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Effect of formulation variables on design, in vitro evaluation of valsartan SNEDDS and estimation of its antioxidant effect in adrenaline-induced acute myocardial infarction in rats

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Abstract
Valsartan is a specific angiotensin II antagonist used for the treatment of hypertension. It suffers from low aqueous solubility and high variability in its absorption after oral administration. The aim of this study was to improve the dissolution and thereby the bioavailability of Valsartan through the development of self nano-emulsifying drug delivery systems. Four ternary phase diagrams were constructed to identify the self-emulsification region of Capmul® MCM, Labrafac® M1944, Capryol® 90 and Labrafac® PG together with Cremophore® RH 40 and Transcutol® HP as oil, surfactant and co-surfactant, respectively. The effect of oil type, oil and surfactant concentration on droplet size and in vitro Valsartan dissolution were studied. The protective effect of the optimum formula F5 in adrenaline-induced oxidative stress in rats during myocardial infarction was determined. Formula F5 exhibited globule size of (13.95 nm) with 76.07% ± 1.10 of Valsartan dissolved after five minutes compared to Disartan 80 mg capsules (13.43%). Results revealed a significant reduction (p < 0.05) in serum aspartate transaminase, creatine kinase myocardial band and malondialdehyde levels, while a significant increase (p < 0.05) in serum glutathione in F5. Therefore, self nano-emulsifying drug delivery systems could be considered as a promising approach to improve the dissolution and thereby the bioavailability of Valsartan.

Introduction
The administration of drugs via oral route is usually the most preferred route as about 80% of common pharmaceutical products are being given orally1. The oral delivery of many drug molecules is recurrently associated with complications of low water solubility leading to poor and highly variable oral bioavailability. Different approaches have been attempted to increase water solubility of poorly water soluble drugs, such as conversion of a crystalline molecule to its amorphous state, particle size reduction, co-solvency, micellar solubilization, complexation, solid dispersion and formulation of lipid based systems2.

Lipid-based formulations, predominantly the self-nanoemulsifying drug delivery systems (SNEDDS), illustrate their potential as alternative approaches for the delivery of hydrophobic drugs. SNEDDS formulations are isotropic mixtures of oil, surfactant, co-surfactant together with the drug. SNEDDS emulsify spontaneously to produce fine o/w nanoemulsions with globule size (2–100 nm) when introduced into aqueous phase under gentle agitation provided by the peristaltic motility of gastrointestinal tract. The small size of the formed droplet provides a large interfacial surface area for drug absorption and therefore increases its bioavailability3.

The chosen surfactant in SNEDDS must be able to lower the interfacial tension to a very small value with achieving a high solubilization potency to aid the dispersion process during the preparation of the nanoemulsion4. Co-surfactants also help in decreasing the interfacial tension between oil and water, adjust the flexibility of interfacial membrane and sometimes reduce the amount of the used surfactant5. Advantages of SNEDDS include: ease of preparation, thermodynamic stability, transparent and elegant appearance, enhanced penetration through the biological membranes, increased bioavailability, less inter- and intra-individual variability in drug pharmacokinetics and bypassing the first pass effect. Such advantages are the reason for which such systems are attractive for use in drug delivery6.

Several studies were carried out to formulate poorly soluble drugs into SNEDDS. Absorption and physical stability of antimalarial compound Halofantrine had been improved when it was formulated in the form of SNEDDS7. Cinnarizine SNEDDS formulations, the antihistaminic drug, showed higher dissolution rate and therefore bioavailability compared to that of the marketed tablet, Stugeron8,9. Optimized SNEDDS formulation of anti-hyperlipidimic Gemfibrozil showed a significant increase in dissolution rate compared to conventional tablets9.

Valsartan is an antihypertensive agent which selectively inhibits type I angiotensin II receptor (AT1). It is a poorly...
water soluble drug belonging to Class II, Biopharmaceutical Classification System (BCS). Valsartan is rapidly absorbed following oral administration with a bioavailability of about 23% and mean half-life of 7.5 h. The usual daily dose ranges from 80 to 160 mg. The drug is weakly acidic, pK<sub>a</sub> = 3.9 and 4.7, and therefore, it is poorly soluble in the acidic environment where its absorption window exists.<sup>10</sup>

Acute myocardial infarction (AMI) results from the prolonged myocardial ischemia with necrosis of myocytes due to interruption of blood supply to an area of heart. The metabolic disarrangements that occur during ischemia predispose for the formation of free radicals and reactive oxygen species leading to the formation of lipid peroxides, damage of cell membrane and destruction of antioxidative defense system resulting in the increase of serum malondialdehyde (MDA), AMI markers [aspartate transaminase (AST) and creatine kinase myocardial band (CK-MB)] and decrease of reduced glutathione (GSH).<sup>11</sup>

The activity of the adrenergic system is increased in AMI which is reflected by raised plasma catecholamine concentration. A renin–angiotensin system (RAS) is present in the heart and its activation leads to increased in the formation of local Angiotensin II. Reactive oxygen species will be formed in excessive amounts resulting in excessive myocardial reperfusion injury. Blocking of Angiotensin II receptors by Valsartan may therefore be effective in reducing oxidative stress during AMI by reducing the formation of reactive oxygen species and thereby inhibiting cell damage which was further confirmed by a marked decrease in aspartate transaminase (AST) and creatine kinase myocardial band (CK-MB) levels in serum.<sup>12</sup>

The aim of this study was to develop Valsartan SNEDDS by studying the effect of some formulation variables and to evaluate their ability to incorporate large amounts of water into oil/surfactant mixtures. Each of the four different oils together with each of the three tested surfactants were mixed at fixed ratio (1:1 w/w) and their ability to incorporate large amounts of water into oil/surfactant mixtures. The surfactant has a great effect on the emulsification process, nano-emulsifying region and droplet size. The surfactants were selected based on their ability to incorporate large amounts of water into nano-emulsifying ability of the selected surfactant.<sup>3</sup> The selected components comprising the four different oils together with Cremophore® RH40 and Transcutol® HP as surfactant and co-surfactant, respectively, were mixed to construct the four different phase diagrams. Phase diagrams were constructed by weighing appropriate amounts of each component into small vials, vortex mixing for 10 min and gently stirring with heating at 37 °C to obtain a homogenous isotropic mixture. For each phase, 36 systems were prepared and stored under ambient conditions for 24 h before visual examination.<sup>19</sup> To determine the

Materials and methods

Materials

Valsartan (Novartis Pharma, Egypt); Capmul® MCM EP and Acconon® MCM-2 EP/NF (Abitec Corp.); Capryol™ 90, Labrafil® PG, Labrafil® M 1944 CS, Transcutol® HP, Lauraglycol® 90 and Labrasol® (Gattefosse, France); Cremophore® RH40 (BASF, Germany); Poly Ethylene Glycol 400 (PEG400) (Fluka, Switzerland); hydrochloric acid (Merck, Darmstadt, Germany); methanol and methylene chloride (Analar, India); adrenaline 1 mg/ml S.C. ampoules (commercially available, CID, Egypt); Rat AST ELISA Assay Kit (Cat. No. 201-11-0595, Sunred Biological Technology Co., Shanghai, China); Rat CK-MB ELISA Assay Kit (Cat. No. KT-12247, Kamiya Biomedical Company, Seattle, WA); GSH Assay Kit (Cat. No. 703002, Cayman Chemical, Ann Arbor, MI); MDA Assay Kit (Cat. No. ab118970, Abcam, Cambridge, UK).

Methods

Solubility studies in oils, surfactants and co-surfactants

The solubility of Valsartan in various oils (Capmul® MCM, Capryol™ 90, Labrafil® M 1944 and Labrasol® PG), 10% surfactant solutions (Cremophore® RH40, Labrasol® and Acconon® MCM-2)<sup>13</sup> and co-surfactants (CoS) (Transcutol® HP, Lauraglycol® 90 and Poly Ethylene Glycol 400) was determined by dissolving an excess amount of Valsartan in 2 g of each of the four oils, surfactants and co-surfactants, respectively, in stoppered vials. The mixtures were continuously stirred using vortex mixer (VM-300, Gemmy Industrial Corp., Taiwan) for 10 min and kept at 37 ± 0.5 °C in water bath shaker (Model 25, Precision Scientific, Dallas, TX) for 72 h to attain equilibrium. The equilibrated samples were centrifuged (model 2041, Centurion Scientific, West Sussex, UK) at 3000 rpm for 15 min and the supernatant was filtered through 0.45 µm membrane filter. The supernatant was suitably diluted with methylene chloride in case of Labrafil® M 1944 and Labrafil® PG oils, or with methanol in case of other excipients. Drug content was quantified spectrophotometrically (UV-1800, Schimadzu, Kyoto, Japan) at 250 nm<sup>14</sup> against a suitable blank.<sup>15</sup>

Screening of surfactants

The surfactant has a great effect on the emulsification process, nano-emulsifying region and droplet size. The surfactants were further screened based on their ability to emulsify the tested oils<sup>16</sup>. In our study, the aim to produce SNEDDS that immediately and rapidly spread in the aqueous medium to give o/w nanoemulsion requires the use of high hydrophilic–lipophilic balance (HLB) surface active agents<sup>17</sup>. Three surfactants [Cremophore® RH40 (HLB = 15), Acconon® MC8-2 (HLB = 14) and Labrasol® (HLB = 12)] were screened based on their ability to incorporate large amounts of water into oil/surfactant mixtures. Each of the four different oils together with each of the three tested surfactants were mixed at fixed ratio (1:1 w/w) to avoid the effect of oil or surfactant concentration on the amount of water incorporated, then water was titrated dropwise with continuous stirring using magnetic stirrer (MS-300HS, Misung Scientific Co., Kyungkido, South Korea) until the mixture changed from transparent to turbid. The maximum amount of water that could be incorporated into the oil/surfactant mixture (o/s mix.) served as one of the criteria in the final selection of the surfactant for SNEDDS development being a second confirmatory test. Percentage of water incorporated was calculated as follows:<sup>18</sup>

\[
\text{Percent of water incorporated} = \left( \frac{\text{wt. of added water}}{\text{(wt. of mixture + wt. of added water)}} \right) \times 100
\]

Screening of co-surfactants

Co-surfactants were screened based on their efficacy to improve the nano-emulsification ability of the selected surfactant<sup>1</sup>. The selected surfactant (Cremophore® RH40) was combined with each of the three different co-surfactants (Transcutol® HP, Lauraglycol® 90 and Poly Ethylene Glycol 400) at a S/Cos ratio of (1:1 w/w) to avoid the effect of surfactant or co-surfactant concentration on the amount of water incorporated. Each S/Cos mixture together with each of the four oils was mixed at a ratio of (1:1 w/w). These mixtures were screened as discussed above under screening of surfactants and the Cos which showed the maximum amount of water incorporated was further selected for the construction of ternary phase diagram<sup>19</sup>.

Construction of ternary phase diagram

The selected components comprising the four different oils together with Cremophore® RH40 and Transcutol® HP as surfactant and co-surfactant, respectively, were mixed to construct the four different phase diagrams. Phase diagrams were constructed by weighing appropriate amounts of each component into small vials, vortex mixing for 10 min and gently stirring with heating at 37 °C to obtain a homogenous isotropic mixture. For each phase, 36 systems were prepared and stored under ambient conditions for 24 h before visual examination<sup>19</sup>. To determine the
effect of drug addition on the nanoemulsion boundary, the phase diagrams were also constructed in the presence of Valsartan with constant drug loading of 4% w/w for all the prepared SNEDDS.

Characterization of the different selected Valsartan SNEDDS

Four formulae were selected from each phase diagram and the composition of the different selected SNEDDS (F1–F16) was given in Table 1. The effect of oil type, oil concentration as well as surfactant concentration on the droplet size and in vitro Valsartan dissolution ($Q_{\text{5min}}$) from the selected SNEDDS was studied.

Robustness to dilution. Robustness to dilution was confirmed by diluting the different selected Valsartan SNEDDS 100 times with 0.1 N HCl. The diluted SNEDDS were stored for 12 h and observed for any sign of phase separation or drug precipitation.

Assessment of self-emulsification efficiency. Turbidity measurement is required to identify the efficiency of the self-emulsification and whether the dispersion has reached equilibrium or not. The selected SNEDDS formulae were diluted 100 times with 0.1 N HCl. The percentage transmittance was measured spectrophotometrically at 638 nm using 0.1 N HCl as a blank.

Assessment of self-emulsification time. Self-emulsification time was assessed using a standard USP dissolution apparatus II (Type PTW, Pharma Test, Hainburg, Germany). One hundred milligrams of each of the selected SNEDDS formula containing 4% w/w Valsartan were loaded onto a watch glass and placed in the bottom of a dissolution vessel containing 500 mL 0.1 N HCl at 37 ± 0.5 °C. Gentle agitation was provided by a standard USP Dissolution Tester, Apparatus II (Rotating paddle) at a rotation of 50 rpm over a period of 50 min. Studies were carried out at 37 ± 0.5 °C in 1 L of 0.1 N HCl (pH 1.2) which was chosen as dissolution medium since it provided better discrimination between the different chosen systems. At appropriate time intervals (3, 5, 7, 9, 11, 13, 17, 21, 25, 29, 33, 41 and 49 min), 2 mL sample was taken, filtered through a 0.2 μm filter and analyzed for Valsartan content by measuring the absorbance at the predetermined wavelength of 250 nm against 0.1 N HCl as a blank.

Viscosity determination. The viscosity of each of the selected Valsartan SNEDDS was determined using Anton Paar rheometer (Model MCR 702, Graz, Austria) connected to spindle no PP25. A certain volume of each system was placed on the plate of the rheometer under ambient conditions. The viscosity of each system was determined by plotting the shear stress versus the shear rate values.

Table 1. Composition of the selected Valsartan SNEDDS systems.

<table>
<thead>
<tr>
<th>Formula no.</th>
<th>Capmul®</th>
<th>Labrafac®</th>
<th>Capryol®</th>
<th>Labrafil®</th>
<th>PG</th>
<th><strong>S</strong></th>
<th><strong>Cos</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>80</td>
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<tr>
<td>F2</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>80</td>
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<tr>
<td>F3</td>
<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>80</td>
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<tr>
<td>F4</td>
<td>30</td>
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<td>30</td>
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<td>F5</td>
<td>–</td>
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<td>–</td>
<td>–</td>
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<td>10</td>
<td>70</td>
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<td>F6</td>
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<td>10</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>70</td>
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<td>F7</td>
<td>–</td>
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<td>60</td>
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<td>F8</td>
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<td>60</td>
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<td>F9</td>
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<td>10</td>
<td>50</td>
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<td>F11</td>
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<td>F12</td>
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<tr>
<td>F13</td>
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<td>10</td>
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<td>–</td>
<td>10</td>
<td>50</td>
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<tr>
<td>F14</td>
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<td>–</td>
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<td>10</td>
<td>50</td>
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<tr>
<td>F15</td>
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<td>10</td>
<td>–</td>
<td>–</td>
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<td>50</td>
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<tr>
<td>F16</td>
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</table>

**S** indicates Surfactant (Cremophore® RH40); **Cos** indicates Co-surfactant (Transcutol™ HP).

Globule size, polydispersity index (PDI) and zeta potential determination. The droplet size of the nanoemulsion is a crucial factor in self-emulsification performance because it determines the rate of drug dissolution and thereby its absorption. The polydispersity values give an indication about the uniformity of droplet size within each system. Zeta potential is a very important factor in characterizing the stability of colloidal dispersions. For the smaller droplet, a high zeta potential will confer stability in the solution or dispersion by resisting aggregation.

In vitro dissolution studies. Each 500 mg SNEDDS system, containing 20 mg drug, was filled into capsules size 00. Dissolution studies of Valsartan from the prepared capsules were performed using the USP Dissolution Tester, Apparatus II (Rotating paddle) at a rotation of 50 rpm over a period of 50 min. Studies were carried out at 37 ± 0.5 °C in 1 L of 0.1 N HCl (pH 1.2) which was chosen as dissolution medium since it provided better discrimination between the different chosen systems. At appropriate time intervals (3, 5, 7, 9, 11, 13, 17, 21, 25, 29, 33, 41 and 49 min), 2 mL sample was taken, filtered through a 0.2 μm filter and analyzed for Valsartan content by measuring the absorbance at the predetermined wavelength of 250 nm against 0.1 N HCl as a blank.

All the dissolution data of Valsartan after 5 min ($Q_{\text{5min}}$) from the selected SNEDDS filled into capsules were compared to that of the commercially available Disartan 80 mg capsules. In vitro dissolution was done in triplicates and the average values (±SD) were taken.

A model independent parameter, the mean dissolution time (MDT), was employed for comparison of dissolution profiles of the different Valsartan SNEDDS with the commercially available Disartan 80 mg capsule and it is calculated according to the following equation:

$$\text{MDT}_{\text{in vitro}} = \frac{\sum_{i=1}^{n} t_{\text{mid}} \Delta M}{\sum_{i=1}^{n} \Delta M}$$

where $i$ is the sample number, $n$ is the number of dissolution sample times, $t_{\text{mid}}$ is the time at the midpoint between $i$ and $i - 1$ and $\Delta M$ is the additional amount of drug dissolved between $i$ and $i - 1$. A low MDT value for a drug delivery system means that it has a fast in vitro drug dissolution.

The dissolution efficiency (DE) of a pharmaceutical dosage form, which is the area under the dissolution curve up to a certain
time \( t \), is expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. DE for SNEDDS compared to the marketed product, Disartan 80 mg capsule, after 5 min \((Q_{\text{min}})\) can be calculated from the following equation\(^{30}\):

\[
DE_T = \frac{\int_0^T y_i \, dt}{\int_{100}^T y_i \, T}
\]

Another model independent parameter, the similarity factor “\( f_2 \)”, which is suggested in order to decide whether the difference between the dissolution profiles of the different systems compared to the market product was being significant or not and was calculated by the following equation:

\[
f_2 = 50 \times \log \left\{ \left[ 1 + \frac{1}{n} \sum_{i=1}^{n} \left( R_i - T_i \right) \right]^{0.5} \times 100 \right\}
\]

where \( n \) is number of dissolution sample times, \( R \) and \( T \) are the dissolution of reference and test products at time \( t \). If “\( f_2 \)” value is between 50 and 100, this suggests that the data of two dissolution profiles are similar\(^{31}\). Statistical analysis of the in vitro dissolution results at \( Q_{\text{min}} \) of the different selected Valsartan SNEDDS was computed by one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) for multiple comparisons at \((p<0.05)\) and unpaired Student’s \( t \)-test. Statistical analysis was performed by the aid of statistical package for social sciences (SPSS) version 14.0 computer program.

**Transmission electron microscopy (TEM).** The morphology of the optimized SNEDDS formula (F5) was examined using transmission electron microscopy (TEM) (JEOL, JEM-1230, Japan). Briefly, an aliquot was prepared after its dilution 100 times with distilled water, sufficient quantity of 1% phosphotungstic acid was added and mixed gently. A drop of the mixture was placed onto the carbon-coated grid and drained off the excess. The grid was allowed to dry. Photographs were taken at suitable magnification\(^{32}\).

**Determination of the antioxidant effect of the optimized Valsartan SNEDDS formula F5 in adrenaline-induced AMI in rats.** The antioxidant effect of Valsartan in adrenaline-induced AMI in rats was determined for the optimized SNEDDS formula (F5) which was composed of Labrafil\(^{R} \) M 1944 (10%), Cremophore\(^{R} \) RH40 (20%) and Transcutol\(^{TM} \) HP (70%) with drug loading (4% w/w) and was compared to Valsartan suspension. The study was carried out according to the work published by Huda and Akhter\(^{12}\).

The research protocol was in accordance and approved by Research Ethics Committee, Faculty of Pharmacy, Cairo University (REC-FOPCU). This study was performed on male Wistar Albino rats \((n=20)\), weight range 150–180 g). Rats were housed in standard polypropylene cages five per cage under standard laboratory conditions of temperature, humidity and light with a free access to standard laboratory diet and water \textit{ad libitum}. The rats were divided into four groups containing five rats each. Group I: received distilled water (2 mL) orally through intragastric tube daily for 14 consecutive days, then distilled water was given subcutaneously in a single dose 24h apart for two consecutive days from 15th day and served as normal. Group II: received distilled water (2 mL) orally through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) was given subcutaneously in a single dose 24h apart for two consecutive days from 15th day and served as control. Group III: received Valsartan SNEDDS formula F5 (equivalent to 30 mg Valsartan/kg)\(^{33}\) after its dispersion in 2 mL distilled water orally through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) was given subcutaneously in a single dose 24h apart for two consecutive days from 15th day and served as treatment. Group IV: received Valsartan (30 mg Valsartan/kg) suspended in 2 mL distilled water orally through intragastric tube daily for 14 consecutive days, then adrenaline (2 mg/kg) was given subcutaneously in a single dose 24h apart for two consecutive days from 15th day and served as standard. All the rats were sacrificed 24h after the last dose under light anesthesia by ether. About 2 mL of blood from each rat was collected in a clean and dry test tube by cervical decapitation. The serum was separated by ultra-centrifugation (4000 rpm for 5 min) and collected by micropipette, transferred to labeled test tubes for biochemical study as follows:

**Estimation of AST level:** AST level was estimated according to the method of Sher and Hung\(^{35}\) using rat AST ELISA assay kit. The concentration of enzyme was measured spectrophotometrically at wavelength 340 nm.

**Estimation of CK-MB level:** CK-MB level was estimated using rat CK-MB ELISA assay kit. The concentration of enzyme was measured spectrophotometrically at wavelength 450 nm.

**Estimation of GSH concentration:** The concentration of GSH was determined using GSH assay kit. The method depends on the fact that GSH react with Ellman’s reagent to form a stable yellow color which can be measured spectrophotometrically at wavelength 412 nm\(^{12}\).

**Estimation of MDA level:** MDA level was measured using the thiobarbituric acid reactive substances (TBARS) assay, as described by Mihara and Uchiyama\(^{35}\). The principle of assay depends on the colorimetric determination of a pink pigment product resulted from the reaction and measured using spectrophotometer at 532 nm\(^{36}\).

All quantitative variables were expressed as mean $\pm$ SD. ANOVA was done for statistical analysis. Post-hoc analysis of differences was done by Least Significant Difference (LSD) test.

**Results and discussion**

**Solubility studies in oils, surfactants and co-surfactants**

The equilibrium solubility of Valsartan in different SNEDDS components was investigated and recorded in Table 2. Regarding the different oils, Valsartan had the significantly highest solubility \((p<0.05)\) in Capmul\(^{R} \) (952 $\pm$ 3.03) followed by Capryol\(^{TM} \) 90 (363.43 $\pm$ 3.81), Labrafil\(^{R} \) M 1944 (68.69 $\pm$ 2.91) and Labrafac\(^{R} \) PG (1.46 $\pm$ 2.14) mg/mL, respectively. Concerning Valsartan solubility in different surfactant solutions and co-surfactants, a significantly higher solubility \((p<0.05)\) in 10% Cremophore\(^{R} \) RH40 solution (29.08 $\pm$ 2.54) and Transcutol\(^{TM} \) HP (1732.85 $\pm$ 3.93) mg/mL, respectively. The four different oils were selected to construct the ternary phase diagrams together with Cremophore\(^{R} \) RH40 and Transcutol\(^{TM} \) HP as surfactant and co-surfactant, respectively.

**Screening of surfactants**

It is obvious from Figure 1 that Cremophore\(^{R} \) RH40, the surfactant with the highest HLB value, showed significantly highest \((p<0.05)\) percentage of water incorporated among the other screened surfactants (Labrasol\(^{R} \) and Acconon\(^{R} \) MCM-2) upon mixing in O:S ratio of (1:1 w/w). Cremophore\(^{R} \) RH40 being the surfactant of choice is reported to be safe, nonionic with low chronic toxicity\(^{16}\).

**Screening of co-surfactants**

Figure 2 shows the percentage of water incorporated in mixtures of (S:Cos mix) in a ratio (1:1w/w) together with each of the
different used oils. It is clear that the mixture containing Transcutol\textsuperscript{TM} HP showed significantly ($p < 0.05$) highest percentage water incorporated compared to the other screened co-surfactants (Lauroglycol\textsuperscript{TM} 90 and Poly Ethylene Glycol 400). It is obvious that the addition of co-surfactant led to an increase in the percentage of water incorporated more than using surfactant alone. This might be due to the fact that the addition of co-surfactant facilitates the penetration of the oil phase into the hydrophobic region of the surfactant monomers, thereby further decreasing the interfacial tension leading to an increase in the fluidity of the interface, enabling the take up of the different curvatures required to form nanoemulsions over a wide range of compositions\textsuperscript{37}.

**Characterization of the different selected Valsartan SNEDDS**

**Visual inspection**

Figure 3(a) shows the different SNEDDS upon diluting 100 times with 0.1 N HCl. Only clear and translucent mixtures [grades (A) and (B)] were selected to determine the effect of drug loading (4\% w/w) as illustrated in Figure 3(b). It is clear that grades (A) and (B) systems exhibit high percentage transmittance above 90\%, which indicates good emulsification ability with globule size in the nanometer range\textsuperscript{19}. Visual observations revealed that no phase separation or drug precipitation was detected upon storage of Valsartan-loaded SNEDDS for 24 h at ambient temperature.

**Assessment of self-emulsification efficiency**

It has been reported that the drug incorporated into the SNEDDS may have some effect on the self-emulsifying performance\textsuperscript{38}. In our study, a little reduction in the nanoemulsion region was observed after drug incorporation with SNEDDS composed of Labrafac\textsuperscript{®} PG or Capryol\textsuperscript{TM} 90 as oils with Cremophore\textsuperscript{®} RH40 and Transcutol\textsuperscript{TM} HP as surfactant and co-surfactant, respectively (Figure 3b). This might be due to the fact that the drug existing in the surfactant may interfere with its capacity to decrease the surface tension and hinder co-surfactant intercalation into the molecules of surfactant to form surface membrane, hence influencing the efficient self-nanoemulsion region\textsuperscript{39}. On the other hand, no significant change in the nanoemulsion region was observed after Valsartan addition for Capmul\textsuperscript{®} MCM and Labrafil\textsuperscript{®} M, respectively. None of the prepared systems, loaded with 4\% w/w Valsartan, showed any drug precipitation within the 12 h storage period after dilution for 100 times with 0.1 N HCl.

Generally, efficient emulsification was attained when the S/Cos concentration was 70\% w/w or more and this is in accordance with Oh et al.\textsuperscript{38} and could be explained by the stabilization of the o/w interface upon increasing the amount of surfactant or co-surfactant leading to the formation of a layer around the emulsion droplets and reducing the interfacial energy as well as providing a mechanical barrier to coalescence.

**Assessment of self-emulsification time**

Assessment of self-emulsification time of the selected Valsartan-loaded SNEDDS does not only indicate the time needed for the emulsification process under gentle agitation but also to ensure the dispersion of Valsartan within the dispersed emulsion without further precipitation. For this purpose, the selected Valsartan-loaded SNEDDS previously mentioned in Table 1 were assessed for their self-emulsification time.

Time needed for detecting 90\% of Valsartan in 0.1 N HCl was chosen as the time required for emulsification as illustrated in Figure 4. Generally, systems with low surfactant concentration up to 30\% w/w (F1, F5, F9 and F13) were emulsified in less than one hour.
minute, in contrary to systems with high surfactant concentration (80% w/w) (F2, F6, F10 and F14) where a longer time (more than eleven minutes) was taken for complete emulsification. Statistical analysis of the data revealed a significant difference in self-emulsification time (p<0.05) between the above systems. This might be due to the fact that systems with high surfactant concentration resulted in gel formation upon contact with water, such an effect was responsible for their slow dispersion, while systems with low surfactant concentration did not form gels and, therefore, dispersed immediately40.

In addition, systems with low oil concentration 10% w/w (F3, F7, F11 and F15) were emulsified in less than three minutes on the other hand, systems with high oil concentration 30% w/w (F4, F8, F12 and F16) took more than three minutes to be emulsified. Statistical analysis of the data revealed a significant difference in self-emulsification time (p<0.05) between these systems. This might be due to that increasing the oil concentration led to an increase in system viscosity that required greater shear forces for dispersion and thus slowing down the system emulsification process41 as shown in Table 3.

It was also reported that high concentration of oil (30% w/w) forms a poor emulsion with reduced ability to entrap water upon dilution42-44. Also, systems with low oil concentration (10% w/w) contain higher co-surfactant concentration of 50% w/w more than systems with high oil concentration that contain only 30% w/w co-surfactant concentration and as reported that increasing the co-surfactant concentration improved the drug dissolution which might be due to the penetration of the co-surfactant into the surfactant monolayer interface, which further enhanced the self-emulsification performance of SNEDDS45.

**Content uniformity**

Concerning the content uniformity, the selected Valsartan SNEDDS filled into capsules were analyzed for their drug content. Results were within the official acceptable range of 85–115%. The actual Valsartan content was found to be the percent of the labeled potency with standard deviation of less than 2.28%, this means that all formulae complied with the pharmacopoeial limits46.

**Globule size, polydispersity index (PDI) and zeta potential determination**

The mean particle diameter, polydispersity index and zeta potential of the selected Valsartan SNEDDS are presented in Table 4. All the selected systems produced nanoemulsion with globule size ranged from 11.99 to 134.7 nm47.

Regarding the effect of oil concentration, it was observed that systems containing 30% w/w oil produced nanoemulsion with larger particle size (17.09, 22.13, 21.81 and 134.70 nm) for F4, F8, F12 and F16, respectively, compared to systems containing 10% w/w oil (13.90, 14.72, 18.75 and 118.10 nm) in F3, F7, F11 and F15, respectively. This might be due to that the penetration of oil molecules into surfactant chain affected the interfacial film influencing the surface curvature of the droplet leading to an increase in particle size48.

Systems with high surfactant or co-surfactant concentration produced nanoemulsion with smaller particle size compared to systems with low surfactant concentration as increasing the amount of surfactant or co-surfactant leading to the formation of a layer around the emulsion droplets and reducing the interfacial energy as well as providing a mechanical barrier to coalescence49. Tang et al. declared a linear relationship between the emulsifier content and the average droplet size, where a gradual decrease in droplet size was observed with increasing amount of emulsifier50.

The polydispersity index of all the selected SNEDDS was found to be below 0.3, which indicates a good uniformity in the droplet size distribution51. The charge of the droplets was found to be negative in value due to the presence of free fatty acids with a zeta potential range of −5.15 to −31 mV. Hence, the SNEDDS would not exhibit any agglomeration as the nanoemulsion was stabilized by a negative zeta potential and steric stabilization effect52.

**In vitro dissolution studies**

The *in vitro* dissolution studies of Valsartan from the selected Valsartan SNEDDS filled into capsules were performed in order to ensure the rapid dissolution of Valsartan into the dissolution medium and to give an idea about the self-nanoemulsification efficiency of the developed systems53. All the dissolution data of Valsartan from the selected SNEDDS were compared to that of the commercially available Disartan 80 mg capsules. All statistical analysis was done using one-way ANOVA and unpaired Student’s *t*-test to study the effect of oil type, oil concentration and surfactant concentration on the percentage of Valsartan dissolved after 5 min (*Q*<sub>5min</sub>) from the different SNEDDS filled capsules. The extent of Valsartan dissolved from the different selected Valsartan SNEDDS is illustrated graphically in

Figure 2. Histogram showing the percentage water incorporated into (a) Capmul® MCM, (b) Labrafil® M 1944, (c) Capryol™ 90 and (d) Labrafac® PG as oils: [Cremophore® RH40/Cos (1:1 w/w)] mixtures at a ratio of 1:1 w/w.
Figures 5–8. It is clear that most of the drug was dissolved within the first five minutes.

Regarding the effect of oil concentration, it is obvious that increasing oil concentration from 10% w/w (formulae F3, F7, F11 and F15) to 30% w/w (formulae F4, F8, F12 and F16) significantly retard the drug dissolution ($p<0.05$), which might be due to the fact that increasing the oil concentration led to an increase in the system viscosity that slows down system emulsification.\textsuperscript{41} This is in agreement with Qi et al. who reported that increasing the viscosity was the reason of retardation of drug dissolution.\textsuperscript{52}

Figure 3. (a) Plain SNEDDS and (b) drug-loaded SNEDDS prepared with different oils using Cremophore\textsuperscript{®} RH 40 as surfactant and Transcutol\textsuperscript{™} HP as co-surfactant.

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Also, systems with low oil concentration containing higher co-surfactant concentration (50% w/w) showed higher drug dissolution than systems with high oil concentration composed of 30% w/w co-surfactant. It had been reported that increasing the co-surfactant concentration improved the drug dissolution due to the penetration of the co-surfactant into the surfactant monolayer interface, which further enhanced the self-emulsification performance of SNEDDS.

Regarding the effect of surfactant concentration, statistical analysis of the data revealed a significant difference in $Q_{5\text{min}}$ ($p<0.05$) between formulae (F1, F5, F9 and F13) containing low surfactant concentration (up to 30%) and formulae (F2, F6, F10 and F14) containing high surfactant concentration (80%). This might be due to the fact that, systems with high surfactant concentration were considered as the most viscous systems; as they resulted in gel formation upon contact with water, such an effect was responsible for the slow dispersion of these formulae into nanoemulsions. While systems with low surfactant concentration did not form gels and, therefore, dispersed immediately.

Based on the in vitro dissolution results, formulae F1, F5, F9 and F13 were chosen as the selected formulae since they contain the least surfactant concentration (up to 30%) and showed the highest drug dissolution after five minutes. The dissolution profiles of Valsartan from the four selected formulae were significantly higher than the commercially available Disartan 80 mg capsule at the selected time intervals ($p<0.05$). After 5 min, it was found that $78.99\% \pm 2.24$, $76.07\% \pm 1.10$, $80.09\% \pm 1.81$ and $69.43\% \pm 2.24$ Valsartan were dissolved from F1, F5, F9 and F13, respectively, while only $13.43\% \pm 0.57$ was dissolved from Disartan 80 mg capsule. The faster the dissolution from SNEDDS might be attributed to the fact the drug was present in a solubilized form and upon exposure to the dissolution medium, small droplets were resulted leading to rapid drug dissolution.

Regarding the effect of oil type, formula F13 prepared using Labrafac® PG as oil showed significantly lower percentage ($69.43\% \pm 2.24$ of drug dissolved after 5 min ($p<0.05$), when compared to the corresponding values of formulae F1, F5 and F9. This might be due to that formulae F1, F5 and F9 have much lower globule size (18.30, 13.95 and 15.10 nm) than formula F13 (121.60 nm), as previously mentioned in Table 4, and it is well known that the behavior of drug is due to the globule size which is inversely proportional to the surface area that means the lesser the globule size the more is the surface area and thus the higher the drug dissolution.

A model independent parameter, the mean dissolution time (MDT), was used to characterize the drug dissolution rate from the dosage form and to predict DE of the prepared systems. Lower MDT for the selected SNEDDS formulae F1, F5, F9 and F13

### Table 3. Viscosity of SNEDDS containing low and high oil concentration.

<table>
<thead>
<tr>
<th>SNEDDS</th>
<th>Oil type</th>
<th>Oil concentration (% w/w)</th>
<th>Viscosity (cp ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>Capmul® MCM</td>
<td>10</td>
<td>40.9 ± 0.35</td>
</tr>
<tr>
<td>F4</td>
<td>Capmul® MCM</td>
<td>30</td>
<td>71.6 ± 0.28</td>
</tr>
<tr>
<td>F7</td>
<td>Labrafac® M 1944</td>
<td>10</td>
<td>39.2 ± 0.24</td>
</tr>
<tr>
<td>F8</td>
<td>Labrafac® M 1944</td>
<td>30</td>
<td>48.3 ± 0.26</td>
</tr>
<tr>
<td>F11</td>
<td>Caproyol® 90</td>
<td>10</td>
<td>35.4 ± 0.25</td>
</tr>
<tr>
<td>F12</td>
<td>Caproyol® 90</td>
<td>30</td>
<td>64.6 ± 0.31</td>
</tr>
<tr>
<td>F15</td>
<td>Labrafac® PG</td>
<td>10</td>
<td>36.7 ± 0.21</td>
</tr>
<tr>
<td>F16</td>
<td>Labrafac® PG</td>
<td>30</td>
<td>46.4 ± 0.21</td>
</tr>
</tbody>
</table>

### Table 4. Globule size, polydispersity index (PDI) and zeta potential determination of the selected Valsartan SNEDDS.

<table>
<thead>
<tr>
<th>Formula no.</th>
<th>Particle size (nm)</th>
<th>Polydispersity index (PDI)</th>
<th>Zeta potential (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>18.30</td>
<td>0.23</td>
<td>-31.00</td>
</tr>
<tr>
<td>F2</td>
<td>13.07</td>
<td>0.13</td>
<td>-15.50</td>
</tr>
<tr>
<td>F3</td>
<td>13.90</td>
<td>0.12</td>
<td>-6.16</td>
</tr>
<tr>
<td>F4</td>
<td>17.09</td>
<td>0.18</td>
<td>-9.65</td>
</tr>
<tr>
<td>F5</td>
<td>13.95</td>
<td>0.11</td>
<td>-12.80</td>
</tr>
<tr>
<td>F6</td>
<td>13.13</td>
<td>0.18</td>
<td>-12.30</td>
</tr>
<tr>
<td>F7</td>
<td>14.72</td>
<td>0.13</td>
<td>-12.10</td>
</tr>
<tr>
<td>F8</td>
<td>22.13</td>
<td>0.12</td>
<td>-7.61</td>
</tr>
<tr>
<td>F9</td>
<td>15.10</td>
<td>0.16</td>
<td>-17.70</td>
</tr>
<tr>
<td>F10</td>
<td>11.99</td>
<td>0.09</td>
<td>-11.70</td>
</tr>
<tr>
<td>F11</td>
<td>18.75</td>
<td>0.21</td>
<td>-5.15</td>
</tr>
<tr>
<td>F12</td>
<td>21.81</td>
<td>0.24</td>
<td>-15.00</td>
</tr>
<tr>
<td>F13</td>
<td>121.60</td>
<td>0.14</td>
<td>-20.90</td>
</tr>
<tr>
<td>F14</td>
<td>16.86</td>
<td>0.16</td>
<td>-9.47</td>
</tr>
<tr>
<td>F15</td>
<td>118.10</td>
<td>0.15</td>
<td>-21.40</td>
</tr>
<tr>
<td>F16</td>
<td>134.70</td>
<td>0.20</td>
<td>-20.90</td>
</tr>
</tbody>
</table>
indicates higher DE and faster solubilization of the drug compared to the marketed product Disartan, as shown in Table 5. The DE was used to evaluate the dissolution performance of the different formulations. Results of DE percent after five minutes (Table 5) revealed that DE of Valsartan from the selected SNEDDS was increased fivefold compared to Disartan 80 mg capsule (8.84%/min).

In order to decide whether the difference between the dissolution profiles for the selected SNEDDS compared with the marketed product is significant or not, another model independent parameter, the similarity factor “f₂” was applied. It is clear from Table 5 that all the selected SNEDDS formulae showed significantly different dissolution profiles, “f₂” value <50, from that of marketed product, Disartan 80 mg.

Based on the above results, formula F5 was selected as the optimized SNEDDS formula taken for further investigations as it exhibited the smallest globule size 13.95 nm with 76.07% ± 1.10 of Valsartan dissolved after five minutes.

Transmission electron microscopy (TEM)
TEM image of the optimized SNEDDS formula (F5) post-dilution in distilled water was performed. It could be seen from Figure 9 that spherical globules were formed with mean globule size of 13.8 nm. Furthermore, no signs of drug precipitation were observed inferring the stability of formed nanoemulsion.

Determination of the antioxidant effect of Valsartan selected SNEDDS formula F5 in adrenaline-induced AMI in rats
Table 6 shows the mean values of serum MDA, GSH, AST and CK-MB 24 h after last adrenaline dose injected. MDA level was 0.62 ± 0.11, 1.13 ± 0.32, 0.62 ± 0.09 and 0.91 ± 0.19 µM/mg for
normal, control, treatment and standard groups, respectively. Statistical analysis revealed a significant difference ($p < 0.05$) between the group treated with Valsartan SNEDDS formula F5 compared to the control and standard groups while a non-significant difference ($p > 0.05$) in mean MDA level between the treatment and normal groups.

Table 5. Mean dissolution time (MDT), dissolution efficiency (DE) and similarity factor ($f_2$) of the selected SNEDDS formulae compared to the market product (Disartan).

<table>
<thead>
<tr>
<th>Formulae</th>
<th>MDT (min)</th>
<th>DE$_{5\text{min}}$ (%/min)</th>
<th>$f_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disartan</td>
<td>194.61</td>
<td>8.84</td>
<td>–</td>
</tr>
<tr>
<td>F1</td>
<td>3.77</td>
<td>45.67</td>
<td>9.07</td>
</tr>
<tr>
<td>F5</td>
<td>3.34</td>
<td>43.47</td>
<td>9.43</td>
</tr>
<tr>
<td>F9</td>
<td>2.99</td>
<td>48.17</td>
<td>7.70</td>
</tr>
</tbody>
</table>

Figure 7. *In vitro* dissolution profiles of Valsartan from SNEDDS prepared using Capryol™ 90, Cremophore® RH40 and Transcutol™ HP.

Figure 8. *In vitro* dissolution profiles of Valsartan from SNEDDS prepared using Labrafac® PG, Cremophore® RH40 and Transcutol™ HP.

Figure 9. Transmission electron microscopy (TEM) image of SNEDDS formula (F5) nanoemulsion post-dilution with distilled water.
Antioxidant effect of Valsartan SNEDDS

Table 6. Serum MDA, GSH, AST and CK-MB after adrenaline administration in different groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma MDA (μM/mg) (mean ± SD)</th>
<th>Erythrocyte GSH (μM/g) (mean ± SD)</th>
<th>Serum AST U/L (mean ± SD)</th>
<th>CK-MB ng/mL (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.62 ± 0.11</td>
<td>3.34 ± 0.42</td>
<td>20.40 ± 2.71*</td>
<td>3.30 ± 0.54*</td>
</tr>
<tr>
<td>Control</td>
<td>1.13 ± 0.32*</td>
<td>1.24 ± 0.06*</td>
<td>110.50 ± 2.12*</td>
<td>22.00 ± 2.82*</td>
</tr>
<tr>
<td>Treatment (Valsartan SNEDDS formula F5)</td>
<td>0.62 ± 0.09</td>
<td>2.77 ± 0.52</td>
<td>33.00 ± 4.36</td>
<td>6.47 ± 0.49</td>
</tr>
<tr>
<td>Standard (Valsartan suspension)</td>
<td>0.91 ± 0.19*</td>
<td>2.06 ± 0.43*</td>
<td>44.00 ± 7.00*</td>
<td>9.10 ± 1.03*</td>
</tr>
</tbody>
</table>

*Means that the groups are significantly different (p<0.05) than the treatment (Valsartan SNEDDS formula F5) group.

Twenty-four hours after the 2nd injection of adrenaline, GSH concentration in the control, treatment and standard groups was 1.24 ± 0.06, 2.77 ± 0.52 and 2.06 ± 0.43 μM/g, respectively, compared to 3.34 ± 0.42 μM/g for the normal group. The treatment and normal groups are not significantly different (p>0.05) from each other while they are significantly different (p<0.05) from the control and standard groups.

Regarding AST level, after the 2nd injection of adrenaline by 24 h, it reached 110.50 ± 2.12, 33.00 ± 4.36 and 44.00 ± 7.00 U/L for the control, treatment and standard groups in contrary to 20.40 ± 2.71 U/L for the normal group. It is obvious from statistical analysis that AST level in the treatment group is significantly (p<0.05) lower than its level in the control and standard groups.

The normal group exhibited CK-MB level equal to 3.30 ± 0.54 ng/mL. After the last dose of adrenaline by 24 h, the level of CK-MB was 22.00 ± 2.82, 6.47 ± 0.49 and 9.10 ± 1.03 for the control, treatment and standard groups, respectively. Results revealed that CK-MB level in the treatment group is significantly lower (p<0.05) than its level in the control and standard groups.

These results proved that Valsartan SNEDDS formula F5 has a highly protective effect in adrenaline-induced oxidative stress in rats during myocardial infarction.

Conclusion

The present study showed that formulation of Valsartan SNEDDS could be a promising strategy in improving its dissolution and hence its bioavailability. The optimized SNEDDS formula F5 prepared using Labrafil® M 1944 (10% w/w) as oil together with Cremophore® RH40 (20% w/w) and Transcutol™ HP (70% w/w) as surfactant and co-surfactant, respectively, showed promising results in terms of globule size, zeta potential, DE and antioxidant effect in adrenaline-induced AMI in rats.

Declaration of interest

The authors report no declarations of interest.

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