Solubility Enhancement of Water Insoluble Drug for Ophthalmic Formulation

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Abstract

Product development of ophthalmic preparations has received considerable attention in the last few years. With the increasing emphasis on their sterility, some of the differences between them and parenteral preparations are becoming less evident. Both classes of preparations employ similar added substances and manufacturing procedures. The successful formulation of poorly water-soluble drugs is one of the major problems in pharmaceutical manufacturing. Poorly water-soluble drugs, such as indomethacin, may show low and erratic oral bioavailability due to poor dissolution of the drug in the fluids of the gastrointestinal tract. Indomethacin is a water insoluble drug, so problems of formulating an aqueous eye-drop are well known. Moreover, unstability of Indomethacin aqueous preparations is also a great challenge. In this research work, considering pharmacological importance of drug Indomethacin, we tried to overcome the problem of poor water solubility by making a salt of it and thus formulating an aqueous ophthalmic preparation.

Keywords: ophthalmic preparation, indomethacin salt, aqueous eye drops, poor water soluble drugs.

Introduction

Absorption of drugs into the eye requires good pre-corneal penetration and prolonged contact time with corneal tissues. Ideally, the formulation should be able to sustain drug release and to maintain contact with the eye for extended periods of time. The extent of absorption into the eye is severely limited by the physiological constraints such as reflex tearing and blinking. Further, drug loss occurs due to tear turnover, solution drainage by gravity and binding of drugs to proteins and other components of tear. As a result, typically only 1-2% of the instilled drug is bioavailable. In recent times, great emphasis has been placed on the importance of formulation with the recognition that they can significantly influence the physiologic availability of drugs. It is becoming increasingly evident that the rate of dissolution or release of the drug from the formulation is of paramount importance and that even minor changes unknowingly can greatly influence this property in some cases. In dealing with the formulation of new products, as the variety and complexity of materials and techniques have increased, it has become necessary to apply the best research methods and
tools, in order to develop, produce and control the potent, stable and effective dosage forms.\textsuperscript{1, 2} Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used agents that despite chemically heterogeneity, share similar therapeutic properties and adverse effects. Topical ophthalmic NSAIDs are limited to the relatively water soluble phenylacetic and phenylalkanoic acids as well as indole derivatives, which are more suitable for ophthalmic use. Topical ophthalmic NSAIDs are commonly used in the treatment of post-operative inflammation following cataract extraction and various surgical refractive procedures. They are also used in the prevention and treatment of cystoid macular oedema and for the treatment of allergic conjunctivitis. Local irritant effects of topical ophthalmic NSAIDs include conjunctival hyperaemia, burning, stinging and corneal anaesthesia. A more serious complication involves the association of topical ophthalmic NSAIDs with indolent corneal ulceration and full-thickness corneal melts. In general, ophthalmic NSAIDs may be used safely with other ophthalmic pharmaceuticals. Until clinical evidence dictates otherwise, data supporting theories of potential pharmacodynamic mechanisms of NSAID injury do not alter the favorable benefit-risk ratio of ophthalmic NSAID use when employed in an appropriate and judicious manner.\textsuperscript{3-8}

**Materials and Methods**

Drug Indomethacin was generously gifted by Alchem Laboratories, Bombay. Excipients were obtained from commercial sources and were of analytical grade.

**Determination of solubility of indomethacin**

Indomethacin is a low soluble drug (specifically in water). Its solubility was determined in following solvents and their combinations and also in presence of solubilizing agents, by placing an excess quantity of drug in a vial along with the solvent. The tightly closed vials were then agitated for 24 hr at 30\textdegree{} C ± 2\textdegree{} C. The solutions were filtered through a 0.45\textmu{}m filter and the filtrate was analyzed for drug content on a UV / Vis spectrophotometer (Jasco – V550) after appropriate dilutions.

**Preparation of sodium salt of indomethacin**

After trying different solvents and solubilizing agents, it was clear that for formulating eye drops, it was not possible to achieve a target of 10-mg/ml concentration of indomethacin in desired aqueous medium. So, Indomethacin was converted into its sodium salt for formulation of aqueous eye drops. Following method was tried for the preparation of indomethacin sodium: 50-ml methanol was taken in a round bottom flask kept on water bath. Accurately weighed 5g indomethacin was added to it slowly at 50\textdegree{} C with continuous stirring until a clear solution was obtained. Sodium bicarbonate solution (in 1:1 molar ratio with indomethacin) was added to the above solution drop wise with continuous stirring. The reaction was continued at room temperature with stirring at 350 rpm for 2 hrs. The solvent was evaporated by Buchi’s Rota vapour at 35\textdegree{} C. Solid residue was washed with diethyl ether and filtered indomethacin sodium so obtained was dried overnight under vacuum and weighed. Same method was again tried using sodium methoxide and sodium hydroxide in place of sodium bicarbonate. Sodium salt prepared by all the three methods was characterized.

**Characterization of prepared Indomethacin sodium**

On the basis of data reported in various pharmacopoeias, indomethacin sodium prepared by above three methods was analyzed and characterized by following parameters.

**Solubility:** Solubility of prepared indomethacin sodium was determined in water and methanol at room temperature. The results are recorded in table no.1.

**pH:** Indomethacin is a weak acidic drug. Conversion of indomethacin base into its sodium salt will shift the pH of solution towards alkaline side. The pH of 1% solution of prepared indomethacin sodium is given in table no.1.
Melting point: Melting point of prepared indomethacin sodium was determined by melting point apparatus. As it is a metallic salt, its melting point should be higher than the base. Normally, a salt does not give a sharp melting point because it will char over a wide range of temperature. Melting points are given in table no.1.

Table no. 1 Solubility, pH and melting points of prepared indomethacin sodium

<table>
<thead>
<tr>
<th>Method of preparation</th>
<th>Solubility in water</th>
<th>Methanol</th>
<th>pH of 1% solution</th>
<th>Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using sodium methoxide</td>
<td>Not soluble</td>
<td>Soluble</td>
<td>7 – 7.5</td>
<td>155ºC - 160ºC</td>
</tr>
<tr>
<td>Using sodium hydroxide</td>
<td>Soluble</td>
<td>Soluble</td>
<td>9 – 9.5</td>
<td>260ºC - 270ºC</td>
</tr>
<tr>
<td>Using sodium bicarbonate</td>
<td>Soluble</td>
<td>Soluble</td>
<td>8.5 – 9.0</td>
<td>215ºC - 220ºC</td>
</tr>
</tbody>
</table>

UV/Visible spectroscopy:

The UV spectra of indomethacin base and prepared indomethacin sodium were obtained by scanning solutions of 20 ppm concentration in methanol in the range of 200-400 nm wavelength on Jasco V-550 UV / Vis spectrophotometer. An overlay of indomethacin sodium prepared using sodium bicarbonate over indomethacin base is shown in fig. no.1

Differential scanning calorimetry:

The DSC thermograms are obtained by placing the sample in a calorimeter with a programmed temperature device, so that the temperature will increase from 55.00°C to 340°C at 10.00°C / min. Sample was held for 1.0 min at 55.00°C. The calorimeter also contains a control cell with a known heat capacity. The heat flowing into two cells is monitored and balanced to get a “baseline”. When, there is a thermal event in the sample cell (e.g., melting), there will be an endotherm, which will be registered as a peak, which falls back to baseline when the event is over. The area under the peak can be converted to calories or joules.

The DSC thermograms of indomethacin and prepared indomethacin sodium are shown in fig. no.2 and 3.

**Fig. no. 2** DSC thermogram of indomethacin

**Fig. no. 3** DSC thermogram of indomethacin sodium
IR spectroscopy:
The infrared absorption spectrum of indomethacin and prepared indomethacin sodium were recorded between the wavelengths of 2000 to 400 cm\(^{-1}\) on a Hyper IR, Shimadzu standard detector in 1% KBr. The Peaks of indomethacin base were compared with those of indomethacin sodium.

Potentiometric analysis:
Sample of prepared indomethacin sodium was analyzed quantitatively by potentiometric titration using Titrando 808-auto titre.

First, a solution of indomethacin sodium was prepared by dissolving 200-mg sample in 50-ml methanol. This solution was titrated against 0.1M NaOH to check whether there is any free base left in the sample. But, since a straight curve without any peaks was obtained, this further confirms that, there was no reaction between sample solution and NaOH solution.

Same sample solution was again titrated potentiometrically with 0.1 M HCl to calculate the quantity of indomethacin sodium present in the sample. The titration graphs are shown in fig. no.4

Preformulation studies
Preformulation studies mainly focus on those physicochemical properties of the compound that could affect drug performance and development of an efficacious dosage form. A thorough understanding of these properties may ultimately provide a rationale for formulation design.

Solubility of prepared Indomethacin sodium at different pH:
Phosphate buffers of different pH in the range from 5 to 9 pH were prepared. The solubility of prepared indomethacin sodium in these buffers was determined by adding the drug into the buffer solution gradually in a test tube and after shaking observing the solubility visually.

Stability of Indomethacin sodium at different pH:
After finding out that Indomethacin sodium is more soluble at pH above 6, its stability at pH between 6 to 9 was determined by keeping 1% solutions in phosphate buffers of pH 6 to 9 for 15 days at room temperature. Samples were taken from each solution at regular interval and concentration of sodium indomethacin was estimated spectrophotometrically using UV/Vis spectrophotometer Jasco V-550 after appropriate dilution with methanol. The samples were also visually checked for any sign of physical unstability in this duration. The results are given in a graph of pH against concentration is shown in fig. no.5.
Compatibility Studies:
1% solution of indomethacin sodium was prepared in phosphate buffer. The pH was maintained at 6.8. 5 ml of this solution was taken in glass vials and mixed with 0.9% sodium chloride, tween 80 (1% v/v), benzyl alcohol (1% v/v), benzalkonium chloride (0.025%w/v), chlorobutanol (0.5% w/v), phenyl mercuric nitrate (0.004%w/v), propyl paraben (0.015%w/v) and methyl paraben (0.05%w/v) respectively as per IIG. The glass vials were stoppered with rubber stopper, sealed and stored at different storage conditions to find out physical and chemical compatibility of indomethacin sodium with excipients. A sample of plain indomethacin sodium without any excipient was kept as the control.

Physical compatibility:
Vials were checked at regular intervals for clarity, color change etc.

Chemical compatibility:
Since, there was some turbidity due to precipitation in drug solution containing benzyl alcohol; samples of these solutions were discarded. Other solutions were taken for chemical compatibility studies. The above drug solutions kept under different storage conditions were analyzed for drug content at 320 nm on a UV / Vis Jasco V-550 spectrophotometer after appropriate dilutions with methanol. Absorbance’s of sample solutions were compared with that of control samples. (Initial drug content was 100.80%).

Selection of sterilization method
Membrane filter of 0.2-µm porosity was used for sterilization by filtration in µm aseptic area. The suitability of both methods of sterilization in the present study was tested by determining drug content of representative eye drop formulation of indomethacin sodium (aqueous based) before and after subjecting to sterilization by the respective method. Aqueous base solutions were prepared with 1 % drug and other excipients filled in vials and sealed. These vials were autoclaved for 20 minutes. In some other vials, solutions were filled by filtration through 0.2µm Millex, Millipore filter.

Formulation of indomethacin eye-drops
On the basis of results obtained during preformulation studies following formulations of Indomethacin eye-drops were devised.

Table no. 2 Formulation of indomethacin sodium aqueous drops

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation Code</th>
<th>Ingredients</th>
<th>Quantity</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INDOA1</td>
<td>Indomethacin sodium (equi.to) Tween-80 Methyl Paraben Sodium chloride (equi.to) Phosphate buffer in WFI</td>
<td>1% (w/v) base 1% (v/v) 0.05% (v/v) 0.9% (w/v) q.s to 100% (v/v)</td>
<td>Drug Solubiliser Preservative Tonicity modifier Buffering agent &amp; vehicle</td>
</tr>
<tr>
<td>2</td>
<td>INDOA2</td>
<td>Indomethacin sodium (equi.to) Tween-80 BKC Sodium chloride (equi.to) Phosphate buffer in WFI</td>
<td>1% (w/v) base 1% (v/v) 0.025% (v/v) 0.9% (w/v) q.s to 100% (v/v)</td>
<td>Drug Solubiliser Preservative Tonicity modifier Buffering agent &amp; vehicle</td>
</tr>
<tr>
<td>3</td>
<td>INDOA3</td>
<td>Indomethacin sodium (equi.to) Tween-80 Chlorobutanol Sodium chloride (equi.to) Phosphate buffer in WFI</td>
<td>1% (w/v) base 1% (v/v) 0.5% (v/v) 0.9% (w/v) q.s to 100% (v/v)</td>
<td>Drug Solubiliser Preservative Tonicity modifier Buffering agent &amp; vehicle</td>
</tr>
<tr>
<td>4</td>
<td>INDOA4</td>
<td>Indomethacin sodium (equi.to) Tween-80 Phenyl</td>
<td>1% (w/v) base 1% (v/v) 0.004%</td>
<td>Drug Solubiliser Preservative Tonicity</td>
</tr>
</tbody>
</table>
Formulation method–
Initially isotonic saline phosphate buffer was prepared in a 250-ml flask using water for injection. Weighted quantity of indomethacin sodium was dissolved in it. Preservative was dissolved in a little WFI separately with the help of heating at 60-80°C. Tween 80 and preservative solutions were added to the drug solution with proper stirring. The formulations were filtered through 0.2µm filters in aseptic area and filled in sterilized vials and sealed. pH of all the formulations adjusted between 6.8 and 7.0.

Vials, glassware’s and other equipments used in the preparation of eye drops were sterilized in a hot air oven at 250°C for 2 hr and the WFI was freshly prepared. Preparation of formulation, filtration, filling and sealing were all completed in class 10,000 area under the protection of HEPA-filtered laminar flow.

Packaging of products:
Since indomethacin is reported to be a light sensitive drug, so 10 ml, 20 mm USP type–I flint glass vials were used as containers. For closing these vials, 20-mm Grey bromo-butyl slotted rubber stoppers were used. These packaging materials were tested as per USP.

Evaluation of formulated products
Assay Procedure
1 ml of each formulation was accurately taken and transferred to a 100 ml volumetric flask. It was diluted to 100 ml methanol. 10 ml of this was again diluted with solvent to 100 ml. Two dilutions of each sample were prepared at a time for cross check. Absorbance of each dilution was measured at 320 nm on a Jasco–V-550, UV/Vis spectrophotometer. The results are recorded in table no. 3

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>INDAD1</td>
<td>10.24 mg/ml</td>
</tr>
<tr>
<td>INDAD2</td>
<td>10.05 mg/ml</td>
</tr>
<tr>
<td>INDAD3</td>
<td>10.25 mg/ml</td>
</tr>
<tr>
<td>INDAD4</td>
<td>10.06 mg/ml</td>
</tr>
</tbody>
</table>

Sterility testing
Prepared indomethacin eye drops were tested for sterility as per USP. The test was done by direct inoculation method. Fluid Thioglycollate was used for aerobes and facultative anaerobes while Soyabean Casein Digest Broth was used for aerobes and fungi. Test mixtures were incubated in culture tubes and media was examined for microbial growth. Incubation was continued for a total of 14 days from the original incubation at 32.5 ± 2.5°C. Control samples of aerobes, anaerobes and fungi were prepared in both the medium. All the formulations passed test of sterility.

Stability Study of formulated indomethacin eye drops
The formulated products were subjected to stability studies at different storage conditions as per ICH guidelines. The sealed vials containing different formulations were kept in the temperature / humidity control ovens (Newtronics). Some vials were also kept in inverted position to check any interaction between rubber closure and formulation. The drug content of each formulation was determined after different time intervals and percent residual drug was calculated.
Table no. 4. Stability study protocol for formulated products

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Storage Condition</th>
<th>Period</th>
<th>Limits during stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Physical test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Appearance pH</td>
<td>2-8°C</td>
<td>Initial</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25°C / 60% RH</td>
<td>7 days</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30°C / 65% RH</td>
<td>15 days</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40°C / 75% RH</td>
<td>1 month</td>
<td>5% change</td>
</tr>
<tr>
<td>B</td>
<td>Chemical test assay</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

From the solubility studies, it is clear that we cannot prepare a 1% solution of indomethacin in water using any of the above co-solvents or solubilizing agents. Since, indomethacin sodium prepared by sodium bicarbonate was showing expected results like solubility, pH, melting point, \( \lambda_{\text{max}} \), and UV spectra, it was selected for further studies. The thermograms clearly indicate a shift of endotherm towards higher side in case of indomethacin sodium as compared to that of indomethacin base. The thermogram of indomethacin sodium did not give a sharp peak as the indomethacin base. Also, there was no other peak in the thermogram of prepared indomethacin sodium except a peak of solvent, which confirms that the sodium salt has been formed.

The peaks of IR spectra of indomethacin sodium do not overlap the peaks of IR spectra of indomethacin base. So, two spectrums do not comply with each other and there is a significant shift that indicates that there is no free base present in the sample and it has been converted into indomethacin sodium. Assay of prepared indomethacin sodium was found to be 100.65%, which shows that the base indomethacin was fully converted into its sodium salt. It was observed that indomethacin sodium is more soluble in pH range of 6 to 9. Below pH 6, there was an incompatibility due to precipitation of free indomethacin base. Observations showed that solution of indomethacin sodium was most stable in the pH range of 6.5 to 7.5.

On the basis of compatibility studies these studies it could be inferred that benzyl alcohol is not suitable for indomethacin sodium eye drop formulation. Other excipients tested in the compatibility studied, can be used in the product development of indomethacin eye drops. On the basis of sterility studies it was decided that so both the methods can be used for sterilization of eye drops in the present studies.

Sodium and potassium salts are most simple to prepare. Some stability problems in aqueous preparations and during sterilization may arise but still various research works is going on in this field. Studies are in the stage of clinical trials and many have already applied for FDA registration. As we have seen in this project, sod. IND is stable at a neutral pH, which is easily tolerable by patients. Moreover, it is very soluble in water and buffer, so can be easily stabilized by using other additives like viscosity importing agent, solubiliser, antioxidants etc. Without using all this excipients also, salt was stable in aqueous solution at accelerated temperature / humidity conditions. Anti-inflammatory activity of Sod. IND has been already studied and is equal to indomethacin base.

References

2. Carter, S.J.; Cooper & Gunn’s Dispensing for Pharmaceutical Students, Ophthalmic Products, 634-653.


