DEVELOPMENT AND EVALUATION OF HP-β-CD COMPLEXATION BASED NOVEL OPHTHALMIC GEL FORMULATION OF NEPAFENAC

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ABSTRACT: Nepafenac is an analgesic and anti-inflammatory drug, used in post-operative ocular pain and presently available in the market in the form of 0.1% ophthalmic suspension eye drop due to aqueous insolubility of the drug. The present formulation is not only poor patient compliant due to suspension form but also suffers from a short corneal residence and inadequate ocular bioavailability. The objective of the present research work is to enhance the aqueous solubility of lipophilic drug nepafenac and develop its ocular gel formulation for overcoming the drawbacks of the presently marketed product. The 10% w/v HP-β-CD solution was used to formulate nepafenac in solution form, which resulted in 57.14 fold solubility enhancement of nepafenac in water, facilitating the development of nepafenac eye drop-in solution form. To enhance the pre-corneal residence of the formulation, the drug solution was incorporated into the hydrogel base, i.e. gellan gum capable of ion-activated sol-to-gel transformation in the ocular environment while sodium hyaluronate forms gel that exists in slightly viscous flowable form. The developed formulation was evaluated for their physical appearance, drug content, pH, osmolality, in-vitro gelation property, in-vitro drug release, and ex-vivo trans-corneal drug permeation and all the studied parameters were found satisfactory and acceptable. Trans-corneal drug permeation study revealed that there was approximately 1.38 and 1.48 times enhancement in the drug permeation flux during 24 h study for developed in-situ gel and preformed gel, respectively, as compared to the marketed conventional product.

INTRODUCTION: In the ophthalmic drug delivery systems, protective barriers of the eye lead to the lower absorption of the drug, and it leads to poor bioavailability of the therapeutic drugs. The conventional eye drop formulations suffer from inadequate ocular bioavailability and need to be administered frequently because of their short pre-corneal residence, naso-lacrimal drainage, and poor trans-corneal drug permeation.

Topical formulations like suspension, gels, oily drops, solid inserts, and ointments have been used, but most of them give rise to unacceptable side effects like blurred vision, irritation to the eye. Ocular suspension formulation causes irritation, discomfort due to grittiness and blurred vision to
the eye, leading to increased lacrimation, causing rapid drainage from the eye and thereby limits the corneal contact and ocular penetration of administered drugs, which finally results into inadequate therapeutic efficacy. Nepafenac is a non-steroidal anti-inflammatory pro-drug which converts into amfenac in the ocular biological system used to treat postoperative pain and inflammation in the eye. Presently, it is commercially available in the form of 0.1% ocular suspension drops because of its poor aqueous solubility. Development of an ocular formulation, containing the drug in solution state can overcome such problems.

Various solubilizing agents and techniques have been reported to enhance the solubility of lipophilic drugs for developing topical ophthalmic solutions. HP-β-CD-based inclusion complexation method has also been extensively explored for dissolving the water-insoluble ocular drugs. Application of one drop of 18% HP-β-CD aqueous solution to humans, three times daily for 28 days, is well tolerated in the eye. It is also reported in the literature that in ophthalmic preparations, co-administration of HP-β-CD has been reported to increase corneal penetration, ocular absorption, and the efficacy of poorly water-soluble drugs.

In-situ gel undergoes sol-to-gel transition under required physiological conditions. Gellan gum is an ion-activated polymer that forms gel-forming solutions that exist in liquid form but in the presence of monovalent/divalent ions, it converts into gel. Sodium hyaluronate is a mucoadhesive, viscoelastic gel polymer that has ability to trap and retain large amounts of water and forms gel formulation that exists in slightly viscous flowable form. Hydrogels incorporating ocular drug in dissolved state not only overcomes the drawbacks of conventional products but also impart value addition to the formulation by improved ocular bioavailability and patient compliance.

MATERIALS AND METHODS:
Materials: Nepafenac, hydroxypropyl-β-cyclodextrin (HP-β-CD), and benzalkonium chloride were received as gift samples from M/s Piramal Healthcare Ltd, Pithampur, Madhya Pradesh (India). Gellan gum, sodium hyaluronate, and mannitol were gifted by Sun Pharma Ltd. All chemicals used were of analytical grade.

Methods:
Selection of Solubilizer for Nepafenac: The effect of different solubilizers like PEG-400, transcutol P, tylaxapol, β-cyclodextrin, propylene glycol, cremophor RH 40, tween 20, cremophor EL and various concentration of HP-β-CD on nepafenac solubility were observed to solubilize desired concentration of nepafenac in solution form, i.e., 1 mg/ml (0.1%). The solubility study of nepafenac in different solubilizer was carried out by approximate solubility method and observed that nepafenac achieved more than 1 mg/ml solubility only in 10% HP-β-CD. Equilibrium solubility of nepafenac in 10% HP-β-CD was found to be 2 mg/ml.

It was also found from literature sources that, application of one drop of an aqueous solution containing 18% HP-β-CD to humans, three times daily for 28 days, is well tolerated in the eye. As per European Medicine Agency guidelines, solutions of 12.5% HP-β-CD is found to be nontoxic or non-irritating in rabbit eyes. It is also reported in literature that in the ophthalmic preparations, co-administration of HP-β-CD has been reported to increase the aqueous solubility of lipophilic drugs without affecting their intrinsic ability to permeate biological membranes.

Considering the above evidence, and results, 10% HP-β-CD can solubilize the desired concentration of drug and also a safe concentration for ocular administration. Therefore, 10% HP-β-CD was selected to solubilize 0.1% nepafenac in aqueous solution.

Preparation of Nepafenac / HP-β-CD Inclusion Complex: Inclusion complex of nepafenac with HP-β-CD was formed in solution form. 1 gm of HP-β-CD was accurately weighed and dissolved in 10 ml of phosphate buffer saline (PBS) pH 7.4. 10 mg of nepafenac drug was accurately weighed and dissolved in the above 10% HP-β-CD solution on vortex mixer for 25-30 min.
For the determination of stoichiometry ratio behavior and stability constants of the complex, phase solubility analysis of nepafenac with HP-β-CD was performed.

The solubility of nepafenac in different HP-β-CD molar solutions was determined, and phase solubility diagram was drawn between the solubility of nepafenac and different molar concentrations of HP-β-CD as graphically shown in Fig. 1.

![Phase Solubility Diagram of Nepafenac in HP-β-CD Solution](image)

Fig. 1: Phase Solubility Diagram of Nepafenac in HP-β-CD Solution

The phase solubility diagrams for nepafenac showed the linear increase of drug solubility with increasing concentration of HP-β-CD up to 0.1M. The linearity of the curve confirms AL type of curve, thus showing 1:1 stoichiometry of complexation. The stability constant (K) of the complex can be calculated by the equation as follows

\[
K = \frac{\text{Slope}}{S_0} (1-\text{Slope})
\]

Where \(S_0\) is the solubility of the drug in the PBS 7.4 without HP-β-CD concentration. The slope and intercept were obtained by linear regression of data points. The intercept shows intrinsic solubility of the drug at 37 °C.

The phase solubility curve shows that with the increase in molar concentration of HP-β-CD, the molar concentration of nepafenac is increasing linearly as graphically represented in Fig. 1. The stability constant (K) of nepafenac / HP-β-CD complex was found to be 1135.85 M⁻¹ which indicates that complex will remain stable and can be considered as sufficient for practical applications because complexes with stability constants between 200-5000 are reported to be suitable for practical applications. The phase solubility diagram showed the linear increase in drug solubility.

**Selection of Gelling Agents:** Different polymers such as carbopol 971P, carbopol 940, pluronic F-127, gellan gum, xanthan gum, and sodium hyaluronate were used for screening of gelling agents for hydrogel. Studies were performed to assess the gelling capacity in simulated lacrimal fluid (SLF) and other relevant properties of these polymers. Carbopols are pH-responsive polymers and form gel at physiological conditions, i.e. pH 7.4 and 37 °C temperature. Pluronic F-127 is a temperature-sensitive polymer shows solution to gel transition when temperature is raised from 25 °C to 37 °C. Gellan gum and xanthan gum shows ion-activated phase transition and forms gel with ions. Sodium hyaluronate is a mucoadhesive, viscoelastic gel polymer.

**TABLE 1: Observations of Screening Study of Gelling Agents**

<table>
<thead>
<tr>
<th>S. no</th>
<th>Name of gelling agents</th>
<th>Concentration used</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbopols</td>
<td>0.2-1%</td>
<td>Exhibit desired phase transition behavior at 0.6-1% concentration. It forms gel when pH was raised to 7.4 with NaOH solution but presence of divalent ions in tears led to further dilution of carbopol solution</td>
</tr>
<tr>
<td>2</td>
<td>Pluronic F-127</td>
<td>10-20%</td>
<td>Exhibit desired phase transition behavior at 18-20% concentration which is very high polymeric concentration and may cause irritation to the eye</td>
</tr>
<tr>
<td>3</td>
<td>Gellan gum</td>
<td>0.2-1%</td>
<td>Exhibit desired phase transition behaviour at 0.6-1% concentration in the presence of ions. Clear transparent gel was formed</td>
</tr>
<tr>
<td>4</td>
<td>Xanthan gum</td>
<td>0.2-1%</td>
<td>Exhibit desired phase transition behavior at concentration 0.6%. Forms gel but less transparent than gellan gum gel</td>
</tr>
<tr>
<td>5</td>
<td>Sodium hyaluronate</td>
<td>0.2-1%</td>
<td>Forms clear, transparent and slightly viscous gel at 0.6%</td>
</tr>
</tbody>
</table>

On the basis of observation of the screening studies of gelling agents, sodium hyaluronate and gellan gum were selected to develop ophthalmic hydrogels of nepafenac. Gellan gum forms gel-forming solution while sodium hyaluronate forms a clear preformed gel.
Preparation of Drug Loaded In-situ Gel Formulation: Gel forming solutions are also known as an in-situ gelling system that undergoes sol-to-gel transition phase under required physiological conditions. Gellan gum is an ion activated gel-forming polymer that forms gel-forming solutions that exist in liquid form but it forms gel in the presence of monovalent/divalent ions available in the tear fluid\textsuperscript{14,15}.

The composition of nepafenac in-situ gel formulation is shown in Table 2. 10 gm of HP-β-CD was accurately weighed and dissolved in 50 ml of purified water. 100 mg nepafenac was weighed and dissolved in the above solution of HP-β-CD by shaking over the vortex mixer for 15-25 min to make a clear drug solution. The required quantity of mannitol was dissolved in the drug solution with constant stirring. A quantity of 0.6 gm gellan gum was accurately weighed and dissolved in 30 ml of purified water on magnetic stirrer at 800 rpm for 20 min at 90 °C. Then, it was allowed to cool and hydrate at room temperature for 15-20 min. to produce a clear solution. The drug solution was mixed slowly to gellan gum with continuous mechanical stirring. 10 ml of 0.01% benzalkonium chloride was added to the above mixtures with continuous stirring and final volume was made up to 100 ml using purified water. The method of preparation of in-situ gel is shown in Fig. 2.

Preparation of Drug Loaded Preformed Gel: Sodium hyaluronate is a mucoadhesive, viscoelastic gel polymer that has ability to trap and retain large amounts of water. It is reported in literature that it stabilizes the tear film and promotes epithelial healing. It is also used as ocular lubricant.

Sodium hyaluronate is a polymer that forms gel formulation that exists in slightly viscous flowable form. The composition of nepafenac preformed gel formulation is shown in Table 2.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nepafenac</td>
<td>0.1%</td>
</tr>
<tr>
<td>HP-β-CD</td>
<td>10%</td>
</tr>
<tr>
<td>Mannitol</td>
<td>3.0%</td>
</tr>
<tr>
<td>Gellan gum</td>
<td>0.6%</td>
</tr>
<tr>
<td>Sodium hyaluronate</td>
<td>0.6%</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>0.01%</td>
</tr>
<tr>
<td>Purified water</td>
<td>qs to 100 ml</td>
</tr>
</tbody>
</table>

TABLE 2: COMPOSITION OF DEVELOPED NEPAFENAC IN-SITU AND PREFORMED GEL FORMULATION

![FIG. 2: DIAGRAMMATIC REPRESENTATION OF GEL FORMULATION METHOD](image-url)
10 gm of HP-β-CD was accurately weighed and dissolved in 50 ml of purified water. 100 mg of nepafenac was weighed and dissolved in the above solution of HP-β-CD by shaking over the vortex mixer for 15-25 min to make a clear drug solution.

The required quantity of mannitol was dissolved in the drug solution with constant stirring. 0.6 gm of sodium hyaluronate was accurately weighed and dissolved in 30 ml of purified water on a magnetic stirrer at 800 rpm for 20-30 min at 50 °C and was allowed to cool and hydrate for 15 min to produce a clear solution.

The drug solution was mixed slowly to sodium hyaluronate solution with continuous mechanical stirring. 10 ml of 0.01% benzalkonium chloride was added to this mixture with continuous stirring and the final volume was made up to 100 ml using purified water. The method of preparation of preformed gel is shown in Fig. 2.

**Evaluation of Developed In-situ Gel and Preformed Gel Formulation:**

**pH, Clarity, and Osmolality:** pH of the developed *in-situ* gel, and the preformed gel was measured by pH meter (Cyberscan 510). The clarity of developed formulations was determined by visual examination under proper light alternatively against white and black backgrounds, with the contents set in motion with a swirling action.

The osmolality of the developed formulations was measured by Osmometer (Advanced Instruments Inc.) by taking a 20 μL sample.

**In-vitro Gelation Study of In-situ Gel:** For the estimation of gel formation in physiological conditions, a cylindrical tube of 5 cm length and 1 cm diameter were taken and filled with the SLF. Nepafenac *in-situ* gel containing ponceau red dye was added to it with a conventional dropper and assessed for gel formation.

**Drug Content:** 1 gm of the *in-situ* gel and preformed gel were taken individually in volumetric flasks of 100 ml and were dissolved completely in adequate amount of purified water and filtered. The volume was made up to 100 ml with purified water. Drug concentration was determined after suitable dilutions using a UV spectrophotometer at 378 nm.

**Rheological Studies:** Determination of viscosity of developed formulations was done by Brookfield viscometer (R/S plus Rheometer) by using a C-25 spindle at 25 °C.

**In-vitro Drug Release Study:** *In-vitro* drug release study of the developed *in-situ* gel and preformed gel formulation was carried out and compared to that of the marketed formulation by dialysis bag method. 1 ml study sample was taken in dialysis membrane bag (MCWO 12000-14000) and was dipped in 200 ml PBS (pH 7.4) maintained at 37 ± 0.5 °C at 500 rpm using multi-station magnetic stirrer. 5 ml samples were withdrawn and replaced with the equivalent amount of fresh PBS (pH 7.4) for every hour. The samples were analyzed spectrophotometrically for nepafenac content using a double beam UV-visible spectrophotometer at 378 nm. All the studies were carried out in triplicates.

**Ex-vivo Trans-Corneal Drug Permeation Study:** *Ex-vivo* trans-corneal drug permeation study of the developed *in-situ* gel and preformed gel formulation was carried out on excised goat cornea and was compared to that of marketed formulation using modified Franz diffusion cells with permeation media maintained at 37 °C. The receptor compartment was filled with media and the excised fresh cornea was rinsed with normal saline. It was then placed on top of the receptor compartment of the modified Franz diffusion cell. Then the donor compartment was placed over it and fixed with the help of a stainless steel clip. The whole assembly was maintained at 37 °C. In the donor compartment, 0.5 ml of formulation sample was filled. From the receptor compartment, samples were withdrawn carefully at predetermined time intervals and replaced immediately with equal volume of fresh media. Then cumulative amount of drug permeated was estimated using UV-visible spectrophotometer (Shimadzu 1700). All the experiments were performed in triplicates.

Apparent corneal permeability coefficient \( (P_{\text{app}}) \) was calculated using the following equation:

\[
P_{\text{app}} = \frac{dQ/dt}{1/A} \cdot \frac{1}{C_0} \cdot 3600.0 \cdot C_0
\]

Where, \( dQ/dt \) is slope of the linear portion of the plot between the amounts of the drug in the
receptor chamber (Q) versus time (t), A is an exposed corneal area (1.13 cm²), C₀ is the amount of drug taken in the donor compartment and 3600 represents the conversion of hours to second, Jss is flux at steady state.

RESULTS AND DISCUSSION:
Preparation of Nepafenac Gel Formulation: In the present investigation, nepafenac in-situ gel and preformed gel were prepared efficiently using HP-β-CD and a gelling agent such as gellan gum and sodium hyaluronate. 10% w/v HP-β-CD solution was used to solubilize nepafenac in solution form and resulted in 57.14 fold solubility enhancement of nepafenac in water, facilitating the development of nepafenac eye drop-in solution form. Both formulations were formulated by incorporating the nepafenac solution in hydrogel base to enhance the pre-corneal residence of the formulation.

In-situ gel formulation prepared using gellan gum that is capable of ion-activated sol-to-gel transformation in the ocular environment. Flow characteristics of gellan gum solution were observed by preparing different concentrations of gellan gum solution in purified water and evaluated for physical properties and observed that concentration range of 0.1-0.5% was easily flowable, 0.6%-0.7% was moderately flowable and 0.8-1.0% solutions were viscous and not flowable. Considering the flow characteristics of different concentration of gellan gum solutions, the concentration range of 0.1-0.7% were selected and their gel-forming ability in SLF was tested in order to assess the ability of formulation to form gels on the surface of eyeball in presence of tear. It was observed that all the gels were clear and transparent. 0.1-0.2% solution shows no gelation, 0.3-0.5% solution shows immediate gelation while 0.6-0.7% solution shows immediate gelation which persisted for extended period of time. Therefore, on the basis of flow characteristics and gelation studies, 0.6% gellan gum was selected as the optimum concentration for in-situ gel under physiological conditions of the eye.

Preformed gel formulation contains sodium hyaluronate that forms gel exists in slightly viscous flowable form. The solution of sodium hyaluronate in various concentrations was prepared in purified water and assessed for their clarity and flow characteristics. It was observed that 0.1-0.3% solutions were very fluidic; 0.4 to 0.5% solutions were moderate fluidic; 0.6% sodium hyaluronate solution forms slightly viscous gel and flowable; 0.7% solution was viscous but flowable; 0.8% solution was highly viscous but not flowable. All the solutions formed were clear and transparent. Therefore, on the basis of observations of flow characteristics and clarity, 0.6% sodium hyaluronate was selected as the optimum concentration for preformed gel formulation. In both the formulation, mannitol was used to adjust the tonicity of formulation so that it can be isotonic to the eye, while benzalkonium chloride was used as a preservative.

Evaluation of Developed In-Situ and Preformed Gel Formulation: The prepared formulations were evaluated for pH measurement, osmolality, clarity, in-vitro gelation study, drug content estimation, rheological studies, in-vitro drug release, and ex-vivo trans-corneal drug permeation study.

pH, Osmolality and Clarity: The pH of in-situ gel and preformed gel formulation was found to be 6.8 and 7.4, respectively, while osmolality was found to be 292 m Osm/kg and 297 m Osm/kg respectively. Both formulations have approximately same pH and osmolality of the normal eye.
The developed formulations were clear and transparent without any suspended particles as shown in Fig. 3. Hence, both developed formulations were found suitable for administration to the eye on the basis of their pH, osmolality, and clarity.

In-vitro Gelation Study of in-situ Gel Formulation: In-situ gel formulation should undergo a rapid sol-to-gel transition upon installation to the eye. The developed in-situ gel resulted in gel formation instantaneously in the simulated lachrymal fluid and found suitable to administer into the eye.

Drug Content: Drug content of developed in-situ gel and preformed gel were found to be 99.87% and 98.85% respectively. The drug content of both developed formulations of nepafenac gel was found satisfactory that indicating uniform distribution of the drug.

Rheological Studies: The developed formulation should have an optimum viscosity that is easy to administration into the eye. The viscosity of developed in-situ gel and preformed gel formulations were found to be 1063 cps and 1950 cps respectively. The viscosity of in-situ gel after gelation was found to be 1667 cps. There was an increment of viscosity of in-situ gel after gelation. Results showed that the viscosity of both the formulations is suitable for administration into the eye.

In-vitro Drug Release Study: Cumulative percent drug release for developed in-situ gel, performed gel and the marketed product in 24 h are 77.87%, 94.32%, and 70.70% respectively as shown in Fig. 4. Both the formulation exhibiting comparable drug release profile. The poor water solubility of nepafenac leads to slower release of drug in the marketed suspension as compared to developed gel formulation containing drug in the dissolved state.

Ex-vivo Trans-Corneal Drug Permeation Study: The developed in-situ gel and the preformed gel was found to be effectively cross the cornea than marketed formulation. The percent cumulative drug permeated for developed in-situ gel, performed gel and marketed suspension showed 59.63%, 64.60% and 39.85% drug permeation in 24 h respectively as shown in Fig. 5. in 24 h. The apparent corneal permeability coefficient (Papp) for in-situ gel, performed gel, and marketed suspension was found to be 5.8×10⁻⁶ cm/sec, 6.2×10⁻⁶ cm/sec, and 4.2×10⁻⁶ cm/sec respectively; while steady-state flux (Jss) was found to be 0.0029 μg.cm/sec, 0.0031 μg.cm/sec and 0.0021 μg.cm/sec respectively.

There is 1.38 and 1.48 times enhancement in the flux observed for developed in-situ gel and preformed gel formulation, respectively, as compared to the marketed product, which results in a significant enhancement of nepafenac ocular permeation.

CONCLUSION: Nepafenac, an analgesic-anti-inflammatory agent used in the post-operative pain, was successfully formulated as in-situ gel and preformed gel using a gelling agent in combination with HP-β-CD as a solubilizer. HP-β-CD complexation based in-situ gel and preformed gel formulations of nepafenac can be promising.
ophthalmic drug delivery dosage form for topical administration. 10% HP-β-CD solution showed 57.14 fold solubility enhancement of nepafenac in purified water. It is safe and effective to solubilize water-insoluble ocular drugs. The developed in-situ gel and preformed gel are non-greasy formulations having ease of administration whose application could enhance the ocular drug permeation of the formulation; thereby, potentially lowering corneal toxicity may ultimately result in improved patient compliance. The developed in-situ gel and preformed gel consisting of drug in dissolved state in purified water. It is safe and effective to solubilize nepafenac 57.14 fold solubility enhancement of nepafenac in purified water. It is safe and effective to solubilize nepafenac.

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REFERENCES:


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