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Research Article

Spontaneous Emulsification of Nifedipine-Loaded Self-Nanoemulsifying Drug Delivery System

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ABSTRACT. Self-nanoemulsifying drug delivery system (SNEDDS) can be used to improve dissolution of poorly water-soluble drugs. The objective of this study was to prepare SNEDDS by using ternary phase diagram and investigate their spontaneous emulsifying property, dissolution of nifedipine (NDP), as well as the pharmacokinetic profile of selected SNEDDS formulation. The results showed that the composition of the SNEDDS was a great importance for the spontaneous emulsification. Based on ternary phase diagram, the region giving the SNEDDS with emulsion droplet size of less than 300 nm after diluting in aqueous medium was selected for further formulation. The small-angle X-ray scattering curves showed no sharp peak after dilution at different percentages of water, suggesting non-ordered structure. The system was found to be robust in different dilution volumes; the droplet size was in nanometer range. *In vitro* dissolution study showed remarkable increase in dissolution of NDP from SNEDDS formulations compared with NDP powders. The pharmacokinetic study of selected SNEDDS formulation in male Wistar rats revealed the improved maximum concentration and area under the curve. Our results proposed that the developed SNEDDS formations could be promising to improve the dissolution and oral bioavailability of NDP.

KEY WORDS: nifedipine; poorly water-soluble drug; self-emulsifying drug delivery system; spontaneous emulsification.

INTRODUCTION

Oral route is the most convenient and preferred route of drug delivery as it offers a good patient compliance. However, 40% of drugs delivered *via* the oral route have limited therapeutic efficacy due to poor water solubility (1–4). Conventional techniques, such as salt formation, micronization, solubilization using cosolvents, use of permeation enhancers, oily solutions, and surfactant dispersions that were previously employed to increase oral bioavailability revealed limited utility. Although recently developed strategies, such as new solid dispersion technology and inclusion complexes using cyclodextrins (5), exhibit good potential, they are successful in some cases and are specific to drug candidates. The use of self-nanoemulsifying drug delivery system (SNEDDS) is one of the interesting approaches (1,6).

SNEDDS can be defined as an anhydrous form of nanoemulsions. It is an isotropic mixture of oil, surfactant, co-surfactant, and drug, which spontaneously forms thermodynamically stable oil-in-water nanoemulsions (usually with droplet size between 100 and 300 nm) when introduced into aqueous phase under gentle agitation conditions (7). The availability of drug for absorption can be enhanced by presentation of the drug in solubilized form within a colloidal dispersion (4). The benefits of SNEDDS also include possibility of filling them into unit dosage forms (*e.g.*, soft/hard gelatin capsules), preserving physical and chemical stability upon long-term storage, improving the bioavailability of poorly water-soluble drugs, and reducing the blood profile variation in the patients faced with GI problem (8,9). SNEDDS can be prepared based on hydrophilic-lipophilic balance (HLB) of surfactant (*e.g.*, Weerapol *et al.* (10)) or ternary phase diagram (*e.g.*, Xi *et al.* (11) and Zhao *et al.* (12)). By using ternary phase diagram, every ratio of selected surfactant and oil can be compared easily to select the surfactant, co-surfactant, and oil combinations. Upon contact with aqueous medium, the SNEDDS formulations are self-emulsifying and very fine dispersions (or nanoemulsions) are then formed spontaneously (13) because the free energy required to form the emulsion is either low and positive or negative. This dilution behavior is a characteristic for the formulations although the final droplet size is further affected by the digestion process in the body (14). In fact, the volume of aqueous fluid used for dilution of SNEDDS may influence the size of the emulsion droplets

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formed as well as the drug dissolution profile. Mostly, the amount of aqueous fluid used for dilution study was fixed to 100-fold (15,16) or 250-fold (5,17) of the dose. Different aqueous volumes used for dilution would perhaps be the topic for investigation, in order to ensure the robustness to dilution of the SNEDDS formulations.

The process of spontaneous emulsification proceeds through formation of liquid crystals (LC) and gel phases. Release of drug from SNEDDS is highly dependent on LC formed at the interface, since it is likely to affect the angle of curvature of the droplet formed and the resistance offered for partitioning of drug into aqueous media (18). Currently, knowledge about the phase transition during the emulsification process of SNEDDS is rather limited. Small-angle X-ray scattering (SAXS) can be used to probe the structure of nanoemulsions spontaneously formed. Using short wavelengths, $\lambda < 10$ Å, compared with the droplet size, it is possible to obtain accurate measurements of the structure factor. Phase behavior of o/w emulsion as spontaneous emulsion was studied after equilibrating in aqueous medium for 48 h (19,20). However, the spontaneous emulsification of SNEDDS at the time relevant to gastric emptying (1–2 h) has not been reported.

In this study, nifedipine (NDP) was used as a model poorly water-soluble drug (MW of 346.3 g/mol, solubility 5.8 μg/mL in water, pK_a <1, log *P* 2.50) (21). The drug dissolution from its stable crystalline form was reported to be a limiting step during drug absorption process, resulting in a poor drug bioavailability (22). Therefore, the aim of this study was to prepare NDP-loaded SNEDDS by ternary phase diagram and investigate the physical properties and drug dissolution behavior. The effect of volume of water used for diluting SNEDDS on spontaneous emulsification was studied. The relative oral bioavailability of the selected SNEDDS *versus* the NDP powder was also evaluated in male Wistar rats.

MATERIALS AND METHODS

Materials

NDP was purchased from Jintan Xilin Pharmaceutical Raw Material Co., Ltd. (Jiangsu, China). Caprylic/capric glyceride (CCG; Imwitor® 742) was purchased from Sasol Germany (Hamburg, Germany). Polyoxyl 40 hydrogenated castor oil; HLB 14.0–16.0 (Cremophor® RH40, referred as P40) and Polyoxyl 35 castor oil; HLB 12.0–14.0 (Cremophor® EL, referred as P35) were a gift from BASF (Thai) Ltd. (Bangkok, Thailand). Diethylene glycolmonoethyl ether; HLB 4.0 (Transcutol® HP, referred as DGE) was supported form Gattefosse (Saint-Priest Cedex, France). Other chemicals were of reagent or analytical grade and used without further purification. Distilled water was used in all preparations. The simulated gastric fluid USP without pepsin (SGF) was prepared by dissolving 2 g of sodium chloride and 7 mL of hydrochloric acid into distilled water and adjusting volume to 1000 mL, pH to 1.2, and used as test medium.

Construction of Ternary Phase Diagram

The range of the self-emulsifying formulations that could form spontaneous emulsification under dilution and gentle agitation was identified from ternary phase diagram of systems containing oil, surfactant and co-surfactant. A series of self-emulsifying formulations were prepared using various concentrations of oil (CCG, 10–98%, *v*/*v*), surfactant (P35 or P40, 0–90%, *v*/*v*), and co-surfactant (DGE, 2–90%, *v*/*v*), at 25°. The obtained formulations (0.1 mL) were introduced into 19.9 mL (199-fold) of water in a test tube and then mixed gently. The tendency to spontaneously emulsify was observed visually while the progress of emulsion droplets was observed, in triplicate, by laser diffraction particle size analyzer (model LA-950, Horiba Ltd., Japan). The formulations with emulsion droplet size of 100–300 nm, resulting from dilution, were selected for preparing of SNEDDS formulations.

Preparation of SNEDDS Formulations

According to the results from the ternary phase diagram, selected ratios of CCG/P35/DGE and CCG/P40/DGE which provided the droplet sizes between 100 and 300 nm were used for NDP loading (80 mg/mL). The oil, surfactant, and co-surfactant were mixed at 25°C, under light-protected condition, until clear solution was obtained. Then, excess amount of NDP (500 mg) was added to the mixtures and mixed thoroughly. The resultant formulations were shaken at 25°C for 72 h, in dark conditions, before further analysis of NDP content. The formulations with the highest NDP loading (dissolved NDP) were chosen for further investigation.

Analysis of NDP Content

After equilibration for 72 h, the mixtures were centrifuged at 3500 rpm (1166×*g*) for 15 min to remove the undissolved NDP and supernatants were analyzed for NDP content using high-performance liquid chromatography (HPLC; model JASCO PU-2089plus quaternary gradient inert pump, and a JASCO UV-2070plus multiwavelength UV–vis detector, Jasco, Japan) using Luna 5u C18 column (5 μm, 4.6 nm× 25 cm; Phenomenex, USA). The mobile phase composing of water, acetonitrile, and methanol (50:25:25) was filtered through a 0.22-μm membrane filter and degassed in a sonicator bath before use. The flow rate of mobile phase was 1.0 mL/min, and the UV detection wavelength was 235 nm.

Robustness to Dilution

Robustness to dilution is important for SNEDDS formulation to ensure that the nanoemulsion formed has similar properties at different dilutions to achieve uniform drug release behavior and that the drug will not precipitate at higher dilution in the body, which may retard the absorption of the drug from the formulation (7). Robustness of SNEDDS formulation to dilution was studied by diluting them with water at different dilutions (*e.g.*, 0.01–1000-fold) and equilibrating for 30 min before investigation. The sign of phase separation or precipitation was also observed. Droplet size and size distribution of the formed nanoemulsions (n=3) were investigated by photon correlation spectroscopy (model Nano ZS, Malvern, England).

Determination by Small Angle X-ray Scattering

The samples for SAXS determination were prepared by diluting selected SNEDDS containing NDP (80 mg/mL) with various amounts of water or SGF (0.01-, 0.02-, 0.04-, 0.06-, 0.09-, 0.11-, 0.18-, 0.25-, 0.67-, 1.5-, 4-, 99-, 199-, and 302-fold) to perform nanoemulsions. The obtained nanoemulsions were incubated at 25°C for 1 or 2 h. The experiments were performed with SAXS instrument, beamline BL2.2: small/wide angle X-ray scattering (SAXS), installed at Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima, Thailand. The SAXS scattering data were acquired using a large area pixel detector (165 mm diameter CCD; model Mar SX165, Marresearch Ltd., USA) with pixel size of 165×165 mm. The sample was filled into a cell placed between polyimine film (Kapton®, Dupont™, USA) window (at q 0.074–1.100 nm^{-1}). The distance from sample to detector was 2930 mm, and the X-ray energy was 8 keV. The SAXS measurements were performed at 25°C. The raw scattering data were background corrected, integrated, and calibrated using a SAXS Image Tool (SAXSIT) analysis suite, version 3.3 (SLRI, Thailand), which is available at the beamline.

Transmission Electon Microscopic Examination

Selected SNEDDS formulations were examined under transmission electron microscope (model JEM-1230, JOEL

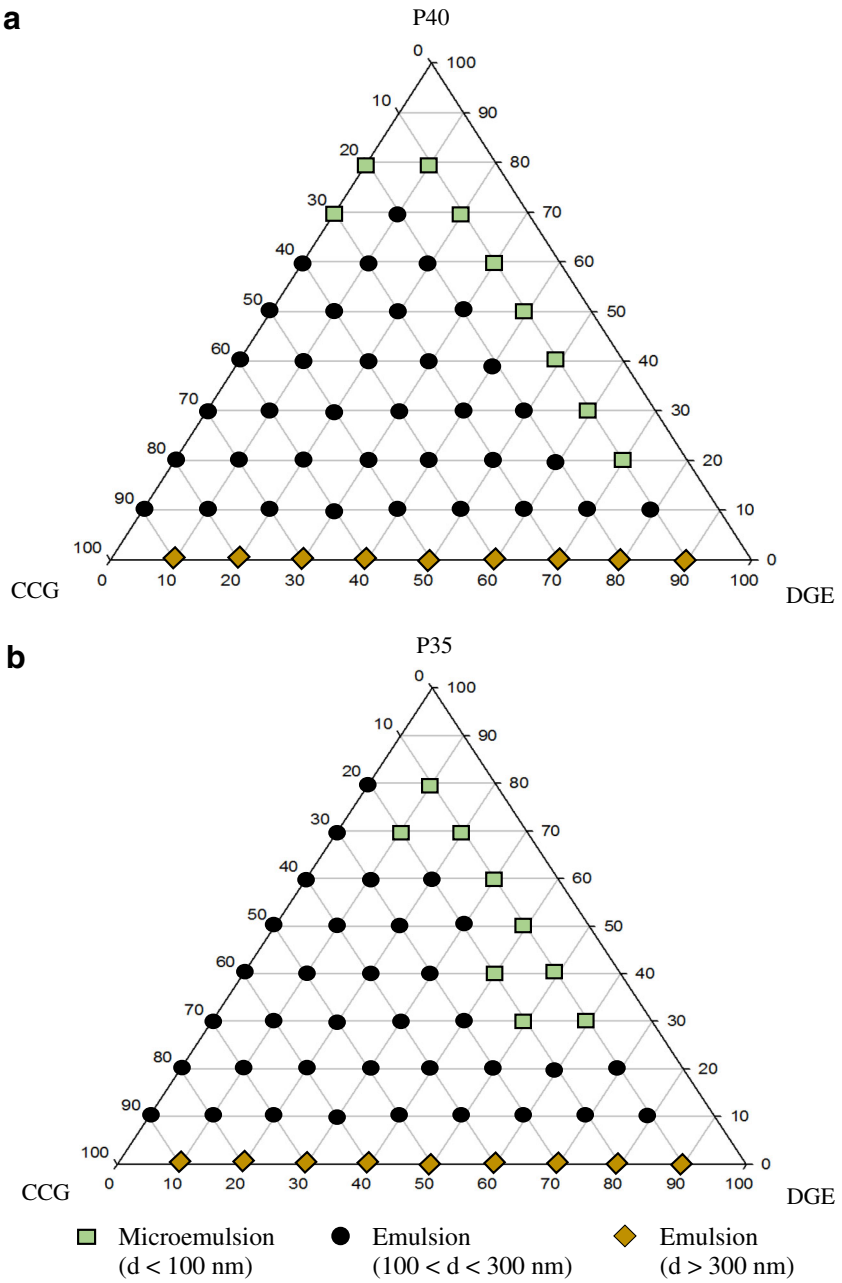

Fig. 1. Ternary phase diagrams of **a** P40, CCG, and DGE and **b** P35, CCG, and DGE. Diagrams were studied at 25°C. *Different symbols* indicate the size of emulsions obtained after dilution in water

corp., Japan) by dispersing in distilled water (199-fold) before dropping and drying on the copper grid. The samples were determined at TEM accelerating voltage of 200 keV.

In Vitro Dissolution Study

The dissolution test was carried out using USP dissolution apparatus II (model PWS3C, Pharma Test, Germany) with 900 mL of SGF (pH 1.2) as a dissolution medium at 37±0.5°C. The paddle speed was adjusted to 50 rpm. Selected NDP-loaded SNEDDS formulations (equivalent to 10 mg of NDP) filled in hard capsules were put into a sinker before placing in a dissolution vessel, which was protected from light. During the study, 5-mL aliquots were removed at predetermined time intervals from the dissolution medium and 5 mL of fresh medium were replaced. Samples were withdrawn from the dissolution vessels at 5, 10, 15, 30, 60, 90, and 120 min and passed through 0.45-μm nylon membrane filters before analysis. The amount of NDP dissolved in the dissolution medium was determined by HPLC, as mentioned above. The dissolution experiments were carried out in triplicate.

Bioavailability Studies

The oral bioavailability of NDP from selected SNEDDS formulation was compared with that of NDP powder. The

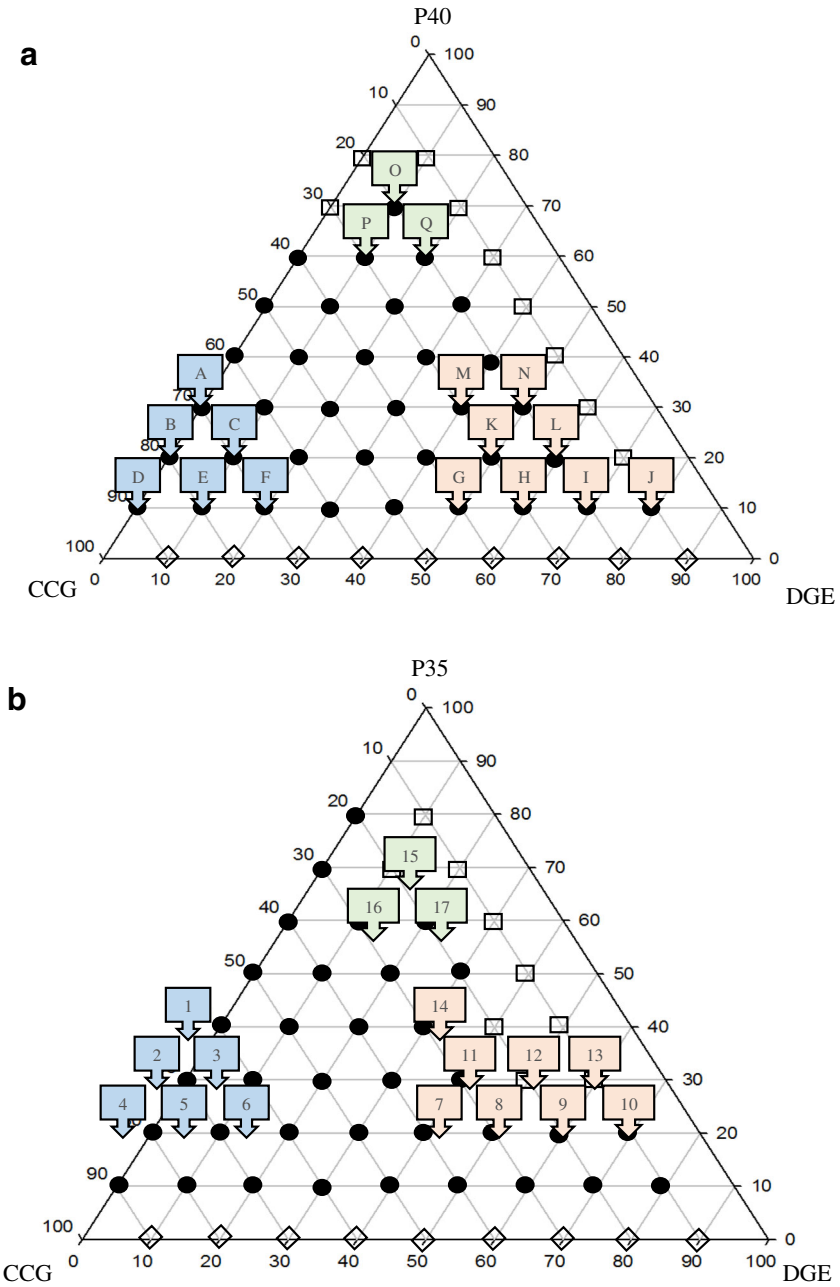

Fig. 2. Ternary phase diagrams showing the ratios of SNEDDS selected for NDP solubility study; **a** ternary phase diagram of P40, CCG, and DGE and **b** ternary phase diagram of P35, CCG, and DGE

in vivo studies were modified form the study of Burapapadh and coworkers (23). The experiments were performed in male Wistar rats (*Rattus norvegicus*, 8 weeks, 300–350 g, Southern Laboratory Animal Facility, Prince of Songkla University, Thailand) and less than 3 rats were stored in a cage, subjected to 12–12-h cycles of light and darkness, with free access to food and water. The rats were fasted for 24 h before experiment in order to avoid food influence on drug absorption. The rats were administrated with the formulation at a dose of 10 mg of NDP/kg body weight (n=5). Before administration to rats, the samples, equivalent to 5 mg of NDP, were dispersed in 1 mL of 0.5% (*w*/*v*) sodium carboxymethylcellulose solution by vortexing the mixtures for 5 min. Then, 1 mL of dispersion composing of NDP (5 mg/mL) was orally administered into rats. Before collecting the blood sample, the catheters were flushed with heparin solution not more than 1 day. Blood samples of approximately 600 μL were collected from the jugular vein at 0, 0.5, 1, 2, 4, 6, 12, and 24 h after dosing, and then placed into microcentrifuge tubes with light protection. The collected samples were centrifuged at 10,000 rpm (3330×*g*) for 5 min, and then plasma was extracted and transferred to a microcentrifuge tube. All samples were kept at −20°C before drug content determination with HPLC.

Prior to HPLC analysis, plasma was removed from the frozen samples and allowed to equilibrate to room temperature. The 200 μL of plasma were placed to another tube and the protein was precipitated by adding 800 μL of acetonitrile and mixed by vortex mixer for a minute. The mixtures were held still for 20 min before evaporation of solvent. The precipitates were dissolved in 150 μL of methanol and then determined the drug content by HPLC. The area under the plasma concentration-time curve (AUC) was calculated. The samples were analyzed by a HPLC (model Agilent 1100 Series HPLC System equipped with a photodiode array detector, Agilent Technologies, USA) using Luna 5u C18 column (5 μm, 4.6 nm×25 cm; Phenomenex®, USA). The analysis methods were applied form the studies reported (24,25). The solvent system is composed of solvent A (100% methanol) and solvent B (0.05% phosphoric acid). A 30-min linear gradient from 70% B to 0% B was applied at a flow rate of 1 mL/min, followed by a 10-min isocratic elution at 0% B and then a 10-min wash at 0% B, before returning to the starting condition. NDP was detected by monitoring of UV absorbance at 235 nm, and it was quantified by comparison of peak areas to a standard curve. All experiments were approved by the ethics committee for the use of laboratory animals, Faculty of Pharmacy, Silpakorn University, under the permission number 001/2013 and monitored by Department of Physiology, Faculty of Science, Prince of Songkla University.

RESULTS AND DISCUSSION

Construction of Ternary Phase Diagram

Ternary phase diagram was constructed in the absence of NDP in order to find the self-emulsifying regions and suitable concentration of oil, surfactant and co-surfactant. Based on previous work (10), P40 and P35 were used as oil phase, CCG was used as surfactant and DGE was used as co-surfactant for constructing different ternary phase diagrams. The spontaneous emulsifying properties were observed visually as well as by particle size analysis. The ternary phase diagrams containing P40/CCG/DGE and P35/CCG/DGE are presented in Fig. 1a, b, respectively. It was found that incorporation of surfactant of at least 10% resulted in clear or slightly bluish emulsions with droplet size between 100 and 300 nm while the surfactant concentration less than 10% resulted in turbid emulsions with droplet size more than 300 nm. Similar results were observed between the two surfactants (P40 and P35). The incorporation of co-surfactant, DGE, within the self-emulsifying region increased the spontaneity of self-emulsifying process. Clear microemulsions with the size less than 100 nm were obtained when concentration of CCG was 10–30% (for P40) or 10–20% (for P35). These formulations were not selected for further investigation because the concentration of DGE used was too high.

Among all formulations having the droplet size between 100 and 300 nm, 17 formulations, for each surfactant, were chosen for NDP loading, as shown in Fig. 2. The drug loading capacity of NDP-loaded SNEDDS formulation was determined (Table I). It

Table I. Solubility of NDP in Different Formulations of SNEDDS (Mean±S.D., n=3)

Formulation	Ratio of P35:CCG:DGE	Solubility (mg/mL)	Formulation	Ratio of P40:CCG:DGE	Solubility (mg/mL)
1	7:3:0	20.8±0.4	A	7:3:0	27.5±1.7
2	7:2:1	22.8±1.9	B	7:2:1	42.9±1.2
3	7:1:2	41.2±3.0	C	7:1:2	45.4±1.3
4	8:2:0	26.4±1.0	D	8:2:0	32.9±1.8
5	8:1:1	31.8±1.0	E	8:1:1	34.3±2.7
6	9:1:0	23.1±3.2	F	9:1:0	18.6±1.3
7	4:1:5	55.0±0.1	G	4:1:5	55.3±0.6
8	3:1:6	34.6±0.1	H	3:1:6	47.7±0.1
9	2:1:7	84.9±0.7	I	2:1:7	92.4±0.4
10	1:1:8	95.8±0.3	J	1:1:8	92.6±0.5
11	3:2:5	65.6±0.5	K	3:2:5	65.4±0.4
12	2:2:6	81.0±0.3	L	2:2:6	79.9±0.3
13	2:3:5	81.5±3.9	M	2:3:5	78.5±0.1
14	3:3:4	57.7±0.1	N	3:3:4	60.8±0.2
15	2:6:2	27.3±3.7	O	2:6:2	29.8±3.9
16	3:5:2	25.6±3.9	P	3:5:2	30.3±4.7
17	2:5:3	29.2±4.4	Q	2:5:3	35.8±5.4

is clearly seen that the formulations with high concentration of surfactant (formulation 1–6 for P35 and formulation A-F for P40) and those with high concentration of CCG (formulation 15–17 for P35 and formulation O-Q for P40) had a low drug-loading capacity (*i.e.*, 18–35 mg/mL). Higher drug loading (35–96 mg/mL) was obtained when the formulation with high concentration of co-surfactant (DGE), *i.e.*, formulation 7–14 and G-N for those using P35 and P40, respectively. The highest drug loading (about 93–96 mg/mL) was found in formulations containing P35/CCG/DGE of 1:1:8 and P40/CCG/DGE of 1:1:8. Zhang *et al.* (26) reported that the formulation containing Labrafil® M1944 CS/P40/DGE of 3:5:3 has the highest drug loading with a mean particle size approximately 140 nm. However, they formulated in a narrow range of P40 (30–70%) and DGE (25–40%). It has been reported that the drug incorporated in the SNEDDS may occasionally have some effects on the self-emulsifying performance and/or emulsion droplet size (27). However, in this study, no significant difference was observed in self-emulsifying performance between the SNEDDS and NDP-loaded SNEDDS formulations. Table II demonstrates the droplet size of SNEDDS formulations (without and with NDP loading) after diluting (199-fold) in water and SGF. It can be seen that, after diluting in water or SGF, the droplet size of SNEDDS was significantly increased. The results are consistent with those of other studies (16,28) that reported the notable increase in droplet size after drug loading in the nanoemulsions. It has been suggested that drug molecules reduce the flexibility of surface film. Drug molecules may also participate at the interface, resulting in closer and more compact interfacial film. Therefore, the spontaneous emulsification of SNEDDS was hindered and nanoemulsions with larger droplet sizes were obtained (16). However, the increase in droplet size was less affected by NDP loading in formulation containing P35. It is possibly due to the less interfacial tension of P35 (42.0 mN/m), compared with that of P40 (44.0 mN/m) (28,29). SNEDDS without and with drug were examined under transmission electron microscope (Fig. 3). The emulsion droplet containing NDP clearly showed the existence of solid phase in emulsion drop. Size of emulsion was confirmed by TEM that round shaped diameter was below 200 nm as shown in TEM images.

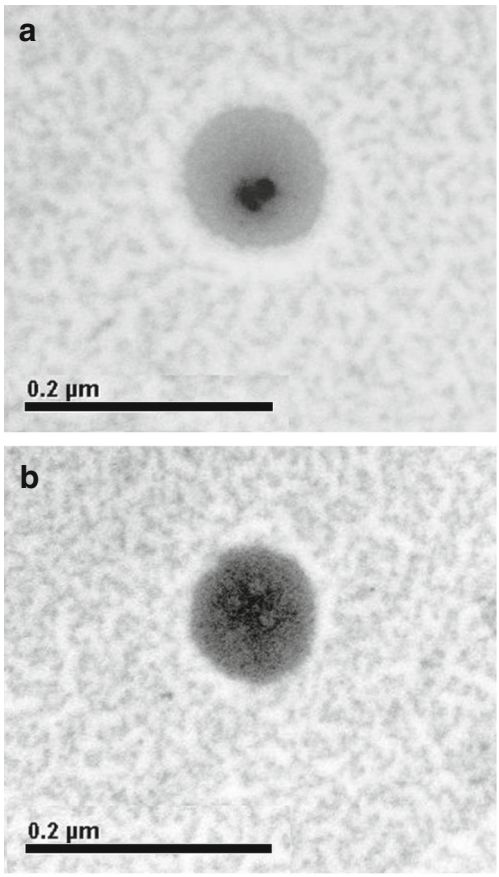

Fig. 3. TEM images of SNEDDS/P35 after diluting in water (199-fold); **a** without drug and **b** with NDP, at a magnification of ×120,000

Robustness to Dilution

Different fold dilutions of selected formulation were exposed to aqueous medium to simulate the *in vivo* conditions where the formulation would come across gradual dilution.

Table II. Droplet Size of SNEDDS Formulations After Diluting in Water and SGF (n=3)

	Size (nm)±S.D. (poly-dispersity index)	
Formulations	In water	In SGF
SNEDDS/P40 (without drug)	127.6±0.9 (0.375)	154.9±0.1 (0.390)
SNEDDS/P35 (without drug)	129.5±0.4 (0.395)	124.8±0.1 (0.315)
NDP-loaded SNEDDS/P40	187.9±5.3 (0.358)	185.4±0.5 (0.352)
NDP-loaded SNEDDS/P35	132.9±0.5 (0.183)	132.3±0.4 (0.178)

SNEDDS/P35 contains P35/CCG/DGE at the ratio of 1:1:8, *SNEDDS/P40* contains P40/CCG/DGE at the ratio of 1:1:8

Table III demonstrates the emulsion droplet size after diluting in different amounts of water. It was found that the droplet size of emulsions decreased when the amount of water increased except that of SNEDDS/P40 diluting with 4-fold water. However, the size of emulsion was still in nanometer

Table III. Droplet Size of SNEDDS Formulations After Diluting in Various Amounts of Water (n=3)

Percentages of water in samples	Folds of water	Size (nm)±S.D. (poly-dispersity index)	
		SNEDDS/P35	SNEDDS/P40
1	0.01	n.d.	n.d.
2	0.02	n.d.	n.d.
4	0.04	n.d.	n.d.
6	0.06	n.d.	n.d.
8	0.09	n.d.	175.9±6.4 (0.390)
10	0.11	n.d.	435.3±2.4 (0.149)
15	0.18	n.d.	498.3±6.9 (0.119)
20	0.25	167.3±21.3 (0.350)	298.7±1.6 (0.166)
40	0.67	558.1±28.8 (0.136)	198.5±1.9 (0.244)
60	1.50	224.5±1.9 (0.193)	130.1±1.0 (0.317)
80	4.00	298.0±8.6 (0.669)	315.7±17.4 (0.669)
99	99.00	132.9±5.3 (0.158)	187.9±5.0 (0.366)
99.5	199.00	129.5±0.4 (0.395)	127.6±0.9 (0.375)
99.67	302.03	114.5±0.0 (0.194)	155.6±0.6 (0.540)

SNEDDS/P35 contains P35/CCG/DGE at the ratio of 1:1:8, *SNEDDS/P40* contains P40/CCG/DGE at the ratio of 1:1:8, *n.d.* not detected (due to the detection limit of the equipment)

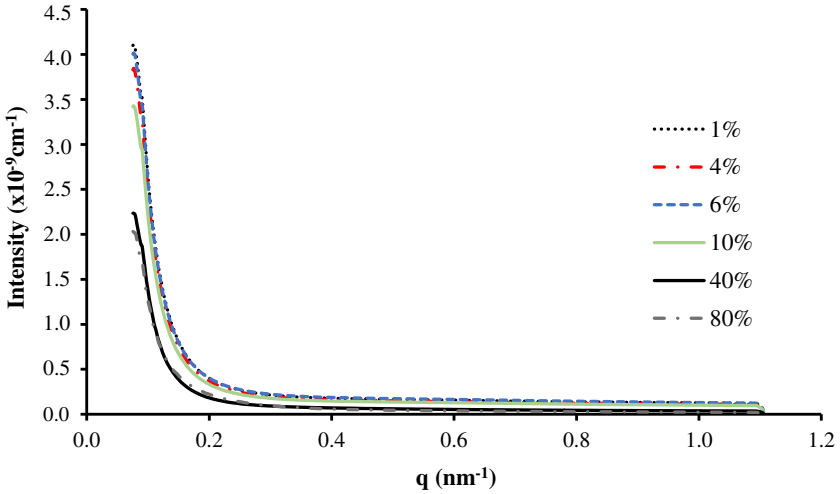

Fig. 4. SAXS curves of NDP-loaded SNEDDS/P35, diluting with different percentages of SGF

range, suggesting their robustness to dilution. At high amount of water, *e.g.*, 500–100-fold, the droplet size was very small and could not be measured by the equipment used. This result agreed with Balakumar *et al.* (30) who reported the acceptable droplet size (nanometer range) after dilution in 50-, 100-, and 1000-fold of water, providing the robustness to dilution.

Small Angle X-ray Scattering

The SAXS curves of SNEDDS/P35 diluting with different amounts of SGF are shown in Fig. 4. In distilled water, the SAXS patterns were similar to those in SGF (data not shown). The ordered structure was not found in this study, suggesting a simple, nano-sized, emulsion without any ordered structure. Formation of nanoemulsions by low-energy method has been related to phase transitions during the emulsification process, involving LC phase (20). Hexagonal and lamellar LC are determined in nanoemulsions composing of 35% water, 65% P35, 10% CCG and 18% water, 72% P35, 10% CCG (20). Our previous study (31) also demonstrated that SNEDDS containing resveratrol, P35, CCG, and water has ordered structure with the lamellar distances (d-spacing) of less than 20 nm. It seemed that the dilution of the prepared SNEDDS in water results in both large oil droplets (200–400 nm) in water and small micelles with the size of 10–20 nm. Surprisingly, this was not found in the case of formulations containing P35 (or P40), CCG and DGE. The DGE as co-surfactant may influence the LC formation. Choi *et al.*(32) reported the effect of short-chain alcohols as co-surfactant on pseudo-ternary phase diagram, the LC region gradually increased in longer carbon chain of alcohol molecule. The LC regions are not observed with shorter chain alcohol (ethanol) in all regions of pseudo-ternary phase diagram.

In Vitro Dissolution Study

The dissolution profiles of NDP, NDP-loaded SNEDDS/P35 and NDP-loaded SNEDDS/P40 are shown in Fig. 5. SNEDDS was immediately dispersed after capsule shell was dissolved within 5 min, suggesting high efficiency of spontaneous dispersion. At 60 min, the SNEDDS formulation provided drug dissolution more than 80% while the dissolution of

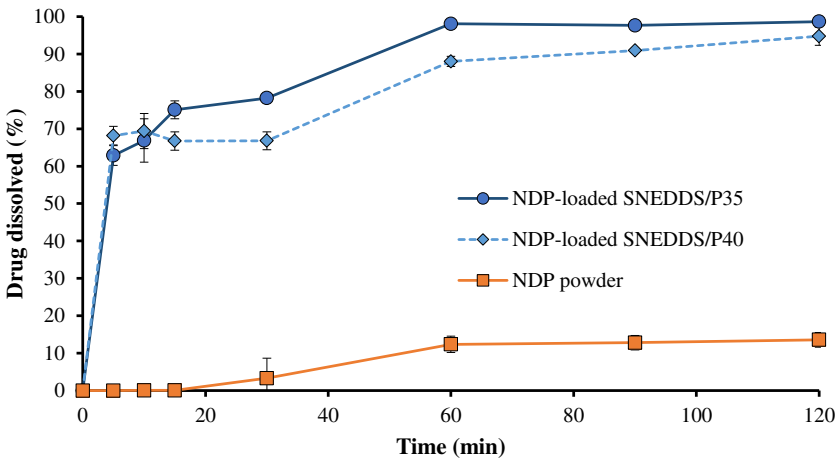

Fig. 5. Drug dissolution profiles of NDP powder, NDP-loaded SNEDDS/P35, and NDP-loaded SNEDDS/P40. The *data* represent the mean±S.D. of results from triplicate experiments

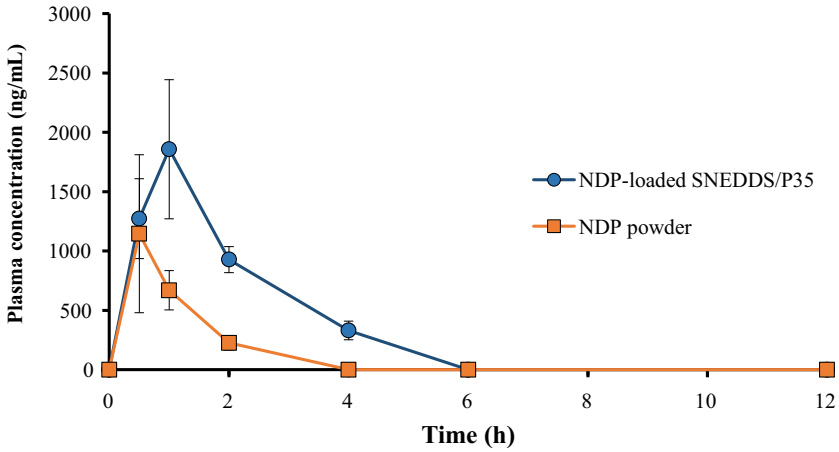

Fig. 6. *In vivo* plasma profiles of NDP-loaded SNEDDS/P35 and NDP powder. (n=5)

NDP powder was less than 20%. This may be due to low free energy required to form an emulsion of self-emulsifying systems that allowed spontaneous formation of an interface between oil droplets and water. Balakrishnan *et al.* (30) suggested that the mixture of oil, surfactant and co-surfactant and water phases swells, the emulsion droplet size decreases and ultimately the drug dissolution increases. Moreover, in this study, the improvement in NDP loading and dissolution was achieved, compared with our previous study (10); the NDP loading was increased from 30 to 80 mg/mL and the NDP dissolution, at 60 min, was increased from about 70% to 88–98%.

Bioavailability Studies

The formulation effect on the oral bioavailability of NDP was evaluated in rats. Figure 6 presents the *in vivo* plasma concentration-time profiles of NDP-loaded SNEDDS/P35 comparing with NDP powder. The NDP-loaded SNEDDS/P35 provided a higher in plasma profile. The C_{max} of NDP-loaded SNEDDS/P35 and NDP powder were 1857.8±585.5 and 1145.9±664.8 ng/mL, respectively. The $AUC_{0-12\ h}$ of NDP-loaded SNEDDS/P35 was higher than NDP powder (2.9-fold). The $AUC_{0-12\ h}$ of NDP-loaded SNEDDS/P35 and NDP powder were 4082.6±621.7 and 1413.4±388.4 ng/mL h, respectively. The results agreed with Kommuru *et al.* (33) who reported that the delivery of lipophilic compounds (CoQ_{10}) is improved by the oral SEDDS. Optimized formulation consists of Myvacet® 9-45 (40%), Labrasol® (50%), and Lauroglycol® (10%). A 2-fold increase in the bioavailability was observed for the SEDDS, compared with a CoQ_{10} powder. In another study reported by Zhao *et al.* (34), SNEDDS for the oral delivery of zedoary turmeric oil, containing zedoary turmeric oil, ethyl oleate, Tween® 80, DGE (30.8:7.7:40.5:21, *w*/*w*), were optimized and tested *in vivo*. Oral administration of SNEDDS in rats, AUC and C_{max} increased by 1.7-fold and 2.5-fold, respectively, compared with the unformulated zedoary turmeric oil.

As described above, dissolution study in SGF (pH 1.2) shows that the NDP-loaded SNEDDS/P35 had significant higher (about 7-fold) drug dissolution than NDP powder (Fig. 5). An increase in bioavailability of SNEDDS, compared with NDP powder, was fairly low (2.9-fold for $AUC_{0-12\ h}$). It is possible that digestion of lipid in the formulation could reduce the solubility of NDP in the gut lumen, which would result in precipitation of the drug and a decrease in the absorption rate (8). These observations are in accordance with the results published by Christiansen *et al.* (35), who reported a small difference in *in vivo* absorption of cinnarizine, when administered as liquid SNEDDS to beagle dogs in fasted state.

CONCLUSIONS

The present study has clearly demonstrated the potential use of SNEDDS for formulating NDP with improved dissolution and oral bioavailability. After SNEDDS was diluted with water, the droplet size of about 120 nm was obtained. The non-ordered structure was proposed after dilution at different percentages of water, according to the scattering experiment by SAXS. The dissolution studies revealed that SNEDDS formulations attributed to higher and faster dissolution of NDP than NDP powders. The selected SNEDDS formulation gave a higher AUC and C_{max} than NDP powder. The present study may serve as an approach for the formulation development of poorly water-soluble drugs in liquid form.

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REFERENCES

1. Neslihan GR, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed Pharmacother. 2004;58(3):173–82. doi:10.1016/j.biopha.2004.02.001.

2. Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev. 1997;25(1):103–28. doi:10.1016/S0169-409X(96)00494-2.
3. Chakraborty S, Shukla D, Mishra B, Singh S. Lipid—an emerging platform for oral delivery of drugs with poor bioavailability. Eur J Pharm Biopharm. 2009;73(1):1–15. doi:10.1016/j.ejpb.2009.06.001.
4. Porter CJ, Pouton CW, Cuine JF, Charman WN. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv Drug Deliv Rev. 2008;60(6):673–91. doi:10.1016/j.addr.2007.10.014.
5. Atef E, Belmonte AA. Formulation and in vitro and in vivo characterization of a phenytoin self-emulsifying drug delivery system (SEDDS). Eur J Pharm Sci. 2008;35(4):257–63. doi:10.1016/j.ejps.2008.07.004.
6. Mu H, Holm R, Mullertz A. Lipid-based formulations for oral administration of poorly water-soluble drugs. Int J Pharm. 2013;453(1):215–24. doi:10.1016/j.ijpharm.2013.03.054.
7. Date AA, Desai N, Dixit R, Nagarsenker M. Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances. Nanomedicine. 2010;5(10):1595–616. doi:10.2217/nnm.10.126.
8. Pouton CW. Formulation of self-emulsifying drug delivery systems. Adv Drug Deliv Rev. 1997;25(1):47–58. doi:10.1016/s0169-409x(96)00490-5.
9. Cole ET, Cade D, Benameur H. Challenges and opportunities in the encapsulation of liquid and semi-solid formulations into capsules for oral administration. Adv Drug Deliv Rev. 2008;60(6):747–56. doi:10.1016/j.addr.2007.09.009.
10. Weerapol Y, Limmatvapirat S, Nunthanid J, Sriamornsak P. Self-nanoemulsifying drug delivery system of nifedipine: impact of hydrophilic–lipophilic balance and molecular structure of mixed surfactants. AAPS PharmSci. 2014;15(2):456–64. doi:10.1208/s12249-014-0078-y.
11. Xi J, Chang Q, Chan C, Meng Z, Wang G, Sun J, *et al*. Formulation development and bioavailability evaluation of a self-nanoemulsified drug delivery system of oleanolic acid. AAPS PharmSciTech. 2009;10(1):172–82. doi:10.1208/s12249-009-9190-9.
12. Zhao Y, Wang C, Chow AH, Ren K, Gong T, Zhang Z, *et al*. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: formulation and bioavailability studies. Int J Pharm. 2010;383(1–2):170–7. doi:10.1016/j.ijpharm.2009.08.035.
13. Solè I, Solans C, Maestro A, González C, Gutiérrez JM. Study of nano-emulsion formation by dilution of microemulsions. J Colloid Interface Sci. 2012;376(1):133–9. doi:10.1016/j.jcis.2012.02.063.
14. Dahan A, Hoffman A. Rationalizing the selection of oral lipid based drug delivery systems by an *in vitro* dynamic lipolysis model for improved oral bioavailability of poorly water soluble drugs. J Control Release. 2008;129(1):1–10. doi:10.1016/j.jconrel.2008.03.021.
15. Wang Z, Sun J, Wang Y, Liu X, Liu Y, Fu Q, *et al*. Solid self-emulsifying nitrendipine pellets: preparation and in vitro/in vivo evaluation. Int J Pharm. 2010;383(1–2):1–6. doi:10.1016/j.ijpharm.2009.08.014.
16. Wang L, Dong J, Chen J, Eastoe J, Li X. Design and optimization of a new self-nanoemulsifying drug delivery system. J Colloid Interface Sci. 2009;330(2):443–8. doi:10.1016/j.jcis.2008.10.077.
17. Iosio T, Voinovich D, Perissutti B, Serdoz F, Hasa D, Grabnar I, *et al*. Oral bioavailability of silymarin phytocomplex formulated as self-emulsifying pellets. Phytomedicine. 2011;18(6):505–12. doi:10.1016/j.phymed.2010.10.012.
18. Chava VP. Are SMEDDS and SNEDDs same? A gimmick or pharmaceutically relevent. Mintage J Pharm Med Sci. 2012;1(1):7–10.
19. Forgiarini A, Esquena J, González C, Solans C. Formation of nano-emulsions by low-energy emulsification methods at constant temperature. Langmuir. 2001;17(7):2076–83. doi:10.1021/la001362n.
20. Sadurní N, Solans C, Azemar N, García-Celma MJ. Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical applications. Eur J Pharm Sci. 2005;26(5):438–45. doi:10.1016/j.ejps.2005.08.001.
21. Yalkowsky S, He Y, Jain P. Handbook of aqueous solubility data. 2nd ed. Tucson: CRC Press; 2010.
22. Huang J, Wigent RJ, Bentzley CM, Schwartz JB. Nifedipine solid dispersion in microparticles of ammonio methacrylate copolymer and ethylcellulose binary blend for controlled drug delivery. Effect of drug loading on release kinetics. Int J Pharm. 2006;319(1–2):44–54. doi:10.1016/j.ijpharm.2006.03.035.
23. Burapapadh K, Takeuchi H, Sriamornsak P. Novel pectin-based nanoparticles prepared from nanoemulsion templates for improving in vitro dissolution and in vivo absorption of poorly water-soluble drug. Eur J Pharm Biopharm. 2012;82(2):250–61. doi:10.1016/j.ejpb.2012.07.010.
24. Jansakul C, Tachanaparuksa K, Mulvany MJ, Sukpondma Y. Relaxant mechanisms of 3,5, 7,3′,4′-pentamethoxyflavone on isolated human cavernosum. Eur J Pharmacol. 2012;691(1–3):235–44. doi:10.1016/j.ejphar.2012.07.019.
25. Zhang QY, Kaminsky LS, Dunbar D, Zhang J, Ding X. Role of small intestinal cytochromes p450 in the bioavailability of oral nifedipine. Drug Metab Dispos. 2007;35(9):1617–23. doi:10.1124/dmd.107.016543.
26. Zhang J, Peng Q, Shi S, Zhang Q, Sun X, Gong T, *et al*. Preparation, characterization, and in vivo evaluation of a self-nanoemulsifying drug delivery system (SNEDDS) loaded with morin-phospholipid complex. Int J Nanomedicine. 2011;6:3405–14. doi:10.2147/ijn.s25824.
27. Li W, Yi S, Wang Z, Chen S, Xin S, Xie J, *et al*. Self-nanoemulsifying drug delivery system of persimmon leaf extract: optimization and bioavailability studies. Int J Pharm. 2011;420(1):161–71. doi:10.1016/j.ijpharm.2011.08.024.
28. Matsaridou I, Barmpalexis P, Salis A, Nikolakakis I. The influence of surfactant HLB and oil/surfactant ratio on the formation and properties of self-emulsifying pellets and microemulsion reconstitution. AAPS PharmSciTech. 2012;13(4):1319–30. doi:10.1208/s12249-012-9855-7.
29. Fraga AK, Souza LFI, Magalhães JR, Mansur CRE. Development and evaluation of oil in water nanoemulsions based on polyether silicone as demulsifier and antifoam agents for petroleum. J Appl Polym Sci. 2014;(131). doi:10.1002/app.40889.
30. Balakumar K, Raghavan CV, Selvan NT, Prasad RH, Abdu S. Self nanoemulsifying drug delivery system (SNEDDS) of rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation. Colloids Surf B: Biointerfaces. 2013;112:337–43. doi:10.1016/j.colsurfb.2013.08.025.
31. Kumpugdee-Vollrath M, Weerapol Y, Svhrader K, Sriamornsak P. Investigation of nanoscale structure of self-emulsifying drug delivery system containing poorly water-soluble model drug. Adv Mater Res. 2014;970:272-8. doi:10.4028/www.scientific.net/AMR.970.272.
32. Choi S-Y, Oh S-G, Bae S-Y, Moon S-K. Effect of short-chain alcohols as co-surfactants on pseudo-ternary phase diagrams containing lecithin. Korean J Chem Eng. 1999;16(3):377–81. doi:10.1007/BF02707128.
33. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int J Pharm. 2001;212(2):233–46. doi:10.1016/S0378-5173(00)00614-1.
34. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates in vitro and in vivo. Int J Pharm. 2004;278(1):119–31. doi:10.1016/j.ijpharm.2004.03.001.
35. Christiansen ML, Holm R, Kristensen J, Kreilgaard M, Jacobsen J, Abrahamsson B, *et al*. Cinnarizine food-effects in beagle dogs can be avoided by administration in a self nano emulsifying drug delivery system (SNEDDS). Eur J Pharm Sci. 2014;57:164–72. doi:10.1016/j.ejps.2013.11.003.