Formulation and Evaluation of Niosomal Drug Delivery System of Ketoprofen

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ABSTRACT

Purpose: Targeted drug delivery systems are used to deliver drugs to specific areas in definite concentration. Ketoprofen, belongs to NSAID, has various side effects associated with oral administration and also has less rate of permeation through skin from topical formulations. With an intention to increase skin permeability of ketoprofen through the skin by the use of vesicular structures called niosomes this study was undertaken. Methodology: In this particular study, niosomes were prepared by thin film hydration technique and ether injection technique. A topical niosomal gel was prepared by incorporating niosomes into 2% carbopol gel. Findings: Formulations prepared by thin film hydration technique, using drug, tween 40 and cholesterol in a ratio of 1:1:1 resulted in better entrapment efficiency and vesicular size in comparison to ether injection method. Evaluation: The niosomal formulations were characterised for vesicle size distribution, SEM and zeta potential. The best formulation (F16) was selected on the basis of drug entrapment efficiency of 83.63 ± 0.11% and in vitro diffusion profile. Conclusion: A comparative ex-vivo permeation study of niosomal gel against marketed gel, 2.5% w/w gel on excised rat abdominal skin model indicated a two-fold increase in permeation in comparison to marketed gel and a three fold increase in permeation in comparison to 2.5% w/w ketoprofen gel formula.

Key words: Niosome, Non-ionic surfactant, Drug entrapment, Novel drug delivery system, Ex vivo permeation.

INTRODUCTION

Topical drug delivery is used to deliver drugs to surface of the skin as well as underneath the skin. The main advantages of topical drug delivery system is that it bypasses first-pass metabolism, avoids effect of gastric pH, effect of enzymes and also effect of gastric emptying, etc. The skin acts as a principle barrier for topical drug delivery. The Stratum corneum plays a crucial role in barrier function for topical drug delivery. Despite major research and development efforts in topical systems and the advantages of these routes, low permeability of Stratum corneum limits the application of topical drug delivery. To overcome these limitations, novel drug delivery system are used which provides control of drug release in the body.

Novel drug delivery attempts to either sustain drug action at a pre-determined rate or maintain a relatively constant effective drug level in the body with concomitant minimization of undesirable side effects. Drug carriers are substances that serve as mechanisms to improve the delivery and the effectiveness of drugs. Drug carriers are used in drug delivery systems to bring about controlled-release technology to prolong in vivo drug actions, decrease drug metabolism, and reduce drug toxicity. The drug carriers used in the drug delivery systems are microparticles, microcapsules, cells, lipoproteins, liposomes, and niosomes etc. These carriers can be made to degrade slowly, to be stimuli responsive and to target at specific sites.

Ketoprofen (RS) 2-(3-benzoylphenyl)-propionic acid (chemical formula C₁₆H₁₄O₃) is one of the propionic acid class of non-steroidal anti-inflammatory drug with

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Research Article
analgesic and antipyretic effects. Inhibition of cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis via the arachidonic acid pathway is responsible for its anti-inflammatory action. Ketoprofen has better therapeutic action when administered orally compared to its topical application. It is less permeable when applied topically. Moreover, oral use of ketoprofen has side effects such as stomach upset, nausea, drowsiness, loss of appetite after oral administration.4

Niosomes, which are non-ionic surfactant vesicle act as a drug carrier to transport drug to the site of action with size ranges between 10-1000 nm, wherein aqueous phase is enclosed in a highly ordered bilayer of non-ionic surfactant with or without cholesterol and dicetyl phosphate. Niosomes can be used for entrapping both hydrophilic and hydrophobic drugs.

Niosomes are preferred over other vesicular system because it has low toxicity, non ionic nature, bio-degrad ability, and better availability of drug at site and good intrinsic skin penetration.5

Therefore, to avoid the side effects, ketoprofen can be incorporated into vesicular structure, which can be used for improved permeation of drug through skin.

MATERIALS AND METHODOLOGY

Ketoprofen ([RS]-2-(3-benzoylphenyl) propanoic acid, was obtained as a gift sample from BEC Chemicals Pvt. Ltd. India. Span 60, Span 40, Tween 60, Tween 40, Cholesterol was procured from Karnataka Fine Chemicals Private Limited, Bangalore, India. Carbopol 934 was obtained from Colorcon Asia Pvt. Limited, Bangalore, India. All the other chemicals used were of analytical grade.

Compatibility studies

FTIR spectroscopy was carried out to check the compatibility between ketoprofen and surfactants used. IR spectra of ketoprofen, 1:1 ketoprofen: surfactant mixture was studied using Bucker Tensor-27 (Bucker, Germany) FTIR (ATR) instrument.

Analytical method

A stock solution of 1 mg/ml of ketoprofen was prepared by dissolving 100 mg of drug in 100 ml of phosphate buffer pH 6.8. From this stock solution, 5 ml was diluted to 50ml using same buffer resulting in 100 μg/ml. λ<sub>max</sub> of the solution was determined by scanning the solution between 200 and 400 nm. The above 100 μg/ml solution was serially diluted in the range of 4-12 μg/ml and the absorbance was measured at observed λ<sub>max</sub> 260 nm.

Preparation of Niosomes

Ether Injection Method

Ketoprofen containing niosome was prepared by this technique using different ratios of nonionic surfactant and cholesterol as given in (Table 1). In this method, surfactant and cholesterol was dissolved in 5 ml of diethyl ether. Similarly, the drug was dissolved in ethanol. The drug solution was mixed with surfactant solution and the same was injected using micro syringe to the previously heated hydrating solution maintained at 60º with continuous stirring resulting in spontaneous vesicles.5

Thin film hydration technique

Niosomes were prepared by a thin film hydration method using a surfactant mixture consisting of (span 40, span 60, tween 60 and tween 40) and cholesterol, at different specified ratios as given in Table 2. Surfactant and cholesterol was dissolved in 8 ml of diethyl ether and the drug was dissolved in 2 ml of ethanol. The mixture was then transferred to a round bottom flask, and the solvent was evaporated under reduced pressure at a temperature 20-25º, using a rotary flash evaporator until the formation of a thin lipid film. The formed film was hydrated with 10 ml of phosphate buffer saline pH 7.4. The hydration was continued for 1 h, while the flask was kept rotating at 55-65º. The hydrated niosomes were sonicated for 20 min using a bath sonicator to obtain niosomal dispersion.6

Evaluation of Niosomes

Drug content and Entrapment Efficiency

Drug content was determined by disrupting the niosomal formulation by propane-1-ol, diluted suitably using phosphate buffer pH 6.8 and analysed for the drug content spectrophotometrically at 260 nm.

The free drug was determined by subjecting the niosomal formulation to centrifugation at 7000 rpm for 30 min to separate the free drug. After centrifugation, the supernatant was collected, and further centrifuged at 7000 rpm for 30 min. A clear solution was separated and the settled niosomes were collected. The collected supernatant was analysed for the drug content spectrophotometrically at 260 nm.7

Entrapment efficiency was calculated using the following formula:

\[
\text{% Entrapment efficiency} = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100
\]

Vesicle Size

Vesicle size of selected niosomal dispersion was determined by optical microscope and vesicle size, shape and
surface property of the selected formula was studied using Scanning Electron Microscope.  

**Zeta potential**

Niosomal dispersion were characterised for zeta potential by dynamic light scattering technology using Malvern Instruments at Aimil Ltd. Bangalore.  

**In vitro diffusion study**

The *in vitro* drug diffusion study was conducted by using Franz diffusion cell assembly. Niosomal formulation was placed on dialysis membrane between donor and receptor compartment of diffusion assembly. The receptor compartment was filled with (Phosphate buffer pH 6.8) which was maintained at 35 ± 1°, magnetically stirred at 50 rpm. The drug content was determined by collecting the receptor fluid (1ml) every h for 24 h, the volume withdrawn was replaced with 1ml of fresh buffer. After suitable dilution, the samples were analyzed spectrophotometrically at 260 nm.  

**Formulation of niosomal gel**

**Formulation of 2.5% ketoprofen gel**

Gel was prepared using carbopol-934 as gelling agent. Required quantity of gelling agent was weighed and dispersed in sufficient quantity of distilled water. This dispersion was neutralized by drop wise addition of triethanolamine till a clear gel was obtained. A 2.5% w/w gel was obtained by dissolving ketoprofen in propylene glycol, and treated in the same way as explained above. Formula of gel is given in Table 3.  

**Incorporation of niosomes to gel base**

Selected niosomal formula (F16) equivalent of 2.5% w/w ketoprofen was incorporated into gel base by gentle mechanical mixing at 25 rpm for 15 min.  

**Evaluation of Niosomal Gel**

**Physical examination**

The prepared gel formulations were inspected visually for colour, homogeneity, consistency, grittiness and spreadability.  

**pH**

The pH of gel was determined using digital pH meter (Digisun Electronics).  

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**Table 1: Formulation of niosomes by ether injection method**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Surfactant</th>
<th>Drug: surfactant: cholesterol</th>
<th>Weight taken (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Span 60</td>
<td>1: 1: 1</td>
<td>100: 100: 100</td>
</tr>
<tr>
<td>F2</td>
<td>Span 60</td>
<td>1: 2: 1</td>
<td>100: 200: 100</td>
</tr>
<tr>
<td>F3</td>
<td>Span 40</td>
<td>1: 1: 1</td>
<td>100: 100: 100</td>
</tr>
<tr>
<td>F4</td>
<td>Span 40</td>
<td>1: 2: 1</td>
<td>100: 200: 100</td>
</tr>
<tr>
<td>F5</td>
<td>Tween 60</td>
<td>1: 1: 1</td>
<td>100: 100: 100</td>
</tr>
<tr>
<td>F6</td>
<td>Tween 60</td>
<td>1: 2: 1</td>
<td>100: 200: 100</td>
</tr>
<tr>
<td>F7</td>
<td>Tween 40</td>
<td>1: 1: 1</td>
<td>100: 100: 100</td>
</tr>
<tr>
<td>F8</td>
<td>Tween 40</td>
<td>1: 2: 1</td>
<td>100: 200: 100</td>
</tr>
</tbody>
</table>

**Table 2: Formulation of niosome by thin film hydration technique**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Surfactant</th>
<th>Drug: Surfactant: cholesterol</th>
<th>Weight taken (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F9</td>
<td>Span 60</td>
<td>1: 1: 1</td>
<td>100:100:100</td>
</tr>
<tr>
<td>F10</td>
<td>Span 60</td>
<td>1: 2: 1</td>
<td>100:200:100</td>
</tr>
<tr>
<td>F11</td>
<td>Span 40</td>
<td>1: 1: 1</td>
<td>100:100:100</td>
</tr>
<tr>
<td>F12</td>
<td>Span 40</td>
<td>1: 2: 1</td>
<td>100:200:100</td>
</tr>
<tr>
<td>F13</td>
<td>Tween 60</td>
<td>1: 1: 1</td>
<td>100:100:100</td>
</tr>
<tr>
<td>F14</td>
<td>Tween 60</td>
<td>1: 2: 1</td>
<td>100:200:100</td>
</tr>
<tr>
<td>F15</td>
<td>Tween 40</td>
<td>1: 1: 1</td>
<td>100:100:100</td>
</tr>
<tr>
<td>F16</td>
<td>Tween 40</td>
<td>1: 2: 1</td>
<td>100:200:100</td>
</tr>
</tbody>
</table>

**Table 3: Formula of 2.5% ketoprofen gel**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (100 gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoprofen (2.5%)</td>
<td>2.5 gm</td>
</tr>
<tr>
<td>Carbopol 934 (2%)</td>
<td>2 gm</td>
</tr>
<tr>
<td>Propylene glycol (10%)</td>
<td>9.6 ml</td>
</tr>
<tr>
<td>Triethanol amine</td>
<td>q.s</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s</td>
</tr>
</tbody>
</table>
Viscosity

Viscosity was determined using Brookfield viscometer by selecting suitable (T-F) spindle by trial and error method at 10 rpm. The viscosity in cps was directly read.15

Spreadability

The Spreadability of the gel formulations was determined by taking two glass slides of equal length. On one glass slide, 1 gm gel was applied. To the other glass slide, weights were added and the time taken for the second glass slide to slip off from the first glass slide was determined. Spreadability coefficient was determined by the formula.16

\[ SC = \frac{M \times l}{t} \]

Where, SC=spreadability coefficient=Mass in gm, l is the length, t is time in min.17

Drug Content

Niosomal gel formulation equivalent to 100 mg ketoprofen was dissolved in 25 ml phosphate buffer pH 6.8, by mechanical shaking for 2 h, diluted suitably and analyzed for drug content spectrophotometrically at 260 nm.18

In vitro Diffusion Study

The in vitro drug release studies were conducted by using Franz diffusion cell assembly. A 100 mg drug equivalent niosomal formula was placed on dialysis membrane between donor and receptor compartment of diffusion cell assembly. The receptor compartment was filled with phosphate buffer pH 6.8 which was maintained at 35°C ± 1°C, magnetically stirred at 50 rpm. The drug content was determined by collecting 1ml of receptor fluid every h. The volume withdrawn was replaced with equal quantity of fresh buffer. After suitable dilution, the samples were analyzed spectrophotometrically at 260 nm.19

Ex vivo Permeation Study

Skin permeation study was carried out by using modified Franz diffusion cell. The experimental protocol was approved by Animal Ethics committee of Krupanidhi College of Pharmacy vide Ref. KCP/IAEC-0007/2013-14 date 31.12.2013. The rat abdominal skin sample was placed between donor and receptor compartment of the diffusion cell, Stratum corneum facing towards donor compartment. Niosomal gel formulation equivalent to100 mg of ketoprofen was applied to Stratum corneum of skin specimen. The receptor compartment was filled

Table 4: Drug content and entrapment efficiency of formulations F1-F8

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug Content (%)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>98.12 ± 0.20</td>
<td>68.52 ± 0.16</td>
</tr>
<tr>
<td>F2</td>
<td>97.4 ± 0.39</td>
<td>72.59 ± 0.15</td>
</tr>
<tr>
<td>F3</td>
<td>97.45 ± 0.42</td>
<td>69.57 ± 0.24</td>
</tr>
<tr>
<td>F4</td>
<td>98.84 ± 0.34</td>
<td>64.59 ± 0.22</td>
</tr>
<tr>
<td>F5</td>
<td>98.89 ± 0.52</td>
<td>73.51 ± 0.23</td>
</tr>
<tr>
<td>F6</td>
<td>97.56 ± 0.36</td>
<td>67.28 ± 0.17</td>
</tr>
<tr>
<td>F7</td>
<td>97.4 ± 0.34</td>
<td>80.20 ± 0.15</td>
</tr>
<tr>
<td>F8</td>
<td>98.66 ± 0.22</td>
<td>79.05 ± 0.05</td>
</tr>
</tbody>
</table>

Table 5: drug content and entrapment efficiency of formulations F9-F16

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug Content (%)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F9</td>
<td>98.19 ± 0.21</td>
<td>69.65 ± 0.2</td>
</tr>
<tr>
<td>F10</td>
<td>96.40 ± 0.32</td>
<td>79.41 ± 0.18</td>
</tr>
<tr>
<td>F11</td>
<td>98.32 ± 0.43</td>
<td>71.61 ± 0.31</td>
</tr>
<tr>
<td>F12</td>
<td>98.87 ± 0.34</td>
<td>68.28 ± 0.22</td>
</tr>
<tr>
<td>F13</td>
<td>97.45 ± 0.49</td>
<td>75.54 ± 0.23</td>
</tr>
<tr>
<td>F14</td>
<td>98.36 ± 0.41</td>
<td>69.45 ± 0.33</td>
</tr>
<tr>
<td>F15</td>
<td>97.53 ± 0.35</td>
<td>84.61 ± 0.24</td>
</tr>
<tr>
<td>F16</td>
<td>98.86 ± 0.25</td>
<td>83.63 ± 0.11</td>
</tr>
</tbody>
</table>

Table 6: Physical evaluation of gel

<table>
<thead>
<tr>
<th>Test</th>
<th>Ketoprofen 2.5% w/w gel</th>
<th>Niosomal gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spreadability</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Washability</td>
<td>Washable</td>
<td>Washable</td>
</tr>
<tr>
<td>Homogenity</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Appearance</td>
<td>Clear</td>
<td>Transparent</td>
</tr>
<tr>
<td>Phase separation</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
with (phosphate buffer pH 6.8) which was maintained at $35 \pm 1^\circ$, magnetically stirred at 50 rpm. The drug content was determined by collection of receptor fluid (1ml) every h and volume withdrawn was replaced with equal quantity of fresh buffer. After suitable dilution, the samples were analyzed for the drug content spectrophotometrically at 260 nm.\textsuperscript{19}

**Stability studies**
Selected niosomal formulation (F16) was packed in tightly closed amber coloured bottles wrapped in aluminium foil and kept at $30^\circ \pm 2^\circ$ at $(65 \pm 5\%$ RH) for 30 days in a stability chamber and also at 2-8\° temperature in a refrigerator. After 30 days the samples were evaluated for % entrapment efficiency and vesicle size.\textsuperscript{20}

**RESULTS AND DISCUSSION**

**Analytical method**
Absorption spectrum of ketoprofen in phosphate buffer pH 6.8 showed $l_{max}$ at 260 nm. Ketoprofen in phosphate buffer was found to follow Beer’s law in 4-12 µg/ml concentration range with regression coefficient of 0.997.

**FTIR Spectroscopy**
In the present study, the possible interaction between ketoprofen and excipient mixtures such as surfactants and cholesterol was carried out. Existence of principle peaks revealed no considerable changes in the FTIR peaks of Ketoprofen when mixed with excipients compared to pure Ketoprofen. (Figure 1, 2).

**Evaluation of Niosomes**

**Drug content**
Drug content was determined for all the niosomal formulations. Average of three readings was considered. The drug content by ether injection method was found to be in the range of 97.4\% ± 0.39 to 98.89\% ± 0.52 and by thin film hydration method it was in the range of 96.40 ± 0.32-98.87 ± 0.34\%. The data are presented in Table 4 & 5 respectively.

**Entrapment efficiency**
Of all the niosomal formulations, tweens showed to have higher entrapment efficiency compared to spans. This is due to the fact that tweens are hydrophilic in nature which has a high HLB value in comparison to span. Therefore, hydrophobic drugs may be better entrapped in comparison to span. The niosomes prepared by thin film hydration method had higher entrapment efficiency than ether injection method. The data are presented in Table 4 & 5 respectively.

**Vesicular size**
The vesicle size was found to be $2.09 \pm 0.13\text{ - }4.35 \pm 0.18$ nm for ether injection method and $1.07 \pm 0.02\text{ - }3.33 \pm 0.10$ nm for thin film hydration method. The average vesicle size of selected formulation was found to be around 585 nm (Figure 3).

**Optical microscopy**
Optical microscope view of selected formulation F16 is presented in Figure 4.

**SEM study**
The surface morphological studies of selected formulation (F16) indicates (Figure 5), presence of almost spherical vesicles with even external surface.

**Zeta potential**
The zeta potential value of prepared niosome was found to be 43.9 mV. (Figure 6) A higher zeta potential is an indicative of a stable colloidal system.

**In vitro diffusion study:**
The release study suggests that 50\% of drug was released in 12 h which may be due to improper bursting of niosome vesicles. After that the release was controlled because the niosome began to obtain stability. The release also depends on the entrapment efficiency. As evident from Figure 7 & 8, formulations with more entrapment efficiency showed more release percentage compared to formulations with less entrapment efficiency.

**Evaluation of Niosomal Gel**

**Physical evaluation:** The observations are given in Table 6.

**pH of the gel**
The pH of the gel was found to be 6.9 which falls in range of the skin pH.

**Drug content**
The drug content of the niosomal gel was found to be 94.35\% ± 0.86.

**Spreadability**
The spreadability of the formulation was found to be in the range of $(12.51 \pm 0.70 \text{ to } 15.43 \pm 0.79)$ g cm/sec.

**Viscosity**
Viscosity of the niosomal gel prepared using carbopol 934 was found to be 6170 cp.

**In vitro diffusion study**
The drug release from niosomal gel was 78.9\% at the end of 12 h in (Figure 9) comparison to selected niosomal formula F16, whose drug release was 56.67\% in 12 h.
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Figure 1: IR spectra of ketoprofen

Figure 2: IR spectra of ketoprofen niosomal formulation

Figure 3: Vesicle size distribution of niosomes

Figure 4: Microscopic view of niosomal formulations (F 16) at 10X

Figure 5: SEM of niosome formulation (F16)

Figure 6: Zeta potential of noisomal formulation F16

Figure 7: In vitro diffusion study of Niosomal formulations (F1-F8)

Figure 8: In vitro diffusion study of formulation (F9-F16)
Ex vivo permeation study

Comparative ex vivo permeation study of niosomal gel against 2.5% ketoprofen gel and marketed gel showed that niosomal gel has a higher rate of permeation compared to marketed gel and 2.5% ketoprofen gel. The increased rate of permeation from the niosomal gel may be due to the permeation enhancing effects of niosomes by altering the structural integrity of the skin and altering the physical state of water in the skin. The data is presented in Figure 10.

Stability studies

The best formulation was stored at 2-8° in refrigerator, 30 ± 2° temperature and 60 ± 5% RH, for a period of one month. Sedimentation of the particle was found at 30 ± 2° (RH 60 ± 5%). The vesicle size 0.59 ± 0.01 μm and entrapment efficiency 84.61% ± 0.24 of the best formulation showed that there was no significant change in these parameters when stored at 2-8°. The particle size was found to be increased 2.43 ± 0.12 μm and entrapment efficiency was found to get decreased 64.13 ± 2.02 when stored at 30 ± 2° (RH 60 ± 5%) as compared to the initial results. Thus, it can be concluded that 2-8° and ambient humidity are the most suitable for storage of prepared niosomes of ketoprofen.

CONCLUSION

Ketoprofen being a non steroidal anti-inflammatory drug is associated with adverse effects when administered via oral route and is less permeable through skin. Therefore being incorporated into non ionic surfactant vesicles, by the virtue of altering the structural integrity of the skin might have resulted in a twofold increase in the permeability. Thin film hydration could be used for incorporation of hydrophobic drug since it has higher drug loading efficiency. Use of hydrophilic surfactants resulted in better entrapment efficiency in comparison to lipophillic surfactants. So by incorporating into a suitable gel base, niosomes could be a promising and economical carrier in topical drug delivery.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

ABBREVIATION USED

RH: Relative humidity; SC: Spreadability coefficient; CPS: Centipoise; HLB: Hydrophilic lipophilic balance; FTIR: Fourier transformation infrared spectroscopy.