Formulation and evaluation of nanoemulsion of amphotericin B

Harika K*, Subhashis Debnath, M Niranjan Babu

Department of Pharmaceutics, Seven Hills College of Pharmacy, Venkatramapuram, Tirupathi- 517561, Andhra Pradesh, India.

Abstract

Amphotericin B is a synthetic antifungal drug. It is insoluble in water and having high permeability through stomach. This results in poor bioavailability after oral administration. Therefore nanoemulsion containing Amphotericin B was formulated to increase its solubility and bioavailability. Aqueous titration method was used to formulate nanoemulsions and pseudo-ternary phase diagrams were developed to know the nanoemulsion region. Tween 20 was selected as the surfactant and ethanol was selected as cosurfactant for the formulation and mixed in different volume ratios like 1:0 to 1:4 and 2:1 to 4:1. Based on the solubility study sesame oil was optimized as an oil phase. To construct the phase diagram, oil (sesame oil) and smix were mixed thoroughly with the help of magnetic stirrer in different volume ratios from 1:7 to 7:1. Formulated nanoemulsions were then evaluated for drug release, dispersibility, viscosity, surfactant concentration, electroconductivity and TEM analysis. The in vitro studies revealed that nanoemulsion formulation that is NE2 shows better drug release profile (99.58%) when compared with suspension of Amphotericin B (45.56%).

Keywords: Amphotericin B, Nanoemulsion, Surfactant, Oil, Co-surfactant.

INTRODUCTION

Since from the past years, the oral drug delivery system has been taken to a new extent with the increasing application of lipid as a carrier for the delivery of poorly water soluble lipophilic drugs. In the discovery, about 40% of exciting new molecular entities (NMEs) exhibit low solubility in water leading to poor bioavailability, high intersubject/intrasubject variability and deficient in dose proportionality. Furthermore, oral delivery of numerous drugs is hindered owing to their high hydrophobicity. Therefore, producing suitable formulations is very important to improve the solubility and bioavailability of such drugs. Formulation and development of poorly water soluble drugs candidate continue to be a challenge to formulation scientists mainly because of the emerging new drug discovery programs. The various options available to overcome the hurdle including micronisation, salt formation, use of microspheres, solid dispersions, co-grinding, complexation, lipid- surfactant based formulations and many others. The lipid based formulation approach has attracted wide attention in order to enhance drug solubilization in the gastrointestinal tract (GIT) and to improve the oral bioavailability [1-2].

Thus in the research work a novel o/w nanoemulsion formulation is tried which enhances the oral bioavailability of the poorly water soluble drug amphotericin B. The objectives of the present work include development and characterization of o/w nanoemulsion containing Amphotericin B, improve oral bioavailability by lymphatic transport and reduce hepatic first-pass metabolism and to reduce the dose required to produce some pharmacological effect where by dose related side effects can be reduced [3-4].

MATERIALS AND METHODS

Materials

Amphotericin B was gifted by SRL Pvt Ltd, Maharashtra, Sesame oil from Genius Nature Herbs Pvt Ltd, Coimbatore, Ethanol was supplied by Changshu Yangyuan Chemicals, Hyderabad, Tween-20 from SDFCL S define-chemicals Ltd, Mumbai, Methanol from MERCK Chemicals, Mumbai, Potassium dihydrogen phosphate from MERCK chemicals, Hyderabad, Sodium hydroxide was

To whom correspondence should be addressed:
Harika K
Email: harikapharma11@gmail.com
brought by Sdfine Chemicals Ltd, Hyderabad. And the solvents used are of analytical grade.

**Methods**

**Solubility Studies**

The solubility studies have to be done to detect the oil that solubilises maximum amount of drug. The solubility of the amphotericin B was determined in various oils by adding an excess amount of drug to 1 ml of selected oils (soyabean oil, sesame oil, sunflower oil) in stoppered vials. The vials were kept at 25± 0.5°C in Wrist action shaker for 72 hours to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000rpm for 15 min. The supernatant was taken and filtered through a 0.45µ membrane filter and concentration of amphotericin B was determined in the oils after dilution using UV-Visible spectrophotometer at 416nm [5-7].

**Compatibility Study**

Compatibility studies have to be done all the excipients along with the drug that is used to formulate nanoemulsion. The compatibility studies was done using Bruker FTIR spectrophotometer. Compatibility studies were used for detection of any possible chemical interaction between the drug, oil, surfactant and the cosurfactant. A physical mixture of drug, oil, surfactant and cosurfactant was prepared and mixed with suitable quantity of potassium bromide. About 100 mg of this mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure. It was scanned from 4000 to 400 cm⁻¹ in a Bruker FTIR spectrophotometer. The IR spectrum of the physical mixture was compared with those of pure drug, oil, surfactant and cosurfactant and matching was done to detect any appearance or disappearance of peaks [9].

**Pseudo-Ternary Phase Diagram Study**

Constructing pseudo-ternary phase diagrams is time consuming, particularly when the aim is to accurately delineate the phase boundary. Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous. The relationship between the phase behaviour of a mixture and its composition can be captured with the aid of a phase diagram. Sesame oil (oil), Tween 20 (surfactants), and ethanol (co-surfactant) were selected to study the phase diagrams in detail. Pseudo-ternary phase diagrams were constructed separately for each smix ratio to identify the o/w nanoemulsion regions [10]. The pseudo-ternary phase diagrams were developed using the aqueous titration method. Surfactant (Tween 20) and co-surfactant (Ethanol) were mixed (Smix) in different volume ratios (1:1,1:2,1:3,1:4,2:1,3:1,4:1). These Smix ratios were chosen to reflect the increasing concentration of the co-surfactant with respect to surfactant and increasing concentration of surfactant with respect to co-surfactant for the detailed study of the phase diagrams in the nanoemulsion formulation. Sesame oil optimized as an oil phase based on the solubility study. For each phase diagram, oil (Sesame oil) and specific smix ratios were mixed thoroughly in different volume ratios from 1:7 to 7:1. 13 different combinations of oil and smix (1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1) were made for the study to delineate the boundaries of the phases precisely formed in the phase diagrams. Aqueous phase was slowly titrated for each combination of oil and smix separately. 5ml of aqueous phase was added at each interval upto 50ml under magnetic stirring and visually observed for phase clarity and flowability. Calculations for the ratios of oil and smix were also done. The physical state was plotted on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second representing the oil phase, and third representing a mixture of surfactant and cosurfactant (SMIX) at a fixed volume ratio [9-10].

**Selection of formulations**

Based on the NE region of each phase diagram of different formulations are selected and incorporated into the oil phase on the following basis.

1. 10 mg of amphotericin B was selected as the dose for incorporation into the oil phase.
2. For convenience 2 mL was selected as the NE formulation.
3. The oil conc. should be such that it solubilizes the drug (single dose) completely. 10 mg of amphotericin B will dissolve easily in 0.2 mL of oil (10% of 2 mL).
4. From each phase diagram, different concentration of oils were selected at a difference of 5% (10%,15%,20%,25%,etc) from the NE region.
5. For each 5% of oil selected, the formula that used the minimum concentration of smix for its NE formulation was selected from the phase diagram.

EVALUATION OF NANOEMULSION

Thermodynamic stability tests
Selected formulations were subjected to different thermodynamic stability tests.

Heating cooling cycle
Between refrigerator temperature 4°C and 45°C of six cycles with storage at each temperature of not less than 48 h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation.

Centrifugation
Those formulations that passed were centrifuged at 3500 rpm for 30 min by using centrifuge. The formulations that did not show any phase separated were taken to further tests.

Freeze thaw cycle
Between –21°C and +25°C three freeze thaw cycles with storage at each temperature for not less than 48 h was done for the formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility tests.

Dispersibility tests
Dispersibility tests were done using a dissolution apparatus 2. 1mL of each formulation was added to 500 mL of water at 37±0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. In vitro performance of the formulation was visually assessed using the following grading system:

Grade A: Clear or bluish appearance rapidly forms within 1 min.
Grade B: Slightly less clear emulsion having a bluish white forms rapidly.
Grade C: Fine milky emulsion that formed within 2 min.
Grade D: Dull, greyish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min).
Grade E: Poor or minimal emulsification with large oil globules present on the surface.

The formulations that passed the thermodynamic stability and also dispersibility tests in Grade A and B were selected for further studies. The selected formulations were prepared by dissolving 10 mg (single dose) of amphotericin B in oil (10%, 15%, 20%, 25% etc.). Respective smix ratio was added to the oil, mixed using magnetic stirrer and aqueous phase was added. The resulting mixture gave nanoemulsion.

Viscosity determination
The viscosity of the formulations (0.5 g) was determined as such without dilution using Brookfield DV-II ultra+ viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using spindle # CPE 40 at 25±0.5°C. The software used for the calculations was Rheocalc V2.6.

Electroconductivity study
For the conductivity measurements, the tested nanoemulsions were prepared with a 0.01 N aqueous solution of NaCl instead of distilled water. The test was measured by an electroconductometer (Conductivity meter 305, Systronic).

Refractive index and percent transmittance
The refractive index of the system was measured by an Abbe refractometer (Bausch and Lomb optical company, NY) by placing 1 drop of nanoemulsion on the slide. The percent transmittance of the system was measured at 650 nm using a UV spectrophotometer (Shimadzu, Japan) keeping distilled water as blank [11-12].

Drug content
The drug content was calculated by UV visible spectrophotometer. The formulation was diluted to required concentration using methanol as solvent and the absorbance was measured at 416 nm against a solvent blank. The drug content was calculated as:

Drug content = \( \frac{\text{Analyzed content}}{\text{Theoretical content}} \times 100 \)

Transmission electron microscopy (TEM)
TEM analysis should be done for the formulation nanoemulsion to determine the globule size of oil present in the nanoemulsion. This can be done by TOPCON 002B operating at 200 kV capable of point to point resolution. To perform the TEM observations, the nanoemulsion formulation was diluted with water (1/100). A drop of the diluted nanoemulsion was then directly deposited on the holey film grid and observed after drying.
In vitro drug release

In vitro drug release for the nanoemulsion formulation should be done in order to measure and detect the formulation that release the maximum amount drug (amphotericin B) release from the nanoemulsion formulation. This test was performed in 500 mL of Phosphate buffer pH 7.4 using USP Dissolution apparatus Type II at 75 rpm and 37±0.5°C. 2 mL of nanoemulsion formulation containing single dose 10mg of amphotericin B was placed in a dialysis bag (Himedia dialysis membrane 150). Samples (5mL) were withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8 h) and an aliquot amount of phosphate buffer was replaced. The release of drug from nanoemulsion formulation was compared with the conventional tablet formulation (ZOCOR™) and the suspension of pure drug. The samples were analyzed for the drug content using UV-Visible spectrophotometer at 416nm [14].

RESULT AND DISCUSSION

Solubility studies

The solubility of Amphotericin B was found to be highest in Sesame oil (40.83±0.3µg/ml) as compared to other oils (Table 1). This may be attributed to the polarity of the hydrophobic drug that favour their solubilization in the oil. Thus, Sesame oil was selected as the oil phase for the development of the formulation.

Compatibility studies

The spectra obtained from IR studies at wavelength from 4000 cm⁻¹ to 400 cm⁻¹. After interpretation of the above spectra it was confirmed that there was no major shifting, loss or appearance of functional peaks between the spectra of drug, oil, physical mixture of drug and oil and surfactants, cosurfactants. From the spectra it was concluded that the drug was encapsulated into the oil without any chemical interaction.

Pseudo-Ternary Phase diagram study

When cosurfactant was added with surfactant in equal amount When cosurfactant was added with surfactant in equal amount (Smix ratio 1:1, Fig. 1(A)), the nanoemulsion region in the phase diagrams increased and maximum oil that could be solubilized was 10% w/w using Smix concentration of 13% w/w. This may be attributed to the fact that the addition of co-surfactant may lead to greater penetration of the oil phase in the hydrophobic region of the surfactant monomer thereby further decreasing the interfacial tension, which will lead to increase in the fluidity of the interface thus increasing the entropy of the system. With further increase in cosurfactant i.e. smix ratio 1:2, 1:3 and 1:4 [Fig. 1(B),(C),(D)], it was observed that nanoemulsion area was found to increase and percentage of oil solubilized was 15%,30% and 35% w/w respectively.

When surfactant concentration was increased with respect to co-surfactant [Smix ratio 2:1, Fig. 1(E)] it was seen that nanoemulsion area was increased compared to 1:1 and nearly 15% w/w/ oil could be solubilized with the smix concentration of 25% w/w. When the surfactant was further increased to 3 parts is to 1 part of cosurfactant[Fig. 1(F)], the nanoemulsion area increased and the maximum oil that could be solubilized was 20% w/w. When the smix ratio was 4:1 [Fig. 1(G)], nanoemulsion area was found to increased further with 30% w/w of oil being solubilized at smix concentration of 12% w/w.

Selection of formulations from phase diagrams

Hundreds of formulations can be prepared from the nanoemulsion region of the phase diagram. While going through pseudoternary phase diagrams, oil could be solubilized upto the extent of 40% w/w. Therefore, from each phase diagram different concentrations of oil that formed a nanoemulsion was selected at 5% intervals (10%, 15%, 20%, 25%, 30%, 35%, 40%). So that, largest number of formulations could be selected covering the nanoemulsion area of the phase diagram (Table 2). For each percentage of oil selected, only those formulations were taken from the phase diagram which used minimum concentration of smix.

Evaluation of nanoemulsion

Thermodynamic stability tests

Nanoemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates nanoemulsions from emulsions that have kinetic stability and will eventually phase separate. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests (Table 2), were taken for dispersibility test.
Dispersibility tests
From (Table 2) formulations that passed dispersibility test in Grade A and B were taken for further study, as Grade A and B formulations will remain as nanoemulsions when dispersed in GIT.
From (Table 3) optimized formulations were taken for globule size analysis, viscosity, electro conductivity, refractive index and in vitro release studies.

Viscosity determination
The viscosity of the optimized formulations was determined. The values are shown in (Table 4). It was observed that viscosity of all the formulations is less than 9 cP. Formulation NE2 has the minimum viscosity (4.56 cP), perhaps because of its higher aqueous content. Lower viscosity is an ideal characteristic of the o/w nanoemulsion.

Electroconductivity test
Conductivity of the optimized formulations was found in range of 454.2-552.3 µS/cm (Table 4). From the viscosity and the Electroconductivity study it can be concluded that the system is of o/w type.

Refractive index and transmittance
The refractive index of the developed system was similar to the refractive index of the water (1.333). In addition, the developed system showed percent transmittance > 97%.

Drug content
Drug content of the optimized formulations was found in range of 97.85-99.56%. The drug content varied for upto 3.09% between formulations NE1 to NE5.

Transmission Electron microscopy
TEM analysis for the formulation has to be done using zetasizer. A “positive” image is seen using TEM. Some droplet sizes were measured using TEM, as it is capable of point to point resolution. The droplets in the nanoemulsion appear dark and the surroundings are bright (Fig. 2).

In vitro Release studies
The highest release i.e. 99.58 % was obtained in case of formulation NE2 can be detected by performing Dissolution studies from 5 different nanoemulsion formulations (NE1 to NE5), and simple drug suspension, having same quantity 10mg amphotericin B (Table 5, Fig. 3) and the amount of drug release is compared.

Table 1. Solubility of Amphoteiricin B in different oils (n=3)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>OIL</th>
<th>SOLUBILITY (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soyabean oil</td>
<td>8.82±0.3</td>
</tr>
<tr>
<td>2</td>
<td>Sunflower oil</td>
<td>2.94±0.1</td>
</tr>
<tr>
<td>3</td>
<td>Sesame oil</td>
<td>40.83±0.3</td>
</tr>
</tbody>
</table>

Table 2. Thermodynamic stability and dispersibility tests of different formulations selected from phase diagrams at a difference of 5%W/W of oil.

<table>
<thead>
<tr>
<th>SMIX RATIO</th>
<th>OIL (%)</th>
<th>SMIX (%)</th>
<th>AQUEOUS (%)</th>
<th>H/C</th>
<th>CENT</th>
<th>Freeze Thaw</th>
<th>DT grade</th>
<th>INF.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>5</td>
<td>15</td>
<td>80</td>
<td>X</td>
<td>X</td>
<td>✓</td>
<td>A</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>18</td>
<td>72</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>B</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>13</td>
<td>72</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>B</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>18</td>
<td>63</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
<td>C</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>19</td>
<td>56</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
<td>C</td>
<td>FAIL</td>
</tr>
<tr>
<td>1:2</td>
<td>5</td>
<td>14</td>
<td>81</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>B</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12</td>
<td>78</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>B</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>13</td>
<td>72</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>D</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>16</td>
<td>64</td>
<td>✓</td>
<td>✓</td>
<td>X</td>
<td>C</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>18</td>
<td>57</td>
<td>✓</td>
<td>X</td>
<td>✓</td>
<td>A</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>22</td>
<td>48</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>C</td>
<td>FAIL</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>25</td>
<td>40</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>C</td>
<td>FAIL</td>
</tr>
</tbody>
</table>
Harika K et al., Formulation and evaluation of nanoemulsion of amphotericin B

Table 3. Optimized formulations selected from phase diagram at difference of 5%W/W of oil having least smix concentration.

<table>
<thead>
<tr>
<th>CODE</th>
<th>Smix ratio(ml)</th>
<th>Oil (%)</th>
<th>S (%)</th>
<th>CoS (%)</th>
<th>Aqueous (%)</th>
<th>Oil:Smix ratio</th>
<th>Dispersibility grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE1</td>
<td>1:3</td>
<td>5</td>
<td>13</td>
<td>82</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>14</td>
<td>76</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>16</td>
<td>79</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>17</td>
<td>63</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>17</td>
<td>58</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>21</td>
<td>49</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>27</td>
<td>38</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>37</td>
<td>23</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

Table 4. Characterization of optimized formulations (NE1 to NE5)

<table>
<thead>
<tr>
<th>Code</th>
<th>Conductivity(µS/cm)</th>
<th>Refractive index</th>
<th>Transmittance</th>
<th>Drug content (%)</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE1</td>
<td>483.0</td>
<td>1.23</td>
<td>98.32</td>
<td>98.48</td>
<td>6.32</td>
</tr>
<tr>
<td>NE2</td>
<td>552.3</td>
<td>1.34</td>
<td>97.85</td>
<td>97.86</td>
<td>4.56</td>
</tr>
</tbody>
</table>

Heating cooling cycle (H/C), Centrifugation (CENT), Dispersibility test (DT), Inference (INF).

Table 3. Optimized formulations selected from phase diagram at difference of 5%W/W of oil having least smix concentration.

Table 4. Characterization of optimized formulations (NE1 to NE5)
<table>
<thead>
<tr>
<th></th>
<th>NE3</th>
<th>NE4</th>
<th>NE5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (Hrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>6.86±0.4</td>
<td>8.22±0.02</td>
<td>4.59±0.3</td>
</tr>
<tr>
<td>0.5</td>
<td>12.77±0.1</td>
<td>14.13±0.1</td>
<td>10.95±0.4</td>
</tr>
<tr>
<td>1</td>
<td>22.77±0.2</td>
<td>25.49±0.03</td>
<td>17.29±0.3</td>
</tr>
<tr>
<td>2</td>
<td>35.04±0.3</td>
<td>38.22±0.09</td>
<td>30.04±0.4</td>
</tr>
<tr>
<td>4</td>
<td>46.40±0.1</td>
<td>52.76±0.1</td>
<td>43.22±0.4</td>
</tr>
<tr>
<td>6</td>
<td>67.31±0.1</td>
<td>69.58±0.2</td>
<td>63.22±0.1</td>
</tr>
<tr>
<td>8</td>
<td>75.49±0.2</td>
<td>79.13±0.2</td>
<td>70.04±0.1</td>
</tr>
<tr>
<td>10</td>
<td>83.22±0.4</td>
<td>88.67±0.4</td>
<td>80.99±0.2</td>
</tr>
<tr>
<td>12</td>
<td>97.31±0.6</td>
<td>99.58±0.1</td>
<td>92.76±0.3</td>
</tr>
</tbody>
</table>

Table 5: Comparative *In vitro* release data for various formulation (n=3)

Fig 1. Pseudoternary phase diagrams indicating o/w nanoemulsion region at different smix ratios
SUMMARY AND CONCLUSION
This thesis deals with the investigations carried out on the preparation and characterisation of oil-in-water nanoemulsion containing Amphotericin B with minimum surfactant concentration that could improve its solubility and oral bioavailability. Higher drug release, optimum globule size, minimum polydispersity, lower viscosity, lower surfactant concentration, high electroconductivity and higher bioavailability has been optimized as NE formulation of Amphotericin B containing sesame oil as oil phase, Tween20 surfactant and ethanol as cosurfactant respectively.

REFERENCES
