controlled temperature programmed and the difference in energy inputs between the sample and reference material is measured as a function of temperature. Both the sample and reference are maintained at the same temperature throughout the experiment. The temperature programmed for DSC analysis is designed such that the sample holder temperature increases linearly as a function of time.

l) **Sterilization of ocular in situ gels**

The optimized in situ gels were sterilized by gamma radiation using the Cobalt-60 isotope as source of radiation at ISOMED at Trombay, Mumbai. These sterile formulations were used for ocular irritancy study.
m) Ocular irritancy test

The ocular irritation study was performed on rabbits using optimized in situ gel of Brinzolamide. After administration of the formulation, the rabbit eyes were inspected visually at specific time intervals. Approval of the Institutional Animal Ethic Committee was obtained prior to the commencing of the study. The approval number is CPCSEA/IAEC/BNCP/P-47/2014. In situ Gel of Brinzolamide was instilled in right eye into the lower cul-de-sac and left eye considered as control. Both eyes of rabbit under test were examined periodically for erythema, edema, and lacrimation and for any sign of irritation before treatment and 30 min, 1hr, 24 hrs, 48hrs, 72 hrs, and 1 week after insertion.

n) Stability studies on developed formulations:

The stability studies on optimized in situ gel were conducted according to ICH guidelines for a period of three months. In situ gels are being stored in OSD containers by Aptar Pharma. The samples were kept at the following conditions as per ICH guidelines: 5°C ± 3°C, 25°C ± 2°C / 60 ± 5 % RH and 40°C ± 2°C / 75 ± 5 % RH.

The samples were withdrawn periodically at time intervals of 1st, 2nd and 3rd month and evaluated for various parameters.

- Physico-chemical properties,
- Viscosity (before & after gelation), pH for in situ gel
- Drug content
- Diffusion profiles.

RESULTS & DISCUSSION

UV method development in simulated tear fluid pH 7.4
Evaluation of in situ gels:

**Table 2: Evaluation of In situ gels prepared on parameters like clarity, pH, and Drug content**

<table>
<thead>
<tr>
<th>FORMULATION CODE</th>
<th>Clarity of solution</th>
<th>pH</th>
<th>*Amount of drug present (mg)</th>
<th>*% Drug content AM ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Clear thick</td>
<td>6.08</td>
<td>0.461 ± 0.003</td>
<td>92.18 ± 0.45</td>
</tr>
<tr>
<td>P2</td>
<td>Clear</td>
<td>5.6</td>
<td>0.477 ± 0.003</td>
<td>95.40 ± 0.87</td>
</tr>
<tr>
<td>P3</td>
<td>Clear</td>
<td>7.1</td>
<td>0.490 ± 0.004</td>
<td>98.04 ± 0.75</td>
</tr>
<tr>
<td>P4</td>
<td>Clear &amp; thick</td>
<td>5.72</td>
<td>0.434 ± 0.003</td>
<td>86.72 ± 0.96</td>
</tr>
<tr>
<td>P5</td>
<td>Clear</td>
<td>6.8</td>
<td>0.452 ± 0.002</td>
<td>90.45 ± 0.99</td>
</tr>
<tr>
<td>P6</td>
<td>Clear</td>
<td>6.5</td>
<td>0.458 ± 0.002</td>
<td>91.60 ± 0.72</td>
</tr>
</tbody>
</table>

**Table 3: Evaluation of In situ gels prepared on parameters like gelling behavior, viscosity and sol-gel transition temperature**

<table>
<thead>
<tr>
<th>FORMULATION CODE</th>
<th>Gelling Behaviour</th>
<th>*Viscosity before gelation (Pas)</th>
<th>*Viscosity after gelation (Pas)</th>
<th>*Sol-gel transition temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before gelation</td>
<td>After gelation</td>
<td></td>
</tr>
</tbody>
</table>

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* Average of three determinations

Results are mean of three observations ± standard deviation, + gelation within 50-60 seconds, dissolves rapidly, ++ gelation within 60 seconds and remains stable for 3 hours, +++ gelation within 60 seconds and remains stable for 6 hours

a) **Physical parameters**

The formed *in situ* gels were found to be clear & transparent.

b) **Gelling capacity**

Some formulations were found to gelate immediately & remained as gels for extended periods. Formulations showed varied gelation time due to the varying concentration of HPMC K4M &Carbopol.

![Gelling behavior of P3 in situ gel.](image)

Viscosity before gelation was 27- 52 mPas & after gelation was 80 – 160 mPas. The formulations exhibited pseudo plastic rheology, as was observed shear thinning and decrease in viscosity with increased angular velocity. It was observed, the viscosity was directly dependent on the polymeric content. The viscosity values obtained for formulation P3 at different angular velocity is shown in the table. The formulation exhibited pseudoplastic rheology, as evidenced by shear
thinning and decrease in viscosity with increased angular velocity that can be observed in the figure.

![Rheological behaviour of P3](Image)

**Fig. 5: Rheological behavior of P3 in situ gel**

d) **pH**

The pH of the formulations was found to be in the range of 5.6-7. Most of the formulations were towards neutral pH which confirms that it will not cause eye irritation on application.

e) **Drug content**

Drug content for *in situ* gels was found in the range of 86-99%. The results indicated that the drug was uniformly dispersed.

f) **Sol-Gel transition temperature**

The sol-gel transition temperature was found to be in the range of 32- 40°C. The sol-gel transition temperature of formulation P3 was 37°C. Poloxamer solutions are known to exhibit thermoreversible gelation depending on the polymer grade, concentration, and other included formulation components.

g) **In-vitro drug diffusion studies**

The *in-vitro* release studies were carried out for the selected formulations (P3 & P5) using simulated tear fluids (STF pH 7.4) as the dissolution medium. The formulation P3 was found drug diffused from to be about 94% and that for P5 was 87%.
h) **Ex-vivo Corneal Permeation Studies**

Ex-vivo study was carried out on goat cornea using Franz diffusion cell for 24hrs and drug retention on the cornea was calculated.

*Ex-vivo* studies showed about 85-88% of the drug permeated through the cornea in 24 hours for formulation & 10-13% of the drug was retained on the cornea.

**Fig. 7: Ex-vivo permeation profile of optimized in situ gel formulation, P3**
i) Surface morphology (SEM)

Fig. 8: Scanning electron photomicrographs of the Formulation P3, A): 400 X, B): 2000 X

The SEM of in situ gel suggested that the drug was well dispersed in the polymer matrix.

j) Drug – excipient compatibility studies

FTIR

Fig. 9: FTIR spectrum of Brinzolamide
Fig. 10: FTIR spectra of Brinzolamide and HPMC K4M

Fig. 11: FTIR spectra of Brinzolamide and Lutrol
Differential Scanning Calorimetry: DSC analysis was performed using a Shimadzu differential scanning calorimeter (METTLER-TOLEDEO DSC1). Samples (3–4 mg) were placed in flat-bottomed aluminum pan and heated at a constant rate of 10°C/min in an atmosphere of nitrogen in a temperature range of 20–200°C.

DSC thermogram of Brinzolamide was typical of a crystalline substance, exhibiting a sharp endothermic peak at 133°C relative to its melting point, with onset of the peak at 130.98°C and endset at 138.95°C. The thermogram of in situ gel (P3) did not show the endothermic peak of...
brinzolamide at 133°C. The drug sharp characteristic peak was completely broadened and hardly detected in the DSC thermograms of the formulations which indicate the suppression of the drug crystallinity in the formulations. This is an indication of complete drug amorphization and/or well distribution of Brinzolamide in \textit{in situ} gel formulation.

k) **Sterilization:**

The optimized ocusert and \textit{in situ} gel were sterilized by gamma radiation before \textit{in vivo} study using the Cobalt-60 source.

l) **Ocular Irritancy Test:**

Ocular irritation study was performed to determine whether the developed formulation might cause irritation and pain. It was performed on rabbits using optimized P3 in \textit{in situ} gel. There was no sign of redness and non-continuous blinking of the eye. Thus it was concluded that the formulation was non irritant to rabbit eye.

m) **Stability study of optimized \textit{in situ} gel:**

<table>
<thead>
<tr>
<th>Evaluation parameter/ Stability condition</th>
<th>Appearance</th>
<th>pH</th>
<th>Viscosity (before gelation) mPas</th>
<th>Viscosity (after gelation) mPas</th>
<th>Drug content (%)</th>
<th>% drug diffused</th>
</tr>
</thead>
<tbody>
<tr>
<td>8°C ± 2°C/1 month</td>
<td>Clear transparent</td>
<td>7.0</td>
<td>38 ± 0.05</td>
<td>160 ± 0.26</td>
<td>98.5 ± 0.04</td>
<td>93.5 ± 0.05</td>
</tr>
<tr>
<td>2month</td>
<td>Clear transparent</td>
<td>6.8</td>
<td>40 ± 0.45</td>
<td>165 ± 0.78</td>
<td>98.2 ± 0.09</td>
<td>92.8 ± 0.04</td>
</tr>
<tr>
<td>3month</td>
<td>Clear transparent</td>
<td>6.5</td>
<td>45 ± 0.069</td>
<td>162 ± 0.08</td>
<td>97.5 ± 0.45</td>
<td>91.7 ± 0.96</td>
</tr>
<tr>
<td>25°C ± 2°C/60 ± 5 % RH/1 month</td>
<td>Clear transparent</td>
<td>6.5</td>
<td>42 ± 0.12</td>
<td>155 ± 0.78</td>
<td>98.7 ± 0.25</td>
<td>95 ± 0.47</td>
</tr>
<tr>
<td>2month</td>
<td>Clear transparent</td>
<td>6.8</td>
<td>40 ± 0.36</td>
<td>160 ± 0.54</td>
<td>98.1 ± 0.69</td>
<td>94.8 ± 0.12</td>
</tr>
<tr>
<td>3month</td>
<td>Clear</td>
<td>6.8</td>
<td>35 ± 0.09</td>
<td>152 ± 0.04</td>
<td>97.6 ± 0.09</td>
<td>93.4 ± 0.21</td>
</tr>
</tbody>
</table>
CONCLUSION

In situ gelling thermoreversible ophthalmic gel of Brinzolamide was developed using poloxamer 338 and HPMC K4M or Carbopol polymers. The methodology adopted for preparation of in-situ gel solution was very simple and cost effective. From the study conducted, the following conclusions were drawn, by varying the concentration of polymers, it is to obtain the increased residence time and sustained drug release. Among the novel polymeric systems poloxamer 338 was found to be having good thermoreversible gel properties and HPMC K4M as viscosity enhancer in combination with poloxamer 338 with respect to increased duration of action and drug release.

In situ gels formed were evaluated for various parameters like gelling capacity, pH, sol-gel transition temperature, Rheological before and after gelation, drug content. In vitro diffusion & ex vivo permeation studies were carried out. These in situ gelling formulations were free-flowing, transparent, had uniform consistency, and had spreadability at room temperature. Among the formulations, P3 formula containing poloxamer 338 (22%) and HPMC K4M (1%) was clear transparent solution, having pH of 7.1, gelation was within 60 seconds and remains stable for 6 hours, sol-gel transition temperature was 37°C, viscosity before gelation was 37.8 mPas ± 0.48 and after gelation was 155 ± 0.65mPas. Optimized formulation P3 showed controlled release with 94.64 ± 0.554at the end of 24 hrs. Ex-vivo permeation was found to be 89.18%± 0.748 after 24 hrs. The SEM of in situ gel suggested that the drug was well dispersed in the polymer matrix.

The developed formulations are viable alternatives to conventional eye drops by virtue of their ability to enhance bioavailability through longer precorneal residence time and ability to sustain drug diffusion. In situ gels can also decrease the frequency of administration resulting in better patient acceptance.
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(AptarPharma, consumer health care division, eye care, OSD)