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Development of solidified self-microemulsifying drug delivery systems containing L-tetrahydropalmatine: Design of experiment approach and bioavailability comparison

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ABSTRACT

The study first aimed to apply a design of experiment (DoE) approach to investigate the influences of excipients on the properties of liquid self-microemulsifying drug delivery system (SMEDDS) and SMEDDS loaded in the pellet (pellet-SMEDDS) containing L-tetrahydropalmatine (l-THP). Another aim of the study was to compare the bioavailability of l-THP suspension, liquid SMEDDS and pellet-SMEDDS in the rabbit model. By using Central Composite Face design (CCF), the optimum ratio of Capryol 90, and S_{mix} `(Cremophor RH 40: Transcutol HP) in the formulation of SMEDDS was determined. This optimum SMEDDS was absorbed on the solid carrier (Avicel or Aerosil) for the preparation of pellet-SMEDDS by extrusion and spheronization method. The ANOVA table indicated that Avicel was more effective than Aerosil, the traditional solid carrier, in both terms of preservation of dissolution rate of l-THP from the original SMEDDS and pelletization yield. Results obtained from scanning electron microscopy (SEM) indicated that the existence of liquid SMEDDS droplets on the surface of pellet-SMEDDS was due to the absorption on Avicel. The powder X-ray diffractometry proved the amorphous state of l-THP in pellet-SMEDDS. Pharmacokinetic study in the rabbit model using liquid chromatography tandem mass spectrometry showed that the SMEDDS improved the oral bioavailability of l-THP by 198.63% compared to l-THP suspension. Besides, pharmacokinetics study also proved that the mean relative bioavailability (AUC) and mean maximum concentration (C_{max}) of pellet-SMEDDS were not significantly different from the original liquid SMEDDS ($p > 0.05$).

1. Introduction

L-tetrahydropalmatine (THP) also known as rotundine was an alkaloid extracted from a herbal plant, *Stephania Rotunda Menispermaceae*. This herbal drug had a traditional use as an analgesic, anxiolytic and sedative drug (Zhao et al., 2014). The popular dosage form containing l-THP was the conventional tablet. Accordingly, the dissolution rate of l-THP from the tablet was not mentioned in literature as a limiting-bioavailability factor. However, recent studies indicated that l-THP had low aqueous solubility and low oral bioavailability (Li et al., 2011a). Furthermore, other authors (Chao-Wu et al., 2011; Li et al., 2011a) reported that l-THP had pH dependent solubility. The drug was a weak alkali agent thus being soluble in gastric medium but easily precipitated in the intestinal medium. The poorly aqueous solubility of l-THP was also the general property of alkaloids and several other herbal drugs such as curcumin (Zhang et al., 2012), silymarin (Wu et al., 2006) or baicalein (Liu et al., 2012).

Self microemulsifying drug delivery systems (SMEDDS) has been emerging as one of a potential carrier system for improving the bioavailability of poorly soluble herbal drugs (Bi et al., 2016; Chen et al., 2017; Jaisamut et al., 2017a,b; Zhang et al., 2017). For example, Li et al. (2011b) reported that the bioavailability of SMEDDS containing kaempferol extracted from Persimmon leaf was 1.6 times higher than the conventional tablet. Similarly, Liu et al., (2012) concluded that the bioavailability of SMEDDS containing baicalein extracted from the root of *Scutellaria baicalensis* almost doubled that of an aqueous drug suspension. The reason for the bioavailability enhancement of SMEDDS has been discussed extensively in literature. Briefly, SMEDDS had a very

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high surface area (nano size) for drug absorption when SMEDDS was diluted in GI fluid (Kang et al., 2004; Patel and Sawant, 2007). Besides, the components used in SMEDDS like oil, surfactant and cosolvent were well known as solubilizers or permeability enhancers (Pouton, 2000; Porter et al., 2007; Pouton and Porter, 2008; Bala et al., 2016; Yeom et al., 2017) which played the pivotal role in enhancement of drug bioavailability (Rabinow, 2004; Buckley et al., 2013; Hong et al., 2016). Even though SMEDDS was a useful drug carrier, there has been virtually no publication relating SMEDDS containing L-tetrahydropalmatine. Application of SMEDDS for l-THP – a drug having low solubility and a narrow therapeutic range, therefore, should be regarded as a new and rational approach.

SMEDDS was generally filled into soft gelatin capsules as the final dosage form. However, this dosage form exhibited a number of disadvantages such as high manufacturing cost, incompatibility of capsule shell and liquid SMEDDS, and leakage of liquid SMEDDS (Jannin et al., 2008). Consequently, recently much attention has been drawn to solid SMEDDS (Setthacheewakul et al., 2010; Sermkaew et al., 2013; Qi et al., 2014; Krupa et al., 2015; Midha et al., 2016; Yeom et al., 2016). Three main forms of solid SMEDDS were powder SMEDDS, tablet SMEDDS, and pellet SMEDDS, in which the most important component was solid carriers. Some representatives of the solid carrier included silica dioxide (Tan et al., 2013; Chavan et al., 2015; Pandey et al., 2017), dextran (Oh et al., 2011) or microcrystalline cellulose (Setthacheewakul et al., 2010; Hu et al., 2012; Tao et al., 2016). The effect of these solid carriers on the loading amount of SMEDDS was extensively investigated using the trial and error experimental approach, and it was concluded that silica dioxide was the top priority for having very high surface area and the ability to bear the highest amount of liquid SMEDDS. However, other critical output factors of these solid SMEDDS such as the preservation of high dissolution rate of drugs and yield of solidifying process were paid little attention by the authors. The interaction effect of the solid carriers on these critical output factors has also not been well addressed in existing literature. In such context, a modern experimental design, the quality-by-design, has been applied to study the impact of solid carriers on some critical output factors of these solidified SMEDDS, which is expected to offer a comprehensive view on the solidification process of liquid SMEDDS.

Over the past decades, quality-by-design (QbD) has been promoted by the United States Food and Drug Administration (US FDA) as a systematic approach to enhance pharmaceutical development through design efforts (2009). The QbD has two main objectives: (a) to design a process in a way that pharmaceutical manufacture consistently meets critical quality attributes, and (b) to understand and control the impact of formulation components and process parameters on the critical quality attributes. To get an insight into both the main and interaction effects of formulation and process factors, some designs of the experiment (DoE) have been employed. In this particular research, the central composite design was chosen as our DoE, because it can handle many independent variables simultaneously and allows for better estimation of terms of an order than other designs of the experiment.

Taking into account the advantages of QbD, the role of two popular solid carriers including silica dioxide (Aerosil) and microcrystalline cellulose (Avicel) in pellet containing the original liquid SMEDDS was investigated. Pellet-SMEDDS was chosen as the solid dosage form containing SMEDDS, for it offered many advantages like uniform drug absorption, low in inter- and intra-subject variability in drug absorption and clinical response, avoidance of dose dumping, and lower possibility of localized irritation (Rahman et al., 2009; Sinha et al., 2009). Aerosil was known as an irreplaceable solid carrier for SMEDDS while Avicel played a dual role of a solid carrier and a spherical aid to a pellet. By using analysis of variance (ANOVA), the statistical impact of Aerosil and Avicel on pelletization yield and the release rate of l-THP from pellet-SMEDDS were systematically investigated. Application of DoE approach for the development of a solid form containing liquid SMEDDS which could preserve the original advantages of the liquid SMEDDS was the second new point of this study.

In an attempt to make use of the advantages of SMEDDS and pellet, the study first aimed to apply DoE approach to investigate the influences of excipients on the properties of liquid SMEDDS and pellet-SMEDDS. Another aim of the research was to make a comparison between the bioavailability of l-THP suspension, SMEDDS and pellet-SMEDDS in the rabbit model.

2. Materials and methods

2.1. Materials

L-Tetrahydropalmatine was obtained from Xi'an Biotech Development Co., Ltd. (China). Berberine hydrochloride was purchased from Sigma-Aldrich Corporation (U.S.A). Propylene glycol caprylate (Capryol 90) and diethylene glycol monoethyl ether (Transcutol HP) were supplied by Gattefossé (France). Cremophor RH 40, polyvinyl pyrrolidone K 30 (PVP K30) was purchased from BASF (Germany). Polysorbate 80 (Tween 80) was purchased from Croda (U.K). HPLC-grade methanol was purchased from J.T. Baker (U.S.A.). Microcrystalline cellulose (AvicelPH 101) and sodium croscarmellose were purchased from Mingtai Chemical Co., Ltd. (Taiwan). Fumed silica (Aerosil 200) was purchased from Evonik Corporation (Germany). Lactose monohydrate was purchased from Fonterra corporation (New Zealand). Water was purified by reverse osmosis and was filtered in house. All other reagents were analytical grade commercial products.

2.2. Development of SMEDDS

2.2.1. Solubility studies

The solubility of l-THP in different oils, surfactants, co-solvents and aqueous mediums was investigated. An excess amount of l-THP was added to 5 mL of each selected solvents and shaken using an isothermal shaker (Daihan, Korea, Model WCB 30) at 25 °C for 48 h. After being centrifuged at the relative centrifugal force (rcf) of 1972 for 10 min, the supernatant was withdrawn and filtered through membranes 0.45 μm (Sartorius, Germany, Model Minisart RC 25). The concentration of l-THP in the supernatant of each solvent was determined using a validated HPLC method.

Briefly, the sample was mixed with an equal volume of the mobile phase and then 20 μl was injected into the column for analysis. The HPLC system consisted of an isocratic pump (Agilent, U.S.A., Model G1311C), a manual injector (Agilent, U.S.A., Model G1328C), a column thermostat (Agilent, U.S.A., Model G1316A), a multi-wavelength detector (Agilent, U.S.A., Model G1315D). Detector output was integrated and digitalized using the Agilent ChemStation software (Agilent, U.S.A., Model 1200 Series HPLC system). The column used was a C18 column (Zorbax SB, 4.6 × 250 mm, 5μm particle size, Agilent, U.S.A.). The mobile phase consisted of phosphate buffer saline pH 4.5 (0.05M): acetonitrile (70:30, V/V). Its flow rate was 1.5 mL/min and the detector wavelength was 283 nm. The total run time for a sample was about 10 min. All operation was carried out at ambient temperature.

2.2.2. Construction of ternary phase diagrams

To obtain an optimum formula of the SMEDDS which can form a microemulsion upon dilution with water, pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature. Based on preliminary experiments, Capryol 90 was used as the oil phase, Cremophor RH 40 was used as the surfactant, and Transcutol HP was used as the cosurfactant. Surfactant and cosurfactant were mixed in different weight ratios (5:1, 4:1, 3:1, 2:1, 1:1 and 1:2) to obtain S_{mix}. For each phase diagram, oil and a specific S_{mix} ratio (O/S_{mix}) were mixed thoroughly with different weight ratios from 2:8, 3:7, 4:6, 5:5, 6:4 and 7:3 in glass vials. Pseudo-ternary phase diagrams were developed using aqueous titration method. The phase boundary was determined by visually observing the changes in the sample appearance

from turbid to transparent or via versa.

2.2.3. Loading drug into SMEDDS

To find out a suitable SMEDDS, various amounts of drug (Table 2) were added into mixtures of oil and S_{mix}. The total amount of oil and S_{mix} in each SMEDDS formulation was fixed at 1.5 g. These formulations were then diluted with 25 ml of distilled water, and the change of appearance after 72 h storage at room temperature was visually observed. An optimum SMEDDS must have a transparent state without drug precipitation.

2.2.4. Design of experiment for SMEDDS

The design of experiments in formulation settings of SMEDDS was developed using factorial design. The amount of Capryol 90 and S_{mix} were chosen as independent variables. To eliminate any possible errors, all of the conditions relating to the preparation process were kept constant. As shown in Table 3, the screening ranges of Capryol 90 and S_{mix} were 400–800 mg and 700–1100 mg, respectively. In addition, to avoid drug precipitation in SMEDDS, the amount of l-THP loaded in SMEDDS was maintained under 2% in SMEDDS which was equal to 20 mg per one formulation of SMEDDS; the ratio of Cremophor RH 40: Transcutol HP in SMEDDS was 3:1. These two independent formulation variables were simultaneously varied according to Central Composite Face design, which comprised a full or fractional factorial design and star points placed on the faces of the sides. The dependent variables included droplet size and PDI of oil phase after addition of distilled water into SMEDDS and dissolution efficiency of l-THP after 180 min (DE_{180}). The requirement for the optimum SMEDD regarding the droplet size, PDI and DE180 were less than 50 nm, under 0.3 and maximum value, respectively.

2.2.5. Emulsion droplet size measurement

Samples were gently diluted 60 times with ultra-purified water, and measurements were taken at 25 °C. Droplet size distribution of the microemulsion was studied using photon correlation spectroscopy (PCS) with the help of a Malvern Zetasizer (Malvern Instruments, UK, Model Zetasizer Nano ZS90).

2.2.6. Dissolution study

2.2.6.1. Dissolution comparison of different formulations of SMEDDS. To compare the dissolution efficiency of different formulations, SMEDDS containing 20 mg l-THP was diluted with 10 ml of distilled water and added to a dialysis bag (Spectrum® Laboratory, U.S.A, Membrane MWCO 12,000–14,000 Da) was placed into 500 ml of dissolution medium (acid hydrochloride 0.1 N) at 37 °C ± 0.5 °C and under 100 rpm stirring. The dissolution rates of l-THP from samples into the medium were measured using the dissolution apparatus type 2 (Erweka, Germany, Model DT 600). Five milliliters of aliquot was withdrawn at predetermined time intervals of 0.25, 0.5, 1, 1.5, 2, 2.5, 3 h and filtered through membranes 0.45 µm (Satorius, Germany, Model Minisart RC 25). The medium was replaced with 5 ml of fresh medium each time. Withdrawn samples were analyzed using a UV spectrophotometer (Hitachi, Japan, Model U-1800) at 281 nm. Dissolution efficiency (D.E.) of each formulation was calculated by the following equation:

$$DE = \frac{\int_{t_1}^{t_2} y.\, dt}{y_{100}.\ (t_2 - t_1)} \times (100)$$

Where y is the percentage of dissolved product; D.E. is the area under the dissolution curve between time points t_1, and t_2 expresses the percentage of the curve at maximum dissolution, y_{100}, over the same period.

2.2.6.2. Dissolution comparison of l-THP suspension with SMEDDS and pellet-SMEDDS. To compare the dissolution efficiency of SMEDDS and pellet-SMEDDS with raw material, hard capsules with size 0 containing these ones equivalent to 20 mg l-THP was added into 500 ml dissolution medium (acid hydrochloride 0.1 N). After 2 h, the dissolution medium was changed to pH 6.8 by addition of 250 ml Na_2HPO_4 0.4M. The experiment was conducted at 37 °C ± 0.5 °C and under 100 rpm stirring. The dissolution rate of l-THP from samples into medium was measured using the dissolution apparatus type 2 (Erweka, Germany, Model DT 600). Five milliliters of aliquot were withdrawn at predetermined time intervals of 0.25, 1, 2, 2.5, 4, 5 h and filtered through membranes 0.45 µm (Satorius, Germany, Model Minisart RC 25). The medium was replaced with 5 ml of fresh medium each time. Withdrawn samples were diluted by methanol and analyzed using HPLC method.

2.3. Development of pellet-SMEDDS

2.3.1. Preparation of pellet-SMEDDS

Pellet-SMEDDS was prepared by extrusion spheronization technique. The optimum SMEDDS was adsorbed onto the powder mixtures of Avicel PH 101 and/or Aerosil 200 and mixed with other solid excipients (lactose monohydrate, sodium croscarmellose). The percentage amount of lactose monohydrate and sodium croscarmellose in pellet-SMEDDS were 10% and 5%, respectively. A binder solution of PVP K30 was then added to the powder mixture to obtain a suitable wet mass. After 30-min incubation in room condition for absolute absorption of water into microcrystalline chain, this wet mass was extruded through an extruder (Umang Pharmatech, India, Model EXT-65) at 40 rpm and using sieve No 18. The extrudate was spheronized at 600 rpm for 5 min in a spheronizer (Umang Pharmatech, India, Model SPH-250) using a cross-hatch frictional plate with a mm grooved width. The obtained pellets were finally dried in oven at 50 °C for 6–8 h.

2.3.2. Design of experiment for pellet-SMEDDS

To deploy quality by design in formulation settings of pellet-SMEDDS, the design of experiments was one again set up using factorial design. All the preparation process parameters were fixed at the constant levels. The two formulation parameters were the percentage of Aerosil and Avicel in the pellets. The amount of Aerosil and Avicel were adjusted from 0 to 10% and from 30 to 50%, respectively (Table 4). The pelletization yield, dissolution efficiency ($D.E_{50}$), and dissolution rate of l-THP after 10 min ($D.R_{10}$) were selected as dependent variables. The eleven experimental formulations in Table 4 described the coded values of independent variables (Aerosil and Avicel) and the determined results of dependent variables (pelletization yield, dissolution efficiency and dissolution rate of l-THP after 10 min). In this design matrix, the center points, which were formulation No 9, 10, 11, were added in the experimental design for checking curvature.

2.3.3. Pelletization yield measurement

Size distribution of pellet was measured by a set of standard sieves. Pelletization yield was evaluated by the following equation:

$$H = m_1/m_2 * 100\%.$$

Where m_1, m_2 were the weight of pellets in the range of 800 − 1250 µm and the total weight of obtained pellets, respectively.

2.3.4. Dissolution study

To compare the dissolution efficiency of the different formulations, hard capsule with size 0 containing the pellet-SMEDDS equivalent to 20 mg l-THP was put into 500 ml dissolution medium (acid hydrochloride 0.1 N) at 37 °C ± 0.5 °C and under 100 rpm stirring. The dissolution rate of l-THP from samples into the medium was measured using the dissolution apparatus type 2 (Erweka, Germany, Model DT 600). Five milliliters of aliquot was withdrawn at predetermined time intervals of 10, 20, 30, 40 and 50 min then filtered through membranes

0.45 μm (Satorius, Germany, Model Minisart RC 25). The medium was replaced with 5 ml of fresh medium each time. Withdrawn samples were analyzed using a UV spectrophotometer (Hitachi, Japan, Model U-1800) at 281 nm.

2.3.5. Emulsion droplet size measurement

About 3 grams of pellet-SMEDDS was added into 15 ml ultra-purified water and filtered through membranes 0.45 μm (Satorius, Germany, Model Minisart RC 25). Measurements were taken at 25 °C using photon correlation spectroscopy (PCS) with the help of a Malvern Zetasizer (Malvern Instruments, UK, Model Zetasizer Nano ZS90).

2.3.6. Pellet morphology and shape

Morphology and structure of pellet-SMEDDS were studied using scanning electron microscopy (SEM) (Hitachi, Japan, Model FESEM S-4800). The sample was mounted on the stub and sputter coated with gold particles and observed at an accelerating voltage of 0.5–30 kV.

2.3.7. Powder X-ray diffractometry (PXRD)

The crystallinity of l-THP, physical mixture and pellet-SMEDDS were evaluated using an X-ray diffractometer (Siemens, Germany, Model D500) with Cu-Kal radiation and Ni filter. X-ray diffraction data were collected at room temperature in the range of $10^\circ < 2\theta^\circ < 50^\circ$.

2.4. Pharmacokinetics study

The animal study was approved by the Local Animal Use Committee. Nine male rabbits, each weighed 2 kg, were divided into 3 groups of three for use in the pharmacokinetics study. The rabbits were kept in fasting condition one night prior to the day of the experiment. The three samples were the suspension of l-THP in NaCMC 0.5%, liquid SMEDDS and pellet-SMEDDS. The dosage of l-THP used in PK study was 1.5 mg/kg. Blood samples, about 2 ml each, were withdrawn from the ear artery after 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h and supplemented with equal amounts of saline containing heparin 50UI. Plasma was collected by centrifugation of the above blood at 2684 rcf within 10 min and preserved in deep-freezer at −40 °C until the day of analysis.

2.5. LC/MS analysis of l-THP in rabbit plasma

The withdrawn samples were analyzed by liquid chromatography tandem mass spectrometry. An AB Sciex 5500 QQQ mass spectrometer (AB Sciex, USA) coupled with LC- 20AD high-pressure pumps, column compartment and autosampler (Shimadzu, Japan) was used to quantify the analyte. LC separation was obtained by using a Symmetry C18 column (150 × 4.6 mm; 5 μm particle size) and a precolumn (Waters, USA) with a mobile phase composition of 5 mM ammonium acetate and acetonitrile. The gradient program was initially set at 50% acetonitrile for 1 min then increased linearly to 100% acetonitrile over 1 min. After that, the eluent composition was maintained at 100% acetonitrile for 4 min then returned to 50% acetonitrile in 1 min and re-equilibrated for over 3 min. The flow rate was kept constant at 0.5 ml min^{-1}. The total run time was 10 min.

The mass spectrometer was operated in negative ESI mode with the capillary voltage and temperature set at −4500 V and 400 °C, respectively. A Peak Scientific AB-3G gas generator (UK) was used to generate N_2 used as curtain gas and air used as source gas. MS experiments were carried out in multiple reaction monitoring modes with two transitions for each compound. The higher intensities of the precursor-to-product ion transition were used for quantification.

A 500 μL aliquot of the plasma sample was transferred into a 2 mL centrifuge tube. 25 μL of IS solution of 1 μg mL^{-1} (berberine hydrochloride in methanol) was added to the tube, followed by the addition of acetonitrile (0.5 mL). These elements were then mixed by a vortex mixer for 1 min. A mixture of salts (0.2 mg of magnesium sulfate anhydrous and 0.05 mg of sodium chloride) was gradually added to the tube. After mixing for about 1 min, the tube was centrifuged at the maximum speed (16060 rcf) for 10 min. The supernatant was filtered through a 0.45μm membrane and 5 μL of the filtrate was injected into the LC–MS/MS system.

2.6. Data analysis

The data was calculated using Excel (Microsoft, USA) and WinNonlin (Scienfitic Consulting Inc., USA) program. Data were expressed as mean ± S.D and analyzed for statistical significance by one-way ANOVA and Student' *t*-test using Excel (Microsoft 2016, USA).

3. Results and discussion

3.1. Development of SMEDDS

3.1.1. Preformulation study

Despite the fact that L-tetrahydropalmatine was the main alkaloid responsible for clinical indications of *Stephania Rotunda Menispermaceae*, physicochemical information regarding l-THP, especially its solubility in various solvents and bioavailability, has been rather limited. Available literature on l-THP only included some basics such as its molecular structure (Fig. 1), its pKa of 5.34, its two forms of anhydrous and monohydrate (Yang et al., 2015), as well as the melting point of 141 ~ 144°C. Therefore, the main purpose of this part was to determine the drug solubility in various solvents, one of the important preformulation parameters, which were used to screen the suitable excipients for liquid SMEDDS.

Solubility study

As shown in Table 1, l-THP was almost insoluble in water and had pH-dependent solubility. The poor solubility of l-THP in water might result in the drug having low bioavailability, making the preparation of self-microemulsifying drug delivery systems rational. Besides, the amine group in the molecular structure of the drug (Fig. 1) made it a weakly basic compound which could cause not only variability of drug solubility in the gastrointestinal tract but also intra subject variability in the oral bioavailability. This, once again, highlighted the importance of enhancing the solubility of l-THP by using SMEDDS.

Capryol 90 and Labrafac™ lipophile WL 1349 were used to screen the suitable oil phase for SMEDDS. Capryol 90, also known as propylene glycol caprylate consisted of propylene glycol esters of caprylic acid (C8), was mainly composed of monoesters and a small fraction of diesters. Meanwhile, Labrafac™ lipophile WL 1349 consisted of medium-chain triglycerides of caprylic (C8) and capric (C10) acids, often referred to as medium-chain triglycerides for short. Results showed l-THP had the highest solubility in Capryol 90 (86.56 mg/ml). This might be explained by the amphiphilic structure of Capryol 90 (HLB = 5.0), which made it easier to enhance the drug solubility than an oily vehicle

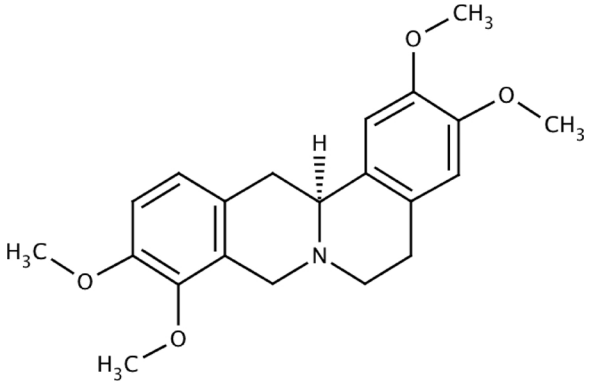

Fig. 1. Structure of L-tetrahydropalmatine.

Table 1
Solubility of l-THP in different mediums (n = 3, Mean ± STDEV).

	Excipients	Solubility (mg/ml) (n = 3, Mean ± STEDV)
Oils	Capryol 90	86.56 ± 0.90
	Labrafac	23.09 ± 0.12
Surfactants	Cremophor RH 40	118.44 ± 0.79
	Tween 80	62.52 ± 0.48
Cosolvents	Transcutol HP	105.62 ± 0.36
	PEG 400	37.81 ± 0.27
	Isopropanol	23.19 ± 0.11
Aqueous mediums	pH = 1.2	72.14 ± 0.33
	pH = 6.8	0.0238 ± 0.00
	Water	0.03875 ± 0.00

like Labrafac™ lipophile WL 1349 (HLB = 1.0).

Regarding the effect of surfactants on drug solubility, Cremophor RH40 increased the drug solubility twice as much as Tween 80 did. That these two water-miscible surfactants contained different ratios of hydrophobic and hydrophilic portion resulted in differences in the solubility of poorly water-soluble drug like l-THP. Specifically, Cremophor 40 was a pegylated castor oil or hydrogenated castor oil and consisted of a mixture of approximately 75% relatively hydrophobic portion (Strickley, 2004). Meanwhile, Tween 80, also known as polysorbate 80, had about 84% hydrophilic portion (ICI Americas, i., 1984; Shah et al., 2017). The fact that Cremophor RH 40 contained more hydrophobic portion than Tween 80 explained its higher effectiveness in solubilizing very hydrophobic drug like l-THP (log P = 3.15).

Transcutol HP was chosen as co-solvent to prepare SMEDDS for offering the highest drug solubility (105.62 mg/ml). Transcutol HP was known as a highly purified diethylene glycol monoethyl ether. Owing to the special structure including the two groups of alcohol and ether, Transcutol HP possessed both polar and nonpolar properties and was considered a very powerful solvent for poorly water-soluble drugs such as l-THP. Furthermore, this property made Transcutol HP easily miscible with both lipophilic solvents (Capryol 90 and Cremophor RH 40) and hydrophilic solvents (in this case, distilled water).

Construction of phase diagram

Based on the solubility test, the main components used in SMEDDS were Capryol 90, Cremophor RH 40 and Transcutol HP. The pseudo-ternary phase diagram was constructed to find out the optimum range of excipients which could form the microemulsion zone with various ratios of surfactant/co-surfactant (S_{mix}). The percentage of distilled water at which turbidity-to-transparency and transparency-to-turbidity transition occurred was used to draw the boundaries of microemulsion zone for the development of SMEDDS. Six phase diagrams with six different ratios of S_{mix} (1:2–5:1) were constructed. The selection of S_{mix} was based on two criteria: (a) the biggest area of the microemulsion, and (b) the lowest amount of surfactant. The determination of the suitable ratio of Capryol 90 was based on the loading capacity of l-THP in SMEDDS with the optimum S_{mix}.

Table 3
Design of experiment to evaluate the impact of oil and S_{mix} to SMEDDS. .

Exp No	Independent variables		Dependent variables		
	X_1 ($m_{oil,}$ mg)[a]	X_2 ($m_{Smix,}$ mg)[b]	Y_1 (Size, nm)	Y_2 (PDI)	Y_3 (DE, %)
1	−1	−1	24.83	0.153	37.88
2	1	−1	124.55	0.547	43.15
3	−1	1	27.39	0.418	30.7
4	1	1	50.49	0.323	34.11
5	−1	0	24.39	0.335	35.37
6	1	0	114.50	0.549	33.73
7	0	−1	81.59	0.495	37.77
8	0	1	24.56	0.206	34.28
9	0	0	32.49	0.221	53.91
10	0	0	34.29	0.244	50.25
11	0	0	33.04	0.238	50.83

[a] X_1 [−1 (400 mg), 0 (600mg), +1 (800 mg)].
[b] X_2 [−1 (700 mg), 0 (900 mg), +1 (1100 mg)].

Table 4
Design of experiment to evaluate the impact of Aerosil and Avicel PH 101 to pellet-SMEDDS.

Exp No	Independent variables		Dependent variables		
	X_3 (Aerosil, %)[a]	X_4 (Avicel, %)[b]	Y_4 (Pelletization yield, %)	Y_5 (Dissolution efficiency, %)	Y_6 (Dissolution rate of l-THP after 10 min, %)
1	1	1	68.57	72.58	57.13
2	−1	1	74.31	83.71	90.30
3	1	−1	28.25	75.25	67.80
4[c]	−1	−1	0	n/a	n/a
5	0	1	52.03	84.11	87.56
6	0	−1	2.73	78.07	84.13
7	1	0	53.03	78.78	79.04
8	−1	0	61.16	86.75	91.49
9	0	0	52.87	83.18	88.18
10	0	0	74.39	82.68	88.74
11	0	0	69.92	77.39	83.76

[a] X_1 [−1 (0%), 0 (5%), +1 (10%)].
[b] X_2 [−1 (30%), 0 (40%), +1 (50%)].
[c] This formulation was excluded from the data analysis due to the 0% of pelletization yield.

Table 2
Effect of percentage of l-THP and oil to the state of SMEDDS after 3 days storage at room condition. .

%Oil % l-THP	20%		30%		40%		50%		60%	
	Initial	After 3 days	Initial	After 3 days	Initial	After 3 days	Initial	After 3 days	Initial	After 3 days
0.33	✓	✓	✓	✓	✓	✓	0	0	0	0
0.66	✓	✓	✓	✓	✓	✓	0	0	0	0
1.00	✓	✓	✓	✓	✓	✓	0	0	0	0
1.33	✓	✓	✓	✓	✓	✓	0	0	0	0
2.00	↓	↓	↓	↓	✓	↓	0	0	0	0
3.00	↓	↓	↓	↓	✓	↓	0	↓	0	↓
7.00	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓

% oil: Percentage of oil to total amount of oil and Smi.
% l-THP: Percentage of l-THP to total amount of oil and Smix.
✓ Transparent emulsion without l-THP precipitation.
0: Slightly blurred/blurred emulsion without l-THP precipitation.
↓ : l-THP precipitation.

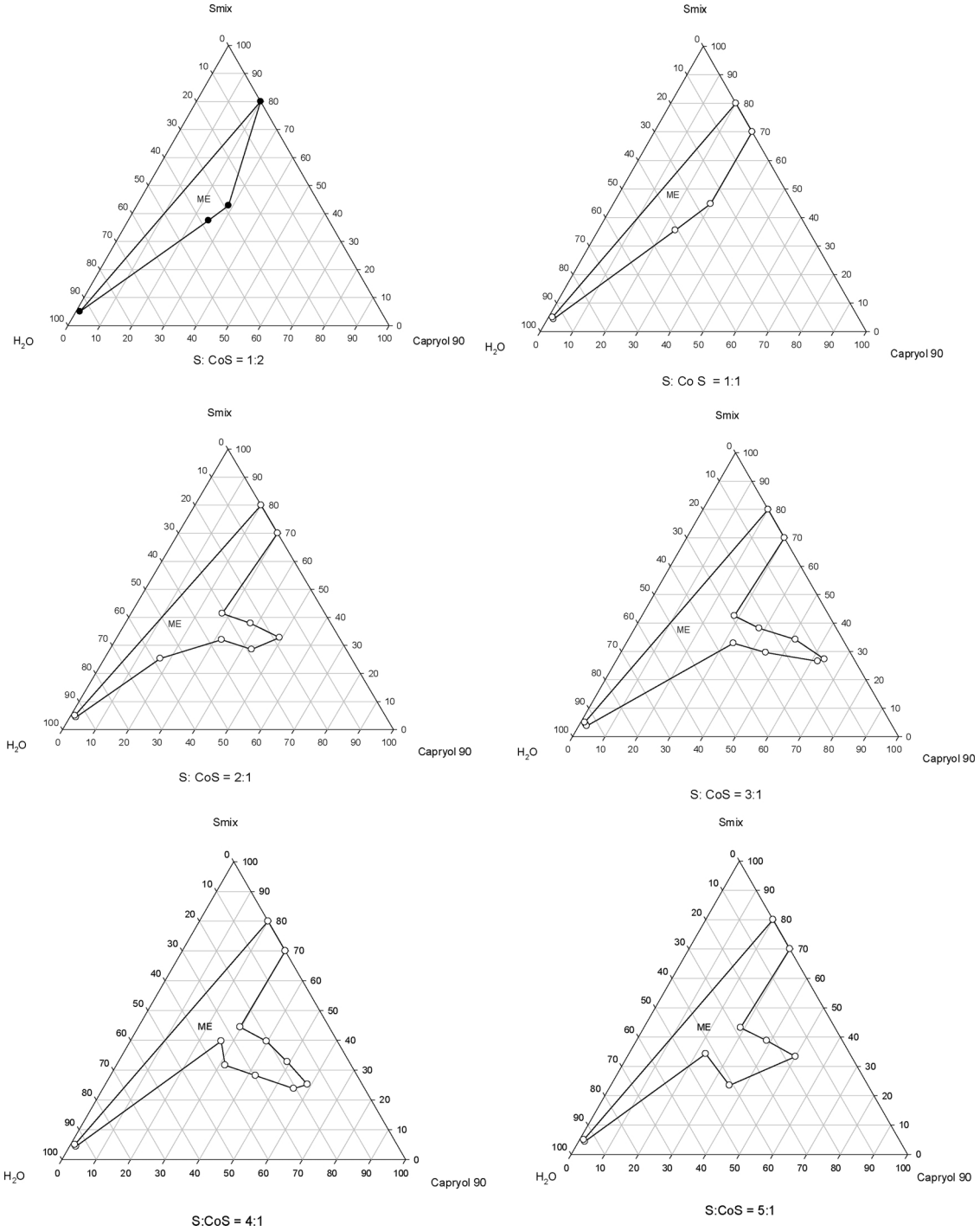

Fig. 2. Pseudoternary phase diagrams of microemulsion consisted of Capryol 90, Cremophor RH 40 (surfactant), Transcutol HP (cosolvent) and water with various ratios of S_{mix} (S: CoS.).

The results in Fig. 2 illustrated the proportional relationship between the microemulsion area and the amount of surfactant. When the ratio of surfactant: co-surfactant was lower than 1:2, the microemulsion was hardly formed because the low level of Cremophor was not enough to decrease the surface tension of oil phase in the aqueous phase. When S_{mix} increased from 1:2 to 3:1, microemulsion area gradually expanded. However, there was not a remarkable change in the microemulsion area as S_{mix} rose from 3:1 to 5:1. It was concluded that the suitable ratio of S_{mix} was 3:1 because this could result in maximum microemulsion existence area while keeping the amount of surfactant (Cremophor RH 40) at a minimum level compared to other ratios (4:1 and 5:1) of S_{mix}. Additionally, at this optimal ratio of S_{mix}, the free energy required to form a stable microemulsion was very low, thus microemulsion could easily form fine oil/water with gentle agitation upon the addition of distilled water into SMEDDS. The microemulsion droplets were covered by an optimum amount of Cremophor RH 40, which decreased the interfacial energy as well as providing a mechanical barrier to coalescence.

Loading l-THP into SMEDDS

The optimum amount of l-THP loaded into SMEDDS was determined by observing the state of the obtained emulsion containing different amounts of l-THP upon the addition of distilled water into different SMEDDS using the increasing percentage of Capryol 90. The obtained emulsion might exist in the three different states including emulsion

with l-THP precipitation, blurred emulsion without l-THP precipitation and transparent emulsion without l-THP precipitation. The visual test was used to determine the initial state of emulsion and their state after 3 days storage in the ambient condition.

Basically, the appearance of drug precipitation in the emulsion resulted from the supersaturation state of l-THP in the emulsion. Results in Table 2 indicated that when 7% l-THP was loaded into SMEDDS, the drug immediately precipitated upon the addition of water into SMEDDS. It was, therefore, concluded that the supersaturation state of l-THP in the emulsion happened as l-THP was around 7%. At the high level of l-THP, the mixture of oil and S_{mix} could not keep the l-THP at the supersaturation for a long time, and a part of l-THP moved to the water phase and started the precipitation process. With the formulation using lower levels of l-THP (2–3%) and more than 40% of Capryol 90, the drug precipitated after 3 days of storage even though the initial emulsions were transparent or slightly blurred. This meant at lower levels of l-THP, a part of this drug still went to the water phase and existed in solubilized molecules. During the 3 days, these solubilized molecules underwent two successive steps of crystallization process including nucleation and crystals growth.

The nucleation rate depended not only on the amount of drug but also the amount of oil phase. When the ratio of Capryol 90 was equal or over 40%, the loaded amount of l-THP respectively increased, and the precipitation rate of l-THP was also slower than that using a smaller amount of Capryol 90. However, the high amount of oil phase might increase the free energy in the interfacial layer of oil and water phase. To reduce this free energy, the oil droplets must coalesce to form the bigger emulsion. As showed in Table 2, 50% of Capryol 90 accelerated the formation of blurred emulsion which was known as the emulsion with the size range of oil droplets higher than 100 nm. This emulsifying system was known as self-emulsifying drug delivery system (SEDDS).

The suitable SMEDDS must load as much l-THP as possible while not undergoing precipitation after 3 days of storage. Based on these criteria, the suitable amount of l-THP was defined as below 2% and the percentage of Capryol 90 was from 20 to 40%. The obtained microemulsion with these ratios of drug and oil had a transparent state without l-THP precipitation after 3 days of storage at ambient condition. However, the exact ratio of the drug, oil, and S_{mix} in SMEDDS must be screened by different kinds of experimental design such as trial-and-error approach or design of experiment (DoE) approach. In order to comprehensively examine the role of oil and S_{mix} in SMEDDS, the DoE approach was applied in this study.

3.1.2. Design of experiment of SMEDDS

By using MODDE 8.0 software (Umetrics, Sweden), there were eleven SMEDDS formulations constructed. The addition of center formulations (No 9, 10, 11) to the experimental design was to measure process stability and inherent variability. The low, medium and high level of input variables were coded in the experimental table by −1, 0, and 1, respectively. The values of output variables were listed in Table 3. The effect of single input variables (amount of Capryol 90 and S_{mix}) and the interaction of these input variables on the three chosen output variables (droplet size, PDI, and DE_{180}) were illustrated by the bar charts (Fig. 3).

First, the effect of Capryol 90 and S_{mix} on the droplet size and PDI of oil phase was illustrated by the direction of the bar in Fig. 3a and b. If the bar representing each factor showed positive value, the impact of this factor on the droplet size and PDI was synergistic and vice versa. Capryol 90 had a synergistic effect on droplet size; meanwhile, S_{mix} had an antagonistic effect on the droplet size. This was confirmed by the fact that the sizes of the two formulations No 2, 6 using a high level (800 mg) of oil were the highest (124.55 and 114.5 nm). Meanwhile, formulations No 1, 5 using a low level (400 mg) of Capryol 90 had the smallest sizes (24.83 and 24.39 nm). The explanation was that the increasing amount of oil phase raised the surface tension between the water phase and oil phase, thus accelerating coalescence of droplet size

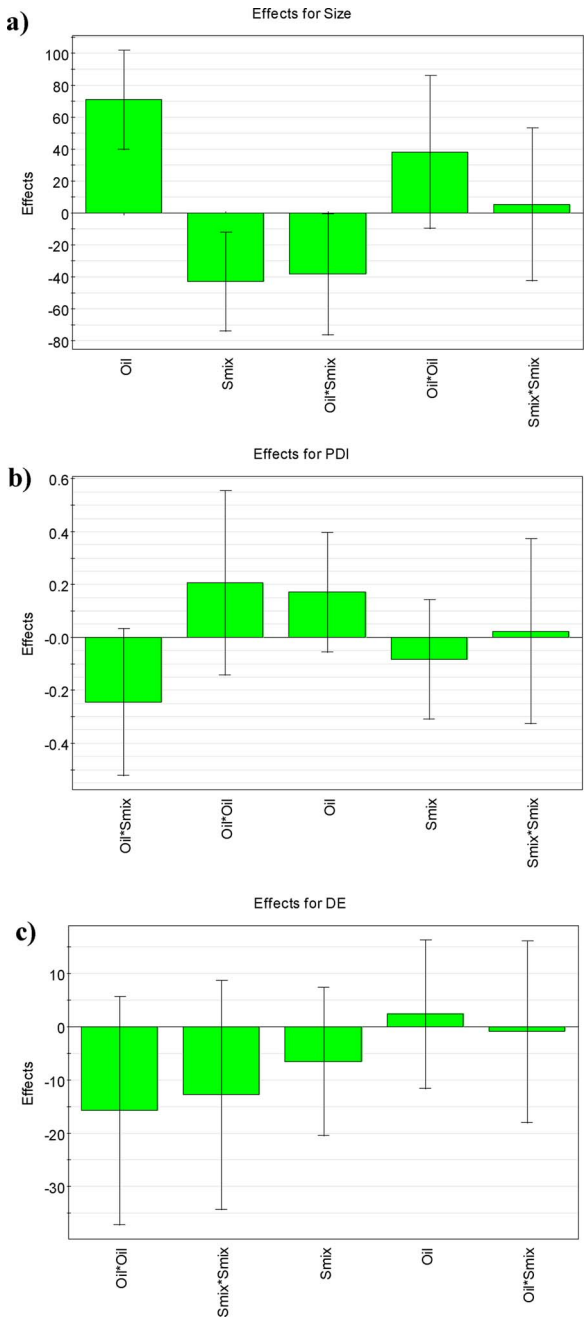

Fig. 3. Effect of oil and S_{mix} to a) droplet size, b) PDI and c) dissolution efficiency of SMEDDS.

of the oil phase.

In contrast, Cremophor RH 40, surfactant, was used to reduce the free energy in the interfacial layer of oil and water phase due to its amphiphilic structure. The cosurfactant, Transcutol HP, was known as a very powerful solubilizer for both polar and nonpolar compounds, since it contained both alcohol and ether group. These advantages of Cremophor RH 40 and Transcutol HP played the main role in the reduction of the droplet size of the oil phase. The formulations No 3, 8 using a high level of S_{mix} (1100 mg) showed the small size of the oil phase (27.39 and 24.55 nm). The results in Fig. 3a indicated an antagonistic effect of interaction between oil and S_{mix} on the droplet size. This proved that S_{mix} had the superior role to oil in the reduction of droplet size. Fig. 3b also showed a similar pattern to Fig. 3a. Accordingly, S_{mix} always played the pivotal role in decreasing size and size distribution of the oil phase upon addition of water into SMEDDS.

The influence of Capryol 90 and S_{mix} on the dissolution efficiency of l-THP was displayed in Fig. 3c. However, the fact that the error bar of each input factor was so high implied that the impact of input factors (oil, S_{mix} or oil*S_{mix}) on the DE_{180} was not of statistical significance. This might be explained by one or both of the following reasons: (1) the screening ranges of oil and S_{mix} did not reflect exactly the role of oil and $S_{mix,}$ and (2) the experiment for the determination of the drug release was not quite suitable for SMEDDS. In this study, the drug diffusion through dialysis membrane was used for the investigation. However, the driving force of drug diffusion was controlled by the concentration gradient between the two sides of the dialysis membrane, the viscosity of medium and physical state of the drug in dialysis chamber. Since the concentration gradient of l-THP was almost similar to all screened formulations, it was more likely that the DE_{180} strongly depended on the two other factors. Both Capryol 90 and Cremophor RH 40 had higher viscosity than the distilled water, the dilution medium of SMEDDS. These high viscosity agents, therefore, might block the diffusion pores in the dialysis membrane. Furthermore, after addition of distilled water to SMEDDS, the drug would mainly stay in the oil phase while a part of it remained in the water phase due to the balance of drug distribution in oil and water phase. The drug diffusing through the dialysis membrane might be the drug in the water phase. After this part of the drug diffused through dialysis pores in the membrane, balance would be re-established between either sides of oil droplet and either sides of dialysis membrane. However, if there are some factors inhibiting diffusion such as the viscosity of the medium, the blockage of the pores, etc., the drug diffusion might change unpredictably. In the present study, the impact of viscosity and the location of a drug in dialysis membranes were not seriously considered, thus it was hard to predict the impact of oil and S_{mix} on $DE_{180.}$

Among the eleven formulations, the center formulation met all the requirements of the output variables, including the highest dissolution efficiency (around 50%), droplet size being less than 50 nm, and PDI under 0.3. Therefore, this formulation, which comprised 39.5% Capryol 90, 59.2% S_{mix} and 1.3% l-THP, was chosen as the optimal liquid SMEDDS for developing pellets containing liquid SMEDDS (pellet-SMEDDS).

3.1.3. Dissolution evaluation of SMEDDS in pH change model

L-Tetrahydropalmatine was a weakly basic compound, thus its dissolution profile depended on pH medium. As shown in Fig. 4, l-THP was quickly soluble in pH 1.2 due to the ionic interaction with acid medium to exist in anionic state. In the case of SMEDDS, l-THP was also completely soluble within 5 min because of both the soluble enhancement property of SMEDDS and ionic interaction with dissolution medium. L-THP now existed in both states of non-ionic and ionic forms. When pH medium was changed to 6.8, the raw material was precipitated. Meanwhile, the dissolution rate of l-THP from SMEDDS was maintained at absolute level at later dissolution time points. This phenomenon proved that SMEDDS inhibited drug re-precipitation in basic medium (pH 6.8). SMEDDS always proved high dissolution efficiency, and the dissolution profile of l-THP from SMEDDS was pH-independent.

3.2. Development of pellet-SMEDDS

The two main components used in the pellets were solid carriers for liquid SMEDDS and spherical aid agents for pellets. A well-known solid carrier was Aerosil, which had high porosity and high surface area to absorb liquid SMEDDS (Jannin et al., 2008; Tan et al., 2013; Chavan et al., 2015). The second important agent in pellets was Avicel, which had the dual roles of spherical aid and a solid carrier. There have been many studies regarding the two excipients in the solid dosage forms; however, only a few made use of quality by design approach to get insights into the positive and negative impacts of these components on the properties of pellet-SMEDDS.

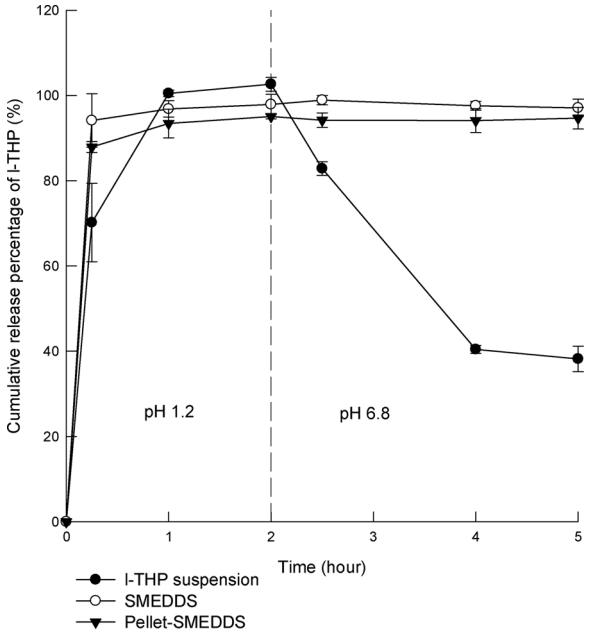

Fig. 4. The dissolution profiles of l-THP from l-THP suspension, liquid SMEDDS and pellet-SMEDDS.

Table 5
Regression results indicating the impact of Avicel and Aerosil to pelletization yield, dissolution efficiency, and dissolution rate of l-THP after 10 min.

	Pelletization yield (%)		Dissolution efficiency (%)		Dissolution rate of l-THP after 10 min (%)	
	Coefficient	P	Coefficient	P	Coefficient	P
Constant	60.60	0.00	81.47	0.00	88.17	0.00
Aer	1.84	0.82	−4.24	0.03	−9.70	0.02
Avi	26.94	0.02	0.03	0.98	−0.98	0.71
Aer*Aer	4.19	0.68	0.72	0.69	−4.81	0.20
Avi*Avi	−25.02	0.06	−3.62	0.10	−8.49	0.05
Aer*Avi	−7.67	0.49	−1.20	0.54	−5.14	0.20

3.2.1. Design of experiment of pellet-SMEDDS

The main effect of a specific formulation factor such as Aerosil or Avicel and the interactions between such factors (Aerosil*Avicel) were displayed in Table 5 and Fig. 5. The main effect of Avicel or Aerosil was the average change of the pelletization yield, dissolution efficiency, and dissolution rate of l-THP after 10 min as these two input variables changed from the low level (−1) to high level (+1). Besides, the interactions between Aerosil and Avicel were defined as half of the difference of the specific response of Aerosil at the low level (30%) and high level (50%) of Avicel. The ANOVA table (Table 5) showed the two important values, including coefficients and p value, which indicated the statistical impact of the main effect of an input factor or the interactions of the input factors on the responses. The contour plots (Fig. 5) visually displayed the effect of Aerosil and Avicel on the pelletization yield, dissolution efficiency, and dissolution rate of l-THP after 10 min.

First, p value representing Avicel under 0.05, proved that this excipient had a significant impact on pelletization yield. Meanwhile, the effect of Aerosil on this response was not remarkable. In this case, pelletization yield (Y_1) was expressed by the following equation: $Y_1 = 60.6 + 26.94Avi$. The positive value of coefficient Avi (26.94) showed that Avicel possessed a synergistic effect on pelletization yield. Avicel was well known as a spheronization aid (Sermkaew et al., 2013); therefore, when a high amount of Avicel was used, pellets were easily formed. The contour plot also illustrated the synergistic influence of this main factor on pelletization yield (Fig. 5a). When the amount of

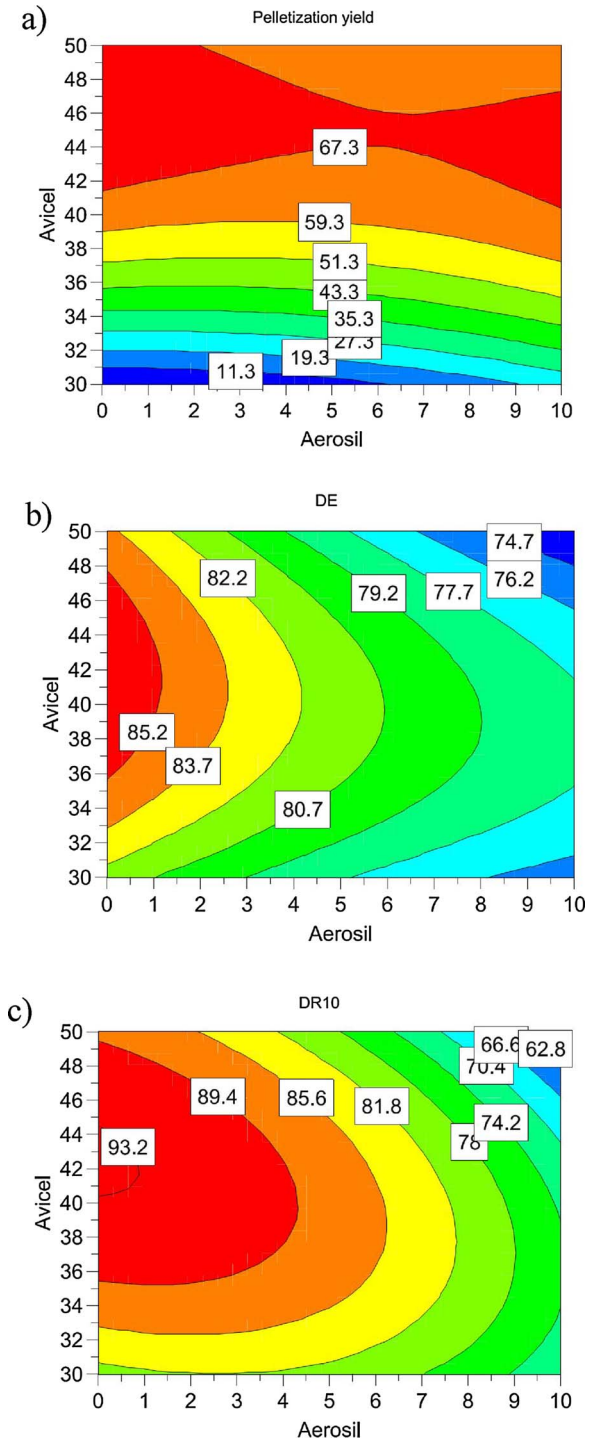

Fig. 5. Contour plots reflect the effect of Avicel and Aerosil on a) pelletization yield, b) dissolution efficiency, c) dissolution rate of RTD after 10 min.

Avicel PH101 rose from 30 to 50%, pelletization yield increased from 11.3 to 67.3% (Fig. 5a). However, the minus value of coefficient Avi*Avi (−25.02) indicated that using a high level of Avicel might have an antagonistic effect on pelletization yield. This result was confirmed in the Fig. 5a, which showed the reduction of pelletization yield when high levels of both Avicel and Aerosil in the pellet formulation were used. It was attributed to the fact that the high level of Avicel might accelerate the formation of big pellets which lied out the sieved range of 0.8–1.25 mm.

Second, the effect of Aerosil and Avicel on the two independent variables of dissolution efficiency and dissolution rate of l-THP after 10 min, which represented the rate and extent of drug dissolution, were examined. As a fumed silica with a very high specific surface area (200 m^2/g), Aerosil 200 has always been used to absorb liquid SMEDDS and solidify the liquid SMEDDS. Most of the previous studies focused on determining the extent to which Aerosil could load liquid SMEDDS (Oh et al., 2011; Tan et al., 2013; Chavan et al., 2015), and most had come up with more or less similar conclusion that the more Aerosil added, the more liquid SMEDDS was loaded in solid dosage forms. However, the rate and extent of drug dissolution after absorption of SMEDDS on Aerosil had not been well addressed in existing literature.

The effects of Aerosil and Avicel on these two variables were also displayed by Table 5 and Fig. 5. As shown in Table 5, p value of Aerosil and Avicel illustrated the statistical impact of these two excipients on the $D.E_{50}$ and $D.R_{10}$, respectively. Only Aerosil had a significant effect on the rate and extent of drug dissolution ($p < 0.05$). The coefficient of Aerosil also indicated that this excipient had an antagonistic effect on dissolution efficiency and dissolution rate after 10 min. As shown in Fig. 3c, when Aerosil increased from 0 to 10%, dissolution rate of l-THP after 10 min reduced from 93.2 to 62.8%. The explanation was that the hydrophobic property of Aerosil inhibited the water uptake inside pellet-SMEDDS (Tan et al., 2013). Besides, the silanol groups on the surface of Aerosil might form the tightening interaction with molecules containing functional groups like −OH, $-NH_2$, −SH or $-SO_2$ (Chavan et al., 2015), causing the reduction of the rate and extent of drug release. In this case, silanol could interact with L-tetrahydropalmatine through hydrogen bond because silanol group had one hydrogen bond donor while L-tetrahydropalmatine had five hydrogen bond acceptors. This may lead to slower drug release from pellet-SMEDDS in terms of the rate and extent of drug release.

The Table 5 and Fig. 5, which indicated the negative effect of Aerosil on the dissolution rate of l-THP, agreed with previous reports (Sermkaew et al., 2013; Chavan et al., 2015). While this called for the use of solid carriers other than Aerosil, which inhibited drug release, the majority of available studies concluded that Aerosil and other silicon dioxide derivatives were irreplaceable solid carriers for liquid SMEDDS because of the high amount of SMEDDS that could be loaded in these excipients. Chavan et al., (2015) found that four different silicon dioxide derivatives including Aerosil 200, Aerosil 300, Aerosil R 972 and Sylysia 350 fcp could bring around 41.7% SMEDDS containing celecoxib. Nevertheless these solid carriers caused a remarkable reduction in the dissolution efficiency of the drug at 120 min (DE_{120}) in comparison to the original SMEDDS, especially Aerosil 200 whose DE_{120} of solid SMEDDS declined about 88.9 folds. Sylysia 350 fcp was eventually chosen as the optimal solid carrier for the resulting lowest reduction of DE_{120} (5.9 times) compared to the other three. Though it was desirable to screen other solid carriers for minimum negative impact on drug release, the authors of the present study decided to use the Sylysia-SMEDDS to evaluate oral bioavailability. The predetermined results of *in-vivo* release study was that the maximum concentration (C_{max}) of Sylysia-SMEDDS reduced about 2.67 folds compared to that of the original SMEDDS.

The discussion suggested that there should be a balance between loading capacity and drug release when selecting solid carriers for liquid SMEDDS. The fact that previous studies paid undue attention to the loading amount of liquid SMEDDS in solid carriers and took little notice of the negative impact of these solid carriers on drug release has blurred the important role of SMEDDS in the enhancement of drug release. If a solid carrier could bear a large amount of SMEDDS, it should have a high surface area and special moieties to keep the SMEDDS bound to its surface, thus reducing drug release.

In the present study, it was not difficult to identify the negative effect of Aerosil on the dissolution rate of l-THP. Table 4 indicated that 10% Aerosil in pellet could bear about 35% SMEDDS while reducing DE_{50} to 7258%. If the amount of Aerosil in pellet formulation increased, the loading capacity of SMEDDS would exceed 35%, but the DE_{50} would also respectively decline. Thus, the amount of SMEDDS was fixed

at 35%, and the impact of different levels of Aerosil and Avicel on the pelletization yield as well as drug release was investigated. The pelletization yield column in Table 4 indicated that pellets containing 5% Aerosil (Exp no 5, 6, 9, 10, 11) or not containing Aerosil (Exp no 2, 8) were still formed. Pellets of only one formulation (Exp no 4), which used both Aerosil and Avicel at low levels (0% Aerosil and 30% Avicel), were not created. When these solid carriers were simultaneously used at low levels, SMEDDS was not absorbed completely and caused the formation of over wetting mass prior to the extrusion and spheronization process.

In contrast, results in Table 5 showed that Avicel PH101 did not significantly affect the dissolution efficiency and dissolution rate of l-THP after 10 min. This spherical aid agent did not have any special moieties, therefore, its interaction with SMEDDS was not tightened. Still, the liquid SMEDDS could absorb the clusters of microcrystalline cellulose by the wetting force. The fact that Avicel coefficient presenting the dissolution rate of l-THP after 10 min was minus values (−0.98) indicated the wetting force slightly inhibited the drug release. However, the insignificant impact of Avicel on the dissolution rate demonstrated that the wetting force was not a strong interaction force. The stronger interactions like hydrogen bond or ionic interaction could not be formed between Avicel and excipients in SMEDDS. Consequently, l-THP was easily released when pellet-SMEDDS was put into dissolution medium.

To minimize undesirable impact on the drug release, Aerosil was removed from pellet formulation, and different levels of Avicel were considered. As shown in Table 4, if Aerosil was excluded from the formulation of pellet-SMEDDS, the minimum amount of Avicel should be around 40% (the middle level). Avicel was the suitable replacement for the traditional solid carrier, Aerosil, in regard to the loading capacity of liquid SMEDDS as well as the maintenance of the drug release profile in comparison to the original liquid SMEDDS. In this study, formulation No. 2 using 50% Avicel (the high level) as the solid carrier was chosen as the optimum pellet-SMEDDS because of the highest pelletization yield obtained (74.31%) and the dissolution rate of l-THP from pellet-SMEDDS which was not significantly different from that of liquid-SMEDDS (Fig. 4). This pellet-SMEDDS consisted of 35% optimum SMEDDS, 50% Avicel, 10% lactose monohydrate, 5% sodium croscarmellose and sufficient PVP 5%. Aerosil was not added into this formulation for the negative effect it produced on all responses.

3.2.2. Properties evaluation of pellet-SMEDDS

To get insights into the effect of solidification process on the SMEDDS, the physiochemical properties of optimum pellet-SMEDDS were investigated. The size of microemulsion before and after added to pellet were 33.26 and 42.08 nm, respectively. Besides, the polydispersity indexes of these microemulsions were 0.238 and 0.328, respectively. The addition of solid excipients changed the optimum ratio of oil, surfactant and cosolvent, thus droplet size and PDI slightly increased after pelletization process.

The morphology of pellets was determined by SEM with different magnifications (Fig. 6). These pellets were spherical and homogeneous in shape. Their surface consisted of solid excipients like Avicel, sodium croscarmellose, and droplets of SMEDDS. After the drying process, liquid SMEDDS adsorbed on the solid excipients and equally distributed on the surface of pellet-SMEDDS. Generally, the droplet size of SMEDDS was around 20–50 nm when measured by SEM, and this result matched that by dynamic light scattering technique.

The powder X-ray diffractometry was used to determine the crystallites of l-THP in pellet-SMEDDS. The results in Fig. 7 showed that l-THP had many crystallized peaks in the range of 10–30 degree. The fact that these peaks still existed in physical mixture reflected the crystallize state of l-THP in the physical mixture. However, the disappearance of these peaks in pellet-SMEDDS proved the amorphous state of l-THP in pellet-SMEDDS. Obviously, due to the high drug dissolution, small droplet size, and an amorphous state, pellet-SMEDDS possessed the high potential to improve the drug bioavailability.

3.3. Pharmacokinetics study

The pharmacokinetics study was conducted in the rabbit model to primarily compare the *in-vivo* release of l-THP from the l-THP suspension, original liquid SMEDDS and pellet-SMEDDS. To analyze the drug concentration in the rabbit plasma, the LC/MS method was developed (Tran et al., 2016). The linear range of LC/MS analysis method was from 5 to 200 ng/mL. The limit of detection (LOD) and limit of quantification (LOQ) were estimated at 0.3 and 1 ng/mL in final solution, respectively. Based on the validated LC/MS analysis method, the obtained pharmacokinetics profiles of these dosage forms in the rabbit plasma were shown in Fig. 8, and the pharmacokinetics parameters were displayed in Table 6. Due to the high dissolution efficiency and small droplet size of SMEDDS, the bioavailability of l-THP from SMEDDS versus l-THP suspension was improved around 198.63%. The AUC_{INF_pred} of l-THP suspension and SMEDDS were 34.38 and 68.29 ng h/ml, respectively. Besides, SMEDDS also increased Cmax of l-THP about 2.35 times in comparison with raw material.

Fig. 8 indicated that the solidified SMEDDS had similar pharmacokinetics pattern to that of liquid SMEDDS. This alkaloid exhibited quick absorption and rapid elimination after oral administration of l-THP suspension, liquid SMEDDS and pellet-SMEDDS. Besides, the fact that l-THP could not be determined in the rabbit plasma 4 h after these two SMEDDS were administered indicated that the solidification of SMEDDS did not retard the drug absorption or elimination.

This pharmacokinetics pattern, quick absorption, and rapid elimination were also seen in other studies that involved pharmacokinetics profile of alkaloids. For example, the bioactive alkaloids presented in Dactylicapnos scandens (D. Don) Hutch. (Papaveraceae), (+) isocorydine and protopine, were also quickly absorbed and rapidly eliminated (Guo et al., 2013). Wang et al .,(2012) used LC–MS/MS to investigate the pharmacokinetic profile of bulleyaconitine A (BLA) in rats. This drug was an aconitine-like alkaloid isolated from Aconitum bulleyanum Diel for treatment of rheumatoid arthritis and chronic pain. The authors also concluded that bulleyaconitine A underwent rapid absorption and elimination from GIT.

In order to make clear the *in-vivo* fate of this alkaloid after oral administration of liquid SMEDDS and pellet-SMEDDS, pharmacokinetics parameters of these two formulations were calculated by WinNonlin Phoenix 6.4 using non-compartment model. Three important parameters including area under the curve (AUC), the maximum observed concentration (C_{max}) and the time of C_{max} (T_{max}) of both formulations were compared by Student's *t*-test. Generally, the PK parameters of pellet-SMEDDS were not significantly different to those of liquid SMEDDS ($p > 0.05$). Specifically, the predicted $AUC_{0-\infty}$ of liquid SMEDDS and pellet-SMEDDS were 68.29 ± 10.63 and 57.82 ± 13.08 (ng.h/ml), respectively. The predicted relative bioavailability of $AUC_{pellet\text{-}SMEDDS(0-\infty)}/AUC_{SMEDDS(0-\infty)}$ was 84.67%. Because this alkaloid was completely eliminated after 4 h, $AUC_{0\text{-}4h}$ of the two formulations was also determined. The $AUC_{0\text{-}4h}$ of liquid SMEDDS and pellet-SMEDDS were 60.40 ± 9.11 and 49.25 ± 13.00 (ng h/ml), respectively. The mean relative bioavailability of $AUC_{pellet\text{-}SMEDDS}(0–4\ h)/AUC_{SMEDDS}(0–4\ h)$ was 81.55%. The non-significant difference ($p > 0.05$) of $AUC_{pellet\text{-}SMEDDS}$ vs. AUC_{SMEDDS} proved that pelletization of SMEDDS did not remarkably change bioavailability of the original liquid SMEDDS. Besides, the relative bioavailability of solidified-SMEDDS was approximately equal to the original liquid SMEDDS, which demonstrated that the solidification of liquid SMEDDS did not strongly interfere with the extent of drug absorption. The strategy to solidify a liquid SMEDDS while maintaining the extent of drug absorption of the original liquid SMEDDS was suitable in terms of the selection of solid dosage kind containing liquid SMEDDS, the experimental design, and selective criteria of solid carriers.

In this present study, the selected solid dosage form containing

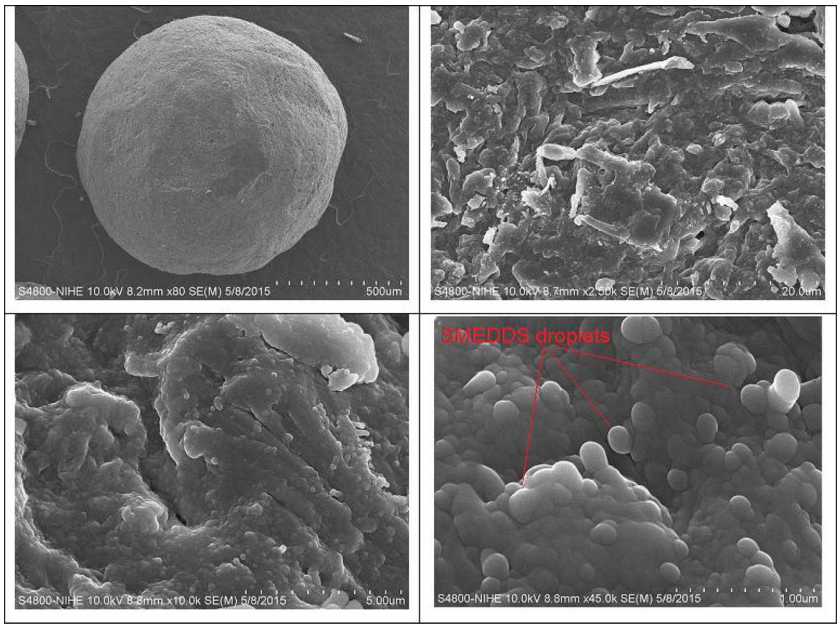

Fig. 6. SEM images of pellet-SMEDDS with different magnification.

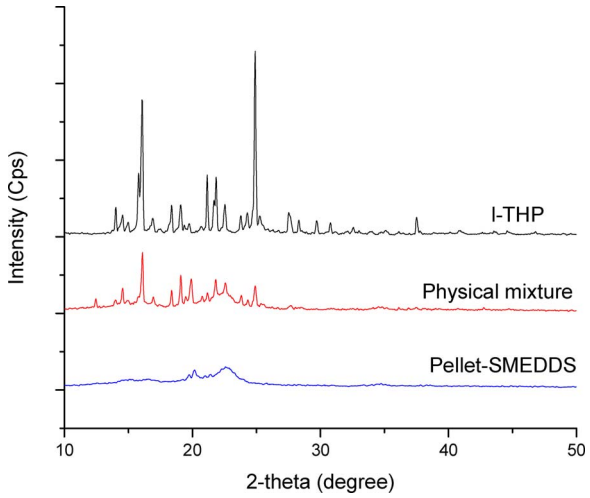

Fig. 7. X-ray diffractograms of l-THP, physical mixture, and pellet-SMEDDS.

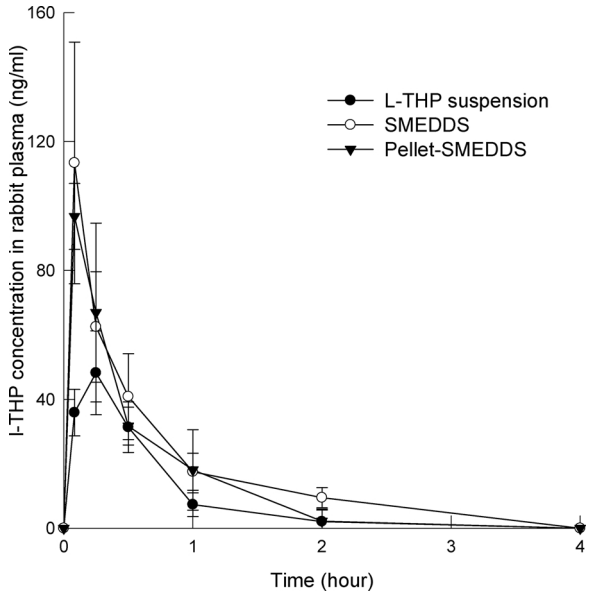

Fig. 8. Pharmacokinetics profiles of l-THP suspension, SMEDDS and pellet-SMEDDS in rabbits at a dose of 1.5 mg/kg (n = 3, Mean ± SE).

liquid SMEDDS was pellet, which was different from other studies (Oh et al., 2011; Tan et al., 2013; Chavan et al., 2015). Accordingly, a powder containing SMEDDS was always a priority choice for the solidification of liquid SMEDDS. The criteria of powder-SMEDDS were free flow and high loading capacity, thus the physical properties of the solid carrier were always high porosity and hydrophobic. Such properties enabled the solid carrier to bear a high amount liquid SMEDDS and minimized the cohesive phenomena of particles resulting from wetting force. Here, the advantage of pellet-SMEDDS versus powder-SMEDDS was that the pellets themselves could easily flow owing to their spherical shape and the size range of 0.8–1.0 mm. Consequently, the hydrophobic solid carriers were not quite essential for pelletization process, and the negative impact of this hydrophobic excipients on the extent of drug release was also minimized.

Instead of using a trial-and-error approach, this study used DoE approach to deeply understand the role of solid carriers in the solidified SMEDDS. The criteria for selecting solid carriers were pelletization yield, disslution efficiency, and dissolution rate of l-THP after 10 min. The latters represented the rate and extent of the drug release. The choice of the most suitable solid carrier must ensure a balance between the two factors of pelletization yield and the drug release. Other studies using trial-and-error approach only gave us a one-way look (Setthacheewakul et al., 2010; Oh et al., 2011; Hu et al., 2012). For example, both Sermkaew (Sermkaew et al., 2013) and Chavan (Chavan et al., 2015) came up with a well-known result that colloidal silicon dioxide and its derivatives could load a large amount of SMEDDS. However, they did not try to reduce or change to other solid carriers that could still maintain the high dissolution rate of the drug from liquid SMEDDS, perhaps because of unwillingness to sacrifice the loading amount of liquid SMEDDS in solid dosage forms. However, the ANOVA table obtained from DoE approach showed the exact role of each solid carrier in solid dosage forms containing liquid SMEDDS. In this study, DoE approach gave us a statistical proof that colloidal silicon dioxide was not quite necessary in the solidified SMEDDS. Furthermore, DoE approach also indicated that microcrystalline cellulose could simultaneously meet two important requirements of a solid dosage form containing liquid SMEDDS, i.e. carrying a large amount of liquid SMEDDS and maintaining high drug release of the original liquid SMEDDS.

Table 6
Pharmacokinetics parameters of l-THP after oral administration of l-THP suspension, SMEDDS and pellet-SMEDDS in rabbits at a dose of 1.5 mg/kg (n = 3, Mean ± SE).

	l-THP suspension	SMEDDS	Pellet-SMEDDS	p value*	90% CI_ (Lower; Upper)[a]
$AUC_{0\text{-}4h}$ (ng.h/ml)	34.89 ± 2.84	60.40 ± 9.11	49.25 ± 13.00	0.26	(41.45; 147.34)
AUC_{INF_pred} (ng.h/ml)	34.38 ± 3.28	68.29 ± 10.63	57.82 ± 13.08	0.28	(43.77; 153.37)
C_{max} (ng/ml)	48.22 ± 13.01	113.37 ± 21.68	96.75 ± 5.92	0.25	(57.72; 134.64)
T_{max} (h)	0.25	0.08	0.08		
Relative bioavailability (%)[b]		198.63	168.18		

* p value of statistical comparison of SMEDDS vs. pellet-SMEDDS by Student's *t*-test.
[a] 90% Confidence Intervals of the ratio ($AUC_{pellet\text{-}SMEDDS}$ /AUC_{SMEDDS} and $C_{max\text{-}pellet\text{-}SMEDDS}$ /$C_{max\text{-}SMEDDS}$) were transformed by the logarithm.
[b] Relative bioavailability of SMEDDDS and pellet-SMEDDS compared to l-THP suspension.

In an attempt to clarify the influence of pelletization of SMEDDS on bioavailability, the bioequivalent study of pellet-SMEDDS vs. original liquid SMEDDS was conducted using parallel design. The lower and upper levels of 90% confidence intervals of the ratio ($AUC_{pellet\text{-}SMEDDS\ (0\text{-}\infty)}$/$AUC_{SMEDDS\ (0\text{-}\infty)}$) and ($AUC_{pellet\text{-}SMEDDS\ (0\text{–}4\ h)}$/$AUC_{SMEDDS\ (0\text{–}4\ h)}$) transformed by the logarithm were (43.77; 153.37) and (41.45; 147.34), respectively. These results indicated that the obtained 90% confidence intervals lied out of the bioequivalent range approved by FDA (80 –125%), and AUCs of pellet-SMEDDS were not bioequivalent to those of liquid SMEDDS. Assumingly, this non-bioequivalence of these two dosage forms was caused by some following reasons. First, the design of pharmacokinetics test was not cross-over study, thus might be subjected to the intra-subject variability in pharmacokinetics parameters. Second, the difference in dosage forms using in PK test (liquid vs. solid), to some extent, might cause differences in the rate and extent of drug release in *in-vivo* condition. Finally, the experimental animals involved only three rabbits, leading to the high standard deviation of AUC values. Even though the bioequivalence of $AUC_{pellet\text{-}SMEDDS}$ vs. AUC_{SMEDDS} was hardly obtained, this result did not contradict to what obtained by the Student's t-test. $AUC_{pellet\text{-}SMEDDS}$ was still not significantly different from AUC_{SMEDDS} ($p > 0.05$), and the strategy for the development of a solidified SMEDDS was still feasible and reliable.

Together with AUC, the maximum observed concentrations (C_{max}) of the alkaloid in the rabbit plasma were calculated. Specifically, the C_{max} of liquid SMEDDS and pellet-SMEDDS were 113.37 ± 21.68 and 96.75 ± 5.92, respectively. The Student's t-test once again was used to prove that $C_{max\text{-}pellet\text{-}SMEDDS}$ was not significantly different from $C_{max\text{-}SMEDDS}$ ($p > 0.05$). It was clear from the result that solidification of SMEDDS by microcrytalline cellulose and pelletization technique was acceptable. These two solutions were not quite novel as compared to the traditional pharmaceutics, but they were the very important factors deciding the success of a solidified SMEDDS. Avicel did not strongly interact with each composition in liquid SMEDDS, and this excipient was also known as a disintegrant in the pellet. Thus, once pellet-SMEDDS contacted to gastrointestinal medium, the pellet was quickly disintegrated and released l-THP as well as the main compositions of SMEDDS including a surfactant (Cremophor RH40), a cosolvent (Transcutol HP), and lipid (Capryol 90). The excipients in SMEDDS, known as solubility and permeability enhancers, quickly accelerated the drug absorption into the blood circulation. Besides, L-tetrahydropalmatine was a weakly basic compound (pKa 5.34), thus its dissolution profile depended on pH medium. The alkaloid was quickly soluble at pH 1.2 due to the ionic interaction with acid medium to exist in the anionic state. However, the balance between ionic and non-ionic form would be immediately established in the stomach medium. The non-ionic form of l-THP would absorb through the epithelium layers presenting in the stomach. As a result, l-THP quickly gained the maximum observed concentration after 5 min of oral administration of the liquid SMEDDS and pellet-SMEDDS.

To solidify the liquid SMEDDS, several solid carriers including dextran, silica dioxide or microcrystalline cellulose had been investigated. Each had their own advantages, but there was little systematical comparison among these solid carriers in terms of the ability to preserve the drug release from the original liquid SMEDDS. Oh et al., (2011) made a comparison of solid SMEDDS using either a hydrophilic carrier (Dextran) or a hydrophobic carrier (silica dioxide). Even though there were no significant differences in dissolution rates of the drug between the solid SMEDDS prepared with silica dioxide and dextran, but that using silica dioxide gave higher plasma concentration of flurbiprofen compared to that using dextran (Oh et al., 2011). The limitation of this study was that the authors did not compare the bioavailability of solid SMEDDS using silica dioxide with the original liquid SMEDDS, thus it was hard to precisely conclude about the advantages or disadvantages of silica dioxide. However, in other studies, Sermkaew et al (2013) and Chavan et al (2015) proved that solid SMEDDS using silica dioxide as the main solid carrier reduced the oral bioavailability of adrographolide and celecoxib. Thus, it could be assumed that both dextran and silica dioxide might decrease the plasma concentration of a drug after using solid SMEDDS compared to that using the original liquid SMEDDS. In order to find out another solid carrier to substitute for silica dioxide and dextran, several investigations using microcrystalline cellulose and its derivatives have been conducted (Setthacheewakul et al., 2010; Qi et al., 2014). They have all come up with the same conclusion as ours that there were no significant differences between the solid SMEDDS using microcrystalline cellulose as solid carrier and the original liquid SMEDDS. However, unlike these studies, which employed a trial-and-error approach to clarify the role of microcrystalline cellulose, we used DoE approach to offer a systematical view of the role of silica dioxide and microcrystalline cellulose in solid SMEDDS. Based on this discussion, it could be briefly concluded that microcrysslline cellulose was the best solid carrier for the liquid SMEDDS in respect of the preservation of the drug release and bioavailability from the original liquid SMEDDS.

4. Conclusion

Liquid and pellet self-microemulsifying drug delivery systems containing L-tetrahydropalmatine were successfully prepared. It was evident that design of experiment was a useful approach for the formulation development of the two novel dosage forms containing l-THP. Avicel was the more suitable solid carrier for pellet-SMEDDS than the traditional solid carrier, Aerosil, with regard to preservation of the drug release rate and the oral bioavailability of l-THP compared to the original liquid SMEDDS.

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