DEVELOPMENT AND CHARACTERIZATION OF NON-IONIC SURFACTANT VESICLES FOR OPHTHALMIC DRUG DELIVERY OF DICLOFENAC POTASSIUM

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ABSTRACT

Non-ionic surfactant vesicles was developed and characterized for ophthalmic drug delivery of Diclofenac potassium. The present research study is a promising approach to improve corneal penetration and bioavailability characteristics. Formulation also found to ensure a good entrainment efficiency and ocular bioavailability of drug in-vitro. Non-ionic surfactant vesicles containing Diclofenac potassium were prepared using surfactant and cholesterol in different ratio by Lipid film hydration technique. Niosomes were characterized For Entrapment efficiency, Particle size analysis, In-vitro drug release and In-vivo studies. The best formulation selected based on above parameters were subjected for sustained release study. Formulation with low cholesterol content which shown 82.1% Entrapment efficiency, 70.01% sustained release over a period of 10 h followed a non-fickian profile with zero order release profile. Scanning electron micrograph indicated that Niosomes have a discrete spherical structure without aggregation. In-vitro study showed an availability of drug in aqueous humor for an extended time period even up to 8 hour and it showed a correlation with the release profile in-vitro. Non-ionic surfactant vesicles are considered the best as it showed good and high Entrapment efficiency and Vitro release with better bioavailability. The proposed method was found to be precise and selective for the development and characterization of Diclofenac potassium Niosomes.

Key words: Diclofenac potassium, corneal penetration, sorbitan mono stearate, Entrapment efficiency, SEM, in vitro release study, HPLC.

INTRODUCTION

The treatment of infections and inflammatory conditions of eyes caused by obligate and intracellular microorganism is difficult because most of the antibiotics have poor intracellular diffusion, low ocular contact time, poor corneal penetration and solubility limitation. The need for drug delivery system with greater intracellular efficacy led to the development of Diclofenac potassium Non ionic surfactant vesicles with good sustained release profile, increased ocular contact time, good entrainment efficiency, improved corneal penetration and bioavailability characteristics.

Niosomes are essentially non-ionic surfactant based multi lamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulted from the organization of surfactant macro molecule as bilayer 1. The present formulation is hydrated mixture of cholesterol and Span 60. They consist of pharmaceutical ingredient and serves as drug carrier 2. They provide controlled ocular delivery by preventing the metabolism of the drug from the enzymes present at the tear/corneal epithelial surface 3. These drug carriers, when administered intravenously are rapidly taken up by cells of corneal surface; inhibit both, leukocyte migration and the enzyme cylooxygenase. Therefore the entrainment of drug within Niosomes has been proposed for the treatment of infections and inflammatory conditions 4.

Moreover, Niosome preparations are chemically stable, precise in chemical composition, cheaper in cost, have low toxicity 5 because of their non-ionic nature and independent of the pH. Diclofenac potassium has low bioavailability, low solubility and short half life that make it a candidate for controlled delivery. They can also widely accept as prophylaxis against cystoid’s macular edema. The aim of the present work is to formulate niosomal drug delivery system of Diclofenac potassium to be applied topically and to evaluate the in-vitro and In-vivo performance of the prepared niosome.

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Materials and Methods

Diclofenac potassium was obtained as a gift sample from Novartis Limited, India. Cholesterol, Span 60, Chloroform and Methanol (L.R grade) were purchased from Loba chemie, Mumbai. All other chemicals used were of analytical grade.

Preparation of Niosomes

Diclofenac potassium Niosomes were prepared by lipid film hydration technique. Span 60 and Cholesterol were weighed and dissolved in chloroform /methanol (2:1) in a 100 ml round bottom flask. A thin lipid film formed under reduced pressure in a rotary flash evaporator. The film then hydrated by 10 ml of phosphate buffer 7.4 at room with gentle shaking. The Niosome suspension further hydrated up to 24 Hrs at 2-8°C. The stabilized vesicles were used for further studies. This phosphate buffer 7.4 was made with 2.38 gm of Di-sodium hydrogen phosphate, 0.19 gm of potassium di-hydrogen phosphate and 8.0 gm of sodium chloride and made up to 1000 ml with distilled water. The formed Niosomes were separated by fractional centrifugation. Various formulations containing different ratio of cholesterol and surfactants were appropriately labelled respectively as shown in the table 1.

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>Ratio (µmol)</th>
<th>Surfactant (mg)</th>
<th>Cholesterol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 1</td>
<td>200:200</td>
<td>86</td>
<td>77.32</td>
</tr>
<tr>
<td>F 2</td>
<td>200:175</td>
<td>86</td>
<td>67.66</td>
</tr>
<tr>
<td>F 3</td>
<td>200:150</td>
<td>86</td>
<td>57.93</td>
</tr>
<tr>
<td>F 4</td>
<td>200:125</td>
<td>86</td>
<td>48.32</td>
</tr>
<tr>
<td>F 5</td>
<td>200:115</td>
<td>86</td>
<td>44.45</td>
</tr>
<tr>
<td>F 6</td>
<td>200:100</td>
<td>86</td>
<td>38.66</td>
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<tr>
<td>F 7</td>
<td>200:85</td>
<td>86</td>
<td>32.8</td>
</tr>
<tr>
<td>F 8</td>
<td>200:75</td>
<td>86</td>
<td>28.19</td>
</tr>
<tr>
<td>F 9</td>
<td>150:200</td>
<td>64.5</td>
<td>77.32</td>
</tr>
<tr>
<td>F10</td>
<td>150:150</td>
<td>64.5</td>
<td>57.93</td>
</tr>
<tr>
<td>F11</td>
<td>150:125</td>
<td>64.5</td>
<td>48.32</td>
</tr>
</tbody>
</table>

Characterization:

The prepared Diclofenac potassium Niosomes were subjected to Entrapment efficiency determination, Particle size analysis, In-vitro release and In-vivo study.

Entrapment Efficiency

Niosome entrapped Diclofenac potassium was estimated by dialysis method. The prepared Niosomes were placed in the dialysis bag 50 (pre-soaked for 24 hrs). Free Diclofenac potassium was dialyzed for 30 minutes each time in 100 ml of phosphate buffer saline pH 7.4. The dialysis of free Diclofenac always complete after 12-15 changes, when no Diclofenac was detectable in the recipient solution. The dialyzed Diclofenac potassium determined by finding out the concentration of bulk of solution by UV spectrophotometer at 275 nm. The samples from the bulk of solution diluted ten times before going for absorbance measurement. The free Diclofenac in the bulk of solution gives us the total amount of un-entrapped drug. Encapsulation efficiency is expressed as the percent of drug trapped.

\[
\text{Total drug} \times 100
\]

\[
\text{% Entrainment} = \frac{\text{Diffused drug}}{\text{Total drug}} \times 100
\]

Particle size analysis

Particle size analysis was carried out using scanning electron microscopy.

In vitro release study

In vitro release study of prepared Niosomes was carried out in dialysis bag method. 0.2 mg equivalent of 0.1% of Niosomal suspension was taken in dialysis bag (Hi media) and the bag was placed in a beaker containing 100 ml simulated tear fluid (pH 7.4 phosphate buffer). The beaker was placed over magnetic stirrer and the temperature was maintained at 37±1°C. 5 ml sample were withdrawn periodically and were replaced by fresh buffer. The sink condition was maintained throughout the experiment. The withdrawn samples were diluted 2 times. The amount of drug release was determined spectrophotometer-metrically at 280 nm keeping phosphate buffer pH 7.4 as blank.

In-vivo study

In-vivo study conducted in the animal model rabbit. Four healthy rabbits were used for the study, 3 drops of 0.1 % niosomal suspension of Diclofenac potassium was instilled in the lower cul-de-sac of each eye. The upper eyelids were gently held closed for 10 seconds to maximize the corneal contact. At the 4th and 8th hour of post dose eyes were anesthetized using 4% Xylocaine solutions. Topically and aqueous humor The mixture was then centrifuged at 3000 rpm for 20 minutes and the supernatant obtained was analyzed for the presence of Diclofenac potassium by HPLC detector, by comparing with the retention time of a standard solution (50µg/ml)

Qualitative estimation of Diclofenac potassium was done by HPLC. Filtered degassed mixture of Methanol and
RESULTS AND DISCUSSION

Diclofenac potassium Niosomes with same proportion of drug and varying proportions of Non ionic surfactant (Span-60) with Cholesterol, were prepared by lipid film hydration technique. Entrapment efficiency determination study suggests that higher entrapment efficiency from vesicle formed of span 60 is predictable because of its higher alkyl chain length. The entrapment efficiency is found to be higher with the formulation no. F6 (82.10) which has said to be the optimum cholesterol surfactant ratio to provide a high entrapment of Diclofenac potassium. The niosomal formulations having high surfactant concentration have the higher entrapment efficiency which may be due to the high fluidity of the vesicles. Very low cholesterol content too found to cause low entrapment efficiency, it may be because of leakage of the vesicles. It’s also observable that very high cholesterol content also has a lowering effect on drug entrapment to the vesicles. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment.

Figure 1: Entrapment efficiency of Diclofenac potassium Niosomes

Entrapment efficiency of various formulations is shown in fig. 1.

Figure 2: Scanning Electron Micrograph of Diclofenac potassium Niosomes

The Scanning electron micrograph of Diclofenac potassium Non ionic surfactant vesicles is shown in fig. 2. It indicated that Niosomes have a discrete spherical structure without aggregation. The particle size differed due to variation in the composition of the formulation. The shrinking of vesicles was observed under the study may be due to drying of vesicles under normal environment condition. The particle size differed due to variation in the composition of the formulation. In-vitro drug release profile has been described in figure is shown in fig. 3. The releases of the drug from various formulations are clearly the attributes of the varying cholesterol and surfactant ratios.

Figure 3: Comparative Assessment of In-vitro drug release profile of the Formulations
Table 2: Regression analysis of models for all formulations

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Zero order</th>
<th>Higuchi’s</th>
<th>Peppa’s</th>
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<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Correlation</td>
<td>Slope</td>
</tr>
<tr>
<td>F₁</td>
<td>5.6826</td>
<td>0.9956</td>
<td>18.2973</td>
</tr>
<tr>
<td>F₂</td>
<td>5.3567</td>
<td>0.9952</td>
<td>17.7922</td>
</tr>
<tr>
<td>F₃</td>
<td>5.7173</td>
<td>0.9974</td>
<td>18.8995</td>
</tr>
<tr>
<td>F₄</td>
<td>6.0543</td>
<td>0.9970</td>
<td>20.2571</td>
</tr>
<tr>
<td>F₅</td>
<td>6.2483</td>
<td>0.9989</td>
<td>28.9314</td>
</tr>
<tr>
<td>F₆</td>
<td>6.5599</td>
<td>0.9967</td>
<td>21.9748</td>
</tr>
<tr>
<td>F₇</td>
<td>6.9329</td>
<td>0.9943</td>
<td>23.4715</td>
</tr>
<tr>
<td>F₈</td>
<td>7.2887</td>
<td>0.9929</td>
<td>25.0848</td>
</tr>
<tr>
<td>F₉</td>
<td>5.0068</td>
<td>0.9945</td>
<td>16.6064</td>
</tr>
<tr>
<td>F₁₀</td>
<td>5.2206</td>
<td>0.9947</td>
<td>17.5716</td>
</tr>
<tr>
<td>F₁₁</td>
<td>5.8270</td>
<td>0.9967</td>
<td>19.6144</td>
</tr>
</tbody>
</table>

All the 11 batches of Diclofenac potassium Niosomes exhibited sustained release for about period of 10 hr. Most of the formulations were found to show a linear release and the formulations were found to provide approximately 60% release within a period of 10 hours. The formulations which have high cholesterol ratio (F₉, F₁₀) were found to sustain the drug release. Cholesterol has a property of abolish the gel to liquid transition of Niosomes, this found to prevent the leakage of drug from the niosomal formulation. The three optimized formulations F₄, F₅, and F₆ were found to give a release of 64.04%, 64.17% and 70.01% respectively over a period of 10 hrs, the higher release from the formulation F₆ may be because of its low cholesterol content. Formulations F₉ and F₁₀ has the lowest release over 10 hours they provide a release of 52.47% and 56.43% respectively, which has the highest cholesterol content. At the end of 10hr formulation F₁ to F₁₁ released about 59.42%, 57.38%, 57.45%, 64.04%, 64.17%, 70.01%, 72.07%, 75.94%, 52.47%, 56.43% and 61.31% respectively. In order to find out the mechanism of drug release, the in-vitro drug release data was graphically treated according to Higuchi’s equation and the graphical fit for the in-vitro data was used to conclude the mechanism of the drug release involved in the delivery system. Correlation value of Higuchi’s plot revealed that the mechanism of drug release is diffusion. The correlation value of zero order plots and correlation value of Higuchi’s plots with peppa’s plots presented in table no. 2. The best formulation selected based on Physico-chemical parameters were subjected for sustained release study is F₆. Separate graphs for F₆ listed below. In vivo study conducted to investigate the ocular availability of drug for a prolonged action after a single dose. The study carried out by comparing the retention time of the standard drug solution to that of the aqueous humor samples extracted sample. The retention time obtained here for the standard is 4.52 minutes, for the samples at 4th and 8th hour are 4.780 and 4.660 minutes respectively. This matching retention time of three samples shows the presence of drug Diclofenac potassium in the aqueous humor sample even after 4th and 8th hour of administration. Thus drug is available in detectable quantities even after 8th hour of administration. This may be because of possible retention of drug in the aqueous humor due to high corneal contact time and permeability provided by the vesicular system. In-vivo study is shown in fig. 4, 5 and 6 respectively.
Figure 4: HPLC Peak for the standard solution

Figure 5: HPLC Peak for the sample at 4th h
CONCLUSION

The method of preparation of Niosomes of Diclofenac potassium was found to be simple and reproducible. The slow and constant release of Diclofenac potassium from Niosomes maintains constant drug plasma concentration thereby increasing therapeutic efficacy and reducing the development of resistance. This study shows that Non-ionic surfactant Niosomes could be a useful carrier for Diclofenac potassium.

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REFERENCES