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Preparation and evaluation of self-nanoemulsified drug delivery systems (SNEDDSs) of matrine based on drug–phospholipid complex technique

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ABSTRACT

To enhance oral bioavailability of matrine, a dedicated and newly emerging drug system called self-nanoemulsifying drug delivery system (SNEDDSs) was developed. Phospholipid complex (MPC) was prepared using solvent-evaporation method to improve the liposolubility of matrine. Solubilization test, infrared spectroscopy (IR) and differential scanning calorimetry (DSC) analysis were employed to confirm the formation of MPC. A rational experimental design was adopted to optimize the properties of SNEDDSs. Eight SNEDDSs prototypes were obtained to form nanoemulsion spontaneously based on optimization experiments. Among them, MPC prepared exhibited excellent solubility. SNEDDSs 2 (composition: Lauroglycol FCC, Cremophor EL and Transcutol HP; ratio: 6:4:1) was selected as the optimal formulation, with a mean droplet size in the range of 65–80 nm and 8.34% of the leakage rate, exhibiting instantaneous emulsion formation with only one flask inversion. Media pH and dilution factor showed no effect on the droplet size. The oral absorption of matrine in rats via SNEDDSs delivery was investigated. C_{max} was increased dramatically from 4.12 to 6.52 and 7.95 μg/mL in case of matrine, MPC and MPC-SNEDDS. In parallel to C_{max}, prolonged T_{max} from 0.39 to 0.50 h, and 3.00 h could be observed. AUC_{0-t} of MPC-SNEDDSs was significantly higher than other two counterparts. In conclusion, the absolute bioavailability of matrine drastically increased from 25% to 84.6% by the formation of MPC-SNEDDS, with an outstanding relative bioavailability of 338%, suggesting its great potential for clinical application.

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1. Introduction

Matrine (structure shown in Fig. 1), one of the major quinolizidine alkaloids from Radix Sophora Flavescentis, is extensively used for the treatment of cancer, viral hepatitis, arrhythmia and skin diseases (Lai et al., 2003; Zhang and Huang, 2004; Liu et al., 2000; Zhang et al., 2001). Recently, it has been demonstrated that matrine has an inhibitory effect on the proliferation of tumor cells and growth of tumors in mice (Chui et al., 2005; Hu et al., 2005).

As well as its pharmacological effects, the pharmacokinetics of matrine has been widely studied (Wu et al., 2003; Wang et al., 2005; Wu et al., 2006; Zhang et al., 2008). However, as water-soluble drug, the absolute bioavailability of matrine was only 31.6%, which was fairly low, compared with that by injection (Zhu et al., 1998). Therefore, it is highly necessary to develop a new dosage form of matrine with improved oral bioavailability.

Previously, nanoemulsions have been employed as the delivery system for hydrophilic drugs such as proteins and peptides. Typically, hydrophilic drugs are present in the aqueous phase either in W/O nanoemulsion (Sarciaux et al., 1995; Constantinides, 1995; Cilek et al., 2005) or O/W microemulsion (Swenson and Curatolo, 1992; Ho et al., 1996). For O/W microemulsion, drugs can leak into environmental aqueous media *in vivo* when it is administered and diluted by gastrointestinal (GI) fluid since the drugs are in the external phase. For W/O microemulsion, phase inversion happens frequently *in vivo* when the preparation is diluted with a relatively large amount of GI fluid, leading to the leakage of drugs into the environment aqueous media. In addition, patient compliance is a concerned issue for W/O nanoemulsion due to the oily nature of the formulation.

Most recently, self-nanoemulsion drug delivery systems (SNEDDSs) have emerged as novel colloidal systems with excellent thermodynamic stability and efficient absorption features. As an isotropic mixture of oil, surfactants and cosurfactants, O/W nanoemulsion could form spontaneously in water (Nazzal et al., 2002; Shah et al., 2007). The resultant nanoemulsion is a thermodynamically stable system with extremely small droplet size, which guarantees efficient absorption of oil droplets.

Usually, SNEDDSs is used to enhance the oral bioavailability of poor water soluble drugs. Most recently, solid dispersion has been utilized as a promising strategy for water soluble drugs to be entrapped into SNEDDSs, such as β-lactamase (BLM) (Venkata

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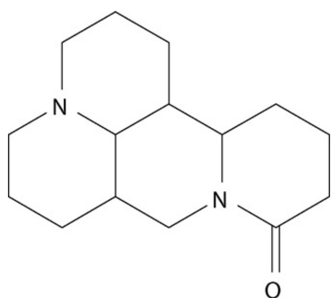

Fig. 1. Structure of matrine.

Ramana Rao and Shao, 2008). However, the study indicated that approximately 50% of BLM loaded into SNEDDSs leaked out of the oil phase into the aqueous phase when the SNEDDSs were mixed with Phosphate buffered saline (PBS) to form nanoemulsions. As they hypothesized, only when the proteins are loaded inside the oil droplets, can the absorption of the proteins be significantly increased (Venkata Ramana Rao and Shao, 2008). Such kind of leakage of proteins from the oil phase into aqueous phase would probably decrease their absorption. Therefore, how to retain water-soluble drugs inside the oil phase in SNEDDSs remains a tough issue that needs to be addressed.

Previous studies have indicated the beneficial role of phospholipids in changing polarity and enhancing the therapeutic efficacy of some molecules of poor oral bioavailability (Carini et al., 1992; Comoglio et al., 1995; Cui et al., 2006; Maiti et al., 2007). In our study, a novel phospholipid based strategy has been established to develop SNEDDSs for matrine delivery, in which phospholipid complexes were formulated to significantly enhance the liposolubility of matrine and drastically facilitate the incorporation of matrine into SNEDDSs.

Therefore, our preliminary objective was to develop novel SNEDDSs for matrine delivery to enhance its oral bioavailability through phospholipids complexes, by which matrine could be loaded into the oil phase easily. Hopefully, this study can highlight the potential application of SNEDDSs in improving oral absorption of water-soluble drugs.

2. Materials and methods

2.1. Materials and animals

The reference substances of matrine and artemisinin were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Free samples of Cremophor EL®, Poloxamer 188 and Cremophor RH40® were generously provided by BASF Corp (BASF, Germany). Peceol®, Labrasol®, Transcutol HP®, Labrafac lipophile WL1349 and Lauroglycol FCC® were purchased from Gattefosse Corp (Shanghai, China). Soya phospholipids were purchased from Panjin pharmaceutical Co., Ltd. (Liaoning Province, China), and the phosphatidyl content was approximately 70% (w/w). HPLC grade methanol was obtained from Tedia Co. Inc. (Fairfield, OH, USA). All other chemicals were of analytical reagent grade. Double distilled water was used for all solutions and dilution. Matrine of analytical grade was supplied by Yanchi Pharmaceutical Factory (Ningxia, China).

Male Wistar rats weighing 180–210 g were supplied by Experimental Animal Center of Guiyang Medical College. The rats were housed at a room temperature of 22 ± 2 °C and a relative humidity of 50 ± 10%. The animals were allowed free access to standard diet and water except wherever indicated. Animals were used and treated as prescribed in the ‘Guide for the care and the use of the laboratory animals’ (NIH Publication No. 92-93, revised 1985) and all the animal studies were approved and supervised by Animal Ethics Committee of Guiyang Medical College.

2.2. Preparation of matrine–phospholipid complex (MPC)

The complex was prepared using matrine and phospholipids at a molar ratio of 1:1. Weighed amount of matrine and phospholipids were put in a 100 mL round bottom flask and 20 mL of dichloromethane was added. The mixture was refluxed at 35 °C for 2 h. The resultant clear solution was evaporated with continuous stirring. The dried residue was collected and placed in desiccators overnight, then crushed in the mortar and passed through a 100 mesh sieve. The formed MPC was transferred into a glass bottle, flushed with nitrogen and stored at room temperature.

2.3. Characterization of matrine–phospholipid complex (MPC)

2.3.1. HPLC analysis

An HPLC system (HP 1100 series, Agilent, USA) equipped with an ODS-C18 column (250 mm × 4.6 mm i.d.) (Waters Corp., Milford, MA) was used for the determination of matrine (Xue et al., 2006). The mobile phase consisted of methanol:double distilled water:0.05 M KH_2PO_4 at a volumetric ratio of 60:40:5, and the pH was adjusted to 3.0 by phosphoric acid. The elution was carried out at a flow rate of 1.0 mL/min at room temperature and the detect wavelength was 210 nm.

Studies were carried out to estimate the precision and accuracy of this HPLC method for the determination of matrine. The standard curve was used to estimate the concentration of matrine and each value represented the mean of triplicate determination.

2.3.2. Solubility studies of MPC

Solubility studies were conducted to verify if the liposolubility of matrine was enhanced after the formation of matrine–phospholipid complexes. Briefly, excess amount of matrine, matrine–phospholipid complexes and physical mixture of matrine and phospholipids were added to 5 mL water or *n*-octanol in sealed glass container at room temperature, respectively. The liquids were horizontally shaken (60 rpm) for 24 h and centrifuged at 5064 × *g* for 10 min. The supernatant was collected and filtered through a filter membrane (0.45 μm). The filtrate (1 mL) was taken into a 10 mL volumetric flask and then diluted to 10 mL with methanol. Aliquot of the resulting solution (10 μL) was injected into HPLC and the concentration of matrine was assayed as described above.

2.3.3. Interaction studies between matrine and phospholipids

IR spectra and DSC were employed to verify the interaction between matrine and phospholipids and to further validate the formation of drug–phospholipids complex. Fourier transform infrared spectrophotometry (FT-IR Spectrometer, BRUKER IFS-55, Switzerland) was used to study the interaction between matrine and phospholipids. The IR spectra of matrine, MPC and physical mixture of matrine and phospholipids were obtained by KBr method. The peak transition onset temperatures were determined and compared on Mettler DSC 30 S (Mettler Toledo, UK) at a speed of 10 °C/min from 0 to 300 °C in nitrogen atmosphere (60 mL/min).

2.4. Preparation of SNEDDSs

Three kinds of oils, four types of non-ionic surfactants (SA) and two types of cosurfactants (CoSA) were used in this study to prepare SNEDDSs. Orthogonal experimental design was used to optimize the formulation of SNEDDSs and to determine the ratios of oil, SA and CoSA. To each oil/SA/CoSA system (1 g) taken in a beaker set up with gentle magnetic stirring (50 rpm), water (100 mL) was added

dropwise. After equilibrium, the mixture was visually observed. The generated sample which has clear or slightly bluish appearance was validated as nanoemulsion. The oil/SA/CoSA systems that formed nanoemulsion spontaneously were further studied.

2.5. Preparation of MPC-loaded SNEDDS

The loading process of MPC into SNEDDSs was done according to the method of Venkata Ramana Rao and Shao (2008) with some modifications. Briefly, fixed amount of MPC (200 mg) was added to 1 mL SNEDDSs in a tube. The tube was shaken for 2 h and then gently vortexed and placed in a sonicator maintained at 25 °C for 120 min, MPC was considered to be totally soluble in the particular type of SNEDDS, when clear oily solution was obtained; otherwise an additional 1 mL of the respective SNEDDSs was added to the tube and then shaken, gently vortexed and sonicated as mentioned above. If clear oily solution still could not be obtained, it was concluded that MPC was not soluble in the particular type of SNEDDS, and therefore was excluded.

2.6. Selection of best MPC-loaded SNEDDSs nanoemulsion

Water (10 mL) was added to 1 g of MPC-loaded SNEDDSs. The mixture was then gently vortexed to form nanoemulsions. Various nanoemulsion formulations were screened for their reduction of leakage of MPC from oil phase detected by gel filtration method. Briefly, MPC-loaded SNEDDSs was mixed with water at the ratio of 1:10 to form nanoemulsion containing 4 mg/mL of matrine. Then 0.5 mL of this nanoemulsion was loaded onto a Sephadex G-50 column (Amersham Pharmaceuticia Biotech ab, Sweden) with water as the elution phase. Fractions of 0.5 mL each were collected and scanned by a UV spectrophotometer (HP 5953, US) in the wavelength range of 210–280 nm. The fractions that had peak absorbance at 210 nm (due to matrine) and no peak at 265 nm (due to SNEDDSs) were deemed to contain matrine alone. The concentration of matrine in these fractions was analyzed by HPLC. MPC solution, matrine and the nanoemulsion prepared by mixing of free MPC solution with blank SNEDDSs (all containing 4 mg/mL matrine) were used as the controls. The formulation of Lauroglycol FCC:Cremophor ELP:Transcutol HP = 6:4:1 was found to give the lowest leakage rate.

To validate the ease of emulsification, 0.5 mL SNEDDSs was slowly added to a 10 mL stoppered volumetric flask that had 10.0 mL water. Emulsification efficiency was observed by noting the number of flask inversions required to give uniform emulsion. This formulation (Lauroglycol FCC:Cremophor ELP:Transcutol HP = 6:4:1) was dispersed within seconds only by one inversions. Hence, it was assumed as the optimal formula and further studied.

2.7. Characterization of MPC-loaded SNEDDS

2.7.1. Morphological characterization

The morphology of MPC-SNEDDSs and MPC were observed by transmission electron microscope (TEM) (HITCHI H-7650 II, Hitachi Ltd., Japan). Samples were diluted with distilled water at a ratio of 1:250 and mixed by gently shaking. Then, a drop of sample after dilution was placed on copper grids. The excess was drawn off with filter paper. Subsequently, samples were stained in 1% of phosphotungstic acid solution for 30 s and then subjected to TEM observation.

2.7.2. Droplet size

The droplet size of the MPC-SNEDDSs nanoemulsion was determined using a Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd., UK). The effect of dilution factors (SNEDDSs:water ratio) and the pH on the droplet size of nanoemulsion were determined. Various aqueous media were used for analysis (SNEDDSs:water ratio = 1:100), such as deionized water, 0.1 M HCl (pH 1.0), acetate buffer (pH 5.5) and PBS (pH 7.4). All experiments were performed in triplicate.

2.7.3. Determination of matrine content in MPC and SNEDDSs

In order to determine drug content in SNEDDSs remained in the oil phase, gel filtration analysis was used to separate the free drug from the delivery system as described in Section 2.6. The fractions that had peak absorbance at 210 nm (due to matrine) and no peak at 265 nm (due to SNEDDSs) were deemed to contain free matrine. These fractions was collected and adjusted to 25 mL with methanol.

Approximately 5 mg of the complex and 20 mg of MPC-loaded SNEDDSs were dissolved in 10 mL methanol and the volume were adjusted to 100 mL with methanol, respectively.

Aliquot of the solutions (10 μL) of the free matrine, total matrine amount in the complex and MPC-loaded SNEDDs were injected into an HPLC system (HP 1100 series) described in Section 2.3.1. The matrine content in MPC (M.C.%) and SNEDDSs (S.C.%) were calculated as follows:

$$\text{M.C.\%} = \frac{\text{matrine amount}}{\text{MPC weight}} \times 100$$

$$\text{S.C.\%} = \frac{\text{total matine amounet} - \text{free matrine amount}}{\text{SNEDDs weight}} \times 100$$

2.8. Bioavailability experiments in rats

2.8.1. Animals and dosing protocols

Male Wistar rats were randomly divided into three groups ($n = 5$) and fasted for 12 h, with free access to water prior to the experiments. Rats received intragastric administration (*o.p.o.*) of various preparations (1 mL each) and intravenous injection (*i.v.*) of 0.5 mL with matrine *via* caudal vein. The *i.v.* and *o.p.o.* doses were 20 mg/kg, respectively. Pharmacokinetic parameters were calculated by computer program DAS 2.0 (Anhui, China).

2.8.2. Chromatography

The plasma concentration of matrine was determined by HP 1100 series LC/MSD G1946D (Agilent, USA). The stationary phase, Lichrosphere-CN analytical column (4.6 mm × 150 mm, i.d., 5.0 μm, Merck), was kept at 35 °C. The mobile phase was a mixture of acetonitrile–water containing 10 mM ammonium acetate (15:85). The flow rate was l.0 mL/min. The following optimized MS conditions were selected: positive ion detection mode with a capillary voltage of 4000 V. The heated drying gas temperature was 350 °C. The nebulizer pressure was set at 35 psi, with a drying gas flow rate of 10.0 L/min. Selective ion monitoring (SIM) was used, and protonated molecules [M^+H] were detected at m/z 249.2 for matrine and 283.3 for internal standard.

2.8.3. Plasma sample preparation and method validation

Blood (500 μL) was taken from the eyeground veins of the rats through the cannulated tube at times 0, 0.17, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 24.0 h after matrine and MPC oral administration, 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h after MPC-SNEDDSs oral administration, or 0, 0.083, 0.17, 0.33, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, 8.0 and 24.0 h after *i.v.* injection, respectively. The plasma obtained after centrifugation (15 min, 5064 × *g*) was stored at −20 °C before analyzed. Internal standard (100 μL) working solution (0.011 mg/mL artemisinin in methanol) was added to 100 μL of plasma sample, and then 200 μL of methanol was added to the mixture and vortexed for 60 s for protein precipitation. The resultant mixture was centrifuged at 10,128 × *g* for 10 min at 4 °C. The

top limpid layer was transferred to an auto-sampler vial for LC–MS analysis.

Matrine from rat serum was separated completely by the extraction process and standard curves ranging from 1 to 40,000 ng/mL were linear (r = 0.998). Minimum detection level of matrine was 0.50 ng/mL. The validation of the method for extraction and quantification from rat serum was done by performing recovery rate experiments. The recover rates of matrine from high, middle and low concentration ranges were 86.25%, 85.47% and 83.32%, respectively. The inter-days relative standard deviations (R.S.D.) were 3.14%, 2.51% and 3.09% respectively and intra-days R.S.D. were 4.18%, 3.64% and 3.88%, respectively. The pharmacokinetic data of matrine in rats were analyzed using standard non-compartment analysis. The area under plasma concentration-time curve (AUC_{0-t}) was estimated by the linear trapezoidal method. The absolute bioavailability (Fa) and relative bioavailability (Fr) were calculated according to the following equations:

$$Fa = \frac{AUC_{ig}}{AUC_{iv}}$$

$$Fr = \frac{AUC_{test}}{AUC_{refernce}}$$

2.9. Statistics

All experiments were performed at least three times. All data were presented as mean ± standard deviation. Difference between groups was evaluated using Student's unpaired t-test. A p value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Content of matrine in complex and SNEDDSs

The content of matrine in the complex and SNEDDSs, as determined by HPLC, were 32.04% (w/w) and 7.12%, respectively. Compared with matrine or physical mixture of matrine and phospholipids, the complex exhibited much higher solubility in n-octanol and lower in water. Meanswhile, the oil–water partition coefficient of matrine was significantly enhanced from 0.17 to 2.86 in the complex (Table 1).

3.2. IR spectra analysis

The IR spectra of matrine, phospholipids, physical mixture and phospholipid complex were shown in Fig. 2. It was obvious that the physical mixture and the complex indicated distinct IR spectra. Compared with the complex, the peaks at 1737 cm^{-1} (due to phospholipid $\nu_{OH-C=O}$) and 1630 cm^{-1} (due to matrine $\nu_{N-C=O}$) were obviously found in the spectrum of physical mixture. However, in the spectrum of their complex, the characteristic absorption peak of matrine was almost masked by that of phospholipids at 1644 cm^{-1} ($\nu_{C=C}$). Moreover, no new peaks were observed in the mixture and complex.

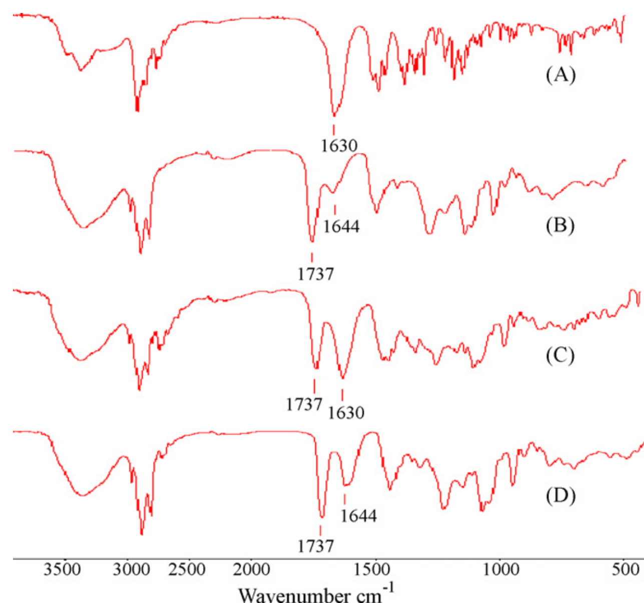

Fig. 2. Infrared spectra of (A) matrine, (B) phospholipids, (C) physical mixture of matrine and phospholipids, (D) matrine–phospholipid complexes.

3.3. DSC analysis

DSC is a fast and reliable method to screen drug-excipient compatibility by providing maximum information about the possible interactions between them. In DSC, an interaction can be concluded by elimination of endothermic peak(s), appearance of new peak(s), changes in peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy. Fig. 3 showed the DSC thermograms of (a) physical mixture of matrine and phospholipid, (b) phospholipid, (c) matrine–phospholipid complex and (d) matrine. Thermogram of matrine exhibited two different peaks. The first one (68.5 °C) was very sharp and it might be caused by

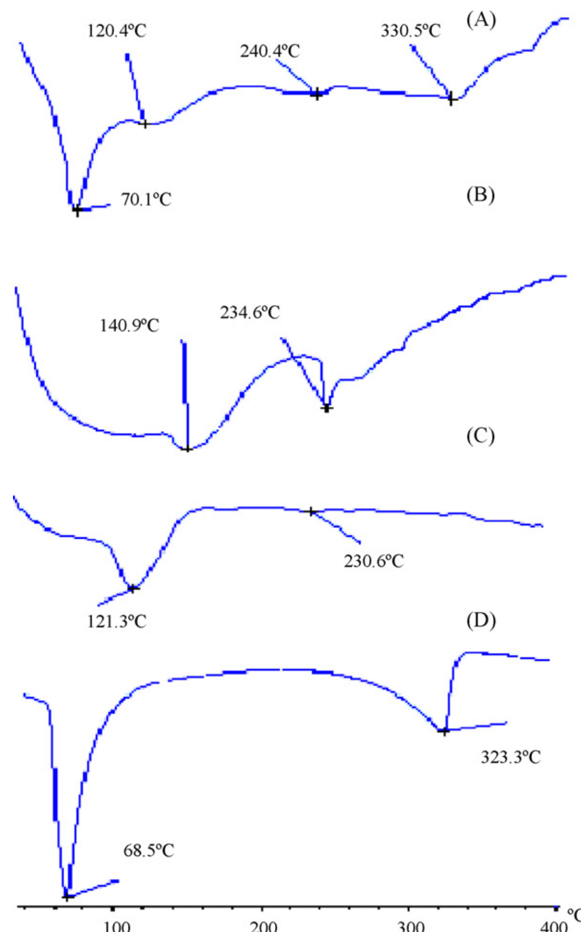

Fig. 3. DSC thermograms of (A) physical mixture of matrine and phospholipids (B) phospholipids (C) matrine–phospholipid complexes and (D) matrine.

Table 1
Solubility of matrine, matrine–phospholipid complex and physical mixture of matrine and phospholipid in water and *n*-octanol at 25 °C (*n* = 3).

Sample	Solubility in water (g/mL)	Solubility in *n*-octanol (g/mL)
Matrine	2.05 ± 0.03	0.34 ± 0.01
Matrine–phospholipids complex	0.84 ± 0.02	2.39 ± 0.05
Physical mixture of matrine and phospholipid	1.84 ± 0.02	1.06 ± 0.08

the phase transition from solid state to liquid state. The second one (323.3 °C) was mild, which was resulted from the decomposition of matrine by heat. Phospholipids showed two different kinds of endothermal peaks. The first endothermal peak (140.9 °C) appeared mild, indicating hot movements of polarity parts of phospholipids molecule. The second endothermal peak appeared at 234.6 °C, which could be possibly owed to the transition from gel state to liquid crystal state and the melting of carbon–hydrogen chain in phospholipids and changes of isomerous or the crystal. Physical mixture of matrine and phospholipids showed that there were four endothermal peaks. The first one was 70.1 °C, in parallel with the endothermal peak of matrine; the others were 120.4 °C and 330.5 °C, respectively, coordinating with the endothermal peaks of phospholipids complex. The peak at 240 °C might be from the phospholipids. It was concluded that matrine would melt and dissolved in phospholipids and phospholipids complex was partially formed when the temperature increased.

DSC of phospholipids complex showed that the endothermal peaks of drug and phospholipid disappeared and the phase transition temperature (121.3 °C and 230.6 °C) emerged due to the formation of the complex which differed from the peak of matrine and phospholipids. It was evident that the original peaks of matrine and phospholipids disappeared from the thermogram of complex and the phase transition temperature was lower than that of phospholipids. Galasso et al. (2006) suggested that both carbonyl carbon and the amide nitrogen were perfectly planar ($\Sigma\alpha \sim 359°$); the lone pair orbital (LPO) of nitrogen lies in the nodal plane of the π system and, therefore, exhibited nearly pure *p*-characteristic. In our cases, after combination of matrine and polarity parts of phospholipids molecule, the carbon–hydrogen chain in phospholipids could turn freely and enwrap the polarity parts of phospholipids molecule, which made the sequential energy decrease between phospholipids aliphatic hydrocarbon chains and resulted in the disappearance of the endothermal peak of phospholipids and decline of the phase transition temperature.

3.4. Development of SNEDDSs formulations

The experimental design involved assigning low, middle and high values for the oil, SA and CoSA. Then based on the coded (1, 2, 3) design shown in Table 2, nine different runs were obtained, which indicated the ratios at which the oil, SA and CoSA should be mixed with each other to evaluate self-nanoemulsion formation.

Table 2
Factors and levels of L_9 (3^3) orthogonal test.

Run #	Oils	Surfactants	Cosurfactants
1	1(4)	1(3)	1(1)
2	1(4)	2(4)	2(2)
3	1(4)	3(5)	3(3)
4	2(5)	1(3)	2(2)
5	2(5)	2(4)	3(3)
6	2(5)	3(5)	1(1)
7	3(6)	1(3)	3(3)
8	3(6)	2(4)	1(1)
9	3(6)	3(5)	2(2)

Factors and levels of L_9 (3^3) orthogonal experimental design was adopted to determine the ratios of oil, SA and CoSA to be evaluated for SNEDDSs formation.

Since there were three kinds of oils, five types of surfactants and two types of cosurfactants, 270 (30 combinations × 9 ratios) potential SNEDDSs of different compositions were obtained. A total of eight SNEDDSs prototypes out of the 270 potential SNEDDSs were able to form nanoemulsions spontaneously upon the addition of aqueous phase. The leakage of matrine from the oil phase into the aqueous phase in this eight nanoemulsion formulations was studied by a gel filtration method. The results (Table 3) showed the leakage rate was lower when Lauroglycol FCC was used as oil than that using Labrafac lipophile WL1349. Meanwhile, better nanoemulsion can be formed when Cremophor EL was employed as surfactants. SNEDDSs 2 and 4 nanoemulsions had the lowest leakage rate of matrine among all the formulations, because high content of oil in the formulation would be beneficial to SNEDDSs. Therefore, SNEDDSs 2 was further characterized.

The rate of emulsification is an important index for the assessment of the efficiency of emulsification. That means the SNEDDSs should disperse completely and quickly when subjected to aqueous dilution under mild agitation. By the comparison of ease of emulsification, it had been showed there was not obvious difference between one time and six times of flask inversion. It was worthy to note that the dispersion exhibited instantaneous emulsion formation with only one flask inversion. The study indicated that this formulation (Lauroglycol FCC:Cremophor ELP:Transcutol HP = 6:4:1) had very good ability to emulsify.

3.5. Leakage of matrine from the oil phase

Gel filtration analysis was used to determine the leakage rate of matrine from the oil phase into the aqueous phase. The method was based on the elution time of matrine in different samples. The results were presented in Fig. 4. The nanoemulsion prepared by blank SNEDDSs (sample 1) was eluted out in fractions 11–14 when free solution of matrine (sample 2) was eluted out in fractions 21–24. In both cases of sample 1 and 2, only one peak was observed in the chromatograms. However, when the MPC solution (sample 3) was analyzed, there were two peaks in the chromatogram: the first peak consisted of fractions 12–14 and the second peak consisted of fractions 21–23. This first peak was very similar as the peak

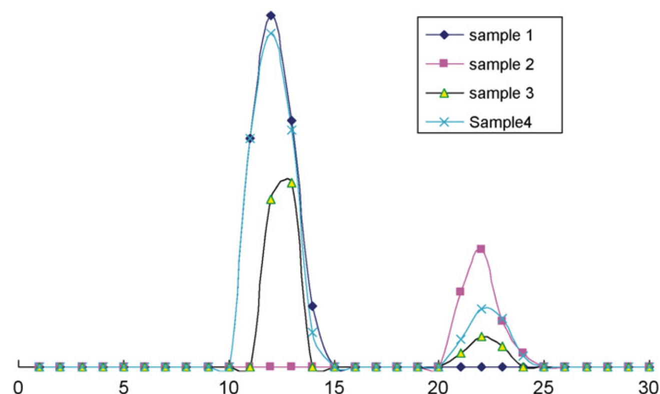

Fig. 4. Gel filtration chromatograms from different samples: sample 1: blank SNEDDS; sample 2: matrine; sample 3: MPC; sample 4: MPC-SNEDDS.

Table 3
Leakage rates of eight SNEDDSs capable of forming nanoemulsion spontaneously.

Run #	Composition O/SA/CoSA	Ratio	The leakage rate of matrine from the oil phase into the aqueous phase (%)
1	Lauroglycol FCC/Cremophor EL/Transcutol HP	5:3:2	27.78 ± 3.8
2	Lauroglycol FCC/Cremophor EL/Transcutol HP	6:4:1	8.34 ± 4.2
3	Lauroglycol FCC/Cremophor EL/Transcutol HP	4:5:3	24.57 ± 5.1
4	Lauroglycol FCC/Cremophor EL/propylene glycol	5:4:3	10.09 ± 4.3
5	Labrafac lipophile WL1349/Cremophor EL/Transcutol HP	5:5:1	23.60 ± 3.4
6	Labrafac lipophile WL1349/Cremophor EL/Transcutol HP	4:4:2	38.13 ± 4.0
7	Lauroglycol FCC/Poloxamer 188/Transcutol HP	6:4:1	47.95 ± 4.8
8	Lauroglycol FCC/Cremophor RH40/Transcutol HP	5:4:3	56.47 ± 3.9

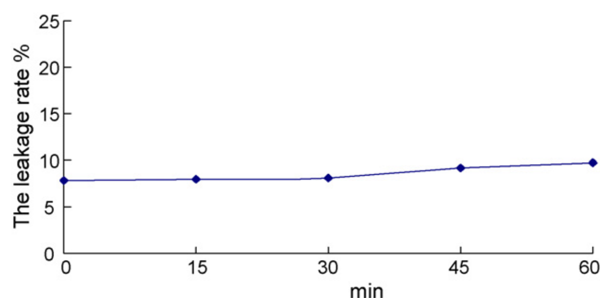

Fig. 5. Stability of MPC-SNEDDSs dilution with water.

with sample 1 because microemulsion was formed due to phospholipids. The second peak represented the matrine duo to solubility of the complex. Obviously, the MPC-SNEDDSs showed two peaks that the first peak represented nanoemulsion and the second peak represented the matrine leaked out of the oil phase.

In order to study the stability of MPC-SNEDDSs dilution with water, the leakage rate was determined on the different time intervals at 0, 15, 30, 45 and 60 min after dilution with water. Fig. 5 showed that the dilution was stable for up to 60 min.

3.6. Characterization of MPC-loaded SNEDDS

3.6.1. Phase diagram and morphological characterization

Fig. 6 shows the pseudo ternary phase diagram for SNEDDS, shadow region represents the nanoemulsion region. The MPC-SNEDDSs could convert to nanoemulsion by dilution with distilled water. TEM picture of MPC-loaded SNEDDSs and MPC were shown in Figs. 7 and 8, respectively. Obviously, SNEDDSs showed spherical morphology with a narrow size distribution in nanometer range.

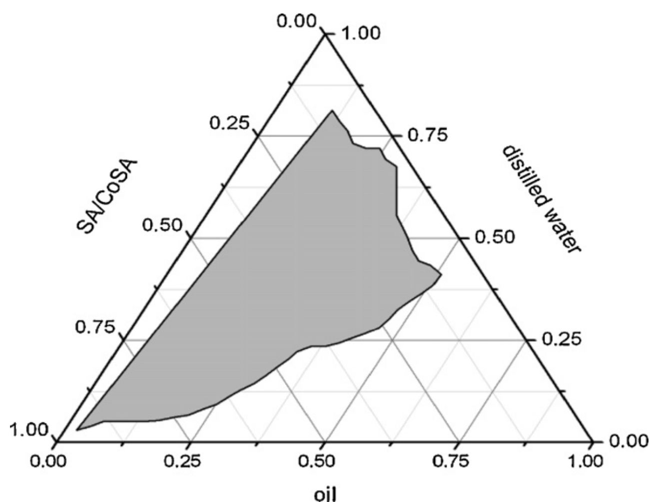

Fig. 6. Pseudo-ternary phase diagram showing nanoemulsion region (oil:lauroglycol FCC; SA:Cremophor EL; CoSA:Transcutol HP and distilled water) at room temperature. The ratio of SA/CoSA was 6:4:1.

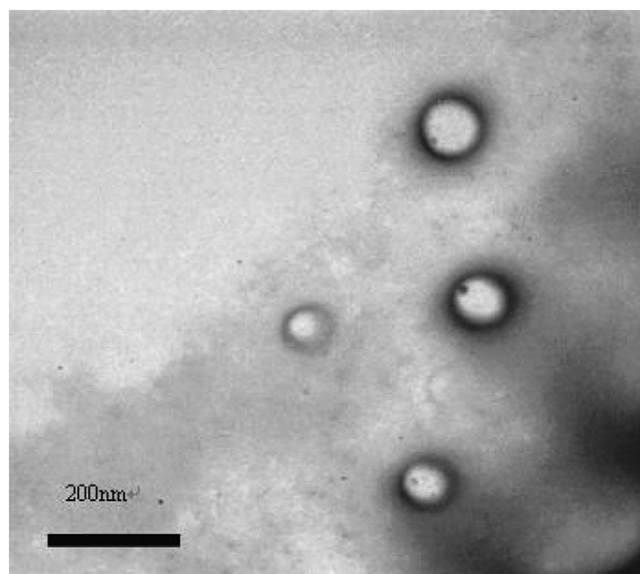

Fig. 7. TEM photo of MPC-loaded SNEDDSs (×42,000).

3.6.2. Droplet size

The droplet size measurement results were shown in Fig. 9. They indicated that the droplet size did not change upon increas-

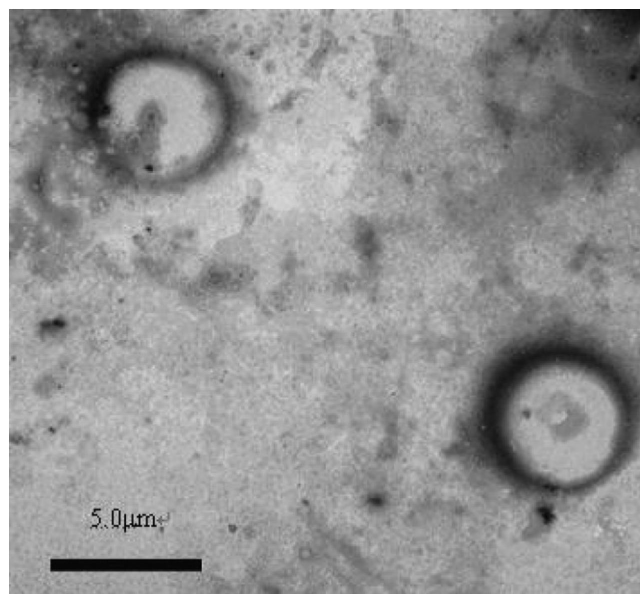

Fig. 8. TEM photo of MPC (×1000).

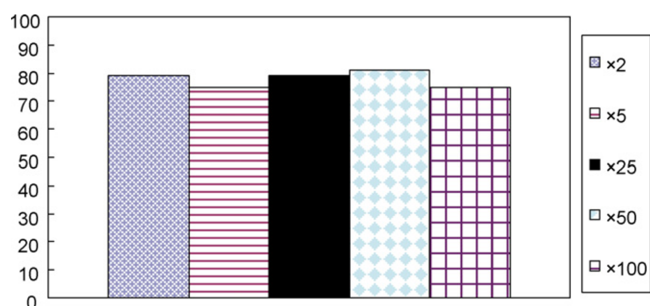

Fig. 9. Effect of the dilution factor on the droplet size of MPC-SNEDDSs (nm, $n = 3$).

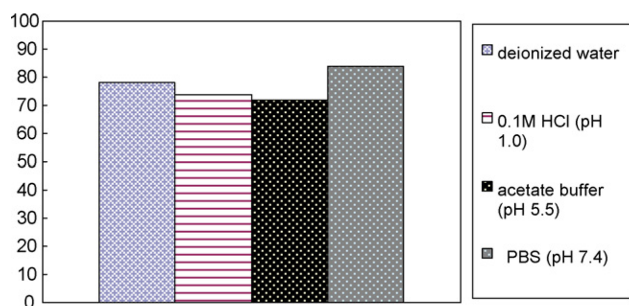

Fig. 10. Effect of pH on the droplet size of MPC-SNEDDS (nm, $n = 3$).

ing dilution factor from 2- to 100-fold. Hence, the dilution factor had no effect on the droplet size and the self-nanoemulsifying behavior, indicating that nanoemulsion was very robust to aqueous volume of the environment. Since the present SNEDDSs preparation was designed mainly for oral delivery, the potential effect of GI tract conditions on the nanoemulsion was evaluated *in vitro*. Fig. 10 showed the measured results of droplet size measurement in various aqueous media. It was clearly that the droplet size of the resultant nanoemulsion formed did not change significantly with pH values at the ratio of 1:100 to form nanoemulsion, suggesting good tolerance of SNEDDSs to the change of environment.

3.7. Pharmacokinetic studies

The plasma concentration of matrine after *i.v.* administration was shown in Fig. 11. The *i.v.* data was used to calculate the absolute bioavailability after oral administration of SNEDDS. The plasma concentration of matrine and the pharmacokinetic profile and parameters after oral administration were shown in Fig. 12 and Table 4, respectively.

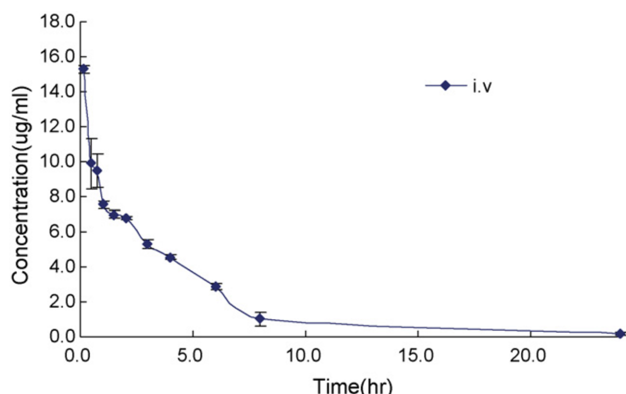

Fig. 11. Plasma concentration–time curve after *i.v.* administration of matrine (20 mg/kg).

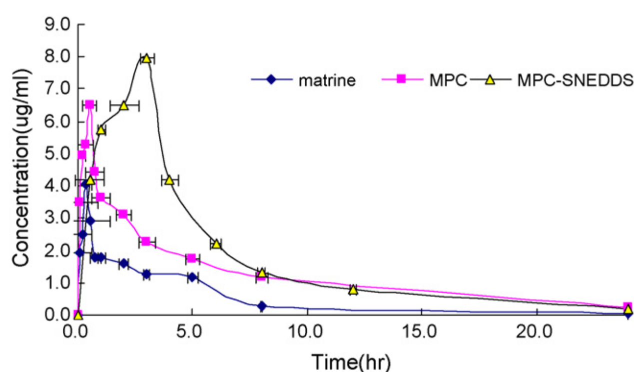

Fig. 12. Plasma concentration–time curve in rats after oral administration of matrine, MPC, MPC-SNEDDSs formulation at a dose of 20 mg/kg.

Table 4 shows the main pharmacokinetic parameters of matrine, MPC and MPC-SNEDDSs in rats. C_{max} increased dramatically from 4.12 to 6.52, and 7.95 μg/mL in case of the MPC-SNEDDSs. In parallel to C_{max}, T_{max} increased from 0.39 h to 0.50 h, and 3.00 h in case of the MPC-SNEDDSs. AUC_{0-t} of MPC-SNEDDSs was significantly higher than the other two. It was worthy to note that MPC also provided good improvement in bioavailability without significant delay in T_{max}. In conclusion, after the formation of MPC-SNEDDS, Fa of matrine could be drastically increased from 25% to 84.6%, with an outstanding Fr of 338%, demonstrating great potential for clinical application.

4. Discussion

Self-emulsifying drug delivery systems (SEDDS) are isotropic mixtures of oil, surfactants, co-surfactants and drugs that form fine oil-in-water emulsion when introduced into aqueous phases under gentle agitation. This is possibly as surfactants strongly localized to the surface of the emulsion droplet, interfacial free energy was reduced and a mechanical barrier to coalescence formed resulting in a thermodynamically spontaneous dispersion. Furthermore, co-surfactants increase interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules (Nazzal et al., 2002). The utility of those formulations comprising drug solutions in a single surfactant are typically limited by slow dispersion of the formulation. However, this may be overcome by the inclusion of a secondary co-surfactant. There are two problems with using pure surfactants. The first is that it often takes a considerable time for surfactants to dissolve, due to the formation of viscous liquid crystalline (or gel crystalline) phases at the surfactants-water interface; the second is the concern that pure surfactants can be irritant and poorly tolerated in the gastrointestinal tract. Therefore, when self- emulsifying formulations can be generated using relatively high proportions of long chain lipids (∼60%, w/w) and lower quantities of surfactant (∼30%, w/w) and co-surfactants (∼10%, w/w), more robust drug solubilization properties are typically apparent on both dispersion and digestion (although drug solubility in the formulation may be relatively modest) (Porter et al., 2008).

To getting an efficient self-nanoemusification, we must select the appropriate composition for SNEDDSs. Three kinds of oils, four types of non-ionic surfactants and two types of cosurfactants were used in this study. We used an experimental design, L_9 (3^3) orthogonal test to minimize the constructed number of phase diagram. Eight SNEDDSs prototypes were obtained to form nanoemulsion spontaneously.

Matrine, as a hydrophilic molecule, cannot be dissolved into the oil phase of the SNEDDSs. In this study, matrine–phospholipid complexes were successfully prepared by a simple, novel reflux

Table 4
Pharmacokinetic parameters of matrine, MPC and MPC-SNEDDSs (20 mg/kg, p.o.) in rats ($n = 5$).

Pharmacokinetic parameters	*M* (i.v.)	*M* (p.o.)	MPC (p.o.)	MPC-SNEDDS (p.o.)
AUC_{0-t} (mg/L h)	50.8 ± 4.25	12.7 ± 0.82	30.5 ± 4.48	43.0 ± 3.50
$AUC_{0-\infty}$ (mg/L h)	52.3 ± 4.67	12.9 ± 0.78	31.8 ± 5.21	44.1 ± 3.76
MRT_{0-t} (h)	4.51 ± 0.49	4.22 ± 0.16	5.81 ± 0.44	5.36 ± 0.10
$MRT_{0-\infty}$ (h)	5.28 ± 0.76	4.65 ± 0.20	6.82 ± 1.16	6.05 ± 0.60
$t_{1/2zh}$ (h)	4.79 ± 0.62	4.08 ± 0.24	5.19 ± 1.18	4.96 ± 1.30
T_{max} (h)	0.17 ± 0.00	0.39 ± 0.10	0.50 ± 0.00	3.00 ± 0.00
CL_z (L/h/kg)	0.77 ± 0.07	3.11 ± 0.19	1.28 ± 0.23	0.91 ± 0.08
V_z (L/kg)	5.30 ± 0.71	18.35 ± 1.97	9.46 ± 1.74	6.49 ± 1.63
C_{max} (mg/L)	15.05 ± 0.23	4.12 ± 0.18	6.52 ± 0.33	7.95 ± 0.35

Data represent the mean ± SD. $^*p < 0.05$, statistical significance: $^*p < 0.05$, vs. 20 mg/kg matrine.

method. Physicochemical investigations showed that matrine formed complexes with phospholipids. Solubility studies indicated the liposolubility of matrine was significantly enhanced by the formation of phospholipids complexes, compared with physical mixture of matrine and phospholipids. MPC prepared was able to dissolve in the eight SNEDDSs prototypes. By investigating the leakage rate, SNEDDSs 2 (composition: Lauroglycol FCC, Cremophor EL and Transcutol ratio: 6:4:1) was selected with mean droplet size in the range of 65–80 nm, exhibiting instantaneous emulsion formation with only one flask inversion.

The results of the leakage rate indicated that approximately 8.2% of matrine loaded into SNEDDSs leaked out from the oil phase into the aqueous phase. Our hypothesis was that only when the drug loaded inside the oil droplets, the absorption could be significantly increased and kept in releasing slowly. Therefore, this kind of leakage of matrine from the oil phase into aqueous phase would decrease its absorption. After formulation of MPC-SNEDDSs, the pharmacokinetic profiles of matrine after oral administration (C_{max} and AUC_{0-t}) indicated that the oral absorption of matrine in rats was significantly increased ($p < 0.05$) by MPC-SNEDDSs nanoemulsion compared with matrine and MPC aqueous solution.

Basically, the poor permeability of matrine across the intestinal epithelial cells is the predominant cause for the low oral bioavailability of matrine, which resulted in little absorption in the GI tract. The bioavailability of matrine was also enhanced by MPC according to the pharmacokinetic results. It was assumed that the biocompatibility and membrane permeability of matrine molecule were essentially improved after formation of MPC ($\log P_{MPC} = 2.86$). Sequentially, the MPC uptake by the intestinal epithelial cells was facilitated. Compared with free matrine, the absorption route of MPC was not changed, but the extent of absorption was increased. Therefore, the T_{max} of MPC was approximately the same with that of matrine. In addition to the absorption route mentioned above, however, MPC-SNEDDSs might undergo the intestinal lymphatic transport (probably via M cells) which would delay the entry of drug into systemic circulation. Meanwhile, it took time to release drug entrapped in MPC-SNEDDSs which might be intact in the initial stage in systemic circulation. Hence, the T_{max} and bioavailability of MPC-SNEDDSs were significantly delayed and enhanced respectively, compared with that of matrine or MPC.

Phospholipids play a major role in drug delivery technology, and the exact absorption mechanism of matrine–phospholipids through small intestine will be studied in our future work.

5. Conclusion

Our present work might provide some new insights and paradigms for enhancing oral bioavailability of water soluble drugs. The present investigation illustrated the potential use of phospholipids complexes for the preparation of SNEDDSs to enhance oral bioavailability of water soluble drugs. Prepared phospholipids complexes have improved the liposolubility of water soluble drugs, which will be much easier to be loaded into SNEDDSs and the leakage of the drug can be decreased in SNEDDS.

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References

Carini, R., Comoglio, A., Albano, E., Poli, G., 1992. Lipid peroxidation and irreversible damage in the rat hepatocyte model: protection by the silybin-phospholipid complex IdB 1016. Biochem. Pharmacol. 43, 2111–2115.
Chui, C.H., Lau, F.Y., Tang, J.C., Kan, K.L., Cheng, G.Y., Wong, R.S., et al., 2005. Activities of fresh juice of Scutellaria barbata and warmed water extract of Radix Sophorae Tonkinensis on anti-proliferation and apoptosis of human cancer cell lines. Int. J. Mol. Med. 16, 337–341.
Cilek, A., Celebi, N., Tirnaksiz, F., Tay, A., 2005. A lecithin-based microemulsion of rh-insulin with aprotinin for oral administration: investigation of hypoglycemic effects in non-diabetic and STZ-induced diabetic rats. Int. J. Pharm. 298, 176–185.
Comoglio, A., Tomasi, A., Malandrino, S., Poli, G., Albani, E., 1995. Scavenging effect of silipide, a newsilybin-phospholipid complex, on ethanol-derived free radicals. Biochem. Pharmacol. 50, 1313–1316.
Constantinides, P.P., 1995. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharm. Res. 12, 1561–1572.
Cui, F.D., Shi, K., Zhang, L.Q., Tao, A.J., Kawashima, Y., 2006. Biodegradable nanoparticles loaded with insulin-phospholipid complex for oral delivery: Preparation, *in vitro* characterization and *in vivo* evaluation. J. Controlled Release 114, 242–250.
Galasso, V., Asaro, F., Berti, F., Pergolese, B., Kovač, B., Pichierri, F., 2006. On the molecular and electronic structure of matrine-type alkaloids. Chem Phys. 330, 457–468.
Ho, H.O., Hsiao, C.C., Sheu, M.T., 1996. Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs. J. Pharm. Sci. 85, 138–143.
Hu, M.J., Zeng, H., Wu, Y.L., Zhang, Y.P., Zhang, S., Qiao, M.M., et al., 2005. Synergistic effects of matrine and 5-fluorouracil on tumor growth of the implanted gastric cancer in nude mice. Chin. J. Dig. Dis. 6, 68–71.
Lai, J.P., He, X.W., Jiang, Y., Chen, F., 2003. Preparative separation and determination of matrine from the Chinese medicinal plant *Sophora flavescens* Ait. by molecularly imprinted solid-phase extraction. Anal. Bioanal. Chem. 375, 264–269.
Liu, J., Shen, H.M., Ong, C.N., 2000. Salvia miltiorrhiza inhibits cell growth and induces apoptosis in human hepatoma HepG(2) cells. Cancer Lett. 153, 85–93.
Maiti, K., Mukherjee, K., Gantait, A., Saha, B.P., Mukherjee, P.K., 2007. Curcumin-phospholipid complex: preparation, therapeutic evaluation and pharmacokinetic study in rats. Int. J. Pharm. 330, 155–163.
Nazzal, S., Smalyukh, I.I., Lavrentovich, O.D., Khan, M.A., 2002. Preparation and in vitro characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiquinone: mechanism and progress of emulsion formation. Int. J. Pharm. 235, 247–265.
Porter, C.J.H., Pouton, Colin, W., Cuine, Jean, F., Charman William, N., 2008. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv. Drug Deliv. Rev. 60, 73–691.
Sarciaux, J.M., Acar, L., Sado, P.A., 1995. Using microemulsion formulations for oral drug delivery of therapeutic peptides. Int. J. Pharm. 120, 127–136.
Shah, R.B., Zidan, A.S., Funck, T., Tawakkul, M.A., Nguyenpho, A., Khan, M.A., 2007. Quality by design: characterization of self-nano-emulsified drug delivery systems (SNEDDs) using ultrasonic resonator technology. Int. J. Pharm. 341, 189–194.
Swenson, E.C., Curatolo, W.J., 1992. Intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. Adv. Drug Deliv. Rev. 8, 39–92.
Venkata Ramana Rao, S., Shao, J., 2008. Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs. Int. J. Pharm. 362, 2–9.

Wang, S.J., Wang, G.J., Li, X.T., Sun, J.G., Ma, R.L., Sheng, L.S., 2005. Simultaneous determination of oxymatrine and its active metabolite matrine in dog plasma by liquid chromatography-mass spectrometry and its application to pharmacokinetic studies. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 817, 319–325.

Wu, X.N., Yamashit, F., Hashid, M., Chen, X.G., Hu, Z.D., 2003. Determination of matrine in rat plasma by high-performance liquid chromatography and its application to pharmacokinetic studies. Talanta 59, 965–971.

Wu, X.L., Hang, T.J., Shen, J.P., Zhang, Y.D., 2006. Determination and pharmacokinetic study of oxymatrine and its metabolite matrine in human plasma by liquid chromatography tandem mass spectrometry. J. Pharm. Biomed. Anal. 41, 918–924.

Xue, R., Shang, B.W., Di, T.Y., 2006. HPLC analysis of determination of matrine by different column. Ningxia Med. J. 28, 518–519.

Zhang, M.J., Huang, J., 2004. Recent research progress of antitumor mechanism of Matrine. China J. Chin. Mater. Med. 29, 115–118.

Zhang, L.P., Jiang, J.K., Tam, J.W., Zhang, Y., Liu, X.S., Xu, X.R., et al., 2001. Effects of matrine on proliferation and differentiation in K2562 cells. Leuk. Res. 25, 793–800.

Zhang, L., Liu, W.T., Zhang, R.W., Wang, Z.W., Shen, Z.D., Chen, X.H., et al., 2008. Pharmacokinetic study of matrine, oxymatrine and oxysophocarpine in rat plasma after oral administration of *Sophora flavescens* Ait. extract by liquid chromatography tandem mass spectrometry. J. Pharm. Biomed. Anal. 47, 892–898.

Zhu, J.P., Xin, S.M., Ma, Z.Q., 1998. The bioavailability study of matrine by intragastric administration. Chin. Tradi. Pat. Med. 28, 2–3.