Sorbitan ester niosomes for topical delivery of rofecoxib

Malay K Das* & Narahari N Palei
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh 786 004, India

Received 29 March 2010; revised 22 February 2011

The aim of the present investigation is to encapsulate rofecoxib in niosomes and incorporate the prepared niosomes into dermal gel base for sustained therapeutic action. Niosomes were prepared by lipid film hydration technique and were analyzed for size, entrapment efficiency and drug retention capacity. Niosomal vesicles were then incorporated into blank carbopol gel to form niosomal gel. The in vitro permeation study across pig skin was performed using Keshary-Chien glass diffusion cell. The size and entrapment efficiency of the niosomal vesicles increased with gradual increase in HLB value of nonionic surfactants used. Maximum drug entrapment was observed with Span 20 with HLB value of 8.6 and drug leakage from vesicles was less at refrigerated condition than at the room temperature. Higher proportion of cholesterol made the niosomal formulation more stable with high drug retention properties. The niosomal gel showed a prolong drug release behavior compared to plain drug gel. Differential scanning calorimetric study of drug loaded gel and pig skin after permeation study confirmed inertness of carbopol gel base toward rofecoxib and absence of drug metabolism in the skin during permeation study, respectively. The niosomal formulations were successfully prepared by lipid film hydration technique using cholesterol and Span as nonionic surfactant. Presence of cholesterol made niosomes more stable with high drug entrapment efficiency and retention properties. The lower flux value of niosomal gel as compared to plain drug gel across pig skin assured the prolong drug release behavior with sustained action.

Keywords: Lipid film hydration technique, Niosomal gel, Niosomes, Rofecoxib, Topical delivery

Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic and hydrophilic drugs and have been well documented for dermal, transdermal and oral drug delivery1-5. Niosomes loaded with drugs for dermal application are aimed to preferentially show interactions with the epidermal tissue without exerting an immediate or strong systemic action6. A higher synovial fluid drug concentrations than the plasma concentrations have been observed with topical application of non-steroidal anti-inflammatory drugs (NSAIDs) over an inflamed joints, which suggests the direct penetration of the drug into the joint6.

Rofecoxib, a selective cyclo-oxygenase 2 (COX 2) inhibitor, was approved in the year 1999 by FDA with a view to reduce the incidence of GI adverse effects as observed with other less selective NSAIDs7. The oral bioavailability of rofecoxib is about 93 % and the effective therapeutic plasma concentration (207-321 ng/ml) is reached at 3-4 days with multiple dose oral administration. But the drug was withdrawn in 2004 after incidence of its GI adverse effects and cardiac toxicities following oral administration. Baboota, Dhalwal and Kohli8 reported that rofecoxib is practically insoluble in water (4.6 mg/L) and a dangerously high drug concentration is achieved in GI tract causing ulceration. It has been reported to demonstrate a significant decrease in gastric ulcerogenic activity of rofecoxib through solid dispersions, which may enhance its dissolution rate leading to a faster onset of action and less GI mucosal toxicities. On the other hand, the above formulations may increase the risk of the systemic cardiac toxicities of rofecoxib. The topical administration of rofecoxib may prevent the systemic side effects by confining drug concentrations / actions to the site of the origin of pain. Das and Ahmed9 reported the anti-inflammatory activity of rofecoxib gel formulation in carrageenan induced hind paw edema model. A maximum of 58.93% inhibition of edema was observed at 6 h as compared to the maximum value of 65.52% inhibition of edema with oral rofecoxib at 4 h. A faster anti-inflammatory activity had been observed with microemulsion gel containing rofecoxib polyethylene glycol 4000 solid dispersions as compared to the conventional gel10. Das and Ahmed11 investigated the enhancing effect of ascorbic acid and
triethyl citrate on the in vitro skin permeation of rofecoxib from gel formulation across rat epidermis for faster anti-inflammatory activity. The topical gel formulations with faster anti-inflammatory activity of rofecoxib may not produce extended action of the drug, which results in unacceptable patient compliance. Therefore, the present investigation is aimed to encapsulate rofecoxib in niosomes and incorporate the prepared niosomes into suitable dermal base to improve therapeutic efficacy with sustained action for patient compliance.

**Materials and Methods**

Rofecoxib was gift sample from Alembic Pharmaceuticals Ltd. (Vadodara, India). Carbopol 940, disodium hydrogen orthophosphate, sodium bromide (Loba Chemie Pvt. Ltd., Mumbai, India); potassium dihydrogen orthophosphate, sodium chloride, propylene glycol, polyethylene glycol 400 (Ranbaxy Laboratories Ltd., New Delhi, India); diethyl ether, glycerin (Qualigens Fine Chemicals Pvt. Ltd., Mumbai, India); cholesterol, triethanolamine, span 20, 40, 60 (CDH Laboratories Ltd., New Delhi, India); ethanol (Bengal Chemicals and Pharmaceuticals Ltd., Mumbai, India); isopropyl alcohol (E Merck Ltd., Mumbai, India) were procured and used in this investigation.

**Preparation and characterization of niosomes**—Niosomes were prepared by lipid film hydration technique\(^1\) with slight modification. The compositions of different niosomal formulations are listed in Table 1. The drug, nonionic surfactants and cholesterol were dissolved in 10 ml of diethyl ether in a 100 ml round bottomed flask. The organic solvent was then removed at room temperature (25 ± 1°C), under vacuum, in a rotary evaporator to obtain a smooth, dry lipid film. The film was hydrated with 5 ml of phosphate buffer saline (pH 7.4) at room temperature for 1 h with gentle shaking. The niosomal suspension was further hydrated at 2-8°C for 24 h.

All the niosomal formulations were viewed under a phase contrast microscope equipped with a camera (100 ×, Leica DMLP, Germany) to observe the shape and lamellar nature of vesicles. The size analysis of the niosomes was performed using an ocular micrometer. The photomicrographs are shown in the Fig. 1.

The prepared niosomes were analyzed for entrapment efficiency by spectrophotometric method after separation of free drug. The drug containing niosomes were separated from untrapped drug by dialysis for 30 min. The niosomes were the disrupted with 50% isopropyl alcohol in phosphate buffer saline (pH 7.4). The drug content in the sample was determined using Hitachi U-2001 UV-VIS spectrophotometer at 260 nm against the appropriate blank. The entrapment efficiency was determined using the equation reported by Ruckmani et al.\(^1\)

The prepared niosomes were analyzed for drug retention capacity. The niosomal formulations were sealed in 15 ml vial and stored at 4 ± 2°C and 25 ± 2°C for a period of 2 months. The samples at each temperature were withdrawn at definite time intervals; analyzed for the residual amount of the drug in vesicles (percent drug entrapment) as described earlier. The entrapment efficiency values after 2 months were compared to that of the freshly prepared formulations.

**Preparation of carbopol gels**—The plain drug gel (GF) was prepared by dispersing 1% w/w carbopol

<table>
<thead>
<tr>
<th>F. N. Code</th>
<th>NS used</th>
<th>NS-Chol.molar ratio (µ mol)</th>
<th>NS-Chol wt qty. (mg)</th>
<th>Entrapment (%)</th>
<th>Vesicle size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-1</td>
<td>Span 20</td>
<td>300:200</td>
<td>103.8:77.32</td>
<td>76.28</td>
<td>11.94±1.24</td>
</tr>
<tr>
<td>NF-2</td>
<td>Span 20</td>
<td>250:250</td>
<td>86.5:96.66</td>
<td>72.43</td>
<td>11.38±1.71</td>
</tr>
<tr>
<td>NF-3</td>
<td>Span 20</td>
<td>200:200</td>
<td>69.2:77.32</td>
<td>64.74</td>
<td>10.93±1.73</td>
</tr>
<tr>
<td>NF-4</td>
<td>Span 40</td>
<td>250:250</td>
<td>100.58:96.66</td>
<td>42.46</td>
<td>10.35±1.57</td>
</tr>
<tr>
<td>NF-5</td>
<td>Span 40</td>
<td>300:200</td>
<td>120.7:77.32</td>
<td>45.67</td>
<td>10.83±1.17</td>
</tr>
<tr>
<td>NF-6</td>
<td>Span 40</td>
<td>200:200</td>
<td>80.46:77.32</td>
<td>36.05</td>
<td>10.24±1.24</td>
</tr>
<tr>
<td>NF-7</td>
<td>Span 60</td>
<td>250:250</td>
<td>107.59:96.66</td>
<td>23.23</td>
<td>10.36±1.50</td>
</tr>
<tr>
<td>NF-8</td>
<td>Span 60</td>
<td>200:200</td>
<td>80.07:77.32</td>
<td>21.63</td>
<td>10.16±1.17</td>
</tr>
<tr>
<td>NF-9</td>
<td>Span 60</td>
<td>300:200</td>
<td>129.11:77.32</td>
<td>28.10</td>
<td>10.55±2.07</td>
</tr>
</tbody>
</table>

Rofecoxib concentration used was 10 mg per batch, NS = Nonionic surfactant, Chol. = Cholesterol
Fig. 1—Photomicrographs of (a) NF-1 (Span 20 : Cholesterol 300:200 µmol); (b) NF-1 after 1 month of preparation; (c) NF-1 after 2 month of preparation; (d) NF-5 (Span 40 : Cholesterol 300 : 200 µmol); (e) NF-9 (Span 60 : Cholesterol 300 : 200 µmol).
940 in a mixture of water and glycerol (30% w/w) with 0.5 % w/w rofecoxib, being kept under magnetic stirring until homogeneous dispersion formed. The dispersion was then neutralized and made viscous by the addition of triethanolamine. The pH of the formulation was observed at 6.62 ± 0.02. The drug content in the prepared gel was assayed using the method reported by Das and Ahmed and the value was found at 97.59 ± 1.09%.

Niosomal gel (NG) was prepared using the same formula. For this purpose the niosomal suspension containing equivalent amount of rofecoxib was centrifuged (7000 rpm at 4°C for 30 min) and the vesicles obtained was incorporated into the previously prepared blank carbopol gel to form niosomal gel.

The photomicrographs of the gel formulations were obtained using a phase contrast microscope (Leica DMLP) and presented in the Fig. 2.

**In vitro permeation study**—The *in vitro* permeation study was performed using Keshary-Chien glass diffusion cell and pig epidermis with intact stratum corneum as membrane. The full thickness pig abdominal skin was collected from slaughterhouse. The method of epidermis preparation was based on the previous report. The epidermis was thoroughly washed with water, dried at room temperature under 25% RH, wrapped in aluminium foil and stored at 4 ± 1°C until further use.

For *in vitro* permeation studies, skins were allowed to hydrate for 1 h before being mounted on the Keshary-Chien diffusion cell with the stratum corneum facing the donor compartment with an effective diffusion area of 2.72 cm². The receptor compartment was filled with 20 ml of aqueous solution of PEG 400 (40% v/v) and receptor phase was maintained at 37 ± 0.5°C to maintain the donor phase temperature at 32-33°C. The gel (1 g) was placed on the stratum corneum side in the donor compartment and covered with aluminium foil to prevent drying out. The amount of drug permeated was determined spectrophotometrically at 260 nm by removing a 1 ml aliquot through a hypodermic syringe fitted with a 0.45 µm membrane filter, at designated time intervals for 8 h. The volume was replenished with the same volume of prewarmed receiver solution to maintain sink conditions. Blanks were run for each set as described above with placebo gel and calculated accordingly. The concentration of drug in the samples was corrected for sampling effects according to the equation reported by Hayton and Chen.

**Differential scanning calorimetry (DSC)**—The DSC analysis of rofecoxib, carbopol 940, drug loaded carbopol gel, pig epidermis before and after...
permeation study were carried out in the heating range of 140-250°C at the rate of 10°C/min using Differential Scanning Calorimetry (DSC 50, Shimadzu, Japan).

Data and statistical analysis—The steady state flux (J, µg/cm²/h) was calculated from the slope of the linear plot of the cumulative amount permeated per unit area (µg/cm²) as a function of time (t, h). The lag time (tₜₑ, h) was determined from the x-intercept of the slope at the steady state. The permeability coefficient (Kₚ, cm/h) was calculated from the ratio of flux to the donor drug concentration.

Data are expressed as mean±SD (n = 3). Statistical comparisons were made using Student’s t-test at a significance level of P < 0.05 using MS-Excel software.

Results

Characterization of niosomes—The prepared niosomes were spherical in shape (Fig. 1). The mean vesicle size of all formulations was between 10.16±1.17 and 11.94±1.24 µm. The mean volume diameters of vesicles and entrapment efficiency of rofecoxib in various niosomal formulations are presented in Table 1.

The effect of different surfactants, and surfactant:cholesterol ratio was studied on entrapment efficiency. It varied between 21.63 and 76.28 %. The encapsulated drug tends to leak out from the bilayer structured vesicles during storage. A significant loss of rofecoxib was noted after incubation of niosomal formulations at 4 ± 2°C and 25 ± 2°C for 2 months and the results of percent drug recovered are recorded in Table 2.

In vitro permeation study—The flux values of plain drug gel (GF) and niosomal gel (NG) were found to be 55.26 ± 2.17 µg/cm²/h and 20.93 ± 1.79 µg/cm²/h, respectively. The lower flux value of niosomal gel indicates its prolong drug release behavior as compared to plain drug gel. The permeability coefficient and lag time of niosomal gel were found to be 4.18 × 10⁻² cm/h and 0.33 h, respectively (Table 3).

The permeation profile of niosomal gel (Fig. 3) show a slower flux in the initial period of the permeation experiments and then rofecoxib permeation rate was higher in all sampling times. Initial rapid release (burst effect) was observed for plain drug gel (Fig. 3) and then constant values were observed for the rest of time of the permeation experiments.

A linear relationship (r > 0.9) existed between the cumulative amount permeated and the square root of time (Fig. 4) indicating that the drug permeation was based on Higuchi diffusion controlled mechanism.

Differential scanning calorimetry (DSC)—DSC thermograms of pure rofecoxib and carbopol, drug loaded gel are shown in Fig. 5. The thermogram of drug loaded gel show a nonsignificant different in the endothermic peaks of rofecoxib (increased from 212.80°C to 213.71°C). The consistency of thermogram of drug loaded gel with that of pure drug indicates that no structural changes occurred for rofecoxib in carbopol gel base.

Discussion

Characterization of niosomes—The main factors affecting the size and size distribution of vesicles are cholesterol and HLB value of surfactants. The niosome volume diameter decreases with increasing the amount of cholesterol content from 200 to 250 µmol. The vesicles with relatively high cholesterol content increases the chain order, stabilizes the bilayers of vesicles.

The mean vesicle size of niosomes increased with gradual increase in the HLB value of the Span in the following order: Span 60 (4.7) < Span 40 (6.7) < Span 20 (8.6). Niosome size and Span hydrophobicity has been attributed to the decrease in surface energy with

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Entrapment efficiency (%)</th>
<th>Drug retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-1 4 ± 2</td>
<td>62.18</td>
<td>81.52</td>
</tr>
<tr>
<td>25 ± 2</td>
<td>39.09</td>
<td>51.25</td>
</tr>
<tr>
<td>NF-2 4 ± 2</td>
<td>60.87</td>
<td>84.04</td>
</tr>
<tr>
<td>25 ± 2</td>
<td>44.03</td>
<td>60.80</td>
</tr>
<tr>
<td>NF-3 4 ± 2</td>
<td>51.92</td>
<td>80.20</td>
</tr>
<tr>
<td>25 ± 2</td>
<td>37.65</td>
<td>58.17</td>
</tr>
</tbody>
</table>

Table 3—Permeation parameters from rofecoxib gel formulations

<table>
<thead>
<tr>
<th>F.N. Code</th>
<th>Cumulative amount permeated (µg/cm²)</th>
<th>Flux (J, µg/cm²/h)</th>
<th>Permeability coefficient (Kₚ, cm/h × 10⁻²)</th>
<th>Lag time (tₜₑ, h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF</td>
<td>544.80 ± 25.31</td>
<td>55.23 ± 2.17</td>
<td>11.05 ± 0.89</td>
<td>–</td>
</tr>
<tr>
<td>NG</td>
<td>166.76 ± 5.34</td>
<td>20.93 ± 1.79</td>
<td>4.18 ± 0.30</td>
<td>0.33</td>
</tr>
</tbody>
</table>
increasing hydrophobicity\textsuperscript{15} resulting in the smaller vesicles. It has been reported that the mean size of niosomes increased with progressive increase in the HLB value of different sorbitan ester surfactants\textsuperscript{13}. This behavior can be explained because the higher the surfactant hydrophilicity, the higher the water uptake into the bilayer. Therefore, vesicle size increases as well as the entrapment of rofecoxib (Table 1).

The entrapment of rofecoxib in niosomes was increased with increase in the HLB value of Span in the following order Span 60 < Span 40 < Span 20\textsuperscript{6}. The maximum drug entrapment was observed with Span 20 with HLB of 8.6. When the molar concentration of Span 20 was increased, the entrapment efficiency of rofecoxib increased from 64.74 to 76.28%. Also the increase in cholesterol concentration up to 50% resulted in increased entrapment efficiency. This increase in entrapment may be due to increase in the vesicle size, lipophilic behavior and crystallinity of the lipid bilayer of niosomes\textsuperscript{16}.

The results from drug leakage study indicate that the percent drug retention in vesicles was significantly different ($P < 0.05$) at $4 \pm 2^\circ C$ and $25 \pm 2^\circ C$. The drug leakage from vesicles was less at refrigerated condition than at the room temperature. This may be due to the higher fluidity of lipid bilayers at higher temperature resulting in higher drug leakage\textsuperscript{16}. The drug retention was more in NF-2 with 84.04% and 60.8% at 4°C and 25 ± 2°C, respectively, after two months compared to NF-1 and NF-3. It indicates that the higher proportion of cholesterol makes the niosomal formulation more stable with high drug retention properties.

\textit{In vitro permeation study}—The lower flux values of niosomal gel results in prolong drug release behavior as compared to plain drug gel (Table 3). The prolonged drug release from niosomal gel may be due to the slower diffusion of drug into the skin. The lower extent of drug permeation via niosome vesicle may indicate that the lipid bilayer of niosomes is rate

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig_3.png}
\caption{Permeation profile of rofecoxib from gel formulations. [Each data point represents the mean ± SEM of 3 experiments.]}\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig_4.png}
\caption{Higuchi plot for rofecoxib permeation from gel formulations.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig_5.png}
\caption{DSC thermograms.}
\end{figure}
limiting in the drug permeation. Thus, a sustained delivery of rofecoxib may be obtained from the niosomal gel formulation.

Topically applied niosome can increase the residence time of drugs in the stratum corneum and epidermis, while reducing the systemic absorption of drug. They are thought to improve the horny layer properties, both by reducing transepidermal water loss and by increasing smoothness via replenishing lost skin lipids. Our study showed that the investigated lipophilic surfactants formed smaller niosomes with unilamellar vesicle structure (Table 1 and Fig. 1). After topical application, the unilamellar vesicles may readily disintegrate on the skin surface and a close contact between skin lipids and vesicle constituents is obtained. Thus, material exchange between vesicles and intercellular lipids may occur, allowing the diffusion of free molecules as well as small membranal fragments into the stratum corneum and localized the drug in the sebaceous structure. A prolong anti-inflammatory action of rofecoxib may be obtained from the niosomal gel formulation. The burst effect with plain drug gel may be due to initial migration of the drug towards the surface of the gel.

Differential scanning calorimetry (DSC)—This investigation confirms the inertness of the vehicle (carbopol) toward the drug; therefore, the rapid permeation of rofecoxib from the prepared gels could be attributed to the absence of interaction between the drug and the vehicle. The DSC thermogram of pig epidermis after permeation study shows the sharp melting peak of rofecoxib (Fig. 5), which indicates the accumulation of crystalline drug in the skin during permeation experiment. It also indicates the absence of drug metabolism in the skin during permeation study. The sharp peak may be due to the presence of moisture in the sample of pig epidermis after permeation study. It is in agreement with the observation of Khan and Kellaway. The broad melting peak in plain drug sample indicates its amorphous nature.

Conclusion

The niosomal formulations were successfully prepared by lipid film hydration technique using cholesterol and Span as nonionic surfactant. The presence of cholesterol made the niosomes more stable with high drug entrapment efficiency and retention properties. The highest entrapment efficiency was observed with Span 20 and it may be concluded that the entrapment efficiency may be improved using surfactant with higher hydrophilicity. The lower flux value of niosomal gel as compared to plain drug gel across pig skin indicated that the lipid bilayer of niosome was rate limiting in drug permeation and thus the prolong drug release behavior with sustained therapeutic action can be obtained. The DSC investigations confirmed the inertness of carbopol gel base toward the drug and absence of drug metabolism and rofecoxib accumulation in the skin during permeation study. The results indicate that the niosomes can be used as novel drug delivery carrier for skin targeting of rofecoxib for its sustained anti-inflammatory action.

Acknowledgement

The authors are grateful to All India Council for Technical Education, New Delhi, for providing financial assistance to N. N. Palei in relation to the present investigation. The authors gratefully acknowledge Alembic Chemical Works Co. Ltd., Vadodora, India, for providing rofecoxib as gift sample.

References

15 Yoshioka T, Sternberg B & Florence A T, Preparation and properties of vesicles (niosomes) of sorbitan monoester (Span 20, Span 40 and Span 80) and a sorbitan trimester (Span 85), *Int J Pharm*, 105 (1994) 1.