FORMULATION AND EVALUATION OF NIOSOMAL GEL FOR TRANSDERMAL DELIVERY OF LAMIVUDINE

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ABSTRACT
The aim of present study is an attempt to formulate and evaluate niosomal gel for transdermal delivery of Lamivudine for potentially treating HIV and AIDS related condition. Lamivudine is an antiretroviral drug for the treatment of acquired immune deficiency syndrome (AIDS) & Hepatitis. The present study involves the preparation and characterization of Lamivudine entrapped niosomes and finding the drug carrier qualities of the niosomes. The formulation F1-F4 which were prepared by varying the concentration surfactant (span 60) by thin film hydration method. The optimized formulation was incorporated into carbopol gel which is prepared by cold mechanical method and they were characterized for different evaluation parameters such as vesicle size, % entrapment efficiency, drug content, in vitro release and the stability studies was carried out at different temperature. The present study demonstrates the controlled drug release after encapsulation of Lamivudine into niosomal gel.

KEYWORDS: Niosomes, AIDS, Lamivudine, Surfactant, controlled drug delivery.

INTRODUCTION
Controlled drug delivery systems have acquired a centre stage in the area of pharmaceutical research and development sector. Controlled drug delivery systems, which release the drug in continuous manner by both dissolution controlled as well as diffusion controlled mechanisms. In recent years it has been shown that the skin a useful route for drug delivery to the systemic circulation. Transdermal drug delivery system includes all topically administered drug...
formulations intended to deliver the active ingredients into the circulation. They provide controlled continuous delivery of drugs through the skin to the systemic circulation. Niosomes are non-ionic surfactant vesicles, capable of forming vesicles & entrapping hydrophilic and hydrophobic molecule.\textsuperscript{[1]}

Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can accommodate drug molecules with a wide range of solubilities. Non-ionic surfactants are comprised of polar and non-polar segments and possess high interfacial activity. The formation of bilayer vesicles instead of micelles is dependent on the hydrophilic–lipophilic balance (HLB) of the surfactant. They have longer shelf life, stability and ability to deliver the drug at the target site in a controlled or sustained manner which enhances bioavailability and they offer several advantages over liposome’s such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs and ability to deliver the drug at the target site in a controlled or sustained manner which enhances bioavailability.\textsuperscript{[2,3]}

Transdermal drug delivery system is a new approach to provide prolonged action of the drug with low toxicity and better patient compliance and thus reduces the side effects caused by oral route. TDDS is one of the lying under the category of controlled drug delivery, in which the aim is to deliver the drug through skin in a predetermined in a controlled rate. For transdermal delivery of drug, stratum corneum is main barrier layer for permeation of drug. Hence, to increase the flux through the skin membrane, different approaches of penetration enhancement are used. Topical applicability of niosomes was further enhanced by developing niosomal gel formulation using carbomers.\textsuperscript{[4,5]}

**MATERIAL AND METHODS**

**Materials**

Lamivudine was obtained as a gift sample from Strides Arco labs ltd, Bangalore. Span 60 and cholesterol, chloroform and methanol were purchased from SD fine chemicals ltd, (Mumbai, India). Phosphate Buffer Saline pH 7.4 (PBS pH 7.4) and Phosphate Buffer Saline pH 6.8 (PBS pH 6.8) were prepared as described in the Indian Pharmacopoeia (1996).

**Method**
Formulation and evaluation of niosomal gel

Niosomes were prepared by thin film hydration technique by using surfactant and cholesterol. The type of surfactant was optimized, keeping drug: cholesterol: surfactant molar ration at 1:0.5:1, 1:1:1, 1:1.5:1 and 1:2:1. Accurately weighed quantities of surfactant (span 60) and CHOL were dissolved in 10 ml chloroform using a 100 ml round bottom flask. The weighed quantity of drug is added to the solvent mixture. The solvent mixture was removed from liquid phase by flash evaporation at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of residual solvent can be ensured by applying vacuum. The dry lipid film was hydrated with 10 ml phosphate buffer saline of pH: 7.4 at a temperature of 60 ± 2°C for a period of 1 hour until the formation of niosomes.[6]

Table: 1 Formulation design of Lamivudine niosomes.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamivudine</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Span-60</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Phosphate buffer pH 7.4 (ml)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Formulation of Niosomal Gel

Based upon the results of formulated drug loaded niosomes, the batches with good entrapment efficiency were selected for further formulation of niosomal gel. For the formulation of niosomal gel, the gel base was prepared by dispersing 1% w/w carbopol 940 in a mixture of water and glycerol (7:3), the dispersion is then neutralized and made viscous by addition of sufficient amount of triethanolamine by cold mechanical method.[6]

Table: 2 Formulation design for niosomal gel.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Ingredients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Niosome formulation</td>
<td>10%</td>
</tr>
<tr>
<td>2</td>
<td>Carbopol 934P</td>
<td>1%</td>
</tr>
<tr>
<td>3</td>
<td>Triethanolamine</td>
<td>QS</td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>QS to 100%</td>
</tr>
</tbody>
</table>

Characterization of niosomes and niosomal gel

Niosomal dispersion of Lamivudine was successfully prepared by thin film hydration method. The formulated niosomes were evaluated for various parameters. The preformulation studies were performed which helps in maximizing acceptable, safe and stable product. Incompatibility studies were performed by FT-IR for Drug – Excipients interaction.[7]

Vesicle size analysis
The vesicel size of niosomes was determined by using optical microscope and SEM. All the prepared batches of niosomes were viewed under microscope to study their size. Size of niosomal vesicles from each batch was measured at different location on slide by taking a small drop of niosomal dispersion on it and average size of niosomal vesicles were determined.\[8\]

**Differential scanning calorimetry**

Niosomal pellets were lyophilized. Differential scanning calorimetric (DSC) thermograms for individual components, Span 80, Cholesterol, as well as the drug powder, were investigated. A heating rate of 5°C/min was employed over a temperature range (30–250°C).

**Particle size distribution and Zeta potential**

Zeta potential of the optimized formulation was measured by instrument zetasizer nano ZS using DTS software (Malvern Instrument Limited, UK) using M3-PALS Technology.\[9\]

**Entrapment efficiency**

Entrapment efficiencies of niosomal formulations were carried out in triplicate by centrifugation method. The niosomal suspension was centrifuged at 8000 rpm for 10 min at 3°C. Then the solid mass was separated from the supernatant and then suitable dilutions were prepared with PBS (pH: 7.4). The drug concentration was assayed by UV-visible spectrophotometer method at 270.6 nm. The percentage of drug entrapment was calculated.\[10\]

\[
\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug} - \text{free amount of drug} \times 100}{\text{Total amount of drug}}
\]

**Drug content**

Lamivudine content in niosomes was assayed by an UV spectrophotometric method. Niosomes containing equivalent to 10 mg of drug were dissolved in a 10 ml of methanol. After suitable dilution absorbance was measured by UV spectrophotometer against blank at \(\lambda\text{max} 270.6 \text{ nm}\) and drug content was calculated.\[11\]

**In vitro release study**

In vitro release pattern of niosomal suspension was carried out in dialysis bag method. Lamivudine niosomal suspension equivalent to 10 mg was taken in dialysis bag and the bag was placed in a beaker containing 100 ml of pH: 7.4 Phospahate buffer. The beaker was
placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was
maintained at 37±0.5°C. 1 ml sample were withdrawn periodically and were replaced by
fresh buffer. The samples were assayed by UV Spectrophotometer at 270.6 nm using
phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and
plotted against time.[12]

Evaluation of niosomal gel[13]
The prepared niosomal gel formulations were subjected to various evaluation parameters.

Physical parameters
Appearance
The prepared gels were examined for clarity, colour, homogeneity, presence of foreign
particles and fibres.

PH
2.5 gm of gel was dispersed in 25 ml distilled water and the pH was examined using digital
pH meter.

Drug content uniformity
Drug content uniformity of niosomal gel was determined by analyzing the drug concentration
in the sample taken from four different points. The gel samples were dissolved in 50 ml PBS
(pH: 6.8) and stirred at 100 rpm to facilitate rupture of the vesicles. Drug (Lamivudine)
content was determined using UV spectrophotometer at 270.6 nm.

Drug release from niosomal dispersion and niosomal gel
In- vitro release studies were performed using diffusion cells. Phosphate buffer pH: 7.4 were
used as receptor fluid. Nylon membrane (0.22 μm) was soaked in phosphate buffer pH: 7.4
for 1 hrs before carrying out the study. 500 mg of niosomal gel (F4) containing 10 mg drug
was placed onto the donor compartment. Samples were collected at 1, 2, 3, 4, 5, 6, 7, 12 and
24 hrs intervals and analyzed by U.V.

Release kinetics
In order to describe the kinetics of the release process of drug in all formulations, various
equations were used, such as Zero order rate equation, first order equation, Higuchi’s model,
Peppas model.

RESULTS AND DISCUSSION
Incompatibility studies

FTIR studies to find out the compatibility of drug with the excipients. FT-IR spectroscopy study was carried out to check the compatibility between the drug Lamivudine and the excipients (Span-60 and Cholesterol) used for the formulation of niosomes. The FT-IR was performed for drug, excipients and physical mixture of drug and excipients. The spectra obtained from FTIR spectroscopy studies at wavelength from 4000 to 400 cm⁻¹.

Fig. 1 FTIR spectra of pure drug Lamivudine

FTIR spectra of Span-60

FTIR spectra of Cholesterol
The prepared niosomes size and shape was studied using Optical microscopic (using 45x magnification) techniques. The studies revealed that niosomes are in round in shape and the size was found to be in the range of 0.79-1.62 μm and the SEM analysis were shown in figures.

The particle size analysis also done by using Malvern particle size analyzer for the optimized formulation of F4 has average particle size was found to be 795.9 nm.

### Table: 3 Average vesicle size F1-F8 formulation

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Average vesicle size in (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.79</td>
</tr>
<tr>
<td>F2</td>
<td>0.86</td>
</tr>
<tr>
<td>F3</td>
<td>1.38</td>
</tr>
<tr>
<td>F4</td>
<td>1.62</td>
</tr>
</tbody>
</table>

Fig. 4 FTIR spectra of physical mixture of Lamivudine, with Excepients

Fig. 5 Photomicrograph of optimized formulation of F4
Zeta potential

Zeta potential is a key factor for evaluation of the stability of colloidal dispersion. The zeta potential was measured for the optimized Formulations F4 was found to be -68.1 mV shown in figure.

Fig. 6 Particle size distribution analysis of formulation F4

Fig. 7 SEM of optimized formulation F4

Fig. 8 Zeta potential analysis of formulation F4
Percent entrapment efficiency
The percentage entrapment efficiency of Lamivudine in different niosomal formulations with different drug, surfactant and cholesterol ratio was determined spectrophotometrically. Highest entrapment efficiency was observed in F1 and F4 with 88.3% and 95.4% respectively. The high drug entrapment may be observed due to increase in the surfactant ratio and was shown in figure.

Drug content
Lamivudine content in niosomes was assayed by an UV spectrophotometric method. The drug content of F1-F4 were found to be 80.01%, 86.31%, 89.65% and 92.10% respectively.

Table-4: Percent Drug content and Entrapment efficiency

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Formulation code</th>
<th>% Entrapment efficiency</th>
<th>% drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>88.3</td>
<td>90.01</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>89.36</td>
<td>86.31</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>90.80</td>
<td>89.65</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>95.40</td>
<td>92.10</td>
</tr>
</tbody>
</table>

![Fig. 9 Entrapment efficiency of formulation F4](image)

In-vitro release studies
*In vitro release* study of Lamivudine from various formulations was conducted for 24 hrs by using dialysis membrane. Cumulative % drug release was plotted against time t, the percent drug release from F1-F4 was observed as follows F1-76.01%, F2- 67.20%, F3-63.31% and F4 -57.33%. The increase in surfactant (span 60) ratio from F1 to F4 causes increase in the drug release and the release was more controlled by increasing the surfactant ratio.
Table-5: in vitro release dissolution profile of F1-F4

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>% Cumulative Drug Release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>10.90</td>
</tr>
<tr>
<td>2</td>
<td>24.51</td>
</tr>
<tr>
<td>4</td>
<td>36.11</td>
</tr>
<tr>
<td>6</td>
<td>43.82</td>
</tr>
<tr>
<td>8</td>
<td>48.20</td>
</tr>
<tr>
<td>10</td>
<td>52.80</td>
</tr>
<tr>
<td>12</td>
<td>58.54</td>
</tr>
<tr>
<td>16</td>
<td>64.13</td>
</tr>
<tr>
<td>20</td>
<td>69.61</td>
</tr>
<tr>
<td>24</td>
<td>76.01</td>
</tr>
</tbody>
</table>

Fig. 9 In-vitro release profile of F1-F4 formulation

Evaluation of niosomal gel

1. **pH and Viscosity Measurements**

   pH of the gel formulations was measured by digital pH meter and found to be in the range of 6.4 which lies in the normal pH range of the skin. No formulation showed variation more than ± 0.16. The viscosity of all the gel formulations was found to be in desirable range.

2. **% Drug content**

   The optimized formulation of F4 shows 91.62% of drug content.

Table 6: Appearance, pH, Viscosity (cps) and Drug content of niosomal gel

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Appearance</th>
<th>pH</th>
<th>Viscosity</th>
<th>% Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4</td>
<td>Off white, opaque, odourless with smooth appearance</td>
<td>6.4</td>
<td>11,450</td>
<td>91.62%</td>
</tr>
</tbody>
</table>
3. In-vitro permeation studies of gels
The optimized formulation of niosomal gel containing a pure drug (F4) showed the cumulative percentage of drug permeation 55.61%.

![Graph showing in-vitro release profile of niosomal gel formulation F4-G1](image)

**Fig. 10 In-vitro release profile of niosomal gel formulation F4-G1**

<table>
<thead>
<tr>
<th>Table 7: Comparative in vitro release profile of Lamivudine niosomal gel</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation code</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>F8-G1</td>
</tr>
</tbody>
</table>

Stability studies
The accelerated stability studies was performed for the prepared optimized formulation (F4) by maintaining the temperature at 40 ± 2°C and 75 ± 5%.

Stability studies of niosomal gel formulation F4 showed that, negligible changes in pH, drug content and % CDR revealed that the formulation are stable on storage.

**Table 7: Accelerated stability studies the optimized formulation F4-G1 at 40 ± 2°C and 75 ± 5%**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration in Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Drug content (%)</td>
<td>96.42</td>
</tr>
<tr>
<td>pH</td>
<td>6.40</td>
</tr>
<tr>
<td>% CDR</td>
<td>76.01</td>
</tr>
</tbody>
</table>
CONCLUSION
Lamivudine loaded niosomes were successfully prepared by thin film hydration method using surfactant and cholesterol. The non-ionic surfactant showed reasonable vesicle size, particle size distribution, drug entrapment, drug content and good permeation of the drug. The niosomal gel showed better pharmacological activity than the Lamivudine plain gel indicating a promising potential of the Lamivudine niosomal gel as an alternative to the conventional dosage forms.

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