Dextran — HPMC Eye Drops As Artificial Tears

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In the present study, a formulation containing a combination of dextran and hydroxypropyl methylcellulose (HPMC) was developed where HPMC, a viscosity-imparting agent, forms a thin film over precorneal tear film while dextran provides mechanical strength to this film. The formulation was developed by studying various polymers. A buffer system was selected to achieve an alkaline pH. Various preservatives were evaluated and a suitable antimicrobial preservative having suitable concentration was selected. Eye drops were evaluated for pH, viscosity, clarity, light stability, dextran, and HPMC content, and sterility. Ocular irritation studies and Schirmer’s test were carried out on rabbits. The final formulation was subjected to accelerated thermal studies. The developed formulation was non-irritant and showed optimum viscosity. It had better film forming properties and improved wetting characteristics than the marketed formulation MOISOL®. The developed formulation has potential as artificial tears in the treatment of dry eye syndrome.

Introduction

Dry eye syndrome is a condition which results from a deficiency of either the aqueous or mucin component of the precorneal tear film. Patients complain of severe ocular pain due to a sensation of dryness in the eyes. The terms dry eye and keratoconjunctivitis sicca (KCS) are most commonly used to indicate problems of the ocular surface connected with the reduction or instability of the precorneal tear film. The dry eye syndrome is most commonly found in individuals who spend most of their time in front of Video Display Terminals (VDTs), e.g., PCs and computer games. The prolonged use of VDTs is associated with a decreased frequency of blinking and an increased rate of tear evaporation. This leads to ocular fatigue which is one of the major symptoms of dry eye. The underlying causes of dry eye can be as follows: (i) aqueous tear deficiency, (ii) mucin abnormalities, (iii) impaired lid function.

Due to persistent disruption of tear film, the symptoms of KCS develop. In KCS, mild degree of conjunctival infection is associated with considerable irritation, photophobia, foreign body sensation, and burning.

Artificial tear formulations fulfill the physicochemical role of a normal tear. Artificial tears effectively lower the surface tension of the tear film, aid the formation of hydrophilic layer that is compatible with absorbed mucin, and enhance tear volume when necessary. Thus the mainstay of treatment of dry eye conditions remains the supplementation of tear production by artificial preparations.

Almost all the artificial tears available in the market contain hydrophilic polymer system. The objective of the present work was to develop artificial tears with hydroxypropyl methylcellulose (HPMC) and dextran, which are hydrophilic polymers. The rationale for their use is that HPMC is a viscosity-imparting agent and it forms a thin film over precorneal tear film, while dextran provides the mechanical strength to this film.

Materials and Methods

For the selection of raw materials, based on the requirements of ideal artificial tears, different pH and tonicity adjusting agents were studied, with special emphasis on hydrophilic polymeric system.
which decides the tear film characteristics, as well as the viscosity.

(A) Formulation Development

Based on tear studies the requirements of an ideal tear substitutes are: (a) Should be isotonic, (b) Should have pH of 7.4 ± 0.1 which is equivalent to tear fluid pH, (c) Should contain preservatives to prevent microbial growth, (d) Should have viscosity in between 25 to 50 cP, and (e) Should be sterilized by suitable method.

Initially, isotonic buffer vehicle was selected. Though the eye is tolerant to tonicity variation, given a choice, isotonicity is always desirable. In the present study, sodium chloride (0.45% w/v) was used as a tonicity adjusting agent. Freezing point method and molecular concentration method were used to calculate the amount of NaCl required. The buffered vehicle for the eye drops was prepared in order to prepare alkaline solution having pH about 7.4. However, pH of the formulation was selected to be 8.1, for achieving the stability of the polymeric system. Borax and boric acid buffer were used as the vehicle for the formulation. It was prepared by dissolving 0.19 g of boric acid, 0.19 g of borax, and 0.35 g of potassium chloride in 100 ml of purified water.

The next step was to select a suitable antimicrobial preservative. Benzalkonium chloride (BKC), phenylmercuric nitrate, and chlorbutanol were considered. PMN was rejected as the use of organic mercurials is restricted to neutral to alkaline solutions and as PMN has been reported to cause mercurialisthes. Chlorbutanol was avoided because of its reported slow action, and formulation and packaging difficulties. BKC (0.01 percent w/v) was selected as the preservative for the system.

Finally the selection of the polymeric system was made. Viscosity of artificial tears may be achieved between 25 to 50 cP by viscosity imparting agents such as polyvinyl alcohol (PVA) and cellulosic polymers such as methylcellulose (MC), hydroxypropylcellulose (HPC), and hydroxypropyl methylcellulose (HPMC). To study different viscosity imparting agents, following formulations with varying polymers and their combinations were prepared (Table 1)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Clarity</th>
<th>pH</th>
<th>Sp. gravity</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Clear</td>
<td>8.16</td>
<td>1.0098</td>
<td>26.75</td>
</tr>
<tr>
<td>B</td>
<td>Clear</td>
<td>8.11</td>
<td>1.0106</td>
<td>68.02</td>
</tr>
<tr>
<td>C</td>
<td>Clear</td>
<td>8.16</td>
<td>1.0096</td>
<td>1.10</td>
</tr>
<tr>
<td>D</td>
<td>Clear</td>
<td>8.15</td>
<td>1.0098</td>
<td>25.49</td>
</tr>
<tr>
<td>E</td>
<td>Clear</td>
<td>8.17</td>
<td>1.0098</td>
<td>71.46</td>
</tr>
<tr>
<td>MOISOL®</td>
<td>Clear</td>
<td>8.30</td>
<td>1.0079</td>
<td>42.30</td>
</tr>
</tbody>
</table>

Table 1 — Evaluation of formulations

Formula A = HPMC K4M alone (0.7 per cent w/v) in borate buffer pH 8.0.
Formula B = HPC MFF (0.7 per cent w/v) alone in borate buffer pH 8.0.
Formula C = Dextran 70 (0.1 per cent w/v) alone in borate buffer pH 8.0.
Formula D = HPMC K4M (0.7 percent w/v) and dextran 70 (0.1 per cent w/v) in borate buffer pH 8.0.
Formula E = HPC MFF (0.7 percent w/v) and
dextran 70 (0.1 per cent w/v) in borate buffer pH 8.0.

(B) Evaluation of Ophthalmic Solutions

The prepared formulations were evaluated for the following:

Clarity

The ophthalmic formulation filled into vials was observed for the presence of metal/glass particles, precipitates, etc., against different background in a well-lit cabinet.

pH

Adequate volume of the formulation was taken in a 50 ml beaker and the pH was recorded on a standardized Systronic pH meter.

Specific Gravity

Specific gravity of the formulation was measured by using specific gravity bottle of 10 ml capacity using purified water as standard.

\[
\text{Sp. gravity of formulation} = \frac{\text{Weight of 10 ml of formulation}}{\text{Weight of 10 ml of purified water}}
\]

Viscosity

Viscosity of the formulation was measured using Oswald's Viscometer No. 3. The viscosity studies were carried at constant temperature of 20°C and purified water was used as a standard. Formula used to determine the viscosity was:

\[
\frac{\eta_1}{\eta_2} = \frac{\rho_1}{\rho_2} \left(\frac{t_2}{t_1}\right)^{1/2}
\]

where \( \eta_1 \) = viscosity of the formulation, \( \eta_2 \) = viscosity of purified water at 20°C = 1.0086, \( \rho_1 \) = Sp. gravity of formulation, \( \rho_2 \) = Sp. gravity of purified water at 20°C, \( t_1 \) = time taken by formulation in seconds, and \( t_2 \) = time taken by purified water in seconds.

Content of Dextran 70 and HPMC

Dextran 70 is an optically active compound. Its optical activity was used to analyze its content. A standard curve of dextran 70 was plotted using a Carl Zeiss polarimeter and the percent content of dextran in the formulation was found out by extrapolation (Figure 1).

Since HPMC and dextran are carbohydrates the anthrone method was used to find out total content of carbohydrates in the formulation. The principle behind anthrone reaction is that all carbohydrates, on treatment with concentrated H\(_2\)SO\(_4\), undergo acid hydrolysis to form glucose, which on heating undergoes dehydration and ring formation to produce a furfural derivative. This derivative produces a colour in the presence of anthrone, which can be detected by UV spectrophotometry. A standard curve was first established using the same ratio of HPMC and dextran present in the formulation. The total carbohydrate content in the formulation was determined by extrapolation from the standard curve (Table 2).

From the total carbohydrate content the amount of dextran obtained from polarimetry experiment was subtracted to give the content of HPMC in the formulation.
Table 2 — Standard curve of HPMC and dextran

<table>
<thead>
<tr>
<th></th>
<th>HPMC (µg/ml)</th>
<th>Dextran 70 (µg/ml)</th>
<th>Total carbohydrate (µg/ml)</th>
<th>Absorbance</th>
<th>Regression Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>42</td>
<td>6</td>
<td>48</td>
<td>0.326</td>
<td>r = 0.9954</td>
</tr>
<tr>
<td>84</td>
<td>84</td>
<td>12</td>
<td>96</td>
<td>0.594</td>
<td>Slope = 0.005708</td>
</tr>
<tr>
<td>126</td>
<td>126</td>
<td>18</td>
<td>144</td>
<td>0.864</td>
<td>Y-intercept = 0.0404</td>
</tr>
<tr>
<td>168</td>
<td>168</td>
<td>24</td>
<td>192</td>
<td>1.070</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>210</td>
<td>30</td>
<td>240</td>
<td>1.478</td>
<td></td>
</tr>
</tbody>
</table>

(C) Stability Studies

Stability of the formulation was studied under the following:

Accelerated Thermal Studies

The selected formulation of dextran-HPMC eye drops was subjected to stability studies at various temperatures, i.e., room temperature, 37°C and 5°C. The eye drops were evaluated at every 15 d for a period of six months for clarity, pH, viscosity, specific gravity, percent content of dextran and HPMC.

Light Stability Studies

The effect of light on the physical and chemical stability was examined by exposing the final formulation in clear glass vials to direct sunlight and checking for clarity and discoloration.

Autoclaving Studies

To check for the stability of the formulation during terminal sterilization, i.e., autoclaving, it was exposed to autoclaving conditions at 121°C and 15 psi for 15, 20 and 30 min, respectively. The higher time points were used in an attempt to force degradation.

Sterility Testing

The developed formulation was tested for the presence or absence of fungi and aerobic and anaerobic bacteria by membrane filtration technique in the presence of positive and negative controls.

(D) Animal Studies

To determine the efficacy of the formulation, studies were carried out on rabbits using the marketed preparation MOISOL® for comparative purpose.

(i) Schirmer's Test

The test was performed by using Whatman filter paper No.4.1 strips of 35 mm by 5 mm dimension. The first 5 mm at one end of the paper strip was bent to work as a hook for eyelids (This portion was not included when the length of the moistened strip was measured). The paper notch was inserted into the conjunctival fornix folded, where it was left in position. The amount of wetting of strip was measured after 5 min and the strip was removed. Since the instillation of paper may produce some reflex tears the eye was anaesthetized by instilling a local anaesthetic solution (xylocaine 0.5 per cent) 10 min prior to the insertion of strip.

(ii) Ocular Irritation Studies

Assessment of ocular irritation potential of ophthalmic solution is an extremely important step in the development of ophthalmic product. Six rabbits weighing 2-3 kg each were used for the study. Each time, 2 drops of the formulation were instilled into the cul-de-sac of left eye of rabbit. Observations were made after 5, 10, and 15 min, respectively of instillation and eyes were observed for redness, swelling, watering, etc. The right eye served as control.
Results And Discussion

Borax and boric acid buffer were used as the vehicle with sodium chloride as the tonicity adjusting agent. Formulations containing HPC (Formulations B and E) were found to have relatively high viscosities, which made instillation into the eye difficult. Formulation containing only dextran (Formulation C) had very low viscosity, which was increased in combination with HPMC.

Formulation D was selected because its viscosity was optimum as compared to other formulations. Also, formulation D was able to produce a stable tear film. As seen in Table 1 the chosen formulation passed the evaluation tests for clarity, pH, viscosity, and specific gravity.

In terms of clarity of the formulation, pH, specific gravity, viscosity and per cent drug content, the formulation was found to be stable during accelerated thermal studies for the test period of six months.

It was observed that light had no detrimental effect on the formulation. It is reported that some fractions of dextran may adopt certain degree of crystallinity when stored at accelerated temperature. However, none of the vials showed any type of precipitation or flake formation.

After autoclaving the HPMC was found to precipitate at high temperature. Hence the vials must be shaken in order to redisperse the HPMC. At room temperature the HPMC goes into solution and vials remain clear.

The results obtained with Schirmer’s test revealed that the wetting effect by MOISOL® on eye was maintained for one-and-a-half hour, whereas the wetting effect produced by developed formulation was maintained for more than 2 h. Also the amount of wetting was more with developed formulation than that with MOISOL®.

Ocular irritation studies revealed that none of the rabbits showed any type of ocular irritation towards formulation as well as towards MOISOL®.

Conclusion

This study was aimed at developing a suitable artificial tear formulation for the treatment of dry eye syndrome. An ophthalmic solution was prepared by using HPMC and dextran polymers, which showed better film forming properties than the marketed preparation.

The instillation of normal saline for dry eyes is of very limited value. Other tear substitutes include vitamin A derivatives, viscoelastic agents, mucolytic agents, and lipid containing formulation. However, artificial tears, due to better tear film forming capacity, ease of formulation, ease of instillation and better patient compliance may be considered as the most suitable treatment for dry eye syndrome.

References