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Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization

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ABSTRACT

Tamoxifen citrate is an antiestrogen for peroral breast cancer treatment. The drug delivery encounters problems of poor water solubility and vulnerability to enzymatic degradation in both intestine and liver. In the current study, tamoxifen citrate self-nanoemulsifying drug delivery systems (SNEDDS) were prepared in an attempt to circumvent such obstacles. Preliminary screening was carried out to select proper ingredient combinations. All surfactants screened were recognized for their bioactive aspects. Ternary phase diagrams were then constructed and an optimum system was designated. Three tamoxifen SNEDDS were then compared for optimization. The systems were assessed for robustness to dilution, globule size, cloud point, surface morphology and drug release. An optimum system composed of tamoxifen citrate (1.6%), Maisine 35-1 (16.4%), Caproyl 90 (32.8%), Cremophor RH40 (32.8%) and propylene glycol (16.4%) was selected. The system was robust to different dilution volumes and types. It possessed a mean globule size of 150 nm and a cloud point of 80 °C. Transmission electron microscopy demonstrated spherical particle morphology. The drug release from the selected formulation was significantly higher than other SNEDDS and drug suspension, as well. Realizing drug incorporation into an optimized nano-sized SNEDD system that encompasses a bioactive surfactant, our results proposed that the prepared system could be promising to improve oral efficacy of the tamoxifen citrate.

1. Introduction

Tamoxifen citrate has been the clinical choice for the antiestrogen treatment of advanced or metastatic breast cancer for more than 20 years (Memisoglu-Bilensoya et al., 2005). Tamoxifen belongs to a class of non-steroidal triphenylethylene derivatives (Fig. 1) and is considered the first selective estrogen receptor modulator. Tamoxifen has a relatively low toxicity and is less harmful than most chemotherapeutics (Shin et al., 2006).

Tamoxifen citrate is a highly lipophilic drug of poor water solubility (Gao and Singh, 1998). Furthermore, its oral bioavailability is mainly affected by the first-pass metabolism and P-glycoprotein (P-gp) pump efflux in the liver and intestine. Tamoxifen is a substrate for the efflux of P-gp, breast cancer resistance protein (BCRP) and multidrug resistance-associated protein (MRP), the members of ATP binding cassette (ABC). The ABC family of transport proteins plays a central role in the defense of organisms against toxic compounds. P-gp, MRP2 and BCRP located within the polarized apical membrane of the intestine, liver and kidney mediate the efflux of xenobiotics and toxins into the intestinal lumen, bile and urine (Shin et al., 2006). The only attempt to improve tamoxifen oral bioavailability encompassed co-administration of tamoxifen with quercetin. The study was based on quercetin dual inhibitory effect on CYP3A4 and P-gp (Shin et al., 2006). In spite of the marked role of the drug in the cancer therapy, no other trials were so far adopted to enhance its oral therapeutic effect.

In recent years, much attention has been focused on lipid-based formulations to improve oral bioavailability of lipophilic drugs. In fact, the most popular approach is the incorporation of the drug compound into inert lipid vehicles such as oils, surfactant dispersions (Nielsen et al., 2008), liposomes (Schwendener and Schott, 1996), microemulsions, nanoemulsions, with particular emphasis on self-emulsifying and self-nanoemulsifying drug delivery systems (SNEDDS) (Gursoya and Benita, 2004). The latter systems comprise isotropic mixtures of natural or synthetic oils with surfactants and co-surfactants. These systems spontaneously emulsify when exposed to GIT fluids to form oil in water nanoemulsion with nanometric droplet size, in the range of 20–200 nm (Mou et al., 2008; Porter et al., 2008). SNEDDS exhibited privileges over other delivery systems. They are characterized by excellent stability, circumventing the stability problem of solid lipid nanoparticles (Mueller et al., 2000) and liposomes (Sharma and Sharma, 1997). Furthermore, SNEDDS would be an efficient, convenient and more patient compliant approach in comparison to o/w nanoemulsion as SNEDDS can be filled in hard gelatin capsules due to their anhydrous nature enabling its administration as unit dosage form (Date

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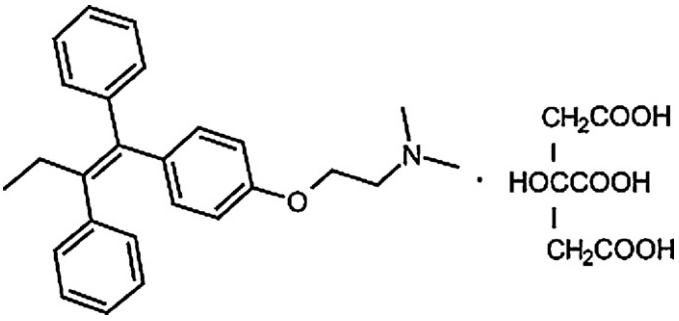

Fig. 1. Chemical structure of tamoxifen citrate.

and Nagarsenker, 2007; Rao and Shao, 2008). Bioavailability from SNEDDS was higher than oils and surfactant dispersions (Nielsen et al., 2008).

SNEDDS are characterized by high solvent capacity, small particle size and excellent 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). Improved drug bioavailability induced by SNEDDS is not merely a matter of solubilization or particle size reduction. The interplay between certain excipients and enzymes or transporters has raised much concern about effect of such systems on drug absorption and metabolism. Reported bioactive excipients encompass Cremophor, Solutol HS-15, Tween 20 and 80, Labrasol, Sucrose monolaurate, Vitamin E-TPGS and Pluronic block copolymers (Chen, 2008). In addition, the drug can be loaded in the inner-phase of SNEDDS and therefore be protected against enzymatic hydrolysis in the gastrointestinal tract. Cefpodoxime proxetil (Date and Nagarsenker, 2007) and proteins (Rao and Shao, 2008) are candidates for drugs that have been successfully protected from presystemic clearance when incorporated in SNEDDS. Furthermore, the drug can be delivered by lymphatic bypass share, restraining hepatic first-pass metabolism of vulnerable drugs (Porter and Charman, 2001). This was apparent in enhanced bioavailability of atorvastatin when incorporated into a self-nanoemulsifying system containing Cremophor RH40. The system was proposed to reduce hepatic clearance of the drug, in addition to increasing its solubility (Shen and Zhong, 2006).

The efficiency of oral absorption of the drug compound from the self-emulsifying formulation depends on many formulation related parameters, such as surfactant concentration, oil/surfactant ratio and droplet size, all of which in essence determine the self-emulsification ability. Thus, only very specific pharmaceutical excipient combinations will lead to efficient self-emulsifying systems. Although many studies have been carried out, there are few drug products on the pharmaceutical market formulated as self-emulsifying formulation, confirming the difficulty of formulating hydrophobic drug compounds into such formulations. At present, there are four drug products, Sandimmune® and Sandimmun Neoral® (cyclosporin A), Norvir® (ritonavir), and Fortovase® (saquinavir) on the pharmaceutical market, the active compounds of which have been formulated into specific self-emulsifying formulations. Significant improvement in the oral bioavailability of these drug compounds has been demonstrated for each case (Porter et al., 2008; Gursoya and Benita, 2004).

Under the aforementioned circumstances, the current work endeavors to design an optimal SNEDD system of tamoxifen citrate. Formula optimization was based on in vitro assessments. The formulation was tailored to compromise between drug solubility in excipients, ease of emulsification and globule size of the dispersion. The system components used were reported for bioactive effects. Selected formulation exhibiting promising in vitro characters is anticipated to improve oral delivery of the drug. The in vivo characteristics of the optimal formulation are currently under investigation.

2. Materials and methods

2.1. Chemicals and reagents

Tamoxifen citrate was obtained from Chemische Fabrikberg, Germany. Glycerol monolinoleate (Maisine 35-1®), propylene glycol monocaprylate (Caproyl 90®), isopropyl myristate (IPM), medium chain triglycerides (Labrafac lipophile® WL 1349), PEG-8 caprylic/capric glycerides (Labrasol®), oleoyl macrogol 6-glycerides (Labrafil® M1944CS) and diethylene glycol monoethyl ether (Transcutol HP®) were kindly donated by Gattefosse Co. (Lyon, France). Apricot kernel oil PEG-6 esters (DUB GPE AB) was a kind gift from Stearinerie Dubois Co. (France). Polyoxy 40 hydrogenated castor oil (Cremophor RH40®) and polyoxy 35 castor oil (Cremophor EL®) were obtained from BASF Co. (Germany). Propylene glycol and Tween 80 were obtained from Al-Nasr Pharmaceutical Co. (Egypt). All other chemicals used were of analytical grade.

2.2. Solubility studies

The solubility of tamoxifen citrate in various buffers, oils, surfactants, and co-surfactants was measured using shake flask method. An excess amount of tamoxifen citrate was added into each vehicle followed by vortex mixing for 30 s (GEMMY vortex mixer; VM-300, Germany). Mixtures were shaken for 48 h at 30 °C in a thermostatically controlled shaking water bath (Kottermann, type 3047, Hanigsen, Germany), followed by equilibrium for 24 h. Mixtures were then centrifuged at 3000 rpm for 10 min and the supernatant was filtered through a Millipore membrane filter (0.45 μl). Samples were suitably diluted with methanol and drug concentration was obtained via UV validated method at 270 nm using methanol as a blank (R^2 = .99057, % Er = 1.5, CV = 2%). The experiment was repeated in triplicates. Results were represented as mean value (mg/ml) ± SEM.

2.3. Preliminary screening of surfactants

Different surfactants for the peroral use were screened for emulsification ability according to the method described by Date and Nagarsenker (2007). Briefly, 300 mg of the surfactants (including Labrasol, Cremophor RH40, Cremophor EL, and Tween 80) were added to 300 mg of the oily phase. The mixtures were gently heated at 50 °C for homogenization of the components. Each mixture, 50 mg, was then diluted with distilled water to 50 ml in a stoppered conical flask. Ease of emulsification was judged by the number of flask inversions required to yield homogenous emulsion. Emulsions were allowed to stand for 2 h and their % transmittance was evaluated at 638.2 nm by UV-160A double beam spectrophotometer (Shimadzu, Japan) using distilled water as a blank. Emulsions were furthermore observed visually for any turbidity or phase separation.

2.4. Preliminary screening of co-surfactants

The selected oily phase and surfactant were used for further screening of the different co-surfactants (Labrafil, Transcutol HP, DUB GPE AB, and propylene glycol) for their emulsification ability. Mixtures of 100 mg of co-surfactant, 200 mg surfactant, and 300 mg oil were prepared and evaluated in a similar fashion as described in Section 2.3.

2.5. Phase diagram study

Ternary phase diagrams of the selected SNEDDS were constructed. The concentrations of oil and surfactant varied from 30% to 70% while that of co-surfactant varied from 0% to 30% (Date and Nagarsenker, 2007). For each diagram, 14 mixtures were prepared. The oily phase was mixed with the surfactant/co-surfactant mixture and the dispersion was homogenized in a shaking water bath at 50 °C for 10 min. From each mixture, 50 mg were diluted to 50 ml with distilled water. Only clear or slight bluish dispersions of particle size 200 nm or lower were considered in the nanoemulsion region of the diagram (Zhang et al., 2008). The particle size analysis was carried out using laser diffraction particle size analyzer (Cilas, model 1064 liquid).

2.6. Preparation of tamoxifen citrate SNEDDS

Following the study and comparison of the constructed ternary phase diagrams, some SNEDDS were selected for drug incorporation and further optimization. Selected formulations are A7 (13% Caproyl, 27% Maisine, 40% Cremophore RH40, 20% propylene glycol), A11 (17% Caproyl, 33% Maisine, 30% Cremophore RH40, 20% propylene glycol) and A13 (20% Caproyl, 40% Maisine, 30% Cremophore RH40, 10% propylene glycol).

Tamoxifen citrate (10 mg) was dissolved in the surfactant/co-surfactant mixture and the dispersion was gently shaken at 60 °C for 5 min. The oily phase was then added and shaking was proceeded for 30 min. Shaking was aided by sonication using Julabo sonicator (USR-3, Ceelbach, Germany) till a clear dispersion was obtained. The prepared SNEDDS were stored in tightly sealed glass bottles at room temperature till used.

2.7. Formula optimization

Based on the former screening, the optimum drug loaded formulation was selected among three SNEDDS (F1, F2, and F3) based on the following optimization criteria.

2.7.1. Robustness to dilution

Robustness of the selected formulations for dilution was assessed by exposing them to 50-, 100- and 1000-fold dilution with water, simulated gastric fluid (0.1N HCl) and simulated intestinal fluid (phosphate buffer pH 7.4). The diluted nanoemulsions were stored for 24 h and monitored for any physical changes (such as precipitation or phase separation).

2.7.2. Globule size analysis

In this section, the effects of dispersing medium type and volume on globule size were investigated. The mean globule size of the selected formulations after 50-, 100-, and 1000-fold dilution in water was compared. In addition, the mean globule size of the formulations after 1000-fold dilution in different media (distilled water, 0.1N HCl and phosphate buffer pH 7.4) was also assessed. Mean globule size was measured using laser diffraction particle size analyzer (Cilas, model 1064 liquid).

2.7.3. Cloud point measurement

The three SNEDDS were compared for cloud point value. Each formulation was diluted with water in the ratio of 1:100 and placed in a water bath with gradual increase in temperature. At the cloud point, the drop in sample % transmittance from the zero point was measured spectrophotometrically (Zhang et al., 2008).

2.7.4. Transmission electron microscopy (TEM)

The morphology of the three SNEDDS was observed by TEM (Jeol, JEM-100 CX electron microscope). After sample dilution with water (1:1000), a sample drop was placed on a copper grid. The excess was drawn off with a filter paper. Samples were subsequently stained with uranyl acetate solution for 30 s.

2.7.5. Drug release study

Drug release from SNEDDS and drug suspensions were assessed using the dialysis bag method (Zhang et al., 2008) with some modifications. In all release experiments, the dialysis bag (MWCO 10,000, Spectrum Medical Industries Inc., USA) was fixed to a 250 ml stoppered glass container containing 200 ml of the medium. The experiment was done in a thermostated shaking water bath equipped at 37 °C and 100 rpm. The drug dialysis was first tested in different media. Drug suspensions in 0.02N HCl (pH 2, 0.06%) and in phosphate buffer (pH 7.4, 0.02%) were prepared according to solubility data and sink conditions. The drug suspension – 5 ml – was added to dialysis bag. The release from different SNEDD formulations – 122 mg in the dialysis bag – in the selected medium was then compared. Finally, release from selected formulation was compared to that from drug suspension under the same sink conditions using the same drug concentration. All samples were measured spectrophotometrically using UV-160A double beam spectrophotometer (Shimadzu, Japan) at λ_{max} = 270 nm using the corresponding proper blank medium. The UV method was validated for both HCl (R^2 = .9898, % Er = 2%, CV = 3%) and phosphate buffer (R^2 = .9964, % Er = 1.5%, CV = 2%). All experiments were performed in triplicate and for each the proper placebo mixture was prepared and compared to its corresponding formulation.

3. Results and discussion

Tamoxifen citrate lipophilicity and vulnerability to enzymatic degradation restrict its oral bioavailability. SNEDDS exhibited potential to improve oral bioavailability of similar lipophilic drug facing metabolic deterrents, such as atorvastatin (Shen and Zhong, 2006) and amphotericin B (Wasan et al., 2009).

SNEDDS spontaneously form nanoemulsions when exposed to GIT fluids. The spontaneous formation of nanoemulsions advantageously presents the drug in a dissolved form. The resultant small droplet size provides a large interfacial surface area for drug release and absorption. In addition, the specific system components promote the intestinal lymphatic transport of drugs. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-gp and/or CYP450 to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid.

In order to prepare an efficient SNEDD system of tamoxifen citrate, the formulation should be tailored for such a drug. Proper type and ratio of oily phase, surfactant mixture and proper globule size should be selected. Furthermore, optimal formulation should posses a cloud point higher than 37 °C and a promising release profile, as detailed in the following sections.

3.1. Solubility studies

The solubility of the drug was tested in four different oily phases (Maisine 35-1, Caproyl 90, isopropyl myristate and Labrafac) that are commonly utilized in SEDDS and SNEDDS formulation (Chen, 2008). Solubilizing capacity of an oily phase is the perspective of consideration regarding oil selection (Pouton and Porter, 2008). Results of solubility studies in oily phases are depicted in Fig. 2. The figure demonstrates that solubility of the lipophilic drug – tamoxifen citrate – was found to be highest in the Maisine 35-1 followed by Caproyl 90. Solubility in both oils was significantly higher than in Labrafac and IPM.

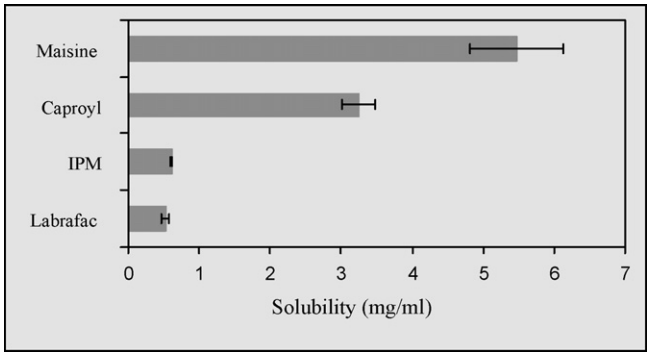

Fig. 2. Solubility of tamoxifen citrate in various oils. Data are expressed as mean ± SEM ($n = 3$).

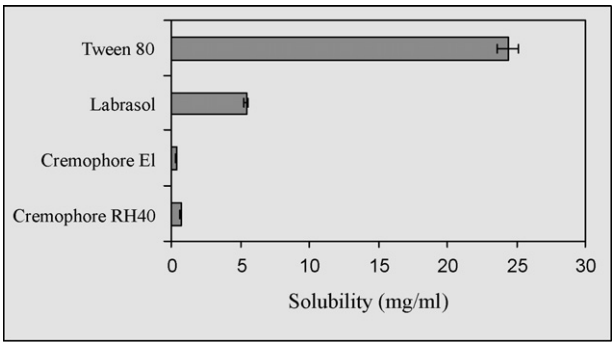

Fig. 3. Solubility of tamoxifen citrate in various surfactants. Data are expressed as mean ± SEM ($n = 3$).

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

3.2. Preliminary screening of surfactants

Non-ionic surfactants are generally considered less toxic than ionic surfactants. They are usually accepted for oral ingestion (Pouton and Porter, 2008). In this study, the four selected non-ionic surfactants (Tween 80, Labrasol, Cremophor EL and Cremophor RH40) were reported to possess bioactive effects. This encompasses effects on tight junction such as Labrasol (Hu et al., 2001), lymphotropic character such as Tween 80 and inhibitory effects on p-gp and CYP enzymes such as Cremophor RH40 and Cremophor El (Chen, 2008). The surfactants were compared for their emulsification efficiencies using different oily phases. It has been reported that well formulated SNEDDS is dispersed within seconds under gentle stirring conditions (Pouton and Porter, 2008). Transmittance values of different mixtures are demonstrated in Table 1. Results inferred that the oily phase Caproyl 90 exhibited the highest emulsification efficiency with all the surfactants employed, with Cremophor RH40 ranking first (98%), requiring only 5 flask inversions (5 s) for homogenous emulsion formation. On the other hand, Maisine 35-1 showed poor emulsification properties with all the surfactants employed, requiring a minimum of 40 flask inversions (40 s) in Cremophor RH40 emulsion. Labrafac exhibited variable emulsification tendency with different surfactants, while isopropyl myristate formed emulsion with only two of the surfactants under study.

Obtained results contend those demonstrated by Rao and Shao (2008). It was reported that oils of medium carbon chain length and higher HLB values such as Caproyl 90 (HLB 6) are better than longer chain length and lower HLB values such as Maisine 35-1, Labrafac and IPM. Labrafac and IPM to form SNEDDS. However, as to drug solubility in Maisine 35-1 was superior to Caproyl 90 (though non-significant), oil mixture seemed to be the optimum selection compromise. In fact, it was reported that use of mixed oils possesses greater solubility in the applied surfactant (Rane and Anderson, 2008). It was also reported that mixed long-chain glycerides (such as Maisine 35-1) are usually recognized as better drug solvents than triglycerides (such as Labrafac) (Porter et al., 2008).

On the other hand, Cremophor RH40 is Polyoxy 40 hydrogenated castor oil utilized in one of the few marketed SEDDS products; Neoral®. The surfactant is supposed to increase lipophilic drug bioavailability not only via solubilization theory but also due to bioactive respects. Cremophor RH40 is a known inhibitor of P-gp and CYP3A, the enzymes incorporated in dimensioned bioavailability of many drug substrates, including tamoxifen citrate (Chen, 2008). Cremophor RH40 was reported to have a role in improving bioavailability of some drugs formulated as self-emulsifying formulations, such as atorvastatin (Shen and Zhong, 2006) and probucol (Nielsen et al., 2008).

Albeit Cremophor El (polyoxy 35 castor oil) was reported to possess similar bioactive effect, the use of Cremophor RH40 for oral ingestion appears more advantageous. It was reported that Cremophor RH40 is less readily digested than Cremophor EL. This effect may be attributed to the difference in reactivity of the saturated backbone of Cremophor RH40. Alternatively, the slightly larger polyethylene oxide content of Cremophor RH40 may have more effectively masked the approach of pancreatic enzymes compared to Cremophor EL. Finally, the latter may simply contain more residual (and digestible) glycerides compared with Cremophor RH40 (Porter et al., 2008). Subjection of Cremophor EL to digestion and hydrolysis was reported to cause drug precipitation and decreased solubilization (Cuiné et al., 2007).

As regarded in Fig. 3, drug solubility in Cremophor RH40 was lower than in other surfactants. Nevertheless, it exhibited the highest emulsification efficiency with all oils utilized. Emulsification ability and bioactive aspects provoked Cremophor RH40 selection for further study.

The aforementioned results suggested the use of a mixture of Maisine 35-1 and Caproyl 90 (M:C) as an oily phase with Cremophor RH40 as a surfactant for further study. Different ratios of the oils (M:C = 1:1, 1:2, and 2:1) have been screened.

3.3. Preliminary screening of co-surfactants

Addition of a co-surfactant to the surfactant-containing formulation was reported to improve dispersibility and drug absorption from the formulation (Porter et al., 2008). In view of current investigation, four co-surfactants, namely propylene glycol, Transcutol HP, DUB GPE AB and Labrafil were compared. As depicted in Table 2, the ratio of Maisine 35-1: Caproyl 90 (1:2) exhibited good emulsification with all co-surfactants, with propylene glycol showing maximum transmittance (98.6%) followed by Transcutol HP (96.4%). Herein, solubility of the drug in different co-surfactants may judge the final selection. Results of solubility study demon-

Table 1
Emulsification efficiency of various surfactants using different oily phases.

Surfactant	%Transmittance			
	Maisine 35-1	Caproyl 90	IPM	Labrafac
Tween 80	40	88	[a]	7
Labrasol	20	83.4	81	82.8
Cremophor RH40	42	98	68	48
Cremophor EL	41	96.8	[a]	23.5

[a] Separated emulsions.

Table 2
Emulsification efficiency of various co-surfactants using Cremophor RH40 as surfactant and different Maisine:Caproyl ratios (1:2, 1:1 and 2:1).

Co-surfactant	%Transmittance		
	1:2	1:1	2:1
Labrafil	90.5	52.3	16.3
DUB GPE AB	76	65	16
Propylene glycol	98.6	89	37.5
Transcutol HP	96.4	93.3	53.6

strated in Fig. 4 inferred higher solubility in propylene glycol. The ratio M:C of 1:1 – on the other hand – exhibited high emulsification efficiency with only Transcutol HP (93.3%). Yet, increasing Maisine 35-1 in the third ratio M:C = 2:1 resulted in poor emulsification efficiency with all co-surfactant mixtures. It is worthy to note that all dispersions exhibited instantaneous emulsion formation with only one flask inversion. This could contend the importance of co-surfactant addition to the surfactant-containing dispersions.

Declining emulsification efficiency with increasing Maisine 35-1 ratio is consistent with the results obtained in surfactant emulsification study. The limited emulsification ability of Maisine 35-1 reflects its high hydrophobic property, which is probably the same reason for its high solubilizing capacity of the drug.

Based on the results of preliminary screening, two distinct systems were selected:

System A: Consisted of Maisine 35-1:Caproyl 90 (1:2) as oily phase (50%)/Cremophor RH40 as surfactant (33%)/propylene glycol as co-surfactant (17%).
System B: Consisted of Maisine 35-1:Caproyl 90 (1:1) as oily phase (50%)/Cremophor RH40 as surfactant (33%)/Transcutol HP as co-surfactant (17%).

Detailed study of the two systems was carried out via ternary phase diagrams.

3.4. Phase diagram study

Based on the results of preliminary screenings, ternary phase diagrams of the selected systems (A&B) were constructed. The detailed composition of phase diagram mixtures IS demonstrated in Tables 3 and 4. The phase diagrams are depicted in Figs. 5 and 6. The shaded region indicates nanoemulsion region. Wider region indicates better self-nanoemulsifying ability. It is noteworthy that surfactant concentration less than 30% resulted in turbid and crude emulsions (data not shown). This could justify the minimum surfactant concentration of 30% (and maximum oil concentration of 70%). These results are consistent with those reported by Date and Nagarsenker (2007). In the current investigation,

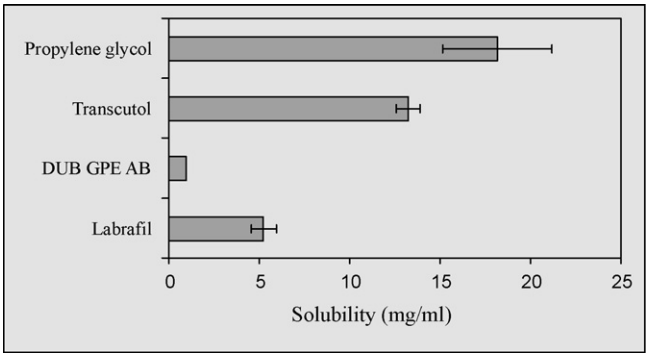

Fig. 4. Solubility of tamoxifen citrate in various co-surfactants. Data are expressed as mean ± SEM ($n = 3$).

Table 3
Composition of SNEDDS constructing phase diagram A.

Formula	Maisine 35-1 (%)	Caproyl 90 (%)	Cremophor RH40 (%)	Propylene glycol (%)
A1	10	20	70	0
A2	10	20	60	10
A3	10	20	50	20
A4	10	20	40	30
A5	13	27	60	0
A6	13	27	50	10
A7	13	27	40	20
A8	13	27	30	30
A9	17	33	50	0
A10	17	33	40	10
A11	17	33	30	20
A12	20	40	40	0
A13	20	40	30	10
A14	23	47	30	0

Table 4
Composition of SNEDDS constructing phase diagram B.

Formula	Maisine 35-1 (%)	Caproyl 90 (%)	Cremophor RH40 (%)	Transcutol HP (%)
B1	15	15	70	0
B2	15	15	60	10
B3	15	15	50	20
B4	15	15	40	30
B5	20	20	60	0
B6	20	20	50	10
B7	20	20	40	20
B8	20	20	30	30
B9	25	25	50	0
B10	25	25	40	10
B11	25	25	30	20
B12	30	30	40	0
B13	30	30	30	10
B14	35	35	30	0

the wider nanoemulsification region of system A phase diagram (Fig. 5) compared to that of system B (Fig. 6) indicated better self-nanoemulsification property of the former system. In both diagrams, mixtures with zero co-surfactant proportion were not easily emulsified. System A yielded nanoemulsion containing as high as 70% oily phase composition. On the other hand, system B produced nanoemulsion till a maximum oil concentration of 40% only. This could be attributed to higher ratio of Caproyl 90 in system A that was therefore selected for further investigation. Three mixtures with oil

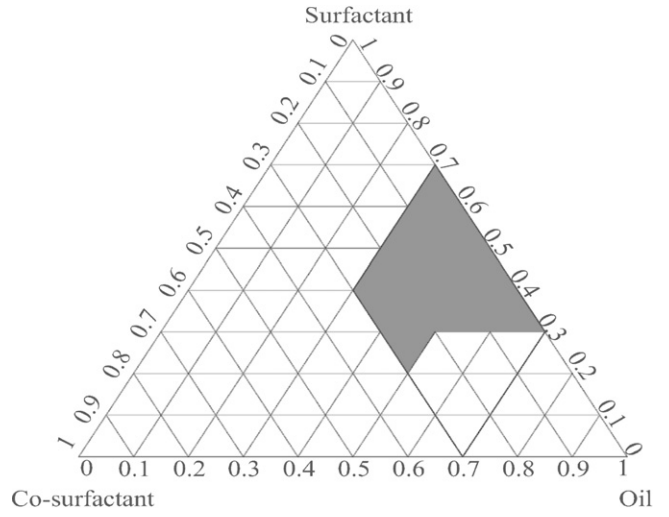

Fig. 5. Ternary phase diagram of system A (Maisine-Caproyl (1:2)/Cremophor RH40/propylene glycol).

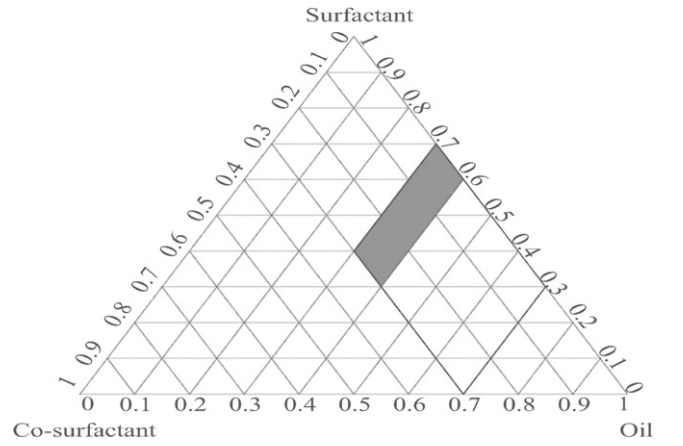

Fig. 6. Ternary phase diagram of system B (Maisine-Caproyl (1:1)/Cremophor RH40/Transcutol HP).

concentrations of 40% (A7), 50% (A11) and 60% (A13) were chosen for drug incorporation and formula optimization. In all mixtures, the surfactant:co-surfactant ratio was unified to 2:1.

3.5. Preparation of tamoxifen citrate SNEDDS

Tamoxifen citrate – 10 mg – was added to the three selected mixtures. Composition of the three formulations (F1, F2, and F3) is shown in Table 5.

3.6. Formula optimization

Selection of an optimum tamoxifen citrate-containing SNEDD formulation was carried out based on the following tests.

3.6.1. Robustness to dilution

SNEDDS formulations were exposed to different folds of dilution in different media in an attempt to mimic the in vivo conditions where the formulation would encounter gradual dilution. Each SNEDD formulation was subjected to 50-, 100- and 1000-fold dilution in water, simulated gastric fluid (0.1N HCl), and simulated intestinal fluid (phosphate buffer pH 7.4). F1 and F2 dispersions showed no signs of precipitation, cloudiness or separation for 24 h. On the contrary, F3 formulation formed immediate turbid dispersions with 50- and 100-fold dilutions. These results confirm robustness of only F1 and F2 formulations to different dilution volumes of various media. Regarding F3, clarity of the dispersion was lost, that may be attributed to higher oil percentage.

Table 5
Composition and physical properties of F1, F2, and F3 SNEDD formulations.

	F1	F2	F3
Composition (%)			
Tamoxifen citrate	1.6	1.6	1.6
Maisine 35-1	13.2	16.4	19.7
Caproyl 90	26.2	32.8	39.3
Cremophor RH40	39.3	32.8	26.2
Propylene glycol	19.7	16.4	13.2
Globule size (nm)			
Water (50-fold)	170 ± 7.07	170 ± 7	900 ± 20
Water (100-fold)	170 ± 7	170 ± 5	490 ± 50
Water (1000-fold)	150 ± 6.9	150 ± 7.07	190 ± 14.1
Phosphate buffer (1000-fold)	140 ± 3	130 ± 5	170 ± 7.07
HCl (1000-fold)	140 ± .7	140 ± 1	200 ± 1
Cloud point (°C)	90	80	90

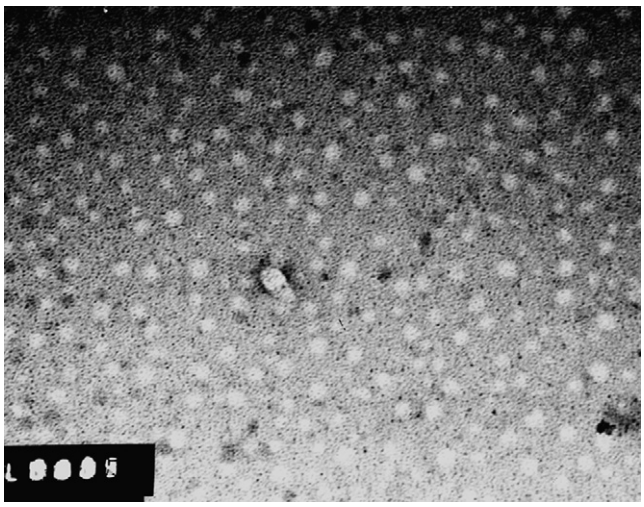

Fig. 7. TEM photograph of F1 formulation (×50,000).

3.6.2. Globule size analysis

Droplet size of SNEDDS is a critical step in the pathway of enhancing drug bioavailability. In this study, the effect of two distinct factors, dispersion medium type and volume, on globule size of different formulations was evaluated. To investigate the effect of dispersion medium type, all formulations were diluted with different media (water, 0.1N HCl and phosphate buffer pH 7.4) to the same volume (1000-fold dilution). Results of globule size analysis are shown in Table 5. Results inferred that diluting medium pH had no effect on globule size. As for dilution volume, results (Table 5) were consistent with that obtained in the previous section. F1 and F2 formulations globule size was not affected by the dilution fold (all below 200 nm). In view of F3 formulation, the globule size was markedly higher with 100 folds (490 nm) and 50 folds (900 nm) as compared to 1000-fold dilution. Results of robustness to dilution and globule size analysis indicated higher particle size and lower stability of F3 when exhibited to gradual dilution.

3.6.3. Cloud point measurement

The cloud point is the temperature above which the formulation clarity turns into cloudiness. At higher temperatures, phase separation can occur due to dehydration of polyethylene oxide moiety of the non-ionic surfactant. Since both drug solubilization and formulation stability will decline with this phase separation, the cloud point of the formulation should be over 37 °C. The cloud point value is affected by factors such as drug hydrophobicity, kind, combination, mixing ratio and amount of each of the oils, surfactants and co-surfactants used (Itoh et al., 2002; Zhang et al., 2008).

In this study, cloud points of all formulations were very high as reported in Table 5. F2 exhibited cloudiness at 80 °C with a drop in transmittance % from 90% to 44%. F3 turned cloudy at 90 °C with a drop in % transmittance from 88% to 58%. In all formulations, cloudiness was reversible after minutes. As for F1, cloudiness did not for persist time enough to be measured. Results contented the stability of all SNEDD formulations towards separation in the GIT temperature.

3.6.4. Transmission electron microscopy (TEM)

The morphology of the three SNEDD formulations was observed using TEM. The photographs depicted in Figs. 7–9 reveal that all particles after dilution possessed spherical shape. As demonstrated, F1 and F2 nearly possessed the same size with F3 showing slightly higher size. This rank was consistent with that obtained in the globule size analysis.

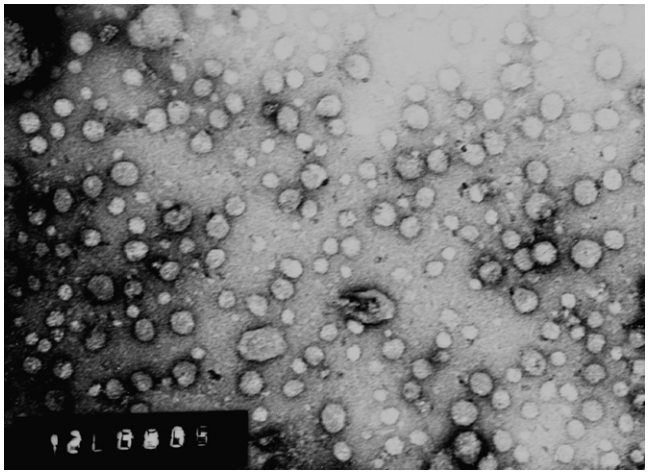

Fig. 8. TEM photograph of F2 formulation (×50,000).

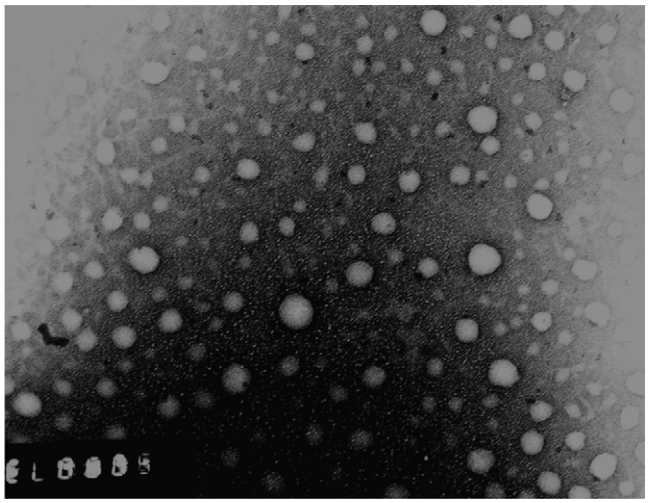

Fig. 9. TEM photograph of F3 formulation (×50,000).

3.6.5. Drug release study

When SNEDDS encounter aqueous medium, different forms of solubilized drug are formed, that encompass free molecular state, drug in nanoemulsion and drug in micellar solution. Under these circumstances, it is necessary to separate free drug molecules from those entrapped in the nanoemulsion droplets or micelles to assess the real release pattern. Thereby, conventional release testing is not adequate to this system. In this field dialysis bag method was recently adopted (Zhang et al., 2008). In our study, the following experiments were carried out. The drug was first assessed for its dialysability in different media. Drug suspensions in 0.02N HCl (pH 2) and phosphate buffer (pH 7.4) were prepared. According to solubility data of the drug in each media (Fig. 10), concentration of suspension was adjusted to create sink conditions (at least 7 folds of saturated solubility). As demonstrated in the release patterns (Fig. 11), drug exhibited very significant decrease in dialysis in HCl compared to phosphate buffer (despite of higher solubility in HCl). Indeed, % drug release in HCl did not exceed 40% after 24 h, whereas the drug was 100% released in phosphate buffer after only 4 h. The conflicting behavior of tamoxifen citrate release with the two media may be attributed to a type of interaction between the drug and the bag material (cellophane) that was enhanced by the acid and restricted the drug release. The aforementioned results suggested the use of pH 7.4 phosphate buffer for assessment of drug release from different SNEDD formulations.

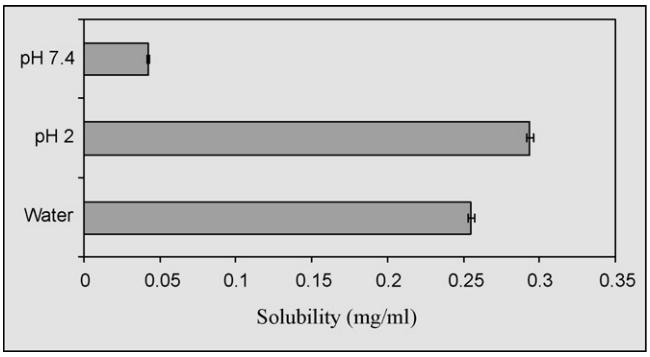

Fig. 10. Solubility of tamoxifen citrate in various pH media. Data are expressed as mean ± SEM, $n = 3$.

Release patterns of different formulations: F1, F2 and F3 in pH 7.4 are depicted in Fig. 12. The patterns reveal that release from F1 and F3 formulations was significantly slower than that from F2 formulations. After 2 h, drug release from F2 formulation was about 75% compared to less than 5% from other formulations. After 6 h, the drug was almost completely released from F2 whereas release from other formulations did not exceed 20%.

F1 formulation (60% surfactant mixture) can be categorized as Type IIIB system according to Lipid Formulation Classification System (LFCS). Such type of systems is characterized by higher percentage of hydrophilic surfactants. This high proportion is usually concomitant with higher probability of surfactant migration into surrounding aqueous media upon dispersion (Porter et al., 2008; Pouton and Porter, 2008). The high surfactant concentration released is supposed to form micelles that trap free drug in side, with subsequent hindrance in drug release. As for F3 (40% surfactant mixture), a coarser emulsion is supposed to be formed inside the bag as revealed from results of robustness to dilution and globule size analysis. The higher globule size formed would lead to slower drug release in comparison to F2. In both cases – F1 and F3 – complete drug release required a lag period to give chance for enough water to dilute the formulation and increase drug release. F2 formulation, on the contrary, exhibited a gradual increase in drug release

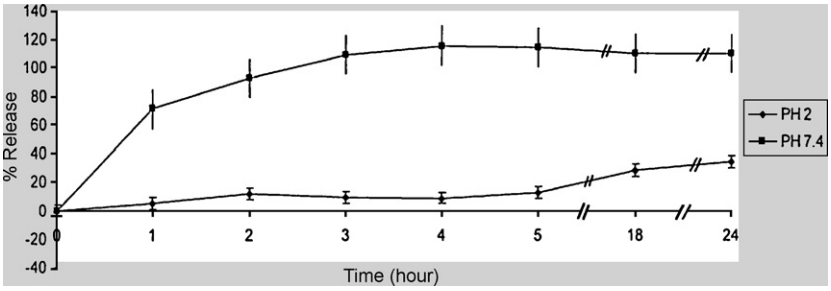

Fig. 11. Release patterns of tamoxifen citrate suspension in pH 2 and 7.4 (mean ± SEM).

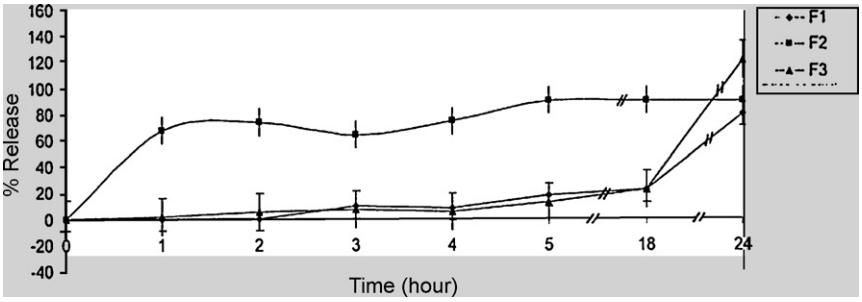

Fig. 12. Release patterns of tamoxifen citrate from different SNEDD formulations in pH 7.4 (mean % ± SEM).

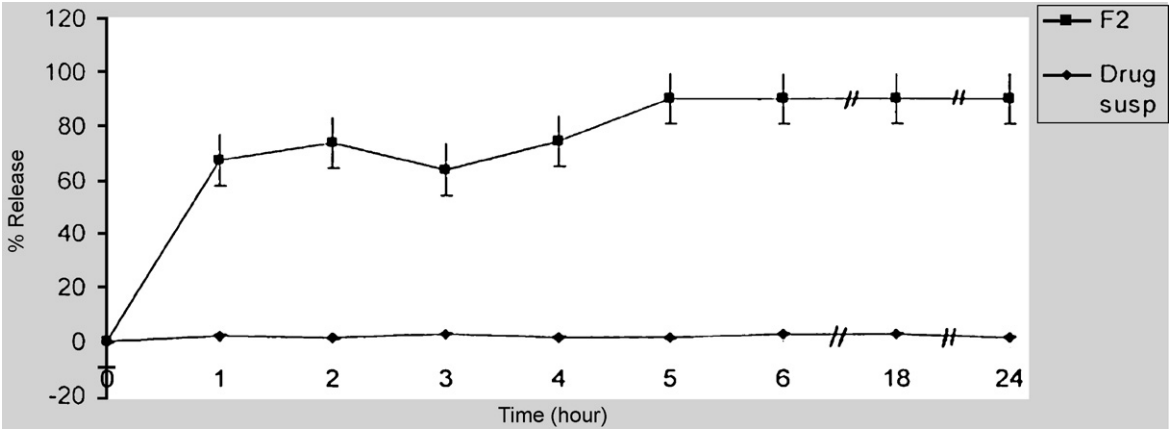

Fig. 13. Release patterns of tamoxifen citrate from F2 SNEDD formulation compared to that from drug suspension (mean % ± SEM).

to reach the maximum at 6 h. This behavior may be attributed to a proper compromise between the oil and surfactant mixture proportions (50:50). The aforementioned results propose the formulation F2 to be the most suitable one for comparison with tamoxifen citrate suspension.

Fig. 13 demonstrates comparison of drug release from F2 SNEDD formulation to that from drug suspension. Both F2 and suspension were carrying the same drug amount (2 mg) and added to the same buffer volume (200 ml). Release patterns reveal that tamoxifen citrate release was significantly higher from SNEDD formulation. Drug release from suspension did not exceed 2% after 24 h, whereas, the drug was 100% released from F2 after 6 h. These results contend the role of SNEDDS formulation to improve tamoxifen citrate solubilization and in vitro release.

4. Conclusion

In the current investigation, self-nanoemulsifying drug delivery systems of tamoxifen citrate were prepared and in vitro evaluated. Following optimization, tamoxifen citrate SNEDD system composed of tamoxifen citrate (1.6%), Maisine 35-1 (16.4%), Caproyl 90 (32.8%), Cremophor RH40 (32.8%) and propylene glycol (16.4%) was selected. The formula was robust to dilution in different media using different dilution folds, exhibiting no signs of precipitation or separation. The globule size was unaffected in all applied media and dilution volumes and lied within appropriate range (130–150 nm). The formula showed cloud point of 80 °C and spherical shaped particles. In vitro release in phosphate buffer (7.4) revealed a gradual release pattern with 100% release at 6 h. SNEDDS of tamoxifen citrate showed a significant increase in release rate compared to the drug suspension under the same conditions. Our results report that the prepared SNEDD system with promising in vitro characteristics is anticipated to solve oral delivery problems of tamoxifen citrate. The in vivo characteristics of the selected formulation are presently investigated.

References

Chen, M.-L., 2008. Lipid excipients and delivery systems for pharmaceutical development: a regulatory perspective. Adv. Drug Deliv. Rev. 60, 768–777.

Cuiné, J.F., Charman, W.N., Pouton, C.W., Edwards, G.A., Porter, C.J.H., 2007. Increasing the proportional content of surfactant (Cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. Pharm. Res. 24, 748–757.

Date, A.A., Nagarsenker, M.S., 2007. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. Int. J. Pharm. 329, 166–172.

Gao, S., Singh, J., 1998. In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J. Control. Release 51, 193–199.

Gursoya, R.N., Benita, S., 2004. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacother. 58, 173–182.

Hu, Z., Tawa, R., Konishi, T., Shibata, N., Takada, K., 2001. A novel emulsifier, Labrasol, enhances gastrointestinal absorption of gentamicin. Life Sci. 69, 2899–2910.

Itoh, K., Tozuka, Y., Oguchi, T., Yamamoto, K., 2002. Improvement of physicochemical properties of N-4472 part I formulation design by using self-microemulsifying system. Int. J. Pharm. 238, 153–160.

Memisoglu-Bilensoya, E., Vurala, I., Bochotb, A., Renoirb, J.M., Ducheneb, D., Hincal, A.A., 2005. Tamoxifen citrate loaded amphiphilic h-cyclodextrin nanoparticles. J. Control. Release 104, 489–496.

Mou, D., Chen, H., Du, D., Mao, C., Wan, J., Xu, H., Yang, X., 2008. Hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs. Int. J. Pharm. 353, 270–276.

Mueller, R.H., Maeder, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery: a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Nielsen, F.S., Petersen, K.B., Ilertz, A.M., 2008. Bioavailability of probucol from lipid and surfactant based formulations in minipigs: influence of droplet size and dietary state. Eur. J. Pharm. Biopharm. 69, 553–562.

Porter, C.J.H., Charman, W.N., 2001. Intestinal lymphatic drug transport: an update. Adv. Drug Deliv. Rev. 50, 61–80.

Porter, C.J.H., Pouton, C.W., Cuine, J.F., Charman, W.N., 2008. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv. Drug Deliv. Rev. 60, 673–691.

Pouton, C.W., Porter, C.J.H., 2008. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv. Drug Deliv. Rev. 60, 625–637.

Rane, S.S., Anderson, B.D., 2008. What determines drug solubility in lipid vehicles: is it predictable. Adv. Drug Deliv. Rev. 60, 638–656.

Rao, S.V.R., Shao, J., 2008. Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs: formulation development. Int. J. Pharm. 362, 2–9.

Schwendener, R.A., Schott, H., 1996. Lipophilic 1-beta-D-arabinofuranosyl cytosine derivatives in liposomal formulations for oral and parenteral antileukemic therapy in the murine L1210 leukemia model. J. Cancer Res. Clin. Oncol. 122, 723–726.

Sharma, A., Sharma, U.S., 1997. Liposomes in drug delivery: progress and limitations. Int. J. Pharm. 154, 123–140.

Shen, H., Zhong, M., 2006. Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin. J. Pharm. Pharmacol. 58, 1183–1191.

Shin, S., Choi, J., Li, X., 2006. Enhanced bioavailability of tamoxifen after oral administration of tamoxifen with quercetin in rats. Int. J. Pharm. 313, 144–149.

Wasan, E.K., Bartlett, K., Gershkovich, B., Sivak, O., Bannoe, B., Wong, Z., Gagnonc, J., Gatesf, B., Leonb, C.G., Wasanb, K.M., 2009. Development and characterization of oral lipid-based amphotericin B formulations with enhanced drug solubility, stability and antifungal activity in rats infected with *Aspergillus fumigatus* or *Candida albicans*. Int. J. Pharm. 372, 76–84.

Zhang, P., Liu, Y., Feng, N., Xu, J., 2008. Preparation and evaluation of self-microemulsifying drug delivery system of oridonin. Int. J. Pharm. 355, 269–276.