INTRODUCTION

Over decades, the oral route is considered as the easiest and most preferred route of drug administration for the chronic treatment of many diseases. Despite the unique advantages of oral drug delivery, the majority of prospective and existing drug molecules pose a challenge to the pharmaceutical scientist limiting their potential uses and increasing the difficulty of formulating low bioavailable products [1]. There are several constraints responsible for this, but a particularly widespread problem is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastrointestinal tract and systemic metabolism by the gut wall and hepatic enzymes [2, 3]. In this case, improved bioavailability can be achieved by the use of delivery systems which can enhance the rate and/or the extent of drug solubilizing into aqueous intestinal fluids. 

Drugs belonging to BCS class II and IV have motivated the formulators for the development of drug delivery technologies to overcome the difficulty in their solubilization by either chemical or mechanical modification of the environment surrounding the drug substance, or physically altering the macromolecular characteristics of aggregated drug particles. Various traditional approaches for solubility enhancement include micronization, solid dispersions and mechanical modification of the environment surrounding the drug substance, or physically altering the macromolecular characteristics of aggregated drug particles. Various traditional approaches for solubility enhancement include micronization, solid dispersions and co-solvency using cyclomixer. Based on solubility studies Capmul M8 NF, Gelucire 44/14 and Transcutol P were selected as oil, surfactant and co-surfactant respectively in order to prepare SNEDDS. Nine different SNEDDS formulations were prepared and subjected to various evaluation tests in order to obtain optimized SNEDDS formulation.

Objective: The primary goal of the present work was to formulate and evaluate self-nano emulsifying drug delivery systems (SNEDDS) of ramipril in order to improve the solubility of this highly lipophilic antihypertensive drug.

Methods: SNEDDS are generally liquid form preparations obtained by homogeneously mixing drug substance with oils, surfactants and co-surfactants using cyclomixer. Based on solubility studies Capmul M8 NF, Gelucire 44/14 and Transcutol P were selected as oil, surfactant and co-surfactant respectively in order to prepare SNEDDS. Nine different SNEDDS formulations were prepared and subjected to various evaluation tests in order to obtain optimized SNEDDS formulation.

Results: The SNEDDS formulations with 16.5-24.75 % of oil, 24.75-68.75 % of surfactant and 12.375-41.25 % of co-surfactant formed thermodynamically stable emulsion with droplet size ranging from 22.6-188.8 nm. Finally, out of 9 different SNEDDS formulations, SN9 formulation was optimized containing 16.5 % of oil, 68.75 % of surfactant and 13.75 % of co-surfactant as it formed a thermodynamically stable emulsion with least globule size (22.6 nm) and without any drug precipitation or phase separation.

Conclusion: Finally, stable, optimized SNEDDS formulation of ramipril was successfully prepared that showed significant improvement in the rate of dissolution of ramipril.

Keywords: Self-nano emulsifying drug delivery system (SNEDDS), Ramipril, Emulsification time, Ternary phase diagram, Simulated Gastric Fluid (SGF).

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The formulations were subjected to heating-cooling, centrifugation, thermodynamic stability studies and subjected to further characterization. Any for 48 h [22].

Formulations which passed the heating-cooling cycles were subjected to centrifugation at 3500 rpm (temp 18 °C) for 15 min using REMI Cooling Centrifuge, and the extent of phase separation was monitored. The stable SNEDDS formulations were selected and subjected to further characterization.

Preparation of SNEDDS

SNEDDS formulations were prepared by dispersing ramipril into the mixture of oil, surfactants and co-surfactants those selected based on solubility studies. The contents were initially mixed by gentle stirring and then subjected to vortex mixing at 37 °C using cyclomixer until the homogenous isotropic mixture was obtained. The obtained SNEDDS were kept at room temperature until used.

Evaluation of SNEDDS

Evaluation of self- emulsification time and stability

Self-emulsifying properties of SNEDDS formulations were performed by visual assessment [21]. For this, about 100 μl of SNEDDS formulation was added dropwise to a beaker containing about 300 ml SGF at 37 °C which was kept under continuous stirring (~ 100 rpm) using magnetic stirrer. The time taken for the emulsion formation for each formulation was recorded. In order to determine the stability of SNEDDS, the formed emulsion was stored at 37 °C and then observed for phase separation and precipitation of the drug, if any for 48 h [22]. The stable SNEDDS formulations were selected and subjected to further characterization.

Thermodynamic stability studies

The formulations were subjected to heating-cooling, centrifugation, and freeze-thaw cycle, where the physical appearances of the formulations were observed at the end of each testing. In heating cooling, all nine formulations were heated at 45 °C and then cooled to 4 °C, with the duration of 24 h at each temperature, for 2 cycles. Then, formulations which passed the heating-cooling cycles were subjected to centrifugation at 3500 rpm (temp 18 °C) for 15 min using REMI Cooling Centrifuge, and the extent of phase separation was monitored. [23]. Finally, only formulations which passed the previous two steps were stored at alternating temperature of 21 °C and 25 °C, with storage at each temperature for not less than 48 h was done for the formulations using REMI Quick Freezer, for 2 cycles [23].

Cloud point measurement

The cloud point measurement was carried out for the formulations as reported earlier [24]. About 50 μl of stable SNEDDS was added to 300 ml of SGF and emulsion was obtained in vitro by using magnetic stirrer. From which, 10 ml of the emulsion sample was taken and heated with a gradual rise in temperature. The cloud point was determined by visual inspection of cloudy appearance in emulsions at a particular temperature.

Robustness to dilution

SNEDDS formulations were subjected to various folds of dilution i.e. 1:5, 1:100, 1:500 and 1:1000 with SGF (pH 1.2). The diluted emulsions were stored for 24 h and monitored for any physical changes such as precipitation or phase separation [25].

Ternary phase diagram

About 100μl of SNEDDS formulation was introduced into a beaker containing 300 ml SGF at 37 °C. The contents were mixed using magnetic stirrer; set at 100 rpm. The existence of SNEDDS that could self-emulsify under dilution and gentle agitation was identified from ternary phase diagrams of systems containing oil, surfactant, and co-surfactant.

Precipitation of drug and phase separation was evaluated by visual inspection of the resultant emulsion after 24 h. The formulations were then categorized as clear (transparent or translucent or transparent with bluish tinge), unclear (turbid), stable (no precipitation at the end of 24 h), or unstable (showing precipitation within 24 h) [21]. Ternary phase diagram was constructed identifying the good self-emulsifying region. Ternary phase diagram was constructed identifying the good self-emulsifying region using Tri-plot v1-4 software [26]. The experiment was conducted in triplicate and similar observations being made between the repeats.

Globule size and zeta potential analysis

The mean globule size (x-average), zeta potential as well as the polydispersity index(PDI) of nanoemulsions formed from stable SNEDDS formulations was determined by photon correlation spectroscopy using nanosized (Nano ZS 90, Malvern instruments, UK). Before analysis, each formulation was diluted to a suitable concentration with filtered double distilled water. Size analysis was performed at 25 °C with an angle of detection of 90 °. All studies were repeated three times and the average values obtained were used.

In vitro dissolution studies

Dissolution studies were carried out using USP Type II Dissolution Apparatus (paddle type) (Electrolab, TD L8, Mumbai, India) in 900 ml of SGF (pH 1.2) without enzyme, the temperature at 37±0.5°C and paddle rotation of 50 rpm. 250 mg of formulation containing 2.5 mg of ramipril (equivalent to a single dose) was encapsulated and then installed to the dissolution medium. At predetermined time intervals 5 ml of sample was withdrawn and replenished with fresh dissolution medium (SGF) to maintain a constant volume. The samples were analyzed spectrophotometrically at 210 nm to detect the amount of drug released at each sampling point. The in vitro drug release data was analyzed by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Solubility Studies

The solubility of ramipril was carried out in various oils, surfactants, co-surfactants and among the excipients screened Capmul PG8 NF, Gelucire 44/14 and Transcutol-P have shown the highest solubility and hence they were selected as oil, surfactant and co-surfactant respectively. All the studies were conducted in triplicate, and the mean of three measurements was recorded. The results were depicted in fig. 1.

Preparation of SNEDDS

The selection of oil, surfactant and the co-surfactant mixture is based on the solvent properties of the vehicle that should allow the drug in solution.

SNEDDS formulations were prepared using Capmul PG8 NF, Gelucire 44/14 and Transcutol P as oil, surfactant and co-surfactant respectively based on solubility study results. Initially, a single dose of ramipril (2.5 mg) was accurately weighed and dissolved in calculated amount of oil, surfactant and co-surfactant in a glass vial. A series of self-emulsifying systems were prepared in each set with varying percent ratios of oil (16.5-49.5 %), surfactant (24.75-68.75%) and co-surfactant (8.25-41.25 %). Here nine different SNEDDS formulations were prepared and coded as SN1, SN2, SN3, SN4, SN5, SN6, SN7, SN8 and SN9 as showed in table 1.
Capmul PG-8 NF is chemically Propylene Glycol Monocaprylate. It is lipophilic in nature, having HLB value 5-6 and is generally practiced to create stable emulsions. Gelucire 44/14 belongs to a group of polyoxy glycerides. Polyoxyglycerides (also named macrogol glycerides by EP) are a well-established class of pharmaceutical excipients for enhancing solubility and bioavailability [27, 28].

**Evaluation of SNEDDS**

**Evaluation of self-emulsification time and stability**

The efficiency of self-emulsifying systems will be measured from the rate of emulsification upon hydration with mild agitation [21]. Surfactant systems in SNEDDS formulation reduce the interfacial tension between oil and aqueous phases resulting in easy dispersion and formation of o/w emulsion. Self-emulsification time of all SNEDDS formulation formulations was shown in table 2.

<table>
<thead>
<tr>
<th>SNEDDS formulation Code</th>
<th>Oil: S mix</th>
<th>Surfactant: co-surfactant (S mix)</th>
<th>Oil (mg)</th>
<th>Surfactant (mg)</th>
<th>Co surfactant (mg)</th>
<th>Drug (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN 1</td>
<td>1:1</td>
<td>1:1</td>
<td>123.75</td>
<td>61.875</td>
<td>61.875</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 2</td>
<td>1:3</td>
<td>1:1</td>
<td>61.875</td>
<td>92.8125</td>
<td>92.8125</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 3</td>
<td>1:5</td>
<td>1:1</td>
<td>41.25</td>
<td>103.125</td>
<td>103.125</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 4</td>
<td>1:1</td>
<td>3:1</td>
<td>123.75</td>
<td>92.8125</td>
<td>30.9375</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 5</td>
<td>1:3</td>
<td>3:1</td>
<td>61.875</td>
<td>139.21875</td>
<td>46.40625</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 6</td>
<td>1:5</td>
<td>3:1</td>
<td>41.25</td>
<td>154.6875</td>
<td>51.5625</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 7</td>
<td>1:1</td>
<td>5:1</td>
<td>123.75</td>
<td>103.125</td>
<td>20.625</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 8</td>
<td>1:3</td>
<td>5:1</td>
<td>61.875</td>
<td>154.6875</td>
<td>30.9375</td>
<td>2.5</td>
</tr>
<tr>
<td>SN 9</td>
<td>1:5</td>
<td>5:1</td>
<td>41.25</td>
<td>171.875</td>
<td>34.375</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Mean of three measurements±SD (n=3).
Thermodynamic stability studies

The resultant emulsions of the formulations were tested for stability at different temperature conditions and rpm. The emulsions were stable during heating and cooling cycle, centrifugation at 3500 rpm and alternative temperature cycles of 21 °C and +25 °C. SN 3, SN 6, SN 8 and SN 9 formulations did not show any phase separation and precipitation which indicated that they were stable at different temperatures and speed of centrifugation (table 4).

Table 4: Results for thermodynamic stability studies of ramipril SNEDDS under different stability conditions

<table>
<thead>
<tr>
<th>SNEDDS formulation</th>
<th>Heating cooling cycle</th>
<th>Centrifugation</th>
<th>Freeze-thaw cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN 3</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>SN 6</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>SN 8</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>SN 9</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
</tbody>
</table>

P=Passed

Cloud point measurement

The temperature at which cloudiness appeared was considered as the cloud point temperature. Higher cloud point values indicate higher thermal stability of emulsions formed from SNEDDS formulation. The cloud point is the temperature above which clarity of emulsion turns into turbid or unclear. In this study cloud point of stable formulations were found in the range of 64-72 °C (table 5).

Table 5: Results for cloud point measurement of ramipril SNEDDS

<table>
<thead>
<tr>
<th>SNEDDS formulation</th>
<th>Cloud point temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SN 3</td>
<td>6±1.0</td>
</tr>
<tr>
<td>SN 6</td>
<td>6±1.0</td>
</tr>
<tr>
<td>SN 8</td>
<td>7±2.0</td>
</tr>
<tr>
<td>SN 9</td>
<td>7±2.0</td>
</tr>
</tbody>
</table>

Mean of three measurements±SD (n=3).

Since both drug solubility, as well as formulation stability, will decrease with phase separation, the cloud point temperature of the formulation should be over 37 °C. Cloud points of all formulations were very high which indicate the stability of these SNEDDS formulations towards separation in the GIT temperature which is about 37 °C. The cloud point value is affected by factors such as drug hydrophobicity, kind, combination, mixing ratio and amount of each of the oils, surfactants and co-surfactants used [24, 29].

Robustness to dilution

SNEDDS formulations which were passed in previous tests were exposed to different folds of dilution in SGF in an attempt to mimic the in vivo conditions where the formulations would encounter gradual dilution. The resultant SNEDDS formulation was stable after dilution up to 50X, 100X, 500X, 1000X with SFG(pH1.2) and there were no signs of drug precipitation and phase separation (table 6). The clarity of dispersion might be due to a higher percentage of surfactant to oil ratio.

Ternary phase diagram

In order to determine the composition of oil and surfactant mix (surfactant to co-surfactant) used for SNEDDS formulation, a ternary phase diagram was constructed, and the efficient emulsification region was identified. Fig. 2 illustrates the formation of a stable emulsion without any phase separation and precipitation of drug with SN3, SN6, SN8 and SN9 formulations. The emulsification time of SNEDDS formulations, the size of globules formed and the stability of the resultant emulsion when introduced into the water with mild agitation not only depend on the type of oil, surfactant, and co-surfactant combination but also on the weight percentage of oil and surfactant/co-surfactant mixture [19]. Depending on the weight percentages of lipid vehicles in stable SNEDDS formulations, phase diagram was plotted using Triplot software. Here in these four stable SNEDDS formulations i.e. SN3, SN6, SN8 and SN9 weight percentage of oil, surfactant and co-surfactant ranges from 16.5-24.75 %, 41.25-68.75 %, and 12.375-41.25 % respectively. Ternary phase diagram including oil (Capmul PG8 NF), surfactant (Gelucire 44/14) and co-surfactant (Transcutol P) was plotted each of them representing an apex of the triangle.

Globule size and zeta potential analysis

Since globule size is one of the prime factors which significantly contribute to the absorption of the drug, the globule size and size distribution after self-emulsification is an important parameter that has to be evaluated. Of formulations from SN3 to SN9, globule size was significantly decreased (table 7). This resulted because of the concentration of surfactant increased, and that of oil decreased from SN3 to SN9. High surfactant levels enabled rapid dispersion of globules in SGF. A surfactant having hydrophilic nature (high HLB) assists information of O/W emulsion rapidly spreading in an aqueous medium. Surfactants are amphiphilic in nature & they can dissolve or soluble relatively high amount of hydrophobic drug compound. This can prevent precipitations of the drug within the GI lumen & for prolong the existence of drug molecules. Since droplet surface area is inversely proportional to diameter, smaller lipid droplets with their associated, greater surface area are thought to facilitate digestion, resulting in more lipid and uniform drug release and absorption [30].

The higher the zeta potential, greater will be the energy barrier to coalescence between oil globules and so higher will be the stability...
of the obtained emulsion. Negative zeta potential values also enable long circulation half-life \textit{in vivo} as described by Jung [31].

Formulation SN9 showed the least globule size with negative zeta potential when dispersed in SGF. Polydispersibility index (P. I) of SN3, SN6, SN8, SN9 formulations was below 0.3 indicating homogeneous dispersion.

\textit{In vitro} dissolution studies

To understand the release behavior of ramipril from stable SNEDDS and pure drug, \textit{in vitro} dissolution test was performed and the cumulative percentage of drug release profiles was depicted in fig. 3.

The amount of ramipril released from stable SNEDDS formulation, SN9 was 97.6±3.1 \% in 60 min and was significantly higher compared to the pure drug (50.98±2.7) (P<0.05) and the final results were demonstrated in table 8. This might be because of small globule size and the eventually higher surface area in case of nanoemulsions compared to that of pure drug, which permitted faster rate of drug release. The reason behind this might be the use of excellent surfactant Gelucire 44/14 (lauroyl macro glyceride) which is good solubilizer as well as a wetting agent having HLB value of 14. This property enhanced solubility and wettability of API \textit{in vitro}. Gelucire also possesses the property of bioavailability enhancement associated with improved \textit{in vivo} drug solubilization which facilitates absorption [32].

<table>
<thead>
<tr>
<th>Table 7: Globule size and zeta potential of ramipril SNEDDS</th>
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<tbody>
<tr>
<td><strong>SNEDDS formulation</strong></td>
</tr>
<tr>
<td>SN 3</td>
</tr>
<tr>
<td>SN 6</td>
</tr>
<tr>
<td>SN 8</td>
</tr>
<tr>
<td>SN 9</td>
</tr>
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P. I: Polydispersity Index

<table>
<thead>
<tr>
<th>Table 8: Cumulative % drug release of ramipril SNEDDS and pure drug</th>
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</thead>
<tbody>
<tr>
<td><strong>Time (min)</strong></td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>45</td>
</tr>
<tr>
<td>60</td>
</tr>
</tbody>
</table>

Mean of three measurements±SD (n=3), *p<0.05 indicate significant difference compared to that of pure drug.

![Fig. 3: Cumulative % drug release of ramipril SNEDDS and pure drug (mean±SD; n=3)](image.png)

CONCLUSION

As per the plan of research work, stable self-nano emulsifying drug delivery system (SNEDDS) of ramipril was prepared successfully which showed improved solubility of the drug. The stable SNEDDS has shown good clarity, thermodynamic stability, the spontaneity of emulsification, robustness to dilution, high cloud point temperatures and good stability towards precipitation and phase separation throughout the examination. Out of which SNEDDS formulation (SN 9) proved to have a high percentage release of ramipril compared to that of pure drug and other SNEDDS formulation. Thus finally, stable SNEDDS formulation, SN 9 was selected as optimized SNEDDS formulation which showed improved solubility of the drug.

CONFLICT OF INTERESTS

Declare none

REFERENCES

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