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Design and optimization of self-nanoemulsifying drug delivery systems of simvastatin aiming dissolution enhancement

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The aim of this work was to improve the in vitro dissolution of simvastatin through development of self-nanoemulsifying tablets. Various modified oils, surfactant and co-surfactant mixtures were used to prepare different self-nanoemulsifying drug delivery systems (SNEDDS) whose composition was optimized using drug-solubility, ternary phase diagram, system stability and droplet size distribution studies. Optimized SNEDDSs, with acceptable surfactant ratio, stability and particle size (nano-range) upon dilution with simulated gastric fluid (SGF, pH 1.2) under gentle agitation conditions, were loaded onto microcrystalline cellulose and nano-size colloidal silicon dioxide powders using loading factor ($L_f$) = 0.2 and excipient ratio ($R_e$) = 20. Prepared powders were compressed into tablets and the in vitro performance of the prepared self-nanoemulsifying tablets was investigated. Results revealed that systems with 10% relatively polar oils ($C_8$), 60% Cremophore® RH 40 (surfactant), and 30% Transcutol® HP (co-surfactant), acquired good self-nanoemulsification properties either in liquid or tableted forms. Prepared self-nanoemulsifying tablets demonstrated significantly higher dissolution rates, compared to direct compression tablets (DCT) and marketed tablet (Zocor®). In conclusion, self-nanoemulsifying tablets were able to introduce simvastatin successfully in a unique immediate-release solid dosage form.

Key words: Simvastatin, self-nanoemulsifying tablets, self-nanoemulsifying drug delivery systems (SNEDDS), droplet size.

INTRODUCTION

Oral route is the major route of drug delivery for the chronic treatment of many diseases due to the convenience and improved patient safety (Wang et al., 2009). To achieve successful therapeutic outcomes, orally-administered drugs should be well absorbed across the gastrointestinal tract (GIT) and should also provide good oral exposure. Thus, improvement of the aqueous solubility of poorly-water soluble drugs presents one of the most important challenges in field of pharmaceutics, because low aqueous solubility will show dissolution rate-limited absorption in vivo and hence poor absorption, distribution, and targeted-organ delivery (Patel and Patel, 2007). Much attention has been focused on techniques used to increase the drug solubility and dissolution properties, such as solid dispersion (Serajuddin, 1999), anti-solvent (Muhrer et al., 2006), complexation with cyclodextrin (Ammar et al., 2006), and lipid-based formulations (Hauss, 2007). In the recent years, there is a growing interest in the lipid-based formulations for the oral delivery of poorly water-soluble drugs. In fact, the most popular approach is the incorporation of the drug compound into inert lipid vehicles such as oils, surfactant

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dispersion (Nielsen et al., 2008), liposomes (Schwendener and Schott, 1996), microemulsions (Araya et al., 2005), nanoemulsions (Shafiq et al., 2007), self-emulsifying formulations (Kommuru et al., 2001), self-microemulsifying formulations (Wu et al., 2006), and self-nanoemulsifying formulations (Villar et al., 2012). Most of them advantageously increase surface area of the drugs to improve solubilization behavior, as well as permeation (Shafiq et al., 2007).

Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of natural or synthetic oils, surfactants, and co-surfactants. These systems spontaneously emulsify when exposed to gastrointestinal fluids to form oil in water nanoemulsion with nanometric droplet size (Elnaggar et al., 2009), while the digestive motility of the stomach and intestine provides the agitation necessary for self-emulsification−dispersion process (Neslihan Gursoy and Benita, 2004). Drug substances with adequate solubility in lipid/surfactants/co-surfactant blends are candidates for this formulation concept (Rane and Anderson, 2008). SNEDDS exhibited privileges over other delivery systems. They are characterized by ease of manufacture, high solvent capacity, small particle size, and excellent physical stability. In addition, they can enhance permeation across the intestinal membrane, reduce or eliminate food effect and enhance drug bioavailability (Rane and Anderson, 2008; Wasan et al., 2009). However, only very specific pharmaceutical excipient combinations will lead to efficient self-emulsifying systems. This could explain that although many studies have been carried out in this field, there are few drug products on the pharmaceutical market formulated as self-emulsifying formulations (Elnaggar et al., 2009). For selection of a successful self-nanoemulsifying vehicle, it is important to assess the drug solubility in various components, the area of self-emulsifying region in the phase diagram, and droplet size distribution following self-emulsification (Kommuru et al., 2001).

Simvastatin is a poorly-water soluble cholesterol-lowering agent; widely used to treat hypercholesterolemia in animals and humans. When given orally, simvastatin (a lactone) undergoes hydrolysis and is converted to the β-hydroxyacid form, a potent competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase; the enzyme that catalyzes the rate-limiting step of cholesterol biosynthesis (Carlucci et al., 1992; Lin et al., 2012). It is a white to off-white, non-hygrosopic, crystalline powder (Ambike et al., 2005). Water solubility of simvastatin is low, approximately 30 μg/ml (Margulis-Goshen and Magdassi, 2009). Several systems for improving simvastatin solubility have been reported in the literature (Kang et al., 2004; Ambike et al., 2005; Patel and Patel, 2007; Patil et al., 2007; Margulis-Goshen and Magdassi, 2009; Thomas et al., 2013).

In this study, self-nanoemulsifying drug delivery systems (SNEDDS) containing simvastatin were formulated with the objective of improving solubility and dissolution rate of the drug. To obtain such formula, the following steps were conducted; (a) preparation and evaluation of self-emulsifying drug delivery systems (SEDDS) composed of different selected oils, surfactants and co-surfactants, (b) incorporation of simvastatin into optimized SNEDDS composed of different ternary systems, (c) identifying optimal drug loaded SNEDDSs through screening the factors influencing droplet size of the generated emulsions after systems’ dilution, (d) incorporation of optimal drug loaded SNEDDSs into tablets. The in vitro release profiles of simvastatin from different self-nanoemulsifying tablets and the tablets prepared by direct compression (DCT) as well as the marketed tablets (Zocor®) were compared. Additionally, the relationship between the simvastatin dissolution rate from self-nanoemulsifying tablets and droplet size of resulting emulsions was investigated.

**MATERIALS AND METHODS**

**Materials**

Simvastatin was kindly provided by Amriya Pharm. Ind. (Alexandria, Egypt). Propylene glycol monocapryrates (Capryol® 90 and Capryol® PGMC), propylene glycol dicaprylocaprate (Labrafac® PG), medium chain triglycerides (Labrafac® Lipidophile WL 1349), polyoxylglycerides (Labrafac® M 1944 CS and Labrafac® M 2125 CS), propylene glycol monolaurates (Lauroglycol® TM 90 and Lauroglycol® TM FCC), glycerol monolinooleate (Maisine® 35-1), glycerolmonolnooleate (Peccel®), and diethylene glycol monomethyl ether (Transcutol® HP) were kindly donated by Gattefosse Co. (France). Polyoxy 40 hydrogenated castor oil (Cremophor® RH 40), and polyoxy 35 castor oil (Cremophor® EL) were kindly gifted by BASF Co. (Germany). Nano-size colloidal silicon dioxide (Cab-o-sil® H-5) was obtained from Cabot Corporation (Germany). Microcrystalline cellulose (Vivapur® PH 102, MCC), and croscarmellose sodium (Ac-Di-Sol®) were kindly supplied by Al Jazeera Pharmaceutical Industries (Riyadh, Saudi Arabia). All other chemicals used were of analytical grade.

**Solubility studies**

Screening of the liquid vehicles was done by determining the equilibrium solubility of simvastatin in different oils, surfactants, and co-solvents/co-surfactants. An excess quantity of simvastatin was added to each (1.5 ml capacity) capped microfuge tube containing 1 g of each of the selected vehicles. After sealing the tubes, vortex mixer was used to facilitate solubilization of the formed mixtures. Formed suspensions were then shaken at 37°C for 48 h in an isothermal shaking water bath, followed by equilibrium for 24 h. Mixtures were then centrifuged at 12,000 rpm for 15 min at 25°C to remove the undissolved simvastatin. The supernatant was separated and adequately diluted with methanol. Tests were repeated in triplicates and the concentration (w/w) of simvastatin in each vehicle was then quantified spectrophotometrically using UV-visible spectrophotometer (Ultraspex 2100 pro, England) at λmax 238 nm based on a pre-constructed calibration curve ($R^2 = 0.999$).

**Preliminary screening of ternary liquid formulae**

This method was used to investigate relative efficacy of different (oil, surfactant, and co-solvent/co-surfactant) combinations to form
microemulsion or nanoemulsion upon dilution. Homogenous ternary mixtures of selected oils, surfactants, and co-solvents/co-surfactants at mass fraction of 1:2:2, respectively, were blended for all the samples. These mixtures were gently heated at 40°C to facilitate homogenizing the components using vortex mixer. From each isotropic mixture, 100 mg were accurately weighed and diluted 200-folds with simulated gastric fluid without pepsin (SGF, pH 1.2), pre-equilibrated at 37°C, and gently mixed by a magnetic stirrer. The clarity of the formed aqueous dispersion was visually assessed. All experiments were repeated in duplicates, with similar observations being made between repeats.

Construction of ternary phase diagrams

The existence of self-nanoemulsifying formulation fields; that could self-emulsify under dilution and gentle agitation to give SNEDDS, were identified from ternary phase diagrams for formulae containing oil, surfactant, and co-surfactant; each of them, representing an apex of the triangle. Ternary phase diagrams with varying compositions of oil, surfactant, and co-surfactant were constructed. For each diagram, thirty-six ternary systems (S1 to S36) with varying concentrations (10 to 80% w/w) of oil, surfactant, and co-surfactant were prepared such that for any system, the total of three components’ concentration were always added to 100%. The oily phase was mixed with the surfactant/co-surfactant mixture and the ternary system was gently heated at 40°C to facilitate homogenizing the components using vortex mixer. From each system, 100 mg were accurately weighed and diluted 200-folds with SGF (pH 1.2), pre-filtered through 0.22-µm membrane filter (Durapore GVWP, Millipore Corp., USA) and pre-equilibrated at 37°C and gently mixed by a magnetic stirrer.

The clarity of the formed aqueous dispersion was visually assessed using the A, B, C, D, or E grading (Figure 1) to identify the type of tested systems (Mahmoud et al., 2009). All experiments were established in triplicates, with similar observations being made between repeats. Type (A) systems are most likely expected to have particle size less than 50 nm and referred as SNEDDS (Khoo et al., 1998).

Determination of simvastatin saturated solubility in different systems

Excess quantity of simvastatin was added to the obtained type (A) ternary systems, for all ternary formulae (P1 to P8) and mixed using vortex mixer. Formed suspensions were then shaken at 37°C, for 48 h in an isothermal shaking water bath, followed by equilibrium for 24 h. Mixtures were then centrifuged at 12,000 rpm for 15 min at 25°C to remove the undissolved simvastatin. The supernatant was separated and adequately diluted with methanol. Tests were repeated in triplicates and the concentration of the simvastatin was then quantified spectrophotometrically at λmax 238 based on a pre-constructed calibration curve (R² = 0.999).

Preparation of simvastatin-loaded SNEDDS

Simvastatin (5% w/w) was added to pre-prepared and homogenized type (A) systems for all ternary formulae (P1 to P8), and mixed using vortex mixer till the appearance of a clear system. Systems containing simvastatin were then shaken at 37°C for 24 h in an isothermal shaking water bath to ensure complete solubilization, then stored at ambient temperature (25°C).

Evaluation of simvastatin-loaded SNEDDS

Droplet size distribution was used to characterize simvastatin-loaded SNEDDS (drug-loaded type (A) systems). From each system, 100 mg were accurately weighed and diluted as mentioned earlier. The droplet size of resulting emulsions was determined by dynamic light scattering (DLS) at a scattering angle of 173° (Zetasizer Nano-ZS, Malvern, UK) at 25°C, employing an argon laser (λ = 633 nm). Formulations that showed immediate precipitation of the drug or cracking were rejected.

Stability was determined by visual inspection of the resultant nanoemulsions for 12 h. Formulations were considered unstable if they showed any drug precipitation or phase separation within this period of time.

Preparation of self-nanoemulsifying tablets

Calculated quantities (Q) of microcrystalline cellulose (Vivapur® PH 102) were loaded with specific weight (W) of the selected liquid simvastatin-loaded SNEDDS using loading factor (L) = 0.2 (Equation 1) (Spireas and Bolton, 1999).

\[
L_f = \frac{W}{Q}
\]

(1)

After mixing, the resulting wet mixture was blended with the pre-calculated quantities (q) of nano-size colloidal silicon dioxide (Cab-o-sil® H-5) to obtain free flowing dry powder, using excipient ratio (R) = 20 (Equation 2) (Spireas and Bolton, 1999).

\[
R = \frac{Q}{q}
\]

(2)

Finally, 5% w/w of disintegrant (Ac-Di-Sol®) was added to the mixture and mixed for 10 min. Prepared powders were compressed into oval, curve faced tablets of desired weight, using medium-oval convex punches (17.5 mm length × 9 mm width) in a rotary tablet press machine (Rimek Mini Press, Model RSB-4, Kanavati Engineering...
The applied compression force was adjusted to achieve tablet hardness of 40 to 70 N. Batches of 100 tablets, containing 5 mg of simvastatin per tablet, were obtained and exposed to further investigations.

Additionally, direct compression tablets (DCT) were prepared for better comparison, where physical mixtures of drug with all excipients without liquid vehicle were prepared and tableted under the same conditions that were used for the preparation of self-nanoemulsifying tablets.

**Evaluation of self-nanoemulsifying tablets**

**Content uniformity**

The uniformity of drug content in different self-nanoemulsifying tablet formulation was determined by accurately weighing 10 tablets of each formula individually. Each tablet was then crushed and dissolved in methanol, then the solution was filtered through a 0.45-µm filter (Durapore HVHP, Millipore Corp., USA), properly diluted, and then simvastatin content was quantified spectrophotometrically at λ

\[ \text{A}_{238} \] (USP, 2006) based on a pre-constructed calibration curve (R^2 = 0.999).

**Reconstitution properties (droplet size analysis)**

Droplet size of the formed nanoemulsions was investigated after tablets’ reconstitution. Each self-nanoemulsifying tablet was gently mixed with 20 ml of SGF (pH 1.2), filtered, and was subjected to droplet size analysis test using Zetasizer Nano-ZS. Z-average of generated nanoemulsions was compared to that of nanoemulsions generated from dilution of liquid simvastatin-loaded SNEDDS with SGF (pH 1.2).

**In vitro dissolution test**

The dissolution of simvastatin from self-nanoemulsifying tablets was performed in 500 ml of SGF (pH 1.2) at 37 ± 0.5°C using the USP Dissolution Tester (Erweka, model DT 600 HH, Germany), Apparatus II (rotating paddle), at a rotation of 100 rpm. Aliquots from the dissolution medium were withdrawn at 5, 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 min. The withdrawn samples were replaced by equal amounts of dissolution medium to maintain a constant volume. The samples were then filtered through 0.45-µm filter (PVDF membrane, Millipore Corp., USA) and were adequately analyzed, after proper dilution, for simvastatin content spectrophotometrically at λ

\[ \text{A}_{238} \] nm based on a pre-constructed calibration curve (R^2 = 0.999).

**RESULTS AND DISCUSSION**

**Solubility studies**

Self-emulsifying formulation consisting of oil, surfactant, co-solvent/co-surfactant and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the drug in solution after in vivo dilution (Singh et al., 2010). Therefore, appropriate vehicles should have good solubilizing capacity of the drug substance. Also, solubility of drug in such vehicles plays an important role in determining stability of formulation, as many formulations undergo precipitation before undergoing in situ solubilization (Parmar et al., 2011).

Solubilizing capacity of an oily phase is the perspective consideration regarding oil selection (Pouton and Porter, 2008). Figure 2 shows the results of simvastatin solubility tested in ten different oily phases that were commonly utilized in SEDDS and SNEDDS formulations (Chen, 2008). The obtained results showed higher drug solubility in Capryol® PGMC, Capryol® 90, Lauroglycol® 90, Peceol® TM, Maisine® 35-1, and Lauroglycol® FCC, compared to Labrafill® M 2125 CS, Labrafil® M 1944 CS, Labrafac® PG, and Labrafac® Lipophile WL 1349; therefore the former ones were chosen for further investigations.

Four nonionic hydrophilic surfactants with hydrophilic-lipophilic balance (HLB) greater than 10; Cremophor® EL (HLB 12-14), Cremophor® RH 40 (HLB 14-16), Tween® 80 (HLB 15), and Tween® 20 (HLB 16.7), were employed in this study. Results represented in Figure 3 show that Cremophor® EL, Tween® 80, and Tween® 20 exhibited better solubility for simvastatin than Cremophor® RH 40, however, it was reported that Cremophor® RH 40 have a high self-emulsification properties with a wide range of oils and also the use of Cremophor® RH 40 for oral ingestion appeared to be more advantageous than Cremophor® EL (Elaggar et al., 2009). Therefore, the four surfactants were subjected to further screening.

The presence of co-solvent/co-surfactant helps in increasing the solvent capacity of the formulation for the drug (Pouton and Porter, 2008). Acting as co-surfactants, they could also decrease the bending stress of interface and allow the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsion over a wide range of compositions. Also, low or negative interfacial tension and fluidization of the hydrocarbon region of the interfacial film is rarely achieved by the use of surfactant alone, therefore usually necessitating the addition of a co-surfactant (Eccleston, 1994; Kawakami et al., 2002). In this study, solubility of drug in co-solvents/co-surfactants (PEG 400, 1, 2-Propylene glycol, Transcutol® HP, and Span® 20) was examined. From the results depicted in Figure 3 it is clear that amongst all the examined vehicles, Transcutol® HP showed the highest capacity to dissolve simvastatin (212 ± 7 mg/g).

Final selection among different oils would secondly be confirmed according to their self-emulsification properties with other ingredients. Regarding surfactants and co-solvents/co-surfactants selection, drug solubility would come second to the main selection perspective: self-emulsification efficiency (Date and Nagarsenker, 2007).

**Preliminary screening of ternary liquid formulae**

The objective of screening different ternary combinations of oils, surfactants, and co-solvents/co-surfactants was to investigate their ability to form large efficient self-nanoemulsification regions on their ternary phase
Figure 2. Solubility of simvastatin in various oils. Data are expressed as mean ± SD (n = 3).

Figure 3. Solubility of simvastatin in various surfactants and co-solvents/co-surfactants. Data are expressed as mean ± SD (n = 3).

Diagrams (Zhang et al., 2008). This ability could be expected for the ternary mixtures (oil: surfactant: co-solvent/co-surfactant, mass fraction 1: 2: 2) that form clear transparent or translucent emulsion after dilution; denoting the formation of self-nanoemulsion or self-microemulsion.

The efficiency of self-nanoemulsification is controlled by multiple variables including, HLB value of surfactant, lipid–surfactant affinity, and viscoelasticity of the emulsion base (Sánchez et al., 2001). Results in Table 1 show that Cremophor® EL, Cremophor® RH 40, and Tween® 80 surfactants exhibited better self-emulsification efficiency than Tween® 20 due to its higher HLB value (HLB 16.7). Cremophor® EL, Cremophor® RH 40, and Tween® 80 also showed different self-emulsification efficiencies with different lipids used in this study; Cremophor® RH 40 showed high self-emulsification efficiency with Capryol™ 90, Capryol™ PGMC, and Maisine™ 35-1, while Cremophor® EL showed good self-emulsification efficiency with Capryol™ 90, Capryol™ PGMC, and Lauroglycol™ FCC; however, Tween® 80 showed good self-emulsification efficiency only with Capryol™ PGMC and Lauroglycol™ FCC which could be attributed to the difference in lipid–surfactant affinity that led to increased adsorption of surfactants onto droplets of specific oily phases rather than others (Sánchez et al., 2001).

As for the selection of co-solvents/co-surfactants, 1, 2-Propylene glycol, PEG-400 and Span® 20 (HLB 8.6) showed
poor self-emulsifying efficiency compared to Transcutol® HP (HLB 4.2) (Table 1) which could be due to their relatively higher hydrophilic properties, which increase the risk of destroying the emulsion (Zhang et al., 2008). These hydrophilic co-solvents/co-surfactants, being soluble in water, was anticipated to enter the water phase upon dilution and redistribute between the water phase and the emulsion-water interface, resulting in a loss of solvent capacity of the vehicle (Patel and Vavia, 2007). In addition, Transcutol® HP provided the highest drug solubility among all vehicles tested in this study (Figures 2 and 3) and was reported to give optimal SNEDDS formulations (Dixit and Nagarsenker, 2008; Basalious et al., 2010). Therefore, Transcutol® HP was selected as a co-surfactant in development of all SNEDDS formulations aiming to improve the drug loading capabilities and form spontaneous fine nanoemulsions.

Results represented in Table 1 also inferred that the oily phase Capryol® PGMC exhibited the highest emulsification efficiency with all the surfactants employed (except Tween® 20). On the other hand, Lauroglycol® 90 and Peceol® showed very poor emulsification properties with all the surfactants employed. Capryol® 90 and Lauroglycol® FCC exhibited variable emulsification tendency with different surfactants, while Maisine® 35-1 formed emulsion with only one surfactant (Cremophor® RH 40). Generally, the polarity of the lipids decreases with an increase in the number and length of the fatty acid chain (Shen and Zhong, 2006). It was reported that lipids with high polarity seem to be adequate to form a nanoemulsion (Kawakami et al., 2002), also oils of medium carbon chain length and higher HLB values are better than longer chain length and lower HLB values to form SNEDDS (El-Naggar et al., 2009). This could interpret the observed higher self-emulsification properties of Capryol® 90 (C₈, HLB 6), Capryol® PGMC (C₈, HLB 5), and Lauroglycol® FCC (C₁₂, HLB 4) compared to Maisine® 35-1 (C₁₈, HLB 4) and Peceol® (C₁₈, HLB 3). Thus, the poor self-emulsification ability of Peceol® reflected its high hydrophobic property. However, this could not explain the poor self-emulsification ability of Lauroglycol® 90 (C₁₀, HLB 5) that could be attributed to the dependence of self-emulsification properties on the lipid-surfactant affinity, as explained earlier. Moreover, the observed higher self-emulsification ability of Lauroglycol® FCC (with monoesters 45 to 70%) compared to Lauroglycol® 90 (with monoesters > 90%) could be due to the branching; as a result of the presence of higher percentage of diesters, that gave more flexible interfacial films (Wang et al., 2009). This could also explain the observed higher self-emulsification ability of Capryol® PGMC (with monoesters > 60%) compared to Capryol® 90 (with monoesters > 90%) with Cremophor® EL and Tween® 80 as surfactants in the presence of Transcutol® HP as co-surfactant. Thus, Capryol® 90, Capryol® PGMC, Lauroglycol® FCC, and Maisine® 35-1 were selected as oily vehicles due to their good solubility and good self-emulsification efficiency with selected surfactants and co-surfactants upon dilution.

Based on the results of preliminary screening, eight distinct ternary formulae were selected (Table 2) according to their visual assessment grades, which were either A or B (Table 1). Detailed study of these eight ternary formulae; possessing various components in eight different combinations, was carried out via ternary phase diagrams.

### Construction of ternary phase diagrams

Based on the results of solubility studies and preliminary screenings, ternary phase diagrams of the selected ternary formulae namely; P1, P2, P3, P4, P5, P6, P7, and P8 (Table 2) were constructed in the absence of simvastatin. These phase diagrams are depicted in Figures 4 and 5. In this study, the effect of the aqueous phase was ignored and considered as a constant factor for simplicity, where only the oil, surfactant and co-surfactant components were used to identify the self-nanoemulsifying region, after dilution of 200-folds with SGF (pH 1.2), on the ternary phase diagrams.

From the results depicted in Figures 4 and 5, it can be seen that, for ternary formulae having the same oil component, according to the size of the self-nanoemulsifying and self-microemulsifying region of the formulations, the self-nanoemulsifying or microemulsifying efficiency of surfactants was suggested to be in the following order: Cremophor® RH 40 > Tween® 80 > Cremophor® EL which could be due to increasing the HLB value of the surfactant being required for forming a good oil-water emulsion (Kommuru et al., 2001). The wider self-nanoemulsifying region observed with ternary formula P4 compared to others could be attributed to the high polarity of the lipid content (Capryol® 90, HLB 6) and its high lipid-surfactant affinity. However, results obtained with ternary formula P8 indicated that apart from HLB value and type of surfactant, other factors such as structure and relative length of hydrophobic chains of lipid (Maisine® 35-1, C₁₈ glyceryl monolinoate) had influence on emulsification efficiency and thereby self-nanoemulsifying and/or self-microemulsifying regions on the phase diagram (El-Naggar et al., 2009).

Results also deduced that varying ratios of oil provides the largest contribution to self-emulsifying efficiency of the systems. It is noteworthy that oil concentration more than 40% resulted in turbid and crude emulsions that were attributed to increase in the emulsion particle size upon increasing oil content (Hong et al., 2006). Also, the increase in surfactant content increased the clarity of the produced emulsion (Nazzal et al., 2002). This could be explained by the fact that the surfactant stabilizes the oil-water interface and its concentration increased at the interface upon decreasing the oily content in the ternary system, thus decreasing the generated emulsion particle size (Levy and Benita, 1990). However, the existence of
Figure 4. Ternary phase diagrams; P1, P2, P3, and P4.
Figure 5. Ternary phase diagrams; P5, P6, P7, and P8.
Table 1. Classification of systems composed of (oil:surfactant:co-solvent/co-surfactant) at mass fraction of (1:2:2) as grade A, B, C, D, or E upon dilution (“200-folds”) with SGF (pH 1.2).

<table>
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<th>Surfactant</th>
<th>Co-surfactant</th>
<th>Oil</th>
<th>Capryol 90</th>
<th>Capryol PGMC</th>
<th>Lauroglycol 90</th>
<th>Lauroglycol FCC</th>
<th>Peceol</th>
<th>Maisne 35-1</th>
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<td>D</td>
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<tr>
<td>PEG -400</td>
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<tr>
<td>Transcutol HP</td>
<td>C</td>
<td>C</td>
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<td>D</td>
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<td>Span 20</td>
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</table>

*Visual grading system: A, denoting the formation of clear microemulsion or nanoemulsion; B, denoting the formation of translucent microemulsion; C, denoting the formation of less clear bluish white emulsion; D, denoting the formation of bright white emulsion and E, denoting that poor emulsion or even no emulsion was formed.

Table 2. Oils, surfactants and co-surfactants grouped in different combinations for construction of ternary phase diagrams.

<table>
<thead>
<tr>
<th>Ternary phase diagram formula</th>
<th>Oil</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Capryol PGMC</td>
<td>Cremophor RH 40</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P2</td>
<td>Capryol PGMC</td>
<td>Cremophor EL</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P3</td>
<td>Capryol PGMC</td>
<td>Tween 80</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P4</td>
<td>Capryol 90</td>
<td>Cremophor RH 40</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P5</td>
<td>Capryol 90</td>
<td>Cremophor EL</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P6</td>
<td>Lauroglycol FCC</td>
<td>Cremophor EL</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P7</td>
<td>Lauroglycol FCC</td>
<td>Tween 80</td>
<td>Transcutol HP</td>
</tr>
<tr>
<td>P8</td>
<td>Maisne 35-1</td>
<td>Cremophor RH 40</td>
<td>Transcutol HP</td>
</tr>
</tbody>
</table>
more turbid diluted systems upon increasing the surfactant concentration to a certain extent was observed in phase diagrams P2, P3, and P7 (Figures 4 and 5), where similar trends were reported in studies for various self-emulsifying systems (Kommuru et al., 2001; Wang et al., 2009; Parmar et al., 2011). This phenomenon could be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase (Pouton, 1997).

Ternary phase diagrams also corroborated the effect of co-surfactant on the nanoemulsifying area. SNEDDS were easily obtained, using the proper ratio and kind of surfactant and co-surfactant as it was reported that the development of a successful self-emulsifying formulation requires a right blend of low and high HLB surfactant necessary for the formation of a stable microemulsion (Craig et al., 1995; Pouton, 2000). Therefore, using a high HLB surfactant; Cremophor® EL (with an average HLB of 13), Cremophor® RH 40, or Tween® 80 (with average HLB of 15), with a low HLB co-surfactant; Transcutol® HP (with an average HLB of 4.2), have proven to give successful self-nanoemulsifying formulations. Furthermore, co-surfactant (Transcutol® HP) was likely to increase interfacial fluidity of surfactant boundaries in the micelles by penetrating into the surfactant film creating void space among surfactant molecules (Constantinides and Scalart, 1997).

Finally, from the results observed during construction of the ternary phase diagrams that were discussed earlier, it was concluded that:

1. Optimum self-nanoemulsifying and self-microemulsifying performance of ternary formulae (oil, surfactant, and co-surfactant) require the employment of surfactants with high HLB values and oils with high polarity and relatively short hydrophobic chain length, provided that the employed oil and surfactant possess a high lipid-surfactant affinity towards each other.
2. Increasing the oil content in the formed systems results in decreasing the self-nanoemulsifying and self-microemulsifying efficiency of the systems (oil concentration > 40% resulted in turbid and crude emulsions), while in most cases, increasing the surfactant content increased the clarity of the produced emulsion.
3. Using a high HLB surfactant with a low HLB co-surfactant in proper ratio, have proven to give successful self-nanoemulsifying formulations.

From each constructed phase diagram, SNEDDS or type (A) systems were identified and selected for further investigation.

**Determination of simvastatin solubility in different SNEDDS**

Simvastatin-loaded SNEDDS should have stability such that it does not undergo precipitation, creaming or cracking upon dilution. However, in many cases, drug tends to precipitate from the formed nanoemulsion; seed crystals start to appear and might grow to large crystalline materials that will precipitate out at the bottom of the vessel (Parmar et al., 2011). Whether nucleation and crystallization would subsequently occur or not in such a system depends on relative levels of drug solubilized versus its saturation concentration in the system (Narang et al., 2007).

Therefore, simvastatin concentration in the prepared systems should be much less than the saturation concentration of simvastatin in these systems. Accordingly, a fixed simvastatin concentration (5% w/w) was selected to be loaded on all type (A) systems as this concentration was less than the minimal simvastatin saturated solubility observed for type (A) systems (approximately 6.5% w/w) (results not shown) and was expected to provide spontaneous emulsification of SNEDDS with lower possibility of drug precipitation upon aqueous dilution. Using fixed simvastatin concentration for all systems was proposed to exclude the effect of varying the drug concentration on the self-emulsifying efficiency of the systems.

It was also noticed that, when simvastatin was used in concentrations above 5% w/w, mixtures showed a bluish white appearance or even phase separation upon aqueous dilution. This observation agreed with the earlier investigations reported by Wang et al. (2009) who have concluded that above specific drug concentration, the nanoemulsion displays a notable increase in droplet size. It has been also suggested that at high drug concentrations, there is a possibility of precipitation of drug particles in the oil in water interface, where these precipitated drug particles could reduce the flexibility of the surfactant film (Park and Kim, 1999) and result in more compact interfacial films hindering the spontaneous emulsification of SNEDDS (Wang et al., 2009). Therefore, systems formulated to have drug solubilization capacity much higher than the required optimum concentration would be expected to show the least propensity for precipitation (Narang et al., 2007).

**Characterization of simvastatin loaded SNEDDS**

**Droplet size analysis**

The droplet size is a crucial factor in self-emulsification performance, because it predicts the rate and extent of drug release as well as in vivo absorption (Constantinides et al., 1994). The smaller droplet size permits a faster release rate and provides a larger interfacial surface area for drug absorption (Liu et al., 2009).

Table 3 shows the z-average diameter and polydispersity index (PDI) of various emulsions generated from simvastatin-loaded SNEDDS (drug-loaded type (A) systems) from the eight studied formulae (P1 to P8).
Table 3. Ternary composition (%w/w), z-average (Z-av) and polydispersity index (PDI) of simvastatin (5% w/w) loaded type (A) systems for ternary formulae (P1 to P8).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
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<th>S15</th>
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<th>S17</th>
<th>S18</th>
<th>S19</th>
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<tbody>
<tr>
<td>Oil (% w/w)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>SAA (% w/w)</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>30</td>
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<td>50</td>
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<tr>
<td>Co-SAA (% w/w)</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
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<td>60</td>
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<td>60</td>
<td>20</td>
<td>30</td>
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<tr>
<td>P1 Z-av (d. nm)</td>
<td>15.07</td>
<td>15.43</td>
<td>15.84</td>
<td>15.84</td>
<td>20.69</td>
<td>18.20</td>
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<td>—</td>
<td>31.63</td>
<td>29.96</td>
<td>29.24</td>
<td>22.67</td>
<td>22.59</td>
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<tr>
<td>P1 PDI</td>
<td>0.050</td>
<td>0.065</td>
<td>0.171</td>
<td>0.049</td>
<td>0.235</td>
<td>0.075</td>
<td>—</td>
<td>—</td>
<td>0.655</td>
<td>0.583</td>
<td>0.532</td>
<td>0.217</td>
<td>0.037</td>
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<tr>
<td>P2 Z-av (d. nm)</td>
<td>31.00</td>
<td>37.14</td>
<td>37.55</td>
<td>62.13</td>
<td>86.89</td>
<td>115.80</td>
<td>94.06</td>
<td>86.88</td>
<td>—</td>
<td>—</td>
<td>142.00</td>
<td>80.48</td>
<td>52.49</td>
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<tr>
<td>P2 PDI</td>
<td>0.663</td>
<td>0.614</td>
<td>0.757</td>
<td>0.489</td>
<td>0.410</td>
<td>0.472</td>
<td>0.414</td>
<td>0.193</td>
<td>—</td>
<td>—</td>
<td>0.409</td>
<td>0.290</td>
<td>0.110</td>
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<tr>
<td>P3 Z-av (d. nm)</td>
<td>61.85</td>
<td>66.17</td>
<td>67.94</td>
<td>78.49</td>
<td>104.40</td>
<td>**</td>
<td>**</td>
<td>—</td>
<td>—</td>
<td>118.90</td>
<td>87.63</td>
<td>60.35</td>
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<tr>
<td>P3 PDI</td>
<td>0.538</td>
<td>0.536</td>
<td>0.491</td>
<td>0.452</td>
<td>0.435</td>
<td>**</td>
<td>**</td>
<td>—</td>
<td>—</td>
<td>0.622</td>
<td>0.639</td>
<td>0.593</td>
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<td>P4 Z-av (d. nm)</td>
<td>6.89</td>
<td>7.14</td>
<td>14.48</td>
<td>15.10</td>
<td>17.15</td>
<td>35.01</td>
<td>36.11</td>
<td>123.80</td>
<td>42.06</td>
<td>38.54</td>
<td>33.46</td>
<td>19.76</td>
<td>27.06</td>
<td>33.09</td>
<td>35.63</td>
<td>21.76</td>
<td>25.45</td>
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<tr>
<td>P4 PDI</td>
<td>0.130</td>
<td>0.070</td>
<td>0.105</td>
<td>0.147</td>
<td>0.282</td>
<td>0.831</td>
<td>0.638</td>
<td>0.392</td>
<td>0.707</td>
<td>0.769</td>
<td>0.780</td>
<td>0.202</td>
<td>0.166</td>
<td>0.410</td>
<td>0.690</td>
<td>0.146</td>
<td>0.078</td>
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<tr>
<td>P5 Z-av (d. nm)</td>
<td>54.51</td>
<td>51.21</td>
<td>59.16</td>
<td>76.98</td>
<td>94.95</td>
<td>151.54</td>
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<tr>
<td>P5 PDI</td>
<td>0.415</td>
<td>0.290</td>
<td>0.256</td>
<td>0.280</td>
<td>0.359</td>
<td>0.762</td>
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<tr>
<td>P6 Z-av (d. nm)</td>
<td>54.19</td>
<td>27.71</td>
<td>26.95</td>
<td>43.40</td>
<td>65.79</td>
<td>99.91</td>
<td>155.10</td>
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<tr>
<td>P6 PDI</td>
<td>0.919</td>
<td>0.564</td>
<td>0.331</td>
<td>0.427</td>
<td>0.286</td>
<td>0.425</td>
<td>0.497</td>
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<tr>
<td>P7 Z-av (d. nm)</td>
<td>—</td>
<td>102.70</td>
<td>88.30</td>
<td>97.42</td>
<td>117.20</td>
<td>146.10</td>
<td>149.40</td>
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<tr>
<td>P7 PDI</td>
<td>—</td>
<td>0.445</td>
<td>0.463</td>
<td>0.381</td>
<td>0.228</td>
<td>0.387</td>
<td>0.517</td>
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<tr>
<td>P8 Z-av (d. nm)</td>
<td>16.08</td>
<td>16.19</td>
<td>16.39</td>
<td>17.02</td>
<td>26.37</td>
<td>53.47</td>
<td>141.10</td>
<td>—</td>
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<tr>
<td>P8 PDI</td>
<td>0.091</td>
<td>0.044</td>
<td>0.047</td>
<td>0.033</td>
<td>0.328</td>
<td>0.381</td>
<td>0.459</td>
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</table>

Surfactant is denoted as SAA, and Co-surfactant is denoted as Co-SAA. *For each formula (P1 to P8), systems whose z-average and PDI are represented as (—) were not type (A) systems, and those represented as (**) showed phase separation and were not able to be examined.

The relationship between HLBmix and the droplet size of the generated emulsions at different oil concentrations: For each formula, there is an optimum HLBmix value of the surfactant/co-surfactant mixtures that should be achieved to obtain nanoemulsions with the smallest droplet size (Wang et al., 2009). Accordingly, proper ratio of surfactant and co-surfactant results in production of formulation in nano-range droplet size (Taha et al., 2004). The HLBmix values of the surfactant/co-surfactant mixtures could be calculated from the following equation:

$$\text{HLBmix} = f_A\text{HLB}_A + f_B\text{HLB}_B$$ (3)
where HLB_A, HLB_B are the HLB values and f_A, f_B are the weight fractions of surfactant and co-surfactant, respectively. This equation shows that the increase in HLBmix was due to the increase in surfactant (high HLB) concentration with simultaneous decrease in co-surfactant (low HLB) concentration.

Figure 6a and b depicts the relationship between the HLBmix and the z-average diameters of the emulsions generated after dilution of simvastatin-loaded type (A) systems. From Figure 6a, it is clear that, at 10% w/w oil, increasing HLBmix in different SNEDDS formulae decreased the z-average diameter of emulsion formed. However, increasing HLBmix above the value of 10 with ternary formulae P6 and P7 containing Lauroglycol\textsuperscript{TM} FCC (oil), cause a slight increase in the z-average diameter. The decrease in droplet size may be the result of more surfactant being available for adsorption and the formation of a more closely packed surfactant film at the oil–water interface, thereby providing stable and condense interfacial film (Zhao et al., 2010; Gupta et al., 2011), as well as the decrease interfacial tension in the system, both of which in favor of the formation of nanoemulsions with smaller droplet size (Wang et al., 2009; Cui et al., 2011). This decrease in droplet size could be also attributed to the decrease in co-surfactant concentration with subsequent decrease in the expansion of the interfacial film supported by the presence of co-surfactant (Gao et al., 1998).

On the other hand, it is clear from Figure 6b that, systems with higher oil content (20 or 30% w/w) exhibited a very different pattern. Where, an increase in z-average diameter was observed with the increase of HLBmix. Similar trends of increasing the emulsion droplet size with increasing the surfactant concentration were reported in studies for various self-emulsifying systems (Kommuru et al., 2001; Wang et al., 2009; Parmar et al., 2011). This could be attributed to that in the presence of this relatively higher oil content, the decrease of co-surfactant ratio increased the bending stress and rigidity of interface and decreased the flexibility of the interfacial film thus lost its ability to take up different curvatures required to form nanoemulsion (Eccleston, 1994; Kawakami et al., 2002), resulting in emulsions with larger droplet size.

These results inferred an important influence of the HLBmix values and the relative proportion of surfactant to co-surfactant on the droplet size of nanoemulsions generated from drug-loaded SNEDDS. However, from the results depicted in Figure 6a and b, it was observed that both the decrease and the increase in z-average diameter (at 10% w/w oil and at 20 to30% w/w oil, respectively) were much less prominent with increasing Cremophor\textsuperscript{®} RH 40 in formulae (P1, P4, and P8) compared to increasing Cremophor\textsuperscript{®} EL in formulae (P2, P5, and P6) or Tween\textsuperscript{®} 80 in formulae (P3 and P7).

Effect of surfactants and oils structures on the droplet size of the generated emulsions: Selection of surfactant based on their structural features over the HLB has also been a subject of discussion. Wang et al. (2009) observed that surfactant molecular structure had a significant effect on the final emulsion droplet size. This observation is in line with the investigations reported by Dai et al. (1997), Malcolmson et al. (1998) and Warisnocharoen et al. (2000).

From Figure 6a and b, it was also observed that, apart from the effect of HLB value on the droplet size of the formulation, there is a significant difference (p < 0.05) in the emulsion droplet size as a function of surfactant molecular structure. Nanoemulsions with the smallest droplet size were prepared from systems of ternary formulae (P1, P4, and P8); containing Cremophor\textsuperscript{®} RH 40 surfactant, while those with the largest droplet sizes were prepared from systems of ternary formulae (P3 and P7); containing Tween\textsuperscript{®} 80 surfactant even though both surfactants possess the same HLB value. Such small droplet size observed for systems containing Cremophor\textsuperscript{®} RH 40 surfactant may be due to the proper arrangement of co-surfactants specifically along with Cremophor\textsuperscript{®} RH 40 in mixed film around oil–water interface which, in turn, is dependent on molecular structure of both surfactant and co-surfactant (Nepal et al., 2010). It was reported that, effective arrangement of co-surfactant in the films would bring marked reduction in interfacial tension and emulsion droplet size (Nepal et al., 2010). Also, it was observed that ternary formulae containing Cremophor\textsuperscript{®} RH 40 surfactant were able to generate nanoemulsions with almost invariable z-average values over a wide range of HLBmix values and surfactant concentrations (Table 3 and Figure 6a and b).

It was reported that the nature of the oil affects emulsion properties as well (Wang et al., 2009). In this study, although Maisine\textsuperscript{™} 35-1 (C_{18} glycerol monolinoate, HLB 4) is less polar compared to other used oils, however, it was able to give nanoemulsions with small droplet size in combination with Cremophor\textsuperscript{®} RH 40 (ternary formula P8). This could be attributed to the presence of unsaturated linoleic acid backbone (two double bonds), as it was reported that lipids with unsaturated chain would increase the fluidity of surfactant film, and reduce the tendency for chance to crystallize or form liquid crystalline mesophases (Trotta et al., 1999).

**Stability of diluted systems**

SNEDDS possess the risk of in vivo drug precipitation upon dilution in GIT which can lead to failure in bioavailability enhancement (Narang et al., 2007). Moreover, the two phases of the formed nanoemulsion have a tendency for separation to reduce the interfacial area, and hence, free energy of the system (Craig et al., 1995). Hence, stability of the drug loaded SNEDDS was evaluated by keeping it under visual inspection for 12 h after aqueous dilution (200 folds with SGF, pH 1.2) for
Detection of any drug precipitation, phase separation or cracking. It was observed that, systems with surfactant concentration less than 40% were unable to give stable nanoemulsions over the period of 12 h.

**Rational of selecting SNEDDS to be formulated as self-nanoemulsifying tablets**

Selection of different SNEDDS formulations from different ternary formulae was based on the following criteria:

1) SNEDDS from ternary formulae (P1, P4, and P8) containing Cremophor® RH 40 surfactant were provoked to be selected due to the preferential properties of Cremophor® RH 40 including that: (a) Cremophor® RH 40 exhibited the highest self-nanoemulsification efficiency and the smallest nanoemulsion droplet size throughout this study. Also, the change in Cremophor® RH 40 concentration had insignificant effect on the droplet size of the generated nanoemulsion over a wide range of surfactant concentration; (b) The use of Cremophor® RH 40 (polyoxy 40 hydrogenated castor oil) for oral ingestion appeared to be advantageous as it was reported that Cremophor® RH 40 is less readily digested and hydrolyzed, thus, masking the approach of pancreatic
enzymes and preventing drug precipitation and decreased solubilization. This effect may be attributed to the low reactivity of the saturated backbone of Cremophor® RH 40, the high polyethylene oxide content, and the low residual of digestible glycrides content (Elnaggar et al., 2009); (c) This surfactant was supposed to increase lipophilic drug bioavailability not only via solubilization theory but also due to bioactive respects. It is a known inhibitor of cytochrome-3A (CYP3A) (Elnaggar et al., 2009), the enzyme incorporated in dimensioned bioavailability of many drug substrates, including simvastatin (De Angelis, 2004); (d) Cremophor® RH 40 was reported to have a role in improving bioavailability of some drugs formulated as self-emulsifying formulations, such as atorvastatin (Shen and Zhong, 2006), probucol (Nielsen et al., 2008) and tamoxifen citrate (Elnaggar et al., 2009), and was utilized in one of the few marketed SNEDDS products; Neoral® (Elnaggar et al., 2009).

2) Generated nanoemulsions from diluted simvastatin loaded SNEDDS should have z-average diameter less than 50 nm and polydispersity index (PDI) in the range of 0.25 ± 0.05 which indicates that the system had narrow size distribution. It was reported that a poor formulation can be distinguished from an adequate formulation by determining PDI (Pouton and Porter, 2008).

3) The surfactant concentration should range between 40% and 60% (w/w) as it was reported that surfactant concentration (30 to 60%) are necessary to form stable SNEDD (Neslihan Gursoy and Benita, 2004; Tang et al., 2008) and as observed in this study, systems with surfactant concentration less than 40% were unstable over 12 h period after dilution.

4) Surfactant to co-surfactant ratios of 2:1 and 1:1 were selected as these ratios were reported to be optimal and found to yield the desired SNEDDS in previous studies (Taha et al., 2004; Zhang et al., 2008). System P4-S18 (oil: surfactant: co-surfactant, of ratio 30:40:30) was also selected for comparative aspects.

5) Systems should be stable and still have the required properties when the formed quantity was scaled up.

Accordingly, six different simvastatin-loaded SNEDDS as shown in Table 4 were found to match these criteria and were selected for further studies.

### Evaluation of self-nanoemulsifying tablets

#### Content uniformity

In this study, a dose of 5 mg simvastatin per tablet was selected for formulating the self-nanoemulsifying tablets. The rational for using such a low dose was the possible higher bioavailability expected by these self-nanoemulsifying formulations (Kang et al., 2004).

It was observable that all tablet formulae complied with the test of simvastatin content uniformity according to the United States Pharmacopoeia, where none of the individual tablets is outside limits of 90 to 110% of the labeled amount of simvastatin (5 mg per tablet) (USP, 2006). It was claimed that usually the processes involved adsorption of liquid formulation onto carriers give uniform drug distribution; therefore, promote good content uniformity (Fahmy and Kassem, 2008). It was also observed that, there was no distinct difference in drug content uniformity between self-nanoemulsifying tablets and direct compression tablets (DCT) observed in this study.

#### Reconstitution properties (droplet size analysis)

Self-nanoemulsification efficiency of prepared tablets was estimated by determining the droplet size distribution of emulsions generated after tablet reconstitution in SGF (pH 1.2). The z-average diameters and PDI of the diluted liquid SNEDDS and reconstituted tablets are presented in Table 5 which reveals that all self-nanoemulsifying tablets were able to generate nanoemulsions in an acceptable droplet size range (<50 nm). As shown in Table 5 and Figure 7, there was no significant difference (p > 0.05) between droplet sizes of nanoemulsions generated from P4-S3 (14.48 nm) liquid SNEDDS and those generated from their corresponding tablet formulations SNT-14 (15.80 nm), also the difference between droplet sizes of nanoemulsions generated from P1-S3 (15.84 nm) liquid SNEDDS and those generated from their corresponding tablet formulations SNT-4 (22.08 nm) was small compared to other formulations. In addition, their low PDI values indicated uniform droplet size distribution of the

### Table 4. Composition of systems selected to be formulated as self-nanoemulsifying tablets.

<table>
<thead>
<tr>
<th>SNEDDS&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Composition (% w/w)</th>
<th>Oil</th>
<th>Surfactant</th>
<th>Co-surfactant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Capryol PGMC</td>
<td>Capryol 90</td>
<td>Maisine 35-1</td>
</tr>
<tr>
<td>P1-S3</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>60</td>
</tr>
<tr>
<td>P1-S12</td>
<td>20</td>
<td>—</td>
<td>—</td>
<td>40</td>
</tr>
<tr>
<td>P4-S3</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>60</td>
</tr>
<tr>
<td>P4-S12</td>
<td>—</td>
<td>20</td>
<td>—</td>
<td>40</td>
</tr>
<tr>
<td>P4-S18</td>
<td>—</td>
<td>30</td>
<td>—</td>
<td>40</td>
</tr>
<tr>
<td>P8-S3</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>60</td>
</tr>
</tbody>
</table>

<sup>a</sup>SNEDDS are denoted as (Ternary formula symbol – System symbol).
Table 5. Z-average (mean emulsion droplet size) and polydispersity index (PDI) of diluted liquid SNEDDS and their corresponding reconstituted self-nanoemulsifying tablets.

<table>
<thead>
<tr>
<th>Liquid SNEDDS</th>
<th>P1-S3</th>
<th>P1-S12</th>
<th>P4-S3</th>
<th>P4-S12</th>
<th>P4-S18</th>
<th>P8-S3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z- average (d nm)</td>
<td>15.84</td>
<td>22.67</td>
<td>14.48</td>
<td>19.76</td>
<td>21.76</td>
<td>16.39</td>
</tr>
<tr>
<td>PDI</td>
<td>0.171</td>
<td>0.217</td>
<td>0.105</td>
<td>0.202</td>
<td>0.146</td>
<td>0.047</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Self-nanoemulsifying tablets</th>
<th>SNT-4</th>
<th>SNT-9</th>
<th>SNT-14</th>
<th>SNT-19</th>
<th>SNT-24</th>
<th>SNT-29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z- average (d nm)</td>
<td>22.08</td>
<td>62.36</td>
<td>15.80</td>
<td>53.27</td>
<td>45.96</td>
<td>39.58</td>
</tr>
<tr>
<td>PDI</td>
<td>0.377</td>
<td>0.487</td>
<td>0.198</td>
<td>0.577</td>
<td>0.561</td>
<td>0.557</td>
</tr>
</tbody>
</table>

Figure 7. Droplet size distribution of nanoemulsions generated from: (a) dilution of liquid P1-S3 SNEDDS and reconstitution of SNT-4 tablet; (b) dilution of liquid P4-S3 SNEDDS and reconstitution of SNT-14 tablet measured using (Zetasizer Nano-ZS).
Figure 8. Dissolution profiles of simvastatin from different liquisolid tablets, DCT, and marketed tablets in SGF (pH 1.2). Data are expressed as mean ± SD (n = 3).

Figure 9. Correlation between z-average of generated nanoemulsions after reconstitution of self-nanoemulsifying tablets and the 10-min dissolution rate of simvastatin from these tablets.

nanoemulsions generated from reconstitution of these tablets. From these results, it was clear that the self-nanoemulsifying tablets SNT-4 and SNT-14 were able to preserve the good self-nanoemulsifying efficiency of their loaded liquid SNEDDS. This could be probably attributed to the low oil content (10%), high surfactant content (60%), and surfactant to co-surfactant ratio (2:1) of the loaded liquid SNEDDS, as well as the nature of their oils content (Capryol™ PGMC and Capryol™ 90) that possess high polarity and relatively short hydrophobic
chain length (C₈). Therefore, these SNEDDS have proven to be able to retain their self-nanoemulsification properties irrespective of physical form change compared to other liquid SNEDDS due to their optimized properties.

**In vitro dissolution test**

The dissolution profiles of different simvastatin self-nanoemulsifying tablets together with the dissolution profile of simvastatin directly compressed tablets (DCT), and that of simvastatin marketed tablets (Zocor®) are presented in Figure 8. Simulated gastric fluid without pepsin (SGF, pH 1.2) was chosen as a dissolution medium to eliminate the possibility of partial conversion of simvastatin into the more soluble open-ring form that may occur at high pH (Margulis-Goshen and Magdassi, 2009). Also, no dissolution-accelerating components or surfactants, such as sodium lauryl sulfate, were added to the media, because these components result in failure to discriminate between different dissolution profiles as the surfactant is the key element in improving dissolution of SEDDS dosage forms (Atef and Belmonte, 2008; Margulis-Goshen and Magdassi, 2009).

Figure 8 shows that dissolution profiles of simvastatin from self-nanoemulsifying tablets produced constantly superior drug dissolution rate (p < 0.01) compared to that of DCT and that of marketed tablets (Zocor®). Within 10 min, only 26 ± 3 and 33 ± 1% of simvastatin was dissolved from DCT and marketed tablets, respectively; however, simvastatin dissolved from self-nanoemulsifying tablets within the same time period reached 84 ± 3% for SNT-14 tablets. This enhancement in simvastatin dissolution rate and extent could be attributed to the formation of nanoemulsion during dissolution process with droplet size in submicron range induced the presentation of simvastatin at a molecular level in the form of nanoemulsion which lead to an increased solubilization and enhanced drug dissolution rate and extent. However, the comparatively slow simvastatin dissolution from DCT and the marketed tablets can mainly be explained by the poor water solubility of the drug (Dixit and Nagarsenker, 2008; Balakrishnan et al., 2009). This finding also supported the hypothesis that nano-sized droplets of emulsion can enhance the release of poorly soluble drugs (Ghai and Sinha, 2011). Also, as shown in Figure 9, there was a good correlation between z-average of generated nanoemulsions after reconstitution of self-nanoemulsifying tablets and the 10-min dissolution rate of simvastatin from these self-nanoemulsifying tablets (R² = 0.889, slop = -1.334). This indicates that the amount of drug dissolved in the aqueous phase at time t, is inversely proportional to the droplet size of the generated nanoemulsions after tablet reconstitution (Atef and Belmonte, 2008). Thus, this rapid drug release was promoted by the larger interfacial areas present in emulsions with smaller drops (Wang et al., 2009).

**Conclusion**

In this study, formulation factors had a distinct influence on the mean droplet size of nanoemulsions generated from SEDDS upon dilution. Cremophor® RH 40 (surfactant) and Transcutol® HP (co-surfactant) have proven to acquire excellent self-nanoemulsifying efficiency. Oil concentration had a great influence on the pattern by which the surfactant and co-surfactant concentrations affect the droplet size of generated nanoemulsions. In addition, self-nanoemulsifying tablets were able to successfully introduce the SNEDDS into the dissolution media where it was efficiently transformed into nanoemulsion by the gentle agitation provided in the dissolution experiment. Prepared self-nanoemulsifying tablets demonstrated significantly higher dissolution rates, compared to DCT and marketed tablets (Zocor®) and expected to increase simvastatin bioavailability. SNEDDS with low oil content (10%), high surfactant content (60%), and surfactant to co-surfactant ratio (2:1), and with relatively polar and short hydrophobic chain length of oils content (C₈) were able to retain their self-nanoemulsification properties irrespective of physical form change (either liquid or tableted form).

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**ABBREVIATIONS**

d, nm, Diameter in nanometer; DCT, directly compressed tablets; DLS, dynamic light scattering; PDI, polydispersity index; SEDDS, self-emulsifying drug delivery systems; SNEDDS, self-nanoemulsifying drug delivery systems; SNT, self-nanoemulsifying tablet formula; Z-av, z-average (cumulants mean droplet size).

**REFERENCES**


Balakrishnan P, Lee BJ, Oh DH, Kim JO, Hong MJ, Je JY, Kim JA, Yoo BK, Woo JS, Yong CS, Choi HG (2009). Enhanced oral...


United States Pharmacopeia (2006). In: USP 29, NF 24, Rockville, Maryland, USA.


