OPTIMIZATION OF FORMULATION PARAMETERS ON OCULAR LOTEPREDNOL Etabonate NANOuspension by Media Milling Method

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ABSTRACT
Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Loteprednol Etabonate (LE) is a steroid belonging to a unique class of glucocorticoids. It is widely used in ophthalmic responsive inflammatory conditions such as allergic conjunctivitis. LE is poorly water-soluble drug and its bioavailability is very low from its crystalline form. Poor water soluble and low permeable drug, the rate of absorption is often controlled by the dissolution rate in the ocular fluid. Therefore, together with permeability, the solubility and dissolution rate of drug are key determinants of its bioavailability.

Over the years, various formulation techniques to enhance the dissolution of poorly soluble substances have been introduced with different degrees of success. Many attempts have been made to enhance drug solubility such as complexing, solid solutions, pharmaceutical salts, and pro drugs for improving bioavailability. The technique of nanosuspension is a new and promising addition towards such a novel aim.

The objective of present study is to develop and characterize Loteprednol Etabonate to enhance ocular bioavailability, solubility and dissolution rate because its marketed product has low bioavailability and its micronisation does not enhance its bioavailability.

Key words: Loteprednol Etabonate, media milling method, ocular nanosuspension, saturated solubility, dissolution rate.

1. INTRODUCTION
Topical application of drugs to the eye is the most popular and well-accepted route of administration for the treatment of various eye disorders. The bioavailability of ophthalmic drugs is, however, very poor due to efficient protective mechanisms of the eye. Blinking, baseline and reflex lacrimation, and drainage remove rapidly foreign substances, including drugs, from the surface of the eye. Moreover, the anatomy, physiology and barrier function of the cornea compromise the rapid absorption of drugs.1,2,3 Numerous strategies were developed to increase the bioavailability of ophthalmic drugs by prolonging the contact time between the preparation, and therefore the drug, and the corneal/conjunctival epithelium. The use of a water-soluble polymer to enhance the contact time and possibly also the penetration of the drug was first proposed by Swan.4 There is no reliable correlation between the performance of ophthalmic vehicles in rabbits and in humans, mainly due to differences in blinking frequency.5,6 Therefore the techniques of nanosuspension that have commonly been used to overcome drawbacks associated with poorly water-soluble drugs, in general includes micronisation, salt formation, use of surfactant and use of pro-drug. Over the last 8-10 years, nanoparticles engineering processes have been developed and reported for pharmaceutical applications. Nanosuspension processes currently prepared by precipitation technique or, pearl milling technique,
either in water or in mixtures of water and water-miscible liquids or nonaqueous media. [7, 8]

Nanosuspensions are drug nanoparticles dispersed in a liquid phase, e.g. water. The drug nanoparticles can also be transferred to a dry product, e.g. by spray-drying, lyophilisation or using nanosuspensions as wetting agents in a granulation process to formulate tablets or pellets. The increase in bioavailability of mucus layer or the eye tissues is due to the special feature of nanosuspensions. Moreover, once-a-day or twice daily nanosuspension formulation should improve patient compliance. [9]

1.1 : Loteprednol Etabonate: [11, 12, 13]

Name of the drug: Loteprednol Etabonate
Category: Ocular anti-inflammatory Corticosteroids

2.1 : Preparation of Loteprednol Etabonate (LE) Nanosuspension by media milling method:

Nanosuspension was prepared by media milling technique. Zirconium oxide beads were used as milling media. In 20ml glass vial, weighed quantity of zirconium oxide beads was taken and 5ml distilled water was added in this vial. Surfactant and drug were incorporated and comminution was carried out on magnetic stirrer for particular period of time.

1) Effect of larger: smaller beads on nanosuspension by media milling method:

Different ratios of larger: smaller beads were tried, and the optimum ratio was found on the basis of achieved particle size. Diameter of smaller beads ranges from 0.4 to 0.7 mm and that of larger beads varies from 1.2 to 1.7 mm. Surface area for milling is greatly affected by ratio of beads (Table 1). Therefore 100% smaller beads were optimized and used for further study.

2) Effect of concentration beads on LE nanosuspension by media milling method:

Three concentrations of milling medium (Zirconium oxide beads) was optimized using 80, 100, and 120% w/v of batch size. Minimum particle size was achieved with 100% w/v of beads (Table 2).

3) Effect of stirring time on LE nanosuspension by media milling method:

Until this stage, the stirring time was kept constant and other parameters were optimized. Those optimized parameters were used during optimization of stirring time (Table 3).

2.2 Factorial design for optimization of key parameters of Loteprednol Etabonate (LE) Nanosuspension by media milling method:

Various formulation and process variables relating to effectiveness and usefulness should be optimized simultaneously when developing pharmaceutical formulations. In case of traditional method of optimization, combined effects of the independent variables are not considered. The difficulties in optimizing a pharmaceutical formulation are due to the difficulty in...
understanding the real relationship between dependent and independent responses. Factorial design has often been applied to optimize the formulation variables with basic need of understanding of interaction of independent variables.

Both particle size and drug release, important features of nanosuspension have been considered to play significant role in the formulation performance, are taken as response or dependant parameters in this study. Concentration of amount of small zirconium oxide beads and stirring time are taken as variable or independent parameters.

**Coded and actual values of independent variables for the formulation of Loteprednol Etabonate nanosuspension:**

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>X&lt;sub&gt;1&lt;/sub&gt;: amount of small zirconium oxide beads (%)</td>
<td>Low: 80, Medium: 100, High: 120</td>
</tr>
<tr>
<td>X&lt;sub&gt;2&lt;/sub&gt;: stirring time (hr)</td>
<td>12, 14, 16</td>
</tr>
<tr>
<td>Transform values</td>
<td>-1, 0, +1</td>
</tr>
</tbody>
</table>

Dependent variables: Y<sub>1</sub>: Particle size (nm), Y<sub>2</sub>: Q<sub>1</sub> (% drug release after 1 hr)

2.3 Characterization of Optimized LE Nanosuspension by Media Milling Method:

For prepared nanosuspension were characterized for the following physico-chemical properties.

**a) Particle size and zeta potential:**
Mean particle size and size distribution of the prepared nanosuspensions were obtained by using Malvern Zetasizer Nano-Series Nano-ZS, which follows principle of LASER light diffraction or also caller Photon correlation spectroscopy (PCS). The computer can provide the mean size and the distribution width of the nanoparticles in the batch (Malvern Zeta Sizer Nano series manual). Diluted nanosuspension was added to the sample cell (quartz cuvette) and put into sample holder unit and measurement was carried out with the help of software. Zeta potential of the optimized formulation was measured using the same instrument.

**b) Saturated solubility:**
Saturation solubility of bulk Loteprednol etabonate powder and nanosuspension formulation was measured in phosphate buffer (pH-8) and in solutions of different concentrations of stabilizers (Poloxamer 407) was determined by addition of an excess of the drug to the phosphate buffer (pH-8). The contents were stirred on magnetic stirrer (Remi, India) at 25°C for 24 h at 300 rpm. After reaching equilibrium, samples were filtered through a 0.45μm membrane filter, suitably diluted with phosphate buffer (pH 8) and analyzed for drug content at the max of 244 nm using a spectrophotometer (Shimazdu-1700,Japan). The individual values for three replicated were measured and their mean values are reported.

c) **Dissolution study:**
LE nanosuspension release profile was taken in modified diffusion cell apparatus. The drug release was determined using a dialysis tube (donor compartment) containing the known quantity (10 ml) of nanosuspension in a water-jacketed beaker containing 300 ml of Simulated Tear Fluid (pH 8) at 37 ± 1°C for 6 hrs. The contents of the beaker were agitated on a magnetic stirrer. LE content was determined by UV method at 244nm (Shimanzu, Japan).

d) **pH measurement:**
pH is the most important parameter for the ophthalmic preparation and it should remain near to neutral side for patient compliance. pH of the final formulation that was was measured using pH meter (Lab India Instrument, Mumbai).

e) **Stability study:**
Stability studies for nanosuspension were conducted at two different storage conditions for 1 month:

1. Room temperature
2. Refrigerated (2 - 8 °C)

Optimized batch of nanosuspension were used for each condition. The particle size and physical appearance are most important parameter for activity and physical stability of any nano sized formulations. In addition to change in particle size, assay was also carried out periodically to determine the stability of drug in the formulation at various storage conditions.

**RESULTS AND DISCUSSION**

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Type of surfactant</th>
<th>Conc. of surf. (w/v)</th>
<th>Conc. of Drug (w/v)</th>
<th>Larger : smaller beads ratio</th>
<th>Conc. of beads (w/v)</th>
<th>Stirring time (hr)</th>
<th>Mean particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Poloxamer 407</td>
<td>0.75%</td>
<td>0.5%</td>
<td>0 : 100</td>
<td>100 %</td>
<td>14 hr</td>
<td>235.1nm</td>
</tr>
</tbody>
</table>

In 20ml glass vial, weighed quantities of zirconium oxide beads were taken and 5ml distilled water was added in this vial. Surfactant and drug was incorporated and combination was carried out on magnetic stirrer for particular period of time.
Table 2: Effect of larger: smaller beads ratio on nanosuspension

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>of surfactant (w/v)</th>
<th>Conc. of drug (w/v)</th>
<th>Larger: smaller beads Ratio</th>
<th>Conc. of beads (w/v)</th>
<th>Stirring time</th>
<th>Mean particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M2</td>
<td>0.75%</td>
<td>0.5%</td>
<td>100:0</td>
<td>100%</td>
<td>14 hr</td>
<td>445.9</td>
</tr>
<tr>
<td>M3</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>50:50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M5</td>
<td>25:75</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M6</td>
<td>0:100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>255.9</td>
</tr>
</tbody>
</table>

As proportion of smaller beads increases, area available for milling also increases. Hence, moving from ratio 100:0 of larger: smaller beads to 0:100 of larger: smaller beads surface area increases. **Batch M6** i.e. 100% of smaller beads gave minimum particle size, due to maximum surface area as well as efficient stirring for the size reduction process (Table2). Therefore 100% smaller beads were optimized and used for further study.

Table 3: Effect of concentration of beads on LE nanosuspension

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Conc. of surf. (w/v)</th>
<th>Conc. of drug (w/v)</th>
<th>Conc. of beads (w/v)</th>
<th>Stirring time</th>
<th>Mean particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M7</td>
<td>0.75%w/v</td>
<td>0.5%</td>
<td>80%</td>
<td>14hr</td>
<td>259.1</td>
</tr>
<tr>
<td>M8</td>
<td>100%</td>
<td></td>
<td>120%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M9</td>
<td>120%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Higher concentration of beads provides larger surface area. Larger concentration than that led to insufficient and ineffective stirring of the contents in the vessel. **Batch M8** with 100% concentration of beads gave maximum size reduction while maintaining stirring efficiency. So, this concentration of beads was optimized and used for further study.

Table 4: Effect of stirring time on the preparation of LE Nanosuspension

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Conc. of surf. (w/v)</th>
<th>Conc. of drug (w/v)</th>
<th>Larger : smaller beads ratio</th>
<th>Conc. of beads (w/v)</th>
<th>Stirring time</th>
<th>Mean particle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimized batch M8</td>
<td>0.75%w/v</td>
<td>0.5%</td>
<td>0 : 100</td>
<td>100%</td>
<td>Initial 14hr</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12 hr</td>
<td>325</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14 hr</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 hr</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18 hr</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 hr</td>
<td>255</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16hr</td>
<td>270.1</td>
</tr>
</tbody>
</table>

14 hr of stirring was found to be sufficient to achieve optimum particle size in (Table 4), as more stirring did not show further significant size reduction as well as better uniformity. As the stirring time increases, there is also a risk of more wear of zirconium oxide into the formulation.

Table 5: Optimized formulation parameters based on preliminary trials

<table>
<thead>
<tr>
<th>Surfactant (Poloxamer 407)</th>
<th>0.75 %w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio of larger : smaller beads</td>
<td>100 % smaller beads</td>
</tr>
<tr>
<td>Vol of beads</td>
<td>100 % w/v</td>
</tr>
<tr>
<td>Concentration of drug</td>
<td>0.5 % w/v</td>
</tr>
<tr>
<td>Stirring time</td>
<td>14hr</td>
</tr>
<tr>
<td>Drug release at 1 hrs(Q1)</td>
<td>90.1%</td>
</tr>
</tbody>
</table>

Table 6: Result of Cumulative percent drug release after 1 hr from Nanosuspension

<table>
<thead>
<tr>
<th>S. No</th>
<th>Time (Minute)</th>
<th>Cumulative % drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>21.2</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>37.32</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>51.32</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>66.32</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>76.38</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>91.45</td>
</tr>
</tbody>
</table>

Table 7: Results of Stability study after 30days

<table>
<thead>
<tr>
<th>Stability condition</th>
<th>Formulation</th>
<th>Initial</th>
<th>After 30days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room temperature</td>
<td>Particle size</td>
<td>240±5±5.36</td>
<td>315±6.98</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>Appearance</td>
<td>No change</td>
<td>No change</td>
</tr>
</tbody>
</table>

Fig 1: Stability Study
Average particle size of optimized batch is 243 shown in (Fig 1). and Zeta potential was found to be -32. shown in (Fig 2). Poloxamer 407, a non-ionic surfactant is used as stabilizer which provide steric stabilization. So, negative zeta potential is attributed to drug nanocrystals. From Reference articles, zeta potential value of ±30mV is sufficient for stability of nanosuspension. In our formulation it is -32 which means it complies with requirement of zeta potential for stability.

**Saturated solubility:**
Saturation solubility of optimized batch of nanosuspension and bulk drug is 2105±16.5 μg/mL and 117±3.7 μg/mL respectively. In other words saturation solubility of nanosuspension was 17.99 times that of bulk drug. This great increase in saturation solubility of loteprednol etabonate due to particle size reduction can be concluded to enhance dissolution and justifying the objective of practical work.

**Dissolution study:**
The dissolution profiles of nanosuspension are presented in above (Table 6). In nanosuspension, more than 50% drug dissolved within 30 minutes and about 90% drug within 60 minute, so nanosuspension enhanced rate of dissolution of Loteprednol Etabonate to great extent.

**PH:**
The pH of LE nanosuspension was 6.6 ±0.3 that was near to the pH of lacrimal fluid. This work has demonstrated the use of 3² factorial designs in the optimizing formulation variables in the preparation of Loteprednol etabonate nanosuspension by media milling technique at laboratory scale and evaluated for particle size, zeta potential, saturated solubility and dissolution study. This methodology could therefore be employed successfully to nanosizing of the drug. The nanosuspension formulation was stable for 1 month at various conditions. The practical work resulted significant enhancement of solubility of Loteprednol Etabonate in Simulated tear fluid (STF-pH-8) using Nanosuspension approach.

**ACKNOWLEDGEMENT:**
We are highly thankful to administration of T. S. Patel College of Pharmacy, Bayad (Gujarat) for their kind assistance and cooperation in completion of this research work. We also appreciate the helping hands of our colleagues and laboratory staff for their continuous help and support. Least but not last, We are also obliged to Sun Pharma for providing free gift sample of Loteprednol Etabonate (LE) and Zirconium Beads and also thankful to BASF Germany for providing us Poloxamer 407 analytical grade.

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