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Preparation and Evaluation of Self-Microemulsifying Drug Delivery System Containing Vinpocetine

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The main purpose of current investigation is to prepare a self-microemulsifying drug delivery system (SMEDDS) to enhance the oral bioavailability of vinpocetine, a poorly water-soluble drug. Suitable vehicles were screened by determining the solubility of vinpocetine in them. Certain surfactants were selected according to their emulsifying ability with different oils. Ternary phase diagrams were used to identify the efficient self-microemulsifying region and to screen the effect of surfactant/cosurfactant ratio (K_m). The optimized formulation for in vitro dissolution and bioavailability assessment was oil (ethyl oleate, 15%), surfactant (Solutol HS 15, 50%), and cosurfactant (Transcutol® P, 35%). The release rate of vinpocetine from SMEDDS was significantly higher than that of the commercial tablet. Pharmacokinetics and bioavailability of SMEDDS were evaluated. It was found that the oral bioavailability of vinpocetine of SMEDDS was 1.72-fold higher as compared with that of the commercial tablet. These results obtained demonstrated that vinpocetine absorption was enhanced significantly by employing SMEDDS. Therefore, SMEDDS might provide an efficient way of improving oral bioavailability of poorly water-soluble drugs.

Keywords self-microemulsifying drug delivery system; vinpocetine; microemulsion; bioavailability

INTRODUCTION

Oral route is the preferred route for chronic drug therapy. However, 40% of new drug compounds exhibit low oral bioavailability and high intra- and intersubject variability because of their poor aqueous solubility properties (Neslihan & Simon, 2004). Thus, for such compounds, dissolution in the environmental lumen is the rate-limiting step in the absorption process. To overcome these problems, various formulation strategies were reported in the literature, including complexation with cyclodextrins, solid dispersions, and coprecipitates (Nazzal, Guven, Reddy, & Khan, 2002; Perng, Kearney, Patel, Palepu, & Zuber, 1998). In recent years, however, much attention has been focused on lipid-based formulations, with particular emphasis on self-emulsifying drug delivery system (SEDDS) and self-microemulsifying drug delivery system (SMEDDS) (Abhijit & Nagarsenker, 2007; Cui, Zhao, Chen, & He, 2005; Wei, Sun, Nie, & Pan, 2005; Zhang, Liu, Feng, & Xu, 2008). SMEDDSs are isotropic mixtures of oil, surfactant, cosurfactant, and drug that form fine oil-in-water microemulsion when introduced into aqueous phases under gentle agitation (Mette, Anette, & Jeanet, 2006). The spontaneous formation of an emulsion leading to drug release in the GI tract advantageously presents the drug in a dissolved form, and the small droplet size provides a large interfacial surface area for drug absorption (Charman et al., 1992; Shah, Carvajal, Patel, Infeld, & Malick, 1994).

Moreover, the emulsion droplets lead to a faster and more uniform distribution of the drug in the gastrointestinal tract, minimizing the irritation from the contact between the drug and the gut wall (Khoo, Humberstone, Porter, Edwards, & Charman, 1998; Shah et al., 1994). The main mechanisms of improving bioavailability include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting *P*-glycoprotein (PGP) and/or CYP450 to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid (Holm, Porter, Müllertz, Kristensen, & Charman, 2002; O'Driscoll, 2002; Wu, Wang, & Que, 2006). Oral absorption of several drugs has been enhanced by SMEDDS employing single or combined mechanism (Six, Verreck, Peeters, Brewster, & Van Den Mooter, 2004; Wu et al., 2006). SMEDDS is a good candidate for the oral delivery of hydrophobic drugs with adequate solubility in oil or oil–surfactant blends.

Vinpocetine was selected as the model compound to represent class II compounds according to the biopharmaceutical classification system and because of its high solubility in lipid- and surfactant-based formulations ensuring a high dosing potential. Vinpocetine (chemical structure is shown in Figure 1)

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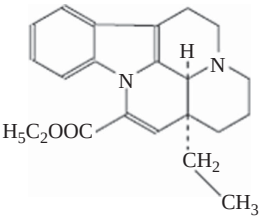

FIGURE 1. The chemical structure of vinpocetine.

has been used for the treatment of disorders arising from cerebrovascular and cerebral degenerative diseases (Chen et al., 2006). Vinpocetine is claimed to improve the cerebral circulation in the ischemia affected area of patients with cerebrovascular disease to decrease platelet aggregation in patients with transient ischemic attack or stroke, to increase red cell deformability in stroke patients, and to have neuroprotective effect and defend against brain ischemia (Chen et al., 2006). Absolute bioavailability of vinpocetine administrated as a 5-mg tablet in humans is only 7% (Szakács, Veres, & Vereczkey, 2001). The low bioavailability of vinpocetine is mainly attributed to its poor aqueous solubility and extensive metabolism during first pass (Szakács et al., 2001). So it is necessary to find a proper approach that will increase drug solubility and protect from degradation by first pass.

The objective of the current investigation was to develop a novel SMEDDS formulation containing vinpocetine to enhance its bioavailability. The oral bioavailability of vinpocetine in SMEDDS was compared with vinpocetine tablets in beagle dogs. Additionally, the effect of components on characteristics and absorption mechanism of the SMEDDS formulation was discussed.

MATERIALS AND METHODS

Materials

Vinpocetine was a generous gift from Harbin Sanlian Pharmaceutical Factory (Heilongjiang, China). Other materials used were Solutol HS-15, Cremophore RH40 (BASF, Ludwigshafen, Germany), Labrafil M 1944CS, Transcutol® P, Maisine 35-1, Gelucire 44/14, Labrasol (Castris, Gattefosse, France), Labrafac lipophile WL1349 (Colorcom Asia Pvt. Ltd., Mumbai, India), ethyl oleate (Beijing Changcheng Chemical Ltd., Beijing, China), Tween 80 (Beijing Yili Chemical Ltd., Beijing, China), PEG-400 (Tianjin Damao Chemical Plant, Tianjin, China), olive oil **(**Shanghai Chemical Plant, Shanghai, China**)**, methanol **(**HPLC grade; Merck, Darmstadt, Germany). All other chemicals used in the study were of analytical grade and were used without further purification. Double-distilled water was prepared freshly whenever required.

Solubility Studies

The solubility of vinpocetine in various oils, surfactants, and cosurfactants was determined. Briefly, an excess amount of vinpocetine was added to various oils, surfactants, and cosurfactants and vortexed to facilitate a proper mixing of vinpocetine in the vehicles. The mixtures were then shaken for 48 h in a water bath shaker (Remi, Mumbai, India) at 37°C, followed by keeping them at ambient temperature for 3 days to get to equilibrium. The equilibrated samples were centrifuged at 2,750 × *g* for 10 min followed by filtration through a membrane filter. The supernatant was then taken and diluted with methanol for quantification of vinpocetine by high-performance liquid performance (HPLC). The amount of vinpocetine in various systems was quantified using an HPLC system consisting of a Shimazu UV-VIS detector (SPD-10AP) and a solvent delivery pump (LC-10ATvp). The chromatographic column was a reverse phase Diamonsil C_{18} column (200 mm × 4.6 mm, 5 μm; Dikma). The mobile phase of in vitro release study consisted of methanol : water : triethylamine at a ratio of 90:10:0.1 (by volume). The flow rate was fixed at 1.0 mL/min and the UV detector was set at λ = 274 nm. The column temperature was maintained at 35°C.

Screening Surfactants for Emulsifying Ability

Emulsifying ability of various oils and surfactants was screened. Briefly, 300 mg of surfactant was added to 300 mg of the selected oil phase. Each system was prepared by weighing the exact quantity of each excipient into a screw cap glass tube followed by vortexing to allow mixing completely. After overnight incubation at 37°C, nonisotropic mixtures were noted and discarded, and isotropic mixtures were assessed for the efficiency of self-microemulsification.

Pseudoternary Phase Diagram Study

Ternary phase diagrams of surfactant, cosurfactant, and oil were plotted: each of them representing an apex of the triangle. Ternary mixtures with varying compositions of surfactant, cosurfactant, and oil were prepared. For any mixture, the total percent of surfactant, cosurfactant, and oil concentrations was always kept at 100%. To investigate the properties of the SMEDDS, a visual experiment to assess the emulsification efficiency (Bachynsky, Shah, Patel, & Malick, 1997) was modified in this article. Formulation (500 mg) was introduced into 500 mL of purified water at 37°C under a gentle agitation (50 rpm). A time of 2 min was used as an evaluation index by Khoo, Humberstone, Porter, Edwards, and Charman (1998) in the emulsification process. The systems were assessed visually in terms of the tendency to emulsify spontaneously and the final appearance of the emulsion. Classification was stratified into "SMEDDS" region that formed clear microemulsions or systems that resulted in the formation of microemulsions which had a bluish white appearance. The tendency to form an emulsion was judged as "SEDDS" region when droplets spread easily in water and formed a fine milky emulsion. And it was judged "bad" region when there was poor or no emulsion formation with immediate coalescence of oil droplets, especially

when stirring was stopped. All studies were repeated triplicate, with similar observations being made between repeats.

Preparation of SMEDDS

A series of SMEDDS formulations were prepared by adding the desired excipients to a glass vessel. Semisolid excipients such as Solutol HS 15 were melted and mixed well prior to sampling and addition. After stirring to yield a homogeneous mixture, vinpocetine in the desired amount was then added, and the resulting mixture was stirred at ambient temperature until a solution was obtained. The mixture was stored at room temperature until used.

Particle Size Analysis

The mean diameter of SMEDDS in the dispersion was determined by photon correlation spectroscopy (PCS) using a laser light scattering instrument (Photal LPA-3000/3100; Ostuka Electris, Tokyo, Japan) at a fixed angle of 90° at 25°C. The particle size analysis data were evaluated by the volume distribution. Before measurement, SMEDDS dispersions were diluted 10-fold with water for size determination. All the measurements were performed in triplicate.

In Vitro Dissolution Test

SMEDDS of vinpocetine was filled in size "0" hard gelatin capsule. In vitro release profile of SMEDDS was performed using USP XXIII apparatus I at 37 ± 0.50°C with a rotating speed of 100 rpm in dissolution media, namely, pH 1.2, 6.8 buffer and water so as to evaluate the effect of pH on in vitro dissolution. At designated time intervals (2, 5, 8, 10, 15, 20, and 30 min), 1 mL of release medium was collected and concentration of vinpocetine analyzed by HPLC. Release percentages were calculated as the ratio of vinpocetine released to total vinpocetine. All the operations were carried out in triplicate.

Bioavailability Studies

Beagle dogs (provided by Shenyang Pharmaceutical University Animals Center), weighing between 10.80 and 13.10 kg (mean ± *SD*, 11.23 ± 0.96 kg), were used for the in vivo study. All animal experiments complied with the requirements of the National Act on the use of experimental animals (People's Republic of China). Six dogs were administered with commercial tablet (15–mg single dose) and SMEDDS form (15-mg single dose orally; mean particle size, 23.5 nm). SMEDDS was administered with hard capsule dosage form (500 mg/capsule containing 5 mg of vinpocetine). The study was conducted according to a two-way crossover design and a washout period of 1 week between the two treatments.

Beagle dogs were fasted for 12 h prior to the experiment, and water was available ad libitum. Approximately 5 mL of blood samples were withdrawn into heparinized tube at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, and 24 h after administration. Blood samples were separated immediately by centrifugation and frozen at −20°C until analyzed. The concentration of vinpocetine in beagle dogs was determined by HPLC as follows: 50 μL of an internal standard (diazepam 10 μg/mL in methanol) solution and 100 μL 0.5 M NaOH were added to 0.5 mL of plasma. After vortex mixing for 3 min, 5 mL of ether anhydrous was added and vortexed at room temperature for 5 min. After centrifugation at $2{,}750 \times g$ for 10 min, the organic layer was transferred to a new tube and evaporated by nitrogen purging and the residue was dissolved in 100 μL methanol. After vortex mixing for 5 min, 20 μL of the sample was used for HPLC analysis. The concentration of vinpocetine in the samples was analyzed using reverse phase HPLC. The mobile phase was composed of methanol : acetonitrile : 0.1% triethylamine water (adjusted to, pH 6.3, with 10% phosphoric acid) in a volume ratio of 40:30:30 (by volume). The solvent was filtered through a 0.45-μm filter and degassed. The flow rate was fixed at 1.0 mL/min and the UV detector was set at λ = 274 nm. The column temperature was maintained at 35°C.

Data Analysis

Maximum plasma concentration (C_{max}) and time of maximum plasma concentration (T_{max}) were obtained directly from the individual plasma concentration–time profiles. The area under the concentration–time curve from time zero to time t ($AUC_{0\to t}$) was calculated using the trapezoidal method. The area under the total plasma concentration–time curve from time zero to infinity was calculated by $AUC_{0\to\infty} = AUC_{0\to t} + C_t/K_e$, where C_t was the vinpocetine concentration observed at last time and K_e is the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration–time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The relative bioavailability F_r at infinity at the same dose was calculated as $F_r = AUC_{SMEDDS,\ 0\to\infty}/AUC_{reference,\ 0\to\infty}$. The mean residence time (MRT) was estimated by $MRT = AUMC_{0\to\infty}/AUC_{0\to\infty}$.

Statistics

The data obtained from different formulations were analyzed by one-way analysis of variance and *t* test using a statistical package for social sciences (Kinetica version 4.4) software. Statistically significant differences were assumed when $p < .05$. All values are expressed as their mean ± *SD*.

RESULTS AND DISCUSSIONS

Solubility Studies

The solubility of vinpocetine in various surfactants and oils was presented in Table 1. Among the various oils that were investigated, ethyl oleate, Maisine 35-1, and Gelucire 44/14 had provided higher solubility than other oils. So these oils

TABLE 1
Solubility of Vinpocetine in Various Oils and Surfactants ($x \pm s$, $n = 3$, 37°C)

Vehicles	Solubility (mg/mL)
Labrafac WL 1394	1.55 ± 0.3
Labrafil M 1944	1.67 ± 0.7
Olive oil	2.03 ± 0.5
Miglyol 812N	3.96 ± 1.3
Maisine 35-1	6.16 ± 2.0
Gelucire 44/14	6.82 ± 1.7
Ethyl oleate	7.10 ± 1.4
Tween 80	1.87 ± 2.1
Cremophor RH 40	3.39 ± 3.6
Labrasol	5.28 ± 1.3
Solutol HS 15	10.41 ± 2.9
Propylene glycol	1.65 ± 0.7
PEG-400	4.71 ± 0.9
Transcutol® P	24.08 ± 4.8

were selected for further investigation. The oil represents one of the most important excipients in the SMEDDS formulation not only because it could solubilize marked amounts of the lipophilic drug or facilitate self-emulsification, but also mainly because it could increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract depending on the molecular nature of the triglyceride (Gershanik & Benita, 2000; Holm et al., 2002; Lindmark, Nikkila, & Artursson, 1995).

A cosolvent was also needed to improve solubility to permit the desired dose to be delivered in a capsule. A lipophilic, nonvolatile cosolvent is less likely to migrate to the shell than solvents such as ethanol and is also more likely to be retained by the oil phase on dilution with aqueous media, thus avoiding precipitation (Constantinides, 1995; Pouton, 2000). Transcutol® P, the purified diethylene glycol monoethyl ether by distillation, was used as a cosurfactant. It provided the highest drug solubility among the tested vehicles (Table 1) and the biggest self-microemulsifying region in the pseudoternary phase diagrams compared with other cosurfactants (data not shown). Transcutol® P was also known to enhance the permeability of drugs (Gao et al., 1998).

From these results, ethyl oleate, Maisine 35-1, and Gelucire 44/14 were selected as oils and Transcutol® P was used as cosurfactant for preparing SMEDDS system of vinpocetine for further studies.

Screening of Surfactant

Phase separation studies clearly distinguished the ability of various surfactants to emulsify various oils. These studies revealed that Solutol HS 15 had very good ability to emulsify ethyl oleate, Maisine 35-1, and Gelucire 44/14, because these mixtures were not separated after 2 h. Phase separation was not observed in the mixture of Labrasol–ethyl oleate. Thus, these mixtures were selected for further investigation.

Although the HLB values of the surfactants used in the investigation were in the range of 13–16, there was a great difference in their emulsifying ability. These studies indicated that Solutol HS 15 and Labrasol had very good ability to emulsify ethyl oleate, whereas Labrasol appeared to be a poor emulsifier for Maisine 35-1 and Gelucire 44/14. It was considered that emulsifying efficiency of the surfactant depended to a great extent on chemical structure of oils investigated besides the HLB, the structure, and chain length of the surfactant itself. This observation was in line with the investigation reported by Malcolmson, Sidhu, Satra, Kantaria, and Lawrence (1998) and Warisnoicharoen, Lansley, and Lawrence (2000).

Pseudoternary Phase Diagram Studies

SMEDDS form fine oil–water microemulsions under gentle agitation when exposed to aqueous media. Surfactants form a layer around the emulsion droplets and reduce the interfacial energy as well as provide a mechanical barrier to coalescence. As the free energy required to form an emulsion is very low, it is a thermodynamically spontaneous procedure (Craig, Barker, Banning, & Booth, 1995).

In pseudoternary phase diagram study, systems consisting of ethyl oleate, Maisine 35-1, and Gelucire 44/14 as oil phase; Solutol HS 15 as emulsifier; and Transcutol® P as coemulsifier were titrated with water, and self-emulsifying formulations were selected by observing regions of infinite dilution. The ternary phase diagrams were shown in Figure 2. From Figure 2A–C, it was evident that ethyl oleate as oil phase provided a larger microemulsifying region as compared with Maisine 35-1 and Gelucire 44/14. This might be due to the differences in the penetration of different oils into the tail region of the surfactant and their subsequent influence on the curvature of the interfacial film, which was consistent with the reported findings (Kommuru, Gurley, Khan, & Reddy, 2006; Malcolmson et al., 1998). So ethyl oleate was introduced to the system as the oil for its relatively good self-microemulsifying efficiency and its potential as promoter for lymphatic transport (Wu et al., 2006).

The phase diagrams of the systems containing different surfactants (Solutol HS 15 or Labrasol)–Transcutol® P–ethyl oleate are shown in Figure 2A and D. The self-microemulsifying region decreased and higher concentrations of Labrasol were required (in comparison with systems containing Solutol HS 15 as emulsifier) for good self-microemulsifying efficiency. In this system, the formulations surrounding the good self-emulsification region exhibited immediate coalescence following the self-emulsification process. Therefore, much higher concentrations of Labrasol were required compared with Solutol HS 15 to form a stable interfacial film in order to stabilize the emulsion. Another potential consideration may be that Transcutol® P decreased the capacity of Labrasol for emulsification of oil and water phases (Ljiljana & Marija, 2008).

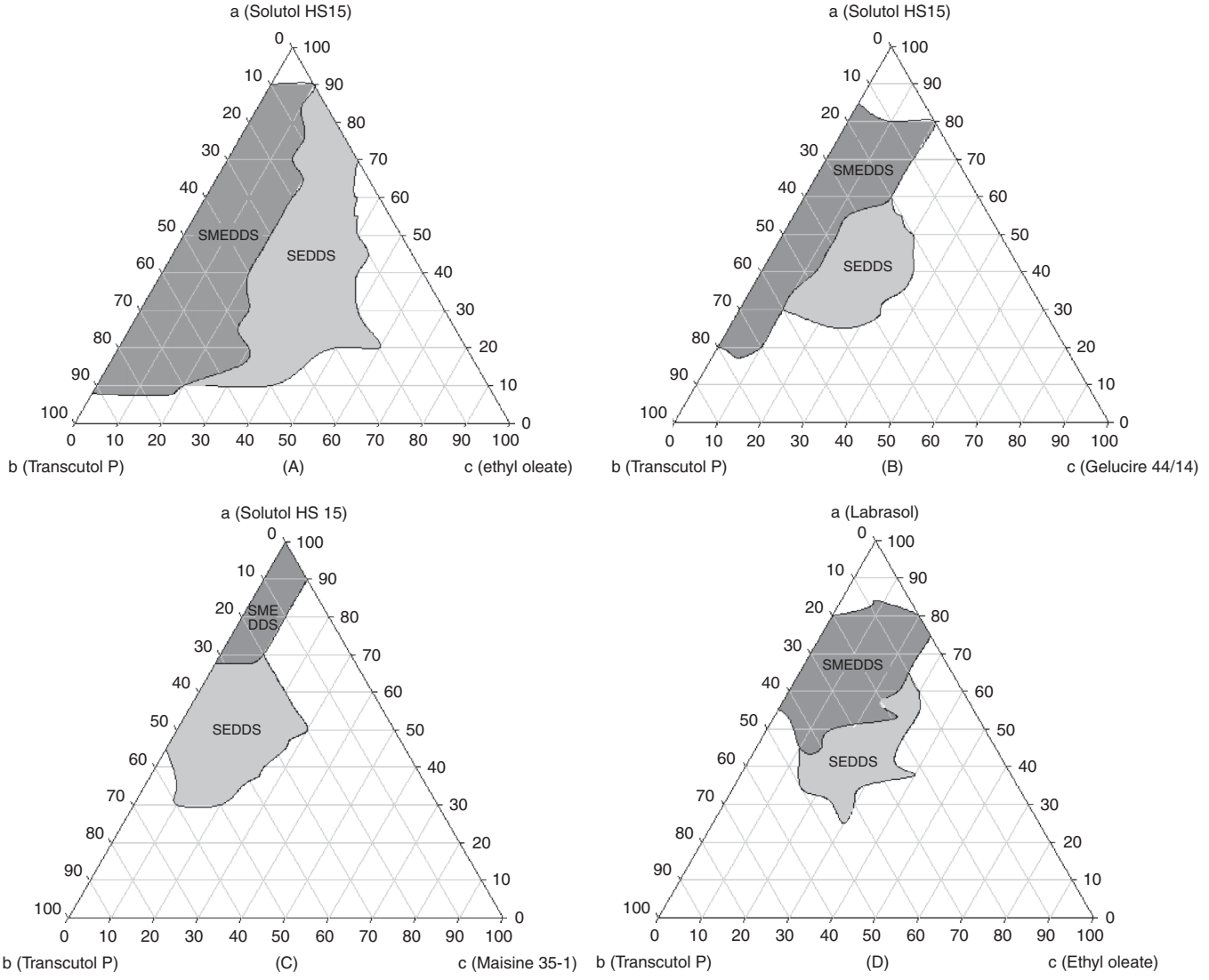

FIGURE 2. Ternary phase diagram (A): a, Solutol HS 15; b, Transcutol® P; and c, ethyl oleate. (B) a, Solutol HS 15; b, Transcutol® P; and c, Gelucire 44/14. (C) a, Solutol HS 15; b, Transcutol® P; and c, Maisine 35-1. (D) a, Labrasol; b, Transcutol® P; and c, ethyl oleate.

In view of current investigation, because of larger self-emulsifying region and greater capacity for incorporation of vinpocetine, Solutol HS 15–Transcutol® P–ethyl oleate system was selected for further studies.

Effect of Ratio of S/CoS (k_m) Value

The ratio of surfactant to cosurfactant [*S*/*CoS* (k_m)] was very essential to stable and efficient SMEDDS formulations. The phase diagrams were constructed at the ratios of surfactant to cosurfactant (k_m) 4:1, 3:1, 2:1, 3:2, 1:1, 2:3, 1:2, 1:3, and 1:4 (wt/wt). The phase diagrams where k_m, oil, and water represented an apex of the triangle were slightly different from that prior described in *pseudoternary phase diagram study*. The region of self-microemulsifying increased with the increasing concentration of Solutol HS 15, whereas the spontaneity of the self-emulsification increased when Transcutol® P ratio was higher. It is according to Cuiné, Charman, and Pouton (2007) and Trotta, Pattarino, and Grosa (1998). When a cosurfactant is added to the system, it further lowers the interfacial tension between the oil and the water interface and also influences the interfacial film curvature, which thereby readily deforms around oil droplet. With the decreasing of k_m, the area decreased slightly. This was not coincidence with Bok, Jin, and Se (2004) that the higher concentration of cosurfactant is, the higher self-emulsifying region will be obtained in the phase diagrams. The maximum self-microemulsifying region was to be at the ratio of 3:2 and 3:1. However, the drug precipitation was observed several hours after dilution with water at the ratio of 3:1. It might be because that vinpocetine had a much better solubility in Transcutol® P, it needed less S_{mix} (the mixture of surfactant and cosurfactant) at the ratio of 3:2 than that of 3:1

to dissolve the same quantity of vinpocetine. This may lead to an increase in the effective concentration of surfactant and cosurfactant available for microemulsion formulation, which may be responsible for the highest area of microemulsion formulation at the ratio of 3:2. Hence, the optimal ratio of surfactant to cosurfactant was selected to be 3:2 and the ethyl oleate content was set at 15% to ensure the solubilization capacity.

Based on our results, a three-component SMEDDS formulation was established: oil (ethyl oleate, 15%), surfactant (Solutol HS 15, 50%), and cosurfactant (Transcutol® P, 35%) regarding its self-microemulsifying ability and solubilization ability.

Analysis of Particle Size

The effect of the formulations on the particle size is shown in Table 2. When the surfactant concentration increased from 30 to 50% (wt/wt), the particle size decreased from 75.1 ± 11.4 to 23.5 ± 4.3 nm. It seemed that the particle size of vinpocetine microemulsion was decreased with increasing surfactant content of SMEDDS. This could be explained by the result of more surfactant being available to stabilize the oil–water interface. Furthermore, the decrease in the droplet size behavior reflected the formation of a better close packed film of the surfactant at the oil–water interface, thereby stabilizing the oil droplets (Levy & Benita, 1990). On the other hand, the mean droplet size may increase with increasing surfactant concentrations to some extent (Feigin, Doronin, Popova, Gribatcheva, & Tchernov, 2001; Song, Chung, & Shim, 2005; Venkatesan et al., 2006). Formula 4 with 60% Solutol HS 15 formed microemulsion with the mean diameter of 39.2 ± 6.9 nm. This phenomenon could be attributed to the interfacial disruption elicited by enhancing water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase (Pouton, 1997).

Meanwhile, the effect of drug loading on particle size of SMEDDS (with or without vinpocetine) was evaluated. The mean particle sizes of SMEDDS containing vinpocetine tended to be larger than those of SMEDDS without vinpocetine in simulated gastric fluid (pH 1.2) without pepsin or simulated intestinal fluid (pH 6.8) at 37°C. When drug loading further increased to 10 mg in 500 mg SMEDDS, particle size increased dramatically. This could be explained that it was beyond the range of colloidal system and no more presented the properties of microemulsion. Meanwhile, the undissolved drug in the formulation affected the mean droplet size to increase.

In Vitro Dissolution Study

In vitro dissolution profile of optimized vinpocetine SMEDDS and the commercial tablet as reference in various dissolution media are presented in Figure 3. In vitro dissolution experiments demonstrated a marked increase in the release percentage for the SMEDDS formulation as compared with the commercial tablet. The dissolution profile of vinpocetine SMEDDS in various dissolution media showed that 100% of vinpocetine was released within 10 min irrespective of the pH value of dissolution medium. However, the release rate of vinpocetine tablet was slow in all three media. It could be concluded that vinpocetine dissolved perfectly in SMEDDS form and released sufficiently because of the small droplet size and the strong solubilization of Solutol HS 15 and Transcutol® P, which permits a faster rate of drug release into aqueous phase, faster than reference, and it could enhance bioavailability.

In Vivo Pharmacokinetic Study

In vivo absorption study was undertaken to determine whether or not the enhanced solubility and in vitro dissolution of vinpocetine in a SMEDDS could increase the GI absorption of drug after oral administration. The plasma concentration profiles of vinpocetine in beagle dogs following oral administration of the conventional tablet and SMEDDS form were compared. Figure 4 presented the mean plasma vinpocetine concentration versus time profiles obtained with commercial tablet and SMEDDS. After administration of vinpocetine tablet, plasma level of vinpocetine was very low, most of which (after 6 h) was below the limit of quantification of the assay, whereas the plasma level of SMEDDS formulation was not detected after 10 h. Therefore, the SMEDDS formulation had a longer in vivo resident time than that of conventional tablet.

The corresponding mean pharmacokinetic parameters for both formulations were presented in Table 3. For SMEDDS formulation, $AUC_{0\rightarrow 24\ h}$ and C_{max} were 564.91 and 248.65 ng/mL,

TABLE 2
Practical Size of Vinpocetine SMEDDS Formulations (n = 3, 25°C)

	Composition (%, wt/wt)				
Formulation No.	Surfactant	Cosurfactant	Oil	Drug (mg)	Mean Droplet Size (nm)
1	30	55	15	5	75.1 ± 11.4
2	40	45	15	5	48.3 ± 5.5
3	50	35	15	5	23.5 ± 4.3
4	60	25	15	5	39.2 ± 6.9

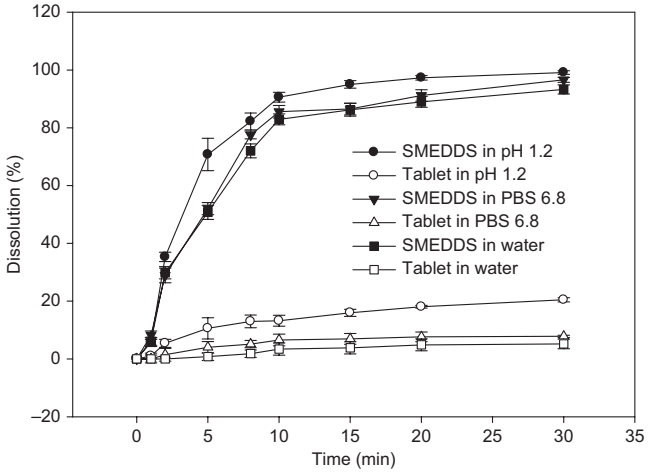

FIGURE 3. Dissolution profiles of Vinpocetine from SMEDDS (●) and the conventional tablet (❍) in pH 1.2; SMEDDS (▼) and the conventional tablet (△) in PBS6.8 and SMEDDS (■) and the conventional tablet (□) in water.

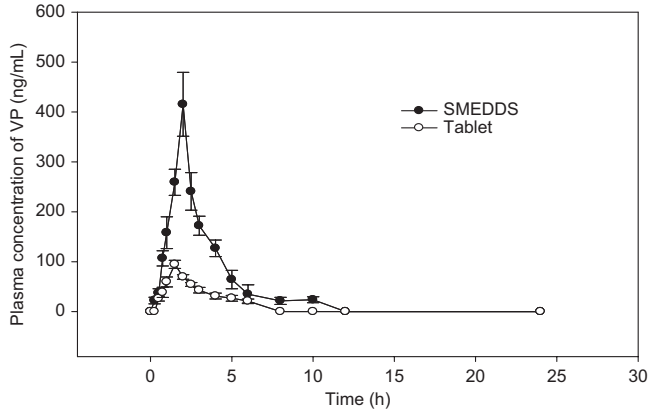

FIGURE 4. Mean plasma concentrations of vinpocetine (n = 6) versus time profiles of vinpocetine following the oral administration of SMEDDS (●) and commercially available tablet (❍) to fasted beagles.

respectively, compared with the conventional tablets whose corresponding data were 327.63 and 92.19 ng/mL, respectively. Higher drug concentration in blood indicates better systemic absorption of vinpocetine from SMEDDS as compared with the commercial tablet. And the relative bioavailability of SMEDDS of the conventional tablet was 172%. Therefore, SMEDDS might be a promising approach to the oral delivery of vinpocetine. However, no significant difference was observed between T_{max} values ($p < .05$), which means that both SMEDDS and tablet of vinpocetine show same rate of absorption.

In dog D, double peaks of maximum concentrations were observed when giving the SMEDDS formulation, and this is a obvious characteristic of enterohepatic circulation (Morazzoni, Montalbetti, Malandrino, & Pifferi, 1993). This may be due to

TABLE 3
Pharmacokinetic Parameters After Oral Administration of SMEDDS and Reference ($x \pm s$, n = 6)

Parameter	Reference	SMEDDS
C_{max} (ng/mL)	92.19 ± 0.27	248.65 ± 0.29*
T_{max} (h)	1.58 ± 0.13	2.0 ± 0.31
AUC (ng/mL h)	327.63 ± 4.04	564.91 ± 3.29*
AUMC (ng h/mL)	1779.3 ± 7.02	2896.09 ± 6.65
MRT	4.35 ± 0.28	3.25 ± 0.62
K_e (h^{-1})	0.24 ± 0.09	0.39 ± 0.13
CL (L/h)	46.78 ± 1.58	33.05 ± 1.42
F_r	—	1.72

*$p < .05$, when compared with the pharmacokinetic parameters of SMEDDS and commercial tablet by the ANOVA test.

the need for mobilization of other components of the chylomicra and very-low-density lipoprotein in the absorption process as suggested by Diplock (1985).

The exciting results we obtained showed that vinpocetine absorption was enhanced significantly by employing SMEDDS. The absorption of vinpocetine from the self-microemulsifying formulation resulted in a 1.72-fold increase in bioavailability (as indicated by AUC and C_{max} values) compared with that of the commercial tablet. This could be because that the increased dispersion of vinpocetine in the SMEDDS could overcome the barrier of solubility-limited absorption as mentioned earlier. For a poorly water-soluble drug, the absorption is often inadequate because of an insufficient dissolution in the gastrointestinal tract. Therefore, the dissolution process might be a critical factor influencing the absorption. When the SMEDDS encounters the gastrointestinal fluid, the spontaneously formed microemulsion will present the drug in a dissolved form, and the drug in the soluble form will significantly enhance absorption.

Like other formulations, reduction in the particle size is a factor for improving the peroral performance of poorly water-soluble drugs. Mechanisms of particle size effect on drug absorption may include improved release and facilitated lymphatic transport (Bachynsky et al., 1997; Shah et al., 1994). In SMEDDS formulations, the particle size range was reduced to less than 100 nm, resulting in an increase in surface area and saturation solubility. In vitro release tests had confirmed that the release velocity from SMEDDS was significantly faster than that of vinpocetine commercial tablet. Additionally, the diffusion of surfactants altered the barrier properties of the aqueous **mucosa** layer and the intestinal mucosa permeability resulting in a high absorption rate of vinpocetine.

In addition, the markedly decreased clearance value in SMEDDS in relation to tablets (33.05 and 46.78 L/h) might be explained by improved lymphatic transport pathway, reduced

metabolism in the liver, and possible lipid protection of drug from enzymatic degradation.

Additional factors that might have contributed to increased oral bioavailability were coadministration of various PGP inhibitors and cytochrome P450 (CYP) 3A inhibitors (Zhang & Benet, 2001). Solutol HS 15, the main component of which is the polyethylene glycol 660 ester of 12-hydroxy stearic acid (Ruchatz & Schuch, 1998), which has been shown to have an affinity to the PGP (Buszello, Harnisch, Müller, & Müller, 2000; Morazzoni et al., 1993), might moderately inhibit the PGP efflux system that has increased vinpocetine transport over the intestinal mucosa, leading to the improved oral absorption of vinpocetine. Solutol HS 15 has been implicated as inhibitors of efflux pumps, but the mechanism of inhibition has not been determined. This could be a nonspecific conformational change caused by penetration of surfactant molecules into the plasma membrane, adsorption of surfactants to the external surface of the efflux pump, or even interaction of small molecules with the intracellular domains of the efflux pump. The chemical heterogeneity of surfactants such as Solutol HS15 will make it difficult to establish a precise mechanism (Flemming, Kamilla, & Anette, 2008). Additionally, the surfactants may have improved the affinity between lipid particles and the intestinal membrane (Song et al., 2005; Venkatesan et al., 2006). Some particles may be taken up into the lymphatic organs and eventually enter the systemic circulation (Holm, Müllertz, Christensen, Hoy, & Kristensen, 2001; O'Driscoll, 2002).

Another advantage of SMEDDS formulations over vinpocetine commercial tablet is the lipid protection of the drug from chemicals, as well as enzymatic degradation, thereby delaying the in vivo metabolism (Miskolczi, Kozma, Polgar, & Vereczkey, 1990). It could therefore be concluded that the drug absorption into the systemic blood circulation and corresponding bioavailability were significantly enhanced by SMEDDS formulation.

CONCLUSION

The optimal formulation of SMEDDS containing vinpocetine (high drug loading and small particle size) was as follows: 50% of Solutol HS 15, 35% of Transcutol® P, and 15% of ethyl oleate. In vitro dissolution studies revealed that release of vinpocetine from SMEDDS was faster than that of the conventional tablet. Also, in vivo studies, SMEDDS showed a significantly greater absorption than the conventional tablet. The relative bioavailability of SMEDDS to the conventional tablet was 172%. Our studies illustrated the potential use of SMEDDS for the delivery of hydrophobic compounds, such as vinpocetine, by the oral route.

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