AAPS PharmSciTech, Vol. 11, No. 2, June 2010 (© 2010)
DOI: 10.1208/s12249-010-9432-x

Research Article

Development of Silymarin Self-Microemulsifying Drug Delivery System with Enhanced Oral Bioavailability

Xinru Li,[1] Quan Yuan,[2] Yanqing Huang,[1] Yanxia Zhou,[1] and Yan Liu[1,3]

Received 26 January 2010; accepted 5 April 2010; published online 20 April 2010

Abstract. The objective of this work was to develop a self-microemulsifying drug delivery system (SMEDDS) for improving oral absorption of poorly water-soluble drug, silymarin. The pseudo-ternary phase diagrams were constructed using ethyl linoleate, Cremophor EL, ethyl alcohol, and normal saline to identify the efficient self-microemulsification region. The particle size and its distribution of the resultant microemulsions were determined using dynamic light scattering. The optimal formulation with the best self-microemulsifying and solubilization ability consisted of 10% (*w*/*w*) of ethyl linoleate, 30% of Cremophor EL, and 60% of ethyl alcohol. The release of silymarin from SMEDDS was significantly faster than that from the commercial silymarin preparation hard capsule (Legalon®). The bioavailability results indicated that the oral absorption of silymarin SMEDDS was enhanced about 2.2-fold compared with the hard capsule in fasted dogs. It could be concluded that SMEDDS would be a promising drug delivery system for poorly water-soluble drugs by the oral route.

KEY WORDS: bioavailability; microemulsion; self-microemulsifying drug delivery system; silymarin.

INTRODUCTION

Silymarin, isolated from chrysanthemum plant milk thistle, was used for the treatment of chronic inflammatory liver disorders such as cirrhosis, hepatitis, and fatty infiltration due to alcohol and toxic chemicals in clinical studies. However, the effectiveness of silymarin was discounted by its poor water solubility and low bioavailability after oral administration. Orally administered silymarin is absorbed rapidly with a t_{max} of about 2–4 h and a $t_{1/2}$ of 6 h (1). Totally, only 20–50% of oral silymarin is absorbed from the gastrointestinal tract after oral administration. In order to improve the dissolution and bioavailability of silymarin, several approaches have been investigated, such as forming silybin–phosphatidylcholine complex (2,3), incorporating into solid dispersion (4), encapsulating in liposomes (5), and solubilizing in self-microemulsifying drug delivery system (SMEDDS) (6,7). One of the silymarin preparations, bearing a brand name Legalon®, showed two- to threefold increase in bioavailability compared with other preparations (8,9). Among them, SMEDDS has recently attracted much attention in pharmaceutical research areas (10–18), benefiting from the success of cyclosporine A formulations—Sandimmune and Neoral®. In addition, high thermodynamic and kinetic stability, low viscosity, and optical transparency make them very attractive in pharmaceutical applications.

SMEDDS is a mixture of drug (usually poorly water-soluble one), oil, emulsifier, and coemulsifier, which form fine oil-in-water (o/w) microemulsion with a particle size of less than 100 nm when exposed to aqueous media under conditions of gentle agitation or digestive motility that would be encountered in the gastrointestinal tract. Oral absorption of several poorly water-soluble drugs has been enhanced by SMEDDS (11,14–16,19). These were attributed to the fact that the specific components of SMEDDS promote the intestinal lymphatic transport of drugs. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-gp and/or CYP450 to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid (20–23).

In the case of silymarin, Woo J.S. *et al.* (6) reported silymarin SMEDDS prepared using glycerol monooleate as oil phase, a mixture of polysorbate 20 and polyoxyethylene-50-hydrogenated castor oil as emulsifier and transcutol as coemulsifier, while the concentrate was not diluted infinitely with excess water, indicating that this SMEDDS was not ideal for an oral delivery system. Wu W. *et al.* (7) reported silymarin SMEDDS consisting of Tween 80, ethyl alcohol, and ethyl linoleate; this design was proven to have small particle size, higher drug loading, and higher oral relative bioavailability. However, in pharmacokinetics study, silymarin solution in PEG 400 and silymarin suspension were used as the reference preparation rather than commercial formulation, and besides, dose (300 mg/kg, expressed as silybin equivalents) was considerably high, and the rabbit used was not the appropriate animal model. These indicated the incredible conclusion about enhancing oral bioavailability

[1] Department of Pharmaceutics, School of Pharmaceutical Sciences, Peking University, Beijing, China.
[2] Beijing Novartis Pharma Ltd, Beijing, China.
[3] To whom correspondence should be addressed. (e-mail: yanliu@bjmu.edu.cn)

for this silymarin SMEDDS. Therefore, there has been an urgent quest to develop an ideal SMEDDS that can solubilize silymarin efficiently and dilute infinitely with excess water.

The objective of this research was to formulate the oral SMEDDS for silymarin. Optimal SMEDDS formulations composed of silymarin/ethyl linoleate/Cremophor EL/ethyl alcohol were selected regarding the self-microemulsifying ability, solubilization ability, and reduced use of surfactant. The physicochemical characteristics of each microemulsion system were investigated for the optimization of SMEDDS. Then, the *in vitro* release characteristics of silymarin from SMEDDS were evaluated compared with commercial silymarin preparation Legalon®. The bioavailability of silymarin SMEDDS was also compared with Legalon® in dogs.

MATERIALS AND METHODS

Materials

Silymarin was obtained from Panjin Pharmaceuticals (Liaoning, China). Silybin standard was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) with a purity of 99.8%. Cremophor EL was purchased from BASF (Germany). Ethyl linoleate was supplied by Aoqi Biopharmaceuticals (Wuxi, China). Deionized water was prepared by a Milli-Q purification system (Millipore, USA). All the reagents were of the highest purity available and used as supplied.

Pseudo-ternary Phase Diagram Study

In our previous work, the solubility of silymarin was studied in a variety of oils, surfactants, and short-chain alcohols, and the best solubilization and self-microemulsifying effect and dispersability were found for ethyl linoleate/Cremophor EL/ethyl alcohol combinations. The pseudo-ternary phase diagrams of oil, emulsifier/coemulsifier, and saline were developed using saline titration method. Briefly, emulsifier was mixed with coemulsifier in fixed weight ratios (K_m=1:1, 2:1, 3:1, and 4:1). For each phase diagrams at a specific ratio of emulsifier/coemulsifier, aliquots of each emulsifier–coemulsifier mixture (S_m) were then mixed with oil. Then, each mixture was titrated with saline under magnetic stirring or vortexing. The equilibrated samples were assessed visually. The phase state was classified into three, that is, clear one-phase with low viscosity, clear one-phase with high viscosity, and multiple phases. The one-phase with low viscosity was separated further into water-in-oil (w/o) or o/w microemulsion phase by simply considering the composition, that is, whether it was oil-rich or water-rich. The clear one-phase with high viscosity was separated further into gel or liquid crystalline by using polarized light microscope (59X, Shanghai, China), and multiple phases were considered as crude emulsion. The physical states were represented on a pseudo-ternary phase diagram with one axis representing water, one representing oil, and the third representing the S_m. The influence of weight ratio of emulsifier to coemulsifier on the area of o/w microemulsion region was investigated on the pseudo-ternary phase diagram.

After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios. In order to form the microemulsion, a series of SMEDDS were prepared as follows.

Preparation of SMEDDS

A series of SMEDDS were prepared simply through mixing the components in each of four formulations (Table I) with varying ratio of oil, emulsifier, and coemulsifier. Briefly, silymarin was dissolved by ethyl alcohol in glass vials. Then, ethyl linoleate and Cremophor EL were accurately weighed into glass vials. The components were mixed by gentle stirring and vortex mixing until a homogeneous mixture formed. The mixture was sealed in a glass vial and stored at room temperature until used.

Determination of Physicochemical Parameters

Solubility

The solubility of silymarin in SMEDDS was determined. An excess amount of silymarin was introduced into 3 mL of each of the selected vehicle, and the mixtures were shaken with shaker at 25°C for 48 h. After reaching equilibrium, samples in triplicate were centrifuged at 500 g for 5 min using a high-speed centrifuging machine (TGL–16G, Shanghai, China) to remove the excess insoluble silymarin. Then, aliquots of supernatant were taken, and the concentration of silymarin was quantified by Shimadzu high-performance liquid chromatography (HPLC) system (SPD–10A, Japan) after dilution with ethyl alcohol.

Ultraviolet (UV) Spectra

Appropriate amount of silymarin, silymarin+ethyl linoleate, silymarin+Cremophor EL, and silymarin SMEDDS were dissolved in alcohol, respectively. The ultraviolet (UV) absorption spectra from 200 to 400 nm of samples were recorded using a UV–VIS spectrophotometer.

Dispersability of Silymarin SMEDDS

A volume of 0.1 mL oil–S_m mixture, prepared as described above, was placed at the bottom of a UV cell, and 1.6 mL of 0.1 M HCl solution (pH1.2, assuming the pH of gastric fluid) was gently added to the mixture as the water phase. The oil–S_m mixture was gradually dispersed into the aqueous phase, and the microemulsion formed spontaneously. The dispersability was observed by monitoring the

Table I. Composition of the Self-Microemulsifying Drug Delivery System Formulations

Drug/excipient	FI	FII	FIII	FIV
Cremophor EL	4.5	6.0	6.75	7.2
Ethyl alcohol	4.5	3.0	2.25	1.8
Ethyl linoleate	1.0	1.0	1.0	1.0
Silymarin solubility (mg mL^{-1})	130.8±2.2	90.8±4.1	80.8±3.3	80.8±3.8
Mean particle size (nm)	22.9±1.8	30.8±1.1	21.7±2.6	21.0±2.4

absorbance change at 450 nm at 37°C by UV spectrophotometer (6010, Shanghai, China).

The Morphology and Particle Size Distribution of Silymarin Microemulsion

A certain volume of silymarin SMEDDS was diluted with 0.1 M HCl solution to a definite volume in a flask and shaken gently to mix thoroughly. The particle size of the soformed microemulsion was determined by dynamic light scattering (DLS; 370 subμm particle sizer, Santa Barbara, CA, USA). The measurement conditions were He-Ne laser; angle, 90°; temperature, 23°C; reflection index, 1.333; and wavelength, 632.8 nm.

The morphology of microemulsion was also observed by transmission electron microscopy (TEM; JEM-1230, JEOL, Japan). To improve the contrast, the samples were treated with a 1 wt.% phosphotungstic acid solution for 2 h, deposited on copper grids, and allowed to dry for 48 h before TEM examination.

In vitro Release Test

The *in vitro* release test of silymarin SMEDDS was performed using a dialysis method (19) in 900 mL of phosphate buffer solution (pH 7.0) containing 0.5% of sodium dodecyl sulfate (SDS), which was based on ChP release test method II. Silymarin SMEDDS was placed in a dialysis bag (MWCO 3500, Spectrum, USA) during the release period to compare the release profile with the commercial hard capsule (Legalon®). At definite time intervals, 3 mL of release medium was taken and immediately replaced with the same volume of fresh release medium. The content of silybin in release medium was analyzed by Shimadzu HPLC system (SPD-10A, Japan). Release percentages were calculated as the ratio of silybin released to total silybin.

Bioavailability Studies

Bioavailability of silymarin SMEDDS was compared with commercial silymarin preparation Legalon®. Male mongrel dogs weighing 10±0.2 kg were fasted overnight prior to the experiment, and water was available *ad lib*. To minimize individual variance and administration time's difference and remove a carryover effect of post drug, six dogs were allocated at random to two treatment groups and administered Legalon® hard capsule and SMEDDS (single dose, silymarin 50 mg/kg) in a crossover design. The washout period between the two treatments was 7 days. The experimental procedures were approved by the intuitional animal ethical committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Blood sample (2 mL) was collected through femoral vein into heparinized tubes before administration and after administration of two preparations at designated time intervals. Blood samples were centrifuged at 3,000 rpm for 10 min using a high-speed centrifuging machine (TGL-16, Shanghai, China), and plasma samples were taken and stored at −20°C. Frozen plasma samples were thawed just prior to extraction procedure at room temperature, thoroughly agitated. Liquid–liquid plasma extraction procedure was as follows: in a 5-mL polypropylene screw-capped conical tube, 200 μL of plasma was added followed by 20 μL of an internal standard (α-naphthol, 10 μg/mL in methanol) and 3 mL of *tert*-butyl methyl ether. After vortex mixing for 1 min, the resultant was centrifuged at 3,000 rpm for 5 min; the organic layer was transferred to another tube and evaporated under a light stream of nitrogen at 50°C. The residue was dissolved by 40 μL of methanol, and 20 μL was injected into an HPLC system (Shimadzu LC-10AT, Kyoto, Japan) equipped with an ultraviolet detector (SPD-10A, Japan) and a reversed phase C18 column (Diamonsil, 5 μm, 4.6×250 mm, Dikma, China) guarded with a refillable precolumn (C18, 2.0×20 mm, Alltech, USA). A mobile phase of methanol/distilled water/glacial acetic acid in a volume ratio of 56/44/0.5 was pumped at a flow rate of 1.0 mL/min. The effluent was monitored at 288 nm.

Quantification was based on area ratio ($A_{\text{silybin}}/A_{\alpha\text{-naphthol}}$), and the area of silybin isomers was calculated as a whole. Straight regression line with correlation coefficient above 0.999 and over the concentration range of 0.011–2.2 μg/mL was obtained. Acceptable results with respect to precision and accuracy were obtained for all analytes. The limit of detection was 22 ng/mL. After storage for 1 month at −20°C, and freeze-thawing for three times, silybin was stable in plasma.

The maximal plasma concentration of drug ($C_{\max}$) and the time to reach maximum plasma concentration ($T_{\max}$) were directly obtained from plasma data. The area under the drug concentration–time curve from zero to 12 h ($AUC_{0-12\ h}$) was calculated according to the linear trapezoidal rule. Other parameters were calculated by WinNonLin (Pharsight, Mountain View, CA, USA).

Data Analysis

All data were shown as mean±SD. The difference between groups was analyzed using the Student *t* test. Difference with $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Pseudo-ternary Phase Diagram Studies

SMEDDS forms fine o/w microemulsions with only gentle agitation, upon its introduction into aqueous media. Since the Gibbs energy required to form microemulsion is very low, the formation is thermodynamically spontaneous. Surfactants (emulsifiers) form a layer around the emulsion droplets and reduce the interfacial energy as well as provide a mechanical barrier to coalescence. For selecting a suitable self-emulsifying vehicle, it is important to assess (a) the drug solubility in various components, (b) the area of self-emulsifying region in the phase diagram, and (c) droplet size distribution following self-emulsification (14).

Pseudo-ternary phase diagrams were constructed to identify the self-microemulsifying regions and to optimize emulsifier to coemulsifier ratio (K_m) and the concentration of oil. The studied systems were composed of ethyl linoleate, Cremophor EL, ethyl alcohol, and saline. The pseudo-ternary phase diagrams with various weight ratios of Cremophor EL to ethyl alcohol were described in Fig. 1. The self-microemulsifying formulations were selected observing regions of infinite dilution. No distinct conversion from w/o to o/w microemulsions was observed. Self-microemulsifying formu-

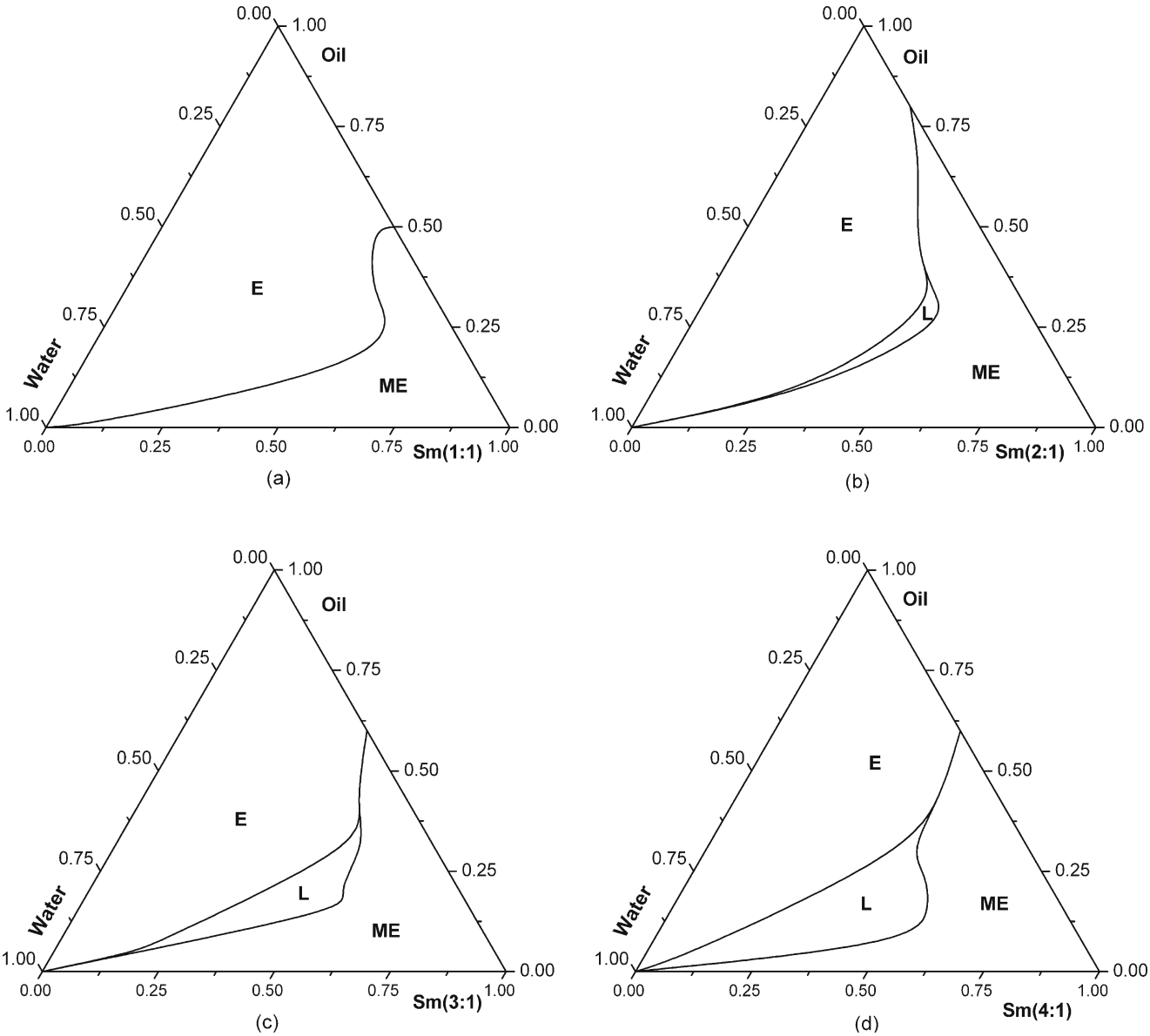

Fig. 1. Pseudo-ternary phase diagrams indicating the efficient self-microemulsification region (K_m=1:1 **a**, 2:1 **b**, 3:1 **c**, and 4:1 **d**). *E* crude emulsion, *L* liquid crystal, *ME* microemulsion

lations could be obtained under the condition of K_m from 1:1 to 4:1, and oil/S_m equaled to 1/9 and 2/8. It was observed that with the increasing of the concentration of the emulsifier in SMEDDS formulation, the area of the self-microemulsification region was changed slightly. Furthermore, the phase studies revealed that the addition of emulsifier/coemulsifier mixture with K_m greater than 2:1 could produce transparent and viscous liquid crystalline observed using cross polarizer, and the area of liquid crystal region increased markedly with the increasing of K_m. Therefore, the much more higher the K_m value (from FI to FIV), the much more poorer the self-microemulsifying ability of the SMEDDS formulation (Fig. 2). Moreover, the best solubilization effect of FI on silymarin was observed (Table I). As seen from Table I, the mean droplet size was smaller, which ranged from 20 to 30 nm; no remarkable difference was observed for all formulations. Based on the results of the self-microemulsifying ability, solubilization ability, and reduced use of surfactant, the optimized formulation (FI: K_m=1:1 and S_m=9:1) was therefore chosen in the subsequent studies.

Physicochemical Properties of Silymarin in Microemulsion

The solubility of silymarin in water, ethyl linoleate, alcohol, and polysorbate 20 was 0.4, 2.1, 225.2, and 131.3 mg/mL (6), respectively, suggesting that silymarin might be not only poorly water-soluble but also poorly oil-soluble. In addition, the solubility parameters of silybin, ethyl linoleate, Cremophor EL, and alcohol calculated according to the group contribution method (24) were 33.12, 16.38, 19.62, and 25.93 $Mpa^{1/2}$, respectively. The higher solubility parameter of silybin was also indicated that it was not lipophilic drug. This solubility property might have been the reason for the poor oral absorption for silymarin. In order to improve the oral absorption for this kind of drugs,

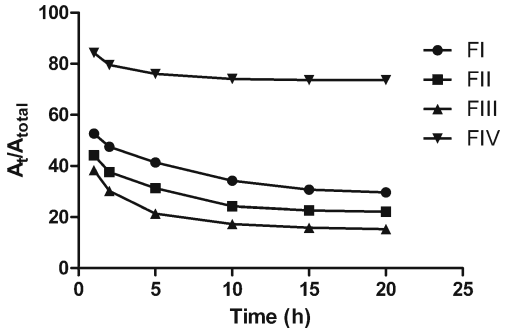

Fig. 2. Dispersion rate of formulation FI into 0.1 M HCl solution assuming the pH of gastric fluid

solubilization is a better method. In the case of our experiment, silymarin was successfully solubilized 327-fold by SMEDDS. In addition, the solubility of silymarin in FI (130.8 mg/mL) was closer to that in polysorbate 20 whose structure was similar with Cremophor EL (130 mg/mL (6)), which demonstrated that solubilization efficiency for silymarin in SMEDDS was similar to that in polysorbate 20 alone, thereby the safety was enhanced due to the fact that low content of emulsifier was used in SMEDDS.

It was reported that the mechanism about solubilization of substance in microemulsion or micelle could be investigated by X-ray, UV, or nuclear magnetic resonance (25–27). As seen from Fig. 3, the maximum wavelength of UV absorption were 287.0, 286.2, 290.0, and 290.6 nm for silymarin+Cremophor EL, silymarin+ethyl linoleate, silymarin SMEDDS, and silymarin+alcohol, respectively, which indicated that characteristic of UV for silymarin in SMEDDS was much more closer to that in alcohol. These results suggested that silymarin was primarily located in the interfacial region that was the location region for alcohol, not in inner phase of microemulsions.

Morphology and Particle Size Distribution of Silymarin Microemulsion

In order to observe the oily droplets, phosphotungstic acid was used to visualize the microstructure of microemulsion obtained after the microemulsification of FI. Figure 4 shows

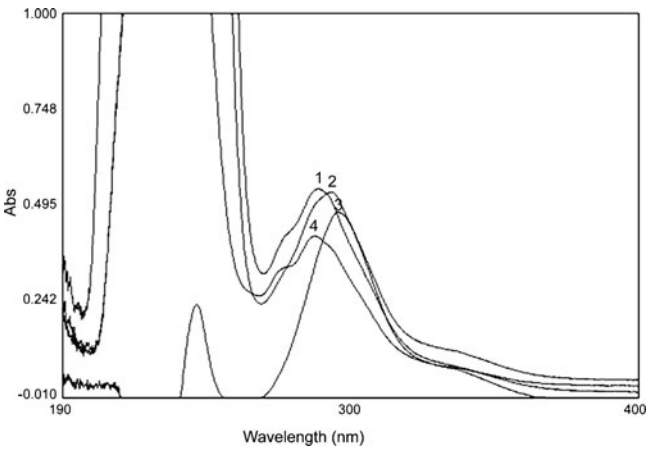

Fig. 3. Ultraviolet spectra of samples (*1* silymarin+cremophor EL, *2* silymarin self-microemulsifying drug delivery system (SMEDDS), *3* silymarin SMEDDS and silymarin+alcohol, *4* silymarin+ethyl linoleate)

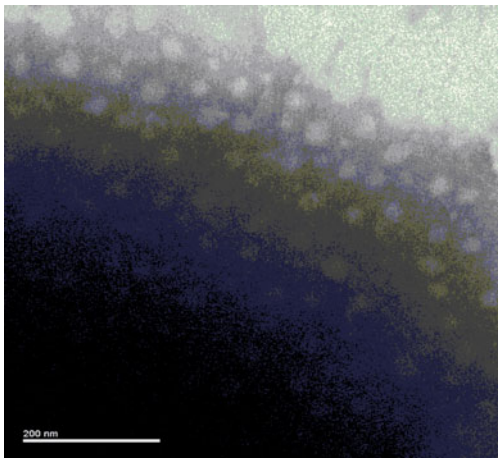

Fig. 4. The transmission electron microphotograph of silymarin microemulsion obtained after the microemulsification of FI

the TEM images of the microemulsion. The homogeneous and spherical droplets in microemulsion were observed.

Particle size of droplets after microemulsification was the most important property of SMEDDS. Mechanisms of particle size effect on drug absorption may include improved release and facilitated lymphatic transport (19,23,28,29). The average droplet size and the size distribution of blank microemulsion and silymarin-loaded microemulsion were measured at 23°C by using DLS method. SMEDDS were diluted with aqueous phase up to 500,000-fold dilution. In the case of blank microemulsion, particle size distribution was narrow, and the mean droplet diameter was 23 nm. The effect of the drug content on droplet size of microemulsions was represented in Fig. 5. It was found that the mean size increased slightly with the increase of drug content up to 40 mg/mL, then particle size increased dramatically up to 30 nm when drug content got up to 80 mg/mL.

In order to evaluate the physical stability of microemulsification, self-microemulsifying behavior was also studied after SMEDDS had been sealed in glass vial and stored at 40°C for 6 months. Particle size measurement showed that no distinguishable difference was observed after storage (data not shown).

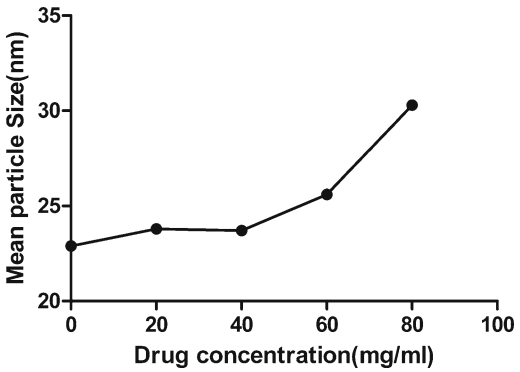

Fig. 5. The effect of the content of silymarin on the particle size of microemulsions

In vitro Release Test

Release study was performed for silymarin SMEDDS, and the release of the hard capsule contents (Legalon®) was also tested as control. The release of silymarin from these dosage forms was evaluated in phosphate buffer solution (pH 7.0) containing 0.5% of SDS. As shown in Fig. 6, 39.6% and 23.9% of silymarin were released from microemulsion and the hard capsule contents within the first 2 h, respectively, and 56.2% and 33.6% within the first 6 h, respectively. These indicated that release of silymarin was greatly enhanced by SMEDDS, suggesting that silymarin dissolved perfectly in SMEDDS form could be released faster than the hard capsule due to the small droplet size, which permits a faster release of drug into aqueous phase, and it could affect the oral bioavailability.

Bioavailability Studies

The *in vivo* study was performed to quantify silybin after oral administration of silymarin SMEDDS and hard capsule (Legalon®). The plasma concentration–time profiles of silybin in dogs following oral administration of SMEDDS and Legalon® were shown in Fig. 7. The non-compartmental pharmacokinetics parameters listed in Table II were calculated based on the observed blood data. $AUC_{0-12\ h}$ and C_{max} were 4.75±0.26 μg·h/mL and 1.85±0.09 μg/mL for SMEDDS and 2.09±0.15 μg·h/mL and 1.06±0.04 μg/mL for Legalon®, respectively, and the relative bioavailability of SMEDDS to Legalon® was 227%. There existed significant difference in C_{max} for the two formulations ($p<0.05$), while in the case of the parameter T_{max}, no significant difference was observed ($p>0.05$) for the two formulations.

In vivo study in the current research indicated that SMEDDS significantly enhanced the values of $AUC_{0-12\ h}$ and C_{max} of the drug as compared with Legalon®, suggesting that silymarin absorption could be improved by SMEDDS. The similar results were obtained in the previous reports. Woo J.S. *et al.* reported that the relative bioavailability of silymarin SMEDDS to Legalon® was 360% after oral administration to SD rats at a dose of 140 mg/kg (6). Wu W. *et al.* reported that the relative bioavailability of silymarin was dramatically enhanced by SMEDDS in an average of 1.88- and 48.82-fold that of silymarin PEG 400 solution and suspension in male rabbits at a oral dose of 300 mg/kg, respectively (7). High bioavailability of silymarin SMEDDS might attribute to its promotion of lymphatic transport through transcellular pathway (29). It was reported that the long-chain oils promote lipoprotein synthesis and subsequent lymphatic absorption (30). Our previous results indicated that good absorption of silymarin SMEDDS was also observed in the middle and distal parts of intestine (29), where there exists abundant Peyer's patch. In addition, the spontaneous formation of microemulsion advantageously presented the drug in a dissolved form and prevented precipitation during dispersion in the gastric fluid and digestion in the intestine fluid, and the resultant small droplet size provided a large interfacial surface area for drug release and absorption.

It also should be kept in mind that most surfactants act as absorption enhancers. The main rate-limiting barrier for drug absorption is the single layer of intestinal epithelial cell. It is thought that high content of surfactants in SMEDDS could increase the permeability by disturbing the cell membrane (20). However, this might not be an important factor due to the fact that the percent of drug absorbed through the small intestine for silymarin-loaded Cremophor EL micelle and Cremophor EL-bile salt mixed micelle were markedly lower than that for SMEDDS in the present study (data not shown). Sha *et al.* (31) had investigated the effect of two novel SMEDDS containing Labrasol on tight junctions of intestinal epithelial cells. They demonstrated that microemulsion could reversibly open the tight junctions of Caco-2 cells with the exception of interaction of the surfactant in microemulsion with the polar head groups of the lipid bilayers, and modifying hydrogen bonding and ionic forces between these groups as well as insertion of the surfactant in microemulsion

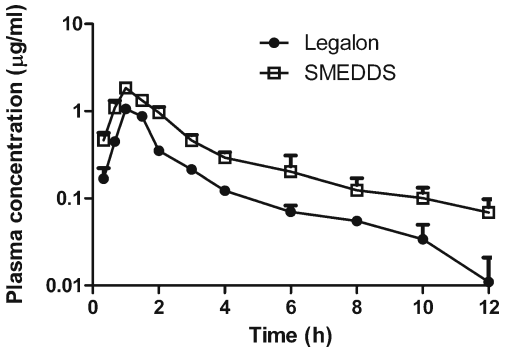

Fig. 7. Plasma concentration profiles of silybin after oral administration of Legalon® hard capsule and silymarin self-microemulsifying drug delivery system in dogs ($n=6$)

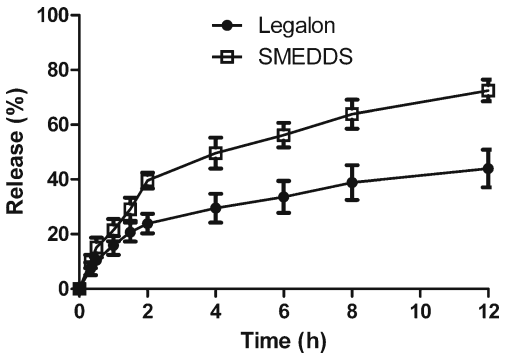

Fig. 6. Dissolution profiles of silymarin from self-microemulsifying drug delivery system and Legalon® capsule contents at 37°C ($n=3$)

Table II. Pharmacokinetic Parameters of Silymarin after Oral Administration of Test Preparations

Parameters	Formulation	
	SMEDDS	Legalon®
T_{max}/h	1.00±0.00	1.00±0.00
C_{max}/μg/mL	1.85±0.09*	1.06±0.04
$T_{1/2}$/h	2.69±0.12*	1.71±0.33
$AUC_{0-12\ h}$/μg·h/mL	4.75±0.26*	2.09±0.15
$AUC_{0-\infty}$/μg·h/mL	5.23±0.21*	2.12±0.07

*$p<0.05$, when compared with Legalon®

between the lipophilic tails of the bilayers, resulting in a disruption of the lipid-packing arrangement.

In addition, for poorly water-soluble drugs with low bioavailability, lymphatic pathway may play an important role in improving bioavailability by lipid-based delivery system, as reported that the total quantity of CI-976, a poorly water-soluble lipid regulator, transported in lymph over 14 h, as a percent of dose administered, was seven times greater for the emulsion as compared to the aqueous suspension (21).

CONCLUSION

The silymarin SMEDDS was developed using ethyl linoleate as the oil phase, Cremophor EL as the emulsifier, and ethyl alcohol as the coemulsifier in this study. The mean droplet size of blank microemulsion system was about 23 nm, and no changes were noted at 40°C for 3 months. *In vitro* release studies revealed that release of silymarin from SMEDDS was faster than that from the hard capsule (Legalon®). In *in vivo* studies for clinical purpose, SMEDDS showed significantly enhanced absorption than Legalon®. The relative bioavailability of SMEDDS to Legalon® was 227%. Therefore, SMEDDS might be a promising drug delivery system for oral administration of silymarin and could increase bioavailability for other poorly water-soluble drugs, as is the case for silymarin.

ACKNOWLEDGMENTS

This work was supported by the National Development of Significant New Drug (New Preparation and New Technology, Grant nos. 2009ZX09310-001) and the National Basic Research Program of China (973 program, no. 2009CB930300).

REFERENCES

1. Pepping J. Milk thistle: silybum marianum. Am J Health Syst Pharm. 1999;56:1195–7.
2. Barzaghi N, Crema F, Gatti G, Pifferi G, Perucca E. Pharmacokinetic studies on IdB 1016, a silybin–phosphatidylcholine complex, in healthy human subjects. Eur J Drug Metab Pharmacokinet. 1990;15:333–8.
3. Schandalik R, Gatti G, Perucca E. Pharmacokinetics of silybin in bile following administration of silipide and silymarin in cholecystectomy patients. Arzneimittelforschung. 1992;42:964–8.
4. Chen W, Xia H, Wu W. Optimized preparation of silymarin dripping pills by a central composite design–response surface method. Chin Trad Herb Drug. 2005;36:679–83.
5. Xiao YY, Song YM, Chen ZP, Ping QN. Preparation of silymarin proliposome: a new way to increase oral bioavailability of silymarin in beagle dogs. Int J Pharm. 2006;319:162–8.
6. Woo JS, Kim TS, Park JH, Chi SC. Formulation and biopharmaceutical evaluation of silymarin using SMEDDS. Arch Pharm Res. 2007;30:82–9.
7. Wu W, Wang Y, Que L. Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. Eur J Pharm Biopharm. 2006;63:288–94.
8. Weyhenmeyer R, Mascher H, Birkmayer J. Study on dose-linearity of the pharmacokinetics of silibinin diastereomers using a new stereospecific assay. Int J Clin Pharmacol Ther Toxicol. 1992;30:134–8.
9. Schulz HU, Schurer M, Krumbiegel G, Wachter W, Weyhenmeyer R, Seidel G. The solubility and bioequivalence of silymarin preparations. Arzneimittelforschung. 1995;45:61–4.
10. Pouton CW. Formulation of self-emulsifying drug delivery systems. Adv Drug Deliv Rev. 1997;25:47–58.
11. Khoo SM, Humberstone AJ, Porter CJH, Edwards GA, Charman WN. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. Int J Pharm. 1998;167:155–64.
12. Kim HJ, Yoon KA, Hahn M, Park ES, Chi SC. Preparation and *in vitro* evaluation of self-microemulsifying drug delivery systems containing idebenone. Drug Dev Ind Pharm. 2000;26:523–9.
13. Pouton CW. Lipid formulation for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. Eur J Pharm Sci. 2000;11:S93–8.
14. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int J Pharm. 2001;212:233–46.
15. Itoh K, Matsui S, Tozuka Y, Oguchi T, Yamamoto K. Improvement of physicochemical properties of N-4472 Part II: characterization of N-4472 microemulsion and the enhanced oral absorption. Int J Pharm. 2002;246:75–83.
16. Holm R, Porter CJH, Edwards GA, Müllertz A, Kristensen HG, Charman WN. Examination of oral absorption and lymphatic transport of halofantrine in triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides. Eur J Pharm Sci. 2003;20:91–7.
17. Grove M, Müllertz A, Nielsen JL, Pedersen GP. Bioavailability of seocalcitol II: development and characterisation of self-microemulsifying drug delivery systems (SMEDDS) for oral administration containing medium and long chain triglycerides. Eur J Pharm Sci. 2006;28:233–42.
18. Cirri M, Mura P, Corvi Mora P. Liquid spray formulations of xibornol by using self-microemulsifying drug delivery systems. Int J Pharm. 2007;340:84–91.
19. Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G *et al.* Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int J Pharm. 2004;274:65–73.
20. Swenson ES, Curatolo WJ. Means to enhance penetration. Adv Drug Deliv Rev. 1992;8:39–42.
21. Haus DJ, Mehta SC, Radebaugh GW. Targeting lymphatic transport and modified systemic distribution of CI-976, a lipophilic lipid-regulator drug, via a formulation approach. Int J Pharm. 1994;108:85–93.
22. Humberstone AJ, Charman WN. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv Drug Deliv Rev. 1997;25:103–28.
23. O'Driscoll CM. Lipid-based formulations for intestinal lymphatic delivery. Eur J Pharm Sci. 2002;15:405–15.
24. Liu JB, Xiao YH, Allen C. Polymer-drug compatibility: a guide to the development of delivery systems for the anticancer agent, ellipticine. J Pharm Sci. 2004;93:132–43.
25. Philipoff W. Micelles and X-rays. J Colloid Sci. 1950;5:169–91.
26. Riegelman S, Allawala NA, Hrenoff MK, Srait LA. The ultraviolet absorption spectrum as a criterion of the type of solubilization. J Colloid Sci. 1958;13:208–17.
27. Folliad KJ, Berke NS. Properties of high-performance concrete containing shrinkage-reducing admixture. Cem Concr Res. 1997;27:1357–64.
28. Gershanik T, Benita S. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. Eur J Pharm Biopharm. 2000;50:179–88.
29. Yuan Q, Li XR, Wang HJ, Li XY, Liu Y. The absorption kinetics of silymarin microemulsion in rat intestine. Acta Pharm Sin. 2004;39:631–4.
30. Charman WN, Stella VJ. Transport of lipophilic molecules by the intestinal lymphatic system. Adv Drug Deliv Rev. 1991;7:1–14.
31. Sha XY, Yan GJ, Wu YJ, Li JC, Fang XL. Effect of self-microemulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells. Eur J Pharm Sci. 2005;24:477–86.