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Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: Formulation and bioavailability studies

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ABSTRACT

The aim of the present study was to develop a self-nanoemulsifying drug delivery system (SNEDDS) for the oral delivery of Zedoary turmeric oil (ZTO), an essential oil extracted from the dry rhizome of *Curcuma zedoaria*. Pseudo-ternary phase diagrams were constructed to identify the efficient self-emulsification regions. ZTO could serve as a partial oil phase with the aid of the second oil phase to enhance drug loading. Increasing the surfactant concentration reduced the droplet size but increased the emulsification time, while the reverse effect was observed by increasing the co-surfactant concentration. Based on the emulsification time, droplet size and zeta potential after dispersion into aqueous phase, an optimized formulation consisting of ZTO, ethyl oleate, Tween 80, transcutol P (30.8:7.7:40.5:21, w/w) and loaded with 30% drug was prepared. Upon mixing with water, the formulation was rapidly dispersed into fine droplets with a mean size of 68.3 ± 1.6 nm and ξ-potential of -41.2 ± 1.3 mV. The active components remained stable in the optimized SNEDDS stored at 25 °C for at least 12 months. Following oral administration of ZTO-SNEDDS in rats, both AUC and C_{max} of germacrone (GM), a representative bioactive marker of ZTO, increased by 1.7-fold and 2.5-fold respectively compared with the unformulated ZTO.

1. Introduction

Zedoary turmeric oil (ZTO), an essential oil extracted from the dry rhizome of *Curcuma zedoaria*, is a mixture of structurally diverse compounds which are volatile and unstable under ambient condition (Yang et al., 2005). Pharmacological and clinical studies indicated that ZTO exhibits a wide array of therapeutic activities, such as hepatoprotection, tumor suppression, antioxidation and anti-bacterial action (Li et al., 2002; Zhao et al., 2006). The main bioactive components in ZTO identified thus far include neocurdione (NCD), curdione (CD), germacrone (GM), curzerene (CZ), furanodiene (FD) and β-elemene (β-E) (Xiao et al., 2007; Li et al., 2002; Hisashi et al., 1998; Mau et al., 2003).

Clinically, ZTO is administered mostly in the form of a glucose injection which is documented in the Chinese Pharmacopoeia (2005) with germacrone (GM) as the official quality control marker for the product. However, severe side-effects have been reported in recent years for the ZTO glucose injection, and they appeared to be linked to the high concentration of the solubilizer (Tween 80) used and the poor stability and clarity of the preparation (Liu et al., 2003; Zhang et al., 2005; Li and Zhou, 2006). Therefore, development of an alternative dosage form for ZTO is urgently required to circumvent all these formulation and safety problems associated with the injection.

A recent review has summarized the formulation strategies that may be used to tackle the formulation challenges with essential oils (Wu et al., 2008). Some of these strategies have yielded encouraging results for ZTO. For instance, a sustained-release self-emulsifying microsphere has been prepared to increase and prolong the oral absorption of ZTO *in vivo* (You et al., 2006). In addition, a chitosan-alginate nanocapsule has been formulated to increase the drug loading and stability of ZTO *in vitro* (Lertsutthiwong et al., 2008). Apart from these two approaches, the self-nanoemulsifying drug delivery system (SNEDDS), which is well known for its potential to improve the aqueous solubility and oral absorption of lipophilic drugs (Pouton, 2000), is also worth considering. SNEDDS is an isotropic mixture composed of oil, surfactant, co-surfactant and drug. It can readily disperse in the aqueous environment of the gastrointestinal tract to form a fine oil-in-water emulsion with a droplet size less than 100 nm under gentle agitation for improving the oral bioavailability of poorly water-soluble drugs (Shah et al., 1994; Constantinides, 1995). Compared to conventional metastable emulsions, SNEDDS is a thermodynamically stable formulation with high solubilization capacity for lipophilic drugs, and also can

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be filled directly into soft or hard gelatin capsules for convenient oral administration. Several SNEDDS-based products, e.g., Sandimmune Neoral® (cyclosporin), Norvir® (ritonavir) and Fortovase® (saquinavir), have been successfully commercialized, which has boosted the interest in increasing the utilization of this formulation strategy (Gursoy and Benita, 2004).

While considerable efforts have been made to develop SNEDDS for solid lipophilic drugs, few of them have considered SNEDDS for liquid lipophilic components, e.g., essential oils in Chinese herbal medicines, to improve their stability and oral bioavailability. Moreover, a liquid formulation such as SNEDDS is probably more suitable to be used for essential oil to prevent or minimize its volatility, which is normally not achievable with the solid preparation. Using ZTO as a model essential oil, the objectives of the present study were (1) to explore the feasibility of using ZTO as the sole or partial oil phase for developing a stable and readily water-dispersible SNEDDS formulation with high drug loading for oral administration; (2) to study the effects of weight ratio of surfactant, co-surfactant and ZTO on the performance of nanoemulsion (in terms of droplet size, emulsification time, zeta potential) *in vitro*; and (3) to evaluate the relative oral bioavailability of the developed ZTO-SNEDDS versus the unformulated oil in rats.

2. Materials and methods

2.1. Materials

ZTO, extracted from *Curcuma wenyujin*, was purchased from Tianren Company (Zhejiang, China). The reference compounds, NCD, CD, GM, CZ, FD and β-E were prepared in-house with a minimum purity of 98% by gas chromatography–mass spectrometry (GC–MS) (Yang et al., 2005). Ethyl oleate, olive oil, soybean oil, Tween 80 and Tween 85 were obtained from Sigma (St. Louis, MO, USA). Labrasol®, Miglyol® 812/840 and transcutol® P were kindly donated by Gattefossé (Cedex, France). Neobee M5 was obtained from Bronson & Jacobs (HK). Cremophor® EL was a gift sample from BASF (Ludwigshafen, Germany). All the excipients and reagents were used as received. All methanol and acetonitrile used were of HPLC grade. Water was freshly prepared using a Milli Q-water purification system (Millipore, MA, USA).

2.2. HPLC analysis

An Agilent 1100 HPLC system with an Agilent C_{18} column (Zorbax ODS, 5 μm, 250 mm × 4.6 mm I.D.) coupled with a guard column (Zorbax ODS, 5 μm, 20 mm × 3.9 mm I.D.) were used. The mobile phase consisting of water (A) and acetonitrile (B) was pumped at a flow rate of 1 ml/min with gradient elution as follows: 45–35% A at 0–15 min, decreased linearly to 20% at 25 min, decreased to 0% at 45 min. Sample (10 μl) was injected into the column for detection of the six components simultaneously at 214 nm with a diode array detector (DAD). All six active components (NCD, CD, GM, CZ, FD, β-E) were well resolved in the HPLC chromatograms (Fig. 1). All standard curves showed excellent linearity with an $R^2 > 0.999$. The RSDs of inter- and intra-day precision and accuracy for the six active components were all less than 3% at low, medium and high concentrations.

2.3. Preparation of SNEDDS for ZTO

2.3.1. Miscibility studies

ZTO (1 ml) was mixed with each of the oil, surfactant or co-surfactant (1 ml) by vortexing for 10 min. The mixture was then equilibrated at room temperature for 24 h. Then resulting mixture was examined visually after centrifugation at 3500 rpm for 10 min.

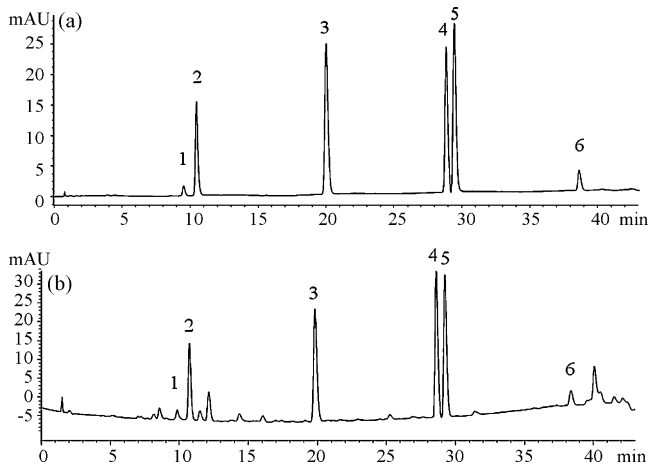

Fig. 1. Representative chromatograms of mixed standard preparations (a); SNEDDS of ZTO (b) (key: peaks 1–6 are NCD, CD, GM, CZ, FD and β-E, respectively).

2.3.2. Construction of pseudo-ternary phase diagrams

A series of self-emulsifying systems were prepared for each of the five formulas (Table 1) with varying weight percentages of oil from 25 to 70%, surfactant from 30 to 75%, co-surfactant (Co-S) from 0 to 25% and drug (ZTO was fixed at a weight ratio of 3:10 to the total amount of oil phase except for the formulation containing ZTO only as the lipid phase) at room temperature. Above mixture (0.1 ml) was gently mixed with 100 ml of distilled water in a glass beaker at room temperature. The tendency to emulsify spontaneously and the progress of emulsion droplets spread were visually assessed using the grading criteria of Khoo and Andrew (1998) and Shafiq et al. (2007). If a clear and slightly bluish or a slightly less clear and bluish white microemulsion (i.e., a transparent or semitransparent dispersed system) is rapidly formed (within 1 min), the corresponding region in the phase diagram will be labeled as "A" to describe the best efficient self-emulsification region. If a bright white emulsion (fine opaque emulsion or coarse emulsion) is formed within 2 min, the region will be labeled as "B" and will still be considered to have met the criterion for self-emulsification. However, if a dull, grayish white emulsion with a slightly oil appearance (i.e., a formulation exhibiting either poor or minimal emulsification with large oil droplets floating on the surface) is formed slowly (i.e., longer than 2 min), the composition for this formulation will be labeled as "C" in the phase diagram.

2.3.3. Effect of different formulae on the droplet size and zeta potential

To investigate the effect of surfactant concentration on the droplet size and zeta potential, formulations without co-surfactant were prepared. The surfactant concentration was raised at increments of 5% while the oil phase concentration was decreased by the same percentage without the co-surfactant to keep the total component concentration constant at 100%. To investigate the effect of co-surfactant, the concentration of co-surfactant was increased at increments of 5% while the surfactant amount was decreased by the same percentage. Except for the formula with ZTO alone as oil phase, the content of ZTO was fixed at the weight ratio of 30% in

Table 1
Compositions of various SNEDDS formulations.

Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
ZTO	ZTO	ZTO	ZTO	ZTO
Miglyol 812	Miglyol 812	Ethyl oleate	Ethyl oleate	
Tween 80	Tween 85	Tween 80	Tween 85	Tween 80
Transcutol P	Transcutol P	Transcutol P	Transcutol P	Transcutol P

total oil phase for the above measurements. In addition, to assess the effect of the ZTO on the droplet size and zeta potential, the level of ZTO was increased from 0 to 100% (weight ratio in the total oil phase) in various formulations.

2.4. Droplet size and zeta potential analysis

The average droplet size and polydispersity index (PDI) of SNEDDS were measured by photon correlation spectroscopy (PCS) using a Malvern Zetasizer (Nano ZS90, Malvern instruments Ltd., UK) with a 50 mV laser. The PDI reflects the uniformity of particle diameter and can be used to depict the size distribution of microemulsion population. The sensitivity range is 10 nm to 5 μm and the data are shown by computer calculation using the Mie equations of light scattering. The measurements were performed at 25 °C at a fixed angle of 90°. The measurement time was 2 min and each run underwent 12 subruns. The formulation (0.1 ml) was dispersed into 100 ml of water under gentle stirring in a glass beaker. Then a 1 ml aliquot was withdrawn and added into a sample cell for droplet size measurement. Each size value reported was the average of at least three independent measurements. Zeta potential measurements were carried out on the same diluted sample using the same equipment and operating conditions, and the zeta potential values were calculated according to the Smoluchowski equation.

2.5. Transmission electron microscopy (TEM) of SNEDDS

Transmission electron microscopy (H-600, Hitachi, Japan) was employed to study the morphology of the resulting nanoemulsion. Prior to the analysis, the SNEDDS samples were diluted 1000 times with water to form an emulsion, stained with 2% (w/v) phosphotungstic acid for 30 s and placed on 400-mesh copper grids with films for observation.

2.6. In vitro dispersibility studies

Dispersibility of ZTO active components from SNEDDS into aqueous media was assessed using a standard dissolution tester with apparatus I (DT 7061000LH, Erweka, Germany) as described in the US Pharmacopeia (USP29/NF24, 2006). Hard gelatin capsules, freshly filled with formulated ZTO (~650 mg; equivalent to 200 mg essential oil) were gently agitated inside a standard stainless steel rotating basket at 100 rpm in 900 ml purified water, simulated gastric fluid (0.1 M HCl, pH 1.2, enzyme-free) or simulated intestinal fluid (phosphate buffer, pH 6.8, enzyme-free) at 37 °C. Samples (3 ml) withdrawn at intervals of 5, 10, 15, 20, 30, 45, 60, 90 and 120 min were filtered using a 0.45 μm Millipore filter and analyzed by HPLC. All dissolution experiments were performed in triplicate.

2.7. Stability study

Short-term stability of the unformulated ZTO in amber glass vials was assessed at 25 °C over a 10-day period. Long-term stability study was performed by storing the ready-to-use SNEDDS formulations in the sealed amber glass vials at 25 °C. The physical stability was determined by monitoring the time-dependent change in the physical characteristics (e.g., phase separation of the essential oil) of the SNEDDS formulations. The chemical stability of the active components was analyzed by HPLC.

2.8. Bioavailability studies

All the animal studies were approved and supervised by the Institutional Animal Care and Use Committee of Sichuan University (Sichuan, China). Male Sprague–Dawley rats (250 ± 20 g) were obtained from Laboratory Animal Center of Sichuan University (Sichuan, China). The rats were housed under standard conditions of temperature, relative humidity and light, and had free access to standard rodent diet and water before the experiment. Rats were fasted overnight prior to drug dosing.

Since GM is the official quality-control marker for the ZTO injection product (Chinese Pharmacopoeia, 2005), the concentrations of GM in rat plasma after oral administration of the formulated or unformulated ZTO were determined by HPLC-DAD. A Waters 2896 HPLC system with a Diamonsil C_{18} column (5 μm, 200 mm × 4.6 mm I.D.) coupled with a guard column (YWG-C_{18}, 10 μm, 10 mm × 6.0 mm I.D.) were employed. The mobile phase consisting of 25% water and 75% acetonitrile (w/w) was eluted at a flow rate of 1 ml/min. UV absorption was measured at 214 nm. Plasma (0.1 ml) was added to acetonitrile (0.15 ml) containing tanshinone IIA (0.1 ml, 20 μg/ml) as internal standard. After vortex mixing for 30 s and subsequent centrifugation at 10,000 rpm for 10 min at 4 °C, 20 μl of the supernatant was injected for HPLC analysis.

GM from ZTO and internal standard in rat plasma were well resolved in HPLC chromatogram. The method showed good linearity ($R^2 > 0.999$) in the range of 0.040–1.60 μg/ml in rat plasma. LOQ and LOD were 40 and 20 ng/ml, respectively. The mean extraction recoveries of GM were 91.8% and 91.3% at 0.10 μg/ml and 0.40 μg/ml, respectively. The inter- and intra-day RSD for GM was less than 3.20% at the above two concentrations. After storage for 72 h at −20 °C and three freeze–thaw cycles, GM remained stable in the plasma.

Bioavailability of ZTO-SNEDDS was compared against the unformulated ZTO. Rats were divided randomly into two treatment groups with six rats each. Unformulated ZTO and ZTO-SNEDDS were administered intra-gastrointestinally at a dose of 500 mg/kg and 1625 mg/kg (equivalent to 500 mg/kg ZTO) in the aqueous solution, respectively. Blood was withdrawn from tail vein into heparinized centrifuge tubes at 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 12, 18, 24 h after oral dosing. Blood samples were centrifuged at 3500 rpm for 10 min to separate the plasma and stored at −20 °C. The plasma concentrations of GM and internal standard were determined using the methods described above. The pharmacokinetic parameters were computed by DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, China) using the trapezoidal rule (for AUC calculation). The relative bioavailability, *F*, of ZTO-SNEDDS was calculated according to Eq. (1).

$$F = \frac{\mathrm{AUC_{SNEDDS}}}{\mathrm{AUC_{ZTO}}} \times 100\% \quad (1)$$

where AUC is the area under the plasma concentration–time curve.

2.9. Statistical analysis

The pharmacokinetic data of the two formulations were compared by the Student's *t*-test. A *p*-value of less than 0.05 was considered as statistically significant.

3. Results and discussion

3.1. Miscibility studies and excipient selection

Excipient screening and formulation optimization of SNEDDS should be based on the following criteria: (1) the formulation composition should be simple and safe (e.g. using the least amounts of surfactant and co-surfactant to produce the nanoemulsion); (2) it should afford a large nanoemulsion area in the phase diagram and no phase separation should be visible after storage for 24 h; (3) it should be able to achieve high drug loading, small and uniform droplet size (i.e., <100 nm, small polydispersity index) and

rapid dispersion upon dilution with an aqueous medium (Wu et al., 2008; Xi et al., 2009).

In the oil phase tested, both the medium and long chain fatty acid glycerides (as represented by miglyol 812/840 and ethyl oleate, respectively) formed homogenous, transparent and flowable mixtures with ZTO, as confirmed by visual observation. However, the two natural oils used, namely, olive oil and peanut oil, could not mix well with ZTO. Neither Captex 200 nor Captex 355 formed transparent mixtures with ZTO. Since both miglyol 812 and ethyl oleate are safe food additives (i.e., with approved GRAS or generally-recognized-as-safe status), and their long aliphatic chains can potentially promote lipoprotein synthesis and lymphatic absorption (Charman and Stella, 1991), they were selected as the oil phase in the present SNEDDS formulation.

Two non-ionic surfactants with high HLB values, namely, Tween 80 (HLB = 15) and Tween 85 (HLB = 10), which are well miscible with ZTO, were chosen for the present formulation. Being less toxic and less affected by pH and ionic changes in the dispersion medium, nonionic surfactants are preferred to ionic surfactants (Constantinides, 1995). In addition to Tween 80 and Tween 85, transcutol P (diethyl glycol monoethyl ether), a cosolvent exhibiting excellent miscibility with ZTO and good flowability (more than 2 droplets/second when being drawn into a syringe positioned vertically), were selected as the co-surfactant to decrease viscosity and increase flowability so as to reduce the emulsification time and droplet size. Neither Cremophor EL nor Lauroglycol FCC formed transparent and freely flowable mixtures with ZTO. Therefore, five formulae based on various compositions of these selected excipients were tested (Table 1) and used to construct the corresponding pseudo-ternary phase diagrams.

3.2. Pseudo-ternary phase diagram

Pseudo-ternary phase diagrams were constructed to identify the self-emulsifying regions for the various SNEDDS formulations. As shown in Fig. 2, Tween 80 formed larger self-emulsifying regions ("A") than Tween 85 with both long-chain (ethyl oleate) and medium-chain (miglyol 812) oils. The better emulsification performance of Tween 80 compared to Tween 85 can be ascribed to a higher HLB value being required for forming a good o/w emulsion (Kommuru et al., 2001). ZTO, being of vegetation origin, is similar to the edible oil or modified vegetable oil and can therefore serve as the oil phase in SNEDDS. However, as shown in Fig. 2e, ZTO alone could afford only a small emulsifying region, suggesting that ZTO is not suitable for use as the sole oil phase in the formulation.

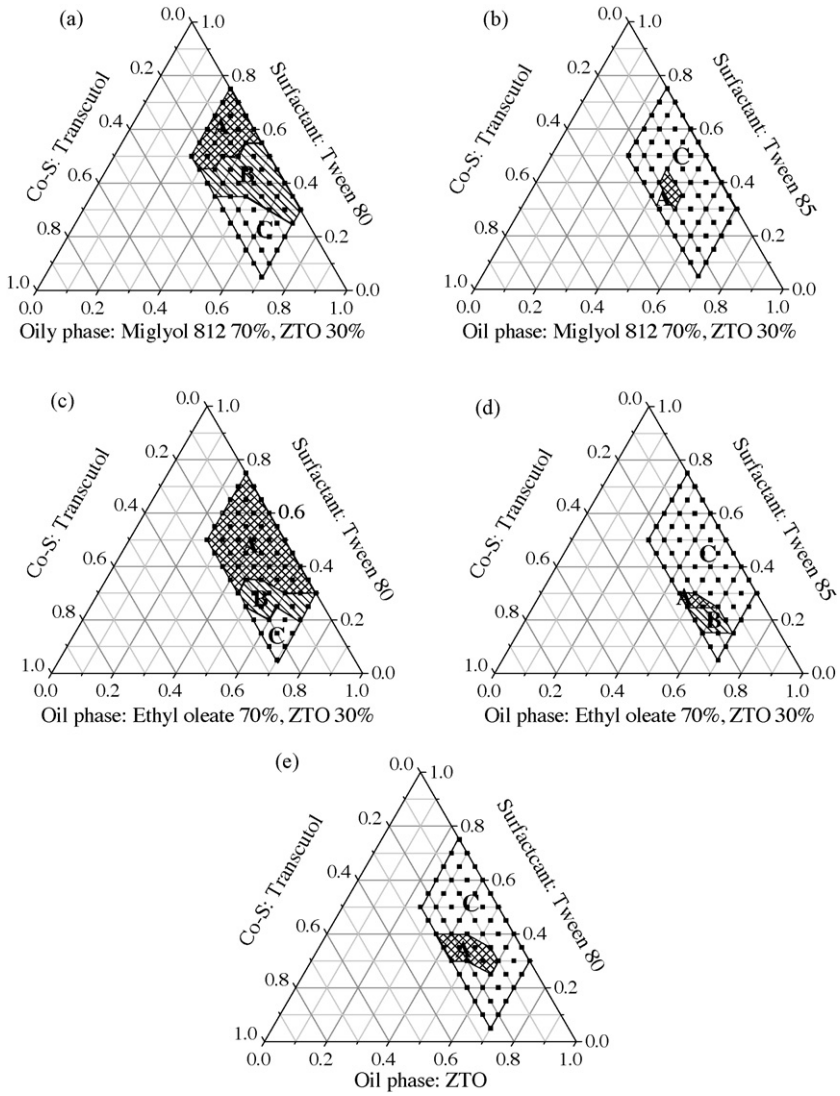

Fig. 2. Pseudo-ternary phase diagram with Miglyol 812 and ZTO (7:3, w/w)/Tween 80/Transcutol P (a); Miglyol 812 and ZTO (7:3, w/w)/Tween 85/Transcutol P (b); ethyl oleate and ZTO (7:3, w/w)/Tween 80/Transcutol P (c); ethyl oleate and ZTO (7:3, w/w)/Tween 85/Transcutol P (d); ZTO/Tween 80/Transcutol P (e).

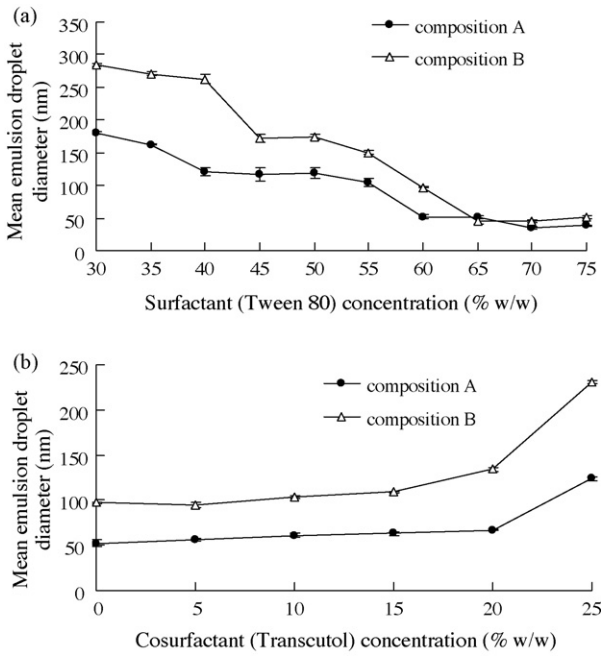

Fig. 3. Effect of surfactant concentration (a) and co-surfactant concentration (b) on the mean emulsion droplet size (key: composition A: ethyl oleate and ZTO (7:3, w/w)/Tween 80; composition B: Miglyol 812 and ZTO (7:3, w/w)/Tween 80, $n = 3$).

Pseudo ternary phase diagrams are normally constructed with the oil phase, surfactant or mixture of surfactant and co-surfactant, and the aqueous phase, which will reveal the regions of liquid crystal, microemulsion (w/o or o/w) and coarse emulsion (Constantinides, 1995; Xi et al., 2009). For simplicity, the present study has ignored the effect of the aqueous phase (1000 times dilution by water), and used only the oil, surfactant and co-surfactant components to identify the self-emulsifying region (Kommuru et al., 2001). In addition, the concentration range of each component (oil, surfactant and co-surfactant) was empirically chosen, i.e., 25–70% oil, 30–75% surfactant and 0–25% co-surfactant (Pouton, 1997).

3.3. Development of the SNEDDs formulations

3.3.1. Effect of excipient concentration on the droplet size and emulsification time

The effect of surfactant concentration on the droplet size of resulting emulsions is shown in Fig. 3a. Results indicated that for the self-emulsifying systems containing ethyl oleate (Fig. 3, composition A), the emulsification time increased from 1 min to 5 min while the droplet size decreased from 180 nm to 38 nm with an increase in surfactant content from 30% to 75%. Such a 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 stronger stabilization (Levya and Benita, 1990). Similar pattern of behaviour was also observed in composition B with a larger droplet size. The results also suggested that ethyl oleate was the 'best' lipid vehicle for the ZTO-SNEDDS since it always afforded the smallest and uniformly distributed droplet size and the largest self-emulsifying region in the corresponding phase diagrams.

The effect of co-surfactant concentration on the droplet size is shown in Fig. 3b. In composition A, the emulsification time decreased from 4 min to 0.5 min while the droplet size increased from 50 nm to 120 nm with an increase of co-surfactant level from 0 to 25%. The increase of droplet size was relatively mild from 0 to 20% (w/w), but in this range, the emulsification time decreased from more than 4 min to 1 min. Similar results were reported by Gao et al. (1998), who demonstrated that an increase in co-surfactant content also increased the droplet size of the microemulsion systems containing Captex® 355, Cremophor® EL, Transcutol® P and saline. These authors further explained that the increased droplet size was due to the expansion of the interfacial film by the co-surfactant present (Gao et al., 1998). Hence, to ensure the formation of small and uniform droplet size and a short emulsification time, as well as to keep the required concentrations of the surfactant and co-surfactant at a minimum, the co-surfactant concentration was fixed at 20%.

Table 2
Effect of ZTO level in oil phase on the mean emulsion droplet size.

ZTO in total oil phase (w/w%)[a]	Droplet size (nm)	Polydispersity index (PDI)
0	182 ± 2.8	0.263 ± 0.036
20	142 ± 2.1	0.243 ± 0.020
30	67.1 ± 1.2	0.225 ± 0.023
40	60.2 ± 1.8	0.234 ± 0.012
50	84.2 ± 0.7	0.168 ± 0.024
60	79.2 ± 1.5	0.085 ± 0.006
80	77.6 ± 1.8	0.089 ± 0.011
100	92.0 ± 3.1	0.522 ± 0.032

[a] Oil (ethyl oleate and ZTO):Tween 80:transcutol P = 40:40:20 (w/w). Data represent mean ± standard deviation (SD), $n = 3$.

As shown in Table 2, the relative percentage of ZTO in the total lipid phase exerted a significant impact on the mean emulsion droplet size. With the same SNEDDS composition (oil:Tween 80:transcutol P = 40:40:20, w/w), an increase in ZTO level from 0 to 40% caused a progressive reduction in droplet size. However, a slightly reverse trend in the droplet size was observed beyond 40% ZTO, and at 100% ZTO (without any incorporated ethyl oleate), the emulsification performance of the oil phase was poor and showed a high PDI value.

3.3.2. Effect of excipient concentration on the zeta potential

Using ethyl oleate as the oil phase (with 30%, w/w, ZTO) and Tween 80 as the surfactant (without co-surfactant), the emulsion obtained was negatively charged and its zeta potential became progressively less negative from −43 to −32 mV when varying the surfactant level from 30% to 75% (w/w) or the total oil phase from 70% to 25%. The co-surfactant level (0–25%) appeared to exert a less significant impact on the zeta potential of the emulsion being formed (from −40 to −35 mV). Moreover, it was found that the absolute zeta potential decreased by as much as 10 mV (from −30 to −20 mV) when the inert oil (ethyl oleate) concentration in the lipid phase was reduced from 100 to 50%. The negative charge on the emulsions is possibly imparted by the free fatty acids present (as contaminants) in the inert oil phase and/or the surfactants and co-surfactants used since the latter materials are mostly derivatives of fatty acids. Because the decrease in negative zeta potential correlated positivley with the decreased amount of inert oil (ethyl oleate) present in the formulation, the fatty acid contaminants in ethyl oleate may be largely responsible for the negative zeta potential of the nanoemulsion produced.

It has been suggested that zeta potential may serve as a partial indicator for the physical stability of the emulsion being formed. High absolute zeta potential values (above 30 mV) should preferably be achieved in most of the emulsions prepared in order to ensure the creation of a high-energy barrier against coalescence of the dispersed droplets (Yang and Benita, 2000). However, this suggested zeta potential cut-off point is only an experience-based value and cannot be reliably used to predict the stability of SNEDDS because a wide range of absolute zeta potential values (i.e., 1.5,

12.5, 45.5 mV) have been reported for SNEDDS in previous studies, most of which did not have any long-term stability assessment for verification purpose (Fatourns et al., 2007; Yang et al., 2004).

Based on the above results, an optimal formulation consisting of ZTO, ethyl oleate, Tween 80 and transcutol P at a weight ratio of 30.8:7.7:40.5:21 (38.5% oil phase, 40.5% surfactant and 21% co-surfactant) was obtained. The droplet size and ξ-potential of this formula upon dispersion in water were 68.3 ± 1.6 nm and −41.2 ± 1.7 mV respectively. For this formula, drug loading is 30%, which is much higher than the commonly reported value for SNEDDs (2.5–10%, w/w) (Gursoy and Benita, 2004), as well as the reported chitosan-alginate nanocapsule of ZTO (10%, w/w). The high drug loading can be attributed to the fact that the drug component (essential oil) itself could serve as a partial lipid phase in SNEDDS. At this optimum composition, the droplet size was 68.3 ± 1.6 nm, 62.1 ± 1.4 nm and 78.2 ± 1.4 nm when dispersed into distilled water, 0.1N HCl (pH 1.2) and phosphate buffer (pH 6.8), respectively, suggesting that the pH of the dilution media has a mild effect on the droplet size of the resulting nanoemulsion. In addition, the droplet size remained small (<100 nm) when the SNEDDS was diluted 50–2000 times with water, suggesting that the formulation will likely yield a nanoemulsion in the GI tract following oral administration.

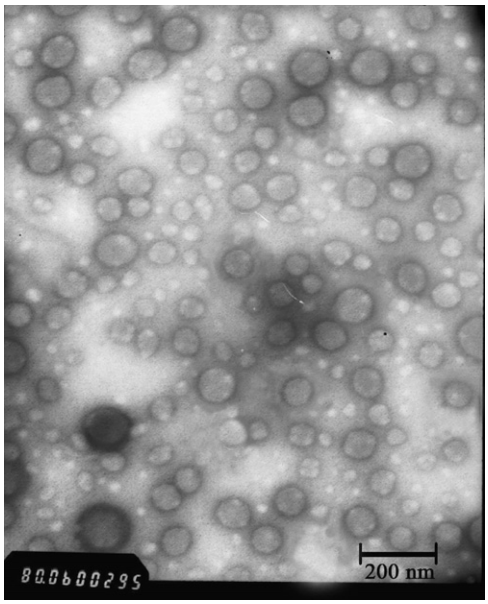

Fig. 4. Transmission electron micrograph of ZTO-SNEDDS. Key (magnification 80K; dilution 1000-fold with water).

3.4. Morphology of ZTO-SNEDDS

As shown in the TEM photograph (Fig. 4), the diluted preparation (nanoemulsion) appears spherical and homogeneous with a large population of the smaller droplet in the size range of less than 100 nm, which is consistent with the distribution data obtained from Malvern measurement (data not shown).

3.5. Dispersibility studies in vitro

In vitro dissolution studies showed that all of the six major active components, namely, NCD, CD, GM, CZ, FZ and β-E, of ZTO were rapidly released (≥90%) from the optimized SNEDDS into the aqueous media (i.e., water, simulated gastric fluid or simulated intestinal fluid) (Table 3). In contrast, direct addition of the unformulated ZTO into the aqueous dissolution medium could only generate large oil globules floating on the surface of the medium.

We have previously reported that GM is passively transported across the Caco-2 cell monolayer with an apparent permeability (~10^{-5} cm/s) comparable to that of the transcellular marker, propranolol, suggesting that permeation across the small intestine is probably not the rate-limiting step for the absorption of GM (Leng et al., 2007). In this study, the active components of ZTO in SNEDDS were found to disperse readily in aqueous media, which would facilitate their absorption *in vivo*. Consequently, oral bioavailability assessment of the formulation in rats was performed to determine if the observed improvement in ZTO dispersibility *in vitro* would lead to better and faster *in vivo* absorption (see Section 3.6).

3.6. Stability

Short-term stability studies on the unformulated ZTO showed that NCD, CD, GM, CZ, FD, β-E were not stable at 25 °C when kept in amber glass vials for 10 days (Table 4). In contrast, the six representative active components of ZTO in SNEDDS remained stable for at least 12 months when stored at 25 °C (Table 5). No new peaks attributable to the degradation products of these active components were observed in the HPLC chromatograms. In addition, no physical phase separation was apparent, reflecting the excellent stability of the developed SNEDDS formulation.

Table 3
Release of active components from ZTO-SNEDDS after 20 min at 37 °C.

Dissolution medium	Cumulative percentage of active component released (%)					
	NCD	CD	GM	CZ	FD	β-E
Water	96.20 ± 5.00	97.89 ± 3.19	89.50 ± 0.83	90.80 ± 1.83	89.63 ± 0.82	90.04 ± 1.37
Simulated gastric fluid (pH 1.2)	98.31 ± 0.61	97.70 ± 1.77	91.19 ± 0.26	94.83 ± 2.89	91.42 ± 1.07	91.42 ± 3.02
Simulated intestinal fluid (pH 6.8)	99.81 ± 0.52	97.88 ± 0.92	92.11 ± 1.36	96.27 ± 1.91	92.35 ± 1.67	91.01 ± 0.60

Data represent mean ± standard deviation (SD), $n = 3$.

Table 4
Short-term stability of unformulated ZTO stored at 25 °C.

Time (days)	Active components in unformulated ZTO (remaining%)					
	NCD	CD	GM	CZ	FD	β-E
0	100	100	100	100	100	100
5	94.43 ± 3.80	96.79 ± 2.18	95.35 ± 1.75	93.82 ± 2.09	85.80 ± 1.07	94.28 ± 1.49
10	92.19 ± 2.98	95.62 ± 1.14	92.28 ± 2.20	87.63 ± 1.59	77.91 ± 0.63	88.99 ± 1.87

Data represent mean ± standard deviation (SD), $n = 6$.

Table 5
Long-term stability of ZTO-SNEDDS stored at 25 °C.

Time (months)	Active components in SNEDDS of ZTO (remaining%)					
	NCD	CD	GM	CZ	FD	β-E
0	100	100	100	100	100	100
1	98.01 ± 1.85	100.86 ± 1.28	101.03 ± 0.95	100.06 ± 1.28	99.59 ± 1.61	98.04 ± 3.27
2	98.90 ± 2.61	100.44 ± 0.67	99.94 ± 0.45	98.20 ± 0.46	98.47 ± 0.36	96.87 ± 1.75
4	97.11 ± 3.85	100.50 ± 3.33	99.35 ± 1.54	101.00 ± 2.10	99.74 ± 1.49	97.46 ± 3.96
6	97.86 ± 3.06	98.57 ± 0.80	99.68 ± 2.06	99.18 ± 1.19	99.20 ± 0.96	98.51 ± 3.24
9	98.85 ± 3.16	100.18 ± 2.02	99.38 ± 1.61	99.80 ± 2.70	98.19 ± 1.17	99.52 ± 2.44
12	97.26 ± 5.58	98.64 ± 0.66	99.42 ± 1.25	97.67 ± 0.84	97.57 ± 1.33	96.62 ± 3.69

Data represent mean ± standard deviation (SD), $n = 6$.

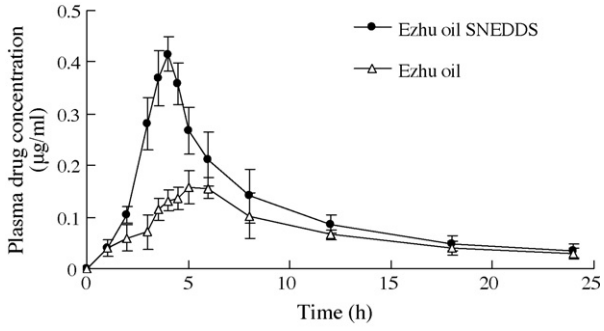

Fig. 5. Plasma concentration of GM after oral administration of unformulated ZTO (500 mg/kg) or ZTO-SNEDDS (1625 mg/kg) to SD rats ($n = 6$).

3.7. Bioavailability study

The plasma concentration versus time profiles of GM in rats for ZTO-SNEDDS and unformulated ZTO following oral administration are presented in Fig. 5. The pharmacokinetic parameters of GM were computed as described in Section 2.8 and tabulated in Table 6. Results showed that the C_{max} and $AUC_{(0-24)}$ of GM in SNEDDS increased by 2.5-fold and 1.7-fold respectively compared to the unformulated ZTO. Additionally, the GM in SNEDDS was absorbed more rapidly and reached its peak concentration sooner ($p < 0.05$).

The above results may be explained by the dispersion of the active components into the aqueous gastrointestinal environment being the rate-limiting step in ZTO absorption. It can be envisaged that following oral administration in rats, the ZTO-SNEDDS will disperse spontaneously to form a nanoemulsion in the gastrointestinal fluid where the active components are present in a solubilized form (i.e. free molecular form incorporated into micelles or dispersed in the microemulsion droplets), and the small droplet size provides a large surface area for drug absorption (Charman et al., 1992; Shah et al., 1994). Such an ultrafine dispersion of the oil will afford rapid and extensive absorption. In addition, high concentration of surfactant in SNEDDS may increase the permeability of the oil across the cell membrane (Penzak et al., 1999), and the lymphatic transport through the transcellular pathway may also contribute to the increased bioavailability (Gershanik and Benita, 2000). On the other hand, the unformulated ZTO will need to be emulsified prior to absorption through the intestinal wall, which accounts for its slower and less extensive absorption.

Table 6
Pharmacokinetic parameters of GM after oral administration of ZTO-SNEDDS and unformulated ZTO in SD rats.

Parameter	ZTO	ZTO SNEDDS
$AUC_{(0-24)}$ (μg/ml h)	1.66 ± 0.21	2.88** ± 0.24
$MRT_{(0-24)}$ (h)	9.60 ± 0.83	8.29* ± 0.56
T_{max} (h)	5.833 ± 1.17	4.08* ± 0.20
C_{max} (μg/ml)	0.17 ± 0.02	0.42** ± 0.03

Data represent mean ± standard deviation (SD), $n = 6$.
* $P < 0.05$ by the Student t-test.
** $P < 0.01$ by the Student t-test.

4. Conclusion

The present study has clearly demonstrated the potential utility of SNEDDS for formulating ZTO with improved aqueous dispersibility, stability and oral bioavailability. In the formulated SNEDDS, the essential oil ZTO itself could serve as a partial lipid phase with the dual advantages of increasing drug loading as well as minimizing the amount of the inert oils required. The present study may serve as a prototype approach for the formulation development of other essential oils or hydrophobic drugs in liquid form.

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