CHAPTER 3

Result

&

Discussion
Preformulation study

3.1.1 Drug

Table 3.1: Characterization of Nystatin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specifications</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Yellowish crystalline powder</td>
<td>Yellowish crystalline powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Very slightly soluble in water, slightly soluble in methanol &amp; Insoluble in chloroform, ether</td>
<td>Very slightly soluble in water, slightly soluble in methanol &amp; Insoluble in chloroform, ether</td>
</tr>
<tr>
<td>Identification</td>
<td>U.V Spectroscopy: at 305nm Absorbance 0.68</td>
<td>Absorbance 0.67</td>
</tr>
<tr>
<td>pH (3%)</td>
<td>Between 6-8</td>
<td>6.5</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>Not more than 5%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Melting point</td>
<td>153-155 °C</td>
<td>153.6 °C</td>
</tr>
<tr>
<td>Particle size</td>
<td>Not less than 98.0% &lt; 80 µ</td>
<td>74 µ</td>
</tr>
<tr>
<td>Assay by U.V.</td>
<td>98-102%</td>
<td>99.45%</td>
</tr>
</tbody>
</table>

- I.R. Spectrum of Nystatin (Agilent microlab)
Nystatin is soluble in methanol and insoluble in chloroform and ether. The maximum absorbance of Nystatin was found to be 304 nm. Percentage purity of Nystatin was determined by U.V. and was found to be 99.45%. All tests complied with monograph also with certificate of analysis.

IR Spectrum showed principal absorption bands similar to standard IR spectrum indicating confirmation of the given sample.

Table 3.3: Characterization of Triamcinolone Acetonide

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specifications</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White crystalline powder</td>
<td>White crystalline powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Practically insoluble in water, very slightly soluble in ether</td>
<td>Practically insoluble in water, very slightly soluble in ether</td>
</tr>
<tr>
<td>Identification</td>
<td>U.V Spectroscopy: at 240 nm, Absorbance 0.37</td>
<td>Absorbance 0.36</td>
</tr>
<tr>
<td>pH (3%)</td>
<td>Between 3-5</td>
<td>5</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>Not more than 5%</td>
<td>4.6 %</td>
</tr>
<tr>
<td>Melting point</td>
<td>300-306 °C</td>
<td>302 °C</td>
</tr>
<tr>
<td>Particle size</td>
<td>Not less than 98.0% &lt; 65 μ</td>
<td>56 μ</td>
</tr>
<tr>
<td>Assay by U.V.</td>
<td>96-104%</td>
<td>97.74 %</td>
</tr>
</tbody>
</table>
**I.R. Spectrum of Triamcinolone Acetonide**

![IR spectrum of Triamcinolone Acetonide](image)

**Figure 3.2: IR spectrum of Triamcinolone Acetonide**

**Table 3.4: Interpretation of IR Spectrum**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3387.65</td>
<td>O-H stretching</td>
</tr>
<tr>
<td>1707.58</td>
<td>C=O stretching</td>
</tr>
<tr>
<td>1614.34</td>
<td>C-H stretching</td>
</tr>
<tr>
<td>1662</td>
<td>Carbonyl groups of aliphatic ester and ketone</td>
</tr>
<tr>
<td>1178.61</td>
<td>1056.08=C-F bond</td>
</tr>
</tbody>
</table>

Triethanolamine is practically insoluble in water, very slightly soluble in ether. The maximum absorbance of TA was found to be 240 nm. Percentage purity of TA was determined by U.V. and was found to be 97.82%. All tests complied with monograph & certificate of analysis.

The IR spectrum of drug was recorded and it showed principal absorption bands similar to standard IR spectrum indicating confirmation of the given sample.
3.1.2 Excipients

Span 60
Melting point of Span 60 was found to be 57\(^0\)C. Saponification value was found to be 146 and Water content was 0.9 %.

Cholesterol
Cholesterol was soluble in acetone. Melting point of cholesterol was found to be 149\(^0\)C.

Stearic acid
Stearic acid was insoluble in water and melting point was found to be 59\(^0\)C.

Carbopol
Carbopol (0.5%w/v) pH was found to be 3.1. Loss on drying of carbopol was 0.57 % and moisture content was found to be 1.8%w/w.

Table 3.5: Evaluation of Carbopol

<table>
<thead>
<tr>
<th>Tests</th>
<th>Limit</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White colour</td>
<td>White colour</td>
</tr>
<tr>
<td></td>
<td>hygroscopic powder</td>
<td>hygroscopic powder</td>
</tr>
<tr>
<td>pH</td>
<td>2.3-3.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Moisture content</td>
<td>Up to 2%w/w</td>
<td>1.5 %</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>Up to 2%w/w</td>
<td>1.9 %</td>
</tr>
</tbody>
</table>

Triethanolamine
Triethanolamine was found soluble in methanol. Specific gravity, melting point and viscosity were found to be 1.123 g/mL, 21°C and 291 cps respectively.
Table 3.6: Monographic Evaluation of Triethanolamine

<table>
<thead>
<tr>
<th>Tests</th>
<th>Specifications</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Clear, colorless to pale yellow-colored viscous liquid having a slight ammoniacal odor</td>
<td>Clear, colorless to pale yellow-colored viscous liquid having a slight ammoniacal odor</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in methanol, acetone, ethyl ether, Carbon tetrachloride, benzene and water</td>
<td>Soluble in methanol, acetone, ethyl ether, Carbon tetrachloride, benzene and water</td>
</tr>
<tr>
<td>Specific gravity (gm/mL)</td>
<td>1.120–1.128</td>
<td>1.11</td>
</tr>
<tr>
<td>Melting range</td>
<td>20–21°C</td>
<td>20 °C</td>
</tr>
<tr>
<td>Viscosity</td>
<td>290 cps</td>
<td>290 cps</td>
</tr>
</tbody>
</table>

All the excipients were found to comply with the standard specifications. The drug and excipients were standardized and found to comply with pharmacoepoeial specifications.

Hence they were used for further development of formulations.
3.1.3 Analytical method development and validation

Figure 3.3: Overlaid spectra of Triamcinolone Acetonide and Nystatin

Table 3.7: Simultaneous Eq. data of Nystatin and Triamcinolone Acetonide

<table>
<thead>
<tr>
<th>Nystatin</th>
<th>Triamcinolone Acetonide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr.no.</td>
<td>Conc. (µg/ml)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Conc. (µg/ml)</th>
<th>Absorb.</th>
<th>304nm</th>
<th>Absorb.</th>
<th>240nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.00</td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.04</td>
<td>0.001</td>
<td>0.32</td>
<td>0.179</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.043</td>
<td></td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.051</td>
<td>0.001</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.059</td>
<td></td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>0.068</td>
<td></td>
<td>0.032</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.4: Regressed standard curve of Nystatin at $\lambda_{\text{max}}$ 304 nm

Figure 3.5: Regressed standard curve of TA at $\lambda_{\text{max}}$ 240nm

Table 3.8: Validation of the proposed method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Nystatin</th>
<th>Triamcinolone Acetonide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity Range (µg/ml)</td>
<td>2 – 10 µg/ml</td>
<td>5 – 25 µg/ml</td>
</tr>
<tr>
<td>Accuracy (% Recovery)</td>
<td>98.99</td>
<td>97.21</td>
</tr>
<tr>
<td>Precision (% Obtained ±S.D.)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>99.08± 0.389</td>
<td>99.08 ± 0.438</td>
</tr>
<tr>
<td>Intra-day</td>
<td>98.91± 0.529</td>
<td>99.97± 0.699</td>
</tr>
</tbody>
</table>
* Indicates the ±Standard deviation value of the % estimation at different Conditions where n=3

Validation of developed analytical method was carried out using International conference on harmonization (ICH) guideline. Validation parameters like precision intra as well as interday, accuracy were determined. Developed and validated method was used for quantitation of Nystatin and Triamcinolone Acetonide in Formulation.

### 3.2 Niosome dispersion

#### 3.2.1 Blank niosome dispersion

**Table 3.9: Observation of blank niosomal dispersion**

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Span 60</th>
<th>Cholesterol</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1</td>
<td>10</td>
<td>10</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-2</td>
<td>20</td>
<td>10</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-3</td>
<td>30</td>
<td>10</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-4</td>
<td>40</td>
<td>10</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-5</td>
<td>50</td>
<td>10</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-6</td>
<td>60</td>
<td>20</td>
<td>Milky white dispersion was obtained</td>
</tr>
<tr>
<td>B-7</td>
<td>70</td>
<td>20</td>
<td>Separation was obtained</td>
</tr>
<tr>
<td>B-8</td>
<td>80</td>
<td>20</td>
<td>Separation was obtained</td>
</tr>
<tr>
<td>B-9</td>
<td>90</td>
<td>20</td>
<td>Separation was obtained</td>
</tr>
<tr>
<td>B-10</td>
<td>100</td>
<td>20</td>
<td>Separation was obtained</td>
</tr>
<tr>
<td>B-11</td>
<td>10</td>
<td>20</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-12</td>
<td>20</td>
<td>20</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-13</td>
<td>30</td>
<td>20</td>
<td>Dispersion was not obtained</td>
</tr>
<tr>
<td>B-14</td>
<td>40</td>
<td>20</td>
<td>Milky white dispersion was obtained</td>
</tr>
</tbody>
</table>
(B1-B29 blank batches contain 0.05% w/v Stearic acid)

Non-ionic surfactant concentration from 0.1-0.3% w/v did not give proper dispersion this may be due to insufficient concentration of non-ionic surfactant to form uniform spherical vesicle. Whereas surfactant concentration more than 0.6% %w/v showed cracking this may be due to precipitation of surfactant.

So Batch no. B6, B14-B16, B24-B26 gave milky white dispersion in which the concentration of surfactant was in the range of 0.4-0.6% w/v and this was taken for the further optimization.
Therefore by carried out blank noisome batches we have optimized Chemical parameters and processing parameters. Chemical parameters i.e. concentration of span 60 & cholesterol and processing parameters include Rate of addition and time of stirring.

### 3.2.2 Drug loaded noisome dispersion

In order to predict the optimum performance of prepared niosomes we have adopted a $3^2$ factorial design approach. Amount of span-60 and cholesterol were selected as two independent variables and different performance indicators were studied to determine the effect of concentration of lipid phase on niosome performance.

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Colour</th>
<th>pH</th>
<th>Odour</th>
<th>Changes after 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-6</td>
<td>Milky white</td>
<td>4.5-5.6</td>
<td>Odourless</td>
<td>No changes</td>
</tr>
<tr>
<td>B14-B16</td>
<td>Milky white</td>
<td>4.5-5.6</td>
<td>Odourless</td>
<td>No changes</td>
</tr>
<tr>
<td>B24-B26</td>
<td>Milky white</td>
<td>4.5-5.6</td>
<td>Odourless</td>
<td>No changes</td>
</tr>
</tbody>
</table>

**Table 3.10: Physical properties of blank noisome**

**Table 3.11: Effect of processing parameter on particle size distribution**

<table>
<thead>
<tr>
<th>Rate of addition</th>
<th>Uniform size distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid addition</td>
<td>Non uniform size distribution</td>
</tr>
<tr>
<td>Drop wise addition during 15 minutes</td>
<td>Uniform size distribution</td>
</tr>
</tbody>
</table>

**Time of stirring**

<table>
<thead>
<tr>
<th>Time of stirring</th>
<th>Uniform size distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 minutes</td>
<td>Non uniform size distribution</td>
</tr>
<tr>
<td>10 minutes</td>
<td>Non uniform size distribution</td>
</tr>
<tr>
<td>15 minutes</td>
<td>Uniform size distribution</td>
</tr>
</tbody>
</table>

Therefore by carried out blank noisome batches we have optimized Chemical parameters and processing parameters. Chemical parameters i.e. concentration of span 60 & cholesterol and processing parameters include Rate of addition and time of stirring.
3.2.2.1 Triamcinolone Acetonide loaded niosome dispersion

Organoleptic properties

The Triamcinolone Acetonide niosomal dispersion was off-white in color, odourless, and fluid in nature. It was stable and did not show sedimentation. pH was found to be in the range of 4.7-5.2. Summarize data of all the nine batches of factorial design is shown in table 3.12.

Table 3.12: Evaluation of TA Niosomal batches

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Appearance</th>
<th>pH</th>
<th>Odour</th>
<th>Drug content (% ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Milky white</td>
<td>4.7</td>
<td>Odourless</td>
<td>99.23±1.75</td>
</tr>
<tr>
<td>T2</td>
<td>Milky white</td>
<td>4.6</td>
<td>Odourless</td>
<td>89.20±0.61</td>
</tr>
<tr>
<td>T3</td>
<td>Milky white</td>
<td>5.1</td>
<td>Odourless</td>
<td>89.13±0.79</td>
</tr>
<tr>
<td>T4</td>
<td>Milky white</td>
<td>4.9</td>
<td>Odourless</td>
<td>101.41±0.90</td>
</tr>
<tr>
<td>T5</td>
<td>Milky white</td>
<td>4.7</td>
<td>Odourless</td>
<td>98.76±1.50</td>
</tr>
<tr>
<td>T6</td>
<td>Milky white</td>
<td>5.2</td>
<td>Odourless</td>
<td>99.52±0.97</td>
</tr>
<tr>
<td>T7</td>
<td>Milky white</td>
<td>5.4</td>
<td>Odourless</td>
<td>97.29±0.59</td>
</tr>
<tr>
<td>T8</td>
<td>Milky white</td>
<td>5.1</td>
<td>Odourless</td>
<td>97.93±1.25</td>
</tr>
<tr>
<td>T9</td>
<td>Milky white</td>
<td>4.8</td>
<td>Odourless</td>
<td>99.45±1.19</td>
</tr>
</tbody>
</table>

Vesicle size

The mean vesicle size of drug loaded niosomes of the different batches according to the factorial design ranged between 2.52 - 3.42 µm. The polydispersvity index (PdI) was in the range of 0.370 – 0.420 for drug loaded niosomes which indicates a narrow vesicle size distribution. The mean vesicle size, PdI & zeta potential of all the nine batches of factorial design are shown in table 3.13.
Table 3.13: Evaluation of TA Niosomal batches

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Polydispersibility index</th>
<th>Particle size (µm)±S.D.</th>
<th>Entrapment efficiency (%)± S.D.</th>
<th>Zeta potential (Mv)± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.411</td>
<td>2.76±0.84</td>
<td>75.52±1.13</td>
<td>-27.77±1.55</td>
</tr>
<tr>
<td>T2</td>
<td>0.389</td>
<td>2.99±0.97</td>
<td>73.60±1.39</td>
<td>-24.84±0.79</td>
</tr>
<tr>
<td>T3</td>
<td>0.420</td>
<td>3.24±0.86</td>
<td>69.05±1.14</td>
<td>-20.29±1.03</td>
</tr>
<tr>
<td>T4</td>
<td>0.385</td>
<td>3.08±0.55</td>
<td>79.60±2.26</td>
<td>-25.44±0.92</td>
</tr>
<tr>
<td>T5</td>
<td>0.325</td>
<td>3.20±0.90</td>
<td>78.09±1.94</td>
<td>-21.07±1.75</td>
</tr>
<tr>
<td>T6</td>
<td>0.370</td>
<td>3.42±0.77</td>
<td>74.84±1.60</td>
<td>-24.57±0.16</td>
</tr>
<tr>
<td>T7</td>
<td>0.387</td>
<td>2.52±1.50</td>
<td>88.24±1.50</td>
<td>-25.69±1.87</td>
</tr>
<tr>
<td>T8</td>
<td>0.404</td>
<td>2.90±0.60</td>
<td>83.24±2.25</td>
<td>-28.27±0.28</td>
</tr>
<tr>
<td>T9</td>
<td>0.395</td>
<td>3.320.61</td>
<td>80.16±1.03</td>
<td>-30.55±0.28</td>
</tr>
</tbody>
</table>

Figure 3.6: Particle size distribution graph of T7 batch

Zeta (ζ) Potential Determination

The values of ζ potential of the drug loaded niosomal formulation were in the range of -20.29 to -30.55mV. Values of ζ potential showed that the drug loaded niosome had sufficient charge and mobility to inhibit aggregation of vesicles.
Differential scanning calorimetry (DSC)

The DSC thermal profile of Triamcinolone acetonide (TA) and TA loaded niosomes (T7) are showed in figure 3.8. DSC thermal profile of pure TA showed a sharp endothermic peak at 308 ± 1.81 °C attributed to melting of TA during DSC run. The DSC thermal profile of lyophilized drug loaded niosomes did not showed peak of pure TA at 308 °C indicating TA entrapment in niosomes which may be in molecular dispersion form or soluble form in lipid bilayer.
Figure 3.8: DCS thermal profile showing characteristic peak of pure Triamcinolone Acetonide and peaks of Niosomal formulation

X-ray diffraction analysis (XRD)
The X-ray diffractogram of pure TA, blank niosomal formulation and drug loaded niosomal formulation (T7) are shown in Figure 3.9. There is sharp diffraction peak appears in spectra of pure TA at 2θ 9.8, 14.1, 23.8, 25.2, 26.1, 26.4 etc., whereas the drug loaded niosomes did not showed peaks of the drug in X ray diffractogram which may be due to entrapment of TA inside the niosomes. It also showed peaks of blank niosomes with decreased intensity. This study reveals that the prepared niosome formulation was less crystalline than pure TA and blank niosomal formulation.

Figure 3.9: XRD spectra of Drug (a), blank Niosomes (b), TA Niosomes (c)
Drug diffusion profile
Drug diffusion from niosomal dispersion and conventional dispersion were found to be 45 % and 62 % respectively. Niosomal dispersion showed sustained released than conventional dispersion. It was confirmed by ex-vivo study by using porcine skin.

Figure 3.10: TA niosome in-vitro diffused profile

Figure 3.11: TA niosome ex-vivo diffused profile
Effect of variables on vesicle size

It was observed that the relative amount of span 60 and cholesterol was found to play an important role in the determining of vesicles size. Vesicle size of drug loaded niosomal batches were found to decrease as concentration of span increases. Hydrophobic TA insert between bilayer of lipid causes considerable cohesion between a polar fraction of the membrane leads to reduction in vesicle size.

![Graph showing the effect of variables on vesicle size of TA niosomes](image)

**Figure 3.12**: Shows the effect of variables on vesicle size of TA niosomes

Equation 2: \( y = 3.2333 + 0.2700X2 - 0.2783X1X1 \)

A good correlation was examined for both variables \( X_1 \) (Span 60) and \( X_2 \) (Cholesterol) in vesicle size of drug loaded niosomes \( (r^2 = 0.8969) \). Observed coefficient values for the drug loaded niosomes gives Eq. (2)

\[
Y = 3.2333 + 0.2700X2 - 0.2783X1X1 \quad \text{--------- Eq. (2)}
\]

The most important parameter, which needs to monitor during niosome preparation its best performance, is the vesicle size and size distribution of niosomes. From the number of reports it was observed that the size and size distribution of the niosome determines there \textit{in-vivo} or \textit{ex-vivo} performance. There are some reports, which showed the effect of niosomes size on the drug release as well as drug deposition in the skin. Thus for the effective delivery the selected method should result in optimum size range and homogeneous population.
**Percent drug entrapment**

Percent drug entrapment (PDE) was expressed as fraction of drug incorporated into niosomes relative to total amount of drug used. In the present study the observed percentage entrapment efficiency for all batches were in the range of 69-88%. Percentage entrapment efficiency it was observed that it was significantly affected by the applied processing variables such as concentration of span 60 as well as cholesterol. It was clearly indicated that with increase in concentration of span 60 percentage entrapment efficiency was increased whereas concentration of cholesterol was inversely related to the percentage entrapment efficiency.

![Figure 3.13: Shows the effect of Variables on % E. E. of TA niosome](image)

Equation 3: \( y = 78.068 + 5.625X_1 - 3.218X_2 \)

When regression was applied to Significant effect of process variables was observed in PDE. To understand the effect of Span 60 and CH concentration on PDE, the coefficient observed for the drug loaded niosomes was fitted in Eq. (1) to generate Eq. (3). A good correlation was observed for both variables \( X_1 \) (Span 60) and \( X_2 \) (CH) in % drug entrapment of niosomes \( (r^2 = 0.9762) \).

\[
Y = 78.068 + 5.625X_1 - 3.218X_2
\]

**Table 3.14: Summary of regression analysis results for measured responses of TA niosome**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
<th>( \beta_{11} )</th>
<th>( \beta_{22} )</th>
<th>( \beta_{12} )</th>
<th>( r^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>% E. E.</td>
<td>78.068</td>
<td>5.625</td>
<td>-3.218</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9762</td>
<td>0.05</td>
</tr>
<tr>
<td>Vesicle size</td>
<td>3.2333</td>
<td>-</td>
<td>0.2700</td>
<td>-2783</td>
<td>-</td>
<td>-</td>
<td>0.8969</td>
<td>0.05</td>
</tr>
</tbody>
</table>
3.2.2.2 Nystatin loaded niosome dispersion

Organoleptic properties

The Nystatin niosomal dispersion was milky-white in color, odourless, and fluid in nature. It was stable and did not show sedimentation. pH was found to be in the range of 4.6-5.4.

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Appearance</th>
<th>pH</th>
<th>Odour</th>
<th>Drug content (%± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>Milky white</td>
<td>4.7</td>
<td>Odourless</td>
<td>99.23 ±1.87</td>
</tr>
<tr>
<td>N2</td>
<td>Milky white</td>
<td>4.6</td>
<td>Odourless</td>
<td>98.62 ±1.01</td>
</tr>
<tr>
<td>N3</td>
<td>Milky white</td>
<td>5.1</td>
<td>Odourless</td>
<td>98.13 ±0.74</td>
</tr>
<tr>
<td>N4</td>
<td>Milky white</td>
<td>4.9</td>
<td>Odourless</td>
<td>101.41 ±0.80</td>
</tr>
<tr>
<td>N5</td>
<td>Milky white</td>
<td>4.7</td>
<td>Odourless</td>
<td>98.76 ±1.91</td>
</tr>
<tr>
<td>N6</td>
<td>Milky white</td>
<td>5.2</td>
<td>Odourless</td>
<td>99.52 ±0.67</td>
</tr>
<tr>
<td>N7</td>
<td>Milky white</td>
<td>5.4</td>
<td>Odourless</td>
<td>97.29 ±0.56</td>
</tr>
<tr>
<td>N8</td>
<td>Milky white</td>
<td>5.1</td>
<td>Odourless</td>
<td>97.93 ±1.19</td>
</tr>
<tr>
<td>N9</td>
<td>Milky white</td>
<td>4.8</td>
<td>Odourless</td>
<td>99.45 ±1.28</td>
</tr>
</tbody>
</table>

Vesicle size

The mean vesicle size of drug loaded niosomes of the different batches according to the factorial design ranged between 182-219 nm. The polydispersvity index (PdI) was in the range of 0.207 – 0.341 for Nystatin loaded niosomes which indicates a narrow vesicle size distribution. The mean vesicle size and PdI of all the nine batches of factorial design is shown in table 3.16. Particle size distribution graph of optimized batch i.e. N7 is shown in figure 3.14.
Table 3.16: Evaluation of Nystatin Niosomal batches

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Polydispersibility index</th>
<th>Particle size (nm)±S.D.</th>
<th>Zeta potential (Mv)±S.D.</th>
<th>Drug entrapment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.325</td>
<td>192 ± 0.84</td>
<td>-27.77±1.55</td>
<td>70.56</td>
</tr>
<tr>
<td>N2</td>
<td>0.289</td>
<td>194 ± 0.86</td>
<td>-24.84±0.79</td>
<td>66.32</td>
</tr>
<tr>
<td>N3</td>
<td>0.341</td>
<td>199 ± 0.97</td>
<td>-23.35±1.87</td>
<td>62.74</td>
</tr>
<tr>
<td>N4</td>
<td>0.285</td>
<td>211 ± 0.90</td>
<td>-25.44±1.86</td>
<td>78.54</td>
</tr>
<tr>
<td>N5</td>
<td>0.225</td>
<td>215 ± 0.77</td>
<td>-28.07±0.75</td>
<td>75.09</td>
</tr>
<tr>
<td>N6</td>
<td>0.207</td>
<td>219 ± 0.80</td>
<td>-31.57±0.16</td>
<td>74.04</td>
</tr>
<tr>
<td>N7</td>
<td>0.267</td>
<td>182 ± 0.59</td>
<td>-29.69±1.03</td>
<td>85.80</td>
</tr>
<tr>
<td>N8</td>
<td>0.304</td>
<td>186 ± 0.61</td>
<td>-28.27±0.28</td>
<td>83.10</td>
</tr>
<tr>
<td>N9</td>
<td>0.259</td>
<td>189 ± 0.55</td>
<td>-30.55±0.28</td>
<td>80.25</td>
</tr>
</tbody>
</table>

Figure 3.14: Particle size distribution graph of N7 batch

Zeta (ζ) Potential Determination

The values of ζ potential of the drug loaded niosomal formulation were in the range of -23.35 to -31.57mV. Values of ζ potential showed that the drug loaded niosome had sufficient charge and mobility to inhibit aggregation of vesicles.
Differential scanning calorimetry (DSC)
The DSC thermal profile of nystatin and nystatin loaded niosomes are showed in figure 3.16. DSC thermal profile of nystatin showed a sharp endothermic peak at 154 ± 1.29 °C attributed to melting of nystatin during DSC run. The DSC thermal profile of lyophilized drug loaded niosomes did not showed peak of pure nystatin at 154 °C indicating nystatin entrapment in niosomes which may be in molecular dispersion form or soluble form in lipid bilayer.

Figure 3.15: A typical graph for zeta potential of N7 batch

Figure 3.16: DCS thermal profile showing characteristic peak of pure Nystatin and peaks of Niosomal formulation
X-ray diffraction analysis (XRD)
The X-ray diffractogram of nystatin, blank niosomal formulation and drug loaded niosomal formulation are shown in Figure 3.17. There is sharp diffraction peak appears in spectra of pure TA at 20 6.5, 15.5, 22.7, 25.6, 26.2, 27.4, 29.7 etc., whereas the drug loaded niosomes did not showed peaks of the drug in X ray diffractogram which may be due to entrapment of nystatin inside the niosomes. It also showed peaks of blank niosomes with decreased intensity. This study reveals that the prepared noisome formulation was less crystalline than pure nystatin and blank niosomal formulation.

Figure 3.17: XRD spectra of Drug(a), blank Niosomes (b), drug Loaded Niosomes (c)
Drug diffusion profile

Drug diffusion from niosomal dispersion and conventional dispersion were found to be 42% and 56% respectively. In *Ex-vivo* study drug diffusion from niosomal dispersion and conventional dispersion were found to be 21% and 34% by using porcine skin. From this study it was confirmed that niosomal dispersion showed sustained released than conventional dispersion.

![Figure 3.18: Nystatin niosome *in-vitro* diffused profile](image)

![Figure 3.19: Nystatin niosome *ex-vivo* diffused profile](image)
Effect of variables on vesicle size

A positive correlation was observed for the variable X2 in case of Nystatin niosomes. Thus with increase in concentration of cholesterol vesicle size was found to be increased in drug loaded vesicles.

![Graph showing the effect of variables on vesicle size of Nystatin niosomes](image)

**Figure 3.20:** Shows the effect of variables on vesicle size of Nystatin niosomes

**Equation 4:** \( y = 215 - 4.6667 X_1 + 3.6667 X_2 - 24.6667 X_1 X_1 \)

Percent drug entrapment

Determination of percentage entrapment efficiency is an important parameter in case of niosomes as it may affect the drug release. Percentage entrapment efficiency is expressed as the fraction of drug incorporated into niosomes relative to total amount of drug used. Entrapment efficiency of niosome was decreased with increase in concentration of cholesterol. In the present study the observed percentage entrapment efficiency for all batches were in the range of 62% - 85%. When regression was applied to percentage entrapment efficiency it was observed that it was significantly affected by the applied processing variables such as concentration of span-60 as well as cholesterol. High percentage entrapment efficiency was observed for the high concentration of span 60 and low concentration of cholesterol.
It was clearly indicated that with increase in concentration of span 60 percentage entrapment efficiency was increased where as concentration of cholesterol was inversely related to the percentage entrapment efficiency.(figure 3.21) Thus to better understand the effect of both the variables on percentage entrapment efficiency response surface plots were generated using eq.

\[ Y = 75.16 + 8.225 X_1 - 2.978 X_2 \]

\[ r^2 = 0.9865 \text{ significant correlation} \]

From above equation it was clearly understood that the variables \( X_1 \) is directly related to the percentage entrapment efficiency and \( X_2 \) is inversely related to percentage entrapment efficiency.

![Figure 3.21: Shows the effect of Variables on % E. E. of Nystatin niosome](image)

**Equation 5:** \( y = 75.16 + 8.225 X_1 - 2.978 X_2 \)

**Table 3.17: Summary of regression analysis results for measured responses of Nystatin niosome**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( \beta_0 )</th>
<th>( \beta_1 )</th>
<th>( \beta_2 )</th>
<th>( \beta_{11} )</th>
<th>( \beta_{22} )</th>
<th>( \beta_{12} )</th>
<th>( r^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entrapment Efficiency (%)</td>
<td>75.16</td>
<td>8.225</td>
<td>-2.978</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9865</td>
<td>0.05</td>
</tr>
<tr>
<td>vesicle size</td>
<td>215</td>
<td>-4.667</td>
<td>3.667</td>
<td>-24.6667</td>
<td>-</td>
<td>-</td>
<td>0.9986</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Thus stable niosomal dispersion of Triamcinolone Acetonide and Nystatin were developed and optimized.
3.3 Niosomal Gel

Rheological characterization

The rheological studies of gels help us to understand the microstructure of the gel, which in turn governs the viscoelastic properties of the gel. The viscoelastic properties of gel play an important role during storage and transportation. Rheological characterizations of gels were carried out using different proportion of carbopol viz. 1%, 1.5% and 2% w/w.

Oscillation stress sweep

The elastic moduli (G’) represent the total energy stored in the elastic bonds of system while viscous moduli (G’”) present total energy expelled from system. The system which showed higher value for LVR and G’ are considered as more elastic. 2 %w/w drug loaded niosomal carbopol gel had shown maximum G’ and LVR as compared to 1% w/w, 1.5 % w/w as shown in figure 3.22 and it was comparatively more stable over applied stress range.

![Stress Vs G']](image)

Figure 3.22: Linear viscoelastic region of 1% gel, 1.5% gel and 2% gel
Figure 3.23 represent the behavior of $G'$ and $G''$ over applied stress for 2% drug loaded carbopol gel. The values for $G'$ was more as compared to $G''$ indicating that the gel has predominant elastic character over applied stress.

![Stress vs G'G''](image)

**Figure 3.23: Behavior of G’ and G” for 2% gel over applied stress**

**Oscillation frequency sweep**

Oscillation frequency sweep measurements give good information on microstructures of the gel system. In this the degree of dependency of $G'$ and $G''$ was examined over the applied frequency range. The ratio of $(G''/G')$ determines the elastic behavior known as loss tangent ($\tan \delta$). A value of $\tan \delta < 1$ indicates the formed structure has good elastic behavior. The prepared gels when analyzed by oscillation frequency sweep as shown in figure 3.24, in that 2% gel showed $\tan \delta$ value less as compared to other gels. This showed that the 2% gel was elastic in nature. Value of phase degree for 2% carbopol gel was found to be 33.50 which indicate that gel was viscous in nature therefore 2% carbopol gel was showed good viscoelastic behavior.
Figure 3.24: Frequency sweep for \( \tan \delta \)

Figure 3.25 indicate that the behavior of \( G' \) over applied frequency range. It was found that System having 2 %w/w carbopol showed independent behavior of \( G' \) over applied frequency. On Other hand system with 1.5% w/w carbopol and 1% w/w carbopol concentration showed a monotonous increase in solid component indicating the partial breakdown of the interconnected network. The initial plateau region was observed for both the gels indicts cross-linked polymer gel linking at lower frequencies.

Figure 3.25: Frequency sweep for \( G' \)

Creep Recovery
The percent creep recovery was higher for the system having 2 %w/w carbopol concentration in comparison with 1.5 % w/w and 1 % w/w system as shown in Figure 3.26. The percent creep recovery for 2%, 1.5% and 1% carbopol gel was found 64.5%, 40.3%, and 19.7% respectively. It clearly indicates that the ability of 2% carbopol gel to recover its original structure after application of stress i.e. 2% carbopol gel was highly stable. Rheological signature of 2% carbopol gel was better when compared to other gel (1%, 1.5% w/w) and hence, was considered for further studies.

![Creep recovery](image)

**Figure 3.26: Creep recovery for 1%, 1.5% and 2% drug loaded gel**

**Table 3.18: Evaluation of conventional gel and niogel**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conventional gel</th>
<th>Niogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apperance</td>
<td>Transparent homogeneous gel</td>
<td>Opaque, homogeneous gel</td>
</tr>
<tr>
<td>Colour</td>
<td>Colourless</td>
<td>Off white</td>
</tr>
<tr>
<td>pH</td>
<td>6.9-7.1</td>
<td>6.4-6.7</td>
</tr>
<tr>
<td>Viscosity</td>
<td>25000 cps</td>
<td>11550 cps</td>
</tr>
<tr>
<td>Spredability</td>
<td>21 gm cm/sec</td>
<td>22.72 gm cm/sec</td>
</tr>
<tr>
<td>Microscopic</td>
<td>No aggregation or lump were</td>
<td>No aggregation or lump</td>
</tr>
</tbody>
</table>
Content Uniformity

Content uniformity of gel from 500 mg of gel across five different locations was found to be 97.21± 1.92% & 98.73 ± 0.96 % of Triamcinolone acetonide and Nystatin respectively. Thus drugs were uniformly distributed throughout gel.

![Content drug uniformity](image)

**Figure 3.27: Content drug uniformity**

*In-vitro* drug diffusion study
**In-vitro** drug release from membrane showed the niosomal gel showed 50.11% & 47.81% nystatin & TA release while conventional gel showed 69.05% & 64.21% nystatin & TA release respectively in 48 hrs.

![Graph showing drug diffusion profile](image)

**Figure 3.28: Drug diffusion profile of Conventional gel and Niosomal gel**

**Ex-vivo** drug diffusion study

Ex-vivo drug release from porcine skin membrane showed the niosomal gel showed 16.41% & 14.95% nystatin & TA release while conventional gel showed 25.39% & 20.41% nystatin & TA release respectively in 48 hrs.

![Graph showing ex-vivo drug diffusion study](image)

**Figure 3.29: Ex-vivo drug diffusion study**
Release kinetic data

The \textit{Ex-vivo} drug diffusion study of developed Niogel through porcine skin followed Higuchi matrix-diffusion mechanism (figure 6.31)

![Zero order](image1.png) ![First order](image2.png)

![Higuchi order](image3.png)

Figure 3.30: Zero order

Figure 3.31: First order

Figure 3.32: Higuchi order

Table 3.19: Permeation data of niogel through Porcine skin Mean ± S.D. (n=3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Niogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flux (J, µg cm⁻²h⁻¹)</td>
<td>0.101±1.23</td>
</tr>
<tr>
<td>Permeability coefficient</td>
<td>1.01 ± 1.03</td>
</tr>
<tr>
<td>P (cm/hr)X 10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>Zero order (R²)</td>
<td>0.958</td>
</tr>
<tr>
<td>First order (R²)</td>
<td>0.965</td>
</tr>
</tbody>
</table>
Drug deposition study

Skin deposition study showed that niosome gave almost two fold increases in drug deposition as compared to conventional preparation.

![Drug deposition in porcine skin](image)

**Figure 3.33: Drug deposition in porcine skin**

3.4 Skin irritation study

The positive control (Formalin) showed erythema or edema. The negative control (placebo gel) did not show any erythema or edema in all the six rabbits on intact and abraded skin at the end of 24 hours and 72 hours. The Primary Irritation Index (P.I.I.) was found to be 0. Therefore; the developed formulations were classified as non-irritant and safe for topical use.
Day 1

Fig 3.34: Photograph of intact & abraded rabbit skin treated with positive control after 24 hours

Fig 3.35: Photograph of intact & abraded rabbit skin treated with negative control after 24 hours

Fig 3.36: Photograph of intact and abraded rabbit skin treated with optimized gel after 24 hours
Day 3

Fig 3.37: Photograph of intact & abraded rabbit skin treated with positive control after 72 hours

Fig 3.38: Photograph of intact & abraded rabbit skin treated with negative control after 72 hours
Fig 3.39: Photograph of intact & abraded rabbit skin treated with optimized gel after 72 hours

Table 3.20: Dermal observations for Niogel formulations after Draize patch test

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Reaction</th>
<th>24 hours</th>
<th></th>
<th>72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
<td>Abraded</td>
<td>Intact</td>
</tr>
<tr>
<td>1.</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6.</td>
<td>Erythema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Edema</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Primary irritation index : 0/24=0.00
3.5 Antifungal activity

Table 3.21: Zone of inhibition (*Candida albicans*)

<table>
<thead>
<tr>
<th>Formulation type</th>
<th>Concentration of drug applied (µg)</th>
<th>Area of Growth inhibited (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niosomal dispersion (ND)</td>
<td>100</td>
<td>2.11</td>
</tr>
<tr>
<td>Niosomal gel (NG)</td>
<td>100</td>
<td>1.19</td>
</tr>
<tr>
<td>Conventional dispersion (CD)</td>
<td>100</td>
<td>1.99</td>
</tr>
<tr>
<td>Conventional gel (CG)</td>
<td>100</td>
<td>1.15</td>
</tr>
<tr>
<td>Without drug (Blank)</td>
<td>-</td>
<td>No inhibition</td>
</tr>
</tbody>
</table>

Figure 3.40: Zone of inhibition of dispersion and gel resp.
Nystatin is broad spectrum antifungal agent has fungicidal activity to \textit{C.albicans}.

Antifungal activity of selected nystatin niosomal dispersion and respective gel were studied against \textit{Candida albicans} 36082. The conventional dispersion and gel were also evaluated for antifungal activity. The zones of inhibition of different formulations are given in the table 3.21

From table as well as figure 3.40 it was clearly indicated that the zone of inhibition obtained by niosomal dispersion was higher as compared to niogel. From these results, it can be interpreted that the drug release was retarded in the gel matrix of niogel. The results obtained using conventional formulations were same as that of niosomal formulation. The blank formulation was not showing any zone of inhibition as shown in figure 3.41. Thus indicating that the solvent used in sample preparation did not interfere in antifungal activity.

Thus, developed formulation showed good antifungal activity against \textit{Candida albicans} 36082.

### 3.6 Stability study

#### 3.6.1 Stability testing of niosomal dispersion

Stability testing was carried out on optimized niosomal dispersion as per ICH guideline

<table>
<thead>
<tr>
<th>Table 3.22: Stability study data at 4-8°C and room temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nystatin Niosomal dispersion</td>
</tr>
<tr>
<td>% Entrapment efficiency</td>
</tr>
<tr>
<td>Time in Days</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>15</td>
</tr>
</tbody>
</table>

---

\textit{JJT, University}
<table>
<thead>
<tr>
<th>Storage Time (min)</th>
<th>% E.E.</th>
<th>Particle Size (nm)</th>
<th>Temp.</th>
<th>% E.E.</th>
<th>Particle Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>80.53</td>
<td>77.28</td>
<td></td>
<td>82.72</td>
<td>80.78</td>
</tr>
<tr>
<td>45</td>
<td>78.88</td>
<td>73.98</td>
<td></td>
<td>80.53</td>
<td>77.28</td>
</tr>
<tr>
<td>60</td>
<td>75.43</td>
<td>70.67</td>
<td></td>
<td>78.88</td>
<td>73.98</td>
</tr>
<tr>
<td>75</td>
<td>73.29</td>
<td>69.45</td>
<td></td>
<td>75.43</td>
<td>70.67</td>
</tr>
<tr>
<td>90</td>
<td>70.92</td>
<td>65.75</td>
<td></td>
<td>70.92</td>
<td>69.45</td>
</tr>
</tbody>
</table>

Figure 3.42: Effect of storage time on % E.E. Nystatin Niosome

Figure 3.43: Effect of storage time on Particle size Nystatin Niosome
3.6.2 Stability testing of niogel

3.6.2.1 Physical evaluation

Table 3.23: Physical specification of Niogel

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification of niogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apperance</td>
<td>Opaque, homogeneous gel</td>
</tr>
<tr>
<td>pH</td>
<td>6.4-6.7</td>
</tr>
<tr>
<td>Colour</td>
<td>Off-white</td>
</tr>
<tr>
<td>Consistency</td>
<td>Viscous-semisolid</td>
</tr>
<tr>
<td>Spredability</td>
<td>27.65 g cm/sec</td>
</tr>
</tbody>
</table>
Table 3.24: Evaluation of Physical parameters

<table>
<thead>
<tr>
<th>Interval</th>
<th>Parameters</th>
<th>0 days</th>
<th>15 days</th>
<th>30 days</th>
<th>60 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Appearance</td>
<td>4-8 °C</td>
<td>Room temp.</td>
<td>4-8 °C</td>
<td>Room temp.</td>
<td>4-8 °C</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Colour</td>
<td>6.5</td>
<td>6.6</td>
<td>6.5</td>
<td>6.6</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Consistency</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td></td>
<td>Spredability</td>
<td>27.6</td>
<td>27.97</td>
<td>27.82</td>
<td>28</td>
<td>27.11</td>
</tr>
<tr>
<td></td>
<td>Homogeneity</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

(NC= No change)

3.6.2.2 Chemical evaluation

Content drug uniformity

Table 3.25: Content drug uniformity (Nystatin)

<table>
<thead>
<tr>
<th>No. of Sample Points</th>
<th>% of Nystatin at 4-8°C</th>
<th>% of Nystatin at room temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>No. of days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99.23</td>
<td>98.76</td>
</tr>
<tr>
<td>30</td>
<td>98.62</td>
<td>98.11</td>
</tr>
<tr>
<td>60</td>
<td>98.13</td>
<td>97.79</td>
</tr>
<tr>
<td>90</td>
<td>98.41</td>
<td>97.93</td>
</tr>
</tbody>
</table>
Figure 3.46: Content drug uniformity at 4-8°C of Nystatin

Fig 3.47: Content drug uniformity at room temp. of Nystatin

Table 3.26: Content drug uniformity (Triamcinolone acetonide)

<table>
<thead>
<tr>
<th>No. of Sample Points</th>
<th>% of TA at 4-8°C</th>
<th>% of TA at room Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of days</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>97.23</td>
<td>97.76</td>
</tr>
<tr>
<td>30</td>
<td>96.62</td>
<td>96.52</td>
</tr>
<tr>
<td>60</td>
<td>96.13</td>
<td>96.29</td>
</tr>
<tr>
<td>90</td>
<td>97.92</td>
<td>97.93</td>
</tr>
</tbody>
</table>
There was no change found in the Appearance and pH of niosomal dispersions kept at, 4-8°C & 25°C ± 2°C60% RH ± 5%). Only slight increase in the vesicle size was observed during the storage conditions. The slight increase in the size may be due to slight fusion of vesicles during stability period.

The gel was found to be off white, translucent, homogeneous and there was no change in the Appearance, pH and Spredability throughout the three months of stability studies at 4-8°C and room temperature. Content drug uniformity showed good uniformity at 4-8 °C compared to room temp. At low temperature the niosomal gel was more stable than the room temperature.

Therefore, it was observed that suitable storage condition for niosomal storage was 4°C.
Dermatophyte is a type of fungus which infect top layer of the skin, nails and hairs. The mainstay of management of fungal infectivity and dermatophytes associated with skin and nail injuries has been oral and topical antifungal drug delivery systems.

Polyene antifungal like Nystatin was found to be effective against many funagal infection as well as molds infection. Furthermore fungal infection is associated with inflammation hence combined therapy of antifungal and anti-inflammatory such as Triamcinolone acetonide is prescribed for the effective treatment. As per BCS classification it is Class-IV drug which has low permeability and low solubility. Due to its toxicity profile it cannot be formulated into injectable formulations.

Niosome carrier released the active pharmaceutical ingredient in sustainable manner and develops niosomal delivery system for fungal infection. Afterwards niogel was developed for improvement of topical applicability of niosomal dispersion to localize API at target site of action.

Preformulation study gives profiles of drugs and other ingredients added in the formulation development. The standardization of drugs and excipients is an integral part of research work. The drug and excipients were standardized and found to comply with pharmacoepeeial specifications. Hence they were used for further development of formulations.

In formulation development of niosomal dispersion, initially blank niosomal formulation was prepared by using various concentrations of Span 60 & cholesterol. The batches were optimized for various processing and formulation parameters.

Optimization of niosomal dispersion is one of the critical process that need consideration of number of factors and ingredients interaction in formulation development. We have adopted a $3^2$ factorial design approach. Quantity of span-60 and cholesterol were preferred as two independent variables and different performance indicators were studied to establish the outcome of concentration of lipid phase on niosome performance. The different parameters studied were vesicle size, size distribution, zeta potential, encapsulation efficiency and \textit{in-vitro} drug release from the obtained data. Niosomal dispersion showed sustained released than
conventional dispersion. It was confirmed by *ex-vivo* study by using porcine skin. Thus stable niosomal dispersion of nystatin and triamcinolone acetonide was developed.

In formulation development and evaluation of niogel initially gels were subjected to Rheological study. Rheological mark of 2% carbopol gel was better when compared to other gel (1%, 1.5% w/w) and hence, was considered for further studies. It was concludes that niosomal gel showed slow release of drug than Conventional Gel. *In-vitro drug* deposition study on excised porcine skin for 48 hrs showed that the Niosomal gel shows more drug deposition than Conventional gel.

Skin irritation study reveals that developed niosomal gel having primary irritation index is zero. Threfore, formulation was found to be safe and non irritant to the skin.

From Antifungal activity study it can be concluded that, zone of inhibition obtained by niosomal dispersion was higher as compared to Niogel. From these results, it can be interpreted that the drug release was retarded in the gel matrix of niogel. The results obtained using conventional formulations were same as that of niosomal formulation. The blank formulation was not showing any zone of inhibition indicating that the solvent used in sample preparation did not interfere in antifungal activity. Thus, developed formulation showed good antifungal activity against *Candida albicans* 36082.

Stability study summarize that Niosomal dispersion and Niogel were more stable at 4-8°C ± 2°C than 25°C ± 2°C, 60% RH ± 5%.

So, it can be concluded that safe, efficacious, non irritant surfactant based topical formulation was successfully developed for treatment of fungal disease.