FORMULATION AND INVITRO EVALUATION OF VESICULAR DRUG DELIVERY SYSTEM ENCAPSULATED WITH ZIDOVUDINE

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ABSTRACT
Proniosomes are dry formulations of surfactant coated hydrophilic carriers, which can be rehydrated by brief agitation in hot water just before use. Zidovudine is a nucleoside analog reverse transcriptase inhibitor used in the treatment of HIV and AIDS related conditions. It has a shorter half life and high intracellular metabolism which makes it a potential candidate for the development of a sustained release formulation. The objective of present study is to formulate and evaluate vesicular drug delivery system of proniosomes encapsulated with Zidovudine. In the present study six formulations of Proniosomes containing zidovudine were prepared by slurry method using dextrin, nonionic surfactants and cholesterol at variable concentrations. All the formulations were evaluated for their preformulation and post- formulation parameters. The FTIR analysis showed no interaction between the drug and other additives. The post-formulation result of entrapment efficiency was found to be inversely proportional to cholesterol concentration in span 60 formulations and directly proportional to cholesterol concentration in tween 60 formulations. The formed Proniosomes were found to be spherical in shape with uniform drug content in all the formulations. The dissolution profiles of formulations containing high concentration of cholesterol showed better controlled release rate over period of 72 hrs. The kinetic release data best fitted into Peppas model suggesting that drug was released by fickian mechanism indicating that the drug was released by diffusion controlled mechanism.

KEYWORDS: Proniosomes, Zidovudine, Dextrin, Cholesterol, Surfactants.

INTRODUCTION
In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and
most recently, genetic engineering. Vesicles can play a major role in modeling biological membranes, and in the transport and targeting of active agents. Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation and perhaps, reduces the toxicity if selective uptake can be achieved. The phagocytic uptake of the systemic delivery of the drug loaded vesicular delivery system provides an efficient method for delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of drugs, especially in case of poorly soluble drugs.\textsuperscript{[1-4]}

Consequently, a number of vesicular delivery systems such as liposomes, transferosomes, pharmacosomes, niosomes/proniosomes etc, were developed. From early 1980s, niosomes have gained wide attention by researchers for their use as drug targeting agents, drug carriers to have variety of merits while avoiding demerits associated with the conventional form of drugs. Niosomes were studied as better alternatives to liposomes for entrapping both hydrophilic and hydrophobic drugs. From a technical point of view, niosomes are promising drug carriers as they possess greater chemical stability and lack of many disadvantages associated with liposomes such as high cost and the variable purity problems of phospholipids. The additional merits with niosomes are low toxicity due to non ionic nature, no requirement of special precautions and conditions for formulation. Moreover it is the simple method for the routine and large-scale production of niosomes without the use of unacceptable solvents. However, stability is a prime concern in the development of any formulation and even though, niosomes have shown advantages as drug carriers, such as being low cost and chemically stable as compared to liposomes. They too, are associated with problems related to physical stability, such as fusion, aggregation, sedimentation, and leakage on storage. Proniosomal concept minimizes these problems.\textsuperscript{[5-17]}

Proniosomes are dry formulation of water soluble carrier particles that are coated with surfactant which can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The resulting niosomes are very similar to conventional niosomes and more uniform in size. The proniosome approach minimizes problems associated with the liposome and niosomes by using dry, free-flowing product, which is more stable during sterilization and storage. Ease of transfer, distribution, measuring, and storage make proniosomes a versatile delivery system with potential for use with a wide range of active compounds. In general a limited number of studies are available which deal with the preparation and evaluation of proniosomes. \textsuperscript{[18-24]}
Zidovudine is a nucleoside analog reverse transcriptase inhibitor (NRTI). The pharmacokinetic profile and intracellular metabolism of zidovudine provide a strong rationale for the development of a sustained release formulation. Orally administered zidovudine is rapidly absorbed, and 65% of the dose is bioavailable, but its short half-life (1 hr) necessitated dosing every 4 to 6 hours in the initial clinical trials. With current dosing regimens, which employ twice or thrice daily administration, plasma zidovudine concentrations are below optimal anti-retroviral concentrations (1μM) for more than half of the dosing interval.\[25-28\]

To minimize problems associated with frequent dosing an attempt is made in present study to develop proniosomal concept to extend the drug release for longer duration of time by encapsulating zidovudine in surfactant vesicles and evaluate their invitro characteristics.

**MATERIALS AND METHODS**

Zidovudine was obtained as a gift sample from Strides Arco Labs Bangalore, Tween 60 and Span 60 were obtained from Rolex chemical Industries Mumbai, Dicetyl Phosphate was obtained from Sigma Aldrich Germany. All the materials used in the study were of analytical grade.

**Preparation of Proniosomes: Slurry Method**\[29-32\]

Surfactant solution was prepared in organic solvent mixture (methanol: isopropyl alcohol - 4:1) by dissolving adequate quantities of the drug, surfactant, cholesterol and Dicetyl Phosphate. Dextrin powder 600 mg as carrier is taken in a 100 ml round-bottom flask and the entire volume of surfactant solution (10 ml) was added directly to the flask to form slurry. The flask was attached to the rotary evaporator and vacuum was applied until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and kept under vacuum overnight. Proniosome powder was stored in sealed containers at 4° C for further use (Table 1).

**Characterization of Proniosomes**

**FTIR Studies**\[33\]

Drug polymer interactions were studied by FT-IR spectroscopy. Appropriate quantity of Zidovudine alone, mixture of drug and excipients, drug encapsulated proniosomes were weighed and mixed properly with potassium bromide uniformly. A small quantity of the powder was compressed into a thin semitransparent pellet by applying pressure. The IR-
spectrum of the pellet from 500–8000 cm\(^{-1}\) was recorded taking air as the reference and compared to study any interference.

**Table 1: Formulation design of Zidovudine Proniosomes with variable concentrations of Surfactant and Cholesterol**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Surfactant</th>
<th>Zidovudine (mg)</th>
<th>Dextrin (mg)</th>
<th>Surfactant (mg)</th>
<th>Cholesterol (mg)</th>
<th>Dicetyl Phosphate (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNS 1</td>
<td>Span 60</td>
<td>200</td>
<td>600</td>
<td>120</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>PNS 2</td>
<td>Span 60</td>
<td>200</td>
<td>600</td>
<td>140</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>PNS 3</td>
<td>Span 60</td>
<td>200</td>
<td>600</td>
<td>160</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>PNT 1</td>
<td>Tween 60</td>
<td>200</td>
<td>600</td>
<td>120</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>PNT 2</td>
<td>Tween 60</td>
<td>200</td>
<td>600</td>
<td>140</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>PNT 3</td>
<td>Tween 60</td>
<td>200</td>
<td>600</td>
<td>160</td>
<td>30</td>
<td>10</td>
</tr>
</tbody>
</table>

**Measurement of Angle of Repose\[^{34}\]**

The angle of repose of dry proniosome powder was measured by funnel method. Briefly, the proniosomes powder was poured into a funnel which was fixed at a position so that the outlet orifice of the funnel is 10 cm above a level black surface. The powder flowed down from the funnel to form a cone on the surface, and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

\[
\theta = \tan^{-1}\left(\frac{h}{r}\right)
\]

**Determination of Viscosity**

Viscosity of the formulations was determined using an Ostwald viscometer at room temperature.

**Osmotic Shock\[^{35}\]**

The effect of osmotic shock on niosomal formulations was investigated by monitoring the change in vesicle diameter after incubation of niosome suspensions in media of different tonicity like 1 M NaCl (hypertonic), 0.9% NaCl (normal) and 0.5% NaCl (hypotonic). Suspensions were incubated in these media for 3 hours and the change in vesicle size was measured by optical microscopy with a calibrated eyepiece micrometer.

**Preparation of Niosomes from Proniosomes**

Prepared proniosomes powder is weighed and filled in vials. Saline buffer pH 7.4 is added to the vials and which are then attached to a vortex mixer and agitated for 2 min to get niosomal suspension.
Characterization of Niosomes prepared from Proniosomes

**Drug Content**\(^{[36]}\)

Proniosomes equivalent to 50 mg were taken into a standard volumetric flask. They were lysed with 25 ml of methanol by shaking for 15 min. The clear solution was diluted to 100 ml with methanol. Then 10 ml of this solution was diluted to 100 ml with saline phosphate buffer 7.4. Aliquots were withdrawn and the absorbance was measured at 267 nm and drug content was calculated from the calibration curve.

**Transmission Electron Microscopy (TEM)**\(^{[37]}\)

A drop of the sample was placed onto a carbon-coated grid and allowed to dry to a thin film. Before drying of this film on the grid, it was negatively stained with 1% phosphotungstic acid. For this, a drop of staining solution was pipette onto the film and the excess drained off with filter paper. The grid was allowed to air dry thoroughly and then examined using a transmission electron microscope with an accelerating voltage of 80 kV.

**Vesicular Size Distribution and Average Particle Size Determination**\(^{[38]}\)

The size, shape, and lamellar nature of vesicles in the formulations were observed by optical microscope (compound microscope) with a calibrated eyepiece micrometer. A suspension of proniosomes was prepared in saline buffer pH 7.4. A drop of the suspension was mounted on a slide and observed under the microscope. About 200 niosomes were measured individually with the help of eye piece micrometer, average was taken and their size distribution range, mean diameter were calculated.

**Microphotography**

All the proniosomal formulations were converted to niosomal suspensions and viewed under optical microscope to observe the shape and the lamellar nature of vesicles. Microphotographs were taken with Nikon D-500 8 megapixel camera. The z-average diameter of sonicated vesicles was determined by dynamic light scattering using a Zetasizer, Nano ZS 90 (Malvern instruments). For the measurement, 100 µl of the formulation was diluted with an appropriate volume of PBS, pH 7.4 and the vesicle diameter and particle size distribution were determined.

**Entrapment Efficiency**\(^{[39]}\)

Zidovudine niosomal formulations were centrifuged at 15,700×g for 90 min at 4°C using a refrigerated centrifuge to separate niosomes from non-entrapped drug. Concentration of the
free drug in the supernatant was determined by measuring absorbance at 267 nm with a UV spectrophotometer (Shimadzu, UV 1650 PC, Kyoto, Japan). This process was repeated thrice to ensure that free drug was completely removed. The percentage of drug entrapment in niosomes was calculated using the equation.

**Percentage drug entrapment = (Total drug - drug in supernatant)/Total drug X 100**

Percent drug entrapment was confirmed by lysing the niosomes with n-propanol after centrifugation and measuring absorbance at 267 nm.

**Zeta Potential Analysis**[^40]

Zeta potential was measured for selected formulations using large bore capillary cells in the Zetasizer Nano-Zs (Malvern Instruments). One ml of niosomal suspension is diluted with 0.9% (M/V) sodium chloride solution prepared in distilled water for optimal signal intensity. The analysis was performed at a scattering angle of 90° C at a temperature of 25° C.

**Invitro Release Studies**[^41-44]

**Invitro** release was studied using a dialysis bag as a donor compartment. Niosomes containing entrapped zidovudine obtained after centrifugation of 2 ml of the formulation were resuspended in 1 ml of PBS, pH 7.4, and used for the release study. The dialysis membrane was soaked in warm water for 10 min, one end was sealed with a clip, the niosome preparation was pipette into the bag and the bag was sealed with another closure clip to prevent leakage. The dialysis bag was placed in 100 ml of PBS, pH 7.4, at 37±2°C. The medium, which acted as the receptor compartment, was stirred at 100 rpm. Samples of medium (5 ml) were withdrawn hourly and replaced with fresh buffer and zidovudine absorbance at 267 nm was measured using PBS as blank. Results were the mean values of three runs. The mechanism of zidovudine release from niosomal formulations was determined using different mathematical models like zero-order kinetics, first -order kinetics, Higuchi kinetics, and the Korsmeyer-Peppas and Hixson-Crowel models.

**RESULTS AND DISCUSSIONS**

**FTIR Studies**

From the FTIR studies it was evident that there was no chemical interaction between the drug and the excipients used.
**Angle of Repose:** The angle of repose of dry proniosomes was between 24.34±0.16 to 26.51±0.11 indicating that the powder was free flowing. This is consistent with the microscopic observation of proniosome powder, which showed the proniosome surface to be smoother.

**Viscosity**

The Viscosity of the formulations was found to be in the range of 1.54 to 2.98 centipoises. The viscosity increased with increase in concentration of the surfactant in the formulations.

**Osmotic Shock:** In hypotonic solution, all the formulations increased in their size whereas in hypertonic solution, all the formulations shrank uniformly. Formulations incubated with saline showed a negligible change in vesicle size when compared to other media. This shows that zidovudine niosomes could be diluted with normal saline for parenteral use.

**Zeta Potential Analysis**

The formulations PNS1 and PNT1 which were subjected to zeta potential analysis showed a value of +28 mv and +30 mv respectively. This higher charge on the surface of vesicle produce a repulsive force between the vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension.

**Surface Morphology:** TEM analysis and microphotographs revealed that the vesicles are spherical in shape with multilamellar nature (Fig 1 & 2).

![Fig 1: TEM Photograph of Zidovudine Vesicles](image-url)
Entrapment Efficiency

Niosomes prepared from proniosomes using Span 60 and Cholesterol entrapped the drug in the range of 88.35±0.86, 89.46±0.76 and 91.39±0.68 for PNS1, PNS 2 and PNS 3 respectively. Niosomes prepared from proniosomes using Tween 60 and Cholesterol entrapped the drug in the range of 90.84±1.07, 88.29±1.12 and 86.39±1.01 for PNT 1, PNT 2 and PNT 3 respectively (Table 2). Increase in the concentration of cholesterol results in the decrease of entrapment efficiency for niosomes prepared with span 60. Lower the concentration of cholesterol, lower is the entrapment efficiency for niosomes prepared with tween 60. (Fig 3)

Table 2: Results of Formulation Variables of Zidovudine Vesicles

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Particle Size (μm) ± SD</th>
<th>Percentage Yield</th>
<th>Percentage Encapsulation Efficiency ± SD</th>
<th>Percentage Drug Content ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNS 1</td>
<td>7.78± 0.04</td>
<td>98.61</td>
<td>88.35 ± 0.86</td>
<td>94.30 ± 0.11</td>
</tr>
<tr>
<td>PNS 2</td>
<td>7.62± 0.02</td>
<td>97.39</td>
<td>89.46 ± 0.76</td>
<td>95.66 ± 0.14</td>
</tr>
<tr>
<td>PNS 3</td>
<td>7.59± 0.05</td>
<td>95.56</td>
<td>91.39 ± 0.68</td>
<td>96.89 ± 0.19</td>
</tr>
<tr>
<td>PNT 1</td>
<td>7.95± 0.11</td>
<td>98.01</td>
<td>90.84 ± 1.07</td>
<td>97.08 ± 0.21</td>
</tr>
<tr>
<td>PNT 2</td>
<td>7.84± 0.09</td>
<td>97.72</td>
<td>88.29 ± 1.12</td>
<td>94.70 ± 0.26</td>
</tr>
<tr>
<td>PNT 3</td>
<td>7.55± 0.12</td>
<td>96.03</td>
<td>86.39 ± 1.01</td>
<td>93.46 ± 0.29</td>
</tr>
</tbody>
</table>

Drug Content

The drug content was found to be in the range of 93.46 to 97.08%. The drug content of zidovudine proniosomes prepared using cholesterol and span 60 at varied concentrations was
in the range of 94.30±0.11, 95.66±0.14 and 96.89±0.19 for PNS1, PNS 2 and PNS 3 respectively. The drug content of zidovudine proniosomes prepared using cholesterol and tween 60 at varied concentrations was in the range of 97.08±0.21, 94.70±0.26 and 93.46±0.29 for PNT 1, PNT 2 and PNT 3 respectively (Table 2).

**Vesicular Size, Shape and Size Distribution:** The proniosomes were converted to niosomal suspension and the size of the niosomes was measured using an optical microscope with calibrated eyepiece micrometer. From every batch about 200 niosomes were measured for the diameter individually and the average was calculated. The average vesicular size of niosomes of all the batches was measured in the range of 7.55 μm to 7.95μm. The size distribution was in the range of 1 to 16 μm (Fig 4). The result suggests niosomes prepared were of uniform size and spherical in shape. The microphotographs of all proniosomal formulations revealed that the niosomes were spherical in their shape.
In vitro Drug Release Studies

The percentage drug release from proniosomal formulations prepared using span 60 and cholesterol was found to be 74.08±0.21, 78.62±0.41 and 82.58±0.64 for PNS1, PNS 2 and PNS 3 respectively at the end of 72 hours. The percentage drug release from proniosomal formulations prepared using tween 60 and cholesterol was found to be 73.67±0.63, 79.40±0.81and 81.13±0.92 for PNT 1, PNT 2 and PNT 3 respectively at the end of 72 hours. In all the cases, 25% to 30% of drug is released in the first 6 hours, due to initial bursting of improper niosomes in the formulations. However, after 24 hours, the release was steady because the stable niosomes retain the drug and the release was extended up to 72 hours with
sustained action (Fig 5 & 6). The proniosomal formulations with high concentration of cholesterol shows better controlled release compared to other concentrations. The regression coefficient values of all the kinetic models showed that the release was best fitted into Peppas model indicating that the drug was released by fickian release mechanism which is diffusion controlled.

CONCLUSION
By studying all the experimental results Vesicular drug delivery system of proniosomes encapsulated with Zidovudine can be successfully formulated by slurry method. By incorporating variable concentrations of cholesterol and surfactants such as Span 60 and tween 60 in the formulations, the rate of drug release can be controlled for a prolonged period of time. Formulations PNS 1 and PNT 1 showed the best results within all the evaluated parameters and hence considered as ideal batches.

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