STUDY ON NANO-EMULSION SYSTEM FOR POORLY SOLUBLE DRUGS (FENOFIBRATE AND OLanzAPINE)

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ABSTRACT

Nano-emulsion are the drug delivery system which are kinetically stable and it contains biopharmaceuticals. In which one liquid is immiscible into another liquid. 20 to 500nm are the droplet size. Droplet diameters and surface property play a role in the formulation of biological behaviour. Droplets are transparent and did not contains any oil phases. These nano-emulsion are used in cosmetics preparation, pharmaceuticals etc. For the preparation of this formulation high and low energy methods are used. In recent time, high pressure homogenization, ultrasonication and phase inversion temperature is used. In this study, poorly soluble drugs are been evaluated and formulated. Active ingredient is been used in the nano-emulsion. this are used for the preparation of biodegradable coating and film packing. These may enhance the quality, nutritional value and functional properties.

KEYWORDS: nano-emulsion, bioactive compounds, Self-emulsification, Co-surfactant

I. INTRODUCTION

It is a type of emulsion in which the droplet size of emulsion contains the size of 100-600nm. It contains oil, water and emulsifier.¹,² For the small size droplets, the emulsifier addition may create problems due to decreases in interfacial tension. Emulsifier may play most important role.³,⁴ It may stabilize the nano-emulsion by steric hindrance and electrostatic interactions repulsive. This emulsifier is also used as surfactant.⁵,⁶ Protein and lipids show the greater effect on nano-emulsion preparations. Now a days, researchers focus on nano-emulsion.⁷,⁸ There are various methods which are used for the preparation of nano-emulsion like low and high energy methods.⁹,¹⁰ HPH and ultrasonication may use and in low energy, an exploit specific system is used for consuming energy and makes small amount of droplets.¹¹,¹²,¹³ For the formation of low energy nano-emulsions phase inversion temperature and emulsion inversion is used. In modern era, bubble bursting and evaporation ripening is used for the formation of nano-emulsion.¹⁴,¹⁵,¹⁶ These nano-emulsion is kinetically stable and gives more time to sperate the nano-emulsion phases. Ostwald ripening shows the most effect or destabilization on mechanism of nano-emulsion.¹⁷,¹⁸,¹⁹

Oil in water types is basically used in NEs. The core of the particles is oil or water. For human consumptions these nano particles are also used of surfactant.²⁰,²¹ For large scale production these emulsions are mixed with water- immiscible oil phase. It contains aqueous phase in high shear stress and mechanical extrusion process.²²,²³,²⁴ Surfactant phase structure may have the phase behaviour of droplets size at the inversion point by temperature and compositions.²⁵,²⁶ Drug transport may be influenced by large interface aera positivity. It was delivering along with specific targeted sites. In nanomaterial world, Nanoscales has some very interesting physical properties about the reducing droplet sizes. It has optical transparency and have elastic behaviour.²⁷,²⁸ NEs has deformable nanoscale droplets properties. These are ranging from liquid to solid and optical properties ranging from opaque to transparent.²⁹,³⁰ NEs required les surfactant for the nano structure of lyotropic phase of microemulsion.³¹,³²,³³

NEs Preparation:
These contain structural liquids of non-equilibrium system. NEs is prepared by the use of large amount of energy and surfactants. High and low energy method is used for the formulation development. In high energy method, mechanical devices are used for the breakdown of oil and water for the formation of nano-sized droplets. Microfluidizer, ultra-sonicators and high-pressure homogenisers are used. The size of the particles will be depending on the instrument as well as the conditions like temperature and time which were used for the formation of formulation. A large composition is due to the particle size. Stability and colour of emulsion is control by this method. NEs may have industrial scalability. These drugs are heat sensitive. Recently, low energy level emulsification is used for the preparation of NEs. It may be developed due the phase behaviour constituent’s property. It promotes the ultra-small droplets. phase transition, phase inversion temperature and self-emulsification methods are included in that. This method is used because this method is used to develop the small droplets from the stored energy. HLB value may be changes due the parameter changing.

II. MATERIALS AND METHODS

2.1 CHEMICALS
Fenofibrate and Olanzapine of pharmaceutical grade and all grades of polymers were obtained as a gift sample, respectively. Analytical grades chemicals and reagents were used.

2.2 METHOD VALIDATION
In this parameters, accuracy, precision, linearity and range, limit of detection and limit of quantitation, selectivity/specificity and system suitability has been calculated and evaluated as per ICH guidelines.

2.2.1 Accuracy:
Standard solution of fenofibrate and olanzapine is used to carry out this experiment with determination of percentages recovery. In this experiment three different concentration is analysed by eight replicates. The mean, standard deviation (SD) and percentage coefficient of variation (% RSD) were calculated. It may be reported by % and analysed the concentration which was calculated as:

\[ \%\text{Nominal} = \frac{\text{measured concentration}}{\text{actual concentration}} \times 100 \]

2.2.2 Precision:
Inter day and intraday is used in this process. In which six injection is used for three different concentration of fenofibrate and olanzapine. It should be reported as percentage coefficient. It should be analysed concentration which was calculated as:

\[ \%CV = \frac{\text{standard deviation(SD)}}{\text{mean}} \times 100 \]

2.2.3 Linearity:
Linearity for fenofibrate and olanzapine were prepared from the standard solution. Different concentration (5µg/ml to 50µg/ml, 80µg/ml to 192µg/ml and 5µg/ml to 25µg/ml) is used. The calibration curve was plotted. From the calibration curve, slope and regression equation was calculated.

2.2.4 Limit of detection (LOD) and Limit of Quantification (LOQ):
Limit of detection (LOD) and limit of quantitation (LOQ) were shown as 3 and 10 times noise level of three different replicated injections of samples were used.

2.2.5 Specificity:
Specificity method is used to analysing the ability of unequivocally. It should be done by in the presence of compounds. It did not affect the degradants, matrix and impurities. It should be compared by standard retention time and spectra of samples.

2.3 SOLUBILITY OF FENOFIBRATE AND OLANZAPINE
The solubility of fenofibrate and olanzapine is checked by shake flask method by using different concentration of oils. Different types of vehicles are been used in this capryol 90, capmul MCM C8, maisine 35-1,
isopropylmyristate etc. A conical flask is used 1mg of drug is mixed with this vehicles and vortex it properly. It should be store in isothermal shaker for 72h. these sample were centrifuged at 3000RPM for 10 to 15min. after that filtration has been done and the supernatant is been collected and take the observation at 286.4nm under the UV spectroscopy.

2.4 PREFORMULATION STUDIES

Preformulation study were done by determination of particle size, bulk density, tapped density, compressibility index and hausner’s ratio and angle of repose.

2.4.1 Determination of particle size

For particle size determination Sieve method is used. Different sieves are (20, 25, 30, 35, 40, 70 and 100) were selected and stand on each one top. 150 g of powder was placed on the top of the sieve and shake it. After 15 minutes of shaking the amount of particle on each sieve were collected.

2.4.2 Bulk density

A weighed powder were introduced in to the measuring cylinder and then the volume was noted.

2.4.3 Tapped density

A weighed powder were introduced in to the measuring cylinder. The cylinder was hit every 2 seconds from the height of 2.5 cm up to volume plateau.

2.4.4 Compressibility index

Compressibility index helps to explain the flow properties of the powders. It was expressed in percentage.

2.4.5 Hausner’s ratio

Hausner ratio is used for the measurement of powder flow.

2.4.6 Angle of repose

Fixed funnel method were used to measure the angle of repose. Drugs which contain different excipient were prepared and weighed it then transfer into a funnel. A funnel was just touching the apex of the heap of the drug. These powders now allow to flow on the surface freely.

2.5 EVALUATION STUDIES

Tablet was evaluated for hardness, friability, weight variation, thickness, drug content, in vitro buoyancy study, swelling index, in vitro dissolution studies and stability study. Tablets Thickness was calculated by Vernier Calliper. Roche friabilator was used to identify the tablets friability. In weight variation test, 18 tablets were weighted and average weight is calculated by using electronic balance. Thickness was determined by dial calliper. For Drug content analysis, 10 tablets were taken and powdered. The powdered equivalent to 10mg of fenofibrate and olanzapine and dissolve in 0.1N HCL. After that filtration was done and analysis by double beam UV spectrophotometer at 250nm wavelength. For in-vitro studies, 900ml of 0.1N HCL were taken and dissolution of fenofibrate and olanzapine tablets done at 37 ± 0.5 °C at 50rpm. The samples were taken out at every 1 hour time interval. The samples were tested by using spectrophotometrically at 250nm wavelength. In stability study, the optimized formulation was packed in amber-coloured bottle, which was tightly plugged with cotton and capped. It was then stored at 25° C / 60 % RH for 6 weeks. According to ICH guidelines, the stability studies of the tablets were carried by storing tablets in solubility chamber. For Long-Term Testing: 25°C ± 2°C / 60 % RH ± 5 % for 12 months. For Accelerated Testing: 40°C ± 2°C / 75 % RH ± 5 % for 6 months.

III. RESULTS AND DISCUSSIONS

3.1 Method validation

Optimization of the chromatographic condition were depending on buffer concentration, pH strength and ACN concentration. It should give the good separation of fenofibrate and olanzapine. the mobile phase of orthophosphate: acetonitrile (50:50) v/v, orthophosphate: acetonitrile (50:50) v/v and orthophosphate: acetonitrile (50:50) v/v. fenofibrate and olanzapine retention time is 7.8, 4.2 and 5.6 min. The peak is been shifted to void
volume and increase the acetonitrile concentration. loss of resolution of acetonitrile is been Decreased. peak splitting is done by increase or decrease in the buffer concentration.

3.1.1 Accuracy:
Mean percent recovery for fenofibrate and olanzapine were found to begreater than 97.2, 96.6 and 96.2% respectively, while the percentage coefficient of variation (\% CV) values for fenofibrate and Olanzapine were less than 1.5\% indicating accuracy method.

3.1.2 Precision:
Precision results shown in table 1. The percentage coefficient of variation values for fenofibrate and olanzapine were less than 1.06\% and olanzapine was less than 0.68 \% which indicates that the proposed method is precise.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Drugs</th>
<th>Conc ((\mu)g/mL)</th>
<th>Mean (±)</th>
<th>SD (±)</th>
<th>% C.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fenofibrate</td>
<td>5</td>
<td>80.1</td>
<td>0.66</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>126.7</td>
<td>0.46</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>190.3</td>
<td>1.06</td>
<td>0.56</td>
</tr>
<tr>
<td>2.</td>
<td>Olanzapine</td>
<td>5</td>
<td>4.87</td>
<td>0.03</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>15.75</td>
<td>0.05</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>24.89</td>
<td>0.03</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Table 1. Precision of drugs

3.1.3 Linearity:
Calibration curve for the drug fenofibrate was developed. Linearity and peak area also calculated. It was observed that the linearity and Peak area is 80\(\mu\)g/mL to 1920\(\mu\)g/mL. The linear regression equation is \(y=19555x\) with \(R^2=0.999\).

Calibration curve for the drug olanzapine was developed. Linearity and peak area also calculated. It was observed that the linearity and Peak area is 6\(\mu\)g/mL to 26\(\mu\)g/mL. The linear regression equation is \(y=50297x\) with \(R^2=0.999\).

3.1.4 Limit of detection (LOD) and Limit of Quantification (LOQ):
Limit of detection for the fenofibrate and olanzapine were found to be 2, 4 and 6\(\mu\)g. Limit of quantification for fenofibrate and olanzapine were found to be 6, 12 and 18\(\mu\)g.

3.1.5 Specificity:
Specificity of the method is done by comparing with the response and retention time of the standard and the sample. good separation is occurred at the standard and the sample.
3.2 SOLUBILITY STUDIES OF DRUGS

Solubility is an important criterion in formulation of NEs. It will remain in liquid form solubilized in oil phase. The oil phase is been selected which show greater solubility. It was evident that capryol 90 shows maximum solubility of fenofibrate and olanzapine. Hence capryol 90 and oleic acid were selected for the formulation of NEs. The solubility is increased due to the more affinity towards the oils.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Fenofibrate (mg/mL)</th>
<th>Olanzapine (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAPRYOL 90</td>
<td>181.24±0.65</td>
<td>42.2±1.99</td>
</tr>
<tr>
<td>CAPMUL MCM</td>
<td>111.67±0.99</td>
<td>33.1±0.99</td>
</tr>
<tr>
<td>OLEIC ACID</td>
<td>116.96±1.65</td>
<td>116.95±1.65</td>
</tr>
</tbody>
</table>

Table 2. Solubility Studies of Drugs

3.3 PREFORMULATION STUDY

The bulk density, tapped density, angle of repose, hausner’s ratio and carr’s compressibility index of tablets of different batch were showed in table 3.
### Table 3. Pre-formulation parameter of blend powder of fenofibrate and olanzapine

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Hardness (Kg/cm²)</th>
<th>Friability (%)</th>
<th>Uniformity of weight (%)</th>
<th>Swelling index in 1 hour (%)</th>
<th>Buoyancy lag time (minutes)</th>
<th>Duration of floating (hours)</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>4.3±0.65</td>
<td>0.94</td>
<td>1.28±0.09</td>
<td>12.3</td>
<td>1.2±0.91</td>
<td>&gt;9</td>
<td>98.4</td>
</tr>
</tbody>
</table>

#### 3.4 Evaluation Test

The hardness, friability and weight variation of tablets of different batch were showed in Table 4.
<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>4.2±0.50</td>
<td>0.90</td>
<td>1.56±0.09</td>
<td>12.9</td>
<td>1.2±0.89</td>
</tr>
<tr>
<td>F3</td>
<td>4.4±0.34</td>
<td>0.87</td>
<td>1.45±0.06</td>
<td>13.2</td>
<td>1.46±0.93</td>
</tr>
<tr>
<td>F4</td>
<td>4.5±0.18</td>
<td>0.90</td>
<td>1.52±0.07</td>
<td>13.5</td>
<td>1.28±0.78</td>
</tr>
<tr>
<td>F5</td>
<td>4.7±0.21</td>
<td>0.89</td>
<td>0.475±0.09</td>
<td>13.4</td>
<td>1.45±0.69</td>
</tr>
<tr>
<td>F6</td>
<td>4.6±0.27</td>
<td>0.88</td>
<td>0.485±0.05</td>
<td>13.1</td>
<td>1.39±0.87</td>
</tr>
<tr>
<td>F7</td>
<td>4.5±0.26</td>
<td>0.97</td>
<td>0.460±0.02</td>
<td>13.6</td>
<td>1.37±0.92</td>
</tr>
<tr>
<td>F8</td>
<td>4.6±0.64</td>
<td>0.86</td>
<td>0.461±0.05</td>
<td>13.3</td>
<td>1.42±0.86</td>
</tr>
<tr>
<td>F9</td>
<td>4.7±0.44</td>
<td>0.93</td>
<td>0.464±0.03</td>
<td>12.6</td>
<td>1.44±0.90</td>
</tr>
<tr>
<td>F10</td>
<td>4.6±0.34</td>
<td>0.92</td>
<td>0.461±0.02</td>
<td>12.8</td>
<td>1.45±0.86</td>
</tr>
<tr>
<td>F11</td>
<td>4.2±0.76</td>
<td>0.95</td>
<td>0.478±0.01</td>
<td>12.5</td>
<td>1.43±0.76</td>
</tr>
<tr>
<td>F12</td>
<td>4.6±0.40</td>
<td>0.84</td>
<td>0.462±0.07</td>
<td>12.3</td>
<td>1.38±0.82</td>
</tr>
<tr>
<td>F13</td>
<td>4.4±0.34</td>
<td>0.87</td>
<td>1.52±0.07</td>
<td>13.5</td>
<td>1.46±0.93</td>
</tr>
<tr>
<td>F14</td>
<td>4.5±0.18</td>
<td>0.90</td>
<td>0.475±0.09</td>
<td>13.4</td>
<td>1.28±0.78</td>
</tr>
<tr>
<td>F15</td>
<td>4.7±0.21</td>
<td>0.89</td>
<td>0.485±0.05</td>
<td>13.1</td>
<td>1.45±0.69</td>
</tr>
<tr>
<td>F16</td>
<td>4.6±0.27</td>
<td>0.88</td>
<td>0.460±0.02</td>
<td>13.6</td>
<td>1.39±0.87</td>
</tr>
<tr>
<td>F17</td>
<td>4.5±0.26</td>
<td>0.97</td>
<td>0.461±0.05</td>
<td>13.3</td>
<td>1.37±0.92</td>
</tr>
<tr>
<td>F18</td>
<td>4.6±0.64</td>
<td>0.86</td>
<td>0.464±0.03</td>
<td>12.6</td>
<td>1.42±0.86</td>
</tr>
</tbody>
</table>

Table 4. evaluation parameters of fenofibrate and olanzapine

3.5 Dissolution Study:

_in vitro_ drug release data were shown in the figures. The plots of % Cumulative drug release v/s Time (hr) in different batches.
In vitro dissolution studies were shown in the formulations 5 and 6. It was compared with pure drug. Residence time and maximum absorption in small intestine. It may enhance the solubilization process. The GI tract is been limited. The between the small intestinal is 3.9 to 5 hours. pH 6 is been used for the dissolution medium. 96% and 96% compatibility is seen in fenofibrate and olanzapine drug. The pure drug is been 12 to 13% pure. The
drug release rate is been compared by both pure drug and marketed drugs. Small droplet size and PDI is been used for this. It my increases the surface area with maximum release of 15 to 20mins. All drugs are found in the solution after 120min. NEs did not show the precipitation. In vitro study NEs may be preserved. It may enhance the dissolution of drugs.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>NEs</th>
<th>Pure drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>94.37±0.58</td>
<td>11.55±0.19</td>
</tr>
<tr>
<td>30</td>
<td>95.40±0.98</td>
<td>12.57±0.28</td>
</tr>
<tr>
<td>45</td>
<td>95.41±0.20</td>
<td>13.79±0.46</td>
</tr>
<tr>
<td>60</td>
<td>96.37±0.78</td>
<td>14.54±0.72</td>
</tr>
<tr>
<td>90</td>
<td>98.23±0.90</td>
<td>16.84±0.64</td>
</tr>
<tr>
<td>120</td>
<td>99.64±0.58</td>
<td>19.33±0.61</td>
</tr>
</tbody>
</table>

Table 5. In vitro dissolution data of fenofibrate

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>NEs</th>
<th>Pure drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>94.09±0.17</td>
<td>17.28±0.37</td>
</tr>
<tr>
<td>30</td>
<td>95.28±0.69</td>
<td>12.54±0.16</td>
</tr>
<tr>
<td>45</td>
<td>95.54±1.94</td>
<td>14.96±0.92</td>
</tr>
<tr>
<td>60</td>
<td>96.98±1.98</td>
<td>15.37±0.37</td>
</tr>
<tr>
<td>90</td>
<td>98.27±0.88</td>
<td>16.29±0.23</td>
</tr>
<tr>
<td>120</td>
<td>99.34±0.11</td>
<td>21.43±0.93</td>
</tr>
</tbody>
</table>

Table 6. In vitro dissolution data of olanzapine

3.7 STABILITY STUDIES:

For optimized formulation stability study were performed for 6 weeks. hardness, drug content, floating properties, and dissolution study were done after 6 week. Comparision of harmness, drug content and floating properties.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 week</th>
<th>6th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>3 kg</td>
<td>3 kg</td>
</tr>
<tr>
<td>Drug content</td>
<td>49.89 ± 0.48 mg</td>
<td>49.50 ± 0.39 mg</td>
</tr>
<tr>
<td>Floating lag time</td>
<td>20 ±4 second</td>
<td>21 ±3 second</td>
</tr>
<tr>
<td>Total floating time</td>
<td>10 hour</td>
<td>10 hour</td>
</tr>
</tbody>
</table>

Table 7.Evaluated data at 0 and 6th week

IV. DISCUSSION

On the basis of test and study the identification test show the drug complied. Drugs are not interacted with excipients mixture. 4100 is the viscosity. This is best for integrity of mixture. Carr’s index and Hausner ratio are used for the drug and excipients.in formulation, the direct compressed process is been suitable. All batches of table are in desirable physical characteristics. it has short lag time. Polymers presence show the short lag time. It has been used to minimize the lag time. Long floating time of 24minutes is exhibited. Polymers shows the high
and low level. It may prevent the entry to the media with high level of polymers. It also prolongs the lag time. Literature shows the concentration of polymers. Maximum floating time 10 hours is been found in all batches. Some tablets show short time duration of 3 to 4 hours, polymer may increase the one factor plot. At high amount of polymers matrix show the increased integrity. Faster erosion of tablet may show the TFT decreases.

V. CONCLUSION

Nano emulsion preparation was optimized by study and performing solubility study of the drugs (fenofibrate and olanzapine) in different oils. In solubility studies, oleic acid and capryol 90 shows the most solubility. It did not show any precipitation in aqueous layer. Pseudoternary phase diagram is used for the optimization of NE composition. It may form maximum concentration to minimum concentration of selected thermodynamically stability study. The droplet size, PDI, zeta potential, viscosity, refractive index, %transmission and conductivity of the selected formulations was found to be 34.80nm,0.241, 0.857mV, 19.60cPs, 1.382, 99.65, 370.7μS/cm and 24.45nm, 0.230, 0.789mV, 19.99cPs, 1.385, 99.55, 391.55μS/cm is shown in formulations. Zetasizer is used in SEM and TEM report. droplet size analysis, PDI and zeta potential is used for the thermodynamic stability tests and dispersibility tests. For in vitro and in vivo studies, capryol 90, SCoS is used for this process. These drugs show the significant behaviour as compare to pure and marketed formulation. The maximum release of fenofibrate and olanzapine is 99.38%, 65.86% and 25.83%, 68.33%. The bioavailability may be enhanced by NEs from AUC, Cmax and Tmax formulations. NEs formulation bioavailability will be increased as compared to marketed formulation and pure drug suspension.

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