**In vitro Dynamics of Ibuprofen Incorporated Proniosomal Gel**


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**ABSTRACT**

**Aim:** The research was aimed to encapsulate the ibuprofen with proniosomal gel and facilitate ibuprofen release in sustained manner for sustained drug release. **Methods:** Different proniosomal gels of ibuprofen were formulated with Span 20/Span 80 and soya lecithin using the method described in literature. In all formulations cholesterol concentration was kept constant. The prepared proniosomal gels were evaluated for chemical incompatibility by FT-IR, vesicle size analysis, encapsulation efficiency, *in vitro* drug permeation and *in vitro* drug release kinetics were performed. **Results:** The principal absorption peaks of ibuprofen were retained in the proniosomal gels indicating that there was no interaction between ibuprofen and excipients. Vesicular diameter markedly depended on the type of the non-ionic surfactant used. As the outer diameter depends on the HLB value of surfactant, the vesicular diameter was less for proniosomes prepared using Span 80 (low HLB). The encapsulation efficiency was more for the proniosomal gels prepared using Span 20. Proniosomal gel prepared using Span 80 showed higher flux across the membrane due to its leaky membrane. The order of ibuprofen release from the proniosomal gel was PN2 > PN4 > PN1 > PN3. **Conclusion:** The optimized proniosomal gel formulation PN3 containing Span 20 exhibited prolonged ibuprofen release profiles. Fickian diffusion mechanism was observed with the PN3 formulation which was due to the sustained release property. The results indicated that the proniosomal gel would be an effective transdermal delivery system for ibuprofen.

**Keywords:** encapsulation efficiency, permeation, flux, fickian diffusion, transdermal delivery.

**INTRODUCTION**

Drug delivery using colloidal particulate carriers such as liposomes and niosomes have distinct advantages over conventional drug delivery systems. The colloidal particulate systems act as drug reservoir and can adjust the drug release rate by modification of the particle composition or the surface characteristics. Niosomes are microscopic non-ionic surfactant vesicles with spherical, unilamellar, bilayered, multilamellar and polyhedral structures. Niosomes can be developed by the hydration of non-ionic surfactants viz. alkyl or dialkyl polyglycerol class, ester linked sorbitan, polysorbates with or without incorporation of cholesterol. Niosomal delivery system is a potential alternative to liposomal delivery for many drug candidates, due to its highest chemical stability offered over liposomes. Additionally, variable purity of phospholipids used in liposomes and their higher cost could be effectively addressed by niosomes.

Though niosomes are advantageous over liposomes, aqueous niosomal dispersion has posed some unanswered questions such as instability associated with aggregation, fusion and hydrolysis of the encapsulated drug. Thus, niosome has limited shelf life. In order to circumvent stability associated problems, proniosomes were initiated. ‘Proniosomes’ are dry, free-flowing granular product which can form multilamellar dispersion upon hydration. The proniosomes offer ease of storage, transportation...
and extended shelf-life over niosomal dispersions. Proniosomes can deliver the active drug moieties very effectively through the transdermal route.\textsuperscript{5,8} Ibuprofen [2-(4-isobutylphenyl) propionic acid] is a non-steroidal anti-inflammatory drug (NSAID) that inhibits both the isoforms of cyclo-oxidase enzyme. It is used in the treatment of inflammation, osteoarthritis, rheumatoid arthritis, primary dysmenorrhea, mild to moderate pain and fever.\textsuperscript{9} Being class II, ibuprofen has elimination half-life of 2 h and cause ulceration, bleeding and perforation in the GI tract which limits its oral use. Sustained release dosage form of ibuprofen that may have a potential to keep therapeutic level in plasma for prolonged period may evade its toxic effects on GIT and will improve its area of application.\textsuperscript{10} Transdermal delivery of ibuprofen is the best way to avoid the post-oral administration problems of GIT. Moreover, it can reduce the frequency of administration and improve patient compliance.\textsuperscript{11}

Therefore, the present study was aimed to develop ibuprofen proniosomal delivery systems using non-ionic surfactants Span 20 and Span 80, cholesterol and soya lecithin. The prepared systems hypothesized to have sustained release for ibuprofen over a period of time. The proniosomes prepared were evaluated for the drug content, entrapment efficiency, homogeneity and \textit{in vitro} drug release. The effect of surfactants and the soya lecithin on the drug encapsulating capacity as well as on the sustained action was also evaluated.

**MATERIALS AND METHODS**

Ibuprofen was obtained as a gratis from M/s. A-Z Pharmaceuticals, Chennai. Span 20 and Span 80 were obtained from M/s. Loba Chem Ltd., Mumbai. Cholesterol was procured from M/s. HiMedia Lab Pvt Ltd., Mumbai. Soya lecithin (phospholipoin 80H) was obtained as a gift sample from Lipoids (Ludwigshafen), Germany. All other chemicals used were of analytical grade and were used without any chemical modifications.

**Preparation of proniosomal gel**

Accurately weighed quantities of ibuprofen, non-ionic surfactants, soya lecithin and cholesterol were taken in a clean and dry wide mouthed glass vial and 2.5 ml of ethanol was added. The open end of the vial was covered with a lid to avert loss of solvent and heated to 65±3°C until the surfactant mixture dissolved completely. Then 1.6 ml of phosphate buffer pH 7.4 (aqueous phase) was added to above mixture and heated to get homogeneous dispersion. Then it was allowed to cool until the dispersion is converted to proniosomal gel.\textsuperscript{12} The composition of various proniosomal gels were listed in Table 1.

**Fourier transform infrared spectroscopy**

Fourier Transform Infrared (FTIR) spectra of ibuprofen, PN1 and PN2 were recorded in a Thermo-IR 200 FTIR spectrophotometer. Potassium bromide pellet method was employed and background spectrum was collected under identical conditions. Each spectrum was derived from 16 single average scans collected in the range of 400–4000 cm\textsuperscript{−1} at the spectral resolution of 2 cm\textsuperscript{−1}.

**Physical appearance**

All the gel formulations were tested for their homogeneity by visual inspection. They were tested for their appearance and presence of any gritty aggregates.

**Vesicle size analysis**

The proniosomal gels were diluted with phosphate buffer pH 7.4 and were observed under polarized binocular microscope to determine the vesicle size. The size distribution range and mean diameter were also calculated.

**Polarized photography**

The prepared proniosomal gels were diluted using phosphate buffer pH 7.4 and spread in a cavity slide and covered with a cover slip. The slide was observed under microscope with and without polarized light (Olympus, BX 51-P). Photomicrographs were taken at suitable magnifications.

**Encapsulation efficiency**

The proniosomal gel in the glass tube was diluted with 10 ml phosphate buffer pH 7.4. The aqueous suspension was sonicated for 20 min. The ibuprofen containing niosomes were separated from the un-entrapped

| Table 1: Composition of Proniosomal Gels in mg |
|-----------------|-----------|-----------|-----------|-------|-------|
| Formulation code | Ibuprofen | Cholesterol | Soyalecithin | Span 20 | Span 80 |
| PN1              | 1000      | 200        | 1800       | 1800   | –      |
| PN2              | 1000      | 200        | 1800       | –      | 1800   |
| PN3              | 1000      | 200        | 900        | 1800   | –      |
| PN4              | 1000      | 200        | 900        | –      | 1800   |
ibuprofen by centrifuging at 10,000 rpm for 30 min. The supernatant liquid was collected and assayed at 225 nm. The percentage of drug encapsulation (EE (%)) was calculated by the following equation:

\[
EE\% = \frac{C_t - C_f}{C_t} \times 100
\]

where, \(C_t\) is the concentration of total ibuprofen and \(C_f\) is the concentration of free ibuprofen.

**Drug content**

Ibuprofen content in the prepared proniosomal gels was determined by dissolving a known quantity of the gels in methanol. The contents were passed through 0.4 µm membrane filter. The filtrate was assayed upon dilution with phosphate buffer pH 7.4.

**In vitro permeation studies**

The permeation of ibuprofen from proniosomal formulations was determined by using vertical Franz diffusion cell. Receptor compartment containing phosphate buffer pH 7.4 was constantly stirred at 50 rpm using magnetic stirrer and the temperature was maintained at 37±2°C. The aliquots were withdrawn at 1, 2, 3, 4, 5, 6, 7 and 8 h. Soon after withdrawal of the aliquots, the receptor compartment was replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed for ibuprofen content using a UV-visible spectrophotometer at 225nm. The flux (J) was determined as the angular coefficient of the curve obtained by plotting the cumulative amount of the penetrated drug versus time. The permeability coefficient (\(k_p\)) of ibuprofen was calculated using the following equation:

\[
k_p = \frac{1}{C}
\]

where, \(C\) is the initial concentration of drug in the formulation applied to the membrane.

**Transmission electron microscopy (TEM)**

Morphology and structure of the optimized proniosomal gel was studied using transmission electron microscopy (TEM) TOPCON 002B operating at 200 kV and capable of point-to-point resolution.

**RESULTS AND DISCUSSION**

**FTIR**

The FTIR spectra (Fig. 1) of the ibuprofen, PN1 and PN2 showed major absorption peaks of ibuprofen at 2961.01 cm\(^{-1}\), 2900.79 cm\(^{-1}\), 2728.06 cm\(^{-1}\), 1717.17 cm\(^{-1}\), 1501.68 cm\(^{-1}\), 1458.12 cm\(^{-1}\), 1317.22 cm\(^{-1}\), 1248.81 cm\(^{-1}\), 1227.50 cm\(^{-1}\), 935.12 cm\(^{-1}\), 863.34 cm\(^{-1}\), 777.39 cm\(^{-1}\), 586.57 cm\(^{-1}\) indicating the presence of CH\(_3\) asymmetric stretching, hydroxyl stretching, CH\(_2\) symmetric stretching, C=O Stretching vibration of –COOH group, Aromatic C-C stretching, CH-CO deformation, O-H in plane deformation, C-H in plane deformation, C-C stretching, CH\(_3\) rocking vibration, C-H out of plane vibration, CH\(_2\) rocking vibration and C-C deformation respectively. Appearance of extra peaks at 3059.19 cm\(^{-1}\) in both the formulations and appearance of peaks near 2153 cm\(^{-1}\) and 2357 cm\(^{-1}\) for PN2 may be due to any of Span, cholesterol and lecithin.

**Physical appearance**

All the formulations were homogenous with glossy appearance.

**Vesicle size analysis**

Vesicle diameter was determined by diluting the proniosomal gel with phosphate buffer pH 7.4. The effect of various concentrations of surfactants (Span 20 and Span 80) on the vesicle size of proniosomes was determined by keeping the cholesterol concentration as constant. The vesicular diameter values of ibuprofen loaded proniosomal gels are shown in Table 2. It was observed that the vesicular diameter of proniosomes prepared with Span 80 was less compared to that of Span 20. The outer dimensions and size of ibuprofen proniosomes are depended on HLB values of Span...
20/80. The lower the HLB value of surfactant, the smaller initial size of the niosomal vesicles which is a result of decreased surface free energy with increase in hydrophobicity as it was observed from Span 80 with HLB 4.3 showed less vesicular diameter for PN2 and PN4 compared to proniosomes (PN1 and PN3) prepared with Span 20 with HLB 8.6. Reduction in vesicular diameter was observed with increase in soya lecithin concentration which is believed to be due to increased hydrophobicity. The order of average vesicular diameter is PN3>PN1>PN4>PN2. The results imply that type of non-ionic surfactant and the concentration of the soya lecithin have a profound influence on vesicular diameter of proniosomes.

**Encapsulation efficiency**

The niosomes formed from Span 20 containing proniosomal gel has showed better entrapment compared to that of Span 80 as shown in Table 2. This is due to that the entrapment efficiency depends on the structure of the surfactant. Longer the saturated alkyl chain of the surfactant, greater is the entrapment efficiency. Span 80 possess unsaturated alkyl chain which made the chains bend and more permeable when it forms membrane which ultimately leads to lowest entrapment efficiency.\(^\text{14}\)

It suggests that the alkyl chain of surfactant is a crucial factor in entrapment efficiency and saturated chain produces higher entrapment compared to unsaturated chain. Effect of soya lecithin on entrapment efficiency was not significant.

**Microscopic images**

Microscopic images were taken to find the vesicle morphology. From the images (Fig. 2) it was evident that the vesicles in the proniosomal gel were discrete and almost spherical in shape. PN1 and PN2 show discrete spherical particles with various sizes, PN3 shows discrete particles of various size, PN4 shows discrete particles out of which majority of vesicles are spherical and some of them possess fractured/roughen edges. The colour variation of the photographs is due to the concentration variation of soya lecithin in the formulations. The difference in the size of the vesicles is purely due to the variation in the magnification.

**Drug content**

Drug content determines the amount of ibuprofen in the prepared proniosomal gels. Results showed the more uniformity of the drug in the proniosomal gels and indicated the less drug loss in formulations as shown in Table 2. The drug content was highest in PN1 formulation which could be due to non-ionic surfactant Span 20.

**In vitro permeation of ibuprofen from the proniosomal gel**

The permeation of drug from proniosomal gels containing Span 80 is high compared to that of Span 20. This was due to unsaturated alkyl chain structure in Span 80 that led to leakier niosomal membrane. Hence, fast drug release was observed in case of ibuprofen proniosomal gels prepared using Span 80. In addition, lecithin acted as penetration enhancer. Hence increase in concentration of lecithin led to enhanced drug release with the same concentration of span 20/span 80 as it was observed from the flux and permeability coefficient (Table 2, Fig. 3). Sustained drug release pattern was observed with ibuprofen proniosomal gel prepared using Span 20. The percent drug release at the end of 8 h was found to be 86.3, 94.1, 82.4 and 90.3% for PN1, PN2, PN3 and PN4 respectively (Fig. 4).

**Transmission electron microscopy**

The optimized proniosomal gel (PN3) was examined microscopically using Transmission Electron Microscopy (TEM). TEM showed that the particles had spherical, uniform shapes. A dense, well-distributed pattern was observed in PN3 (Fig. 5).

**Release kinetics**

The best fit model for PN1 and PN3 was found to be Higuchi release model with correlation coefficient ‘r’

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**Table 2: Drug Content, Flux and Permeability Coefficient of the Proniosomal Gel**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Vesicle size (µm)</th>
<th>% Drug content</th>
<th>Flux (µg cm(^{-2}) h(^{-1}))</th>
<th>Permeability coefficient ((k_p)) (cm h(^{-1}))</th>
<th>% Entrapment efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN1</td>
<td>12.69±0.14</td>
<td>85±0.71</td>
<td>98.02±0.23</td>
<td>4.90×10(^{-4})</td>
<td>76.7±0.96</td>
</tr>
<tr>
<td>PN2</td>
<td>6.66±0.63</td>
<td>64±0.65</td>
<td>170.88±0.67</td>
<td>8.54×10(^{-4})</td>
<td>63.5±0.88</td>
</tr>
<tr>
<td>PN3</td>
<td>13.65±0.24</td>
<td>81.2±0.94</td>
<td>88.42±0.41</td>
<td>4.42×10(^{-4})</td>
<td>75.2±0.28</td>
</tr>
<tr>
<td>PN4</td>
<td>9.98±0.57</td>
<td>61.2±0.89</td>
<td>146.49±0.97</td>
<td>7.32×10(^{-4})</td>
<td>60.6±0.79</td>
</tr>
</tbody>
</table>

Values are expressed in mean±S.D. (n = 3)
values 1.00 and 0.999 describing the Fickian diffusion pattern as shown in Table 3. Whereas PN2 and PN4 exhibited both Hixson-Crowell with r values 0.9978 and 0.9872 respectively indicating the release rate is limited by drug particles dissolution rate rather than by diffusion. The ‘n’ value ranging between 0.8–0.99 indicates non-fickian diffusion and suggests that more than one type of release phenomenon is involved.

**CONCLUSION**

The *in vitro* permeation of ibuprofen from the proniosomal gels of various compositions was studied. Ibuprofen was effectively incorporated into proniosomal gels. The higher encapsulation efficiency was found in a formulation containing span 20. Soya lecithin has no profound effect on entrapment efficiency. Ibuprofen proniosomal gels containing span 20 have exhibited prolonged drug
release profiles. Thus, it is concluded that the proniosomal gel approach is said to be a promising drug delivery system for BCS class II drugs such as ibuprofen and especially this method is easy to prepare, economic and reproducible. Further studies are required to explore the suitability of proniosomal gel drug delivery approach for various therapeutic drug candidates with commercial viability.

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CONFLICTS OF INTEREST

The authors report that this article content does not have any conflicts of interest.

REFERENCES