AAPS PharmSciTech, Vol. 9, No. 1, March 2008 (© 2008)
DOI: 10.1208/s12249-008-9040-1

Research Article

Themed Issue: Oral Controlled Release Development and Technology
Guest Editors: Stephen Howard and Jian-Xin Li

Process Optimization, Characterization and Pharmacokinetic Evaluation in Rats of Ursodeoxycholic Acid–Phospholipid Complex

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Received 8 July 2007; accepted 4 January 2008; published online 7 February 2008

Abstract. The purpose of this research was to study whether the bioavailability of ursodeoxycholic acid could be improved by administering ursodeoxycholic acid–phospholipid complex (UDCA–PLC) orally to rats. A central composite design approach was used for process optimization in order to obtain the acceptable UDCA–PLC. The physicochemical properties of the complex obtained by optimal parameters were investigated by means of scanning electron microscopy and X-ray diffraction. The pharmacokinetic parameters and bioavailability studies were conducted in rats of UDCA after oral administration of UDCA–PLC and UDCA tablet. Multiple linear regression analysis for process optimization revealed that the acceptable UDCA–PLC was obtained wherein the optimal values of X_1, X_2 and X_3 were 3, 60°C and 3 h, respectively. The XRD studies of UDCA–PLC obtained by the optimal parameters demonstrated that UDCA and phospholipids in the UDCA–PLC were combined by non-covalent bonds, not form new compounds. But pharmacokinetic parameters of the complex in rats were T_{max} 1.6 h, C_{max} 0.1346 μg/ml, $AUC_{0-\infty}$ 11.437 μg·h/ml, respectively. The relative bioavailability of UDCA of UDCA–PLC was increased by 241%,compared with the reference ursodeoxycholic acid tablet.

KEY WORDS: characterization; optimization; pharmacokinetics; phospholipid complex; ursodeoxycholic acid.

INTRODUCTION

Ursodeoxycholic acid (3a,7b-dihydroxy-5b-cholanoicacid,UDCA, see Fig. 1) was discovered as the principal bile acid in the polar bear by Hammersten in 1902 (1). UDCA suppresses biliary secretion of cholesterol and inhibits its intestinal absorption in humans (2,3). It is used for the dissolution of cholesterol-rich gallstones in patients with functioning gallbladders (4,5), and in the treatment of primary biliary cirrhosis. Furthermore, it has been tried in the treatment of some chronic liver diseases associated with cholestasis such as liver disease in cystic fibrosis, cholestasis associated with pregnancy, sclerosing cholangitis, chronic active hepatitis and viral hepatitis. UDCA has also shown some promise in the treatment of non-alcoholic steatohepatitis and refractory graft-*versus*-host disease of the liver in transplant patients (6). However, a severe limitation exists and is imputable to the poor or very poor absorption of these active constituents when administered orally or by topical application.

The bioavailability of poorly soluble drugs when administered orally as solid dosage forms is notoriously low. There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastro-intestinal tract. There are numerous advantages of phospholipids in addition to solubilizing property while considering them for a carrier system. It was reported that some poor soluble drugs combined with phospholipids could result in increase of oral bioavailability and improvement of the biological effect, such as silybin (7), flavanolignans (8), glycyrrhetinic acid (9), bilobalide (10).

The objectives of this study were: (1) to improve oral bioavailability of UDCA, UCDA–PLC was prepared by a simple method. A central composite design approach was used for optimization of process variables on the yield (%) of UDCA "present as a complex" in the complex. The joint influence of the independent variables, phospholipid-to-drug ratio (X_1), reaction temperature (X_2, °Celsius) and the reaction time(X_3, hours) on the dependent variable the yield (Y, %) of UDCA "present as a complex" was investigated; (2) the physicochemical characters of UDCA–PLC were evaluated, such as SEM and XRD. The solubility and

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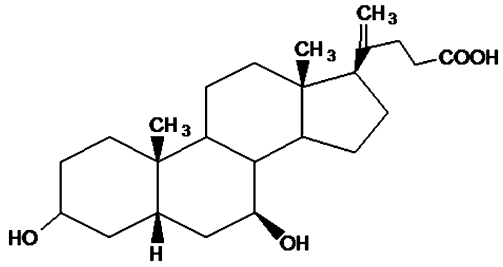

Fig. 1. The chemical structure of UDCA

dissolution studies of UDCA–PLC were performed in order to evaluate the improved solubility and dissolution properties of UDCA–PLC in comparison with pure UDCA and the physical mixture; (3) In order to evaluate the improved bioavailability of UDCA–PLC, the pharmacokinetics and bioavailability of UDCA in rats were studied after oral administration of two formulations: ursodeoxycholic acid tablet and UDCA–PLC. Because of the poor UV absorption of UDCA, the blood concentration of UCDA was assayed by HPLC after derivatization.

MATERIALS AND METHODS

Materials

UDCA was purchased from TianJin-Tai-ping-yang Ltd. (Tianjin, China), purity 99.13%, and phospholipid was purchased from Hua-Qing-Mei-Heng Ltd. (Tianjin, China), and the phosphatidylcholine content was approximately 82% (*w*/*w*). Ursodeoxycholic acid Tablet (included in Chinese Pharmacopeia 2005 edition) was purchased from Jing-Wei pharmacy market(Beijing, China). The other chemical reagents were of analytical grade or better.

Preparation of UDCA–PLC

The phospholipids and UDCA (as a ratio of 1:1, 1.4:1, 2:1, 2.6:1 and 3:1, respectively) were placed in a 100 ml round-bottom flask and dissolved in tetrahydrofuran (30 ml/mg UDCA). The tetrahydrofuran(60 ml) was used as reaction medium. The reaction temperature of the complex was controlled to 40°/44°/50°/56°/60°C using water bath (DSY-2-2, Aiqixia apparatus center, China) and was maintained at the specified temperature for a reaction time of 1/1.4/2/2.6/3 h. After then the tetrahydrofuran was evaporated off under vacuum at 60°C for 10 h, the dried residues were gathered and placed in desiccators overnight, then crushed in the mortar and sieved with a 100 mesh. The resultant UDCA–PLC was transferred into a glass bottle, flushed with nitrogen and stored in the room temperature. All the above-mentioned steps were performed under aseptic conditions.

The Yield of UDCA "Present as a Complex" (%)

The UDCA–PLC prepared as above description was dispersed in sufficient chloroform(5 ml/mg UDCA–PLC).The complex and phospholipids both were easily dissolved in the chloroform (11), but the UDCA was practically insoluble in the chloroform. The UDCA non-complexed was sedimentated and separated to assay. The Yield of UDCA "present as a complex" (%) was determined using following formula equation (1):

$$\text{The Yield} = {}^{(a-b)}/_{a} \times 100\% \qquad (1)$$

Where, *a* was the content of *UDCA* "present as a complex", *b* was the content of UDCA "no-present as a complex" in the complex.

A HP 1100 chromatographic system consisting of a quaternary pump (G1100A QuatPump, Agilent), degasser (G1100A, Agilent), diode array detector (G1100A DAD, Agilent), and HP Chemstation Data system (Agilent Technologies, Palo Alto, CA) was used. Chromatographic column KromasilC18 column (250×4.6 mm, 5 μm) was used for chromatographic separation. The mobile phase was acetonitrile: 0.025 mol/l phosphoric acid solution=45:55(*v*/*v*, pH=3.0). The flow rate was 1.0 ml/min. The detector wavelength was 210 nm at room temperature.

Central Composite Design

To reduce the number of trials and attain the highest amount of information on product properties, the screening was planned applying a circumscribed central composite design to systematically study the joint influence of the effect of independent variables phospholipid-to-drug ratio (X_1), reaction temperature (X_2, °Celsius) and the reaction time (X_3, hours) on the dependent variable the Yield. In this design, three factors were evaluated and experimental trials were performed at all 20 possible combinations (12,13). A statistical model incorporating interactive and polynomial terms was used to evaluate the response employing the formula equation (2):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \qquad (2)$$

Y was the dependent variable, b_0 was the arithmetic mean response of the 20 runs, and b_i was the estimated coefficient for the factor X_i. The main effects (X_1 and X_2) represented the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2, X_2X_3, X_1X_3) showed how the response changes when three factors were simultaneously changed. The polynomial terms (X_1^2, X_2^2 and X_3^2) were included to investigate nonlinearity. The level values of three factors and the composition of the central composite design batches 1 to 20 were shown in Tables I and II.

Table I. Factor Levels for the Experimental Design

	Levels				
Factors	−1.732	−1	0	+1	+1.732
X_1 (*W*/*W*)	1.0	1.4	2.0	2.6	3.0
X_2 (°C)	40.0	44.2	50.0	55.8	60.0
X_3 (h)	1.0	1.4	2.0	2.6	3.0

Table II. Composition of Central Composite Design Batches (n=3, mean±SD)

Batches	X_1	X_2	X_3	Yield (%)
1	−1	−1	−1	53.84±1.34
2	+1	−1	−1	69.69±1.46
3	−1	+1	−1	59.65±1.52
4	+1	+1	−1	71.18±2.01
5	−1	−1	+1	76.39±0.72
6	+1	−1	+1	79.72±1.14
7	−1	+1	+1	81.08±0.67
8	+1	+1	+1	85.12±1.34
9	−1.732	0	0	54.13±1.21
10	+1.732	0	0	82.52±0.75
11	0	−1.732	0	74.19±1.31
12	0	+1.732	0	80.17±1.17
13	0	0	−1.732	60.12±1.61
14	0	0	+1.732	78.85±0.57
15	0	0	0	76.97±0.82
16	0	0	0	75.91±1.03
17	0	0	0	75.98±1.17
18	0	0	0	76.42±0.92
19	0	0	0	76.48±1.03
20	0	0	0	75.17±1.21

Characterization of UDCA–PLC

Scanning Electron Microscopy (SEM)

Phospholipids complex 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).

X-ray Diffractometry (XRD)

The X-ray diffractogram (D/max-r A, Rigaku Denki, Japan) was scanned with the diffraction angle increasing from 5° to 50°, 2θ angle, with a step angle of 0.04° and a count time of 1 s.

Solubility Studies

Solubility determination of UDCA of UDCA or phospholipids complex was carried out by adding excess of UDCA material or phospholipids complex to 6 ml of water or *n*-octanol in sealed glass containers at 25°C. The liquids were agitated for 24 h, then centrifuged to remove excessive UDCA (15 min, 4,000 rpm). The supernatant was filtrated through a 0.45 μm membrane. The concentration of UDCA was assayed as the previous described method.

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, 1,000 ml) was continuously stirred at 100 rpm. Phospholipids complex which was equivalent to 3.6 g of UDCA 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 membrane, 10-ml fresh mediums were added into the flask.

Pharmacokinetics Study *In Vivo* of UDCA–PLC

Experiment Design

Male Wistar rats weighing 200–220 g were obtained from National Resource Center for Rodent Laboratory Animal. Each rat was housed at ambient temperature 20–25°C, and 45–55% relative humidity. Every rat was fed a standard diet and made to fast during 24 h prior to experiment.

The rats were divided into two groups (n=6/group/timepoint). Two ursodeoxycholic acid preparations were compared in the study: a test preparation of UDCA–PLC and a reference preparation of ursodeoxycholic acid tablet (UDCA Tablet). Under ether anesthesia, jugular vein blood samples were collected from both groups of rats into centrifuge tubes at the predetermined time point (0.5, 1, 1.5, 2,2.5,3, 4, 6, 8,10, 12,14 h) after oral administration of UDCA preparations at a dose equivalent to 20 mg/kg of UDCA. Blood was allowed to clot at room temperature for about 1 h, centrifuged at 8,000 rpm for 10 min and serum was separated and kept at −20°C prior to analysis.

Sample Preparation and Solid Phase Extraction (SPE)

Cholic acid (CA, 500 ml 10^{-4} M, i.e. 60 nmol of internal standard) in methanol was transferred into a 9 ml tube and evaporated gently (45°C, stream of nitrogen) to dryness. A serum sample (1 ml) containing UDCA was then added and the tube content mixed and diluted with 5 ml of the phosphate buffer (pH 7.5).SPE columns (Supelclean LC-18) were activated on the SPE vacuum manifold with 2 ml of methanol followed by 3 ml of UHQ water (flow rate 3 ml/min).The serum spiked with the internal standard and diluted with the phosphate buffer was passed through SPE column with a flow rate of 1 ml/min. After washing the SPE column with 2 ml of UHQ water and 2 ml of 20% methanol (flow rate of 3 ml/min), the captured Analytes (UDCA and CA) eluted from the column with 3 ml of methanol (flow rate 1 ml/min).The methanolic eluate was evaporated (45°C, stream of nitrogen) to dryness.

Derivatization Procedure

2-Bromo-2-acetonaphthone (130 ml of 10^{-3} M) and *N*,*N*-diisopropylethylamine (260 μl of 10^{-3}, both in acetonitrile solutions) were added into the tube with the dried serum eluate. The mixture was allowed to react at 60°C for 20 min. Following reaction, the remaining acetonitrile was evaporated (45°C, stream of nitrogen) and the dry residue (a mixture of UV-absorbing naphthacyl esters of bile acids, the derivatizing agent and the amine) was reconstituted in 200 ml of the mobile phase and transferred into a vial containing a glass insert (230 ml volume).

Chromatography

Concentrations of UDCA in the sample were determined using a HPLC apparatus (Agilent, USA) equipped

Table III. Results of Regression Analysis

Response	b_0	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}	R^2
Full model (FM)	−53.8119	57.7109	0.3465	47.1196	−7.4239	0.5086	−6.2847	0.3487	−6.9479	−4.5125	0.985
Reduced model (RM)	−53.8119	57.7109	0.3465	47.1196	−7.4239	–	−6.2847	–	−6.9479	–	0.912

– Indicates the term was omitted in Reduced Model

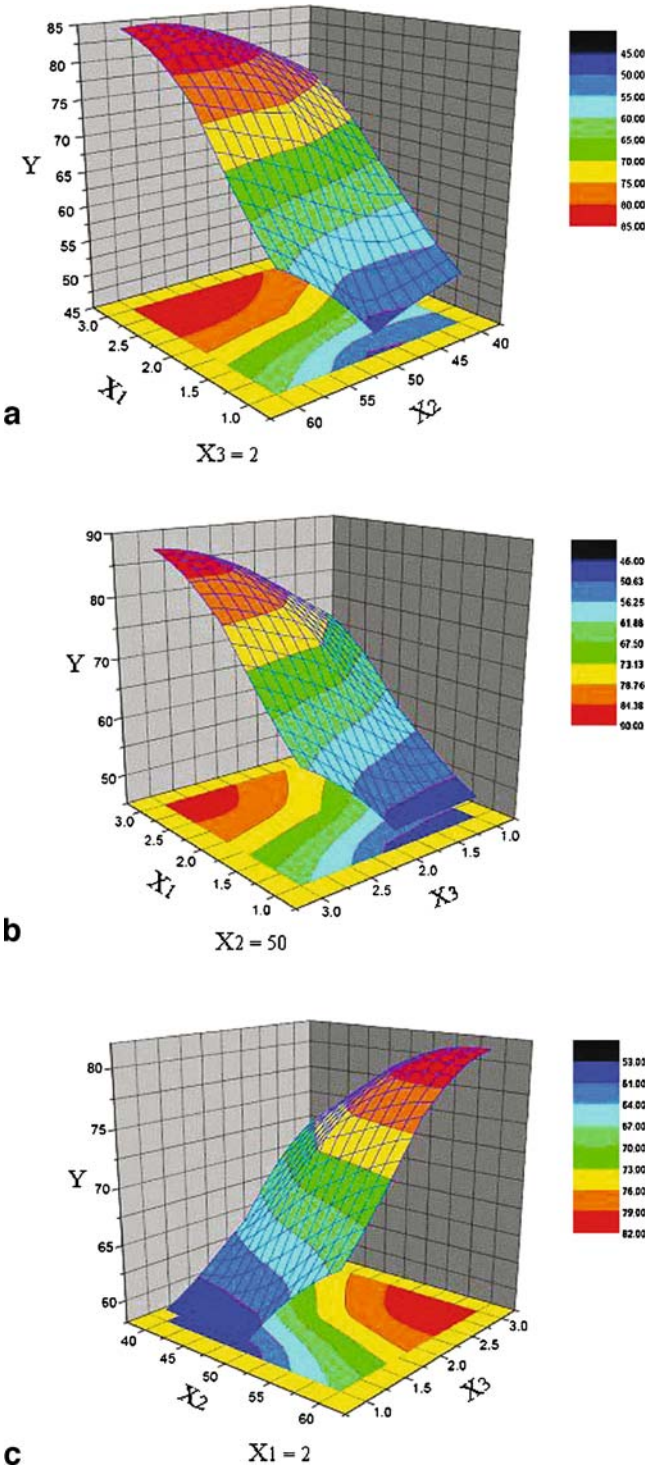

Fig. 2. The response surface plot and contour plots based on the yield (Y, %) as a function of the quantity ratio of phospholipids and UDCA (X_1), reaction temperature (X_2, °Celsius) and reaction time (X_3, hours)

with Kromasil C18 column (250×4.6 mm,5 μm) and C-18 precolumn. The mobile phase was acetonitrile/H_2O (65:35). The flow rate was at 1.0 ml/min, the column temperature was room temperature, the volume of the injected sample was 20 μl and the detector wavelength was 245 nm.

Pharmacokinetics and Statistical Analysis

The pharmacokinetic parameters were evaluated. The zero-order moment area under the blood concentration–time curve was calculated by the trapezoidal rule. The pharmacokinetic parameters and AUC_{0-24} were computed by software program 3p87 (Version 1987, Chinese Pharmaceutical Association, China). The differences among pharmacokinetic parameters in test groups were estimated by multiple comparison tests with software named SPSS (Version 10.0, SPSS Inc. USA).

RESULTS AND DISUSSION

Preparation of UDCA–PLC

Preliminary investigations of the process parameters revealed that factors phospholipid-to-drug ratio (X_1), reaction temperature (X_2, °Celsius) and the reaction time(X_3, hours) highly influenced the yield of UDCA "present as a complex" in the complex. The yield (%) for the 20 batches showed a wide variation of 53.84% to 85.12% (Table III). The fitted polynomial equations (full and reduced model) relating the response yield (%) to the transformed factors are shown in Table III. The polynomial equations could be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e., positive or negative. The significance level of coefficient b_{12}, b_{22}, b_{23} and b_{23} were found to be P=0.2337, 0.3694, 0.1648 and 0.1233, respectively. So those were omitted from the full model equation to generate the reduced model equation. The coefficients b_1, b_2, b_3, b_{11},b_{13} and b_{33} were found to be significant when P was less than 0.05 and thus, were retained in the reduced model. The correlation coefficient R^2 (0.985) of the reduced model was significantly more than that (0.872) of the full model. Multiple linear regression analysis (Table III) revealed that

Table IV. Model-Predicted and Observed Values of the Yield (%) of UDCA "Present as a Complex" in the UDCA–PLC Prepared According to the Optimal Parameters

Predicted Yield (%)	Observed Yield (%)	Bias(%)[a]
95.56	97.65±1.24	−2.19

[a] Bias was calculated according to equation: bias/%=(predicted value−observed value)/predicted value×100%

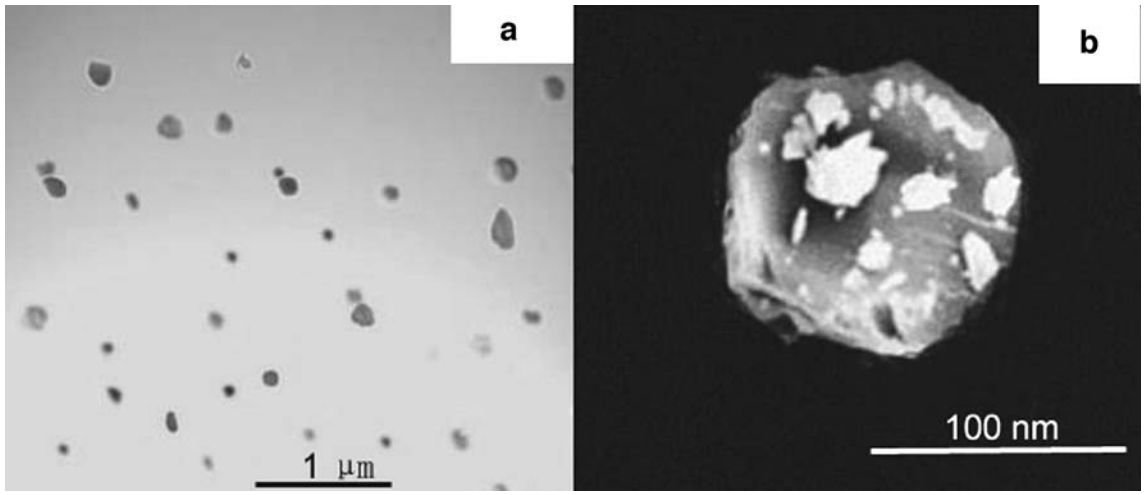

Fig. 3. Scanning electron micrographs of UDCA–PLC **a** at ×100 magnification and **b** at ×2,000 magnification

the coefficient b_1, b_2, b_3 was positive. This indicated that the yield increased on increasing X_1, X_2, X_3.

The response surface and contour plots (Fig. 2) clearly indicated that X_1, X_2 and X_3 strongly influenced the yield (%). The change in the yield (%) as a function of X_1, X_2 and X_3 was depicted in the form of response surface plot and contour plots (Fig. 2) based on central composite design. The data of all the 20 batches of central composite design were used for generating interpolated values using Origin software (Systat Software Inc., Version 7.5). High level of X_1, X_2 and X_3 were found to be favorable conditions for obtaining higher yields (%).

Taken together with multiple linear regression model, it was concluded that optimal values of the quantity ratio of phospholipids and UDCA (X_1), reaction temperature (X_2, °Celsius) and reaction time(X_3, hours) obtained from response surface were 3, 60°C and 3 h, respectively.

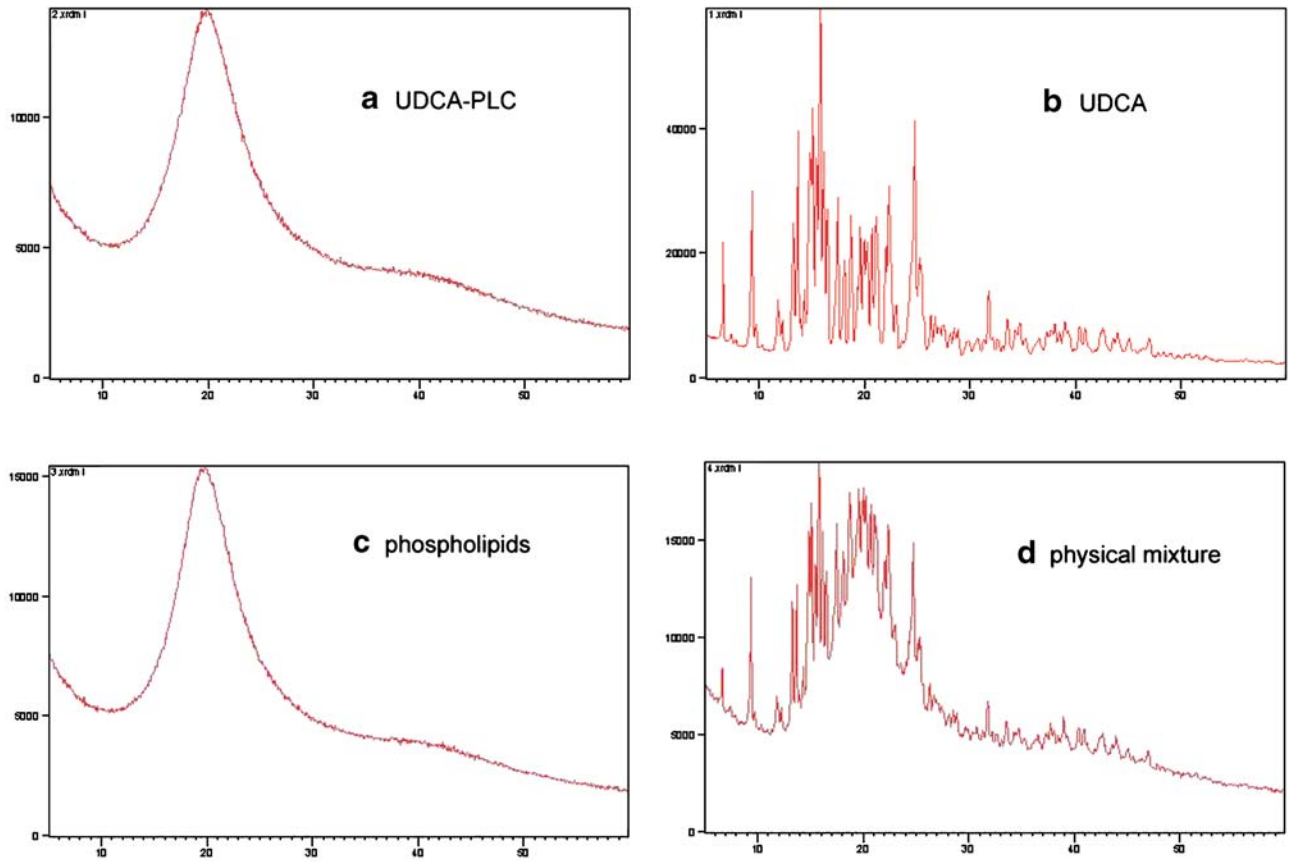

Fig. 4. X-ray diffractometry spectra of **a** complex, **b** UDCA, **c** phospholipids, **d** physical mixture

Table V. Apparent Solubility of UDCA, Physical Mixture and UDCA–PLC in Water and *n*-Octanol at 25°C, Respectively(Mean±SD, n=3)

Samples	Apparent solubility in water (mg ml^{-1})	Apparent solubility in *n*-octanol (mg ml^{-1})
UDCA	0.360±0.007	0.222±0.006
Physical mixture	0.886±0.011	1.299±0.037
UDCA–PLC	4.988±0.039	38.275±0.068

Validation of Model Optimization

In order to evaluate the optimization capability of the models generated according to the results of the circumscribed central composite design, the UDCA–PLC were prepared using the optimal process variable settings that X_1, X_2 and X_3 were equal to 3, 60°C and 3 h, respectively. The yield of UDCA "present as a complex" in the complex obtained with predicted models were shown in Table IV. The results showed good agreement on preparation properties with theoretical predictions.

Characterization of UDCA–PLC

Scanning Electron Microscopy

The surface morphology of UDCA–PLC as examined by SEM was illustrated in Fig. 3. Phospholipids complex which was made up of phospholipids and drugs appeared as amorphous particles. When at ×2,000 magnification, it could be seen that phospholipids did not exit on the appearance of drug but drugs uniformly dispersed in phospholipids and formed the structure of spherical particles.

X-ray Diffractometry

Figure 4 showed the powder X-ray diffraction patterns of UDCA, phospholipids, their physical mixture and the complex. The UDCA powder diffraction pattern shown in Fig. 4c displayed partial sharp crystalline peaks, which was the characteristic of a molecule with some crystallinity. In contrast, phospholipids shown in Fig. 4b were amorphous lacking crystalline peaks. Compared with that of the physical mixture, the crystalline peaks had disappeared in the complex shown in Fig. 4a. It was concluded that UDCA in the phospholipids lipid matrix presented as either molecularly dispersed or amorphous form. However, as seen in Fig. 4d, some crystalline drug signal was still detectable in the physical mixture of UDCA and phospholipids.

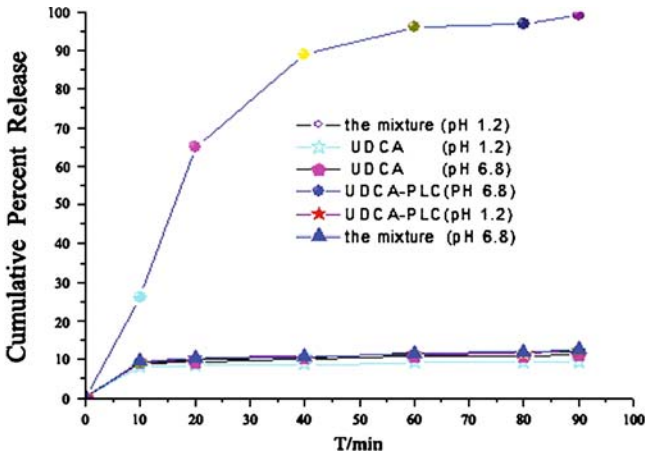

Fig. 5. Dissolution behaviors of UDCA–PLC, the physical mixture and UDCA material in HCl (pH 1.2) and phosphate buffer saline (pH 6.8), respectively

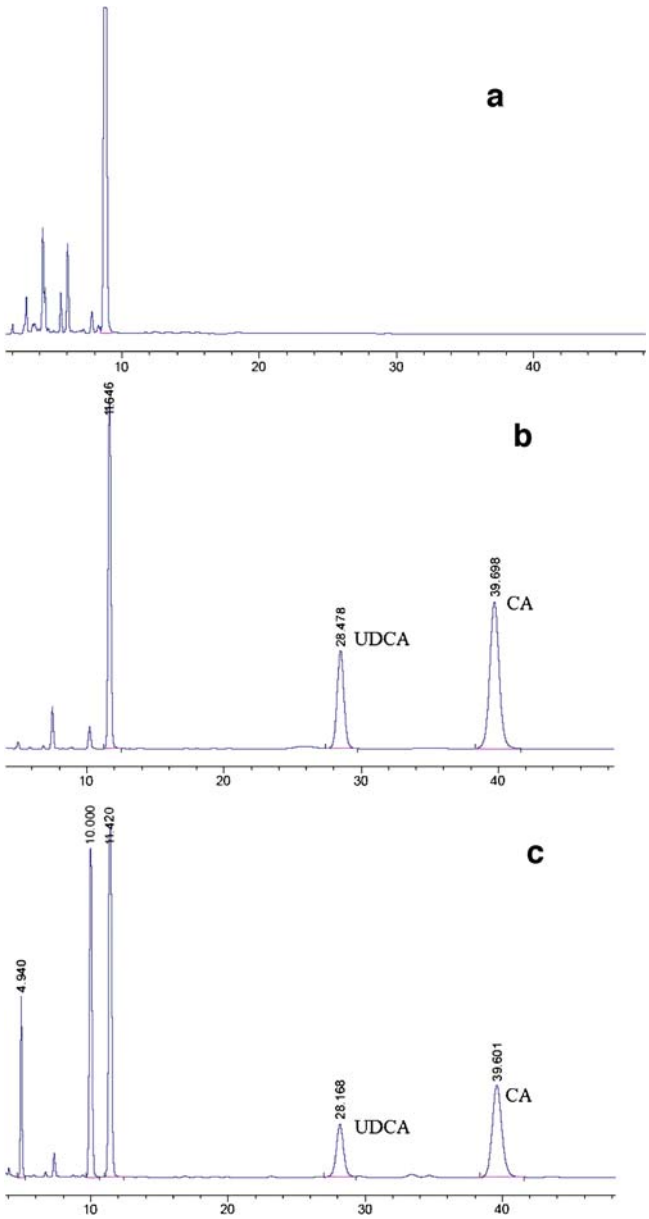

Fig. 6. Typical HPLC of blank rat serum (**a**); blank rat serum spiked with UDCA and internal standard (**b**); a sample after oral administration of UDCA–PLC (**c**)

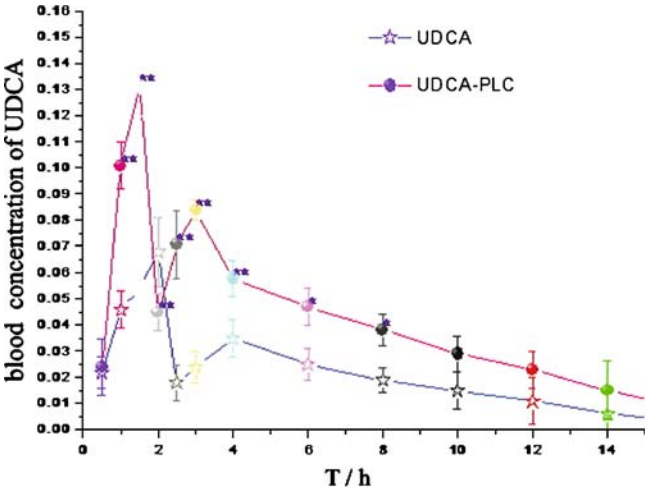

Fig. 7. Mean serum concentration–time curve (μg/ml) of UDCA in rats after oral administration of UDCA–PLC (*closed circle*) and UDCA Tablet(*open star*) equivalent to 20 mg/kg of UDCA, respectively. (Mean±SD, n=6/group/time point).*Double asterisk* $p<0.01$, *single asterisk* $p<0.05$

Solubility Study

Table V showed the solubility of UCDA, physical mixture and UCDA–PLC in water or *n*-octanol. The data showed the solubility of UCDA–PLC in *water* was about 14 multiples more than that of UCDA, the solubility of UCDA–PLC in *n*-octanol was about 190 multiples more than that of UCDA. These were due to the strong dispersibility or/and amorphous form of the phospholipids complex and polar group of UDCA were masked by phospholipids.

Dissolution in HCl (pH 1.2) and Phosphate Buffer Saline (pH 6.8)

Figure 5 showed the dissolution profile of UDCA from phospholipids complex, the physical mixture and UDCA material in 0.1 N HCl (pH 1.2) and phosphate buffer saline (pH 6.8), respectively. The dissolution of UDCA from phospholipids complex in pH 6.8 phosphate buffer saline was not complete until 80 min, the amount about 3.586 g; however, at about 40 min, the dissolution in 0.1 N HCl was completed, and the amount only 0.341 g. Figure 5 showed that the curves of phospholipids complex dissolution procedure were greatly influenced ($p<0.05$) by the pH of media, and the dissolution amount of UDCA was increased with increasing of the pH of media.

Pharmacokinetics Study of UDCA–PLC *In Vivo*

Solid phase extraction (SPE) methods have been routinely used for sample preparation from various body fluids containing bile acids (14). Bile acids did not show significant UV absorption. Neither did bile acids have fluorescent or electrochemical properties suitable for their sensitive and selective detection. UV detection at 200 nm was non-specific due to biological matrix interference. Pre-column labelling of bile acids with either a chromophore or fluorophore was usually necessary (15).

According to the literary sources (16) and in our experience, the most effective way to remove bile acids from blood samples was solid phase extraction (SPE) of a diluted serum. For the enhancing of the detector response and the specificity of the bile acid determination, a pre-column derivatization procedure based on the esterification of the carboxylic group with 2-bromo-2%-acetonaphthone (UV-absorbing chromophore) was developed (17). The derivatization procedure was performed in acetonitrile in the presence of disopropylethylamine (a scavenger of protons). Under these conditions the typical HPLC analyses were shown in Fig. 6. The analytical procedure involving the solid phase extraction, derivatization and HPLC analysis of the derivatized UDCA and CA (internal standard) was accurate and precise. The calibration curve was found to be linear $y=0.9444x+0.1495$ ($r=0.9992$, where x was the concentration ratio of UDCA to CA and y was the corresponding peak–area ratio UDCA/CA) in the 0.0086–0.146 μg/ml range. The accuracy and precision of UDCA determination (using CA as the internal standard) in spiked serum samples were found to be 102.96% and 7.6%, respectively. The mean UDCA plasma recovery was 93.6±1.24%. The R.S.D. in days were 3.18, 3.52 and 3.29%, respectively, the R.S.D. intra-days were 3.41, 3.74 and 3.45%, respectively, which showed the recoveries and RSD in days or intra-days were satisfying.

The mean serum concentrations of UDCA–PLC and UDCA Tablet were showed in Fig. 7 after oral administration. The pharmacokinetic parameters such as C_{max}, T_{max}, AUC_{0-24}, $AUC_{0-\infty}$ were given in Table VI. The results suggested that some of the pharmacokinetic parameters of UDCA–PLC were significantly different from those of UDCA Tablet. All formulations of UDCA–PLC showed higher C_{max} and higher $AUC_{0-\infty}$ values than those of reference. The relative bioavailability of UDCA–PLC ($AUC_{0-\infty}$) compared with UDCA Tablet ($AUC_{0-\infty}$) was 241%. The increase of the relative bioavailability of UDCA–PLC after oral administration might be due to the following reasons: Phospholipids were an important component of cell membrane, having the actions of keeping cell membrane fluidity. Compared with those of

Table VI. The Main Pharmacokinetic Parameters (mean±SD) of UDCA–PLC and UDCA Tablet in Rats (n=6/group/time point), Respectively[a]

Parameters	UDCA Tablet	UDCA–PLC
AUC_{0-24} (μg·h/ml)	3.308±0.859	7.846±0.518**
$AUC_{0-\infty}$ (μg·h/ml)	4.736±0.417	11.437±0.464**
T_{max} (h)	1.9±0.2	1.6±0.3
C_{max} (μg/ml)	0.0576±0.0137	0.1346±0.0313*

[a] Statistical significances with the UDCA phospholipids complex *vs* UDCA tablet are **$p<0.01$ and *$p<0.05$

UDCA, the P of UCDA–PLC in *n*-octanol–water was significantly increased. The lipophilicify of UCDA–PLC was effectively increased. In this case, improved bioavailability could be achieved by the use of delivery systems, which could enhance the rate and/or the extent of drug solubilizing into aqueous intestinal fluids. Phospholipids played a major role in drug delivery technology.

From the above profile, the C_{max} was 0.1346 μg/ml after oral administration of UDCA–PLC with a T_{max} of about 1.6 h. However, the average value of C_{max} was 0.0576 μg/ml after oral administration of UDCA Tablet with a T_{max} of about 1.9 h. The bioavailability of UDCA–PLC *in vivo* was significantly increased comparing to those of UDCA Tablet ($p<0.05$). And the $AUC_{0-\infty}$ of UDCA–PLC was 2.41 multiples than that of UDCA Tablet ($p<0.05$). The blood concentrations of UDCA of rats after oral administration of UDCA–PLC were consistently higher at sampling time points (such as 10 h after administration).

The pharmacokinetic data were simulated by non-linear least squares. The results showed that the open two-compartment model was fitted to UDCA–PLC and UDCA Tablet serum concentration–time course *in vivo* of rats. The presence of secondary peaks in the serum concentration–time profile was typical for drugs with enterohepatic recirculation. These are consistent with those from previous study (5).

CONCLUSIONS

In this paper, the results of central composite design study confirmed that the values of phospholipid-to-drug ratio (X_1), reaction temperature (X_2, °Celsius) and the reaction time(X_3, hours) were significantly influenced the dependent variable the yield (%) of UDCA "present as a complex" in the complex. The solubility and dissolution studies indicated that UDCA–PLC displayed a satisfactory solubility and dissolution. The UDCA–PLC markedly improved the bioavailability of UDCA *in vivo* of rats. So the phospholipids complex systems are preferred as drug carriers for poorly soluble drugs to overcome these drugs' oral bioavailability problems.

ACKNOWLEDGEMENTS

The authors wish to thank the Chinese Medicine Modernization Foundation of Shanghai City for financial support (No. 05DZ19711).

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