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Development of novel amisulpride-loaded liquid self-nanoemulsifying drug delivery systems via dual tackling of its solubility and intestinal permeability

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ABSTRACT

Objective: The aim of the current investigation was at enhancing the oral biopharmaceutical behavior; solubility and intestinal permeability of amisulpride (AMS) via development of liquid self-nanoemulsifying drug delivery systems (L-SNEDDS) containing bioenhancing excipients.

Methods: The components of L-SNEDDS were identified via solubility studies and emulsification efficiency tests, and ternary phase diagrams were constructed to identify the efficient self-emulsification regions. The formulated systems were assessed for their thermodynamic stability, globule size, self-emulsification time, optical clarity and in vitro drug release. Ex vivo evaluation using non-everted gut sac technique was adopted for uncovering the permeability enhancing effect of the formulated systems.

Results: The optimum formulations were composed of different ratios of Capryol™ 90 as an oil phase, Cremophor® RH40 as a surfactant, and Transcutol® HP as a co-surfactant. All tested formulations were thermodynamically stable with globule sizes ranging from 13.74 to 29.19 nm and emulsification time not exceeding 1 min, indicating the formation of homogenous stable nanoemulsions. In vitro drug release showed significant enhancement from L-SNEDDS formulations compared to aqueous drug suspension. Optimized L-SNEDDS showed significantly higher intestinal permeation compared to plain drug solution with nearly 1.6–2.9 folds increase in the apparent permeability coefficient as demonstrated by the ex vivo studies.

Conclusions: The present study proved that AMS could be successfully incorporated into L-SNEDDS for improved dissolution and intestinal permeation leading to enhanced oral delivery.

Introduction

Amisulpride (AMS) is a benzamide atypical antipsychotic drug molecule that acts selectively on both dopamine D2 and D3 receptor types with low affinity for any other receptor system leading to remarkable reduction in its side effects [1]. Additionally, AMS is characterized by its low risk of extrapyramidal symptoms and a unique ability in improving both positive and negative symptoms of schizophrenia [2].

Despite the distinctive neurochemical and pharmacological profiles of AMS, it suffers from two major drawbacks; pH dependent solubility and poor intestinal permeability leading to low oral bioavailability (approximately 48%). Regarding solubility, being a weakly basic drug of pKa =8.99 [3], AMS is highly soluble in acidic medium (stomach pH conditions) but suffers from poor solubility in neutral or high pH medium (intestinal pH conditions). This pH dependent solubility might cause drug precipitation lately after oral administration when reaching the intestinal medium leading to reduced drug absorption. Moreover, AMS suffers poor intestinal permeability as it has been identified as a substrate for the intestinal P-glycoprotein (P-gp) efflux pump [4]. This barrier can be considered also as a main cause for the reduced oral bioavailability and clinical effectiveness of AMS.

Self-nanoemulsifying drug delivery systems are uniform mixtures of oil, surfactant, co-surfactant and the investigated drug, which upon contacting the aqueous gastrointestinal fluids, rapidly emulsify into o/w nanoemulsion with minute droplet sizes and very high interfacial area. The formed nanoemulsions can bypass the dissolution step and introduce the drug to the GIT in a completely solubilized state ready for absorption [5]. Moreover, some of the excipients used in SNEDDS formulation such as Tween® 80, Cremophor® RH40, Cremophor® EL, Labrasol® and pluronics are claimed to be ‘bioenhancers.’ Such bioenhancing properties arise from their inhibitory effect on the intestinal P-gp efflux pump activity leading to enhanced permeability and oral bioavailability of poorly absorbable drugs [6]. Ghai and Sinha succeeded in improving the solubility and permeability of the β1-adrenoreceptor blocker talinolol using SNEDDS comprised of triacetin as the oily phase, Brij-721 as surfactant and ethanol as co-surfactant [7]. Also, Bandyopadhyay et al. [8], formulated SNEDDS using Maisine 35-1 as long chain triglyceride (LCT) or Capryol™ 90 as medium chain triglyceride (MCT) together with Labrasol® and Tween® 80 as surfactants for improving the bioavailability of ezetimibe through enhancing the poor solubility and permeability of the drug.

Literature lacks any evidence about the role of L-SNEDDS in solving both solubility and intestinal permeability problems of AMS. Therefore, the present study targets the formulation, in vitro and ex vivo evaluation of stable L-SNEDDS of AMS for improving its oral delivery.
Materials and methods

Materials

Amisulpride pure drug was a gift sample from Al Andalous for pharmaceutical industries (Cairo, Egypt). Capryol™ 90 (propylene glycol monocaprylate), Labrafac™ Lipophile WL1349 (caprylic/capric triglyceride), Labrasol™ (PEG-8 caprylic/capric glycerides), Labrafil™ M2125CS (linoleoyl macrogol-6 glycerides), Pecceol™ (glyceryl monooleate) and Transcutol® HP (diethylene glycol monoethyl ether) were kind gifts from Gattefosse (Saint-Priest, Lyon, France). Miglyol® 812 (caprylic/capric triglyceride) and Miglyol® 840 (propylene glycol dicaprylate/dicaprate) were purchased from Sasol (Witten, Germany). Cremophor® RH40 (polyoxy-40 hydrogenated castor oil) was purchased from BASF (Münster, Germany). Cremophor® EL (polyoxy-35 hydrogenated castor oil) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Isopropyl myristate (IPM) was purchased from Loba Chemie (Mumbai, India). Propylene glycol (PG), Polyethylene glycol 400 (PEG 400) and Tween® 80 were purchased from El-Nasr pharmaceutical chemicals Co. (Cairo, Egypt). Dialysis tubing cellulose membrane (Molecular weight cut off of 14,000) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade and used without further purification.

Determination of saturated solubility of AMS

The equilibrium solubility of AMS in various oils, surfactants and co-surfactants was determined. Excess AMS powder was added on 2 g of each of the investigated vehicles in a stoppered glass tube, followed by 30 s vortexing (BenchMixer; Benchmark Scientific Inc., Edison, NJ, USA). The tubes were then left at 37 ± 0.5 °C for 48 h in a thermostatically controlled shaking water bath (Memmert; WNB 14, Jakarta, Indonesia), then centrifuged at 4000 rpm for 5 min. Samples were then filtered on a 0.22 μm membrane filter and filtrates were suitably diluted with ethanol and measured spectrophotometrically at 277.4 nm using ethanol as a blank (UV-1800; Shimadzu, Kyoto, Japan).

Emulsification ability screening of surfactants and co-surfactants

Different surfactants were screened for their emulsification ability according to the method formerly described by Date and Nagarsenker [9]. Briefly, 300 mg of each surfactant was added to 300 mg of the oil phase and the mixtures were gently heated at 300 mg of the oil phase and the mixtures were gently heated at Nagarsenker [9]. Briefly, 300 mg of each surfactant was added to the homogenous blends of oil, surfactant and co-surfactant, followed by magnetic stirring at 125 rpm and 50 °C (Stuart, SB162; Stone, Staffordshire, UK) till formation of a completely clear system. Prepared drug-loaded L-SNEDDS were stored at room temperature for upcoming evaluation.

Evaluation of AMS-loaded L-SNEDDS

Assessment of thermodynamic stability and globule size analysis

Thermodynamic stability of the formulated AMS-loaded L-SNEDDS was assessed by visual observation of any signs of phase separation or drug precipitation after centrifugation for 30 min at 3500 rpm, followed by six cooling heating cycles (4 °C and 45 °C) and finally three freeze thaw cycles (−21 °C and +25 °C). Storage period at each temperature was not less than 48 h [13].

The mean globule size and polydispersity index (PDI) of L-SNEDDS were determined by photon correlation spectroscopy using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) after 100 times dilution with aqueous buffers (0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4)). Data were statistically analyzed using one-way ANOVA, followed by Post Hoc least significant difference test (LSD) to detect the source of the difference (SPSS 23.0, Chicago, IL, USA). p Values less than .05 were considered to be significant.

Transmission electron microscopy

Morphology of nanoemulsion droplets was studied using JEOL electron microscope (JEM-2100, Peabody, MA, USA). Formula (F2) was selected for examination under microscope as it showed acceptably minute globule size and low PDI values compared to other formulations. L-SNEDDS formula was diluted 100 times with distilled water, then 1–2 drops were taken onto TEM grid (400-mesh carbon coated grids). The sample was then negatively stained using a drop of 1% phosphotungstic acid for 30 s.

Determination of self-emulsification time

The rate of emulsification was determined by adding 1 g of AMS-loaded L-SNEDDS to 200 ml 0.1 N HCl (pH 1.2) at 37 °C and distilled water, followed by immediate visual observation for investigating self-emulsification ability and presence of any phase separation [11]. The diluted nanoemulsions were left for 24 h at 25 °C and reassessed regarding the previous criteria for stability purpose. Only dispersions with clear or slightly bluish appearance were regarded as the nanoemulsion region of the diagram [12]. The ternary phase diagrams were plotted using SigmaPlot® software (Version 12.5; Systat Software Inc., San Jose, CA, USA).
Figure 1. Ternary phase diagrams showing the self-nanoemulsification regions for systems composed of Capryol™ 90 as an oil, Transcutol® HP as a cosurfactant, and different surfactants (a) Cremophor® RH40, (b) Cremophor® EL, (c) Tween® 80, (d) Cremophor® RH40:Tween® 80 (1:1), (e) Cremophor® EL:Tween® 80 (1:1). Areas of nanoemulsion regions are shaded.
magnetically stirred at 50 rpm. The time required for complete disappearance of the preconcentrate and formation of homogenous single phase system was assessed visually [14].

**Spectroscopic characterization of optical clarity**

Optical clarity of the aqueous nano-dispersions produced from SNEDDS emulsification was measured spectrophotometrically. AMS-loaded L-SNEDDS were diluted to 100 times with aqueous buffers of different pH (0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4)). The absorbance of each solution was measured at 400 nm using distilled water as a blank [14]. Data were statistically analyzed using one-way ANOVA, followed by Post Hoc LSD to detect the source of the difference (SPSS 23.0, Chicago, IL, USA). Values less than .05 were considered to be significant.

**In vitro drug release study**

The selected AMS-loaded L-SNEDDS (F1, F2, F3, F4, F8, F13 and F14) (50 mg/g) were placed, with 5 ml of the dissolution medium (0.1 N HCl (pH 1.2) or phosphate buffer (pH 7.4)) rotated at 100 rpm and immersed in 900 ml freshly prepared dissolution medium (0.1 N HCl (pH 1.2) or phosphate buffer (pH 7.4)) rotated at 100 rpm and maintained at 37 ± 0.5 °C. 5 ml samples were withdrawn at regular time intervals (0.25, 0.5, 1, 1.5, 2, 4, 6 and 8 h) and replaced by an equal amount of fresh dissolution medium in order to maintain sink conditions. Withdrawn samples were filtered through 0.22 μm membrane filter, suitably diluted and analyzed for the drug content using UV spectrophotometer at 280 nm using the buffer as blank. The release patterns of AMS from the tested L-SNEDDS were compared to that of the drug aqueous suspension (dialysis bag containing 50 mg AMS powder in 5 ml dissolution medium) using the similarity factor (f<sub>2</sub>) according to the following equation:

\[
f_2 = 50 \times \log \left[1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} \left(\frac{R_t - T_t}{C_1} - C_2\right)^2 \times 100\right]
\]

**Table 1.** Formulation composition and evaluation of L-SNEDDS selected from the optimum phase diagram.

<table>
<thead>
<tr>
<th>L-SNEDDS formula number</th>
<th>Composition</th>
<th>Drug loading capacity (mg/g)±SD</th>
<th>Globule size (nm) ± SD</th>
<th>Self-emulsification time (s)±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capryol&lt;sup&gt;1M&lt;/sup&gt; 90 % (w/w)</td>
<td>Cremophor&lt;sup&gt;2&lt;/sup&gt; RH40% (w/w)</td>
<td>Transcutol&lt;sup&gt;3&lt;/sup&gt; HP % (w/w)</td>
<td>pH 1.2</td>
</tr>
<tr>
<td>F1</td>
<td>10</td>
<td>20</td>
<td>70</td>
<td>69.79± 1.82</td>
</tr>
<tr>
<td>F2</td>
<td>10</td>
<td>40</td>
<td>50</td>
<td>64.12± 0.93</td>
</tr>
<tr>
<td>F3</td>
<td>10</td>
<td>60</td>
<td>40</td>
<td>59.88± 1.57</td>
</tr>
<tr>
<td>F4</td>
<td>10</td>
<td>70</td>
<td>30</td>
<td>53.93± 0.45</td>
</tr>
<tr>
<td>F5</td>
<td>10</td>
<td>80</td>
<td>20</td>
<td>49.65± 0.57</td>
</tr>
<tr>
<td>F6</td>
<td>10</td>
<td>30</td>
<td>50</td>
<td>54.38± 0.55</td>
</tr>
<tr>
<td>F7</td>
<td>10</td>
<td>50</td>
<td>20</td>
<td>53.68± 1.93</td>
</tr>
<tr>
<td>F8</td>
<td>20</td>
<td>30</td>
<td>50</td>
<td>51.62± 1.31</td>
</tr>
<tr>
<td>F9</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>48.56± 0.70</td>
</tr>
<tr>
<td>F10</td>
<td>20</td>
<td>50</td>
<td>30</td>
<td>44.73± 1.46</td>
</tr>
<tr>
<td>F11</td>
<td>20</td>
<td>60</td>
<td>20</td>
<td>42.57± 2.10</td>
</tr>
<tr>
<td>F12</td>
<td>20</td>
<td>70</td>
<td>10</td>
<td>39.79± 0.88</td>
</tr>
<tr>
<td>F13</td>
<td>30</td>
<td>40</td>
<td>30</td>
<td>52.13± 1.41</td>
</tr>
<tr>
<td>F14</td>
<td>30</td>
<td>50</td>
<td>20</td>
<td>51.55± 1.82</td>
</tr>
<tr>
<td>F15</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td>52.57± 1.52</td>
</tr>
<tr>
<td>F16</td>
<td>40</td>
<td>40</td>
<td>20</td>
<td>39.55± 1.18</td>
</tr>
<tr>
<td>F17</td>
<td>40</td>
<td>50</td>
<td>10</td>
<td>38.71± 0.83</td>
</tr>
</tbody>
</table>

SD: standard deviation from the mean; NA: data not available due to low drug loading of less than 50 mg/g. Data are the mean values (n = 3) ± SD. Bold italic values in Table 1 indicate drug loading of more than 50 mg/g.

**Ex vivo intestinal permeability study**

**Non-erverted gut sac model**

*Ex vivo* intestinal permeation studies of AMS-loaded L-SNEDDS were carried out using the non-erverted gut sac technique. The study protocol adhered to the European community standards and the legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU) (European Union Law, 2010) and was approved by the Research Ethical Committee of the Faculty of Pharmacy, Cairo University (approval number PI-959). Overnight-fasted male Wistar rats (200–250 g) were sacrificed by spinal dislocation, followed by removal of the intestine from the upper end of the duodenum to the lower end of the ileum, then manually stripping the mesentery [15]. The removed intestinal part was carefully washed with ice-cold Krebs solution (pH 6.5) using a blunt-ended syringe [16], then divided into 8 ± 0.5 cm long tubes with 3.0 ± 0.5 mm diameter. The prepared tubes were tied from one end using braided silk suture and then filled with 1 ml of selected L-SNEDDS formula (equivalent to 1 mg of AMS) via a blunt needle. The other end of the tube was then tied forming a closed sac which was immersed in 40 ml oxygenated Krebs solution pre-warmed to 37 °C in a jacketed glass assembly. 3 ml samples were withdrawn from the serosal medium (Krebs solution) at predetermined time intervals (20, 40, 60, 80, 100 and 120 min) and replenished with equal volume of fresh Krebs solution. The collected samples were filtered through 0.22 μm membrane filter and the amount of AMS permeated from mucosal to serosal medium was determined by measuring the withdrawn samples using HPLC method. The same experiment was repeated (n = 3) using similar concentration of AMS aqueous Krebs solution.

The concentration of AMS in the withdrawn samples was analyzed by HPLC system using slightly modified HPLC method for AMS determination developed by Devadasu and Ravisankar and was previously validated in terms of specificity, precision, linearity, accuracy, recovery, robustness and system suitability [17]. The HPLC instrument (LC-2000 plus; Jasco Inc., Tokyo, Japan) was equipped with a reversed-phase C18 column (25 cm × 4.6 mm; particle size of 5 μm). The isocratic mobile phase (10 mM phosphate buffer, 0.1 N HCl (pH 1.2) or phosphate buffer (pH 7.4)) was rotated at 100 rpm and maintained at 37 ± 0.5 °C. 5 ml samples were withdrawn at regular time intervals (0.25, 0.5, 1, 1.5, 2, 4, 6 and 8 h) and replaced by an equal amount of fresh dissolution medium in order to maintain sink conditions.
buffer pH 4.0: Acetonitrile; 80:20) was run at a flow rate of 1.0 ml per minute at room temperature and the column effluent was monitored using an ultraviolet (UV) detector set at 280 nm (UV-2070 Plus; Jasco Inc., Tokyo, Japan). The injection volume was 20 μl and the system was equipped with an interface operated by ChromNAV data system software (v 2.0; Jasco Inc., Tokyo, Japan). Under a concentration range of 0.1–20 μg/ml, retention time was 5.6 ± 0.3 min and the calibration curve equation of peak area against AMS concentration ($R^2 = 0.999$) was:

$$Y = 59067.6327X + 9561.5465$$

**Calculation of apparent permeability coefficient ($P_{app}$)**

The apparent permeability coefficient ($P_{app}$) of plain drug solution and AMS-loaded L-SNEDDS was calculated from the following equation [18]:

$$P_{app} = \left( \frac{dQ}{dt} \right) \times \left( \frac{1}{A - C_0} \right) \text{(cm/s)}$$

where $dQ/dt$ is the steady-state rate of drug entrance into the acceptor solution, $A$ is the surface area of the prepared intestinal sacs, and $C_0$ is the initial concentration of AMS inside the sacs. $dQ/dt$ was calculated by taking the slope of the linear portion of the graph plot between the cumulative amounts of drug permeated (μg) through the sac and the time of permeation (minutes). Considering the intestinal sacs are cylindrical in shape with 8 cm length, 1 ml volume and 0.30 cm inner diameter, the surface area ($A$) is calculated to be 7.54 cm$^2$. Statistical analysis of the intestinal permeation results was carried out using the Paired Student’s t-test ($p < .05$).

**Results and discussion**

**Determination of saturated solubility of AMS**

The drug loading per formulation is a very critical factor in the development of nanoeulsion systems for poorly soluble drugs, which is dependent on the drug solubility in various formulation components (oil, surfactant and co-surfactant). The solubilizing capacity of oil is considered as a controlling criterion regarding its selection for nanoeulsion formulation [19]. Results presented in Table 2 revealed that Capryol™ 90 had the highest solubilizing capacity among the tested oils, which could be attributed to the polarity of AMS molecule that might lead to more favorable solubilization in small/medium chain oils, and in mono-glycerides rather than di- or tri-glycerides [20]. Therefore, Capryol™ 90 was chosen as the oil phase for further investigation.

Regarding surfactants, Table 2 demonstrated that Labrasol® had the highest drug solubility, followed by Tween® 80, Cremophor® RH40, and finally Cremophor® EL. Moreover, Transcutol® HP proved superiority in AMS solubilization (68.38 mg/g) compared to the other tested co-surfactants (Table 2).

Since the selection of surfactants and co-surfactants should depend on their emulsification efficiency not only on their drug solubilizing ability [9], screening them for their emulsification power was a must.

**Emulsification ability screening of surfactants and co-surfactants**

Non-ionic surfactants were selected in this investigation since they are generally regarded as safe and considered less toxic than ionic surfactants. They also have lower CMCs which make them more preferable for oral ingestion, they are less affected by pH and ionic strength changes, and are biocompatible [19,20].

Moreover, the four nonionic surfactants selected in this study (Labrasol®, Tween® 80, Cremophor® RH40 and Cremophor® EL) offer reported bioactive effects such as the inhibitory effects on P-gp and CYP enzymes possessed by Cremophor® RH40 and Cremophor® EL [6,21].

After selection of Capryol™ 90 as the oily phase of highest drug solubility, the goal was to identify the surfactant having the highest emulsification efficiency with the chosen oil phase. Table 3 represents variable transmittance values (%) of different oil-surfactant mixtures proving that the tested surfactants have variable emulsification efficiencies towards Capryol™ 90. The largest number of flask inversions and the least UV transmittance (%) were reported for Labrasol®, while on the other hand, very little number of flask inversions and high UV percent transmittance were reported for Cremophor® RH40, Cremophor® EL, and Tween® 80 (99.77%, 98.86% and 90.16% respectively). Although, AMS solubility in the three surfactants Cremophor® RH40, Cremophor® EL and Tween® 80 was lower than in Labrasol® as shown in Table 2, nevertheless, their higher emulsification efficiency motivated their selection for further investigation.

### Table 2. Saturated solubility of AMS in various vehicles.

<table>
<thead>
<tr>
<th>Type of vehicle</th>
<th>Solubility (mg/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMS aqueous solubility</td>
<td>0.18 ± 0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oils</th>
<th>Solubility (mg/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capryol™ 90</td>
<td>29.28 ± 0.66</td>
</tr>
<tr>
<td>Pecol™</td>
<td>17.67 ± 0.15</td>
</tr>
<tr>
<td>Miglyol® 840</td>
<td>0.93 ± 0.26</td>
</tr>
<tr>
<td>Miglyol® 812</td>
<td>0.80 ± 0.21</td>
</tr>
<tr>
<td>Labrafac™ Lipophile WL1349</td>
<td>0.74 ± 0.14</td>
</tr>
<tr>
<td>Isopropyl myristate</td>
<td>0.21 ± 0.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>Solubility (mg/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labrasol®</td>
<td>38.07 ± 0.67</td>
</tr>
<tr>
<td>Tween® 80</td>
<td>35.83 ± 0.50</td>
</tr>
<tr>
<td>Cremophor® RH40</td>
<td>26.65 ± 0.46</td>
</tr>
<tr>
<td>Cremophor® EL</td>
<td>17.13 ± 0.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Co-surfactants</th>
<th>Solubility (mg/g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcutol® HP</td>
<td>68.38 ± 1.54</td>
</tr>
<tr>
<td>PEG 400</td>
<td>44.86 ± 0.26</td>
</tr>
<tr>
<td>PG (Propylene glycol)</td>
<td>27.93 ± 0.30</td>
</tr>
<tr>
<td>Labrafil® M2125CS</td>
<td>5.28 ± 1.68</td>
</tr>
</tbody>
</table>

Data are the mean values ($n = 3$) ± SD. SD, standard deviation from the mean.

### Table 3. Percent UV transmittance and number of flask inversions for emulsions prepared using Capryol™ 90 as oily phase.

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>UV transmittance (%)</th>
<th>Number of flask inversions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cremophor® RH40</td>
<td>99.77 ± 0.19</td>
<td>1</td>
</tr>
<tr>
<td>Cremophor® EL</td>
<td>98.86 ± 0.52</td>
<td>1</td>
</tr>
<tr>
<td>Tween® 80</td>
<td>90.16 ± 0.82</td>
<td>3</td>
</tr>
<tr>
<td>Labrasol®</td>
<td>50 ± 1.41</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

Data are mean values ($n = 3$) ± SD. *Medians of number of flask inversions required for emulsion formation.
Addition of a co-surfactant to the surfactant-containing formulations was reported to improve dispersibility and drug absorption, and obtain nanoemulsion systems at low surfactant concentration (to minimize the toxicity concerns) [5]. Three co-surfactants (Transcutol® HP, PEG 400 and PG) were tested with combination of Capryol™ 90 as the oily phase, three surfactants (Cremophor® RH40, Cremophor® EL and Tween® 80), and Transcutol® HP as co-surfactant. Five ternary phase diagrams were constructed, with their compositions shown in Figure 1(A–E). The ternary phase diagrams revealed the optimum contents and ratios that could be used for the preparation of stable L-SNEDDS. Shaded regions on the diagrams indicate the nanoemulsion regions; larger shaded regions indicate better self-nanoemulsifying ability.

With all the systems having Capryol™ 90 as oily phase and Transcutol® HP as co-surfactant, system (A) having Cremophor® RH40 as a surfactant, showed the largest nanoemulsion region as it could incorporate up to 40% (w/w) of the oily phase with the production of a clear and homogenous system. System (B), having Cremophor® EL, showed smaller nanoemulsion region, while system (C), having Tween® 80, showed the smallest nanoemulsion region. This confirms the previous emulsification power results of different surfactants with Capryol™ 90, which was descending from Cremophor® RH40 (highest), to Cremophor® EL, then Tween® 80 (least) (Table 3).

1:1 mixtures of either Cremophor® RH40 or Cremophor® EL with Tween® 80 (systems D and E) were tested in order to investigate if this will lead to significant improvement in the nanoemulsification ability of the mixture compared to the single surfactant. However, the results presented in Figure 1 illustrated less oil incorporated into the systems compared to using the corresponding surfactant alone (Cremophor® RH40 or Cremophor® EL). This also may be attributed to the higher emulsification ability of any of these surfactants with Capryol™ 90 than Tween® 80 (Table 3).

Furthermore, all the preconcentrates that gave clear nanoemulsion after dilution showed no phase separation after a period of 24 h storage at 25°C. Conclusively, system A (Capryol™ 90/Cremophor® RH40/Transcutol® HP) showed the largest nanoemulsion region compared to the other prepared systems, and so was selected as optimum system for further investigation.

**Construction of ternary phase diagrams**

The self-nanoemulsifying formulations’ fields that could readily emulsify upon dilution and gentle agitation were identified from the ternary phase diagrams of the multiple systems based on Capryol™ 90 as the oily phase, three surfactants (Cremophor® RH40, Cremophor® EL and Tween® 80), and Transcutol® HP as co-surfactant. Five ternary phase diagrams were constructed, with their compositions shown in Figure 1. The ternary phase diagrams revealed the optimum contents and ratios that could be used for the preparation of stable L-SNEDDS. Shaded regions on the diagrams indicate the nanoemulsion regions; larger shaded regions indicate better self-nanoemulsifying ability.

**Determination of drug loading capacity of L-SNEDDS**

Seventeen self-nanoemulsifying formulations observed from the nanoemulsion region of system A (Capryol™ 90/Cremophor® RH40/Transcutol® HP) were assessed for their AMS-loading ability as indicated by the drug saturated solubility in these formulations (Table 1). Generally, it was observed that for each oil concentration, increasing Transcutol® HP concentration was associated with corresponding increase in AMS saturated solubility in the system, although Cremophor® RH40 concentration declined in the same manner. This confirms the dominating solubilizing power of the co-surfactant rather than the surfactant in these systems as presented in Table 2.

Moreover, Table 1 clarifies that all the formulated L-SNEDDS solubilized more than 30 mg/g AMS. However, only seven of the formulations, F1, F2, F3, F4, F8, F13 and F14, succeeded in solubilizing more than 50 mg/g (50 mg is the target dose) and therefore, selected for further investigation.

**Evaluation of AMS-loaded L-SNEDDS**

**Assessment of thermodynamic stability and globule size analysis**

All the seven tested L-SNEDDS (F1, F2, F3, F4, F8, F13 and F14) proved to be thermodynamically stable with no signs of drug precipitation after centrifugation, heating–cooling and freeze–thaw cycles.

Globule size measurement is a very critical step in L-SNEDDS evaluation as it gives a strong indication about the self-emulsification effectiveness which impact the rate and extent of drug dissolution, as well as, drug absorption. Due to considerable pH variations along the GIT, it was rational to observe the consequence of different media pH (1.2 and 7.4) on the formulated SNEDDS. From the globule size analysis results (Table 1), it was observed that all the prepared systems possessed globule size <30 nm at different pH values which fulfill the SNEDDS criteria of having a mean globule size value <200 nm. This might be attributed to the use of the proper surfactant/co-surfactant mixture which reduced the free energy of the system, giving small globule size [23]. It was also noted from Table 1 that the pH change did not show significant effect on the globules sizes (p >.05).

Additionally, Table 1 showed that increasing oil concentration from 10% (F1, F2, F3 and F4) to 20% and 30% (F8, F13 and F14)
resulted in a significantly larger globule size ($p < .05$). Similar results were observed with Chen et al. [24], where they observed that further addition of oil lead to a significant increase in the mean globule size. This could be related to the expansion of oil droplets upon more oil addition [25].

Besides the globule size analysis, PDI was used for the assessment of the investigated diluted systems. PDI values are very useful in expressing the heterogeneity of globule size distribution in the formed nanoemulsions [26]. PDI values at pH 1.2 ranged from 0.092 to 0.244 with the formulations F13, F2 and F8 having the least PDI values of 0.092, 0.168 and 0.193 respectively. Also, in most of the formulations, changing pH from 1.2 to 7.4 resulted in a significant increase in PDI values. This could be attributed to the basic nature of AMS that might lead to slight precipitation at higher pH values causing a broader range of globule size distribution.

**Transmission electron microscopy**

Morphology of the diluted L-SNEDDS was observed using TEM. Photomicrograph in Figure 2 revealed the uniform size distribution and spherical shape of the nanoemulsion globules indicating a stable state of the formed nanoemulsion. The sizes of globules observed in TEM were consistent with the data produced from the globule size analysis.

![Figure 2](image_url). Transmission electron micrograph of the formulated L-SNEDDS (F2) after 100 times dilution with distilled water.

**Determination of self-emulsification time**

The rate of emulsification is considered an important index for the assessment of self-emulsification efficiency [22]. Usually, the formation of liquid crystals and gel phases are the first steps of the emulsification process that considerably influence globule formation and accessibility of the interface for drug to partition [27,28]. As presented in Table 1, self-emulsification time results revealed that all the seven tested formulations possessed rapid self-emulsification time ranging from 30 to 45 s, which is expected to improve the drug absorption.

**Spectroscopic characterization of optical clarity**

Lower absorbance should be obtained with optically clear solutions because cloudier solutions will scatter more of the incident radiation, resulting in higher absorbance [29]. To assess the optical clarity quantitatively, UV–VIS spectrophotometer was used to measure the amount of light of a given wavelength transmitted by the solution. All the formulations showed very low (almost negligible) absorbance values ranging from 0.007 to 0.098 at different pH values (1.2 and 7.4). This indicates that highly clear and stable systems were obtained by aqueous dilution of the prepared SNEDDS, which might be attributed to the small globule sizes of the prepared formulations (<30 nm).

**In vitro drug release study**

The percentage dissolution-time profiles of the nanoemulsion precursors are shown in Figure 3. In 0.1 N HCl (pH 1.2), Figure 3(A), approximately 70% or more of AMS was released after 4 h from all the tested systems. Also, no significant difference in drug release was observed between the tested formulations and the aqueous drug suspension, with $f_2$ values of 52.21, 60.8, 63.61, 59.57, 74.66 and 67.63 for the formulations F1, F2, F3, F8, F13 and F14, respectively, each compared to the aqueous drug suspension. Such good drug release profiles in 0.1 N HCl could be due to the basic nature of AMS that makes it dissolve completely in the acidic pH.

However, results of drug release at pH 7.4 (Figure 3(B)) proved significantly higher drug release from the prepared L-SNEDDS compared to the aqueous drug suspension, with approximately 22–32% increase in AMS release after the 8 h period. The $f_2$ values at pH 7.4 were 36.06, 31.65, 40.77, 39.64, 37.52, 33.45 and 39.29 for the formulations F1, F2, F3, F4, F8, F13 and F14, respectively, compared to the aqueous drug suspension. These findings suggest that AMS-loaded L-SNEDDS could be used as a useful tool for presenting the drug in a solubilized form into the absorption sites.

![Figure 3](image_url). Release profiles of AMS from L-SNEDDS compared to drug suspension in pH 1.2 (a) and 7.4 (b) at 37 ± 0.5 °C.
and avoiding its precipitation at neutral or basic pH medium inside the GIT.

**Ex vivo intestinal permeability study**

A main objective of the current study was to improve the intestinal permeability of AMS as it is proven to be a substrate for the P-gp efflux pump, which might be responsible for the poor oral bioavailability of the drug [4]. Therefore, an *ex vivo* intestinal permeability model was applied in order to investigate AMS permeation–enhancing impact of the formulated L-SNEDDS.

Since no significant differences were observed in the *in vitro* drug release between all the seven tested self-nanoemulsifying formulations in either pH 1.2 or pH 7.4, the selection of formulations for *ex vivo* study was based mainly on safety concerns through selection of formulations with minimum amount of surfactant. Therefore, the self-nanoemulsifying formulations containing the least surfactant concentrations at each oil category (10, 20 and 30%) were selected; three formulations which are F2 (10% Capryol™ 90, 30% Cremophor® RH40 and 60% Transcutol® HP), F8 (20% Capryol™ 90, 30% Cremophor® RH40 and 50% Transcutol® HP) and F13 (30% Capryol™ 90, 40% Cremophor® RH40 and 30% Transcutol® HP). (An exception was made for F2; selected instead of F1 in the 10% oil-SNEDDS category as it had lower PDI values).

*Ex vivo* absorption models including everted and non-everted gut sac techniques are widely used as more simple procedures for investigating various drug transport mechanisms and predicting *in vivo* drug absorption in humans [30]. Non-everted gut sac technique was adopted in this study as it is characterized by several advantages including more simple procedure, less test sample required, easier successive collection of serosal samples and less morphological damage to the intestinal tissue compared to the everted gut sac method [31].

Results of the intestinal permeation study (Figure 4(a)), illustrated that the three tested L-SNEDDS formulations (F2, F8 and F13) exhibited significantly higher intestinal permeability compared to plain drug solution (*p* < 0.05). After the 120 min-period, approximately 252.99, 410.79 and 427.43 mg AMS were permeated from F2, F8 and F13, respectively, compared to only 144.90 mg permeated from the plain drug solution. The increase in AMS permeability among the three tested formulations might be related to the increase in the oil percentage which could have a role in facilitating drug penetration across the lipophilic intestinal barrier.

The apparent permeability (P_{app}) calculated and presented in Figure 4(b), proved that P_{app} of plain drug solution (2.855 × 10^{-6} cm/s) was significantly improved by nearly 1.6 times using F2 (4.643 × 10^{-6} cm/s), 2.9 times using F8 (8.412 × 10^{-6} cm/s), and 2.8 times using F13 (8.229 × 10^{-6} cm/s).

Such significant improvement in AMS permeation from the L-SNEDDS formulations compared to the plain drug solution could be ascribed to (a) Formulation of AMS into L-SNEDDS, delivering the drug to its absorption sites in a completely solubilized form, (b) Presenting the drug in minute nanometric size droplets, which positively affected the permeability of AMS and (c) The use of bioenhancing surfactants and co-surfactants (Cremophor® RH40 and Transcutol® HP) in the formulation that probably improved the intestinal permeation of AMS through the inhibition of the intestinal P-gp efflux pump activity.

**Conclusions**

Amisulpride solubility and intestinal permeability were significantly improved through successful incorporation of the drug into L-SNEDDS containing bioenhancing excipients (Cremophor® RH40 and Transcutol® HP). The formulated systems with droplet sizes of less than 30 nm proved a promising effect in delivering AMS into the GIT in a soluble form with no risk of precipitation, while inhibiting the intestinal P-gp efflux pump activity as proved by the *ex vivo* studies. Further studies for the development and *in vivo* evaluation of solid dosage forms of the optimized AMS-SNEDDS are carried out in another part of this work, which is valuable for providing patient acceptable AMS dosage forms for more effective treatment and enhanced oral bioavailability.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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