CHAPTER 6
PREPARATION AND EVALUATION
OF NIOSOME-BASED GEL FORMULATIONS
6. PREPARATION AND EVALUATION OF NIOSOME-BASED GEL FORMULATIONS

In the formulation of topical dosage forms, great attention has been devoted to new structures, which can ensure either adequate localization of the drug within the skin to enhance the local effect or can increase the penetration through the stratum corneum and viable epidermis for a systemic effect. For this purpose vesicular system, like liposomes and niosomes have been investigated by several groups (Masini et al., 1993; Vutla et al., 1996; Fang J Y. 2001) [1-3]. Vesicles, consisting of one or more surfactant bilayers enclosing aqueous spaces, have been of particular interest because they offer several advantages over liposomes, with respect to chemical stability, lower costs and availability of materials. Applied on the skin, niosomes may act as a solubilizing matrix for poorly soluble drugs, penetration enhancers, as well as a local depot for sustained drug release.

Niosomes can be applied to the skin as liquids or gels. For topical and transdermal application of niosomes in gel form, hydrophilic polymers are considered to be suitable thickening agents. It has already been proven that niosomes and liposomes are fairly compatible with polymers derived from cross-linking poly (acrylic acid) polymers [4]. Therefore it seems logical to choose a gel base made up of Carbopol resin as a vehicle for niosomes incorporation.

Transdermal delivery of drug from niosomes may influence by composition of niosomes [5], total lipid concentration, total drug encapsulated, nature of drug [6], dehydration of vesicles, and biophysical factors [7]. Hence, this investigation aims to study the effect of composition of niosomes and different niosome-based gel formulations of piroxicam, ketoprofen and aceclofenac on in vitro permeation through rat skin followed by in vivo studies.
6.1 Materials and Equipments

Spans and Tweens (National Chemicals Ltd, India)
Soya lecithin and egg lecithin (Sigma chemicals co)
Dialysis tube [DM-70; Capacity: 2.41 ml/cm, width: 29.31 mm, Avg. diameter 17.5 mm and molecular weight cut off: 12000 to 14000] (Himedia, Mumbai).
Cholesterol (S D Fine chemicals ltd, India)

Equipments

Rotary flash evaporator (EIE-R, Ahmedabad)
Vortex mixture (Remi Equipments, Mumbai, India)
Sonicator (Magna Pak 250, Libra Ultrasonic, Kolkata)
Magnetic stirrer (MS-500, Remi Equipments, Mumbai, India)
Franz diffusion cell (Fabricated)
Digital caliper (Aerospace, India)
Particle size analyzer (Laser diffraction particle size analyzer, Sympatec, Germany)
U V Spectrophotometer (UV 1601 Shimadzu, Japan)

6.2 Preparation of Niosome-based gel formulations

Niosome-based gel formulations were prepared by two different approaches namely; niosomal gel and proovesicular surfactant gel.

6.2.1 Preparation of polymeric gel dispersion

Carbopol 980 (as a gelling agent at concentration of 0.9% w/w) was dispersed in distilled water to prepare an aqueous dispersion. The dispersion was allowed to hydrate for 4-5 hrs.
6.2.2 Preparation of niosomal gel

Selected batches of niosomes and proniosome-derived niosomes were centrifuged at 20,000 rpm and 4 °C temp for 30 minutes to obtain the pellets. These pellets were incorporated to aqueous dispersion of carbopol and the resultant dispersion after uniform mixing was neutralized and made viscous by the addition of triethanolamine (5% w/v) to obtain a translucent gel.

6.2.3 Preparation of Proniosomal gel (Provesicular surfactant gel)

Initially provesicles were prepared by a modified method as reported by Vora et al [8]. Surfactant, cholesterol and drug were dissolved in chloroform for piroxicam and aceclofenac and diethyl ether for ketoprofen in a clean and dry, wide mouth glass tube. The glass tube was attached to rotary flash evaporator to evaporate the chloroform at 80 °C under vacuum (600 mm of Hg) resulting in a formation of a gel. the tube was removed after releasing the vacuum from the evaporator and subjected to vigorous mechanical agitation for 2 min on vortex mixer for collapsing gel to fluid. The tube was again attached to a rotary flash evaporator for the removal of the remaining solvent. Then absolute ethanol and phosphate buffer pH 7.4 were added in the tube and the mixture was further warmed in the water bath at 68 °C for about 3 min so that solution was obtained. Then mixture was allowed to cool to room temperature until the dispersion was converted to provesicles. All the batches were prepared in triplicate such that the final ratio of surfactant: ethanol: PBS pH 7.4 was 1:1:1 w/v/v. This obtained provesicles equivalent to give 1% w/w of drug in final gel were mixed in to an aqueous dispersion of carbopol for the preparation of proniosomal gel.
6.3 Characterization of niosomes and provesicles

Niosomes were characterized for following attributes as describe earlier under section 4.3:

- Percentage drug entrapment
- Vesicle size

Provesicles were hydrated in a glass test tube using 8 ml of phosphate buffer pH 7.4 by vortexing on a mixer for 2 minutes to obtain a niosomal dispersion, which was then sonicated by a probe type sonicator two times each for 30 seconds. These niosomes were characterized for same attributes as mentioned above.
6.4 In vitro evaluation of niosome-based gel formulations

6.4.1 In vitro release studies:

In vitro release studies were carried out by taking 1 gram of gel formulations into dialysis tubing and placed in a beaker containing 250 ml PBS pH 7.4 at 37 ± 1°C. The beaker was placed over a magnetic stirrer and stirred at constant speed. Aliquots of samples were withdrawn at specified time intervals and replaced with equal volume of fresh receptor fluid every time. Samples withdrawn were analyzed by UV spectrophotometer. In vitro release rate studies were done for different formulations and effects of variation in composition on release rate were studied.

6.4.2 Animal Selection

White albino rats of either sex weighing 220 ± 20 g (7-9 weeks old) were used. All animals were housed in polypropylene cages with free access to palletized chow and tap water. The in-vitro and in-vivo studies (animal experiments) were carried out under the guidelines compiled by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal, Ministry of Culture, Government of India) and all the study protocols were approved by the local institutional Animal Ethics Committee.

6.4.3 Skin membrane preparation

The abdominal hair of albino rats (wistar strain), weighing 220 ± 20 gm, was shaved using hand razor. After anesthetizing the rats with ether, the abdominal skin was surgically removed from the animal, and adhering subcutaneous fat was carefully cleaned. To remove extraneous debris and leachable enzymes, the dermal side of the skin was kept in contact with a saline solution for 1 h before starting the permeation experiment.

6.4.4 In vitro skin permeation studies

The permeation of drug from niosome-based gel formulations were determined by using Franz diffusion cell. The excised rat skin was mounted on the receptor compartment with
the stratum corneum side facing upwards into the donor compartment. The donor compartment was filled with the niosome-based gel formulation. A 20 ml of pH 7.4-phosphate buffer was used as receptor medium to maintain a sink condition. The available diffusion area of cell was 2.84 cm². The receptor compartment was maintained at 37°c, with magnetic stirring at 600 rpm. The samples from the receptor compartment were withdrawn at predetermined time intervals and immediately replaced by an equal volume of fresh buffer solution. Initial experiments confirmed the maintenance of sink condition by this procedure. The samples withdrawn from the receptor compartment were then analyzed by using UV spectrophotometer.

6.4.5 Pharmacological response

The anti-inflammatory activity was compared by the carrageenan induced rat paw edema method (9-10). The studies were conducted on albino rats (wistar strain) of either sex, weighing 220 ± 20 gms. The animals in each group (n = 3) were selected so that the average body weight among the groups was as close as possible. Inflammation was produced in the rats using 0.1 ml of 1% w/v carrageenan suspension in saline. This was injected into the plantar surface of the rats' left hind paw. Thirty minutes later, 0.8 gm gel formulation was applied topically on the edematous paw by gently rubbing with an index finger. Topical activity of the various formulations was evaluated by measuring an increase in the hind paw thickness with the help of digital calipers before (Zero time) and 1, 2, 3, 4 and 6 h after carrageenan administration. The percentage of paw thickness increase from time Zero was calculated and compared with that found in animals treated with carrageenan alone as a control group (untreated).

6.4.6 Statistical analysis

All the data were expressed as mean of three experiments ± the standard deviation (SD). The results of in vitro release and permeation studies were evaluated using t-test and the results of paw edema test were analyzed by repeated-measures analysis of variance (ANOVA), with P<0.05 considered statistically significant.
6.5 Niosome-based gel formulations of Piroxicam

Niosomal gel

Batches with an optimum formula for the both piroxicam niosomes (NPO) and proniosomes-derived niosomes (dry granular) (PPO) were selected for the study. Along with the optimized formulation batches NP4, NP8 and NP6 were also selected for the evaluation to study the effect of different molar ratio of surfactant to cholesterol. Niosomal gel was prepared by using all these selected batches of niosomes or proniosome-derived niosomes as per method described in section 6.2.2.

Proniosomal gel

Provesicular surfactant gels were prepared as per the method described in section 6.2.3 with different types of surfactant as per the composition given in the Table 6.1. Provesicles after transforming to niosomes were characterized for the PDE and MVD. These obtained provesicles were incorporated in the carbopol dispersion to prepare a provesicular surfactant gel formulation.

Results and Discussion

Results of the PDE and MVD of the selected niosomes and proniosome-derived niosomes batches are recorded in the section 4.4.4 and 8.4.8. Results of the PDE and MVD of all the batches of provesicles are recorded in the Table 6.1. As shown in Table 6.1 all the batches of niosomes derived from provesicles exhibited a high value of PDE. It is evident from the Table 2 that the provesicles formulations containing soya and egg lecithins exhibited a high value of PDE than the provesicles containing only span 60 and cholesterol. This may be because the provesicles formulations containing soya or egg lecithin exhibited liquid crystalline state (8).
Table 6.1: Compositions of prepared piroxicam provesicles with their PDE and MVD value after transforming to niosomes

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Composition of surfactants</th>
<th>Molar ratio of Surfactants</th>
<th>PDE (pm)</th>
<th>MVD (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG1</td>
<td>Span 60: cholesterol</td>
<td>2:1</td>
<td>74.6 ± 2.87</td>
<td>4.85</td>
</tr>
<tr>
<td>PG2</td>
<td>Span 60: cholesterol</td>
<td>1:1</td>
<td>76.46 ± 4.34</td>
<td>5.12</td>
</tr>
<tr>
<td>PG3</td>
<td>Span 60: cholesterol</td>
<td>1:2</td>
<td>72.56 ± 3.98</td>
<td>5.68</td>
</tr>
<tr>
<td>PG4</td>
<td>Span 60: cholesterol</td>
<td>9:1</td>
<td>82.57 ± 4.56</td>
<td>5.59</td>
</tr>
<tr>
<td>PG5</td>
<td>Span 60: Soya lecithin: cholesterol</td>
<td>4.5:4.5:1</td>
<td>86.24 ± 6.45</td>
<td>4.74</td>
</tr>
<tr>
<td>PG6</td>
<td>Span 60: Egg lecithin: cholesterol</td>
<td>4.5:4.5:1</td>
<td>87.8 ± 5.72</td>
<td>5.96</td>
</tr>
</tbody>
</table>

**In vitro release study**

In order to evaluate the effect of niosomal and provesicular surfactant gel on the piroxicam release rate, the release of piroxicam from plain gel (piroxicam dispersed in to carbopol dispersion) and Marketed gel (Pirox gel, Cipla, India) were also estimated. Figure 6.1 shows the in vitro release profile of the piroxicam from the different niosomal gel formulations and Figure 6.2 shows the in vitro release profile of the piroxicam from the different provesicular surfactant gel, plain gel and market gel formulations. It was found that only about 38.12% to 55.84% of the encapsulated drug released during a period of 8 hrs from the various niosomal and provesicular surfactant gel formulations. No lag phase could be observed because of the minimum sampling time of 1 hrs. The drug release study revealed that an initial release of the drug from the provesicular surfactant gel formulations was higher than the niosomal gel formulations.
Figure 6.1: In-vitro release profile of piroxicam from different piroxicam niosomal gel formulations across the semipermeable membrane.

Figure 6.2: In-vitro release profile of piroxicam from different piroxicam provescular surfactant, marketed and plain gel formulations across the semipermeable membrane.
Comparing the release profile among the different niosomal gels, NP4 and NPO gel formulations exhibited a high initial release of the piroxicam but showed less cumulative amount of drug release compared to niosomal gel prepared with equal molar ratio of surfactant to cholesterol.

Initial high release from these formulations may be explained by the more leaky nature of niosomes as all these batches were prepared with less proportion of cholesterol. Slow release of drug in the later stage and less cumulative release from this gel may be due to affinity of the remaining drug for the lipid ambience, which can now not release the drug molecules to a higher extent.

Similar behavior was also observed with the provesicular surfactant gel formulations PG1 and PG4, which are having less proportion of cholesterol in its composition. Proniosomes containing soya and egg lecithins show a high PDE value but drug release from their gel formulations was significantly lower (t-test, P<0.05) than the proniosomal gel prepared only with the span 60 and cholesterol. This may be due to the lamellar liquid crystalline structure present in these proniosomes. Lamellar structures of lyotropic liquid crystals have been reported to provide a major barrier to drug release [8, 11]. Furthermore, the release from the PG5 proniosomal gel was higher than the PG6 probably because of the composition of soya lipids resulting into better penetration enhancing properties than egg lecithin formulations [12]. soya lecithins have been reported to contain unsaturated fatty acids, oleic and linoleic acid, which have penetration enhancing properties of their own as compared to egg lecithin that contains, saturated fatty acids [13]. These unsaturated fatty acids reverses the physiological process of barrier formation and reduction in the quantity of cholesterol in stratum corneum which results in a beneficial increase in the fluidity of lipid barrier [13]. Among the different niosomal and proniosomal gel; NP5, PPO, NP4, PG2, PG4, PG5 and PG6 were selected for the in vitro skin permeation study.
**In vitro skin permeation study**

In vitro release or permeation studies provide valuable information as to the formulation behaviour in-vivo. The release rates of the Fick’s low equation for the niosomal and provesicular surfactant gel formulations across the synthetic membrane, calculated from the slope of the linear portion of the cumulative drug release versus time plot (4 – 6 h) were found to be significantly higher than its flux across the rat skin (t-test, \( P < 0.05 \)), indicating the barrier properties of skin for the drug (Figure 6.3). The permeability coefficient \((K_p)\) was calculated from the steady state transdermal flux and the applied concentration in the donor compartment \((C_{\text{donor}})\) as follows:

\[
K_p = \frac{J}{C_{\text{donor}}} \quad (1)
\]

The steady state transdermal flux, cumulative amount of drug permeating across the skin, permeability coefficients and penetration enhancement ratio of different formulations are given in the Table 3.

The cumulative amount of piroxicam release from the provesicular surfactant gel formulations studies here showed a linear relationship with square root of time indicating a diffusion controlled model (Higuchi release kinetics). However, after 4 hr, high correlation coefficients were obtained for the zero-order drug release kinetics for niosome-based gel formulations, which suggests that niosomes acted as reservoir systems for a continuous delivery of an encapsulated piroxicam.

No lag phase or small lag time with our formulation was observed in the release study perhaps due to penetration enhancing properties of the surfactant, alcohol and increase in solubility of the free drug in the stratum corneum lipid. An n-octanol-aqueous buffer pH 7.4 partition coefficient of piroxicam is 1.8 (14), therefore, in a niosomal formulation essentially the drug will be intercalated within the bilayers and interior, hence the drug release occurs through the interfacial escape of drug from the bilayers.
Provesicles are transformed to niosomes due to hydration during the incorporation into a polymeric gel matrix and at the skin interface before releasing the drug, resulting in a portion of the unentrapped drug, which in presence of surfactant and alcohol penetrate faster. This may result in an initial higher permeation of the drug from the provesicular surfactant gel than the niosomal gel. Slower initial permeation of the piroxicam from the various niosomal gels could be explained by the slower diffusion of vesicles through the carbopol matrix layer. Swollen carbopol controlled the vesicle diffusion and consequently drug permeation, which may be the rate-limiting step in the diffusion process.

![Graph showing steady state flux and release rate of piroxicam from various gel formulations.](image)

Figure 6.3: The steady state flux and release rate of piroxicam from various gel formulations.

The transdermal permeation of piroxicam from different niosomal and provesicular surfactant gels were significantly higher (t-test, P < 0.05) than that from the plain gel. Surfactant in formulation always acts as a permeation enhancer (8, 15), which might partly contribute to the enhancement of piroxicam permeation from niosomal and provesicular surfactant gel.
Table 6.2: Cumulative amount, steady state flux and enhancement ratio of piroxicam permeation across excised rat skin from different gel formulations.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Cumulative amount (mg/cm²)</th>
<th>Steady state flux (µg/cm² h⁻¹)</th>
<th>Kp (cm/h)</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP4</td>
<td>235.08</td>
<td>25.06</td>
<td>3.61 X 10⁻³</td>
<td>1.84</td>
</tr>
<tr>
<td>NP5</td>
<td>262.58</td>
<td>29.89</td>
<td>3.74 X 10⁻³</td>
<td>2.20</td>
</tr>
<tr>
<td>PPO</td>
<td>257.24</td>
<td>28.47</td>
<td>3.75 X 10⁻³</td>
<td>2.09</td>
</tr>
<tr>
<td>PG2</td>
<td>212.48</td>
<td>25.02</td>
<td>3.27 X 10⁻³</td>
<td>1.84</td>
</tr>
<tr>
<td>PG4</td>
<td>260.17</td>
<td>25.36</td>
<td>3.07 X 10⁻³</td>
<td>1.87</td>
</tr>
<tr>
<td>Marketed</td>
<td>196.25</td>
<td>19.23</td>
<td>1.92 X 10⁻³</td>
<td>1.42</td>
</tr>
<tr>
<td>Plain gel</td>
<td>139.84</td>
<td>13.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There are several mechanisms, which can be used to explain the ability of niosomes to modulate drug transfer across the skin (3, 16-17), including (i) adsorption and fusion of niosomes at the surface of the stratum corneum and generating a high local concentrations which results in increased thermodynamic activity of the drug in the upper layers of the stratum corneum, (ii) the effect of vesicles as the penetration enhancers to reduce the barrier properties of stratum corneum, and (iii) the lipid bilayers of niosomes as the rate-limiting membrane barrier for drugs. So, interaction between the skin and vesicles may be an important consideration in the improvement of transdermal drug delivery. Based on the data obtained for the in-vitro release and permeation experiments, niosomal gel NP4 and NP5 (niosomal gel PPO was not selected for the in vivo study due to similar composition of PPO and NP5) and proniosomal gel PG2 and PG4 were selected for the in vivo studies.
In-vivo studies are important to evaluate the therapeutic performance of the developed formulations. The rats were divided in seven groups each consisting of three rats. It has been reported that carrageenan-induced paw edema response can be divided into two different phases. The first phase is observed due to the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin in the inflammatory area up to 1 h after carrageenan injection. In the second phase, the macrophages release interleukin-1 to induce accumulation of polymorphic nuclear cells into the inflammatory area 3-5 h after carrageenan injection. This then releases the lysosomal enzymes and active oxygen to destroy connective tissues and induce paw swelling. Inflammation in control rats after carrageenan injection was observed as marked increase in paw thickness and reached a peak of swelling after 4 h. The results of the rats paw edema test are recorded in Table 4 and evaluated using repeated-measures ANOVA. Two different factors were examined. Factor 1 was the time repeated and as composed of 5 levels (1, 2, 3, 4 and 6 h) and factor 2 was formed by 6 different formulations (NP4, NP5, PG2, PG4, Plain gel and Marketed gel).

According to the results of ANOVA, there was significant difference in the increase in paw thickness among all formulations studied. The interaction between formulations and time was found to be significant ($P < 0.05$). Hence, the differences in paw thickness among the formulations for each hour were not similar. The Duncan test, used as a post hoc analysis, showed that when all formulations were compared with the control, a significant difference ($P < 0.05$) was found. Table 5 shows pair wise comparison of all the formulations for paw thickness at each hour.
Table 6.3: Differences among piroxicam gel formulations in the increase of rat paw thickness for each hour*

<table>
<thead>
<tr>
<th>Gel</th>
<th>Control</th>
<th>Plain gel</th>
<th>NP4</th>
<th>NP5</th>
<th>PG2</th>
<th>PG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain gel</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NP4</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NP5</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PG2</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PG4</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>2,3</td>
<td>-</td>
</tr>
<tr>
<td>Marketed</td>
<td>1, 2, 3, 4, 6</td>
<td>2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2,5</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.4: Increase in paw thickness (%) after locally applied different piroxicam gel formulations in carrageenan induced rat paw edema model.

* Each value represents the mean ± SD (n=3).
When we compare the increase of paw thickness of among the different gel formulations following conclusions can be made. Increase in paw thickness at different hours for marketed gel formulation was found to be significantly lower than plain gel but significantly higher than the niosomal gel NP4 and NP5 (Table 6.3). Figure 6.4 shows the percentage increase of paw thickness after subplantar injection of carageenan for various piroxicam gel formulations.

Although, no significant difference was observed between the formulations NP4, NP5 and PG2 (except 3 h for NP5 and PG2), according to both in vivo and in vitro permeation studies, niosomal gel NP5 showed the best permeation and effectiveness (3.74 X 10⁻³) (Table 6.2, 6.3 and Figure 6.4).
6.6 Niosome-based gel formulations of ketoprofen

**Niosomal gel**

Batches with an optimum formula for the both ketoprofen niosomes (NKO) and proniosomes-derived niosomes (dry granular) (PKO) were selected for the preparation and evaluation of niosome-based gel formulations. Along with the optimized formulation batches NK5 and NK6 were also selected for the in vitro evaluation to study the effect of total lipid on the permeation of ketoprofen. Different niosomal gels were prepared by using all these selected batches of niosomes or proniosome-derived niosomes as per method described earlier in section 6.2.2.

**Proniosomal gel**

Provesicular surfactant gel (PGK) was prepared with the same composition as NKO, as per method described in section 6.2.3 and evaluated as per method given in the section 6.3.

**Results and Discussion**

PDE and MVD of PGK batch were found to 71.28% and 4.32 μm respectively.

**In vitro release study**

In order to evaluate the effect of niosomal gel on the ketoprofen release rate, Plain gel was also evaluated. Figure 6.5 shows the in vitro release profile of the ketoprofen from the different niosomal gel formulations and Plain gel formulation. It was found that 48.6% to 69.28% of the encapsulated drug released during a period of 8 hrs from the various niosomal and provesicular surfactant gel formulations. The drug release study revealed that the release of the drug from the provesicular surfactant gel formulations was significantly higher (t-test, P<0.05) than the niosomal gel formulations. Higher release of the ketoprofen from the provesicular surfactant gel may be due to solubilization and penetration enhancing effect of ethanol present in the formulation. Lower release rate
from niosomal gel systems compared to Plain gel could be a result of the influence of the gel matrix followed by slower drug penetration.

Figure 6.5: In-vitro release profile of ketoprofen from different ketoprofen gel formulations across the semipermeable membrane.

When comparing the release profile among the different niosomal gel, NKO and PKO gel formulations exhibited a significantly higher release of the ketoprofen then the NK5 and NK6 niosomal gel. Lipid concentration was higher in case of NK5 and NK6 niosomes compared to NKO and PKO niosomes batches, which were prepared at low lipid concentration. Slower release of the ketoprofen from NK5 and NK6 niosomal gel formulations may explain by the following hypothesis. Being an amphiphilic molecule (log p = 0.98) ketoprofen exists in both aqueous core and lipid layer of the niosomes hence, lipid layer of the niosomes may act as a diffusion (or release) barrier for ketoprofen, and the release of ketoprofen affected by the lipid concentration and composition of niosomes which may result in niosomes of different bilayers thickness.
and partitioning from lipid layer to the release medium. Obtained results indicate that the high lipid concentration in the niosomal gel formulation retard the release of the drug. PGK, NKO and PKO gels were selected for the further in vitro skin permeation studies owing to their high drug release profile.

**In vitro skin permeation study**

The steady state transdermal flux of the Fick's law equation for the different gel formulations across the rat skin was calculated from the slope of the linear portion the cumulative drug permeated verses time plot (2-6 h).

The permeability coefficient ($K_p$) was calculated from the steady state flux and the applied concentration in the donor compartment ($C_{donor}$) as per equation 6.1. The steady state transdermal flux, cumulative amount of drug permeated across the skin, permeability coefficients and penetration enhancement ratio of different formulations are listed in the Table 6.4. High value of permeability coefficient ($K_p$) for the NKO and PKO formulation may be due to less amount of drug present in the donor compartment. High cumulative amount of drug permeated from the PGK gel formulation can be explained by solubilization effect of ethanol and penetration enhancement effect of both ethanol and surfactant.

**Table 6.4: Cumulative amount, steady state flux and enhancement ratio of ketoprofen permeation across excised rat skin from different gel formulations.**

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Cumulative amount (µg/cm²)</th>
<th>Steady state flux (µg/cm² h⁻¹)</th>
<th>$K_p$ (cm/h)</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK</td>
<td>466.96</td>
<td>57.42</td>
<td>4.65 X 10⁻³</td>
<td>1.48</td>
</tr>
<tr>
<td>NKO</td>
<td>431.21</td>
<td>53.79</td>
<td>6.45 X 10⁻³</td>
<td>1.38</td>
</tr>
<tr>
<td>PKO</td>
<td>403.65</td>
<td>50.81</td>
<td>5.74 X 10⁻³</td>
<td>1.31</td>
</tr>
<tr>
<td>Plain gel</td>
<td>346.48</td>
<td>38.12</td>
<td>3.81 X 10⁻³</td>
<td>-</td>
</tr>
</tbody>
</table>
The cumulative amount of ketoprofen release from the provesicular surfactant gel formulations studies here shows a linear relationship with time indicating a zero-order drug release kinetics for niosome-based gel formulations. This suggests that niosomes act as reservoirs.

**In vivo studies**

The rats were divided in four groups each consisting of three rats. The results of the rats paw edema test are recorded in Table 4 and were evaluated using repeated-measures ANOVA. Two different factors were examined. Factor 1 was the time repeated and composed of 5 levels (1, 2, 3, 4 and 6 h) and factor 2 was formed by 4 different formulations (PGK, NKO, PKO and Plain gel).

According to the results of ANOVA, there was significant difference in the increase in paw thickness among all formulations studied (P<0.05). The interaction between formulations and time was also found to be significant (P < 0.05). Hence, the differences in paw thickness among the formulations for each hour were not similar. The Duncan test, used as a post hoc analysis, showed that when all formulations were compared with the control, a significant difference (P < 0.05) was found at all points of time. Table 6.5 shows pair wise comparison of all the formulations for each hour of paw thickness.
Table 6.5: Differences among ketoprofen gel formulations in the increase of rat paw thickness for each hour*

<table>
<thead>
<tr>
<th>Gel</th>
<th>Control</th>
<th>PGK</th>
<th>NKO</th>
<th>PKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NKO</td>
<td>1, 2, 3, 4, 6</td>
<td>2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PKO</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Plain gel</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 3, 4</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers show significant difference (P<0.05) at hours between the two different formulation crossed.

Figure 6.6: Increase in paw thickness (%) after locally applied different ketoprofen gel formulations in carrageenan induced rat paw edema model.

* Each value represents the mean ± SD (n=3).
Figure 6.6 shows the percentage increase of paw thickness after subplantar injection of carageenan for various gel formulations. All the formulation tested showed significant difference in paw thickness compare to control (untreated). Provesicular surfactant gel formulation PGK showed significant reduction in increase in paw thickness after carageenan injection compared niosomal gel and Plain gel. Hence, PGK gel was found to be superior among all the formulation studied according to both in vivo and in vitro permeation studies (Figure 6.6, Table 6.4 and 6.5).
6.7 Niosome-based gel formulations of Aceclofenac

**Niosomal gel**

Batches with an optimum formula for the both aceclofenac niosomes (NAO) and proniosomes-derived niosomes (PAO) were selected for the preparation and evaluation of niosome-based gel formulations. Along with the optimized formulation batches NA5 and NA6 were also selected for the in vitro evaluation to study the effect of amount of drug on the permeation of aceclofenac. Niosomal gel was prepared by using all these selected batches of niosomes and proniosome-derived niosomes as per method described in section 6.2.2.

**Proniosomal gel**

Provesicular surfactant gel (PGA) was prepared with the same composition of NAO as per method described in section 6.2.3 and evaluated as per method given in the section 6.3 and 6.4.

**Results and Discussion**

PDE and MVD of PGA batches were found to 63.6% and 5.12 μm respectively.

**In vitro release study**

In order to evaluate the effect of niosomal gel on the aceclofenac release rate, Plain gel was also estimated. Figure 6.7 shows the in vitro release profile of the aceclofenac from the different niosome-based gel formulations and Plain gel formulations. It was found that 61.4% to 73.52% of the encapsulated drug was released during a period of 8 hrs from the various gel formulations.

The drug release study revealed that the release of the drug from the provesicular surfactant gel formulations (PGA) and from plain gel was significantly higher (t-test, P<0.05) than the niosomal gel formulations. Higher release of the aceclofenac from these gel formulations may be due to free drug present in the system.
Niosomal gel formulations NA6, NA5, NAO and proniosomal gel PGA were selected for the further in vitro skin permeation study due to higher total amount of drug released from these formulations.

**In vitro skin permeation study**

The steady state transdermal flux of the Fick's law equation for the different gel formulations across the rat skin was calculated from the slope of the linear portion the cumulative drug permeated verses time plot (4-8 h). The steady state transdermal flux, cumulative amount of drug permeated across the skin, permeability coefficients and penetration enhancement ratio of different aceclofenac gel formulations are listed in the Table 6.6.
High value of permeability coefficient (Kp) for the NAO and NA5 formulation may be due to less amount of drug present in the donor compartment. High cumulative amount of drug permeation from the NA6 gel formulation may be due to more total amount of drug encapsulated in the niosomes.

Table 6.6: Cumulative amount, steady state flux and enhancement ratio of aceclofenac permeation across excised rat skin from different gel formulations.

<table>
<thead>
<tr>
<th>Batch code</th>
<th>Cumulative amount (µg/cm²)</th>
<th>Steady state flux (µg/cm² h⁻¹)</th>
<th>Kp (cm/h)</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA6</td>
<td>260.8</td>
<td>24.1</td>
<td>2.41 x 10⁻³</td>
<td>1.36</td>
</tr>
<tr>
<td>NA5</td>
<td>223.2</td>
<td>22.91</td>
<td>3.00 x 10⁻³</td>
<td>1.29</td>
</tr>
<tr>
<td>NAO</td>
<td>249.6</td>
<td>25.98</td>
<td>3.17 x 10⁻³</td>
<td>1.47</td>
</tr>
<tr>
<td>PGA</td>
<td>244.9</td>
<td>22.77</td>
<td>1.8 x 10⁻³</td>
<td>1.29</td>
</tr>
<tr>
<td>PLAIN</td>
<td>168.4</td>
<td>17.71</td>
<td>1.77 x 10⁻³</td>
<td></td>
</tr>
</tbody>
</table>

The amount permeated of the drug from the different niosomal gel formulations (NA6, NA5 and NAO) shows a linear relationship with square root of time up to 3 hours. Hence, drug permeation rate can be expressed by Higuchi diffusion model from these formulations. However, after the 3rd hour, high correlation coefficients were obtained for the zero-order drug release kinetics for niosomal gels.

Also, the cumulative amount of aceclofenac permeating from the proniosomal gel formulations studies here shows a linear relationship with time indicating a zero-order drug release kinetics. High cumulative amount of drug permeation from the NA6 and NAO gel formulations may be due to more total amount of drug encapsulated in the niosomes. For the in vivo evaluation, the gel formulations NA6, NAO and PGA gel formulations were selected due to their high cumulative amount of drug permeability.
In vivo studies

The rats were divided in four groups each consisting of three rats. The results of the rats paw edema test are recorded in Table 6.7 and evaluated using repeated-measures ANOVA. Two different factors were examined. Factor 1 was the time repeated and composed of 5 levels (1, 2, 3, 4 and 6 h) and factor 2 was formed by 4 different formulations (NA6, NAO, PGA and Plain gel).

According to the results of ANOVA, there was significant difference in the increase in paw thickness among all formulations studied (P<0.05). The interaction between formulations and time was also found to be significant (P < 0.05). The Duncan test, used as a post hoc analysis, showed that when all formulations were compared with the control, a significant difference (P < 0.05) was found at all points of time. Table 6.7 shows pair wise comparison of all the formulations for each hour of paw thickness.

Table 6.7: Differences among aceclofenac gel formulations in the increase of rat paw thickness for each hour*

<table>
<thead>
<tr>
<th>Gel</th>
<th>Control</th>
<th>Plain gel</th>
<th>NA6</th>
<th>NAO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain gel</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NA6</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NAO</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>2, 3, 4</td>
<td>-</td>
</tr>
<tr>
<td>PGA</td>
<td>1, 2, 3, 4, 6</td>
<td>1, 2, 3, 4, 6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Numbers show significant difference (P<0.05) at hours between the two different formulation crossed.
Figure 6.8: Increase in paw thickness (%) after locally applied different aceclofenac gel formulations in carrageenan induced rat paw edema model.

* Each value represents the mean ± SD (n=3).

Figure 6.8 shows the percentage increase of paw thickness after subplantar injection of carrageenan for various aceclofenac gel formulations. Niosomal gel formulation NA6 showed highest reduction in the paw thickness for all points of time. Although NA6 and PGA gel formulations are not significantly different (P>0.05), gel formulation NA6 exhibited better permeation and efficacy according to both in vivo and in vitro permeation studies (Figure 6.8, Table 6.6 and 6.7).
6.8 Comparisons

Niosome-based gel formulations were prepared by two different methods. When the results of permeation profile of the piroxicam from the different niosome-based gel were compared, provesicular surfactant gel was found to have high permeation of the drug initially may be due to presence of free drug, while less cumulative amount of the drug released at the end of 8 h than niosomal gel.

All the niosome-based gel formulations showed significant reduction in the paw thickness compared to the control and plain gel. When the results of reduction in paw thickness for piroxicam, ketoprofen and aceclofenac niosome-based gel formulations (for each drug formulations which give the best response) were compared, PGK (provesicular surfactant gel) showed significantly more reduction in thickness of paw than the piroxicam (NP5) and aceclofenac (NA6) gel formulations. This may be due to the presence of the free drug and alcohol in the provesicular surfactant gel. Here alcohol serves as solubilizing agent and penetration enhancer for drug.
6.9 Conclusion

The in vitro permeation and in vivo reduction in paw thickness of piroxicam, ketoprofen and aceclofenac from niosome-based gel formulations with different contents and types of non-ionic surfactant and drug were evaluated in this study. Drugs included in the niosome-based gel formulations were entrapped within the lipid bilayers and aqueous core. Experimental data and theoretical analysis support the concept that the direct transfer of drug from vesicles to skin occurs only when the drug is intercalated within the bilayers (Weiner et al., 1989) [18]. Two types of interaction between the skin and niosomes may induce the penetration enhancing effect on niosome-based gel formulations: (1) adsorption and fusion of drug entrapped niosomes onto the surface of the skin leads to a high thermodynamic activity gradient of the drug–stratum corneum interface; (2) the effect of niosomes on stratum corneum may cause changes in drug permeation kinetics due to an impaired barrier function of the stratum corneum for the drug (Touitou et al., 1994; Schreier and Bouwstra., 1994) [6, 16]. These studies clearly show that niosome-based gel formulations containing NSAIDs significantly enhance the penetration of drug as compared to plain gel formulation.

Niosomes based gel formulations have shown great potential, for NSAIDs for their use in topical application. Besides providing enhanced permeation of NSAIDs the proposed system has added advantages like stability, possess high entrapment efficiency and is easy to scale up as the process is simple. Thus, niosome-based gel formulations are promising formulations for delivery of NSAIDs by topical route. Further work by clinical studies will prove them as efficient formulations for delivery of NSAIDs.
6.10 References

1. Masini V. Cutaneous bioavailability in hairless rats of tretinoin in liposomes or gel. *J. Pharm. Sci.* 1993;82: 17–21


