Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
International Journal of Pharmaceutics 319 (2006) 162–168
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Preparation of silymarin proliposome: A new way to increase oral bioavailability of silymarin in beagle dogs

Xiao Yan-yu, Song Yun-mei, Chen Zhi-peng, Ping Qi-neng *

Department of Pharmacy, China Pharmaceutical University, Nanjing 210009, PR China

Received 19 January 2006; received in revised form 16 March 2006; accepted 28 March 2006
Available online 1 April 2006

Abstract

The aim of the present study was to find a method to increase oral bioavailability of silymarin, that is to say, by the preparation of silymarin proliposome and to compare the pharmacokinetic characteristics and bioavailability after oral administration of silymarin proliposome and silymarin in beagle dogs. Silymarin proliposome was prepared by the film-deposition on carriers. After the proliposome was contacted with water, the silymarin liposome suspensions formed automatically. The tests of physicochemical properties including SEM, TEM, encapsulation efficiency, dissolution studies, particle size of the reconstituted liposome and stability of the silymarin proliposome were determined by laser-particle-sizer, HPLC, etc. The concentrations of silymarin in plasma of beagle dogs and its pharmacokinetic behaviors after oral administration of silymarin liposome suspensions and silymarin were studied by RP-HPLC. The pharmacokinetic parameters were computed by software program 3p97. The encapsulation efficiency of silymarin liposome could be more than 90%, with an average particle size of about 196.4 nm and the proliposome appeared a very stability at 40 °C during 3 months. It was found that mean plasma concentration–time curves of silymarin after oral administration of liposome suspensions and silymarin in beagle dogs were both in accordance with open two-compartments model and first-order absorption. Pharmacokinetic parameters of silymarin proliposome and silymarin in beagle dogs were T_{max} both 30 min; C_{max} 472.62 and 89.78 ng mL^{-1}; and AUC0-∞ 2606.21 and 697 ng mL^{-1} h, respectively. The high bioavailability of silymarin proliposome could be obtained by oral administration. Silymarin proliposome was stable and did enchance the gastrointestinal absorption of silymarin.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Silymarin; Proliposome; Oral bioavailability; Beagle dogs; Pharmacokinetics

1. Introduction

Silymarin, the seeds extract of the milk thistle (*Silibum marianum* Gaertn), has been widely used to maintain liver health and treat liver disorders, reported by Kvasnicka et al. (2003) and Tedesco et al. (2004). Silybin is the main biologically active component in silymarin and largely responsible for the antihepatotoxic activity. Silymarin is slightly soluble in water and in oil, the poor permeation across the intestinal epithelial cells and minor absorption in rats gastrointestinal (GI) tract has been reported by Barzaghi et al. (1990) and Morazzoni et al. (1992).

Some drugs could not be well absorbed, but they could pass the GI tract when entrapped in liposomes, reported by Arien et al. (1993) and Arien and Dupuy (1997). However, liposome poses many problems in terms of physicochemical stability, such as aggregation, sedimentation, fusion, phospholipids hydrolysis and/or oxidation. Furthermore, problems in the sterilization and large-scale production of liposome remain to be solved (Klein, 1970; Larrabee, 1979; Wong and Thompson, 1982; Ab-Zaid et al., 1985; Lentz et al., 1987). In order to improve the stability of liposome, Mayer et al. (1986) and Payne et al. (1986) introduced a novel method to prepare liposome using the concept of proliposome. Proliposomes are defined as dry, free-flowing particles that immediately form a liposomal suspension when contact with water. Because of the solid properties, the stability problems of liposome can be resolved without influencing their intrinsic characteristics.

In recent years, many researches (Zhong et al., 2003) have been reported concerning preparations with the aim to enchance the bioavailability of silymarin, such as silymarin β–cyclodextrin inclusion complexes (Arcari et al., 1992),

* Corresponding author. Tel.: +86 25 8532 4872; fax: +86 25 8330 1606.
E-mail addresses: pingqn@cpu.edu.cn, czpcpu@sina.com (P. Qi-neng).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.03.037

silymarin solid dispersions (Li and Hu, 2004), silybin-*N*-methylglucamine (Zhang and Wu, 1984), silybin–phospolipid complex (Yanyu et al., 2006) and so on, however no study on silymarin proliposome has been reported yet.

Proliposome could be prepared by many methods including crystal-film method (Wei and Lu, 2003), film-deposition on carriers method (Junping et al., 2000; Song et al., 2002), fluided-bed method (Yang et al., 1993), powder bed grinding method (Ye and Liang, 2002), freezing and drying method (Zhang and Zhu, 1999; Lin et al., 2004) and spray drying method (Chen et al., 1997; Chu and Gu, 2002). In the procotol, based on our laboratory conditions we chose the film-deposition on carriers method to prepare silymarin proliposome.

The purpose of this study was to prepare silymarin proliposome and study its oral bioavailability in beagle dogs.

2. Materials and methods

2.1. Materials

Silymarin, containing 30% silybin (w/w), was purchased from Pan-jing-ge-ling-en biology technique Ltd., and phospholipid which contains approximately 82% (w/w) of the phosphatidyl was purchased from Tai–wei-yao-ye Ltd. Mannitol was purchased from Shanghai Hong Guang factory. The other chemical reagents were of analytical grade or better.

Twelve male beagle dogs, similar in age (2 years) and weight (10.0 ± 0.5 kg), were housed in individual cages and received a standard diet and water ad libitum. All the animals were clinically healthy and haematologically and biochemically normal throughout the experimental period. Food, but not water, was withheld for 24 h before and after drug administration.

2.2. Preparation of silymarin proliposome

The proliposome was prepared according to the method of literature (Song et al., 2002) with minor modification. Four grams of mannitol powders (sieved with a 100 mesh) were placed in a 100 ml round-bottomed flask rotated under vacuum condition for 30 min at 80–90 rpm in water bath at 90 °C. Silymarin (300 mg) and phospholipids (1.5 g) were dissolved in 10 ml mixed organic solvent (methanol: chloroform = 2:1, v/v), and a 0.5 ml aliquot of the organic solution was introduced into the round-bottomed flask at 37 °C. The rotation of the flask was continued until the solution deposited on the surface of mannitol powders and the solvent was then completely evaporated. The process was repeated until the solution was used up. After the last loading, the silymarin-loaded mannitol powders (i.e., proliposomes) were placed in a desiccator overnight, and then sieved with a 100 mesh, the collected powders were transferred into a glass bottle, flushed with nitrogen, sealed and stored at the room temperature.

The silymarin control was prepared according to the same procedure except adding phospholipids.

2.3. Determination of the content of silymarin in silymarin proliposome

The content of silymarin (based on silybin) in silymarin proliposome was determined as follows. Approximately, 1 mg of the silymarin proliposome was dissolved in 50 ml of a mixed solvent (methanol:distilled water = 60:40,v/v), and a 20 μl aliquot of the resulting solution was injected into a HPLC system. The stationary phase, μBondapak C_{18} column (150 mm × 4.6 mm, 5 μm), was kept at 40 °C. The mobile phase was a mixture of methanol:double distilled water:0.05M KH_2PO_4 = 40:60:5, adjusted to pH 4.0 with phosphoric acid. The flow rate was 1.0 ml/min. Effluent was monitored at 288 nm.

2.4. Scanning electron microscopy (SEM)

Silymarin proliposome powders and mannitol powders were coated with platinum in a sputter coater (JFC-1100, Jeol, Japan), and their surface morphology was viewed and photographed with a Jeol scanning electron microscope (JSM-5310-LV, Jeol).

2.5. Transmission electron microscopy (TEM)

Liposome was formed automatically by dropping distilled water to silymarin proliposome powders and shaking the mixture manually for 2 min. A drop of the resultant liposome suspension was placed onto a carbon-coated copper grid, forming a thin liquid film. The films on the grid were negatively stained by adding immediately a drop of 2% (w/w) ammonium molybdate in 2% (w/v) ammonium acetate buffer (pH 6.8), removing the excess staining solution with a filter paper, and followed by a through air-drying. The stained films were then viewed on a transmission electron microscope (Jeol-200 CX, Jeol) and photographed.

2.6. Dissolution studies

The dissolution studies were carried out according to a dissolution test apparatus of China pharmacopoeia (2005 edition, paddle method). The dissolution flasks were immersed in a water bath at 37 °C. The dissolution medium (pH 1.2 HCl or pH 6.8 phosphate buffer saline, 900 ml) was continuously stirred at 100 rounds/min. The silymarin proliposome which is equivalent to 77 mg of silybin was added on the surface of the stirred dissolution medium at the beginning of the study. At different time intervals, 10-ml samples were withdrawn and filtrated using 0.45 μm cellulose nitrate membrane, 10 ml fresh mediums were added into the flask. The 1 ml filtrate was dissolved in 9 ml of a mixed solvent (methanol:distilled water = 6:4, v/v) and a 20 μl aliquot of the resulting solution was injected into a HPLC and detected at a wavelength of 288 nm, the concentration of silymarin (based on silybin) was measured. Meanwhile, we also measured the solubility of silymarin control in pH 1.2 HCl or pH 6.8 phosphate buffer saline at 37 °C.

2.7. Particle size of reconstituted liposome and encapsulation efficiency of silymarin in the reconstituted liposome

Silymarin proliposome (2 mg) was mixed with 1 ml distilled water and agitated manually for 2 min. The size distribution of the reconstituted liposome was characterized by using a laser particle size analyzer (H-3000, Malvern, England). The encapsulation efficiency (EE, %) of silymarin in the reconstituted liposome was determined by filtrating the reconstituted liposome suspensions using 0.45 μm cellulose nitrate membrane. Because silymarin was slightly soluble in water, and when the proliposome was contacted with water, non-trapped silymarin would be precipitated. The EE (%) of silymarin in the liposome was obtained from between the amount of silymarin in the liposome before filtration (A) and after filtration (B), and then calculated using the expression $(B/A) \times 100$.

2.8. Stability of silymarin proliposomes

Accelerated testing of silymarin proliposome was carried out for about three months at 40 °C by using particle size and encapsulation efficiency of the reconstituted liposome as the indexes.

2.9. Bioavailability of silymarin proliposome in beagle dogs

2.9.1. Chromatography

The plasma concentrations of silymarin (based on silybin) were determined by a HPLC. The stationary phase, μBondapak C_{18} column (150 mm × 4.6 mm, 5 μm), was kept at 40 °C. The mobile phase was a mixture of methanol:double distilled water:0.05M KH_2PO_4 = 50:50:5, adjusted to pH 4.0 with phosphoric acid. The flow rate was 1.0 ml/min. Effluent was monitored at 288 nm.

2.9.2. Assay method

After 100 μl of the plasma sample (see Section 2.9.3 for details) had been thawed, 100 μl of 1 M Na_2CO_3 solution and 500 μl of borate buffer saline (pH 8.0) were added, which was then swirled for 30 s. After 4 ml aether was added to mixture solution above, this mixture solution were swirled for 3 min and then centrifugated (15 min, 4000 r min^{-1}). The organic phase was quantitatively decanted into a clear tapered centrifuging tube and the eluate were evaporated under nitrogen at 37 °C. The residues were resuspended in 100 μl of mobile phase and centrifugated (5 min, 4000 r min^{-1}). Aliquots (20 μl) were injected for HPLC analysis.

The method was validated by adding various quantities of silybin to blank beagle dogs plasmas. The resulting concentrations of silybin were 0.035, 0.1, 0.2, 0.6, 1.0, 1.5 and 2.0 μg ml^{-1}. These calibrations were subjected to the entire analytical procedure, so as to test the linearity, precision and accuracy of the method.

2.9.3. Pharmacokinetics study of silymarin proliposome and silymarin in beagle dogs

Twelve adult male beagle dogs divided into two groups were fasted for 24 h, but allowed to take water freely. Liposome was prepared by dropping distilled water to silymarin proliposome powders and shaking the mixture manually for 5 min. Twenty millilitre liposome suspensions equivalent to 7.7 mg kg^{-1} of silybin were orally administered to a group beagle dogs, The capsule full of silymarin equivalent to 7.7 mg kg^{-1} of silybin were orally administered to another group of dogs. After oral administration of the liposome suspensions and the capsule, respectively, 1 ml blood samples were collected from the jugular vein at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12 h. The plasma obtained after centrifugation (10 min, 4000 r min^{-1}) was immediately stored at −20 °C until analyzed.

Peak concentration (C_{max}) and peak times (T_{max}) were derived directly from the experimental points. The other pharmacokinetical parameters were computed by software program 3p97.

3. Results

3.1. Preparation of proliposome

The silymarin proliposome in the present study were prepared successfully. In this protocol, the ability of proliposome hydration was very poor when using tetrahydrofuran as a solvent, still having insoluble particles in the solution. However, when using anhydrous ethanol as solvent, the required amounts of anhydrous ethanol were more than that of the mixture of methanol and chloroform, which made cost increase and efficiency reduce. Compared with mannitol, the appearance of the proliposome was viscous using sodium chloride and sorbitol as carriers. As a result, mannitol was chosen as the carrier and the mixture of methanol and chloroform was chosen as the mixture solvent. The content of silymarin (based on silybin) in the proliposome was 9.73% (w/w).

3.2. Scanning electron microscopy (SEM)

The surface morphology of proliposome powders and mannitol powders, as examined by SEM is Fig. 1. It was obvious that the surface morphology of the two types of powders were different at ×200 magnification. The typical crystal appearance of mannitol was in focus (Fig. 1a), and for proliposome (Fig. 1b), the phospholipids deposited on the surface of mannitol crystal, which made mannitol figure illegible.

3.3. Transmission electron microscopy (TEM)

The TEM of proliposome after slightly shaking in distilled water are shown in Fig. 2. A uniform suspension appeared and monolayer liposome was formed from the proliposome.

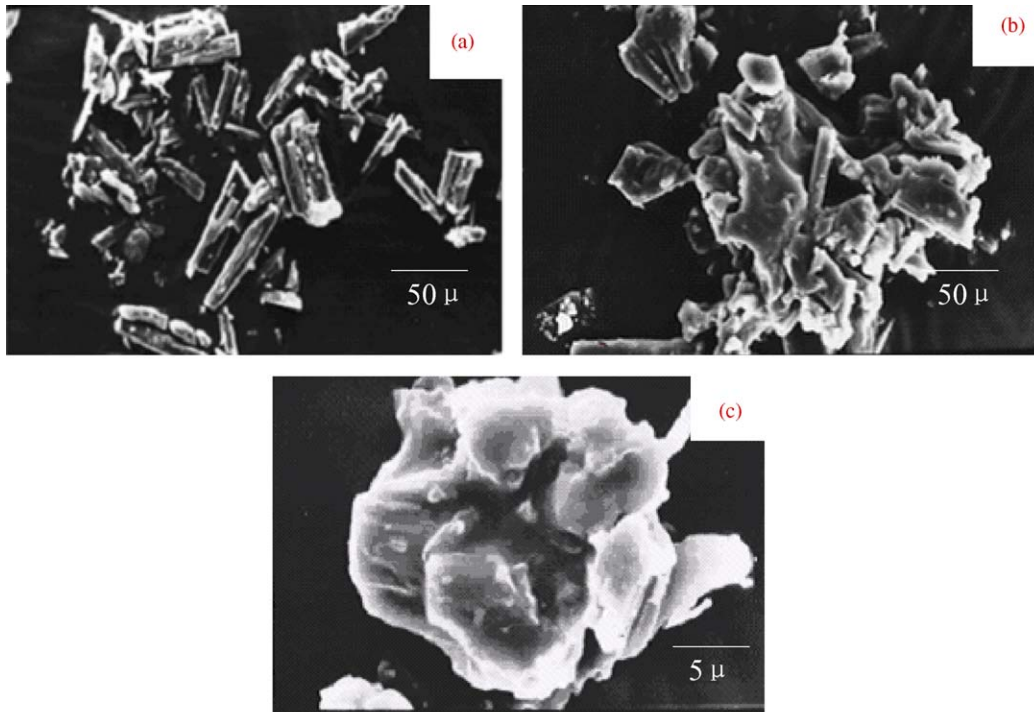

Fig. 1. Scanning electron micrographs of mannitol powders (a) and proliposome powders (b) at ×200 magnification while proliposome powders (c) at ×1000 magnification.

3.4. *Dissolution in HCl (pH 1.2) and phosphate buffer saline (pH 6.8)*

Fig. 3 shows the dissolution profile of silymarin from proliposome and contrast in HCl (pH 1.2) and phosphate buffer saline (pH 6.8), respectively. At about 20 min, the dissolution of silymarin (based on silybin) from proliposome became almost complete regardless of the pH of media. From Fig. 3, we can know that the dissolution of the silymarin proliposome was not influenced by the pH of media. It was considered that the proliposomes formed a liposomal suspension on contact with the media and drugs were entrapped in liposomes. However, for contrast without phospholipids, the dissolution of silymarin was not increased. So, we can get the conclusion that proliposome do enchance the dissolution of silymarin.

3.5. *Particle size of the reconstituted liposome and entrapment efficiency of silymarin in the proliposome*

Particle size analysis revealed the mean particle size of liposome of 196.4 ± 43.7 nm (mean ± S.D., $n = 3$) in the reconstituted liposomal suspension.

The EE (%) of silymarin in the reconstituted liposome was determined as describrd in Section 2.7. Five millilitre of purified water was added to 50 mg of proliposome and the suspension was

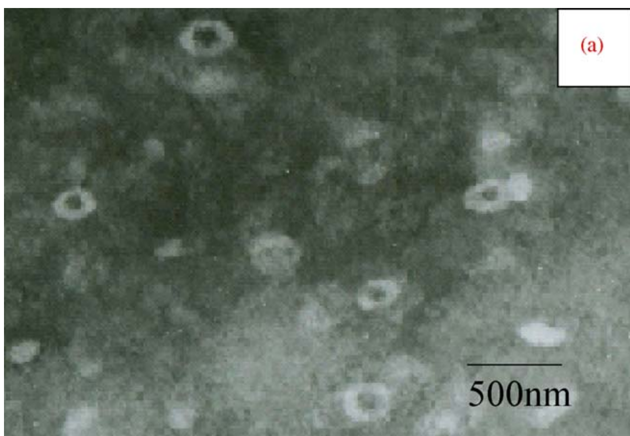

Fig. 2. Transmission electron micrographs of proliposome powders after slightly shaking in distilled water at ×4000 magnification.

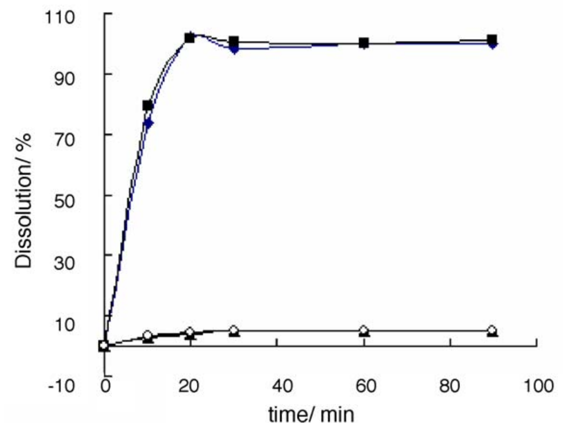

Fig. 3. Dissolution behaviors of silymarin proliposome and contrast in HCl (pH 1.2) and phosphate buffer saline (pH 6.8). (♦) proliposomes in phosphate buffer saline (pH 6.8); (■) proliposomes in HCl (pH 1.2); (◇) contrast in phosphate buffer saline (pH 6.8); (▲) contrast in HCl (pH 1.2).

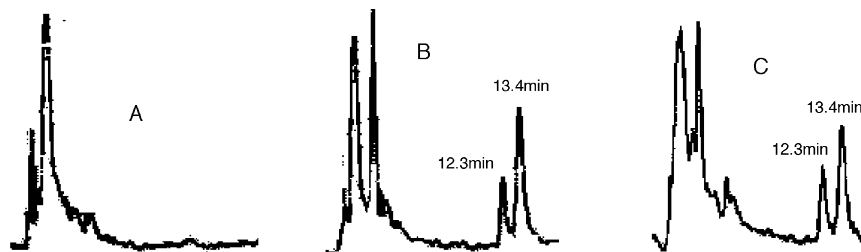

Fig. 4. Typical chromatograms of silymarin: (A) blank beagle dogs plasma; (B) blank beagle dogs plasma spiked with silybin; (C) a sample after oral administration of silymarin liposome suspensions.

shaked 2 min to form liposome. A mean value of 92.56 ± 0.93% (w/w, $n = 3$) was obtained for the EE (%).

3.6. Stability of silymarin proliposome

After 3 months, the appearance of silymarin proliposome still appeared loose, uniform and full, and the silymarin proliposome could immediately form a liposomal suspension on contact with water. Table 1 shows that particle size and entrapment efficiency of silymarin proliposome have no change at 40 °C for about 3 months which helps us to conclude that silymarin proliposome are stable at 40 °C for about 3 months.

3.7. Beagle dogs bioavailability experiments

Silybin in plasma was completely separated under analytical conditions, and because silybin is an isomeric compound, the two peaks were detected at about 12 and 13 min and the sum of the area of the two peaks was used for pharmacokinetic analysis (Fig. 4). Standard curves ranging from 0.035 to 2.0 μg ml^{-1} were linear ($r = 0.9932$). The results attained from the method recoveries of high, middle and low concentrations were, respectively, 82.40, 83.49 and 81.24%, the RSD in days were, respectively, 3.12, 2.56 and 3.74%, the RSD intra-days were, respectively, 4.83, 4.07 and 5.27%, which showed recoveries and RSD in days or intra-days were satisfying, and the lowest detection limit was 20 ng ml^{-1}.

Fig. 5 shows mean plasma concentration–time curves of silybin in beagle dogs after oral administration of silymarin liposome suspensions and silymarin equivalent to 7.7 mg kg^{-1} of silybin ($n = 6$). From the profile, we could know the average value of C_{max} was 472.62 ng ml^{-1} after oral administration of liposomal suspensions with a T_{max} of about 30 min. However, the average value of C_{max} was 89.78 ng ml^{-1} after oral administration of the capsule full of silymarin with a T_{max} of about

Table 1
Stability of silymarin proliposome at the temperature of 40 °C

Time (month)	Average size (nm, $\bar{x} \pm s$, $n = 3$)	Entrapment efficiency (%, $\bar{x} \pm s$, $n = 3$)
0	196.4 ± 15.8	92.56 ± 0.93
1	200.7 ± 20.6	90.92 ± 1.21
2	223.1 ± 17.4	88.54 ± 0.42
3	259.4 ± 22.9	88.76 ± 0.83

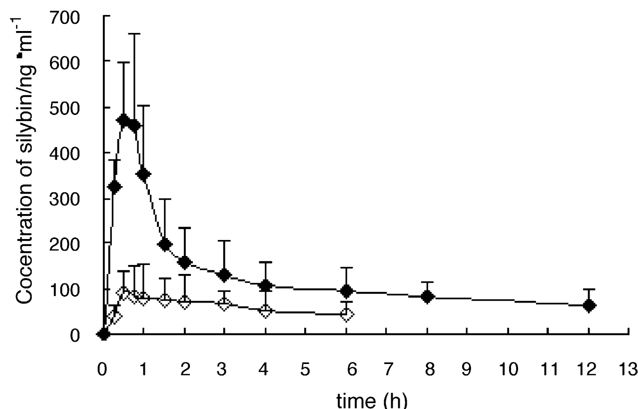

Fig. 5. Mean plasma concentration–time curve of silybin in beagle dogs after oral administration of silymarin liposome suspensions and silymarin equivalent to 7.7 mg kg^{-1} of silybin ($n = 6$). (♦) silymarin liposome and (◊) silymarin.

30 min. The other parameters were obtained by 3p97 procedure (Tables 2 and 3).

The pharmacokinetic data were simulated by non-linear least squares. The results showed that open two-compartments model and first-order absorption were both fitted to proliposome and silymarin material plasma concentration–time curves in beagle dogs.

Table 2
The main pharmacokinetic parameters of silymarin proliposome and silymarin in beagle dogs ($n = 6$)

Parameters	Unit	Silymarin proliposome	Silymarin
A	ng ml^{-1}	1105.32 ± 289.31	62.43 ± 22.74
α		1.85 ± 0.85	5.58 ± 2.07
B	ng ml^{-1}	139 ± 59.43	86.56 ± 42.18
β		0.065 ± 0.025	0.12 ± 0.071
$T_{1/2\alpha}$	h	0.38 ± 0.21	0.12 ± 0.092
$T_{1/2k\alpha}$	h	0.15 ± 0.071	0.069 ± 0.041
$T_{1/2\beta}$	h	1.61 ± 0.46	2.78 ± 2.76
K_a	h^{-1}	4.77 ± 1.27	9.99 ± 2.98
K_{12}	h^{-1}	1.22 ± 0.53	1.29 ± 0.49
K_{21}	h^{-1}	0.37 ± 0.15	4.25 ± 2.31
K_{10}	h^{-1}	0.33 ± 0.17	0.16 ± 0.11
T_{max}	h	0.5 ± 0.21	0.5 ± 0.28
C_{max}	ng ml^{-1}	472.62 ± 126.91	89.78 ± 46.07
AUC	ng ml^{-1} h	2464.62 ± 579.35	717.73 ± 156.93
CL(s)	mg h^{-1} ng^{-1} ml	0.03 ± 0.012	0.11 ± 0.07
V/F(c)	mg ng^{-1} ml	0.095 ± 0.017	0.68 ± 0.38

Table 3
The main pharmacokinetic parameters of silymarin proliposome and silymarin with non-model in beagle dogs ($n = 6$)

Parameters	Unit	Silymarin proliposome		Silymarin	
		T (0–12 h)	T (0–∞)	T (0–6 h)	T (0–∞)
AUC(s0)	ng ml^{-1} h	1519.81 ± 248.92	2606.21 ± 369.42	358.84 ± 106.46	697.04 ± 286.29
AUMC(s1)		6366.73 ± 1012.31	37674.4 ± 2068.43	3758.8 ± 179.85	88306 ± 8075.87
MRT	h	4.19 ± 1.05	14.5 ± 5.86	2.76 ± 0.42	8.09 ± 3.54

Table 4
The comparation of main parameters in human, beagles and rats

	Preparation	Dosage (mg kg^{-1})	C_{max} (ng ml^{-1})	T_{max} (h)	T1/2 (h)	AUC (ng ml^{-1} h)
Human	Silybin–phosphatidylcholine complex	7.2	298 ± 96	1.6 ± 0.3	2.6 ± 1.0	881 ± 207
Human	Silybin solid dispersion	1.6	157.7 ± 114.6	1.3 ± 0.35	2.3 ± 1.4	270.4 ± 98.8
Beagles	Silymarin proliposome	7.7	472.62 ± 126.91	0.5 ± 0.21	1.61 ± 0.46	2464.62 ± 579.35
Rats	Silybin–phosphatidylcholine complex	200	8170 ± 3010	0.33 ± 0.05	0.58 ± 0.15	9780 ± 3290

4. Discussion

According to the definition of proliposome, the carrier must be water-soluble and its particle size must be suitable. Whether the appearance of proliposome was powder, or it was easy to hydrated or not was used to decide which kind of carriers can be chosen. There are serveral reports using the film-deposition on carriers method. In those reports, the microporous sorbitol particles were used as carriers and drug and phospholipids were deposited on the appearance and micropore of sorbitol particles. However, in this investigation we found that mannitol could be as carriers to produce the best results for the proliposomes, which make the appearance of the preparation loose and for sorbitol, viscous.

In this study, we also investigate the pharmacokinetics about oral administration of silymarin proliposome, silymarin and silybin. The results showed that after oral administration of silymarin proliposome compared with silymarin, a pharmacokinetically well defined plasma profile was observed, however, after oral administration of silybin, it did not result in detectable plasma levels in our studies. Morazzoni et al. (1992) had also demonstrated pure silybin had a very low bioavailbility and studied bioavailability of silipide in rats. Barzaghi et al. (1990) had studied pharmacokinetics on silybin–phosphatidylcholine complex in healthy human subjects. Miao et al. (2000) had reported bioavailability of silybin solid dispersion capsule in normal volunteers. The main parameters were compared in Table 4. Weingarten et al. (1985) reported after oral administration liposome could protect the drugs from enzymatic metabolizability. In general circulations, silymarin was mainly enzymatic metabolized by liver microsome, so the preparation of silymarin liposome could protect silymarin from enzymatic metabolizability and get higher bioavailability.

In conclusion, we successfully prepared silymarin proliposome for oral administration by a simple method. However, the mechanism of silymarin liposome through GI tracts needs further studies.

References

Ab-Zaid, S.S., Morii, M., Takeguchi, N., 1985. Effects of freeze-drying and cold storage on the size and membrane permeability of multilamellar liposomes. Maku 9, 43–48.

Arcari, M., Brambilla, A., Brandt, A., Caponi, R., Corsi, G., Di Rella, M., Solinas, F., Wachter, W.P., 1992. A new inclusion complex of silibinin and beta-cyclodextrins: in vitro dissolution kinetics and in vivo absorption in comparison with traditional formulations. Boll. Chim. Farm. 131, 205–209.

Arien, A., Goigoux, C., Baquey, C., Dupuy, B., 1993. Study of in vitro and in vivo stability of liposomes loaded with calcitonin or indium in the gastrointestinal tract. Life Sci. 53, 1279–1290.

Arien, A., Dupuy, B., 1997. Encapsulation of calcitonin in liposomes depends in the vesicle preparation method. J. Microencapsul. 14, 753–760.

Barzaghi, N., Crema, F., Gatti, G., Pifferi, G., Perucca, E., 1990. Pharmacokinetic studies on IdB 1016, a silybin–phosphatidylcholine complex, in healthy human subjects. Euro. J. Drug Metab. Pharmacokinet. 15, 333–338.

Chen, Q.I., Huang, Y.U., Gu, X.Q., Chen, C.Z., Huang, D.N., 1997. A study on the preparation of proliposome by the spray drying method. J. Shenyang Pharm. Univ. 14, 166–169.

Chu, M.Q., Gu, H.C., 2002. The study on the preparation of tanshonones propoliposomes by the spray drying method. Chin. Pharm. J. 37, 32–35.

Junping, W., Maitani, Y., Takayama, K., Nagao, T., 2000. In vivo evaluation of doxorubicin carried with long circulating and remote loading proliposome. Int. J. Pharm. 203, 61–69.

Klein, R.A., 1970. The detection of oxidation in liposome preparations. Biochim. Biophys. Acta 210, 486–489.

Kvasnicka, F., Biba, B., Sevcik, R., Voldrich, M., Kratka, J., 2003. Analysis of the active components of silymarin. J. Chromatogr. A 990, 239–245.

Larrabee, A., 1979. Time-independence changes in the distribution of distearoyl phosphatidylcholine vesicles. Biochemistry 18, 3321–3326.

Lentz, B.R., Carpenter, T.J., Alford, D.R., 1987. Spontaneous fusion of phosphatidylcholine vesicles in the fluid phase. Biochemistry 26, 5389–5397.

Li, F.Q., Hu, J.H., 2004. Improvement of the dissolution rate of silymarin by means of solid dispersions. Chem. Pharm. Bull. 52, 972–973.

Lin, Z.F., Zeng, K., Zhou, Z.G., Li, G.F., Xie, F.M., Zhu, X.L., Zhang, S.Q., 2004. Preparation and characterization of podophyllotoxin-dipalmitoylphosphatidylcholine proliposome. Di Yi Jun Yi Da Xue Xue Bao. 24, 784–786, 788.

Mayer, L.D., Baly, M.B., Hope, K.J., Cullis, P.R., 1986. Techniques for encapsulating bioactive agents into liposomes. Chem. Phys. Lipids 40, 333–345.

Miao, H.J., Liu, G.L., Qian, F., Bao, X.M., Deng, Y.L., Fan, C.H., 2000. Bioavailability of silibinin solid dispersion capsule in normal volunteers. Di Er Jun Yi Da Xue Xue Bao 21, 965–967.

Morazzoni, P., Magistretti, M.J., Giachetti, C., Zanolo, G., 1992. Comparative bioavailability of silipide, a new flavanolignan complex, in rats. Euro. J. Drug Metab. Pharmacokinet. 17, 39–44.

Payne, N.I., Timmis, P., Ambrose, C.V., Warel, M.D., 1986. Proliposomes: a novel solution to an old problem. J. Pharm. Sci. 75, 325–329.

Song, K.H., Chung, S.J., Shim, C.K., 2002. Preparation and evaluation of proliposomes containing salmon calcitonin. J. Control. Release 84, 7–37.

Tedesco, D., Tava, A., Galletti, S., Tameni, M., Varisco, G., Costa, A., Steidler, S., 2004. Effects of silymarin, a natural hepatoprotector, in periparturient dairy cows. J. Dairy Sci. 87, 2239–2247.

Wei, N.N., Lu, B.I., 2003. Preparation, morphology and in vitro release of chitosan coated liposomes of fluorouracil for colon targeting. Acta Pharm. Sinica 38, 53–56.

Weingarten, C., Moufti, A., Delattre, J., Puisieux, F., Ryman, B.E., 1985. Protection of insulin from enzymatic degradation by its association to liposomes. Int. J. Pharm. 26, 251–255.

Wong, M., Thompson, F.H., 1982. Aggregation of dipalmitoylphosphatidylcholine vesicles. Biochemistry 21, 4122–4139.

Yang, Z.J., Hino, T., Kawashima, Y., 1993. Studies on the size of rehydrated new liposome from scutellaria proliposome. J. Chin. Pharm. Univ. 24, 161–164.

Yanyu, X., Yunmei, S., Zhipeng, C., Qineng, P., 2006. The preparation of silybin–phospholipid complex and the study on its pharmacokinetics in rats. Int. J. Pharm. 307, 77–82.

Ye, Z.W., Liang, W.Q., 2002. Preparation of interferon-containing liposomes by the powder bed grinding method. J. ZheJiang Univ. (Med. Sci.) 31, 433–436.

Zhang, J.H., Zhu, J.B., 1999. A novel method to prepare liposomes containing amikacin. J. Microencapsul. 16, 511–516.

Zhang, S.X., Wu, Z.X., 1984. The preparation and quality standard of water-soluble silybin. Pharm. Bull. 19, 7–11.

Zhong, Y.Q., Liu, S.K., Chen, G.S., 2003. The current study about high bioavailability preparations of silymarin. J. Pharm. Pract. 21, 139–140.