FABRICATION AND EVALUATION OF RITONAVIR PRONIOSOMAL TRANSDERMAL GEL AS A VESICULAR DRUG DELIVERY SYSTEM

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ABSTRACT
The aim of the study was formulation and evaluation of a proniosomal transdermal gel as vesicular drug delivery system for improving stability of formulation and sustaining drug release of Ritonavir, an HIV antiviral drug. Initial studies focussed on the formulation parameters that influence the manufacturing process by using non ionic surfactant and then evaluation parameters for optimization of formulation. The drug excipients compatibility studies were carried out by DSC studies and FTIR spectra revealed that the drug and excipients used were compatible with each other. The evaluation of proniosomes shows an entrapment efficiency range of 61.60±2.05 to 91.74±0.54%. The optimized batch shows the encapsulation efficiency 91.74±0.54%, vesicle size 246.48±3nm and drug content 96.41±0.74%. The drug release studies of proniosomal gel was carried out by diffusion through cellophane membrane % drug release was found to be 89.09±1.5%, Rate of spontaneity 14.54±0.95 mm²x1000, pH 7.2±1.45, viscosity 4298±2.05 cp, spreadability 48±0.23 mm. The optimized formulation was evaluated for morphology and structure of proniosomes (SEM) shows spherical in shape, Zeta Potential was found to be -56.21. This study provided the evidence that the proniosomal vesicles are valuable as the transdermal drug delivery for ritonavir to sustaining the drug release and enhance the stability of formulation.

Keywords: Proniosomes, Transdermal Gel, Surfactant, Ritonavir, Vesicular drug delivery system.

INTRODUCTION
Drug delivery system using colloidal particulates carriers such as liposomes and niosomes have distinct advantages over conventional dosage forms. These carriers can act as drug reservoirs and modification of their composition or surface can be used for drug targeting. In disperse aqueous system liposomes have problems associated with degradation by hydrolysis or oxidation as well as sedimentation, aggregation or fusion of liposomes during storage and problems in their sterilisation. Niosomes are unilamellar or multilamellar vesicles are capable of entrapping hydrophilic and hydrophobic solutes. Niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes such as high cost and the variable purity problems of phospholipids. Large scale production of niosomes without the use of acceptable solvents.

Proniosomes are recent development in novel drug delivery system. These are most advanced drug carrier in vesicular system in which these are made up of non-ionic based surfactants, cholesterol and other additives. “Proniosomes are water soluble carrier particles that are coated with surfactant and can be hydrated to form niosomal dispersion immediately”. It minimize problems of vesicular systems such as aggregation, fusion and leakage of drug and provide additional convenience in transportation, distribution, storage and dosing. The vesicles overcomes demerits of liposomes and niosomes. Proniosomes are
hydrated by agitation in hot water for short period of time offer a versatile vesicular delivery concept with the potential for drug delivery via the transdermal route. Ritonavir is selected for current study which is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases. Ritonavir belongs to a group of HIV protease inhibitors (PIs). The drug has a short half life of 3 to 5 hours and is currently administered orally and parentally. However it has to be administered frequently and is associated with numerous side effects due to which an alternate route is required. Transdermal delivery certainly appears to be an attractive route of administration to maintain drug blood levels of Ritonavir for extended period of time.

The aim of this study is to investigate the feasibility of use of proniosome as proniosomal gel of Ritonavir in the transdermal delivery system. Vesicles prepared were characterized by vesicle size determination, scanning electron microscopy, zeta potential analysis, in-vitro drug release studies and stability studies under experimental conditions to investigate the leaking of drug during storage.

**MATERIALS AND METHOD**

**Materials**
Ritonavir was provided as a gift sample from Cipla Ltd, Mumbai. All the other ingredients were of pharmaceutical grade.

**Experimental Methodology**

**Preformulation studies**

**Solubility Studies**
Solubility studies of Ritonavir (RnV) was carried out in various solvents such as water, methanol, isopropanol, phosphate buffer and ethanol.

**Melting Point Determination**
The Melting point was determined by using digital melting point apparatus by taking drug in the capillary.

**Compatibility Studies**

**Infrared absorption spectra**
The IR spectrum of ritonavir was recorded by using KBr pellet method. The pellet was scanned over range of 500-4000 cm\(^{-1}\) in FTIR and the spectrum was obtained.

**DSC**
DSC thermogram of drug, was separately recorded by placing the sample in aluminium pan with a reference pan heated at a rate of 20°C/min over a range of 0-300°C. Inert atmosphere was maintained with purge of nitrogen gas.

**Formulation Development**

**Formulation of Proniosomes**
Proniosomes were prepared by coacervation phase separation method by using different non ionic surfactants. In this method the drug was taken in a clean and dry wide mouthed glass vial and methanol was added to it. All the ingredients were mixed well with a glass rod. The open end of the glass vial was covered and warmed over water bath at 50-60°C for about 5 minutes. Then aqueous phase phosphate buffer pH 7.4 was added and warmed on a water bath until a clear solution was formed.

**Formulation of Proniosomal Transdermal Gel**
The prepared proniosomes batches P1-P8 were incorporated into 1.5% Carbopol blank gel. In this 1.5% w/w of carbopol-934 use for the preparation of gel. The quantity of methyl paraben and propyl paraben equal to 0.2 and 0.02 g respectively in dispersion. The pH of gel was adjusted to 6.8-7.4 by addition of triethanolamine and NaOH. The gel was allowed to stand overnight under vacuum to remove the entrapped air.
Characterisation of Proniosomes

Physical Observation of Proniosomes
The proniosomal dispersion was examined for physical characteristics such as colour and the state of dispersion.

Microscopic Examination
The proniosomal dispersion was examined for the structure and lamellarity under microscope at magnification powers of 10X and 45X and photomicrographs were recorded.

Entrapment Efficiency
The aqueous proniosomal suspension was centrifuged at 25,000rpm at 25°C for 30 min to separate untrapped drug as supernatant. Supernatant was separated, filtered and sufficiently diluted with methanol to determine the concentration of untrapped drug spectrophotometrically at 240 nm. The percentage of drug encapsulation was calculated by equation;

\[
\text{Entrapment efficiency (\%) } = \left[ \frac{(t-f)}{t} \right] \times 100
\]

Where,
- \( t \) is the concentration of total drug.
- \( f \) is the concentration of free drug.

The entrapment efficiency of proniosomes is depend on the vesicle size.

Evaluation of Proniosomal Gel

Organoleptic Characters
The prepared proniosomal transdermal gels were inspected visually for clarity, colour and presence of any particles.

Drug Content
The drug content was determined by dissolving the proniosomal gel sample in ethanol and measuring the absorbance at 240 nm from which the drug content was calculated.

Viscosity
Viscosities of the formulated Proniosomal gels were determined using Brookfield Viscometer (DV-II + Pro) with T-Shaped spindle set using Spindle no. 96 The gel sample was taken in a beaker and the dial reading was noted at 100 rpm 25°C.

\[ \text{pH Measurement} \]

The pH measurement of the proniosomal gels were determined by using a pH meter which was calibrated before use with standard buffer solutions at pH 4 and 7. The quantity of gel equal to 1 g was dispersed in 20 ml distilled water and the pH was measured gives different pH values.

Spreadability
The spreadability of vesicular gel formulation were determined by placing 0.5 g of respective gel within a circle of diameter 1 cm, pre marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for about 15 secs.

Rate of Spontaneity
Rate of spontaneity is defined as number of niosomes formed after hydration of proniosomes for 15 min. 0.2g of proniosomal gel is transferred to bottom of the small Stoppered glass tube and then spread along the walls of the container uniformly. Further, 2 ml saline (0.154 M NaCl) is added carefully and keep aside for 15 min without agitation. A drop of this aqueous layer is withdrawn and placed over Neubau’s chamber and observe number of niosomes eluted from proniosomes in optical microscope.

In-Vitro Drug Release Studies

\[ \text{In-vitro} \]
drug release study of proniosomal transdermal gel was carried out by using dialysis bag as donor compartment. Proniosomal transdermal gel equivalent to 10 mg was taken in dialysis bag, placed in buffer
containing phosphate buffer solution which acted as receptor compartments. A standard dialysis membrane soaked in pH 7.4 for 24 hours before use or soaked in warm water for 10 mins and both the end were sealed with closer clips by adding proniosomal gel formulation. The beaker was kept at magnetic stirrer with 100 rpm and maintained at 37±1°C. At predetermined interval of 12 hrs sample were withdrawn and replaced with 1ml of fresh phosphate buffer solution sink condition was maintained throughout experiment. Sample withdrawn were diluted and analyzed by UV spectrophotometer at 240 nm.17

**Optimization of Proniosomal Transdermal Gel**
The optimization of proniosomal transdermal gel was done by considering all the evaluation parameters discussed above.

**Evaluation of Optimized Formulation**
The optimized formulation were evaluated for following parameters.

*Morphology and structure of proniosomes (SEM)*
The surface morphology (roundness, smoothness, and formation of aggregates) of proniosomes was studied by Scanning Electron Microscopy. Shape for the formulation was determined by scanning electron microscope (Motic image, Kolkatta). The formulation was spread uniformly on glass slide and observed under electron microscope for vesicular shape.18

*Measurement of Vesicle Size*
The mean particle size diameter and size distribution (polydispersity index, PI) was determined by Malvern zetasizer nano. Each sample was run 3 times and analysis was carried out at 25 0°C with an angle of detection 173°.

*Measurement of Zeta Potential*
The zeta potential was analyzed for proniosomal formulation by Malvern Zetasizer in Sinhagad Institute of Pharmacy, Pune. Each sample was run 3 times and the analysis was carried out at 25 0c with an angle of detection 173°C.19,20

*Skin Irritation Study*
Skin irritation test was done on the human skin by applying approximately 1gm of proniosomal transdermal gel on the surface of skin 1cm² and sensitization can be performed on healthy human skin. The proniosomal transdermal gel was to be removed after some time and the skin was observed for any type of irritation.21

*Stability Study*
Stability studies were carried out by storing the optimized formulation at various temperature conditions as per ICH guidelines i.e. At refrigeration temperature (2°-8°C), Room temperature (25°± 0.5°C) for a period of two months. Drug content and variation in the average vesicle diameter were determined before and after the completion of 2 months.22

**RESULT AND DISCUSSION**

**Preformulation Studies of RnV.**

*i. Solubility Studies of RnV*
Solubility studies of RnV was carried out in various solvents. From the result of solubility of RnV in different solvents, it was observed that, RnV was insoluble in water, slightly soluble in isopropanol, soluble in ethanol and soluble in methanol .

*ii. Melting Point Determination*
From the result, melting point of RnV was found to be 121±20°C.

*iii. Compatibility Studies*
The drug-excipient interaction was performed by FT-IR and DSC.

*a) Infrared Absorption Spectra (FT-IR)*
FT-IR spectrum of RnV

The FT-IR spectrum of RnV was measured in solid state as potassium bromide dispersion. The characteristic peaks (cm$^{-1}$) were assigned for respective functional groups. The FT-IR spectra of pure drug and excipients and physical mixture of drug with excipient is shown in figure below.

![Figure 1: FT-IR spectrum of RnV](image1)

The presence of peaks at 672 cm$^{-1}$ and 722 cm$^{-1}$ shows Ar-OH Phenol at plane deformation shows Alcohol/Phenol O-H Stretch, were characteristic to the lecithin used in the formulation.

FT-IR Spectrum of Drug and Excipients Mixture

![Figure 2: FTIR spectrum of drug and excipients mixture](image2)

The presence of peaks at 666 cm$^{-1}$ shows Ar-OH Phenol plane deformation and CH=CH di-substitution of alkene plane deformation, 890 cm$^{-1}$ (Aromatic C-H Bending), 1062 cm$^{-1}$ and 1177 cm$^{-1}$ (C-N stretching, C-O present), 1227 cm$^{-1}$ and (O-H bending in-plane), 1713 cm$^{-1}$ shows Aldehyde C=O Stretch, 2852 cm$^{-1}$ and 2620 cm$^{-1}$ (Carboxylic Acid O-H Stretch), 3146 cm$^{-1}$ shows Alcohol/Phenol O-H Stretch, 3271 cm$^{-1}$ shows Alcohol/Phenol O-H Stretch, 3250 cm$^{-1}$ within range 3300-3250 cm$^{-1}$ shows =CH-H Stretch, were characteristic to the physical mixture of drug and excipients.
IR spectrum of physical mixture of drug with excipients revealed that there was no appreciable change in position and intensity of peak with respect to IR spectrum of pure RnV and lecithin. IR analysis revealed that there was no known chemical interaction between drug and excipients.

b) Differential Scanning Colorimetry (DSC)

Diffential scanning colorimetry is performed for the compatibility study of drug and other excipient at different temperature.

**DSC of Ritonavir**

![Figure 3: DSC of Ritonavir](image)

**DSC of Drug and Excipients Mixture**

![Figure 4: DSC of drug and excipients mixture](image)

Early onset of endotherm for pure RnV (at 128°C) and for physical mixture (at 130°C) endotherm. These results indicated partial amorphisation and solubilisation of RnV due to the processing and absence of any additional peak indicated that there was no interaction between the drug and excipients used in the formulation.

**Formulation of Proniosomes**

The Proniosomes were prepared by Coacervation phase separation method by using different surfactants span 20, 40, 60, 80 and tween 20, 40, 60, 80, cholesterol and Lecithin the detailed procedure were described. The formation of proniosomal dispersion were done successfully.

**Compositions of Preliminary Formulations**
Table 1: Compositions of preliminary formulations

<table>
<thead>
<tr>
<th>Batches</th>
<th>Surfactant Name</th>
<th>Surfactant</th>
<th>Cholesterol</th>
<th>Lecithin</th>
<th>RnV</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Span20</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P2</td>
<td>Span40</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P3</td>
<td>Span60</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P4</td>
<td>Span80</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P5</td>
<td>Tween20</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P6</td>
<td>Tween40</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P7</td>
<td>Tween60</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
<tr>
<td>P8</td>
<td>Tween80</td>
<td>45.5%</td>
<td>5%</td>
<td>45%</td>
<td>1%</td>
</tr>
</tbody>
</table>

Characterization of Proniosomes

i. Physical Observation of Proniosomes

Physical observation of proniosomes shown as given below.

Table 2: Physical observation of proniosomes

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Formulation</th>
<th>Appearance of Proniosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1</td>
<td>White Semi-solid</td>
</tr>
<tr>
<td>2</td>
<td>P2</td>
<td>Brown Transparent Liquid</td>
</tr>
<tr>
<td>3</td>
<td>P3</td>
<td>Creamy White Semi-solid</td>
</tr>
<tr>
<td>4</td>
<td>P4</td>
<td>Creamish Liquid</td>
</tr>
<tr>
<td>5</td>
<td>P5</td>
<td>Creamish Liquid</td>
</tr>
<tr>
<td>6</td>
<td>P6</td>
<td>Brown Liquid</td>
</tr>
<tr>
<td>7</td>
<td>P7</td>
<td>Brownish semisolid</td>
</tr>
<tr>
<td>8</td>
<td>P8</td>
<td>Yellowish gel</td>
</tr>
</tbody>
</table>

These parameters were checked for proniosomes revealed that the proniosomes were white to brown in colour having White Semi-solid, Creamish Liquid in appearance and characteristic odour.

ii. Microscopic Examination

Microscopic examination of proniosomes at 10X magnification power. Photomicrographs were taken for proniosomes by using optical microscope.

Figure 5: Photomicrographs of Formulations P1, P2, P3 and P4 Containing Span 20, 40, 60, 80 as a surfactant

http://www.pharmacophorejournal.com
The photomicrographs of hydrated P1 to P8 proniosomal formulations shown in above figure which is composed of cholesterol, lecithin and span20, 40, 60, 80, tween20, 40, 60, 80. The photographs revealed that the proniosomes were bilayered structure having spherical shape and no aggregation or agglomeration was observed.

**iii. Entrapment Efficiency**

Encapsulation efficiency of proniosomal gel formulations of batches P1-P8 ranged were shown as follows.

![Figure 7: % Entrapment Efficiency of Proniosomal Formulations.](image)

Entrapment efficiency was found to be in range of 80.40% to 92.20% formed from Span and tween surfactants. The cholesterol content of the formulation was increased, the encapsulation of drug also increased. The formulations (P3) containing span 60, soya lecithin, cholesterol showed high encapsulation efficiency compared to other formulations.

**Formulation of Proniosomal Transdermal Gel**

The transdermal proniosomal gel was prepared successfully by using Carbopol as gelling agent. Different batches of proniosomal gel of RnV were prepared by using Spans and Tweens as surfactant. Cholesterol and soya lecithin. Soya lecithin was selected over egg lecithin because the former gives vesicles of larger size, possibly due to differences in the intrinsic composition of soya and egg derived lecithin. The formulations prepared with Spans were smooth and homogenous with gel like appearance. No proper gel was obtained with Tweens. After formulation of proniosomes were dispersed in Carbopol 934 for formulation of proniosomal gel. The prepared proniosomal gel formulations (P1-P8) were kept in dark room or in cool condition for further use and characterisation.

**Evaluation of Proniosomal Gel**

The prepared proniosomal transdermal gel were evaluated for selection and optimization of batch from P1-P8.

**i. Organoleptic Characters**

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Figure 6: Photomicrographs of Formulations P5, P6, P7 and P8 containing Tween20, 40, 60, 80 as a surfactant
These parameters were checked by visual inspection of proniosomal transdermal gel.

Table 3: Organoleptic characters of gel

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Batches</th>
<th>Colour</th>
<th>Odour</th>
<th>Clarity</th>
<th>Particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1</td>
<td>Thick Light brownish</td>
<td>Characteristic</td>
<td>Opaque</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>P2</td>
<td>Pale yellow</td>
<td>Characteristic</td>
<td>Opaque</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>P3</td>
<td>Creamish</td>
<td>Characteristic</td>
<td>Opaque</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>P4</td>
<td>Light brownish gel</td>
<td>Characteristic</td>
<td>Opaque</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>P5</td>
<td>Yellow</td>
<td>Characteristic</td>
<td>Highly thick</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>P6</td>
<td>Yellow</td>
<td>Characteristic</td>
<td>Highly thick</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>P7</td>
<td>Brownish</td>
<td>Characteristic</td>
<td>Opaque</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>P8</td>
<td>Yellowish</td>
<td>Characteristic</td>
<td>Opaque</td>
<td>No</td>
</tr>
</tbody>
</table>

Proniosomal gel of ritonavir prepared with Span (40, 60) have pale yellow semi solid gel like appearance. Proniosomal gel produced from Span 20 have thick sticky gel like appearance and span 80 have light brownish gel like appearance. Proniosomal gel of tween 20 appears as Yellow highly viscous liquid form and proniosomal gel of Tween 80 appears as semisolid crystalline gel and it has characteristic odour.

ii. Drug Content

The drug content of the formulations shown below in table:

Table 4: Drug content

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P1</td>
<td>86.52 ±1.05</td>
</tr>
<tr>
<td>2</td>
<td>P2</td>
<td>88.47 ±0.5</td>
</tr>
<tr>
<td>3</td>
<td>P3</td>
<td>96.41 ±0.74</td>
</tr>
<tr>
<td>4</td>
<td>P4</td>
<td>78.13 ±0.63</td>
</tr>
<tr>
<td>5</td>
<td>P5</td>
<td>76.27 ±0.8</td>
</tr>
<tr>
<td>6</td>
<td>P6</td>
<td>70.43 ±0.1</td>
</tr>
<tr>
<td>7</td>
<td>P7</td>
<td>84.24 ±1.0</td>
</tr>
<tr>
<td>8</td>
<td>P8</td>
<td>89.91 ±3.02</td>
</tr>
</tbody>
</table>

All values represent mean±standard deviations(SD), n=3 The results indicated that the process employed to prepare proniosomes was capable of producing formulations with consistent drug content. The drug content among all the batches was within the range from 65.43 ±0.1% to 96.41 ±0.74% (Table)

Characterization of Proniosomal Formulations for Physical Appearance, pH, Viscosity (cp) And Spreadability

Table 5: Characterization of proniosomal formulations for pH, Viscosity (cp), Spreadability and Rate of spontaneity

<table>
<thead>
<tr>
<th>Batches</th>
<th>Viscosity (cp)</th>
<th>pH</th>
<th>Spreadability Using 50gm</th>
<th>Rate of spontaneity (mm³ x 1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>5580 ±1</td>
<td>6.2 ±2</td>
<td>38±1.05</td>
<td>11.19±1.05</td>
</tr>
<tr>
<td>P2</td>
<td>4500 ±0.05</td>
<td>5.7 ±0.95</td>
<td>46±0.02</td>
<td>15.25±0.45</td>
</tr>
<tr>
<td>P3</td>
<td>4298 ±2.05</td>
<td>7.2 ±1.45</td>
<td>48±0.23</td>
<td>14.54±0.95</td>
</tr>
<tr>
<td>P4</td>
<td>5130 ±1.45</td>
<td>7.1 ±1</td>
<td>45 ±0.05</td>
<td>10.58±0.05</td>
</tr>
<tr>
<td>P5</td>
<td>3536±3.15</td>
<td>6.4±0.05</td>
<td>65±1.05</td>
<td>12.30±0.02</td>
</tr>
<tr>
<td>P6</td>
<td>3452±2</td>
<td>5.9±1.52</td>
<td>53±0.45</td>
<td>10.49±1.8</td>
</tr>
<tr>
<td>P7</td>
<td>3436±1.83</td>
<td>6.8±1</td>
<td>50±3.24</td>
<td>12.90±1</td>
</tr>
<tr>
<td>P8</td>
<td>3246±1.23</td>
<td>5.31±0.34</td>
<td>52±0.23</td>
<td>11.45±0.1</td>
</tr>
</tbody>
</table>

All values represent mean±standard deviations (SD), n=3
iii. In-Vitro Drug Release Studies

The In-Vitro drug release of all 8 batches (P1-P8) were show that the p3 batch sustains the drug release upto 12 hrs. Results are depicted in table and are presented in figure.

![In-vitro drug release study of proniosomal gel of batches P1, P2, P3 and P4](image1)

**Figure 8: In-vitro drug release study of proniosomal gel of batches P1, P2, P3 and P4**

![In-vitro drug release study of proniosomal gel of batches P5, P6, P7 and P8](image2)

**Figure 9: In-vitro drug release study of proniosomal gel of batches P5, P6, P7 and P8**

Formulations which have higher cholesterol content (P1-P8) showed less drug release over a period of 12 hrs. Increase in cholesterol amount and use of different non ionic surfactant resulted in a more intact lipid bilayers as a barrier for drug release and decreased its leakage by improving the fluidity of the bilayers membrane and reducing its permeability, which led to lower drug elution from the vesicles and sustaining drug release.

The in-vitro drug release studies performed showed that P1-P8 released the drug shown in table for 12 hrs. The order of drug release was as follows: P3>P8>P7>P2>P1>P4>P5>P6. The proniosomal gel formation by using span 60 sustaining drug release and by considering all the evaluation parameter batch P3 shows best result and consider it as optimized formulation.

**Optimization of Proniosomal Transdermal Gel**

Optimization of proniosomal transdermal gel Formulation was based on the basis of evaluation parameters batch P3 was found to optimized batch and further it get evaluated for other parameters.

**Evaluation of Optimized Formulation**

Optimized formulation were evaluated for following parameters such as morphology of niosomes derived from proniosomal gel was studied using Scanning Electron Microscopy, Zeta potential, Skin irritancy test and Stability studies.
i. Morphology and Structure of Proniosomes (SEM)

Scanning Electron Microscopy were performed to observed the surface morphology of proniosomal gel.

![SEM images of optimized formulation (P3)](image)

**Figure 10:** SEM images of optimized formulation (P3)

SEM imaging of RnV proniosomal transdermal gel revealed that the niosomes produced from hydration of proniosomes were spherical in shape and discrete with sharp boundaries having large internal aqueous space. SEM imaging of proniosomes produced from optimized formulation P3 was shown in above Figure.

ii. Measurement of Zeta Potential

![Measurement of zeta potential](image)

**Figure 11:** Measurement of zeta potential

Zeta potential of formulation was found to be -56.21 mV. The ionization of surface groups may produce a surface charge. Physiologic surfactants are anionic surfactants and upon ionization it produces pH dependent net surface charge. The optimized formulation showed polydispersity index less than 1 which depicts a narrow size distribution. Niosomes prepared with Tween were significantly larger than those prepared with Span since Tween has lower hydrophobicity than Span. The formulation P3 was considered optimized since Span60 produced the vesicles of normal size with higher entrapment of drug. Moreover, it is found that the drug leakage from the vesicles composed of Span 60 is low, due to its high phase transition temperature. Result for Zeta Potential shown in table.

iii. Measurement of Vesicle Size

The mean particle size diameter and size distribution (polydispersity index, PI) was determined by photon cross-correlation spectroscopy

![Graph for determination of vesicle size of batch P3](image)

**Figure 12:** Graph for determination of vesicle size of batch P3

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Vesicle size of RnV proniosomes of P3 batch was found to be 346.48nm, which indicated that vesicles formed with Spans were smaller in size than vesicles formed with Tweens. The relationship observed between niosome size and Span hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity, resulting in the smaller vesicles. Increasing the cholesterol content also contributed an increase in the hydrophobicity, with a subsequent slight reduction in vesicle size.

Skin Irritation Study
Skin irritation test was done on the human skin. The proniosomal transdermal gel is to be removed after some time and the skin is to be observed for irritation, there is no irritation or oedema on the skin it was observed by visually and sensitization was performed.

Stability Study
There was no significant change in relation to drug release and encapsulation efficiency. It shows that proniosomes has better stability.

Table 6: Stability study data of optimized formulation (P3)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Temp. (°C)</th>
<th>Initial Encapsulation efficiency*</th>
<th>Drug release</th>
<th>After 1 months Encapsulation efficiency*</th>
<th>Drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-8°C</td>
<td>91±0.53</td>
<td>89.02±0.53</td>
<td>89.01±0.46</td>
<td>86.62±0.53</td>
</tr>
<tr>
<td>2</td>
<td>25±2°C</td>
<td>90.61 ± 0.23</td>
<td>88.16±0.23</td>
<td>87.32±0.56</td>
<td>88.06± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>45±2°C</td>
<td>89.01 ± 0.56</td>
<td>88.2 ± 0.56</td>
<td>85.73±0.32</td>
<td>83.2 ± 0.56</td>
</tr>
</tbody>
</table>

All values represent mean±standard deviations(SD), n=3

There was no significant change in physical characterization of formulation. It shows that proniosomes has better stability.

Table 7: Physical characterization of batch P3 after 1 month

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Physical Characterization</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Creamish</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>3</td>
<td>Clarity</td>
<td>Opaque</td>
</tr>
<tr>
<td>4</td>
<td>Particles</td>
<td>No</td>
</tr>
</tbody>
</table>

FTIR Spectrum of Proniosomal Formulation
The FTIR study of optimised batch shows that there is not any interaction between drug and other excipient. It shows that the proniosomal transdermal gel is stable.

Figure 13: FTIR spectrum of proniosomal formulation
The presence of peaks at 665 cm$^{-1}$ and 722 cm$^{-1}$ Ar-OH Phenol plane deformation and CH=CH di-substitution of alkene plane deformation, 841 cm$^{-1}$ and 874 cm$^{-1}$ shows Aromatic C-H Bending, 1056 cm$^{-1}$, 1237 cm$^{-1}$ and 1240 cm$^{-1}$ shows C-N stretching, C-O present, 1330 cm$^{-1}$ shows O-H bending in-plane, 1714 and 1732 cm$^{-1}$ shows Aldehyde C=O Stretch, 1905 and 1985 cm$^{-1}$ shows Substitution on benzene ring, 2857 cm$^{-1}$ and 2918 cm$^{-1}$ shows Carboxylic Acid O-H Stretch, 2909 cm$^{-1}$ shows CH$_3$ & -CH$_2$ in aliphatic compound, 2918 cm$^{-1}$ shows Alkyl C-H Stretch, were characteristic to the final formulation of proniosomal gel. IR spectrum of proniosomal transdermal gel showed that there was no appreciable change in position and intensity of peak with respect to IR spectrum of pure RnV and excipients, excipients and physical mixture of drug. Thus there is compatibility between drug and excipients after the formulation.

**CONCLUSION**

For the present study Ritonavir an antiviral BCS class II drug was selected as the drug candidate for the formulation of transdermal gel. The proniosomal gel could be an effective alternative vehicle for delivering drug through transdermal route. The results of present study indicate that Ritonavir proniosomes containing lecithin, cholesterol and surfactants sustained the release of drug over 12 hrs. The proniosomes containing span 60 shows best results, thus it helped to provide a sustained release effect by delivering it transdermally and helped to reduced side effect associated with other system. Thus proniosomal gel system shown potential for delivery of suitable drug candidate Ritonavir.

**REFERENCE**


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