Process optimization, characterization and evaluation in vivo of oxymatrine–phospholipid complex

Peng-Fei Yue [a,b,*], Hai-Long Yuan [c,**], Xian-Yi Li [c], Ming Yang [a], Wei-Feng Zhu [a]

[a] Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China
[b] Key Lab of Modern Preparation of TCM, Ministry of Education, Nanchang 330004, China
[c] 302 Hospital of PLA&PLA Institute of Chinese Materia Medica, Xisihuan Road, Beijing 100039, China

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ABSTRACT

The objective of this study was to prepare oxymatrine–phospholipid complex (OMT–PLC) to enhance oral bioavailability of oxymatrine. A central composite design approach was used for process optimization. The physicochemical properties of the complex obtained by optimal parameters were investigated by means of differential scanning calorimetry (DSC), X-ray diffraction (XRD) and N-octanol/water partition coefficient. Compared with those of the physical mixture or oxymatrine, the hepatocytes permeability of oxymatrine–phospholipid complexes was studied. The concentrations of oxymatrine after oral administration of OMT–PLC at different time in rats were determined by HPCE. Multiple linear regression analysis for process optimization revealed that the acceptable OMT–PLC was obtained wherein the optimal values of X_1, X_2 and X_3 were 3, 60 °C and 3 h, respectively. The oxymatrine and phospholipids in the OMT–PLC were combined by non-covalent bond, not forming a new compound. The better hepatocytes permeability was obtained by the OMT–PLC. Pharmacokinetic parameters of the complex in rats were T_{max} 2.17 h, C_{max} 0.437 μg ml^{-1}, AUC$_{0-\infty}$ 9.43 μg h ml^{-1}, respectively. The bioavailability of oxymatrine in rats was increased remarkably after oral administration of OMT–PLC ($p < 0.05$), compared with those of oxymatrine or the physical mixture. This was mainly due to an improvement of the solubility of OMT–PLC.

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1. Introduction

Oxymatrine (OMT, Fig. 1) is a kind of alkaloid extracted from a Chinese herb (*Sophora alopecuraides* L.) (Lai et al., 2003). Basic and clinical researches suggested that oxymatrine had the following pharmacological effects such as anti-virus, protecting hepatocytes, anti-hepatic fibrosis and immune regulation (Liu et al., 2003; Dong et al., 2002; Xiang et al., 2002; Yang et al., 2002; Chen et al., 2001a; Li et al., 1998). In particular, wide attention is attracted to its inhibitory effect on hepatitis B virus (HBV) in recent years. Oxymatrine has been proved to have distinct anti-virus effect in the treatment of chronic hepatitis B (CHB). However, the slight liposolubility of oxymatrine resulted in the poor permeation across the intestinal epithelial cells and minor the gastro-intestinal (GI) tract absorption in rats (Chen et al., 2001b, 2002; Yu et al., 2002; Wang, 2000). There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow 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. Phospholipids are an important component of cell membrane, having the actions of keeping cell membrane fluidity and treating hepatic disorder. In this paper, OMT–phospholipid complex (OMT–PLC) was studied in order to improve oral bioavailability of OMT.

The objective of this study is: (1) OMT–PLC was prepared by a simple method. To get the acceptable OMT–PLC, a central composite design approach was used for optimization of process variables on the yield (%) of OMT "present as a complex" in the complex. The joint influence of the independent variables, phospholipid-to-drug ratio (X_1), reaction temperature (X_2, °C) and the reaction time (X_3, h) on the dependent variable the yield (y, %) of OMT "present as a complex" was investigated; (2) the physicochemical characters of OMT–PLC were evaluated, such as DSC, XRD. The n-octanol/water partition coefficient (P) study of OMT–PLC was performed in order to evaluate the improved solubility properties of OMT–PLC in comparison with OMT material and the physical mixture; (3) compared with those of the physical mixture or oxymatrine, the hepatocytes permeability of oxymatrine–phospholipid complexes was studied; (4) after oral administration of three formulations:

* Corresponding author at: Jiangxi University of Traditional Chinese Medicine, Nanchang, China.
** Corresponding author at: 302 Hospital of PLA&PLA Institute of Chinese Materia Medica, Beijing, China.
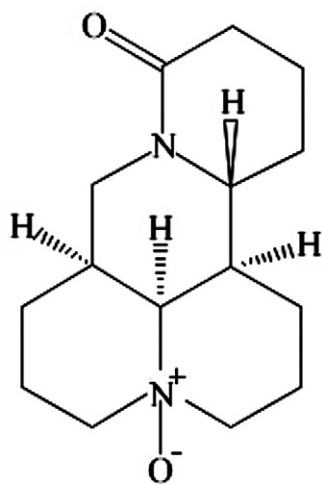E-mail address: ypfpharm@126.com (P.-F. Yue).

Fig. 1. The chemical structure of OMT.

OMT, the physical mixture and OMT–PLC, the pharmacokinetics and bioavailability of OMT in rats were studied. The formulation OMT–PLC, which exhibits comparable to the commercial products or even higher bioavailability, might be clinical candidate for future clinical study.

2. Materials and methods

2.1. Materials

Oxymatrine was purchased from Ningxia-bo-er-tai-li Ltd(Ningxia, China), purity 99.13%, and phospholipid was purchased from Hua-qing-mei-hen Ltd. (Beijing, China), and the phosphatidyl content was approximately 60% (w/w).

2.2. Preparation of oxymatrine–phospholipid complexes

The phospholipids and OMT (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 per mg OMT). 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 40 °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 OMT–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.

2.3. The yield of OMT "present as a complex" (%)

The OMT–PLC prepared as above description was dispersed in sufficient chloroform (5 ml/mg OMT–PLC). The complex and phospholipids both were easily dissolved in the chloroform (Li et al., 2006), but the OMT was practically insoluble in the chloroform. The OMT non-complexed was sedimentated and separated to assay. The yield of OMT "present as a complex (%) was determined using following formula equation (1):

$$\text{The yield} = \frac{a - b}{a} \times 100\% \tag{1}$$

Table 1
Factor levels for the experimental design.

Factors	Levels				
	−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

where a was the content of OMT "present as a complex", b was the content of OMT "no-present as a complex" in the complex.

2.4. Determination of the content of oxymatrine in phospholipids complex

The content of oxymatrine in phospholipids complex was determined as follows. Approximately 5 mg of phospholipids complex were dissolved in 50 ml of solvent A (acetonitrile:dehydrated alcohol = 80:20, v/v), and a 20 μl aliquot of the resulting solution was injected into a HPLC system. The stationary phase, NH_2 column (150 mm × 4.6 mm, 5 μm), was kept at 25 °C. The mobile phase was a mixture of acetonitrile:dehydrated alcohol:3% H_3PO_4 (80:10:10, v/v). The flow rate was 1.0 ml/min. Effluent was monitored at 220 nm.

2.5. 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, °C) and the reaction time (X_3, h) on the dependent variable the yield. In this design, 3 factors were evaluated and experimental trials were performed at all 20 possible combinations (Krogars et al., 2000; Dévay et al., 2000). A statistical model incorporating interactive and polynomial terms was used to evaluate the response employing the formula equation (2):

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

where 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 3 factors were simultaneously changed. The polynomial terms (X_1^2, X_2^2 and X_3^2) were included to investigate non-linearity. The level values of three factors and the composition of the central composite design batches 1–20 were shown in Tables 1 and 2.

2.6. Characterization of OMT–PLC

2.6.1. Differential scanning calorimetry (DSC)

The samples sealed in the aluminum crimp cell were heated at the speed of 5 °C ml^{-1} from 0 to 300 °C in the atmosphere of nitrogen (Dupont 1090B, Dupont, USA). Peak transition onset temperature was determined by means of an analyzer. The peak transition onset temperatures of phospholipids, pure oxymatrine, the mixture of phospholipids and oxymatrine and the oxymatrine–phospholipids complex were compared.

Table 2
Composition of central composite design batches ($n = 3$, mean ± SD).

Batches	X_1	X_2	X_3	Yield (%)
1	−1	−1	−1	62.77 ± 1.34
2	+1	−1	−1	90.51 ± 1.46
3	−1	+1	−1	67.41 ± 1.52
4	+1	+1	−1	95.15 ± 2.01
5	−1	−1	+1	65.57 ± 0.72
6	+1	−1	+1	91.09 ± 1.14
7	−1	+1	+1	68.44 ± 0.67
8	+1	+1	+1	93.82 ± 1.34
9	−1.732	0	0	50.32 ± 1.21
10	+1.732	0	0	94.02 ± 0.75
11	0	−1.732	0	77.54 ± 1.31
12	0	+1.732	0	86.94 ± 1.17
13	0	0	−1.732	84.81 ± 1.61
14	0	0	+1.732	82.64 ± 0.57
15	0	0	0	83.54 ± 0.82
16	0	0	0	84.62 ± 1.03
17	0	0	0	82.83 ± 1.17
I8	0	0	0	83.78 ± 0.92
19	0	0	0	84.32 ± 1.03
20	0	0	0	83.96 ± 1.21

2.6.2. 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.

2.6.3. N-octanol/water partition coefficient (P) of OMT–PLC

P of OMT determination of OMT material, phospholipids complex or the physical mixture was carried out by adding 0.2 g of OMT material, phospholipids complex or physical mixture to a series of 10 ml water solutions (pH 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5) in sealed glass containers at 25 °C, respectively. Each experiment was performed in triplicate. All the 63 liquids were agitated for 24 h and centrifuged to remove excessive residues (15 min, 4000 rpm), respectively. Each liquid was added 10 ml n-octanol and agitated for 24 h. Then they were centrifuged at 4000 rpm for 15 min, respectively. The water phase and n-octanol phase were separated. The water phase and n-octanol phase were filtrated through a 0.45 μm membrane, respectively. The 1 ml filtrate was mixed with 9 ml of methanol and a 20 μl aliquot of the resulting solution was injected into a HPLC and detected as the previous description, the concentrations of OMT were measured, respectively.

P of OMT of OMT material, physical mixture and phospholipids complex were calculated as follows:

$$P = \frac{C_o}{C_w} \tag{3}$$

where C_o was the concentration of OMT material, OMT–PLC or the physical mixture in n-octanol; C_w was the concentration of OMT material, OMT–PLC or its physical mixture in water.

2.6.4. Hepatocytes permeability studies

Rat hepatocytes were prepared by collagenase perfusion as previously described by Fariss et al. (1985). Hepatocyte suspensions (5×10^5 cells ml^{-1}, 24 ml total) were prepared in modified RPMI1640 medium (Fariss et al., 1989). Hepatocyte suspensions were transferred to a 24-well plate. The cells were then placed in a humidified 5% CO$_2$:95% air incubator at 37 °C. After 24 h culture, the medium was replaced with fresh medium and

oxymatrine–phospholipid complexes, the physical mixture or oxymatrine. At the predetermined time-points, aliquots containing cells were withdrawn and centrifuged at $1000 \times g$ for 5 min. Cell pellets were resuspended in 3 ml isotonic Na chloride and immediately centrifuged at $1000 \times g$ for 5 min. The proteinum was sedimented and centrifuged. Aliquots (20 μl) of the supernatant were injected for HPLC analysis.

2.7. In vivo evaluation of OMT–PLC

2.7.1. Rat bioavailability experiments

2.7.1.1. Pharmacokinetic studies of OMT–PLC and oxymatrine in rats. Eighteen male rats (body weight 180–220 g) divided randomly into three groups were fasted for 12 h, but allowed to take water freely. A sample equivalent to 100 mg/kg of oxymatrine phospholipids complex suspended in 2 ml of water was orally administered to one group of rats. The solutions of oxymatrine and physical mixture equivalent to 100 mg/kg of oxymatrine were orally administered to the other group of rats, respectively. The pharmacokinetical parameters were computed by software program 3p87.

2.7.1.2. Plasma sample preparation and validity. The rats were anaesthetized with aether, and 500 μl blood was taken from the eyeground veins. The plasma obtained after centrifugation (15 min, 4000 rpm) was stored at −20 °C until analyzed. When the plasma sample was thawed, 50 μl of cimetidine solution (CMD, 1.4 mg ml^{-1}, internal standard), 100 μl of 1 M Na$_2$CO$_3$ solution and 500 μl of borate buffer saline (pH 8.0) were added, and agitated for 30 s. After 4 ml aether was added to the solution above, this mixture was shaken for 3 min and then centrifuged (15 min, 4000 rpm). The organic phase was quantitatively decanted into a clear tapered centrifuging tube and the eluate was evaporated under nitrogen at 37 °C. The residues were resuspended in 100 μl of mobile phase and centrifuged (15 min, 4000 rpm). Aliquots (20 μl) of the supernatant were injected for HPCE analysis.

2.7.1.3. Capillary electrophoresis. Capillary electrophoresis was performed on a HPCE instrument (Trisep-2010) equipped with a UV detector set at 205 nm (Luo Ming et al., 1999). Separation and analysis were carried out on an uncoated fused-silica capillary tube (50 μm I.D., 56 cm total length and 36 cm from the injection point to the detector) at 25 °C. Before each run, the capillary tube was washed with 0.1 M NaOH for 5 min, bidistilled water for 5 min, and then with the operating buffer tris-hydroxymethyl aminomethane (Tris, 40 mM)–sodium dihydrogen phosphate (10 mM)–4% avantin buffered at pH 7.6 for 5 min. The operating buffer used was degassed by vacuum filtration through a 0.2 μm membrane filter, followed by agitation in an ultrasonic bath. The samples to be analyzed were injected automatically, using the pressure injection mode, in which the sample is pressurized for 3 s. The electrophoresis was performed at 20 kV (about 50 mA) using normal polarity.

3. Results and discussion

3.1. Preparation of oxymatrine–phospholipid complex

Preliminary investigations of the process parameters revealed that factors phospholipid-to-drug ratio (X_1), reaction temperature (X_2, °C) and the reaction time (X_3, h) highly influenced the yield of

Table 3
Results of regression analysis—indicates the term was omitted in reduced model.

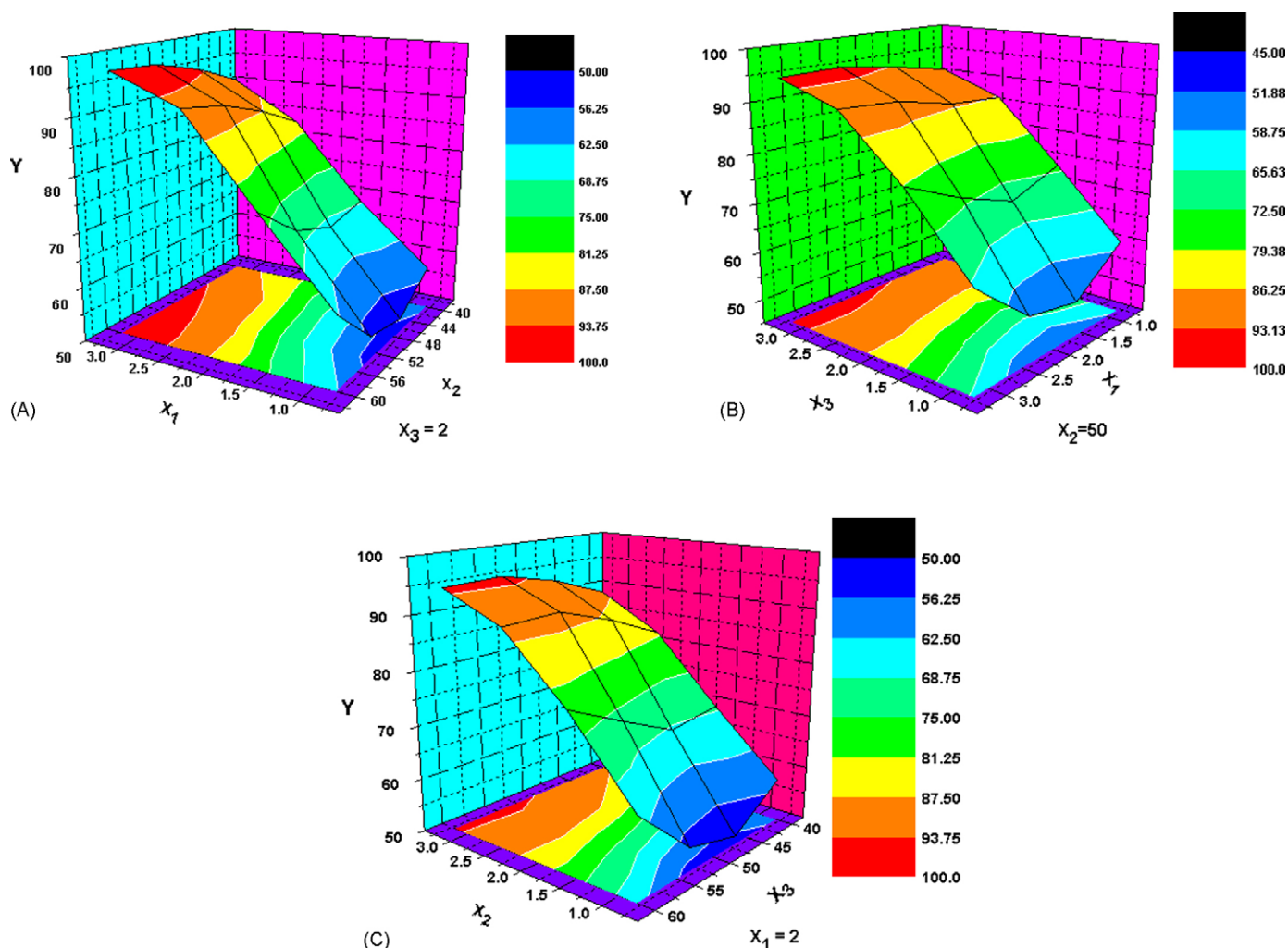| Respose	b_0	b_1	b_2	b_3	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}	R^2
Full model (FM)	−107.634	75.448	3.183	−4.507	−12.9698	−0.0104	1.063	0.0104	−0.3854	−0.0104	0.869
Reduced mode (RM)	−96.098	73.981	3.169	−5.561	−13.079	−0.028	1.776	—	—	—	0.986

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

OMT "present as a complex" in the complex. The yield (%) for the 20 batches showed a wide variation of 50.32–95.15% (Table 3). The fitted polynomial equations (full and reduced model) relating the response yield (%) to the transformed factors are shown in Table 3. 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_{23} and b_{13} were found to be P equals 0.1796, 0.4047 and 0.7948, respectively. So those were omitted from the full model equation to generate the reduced model equation. The coefficients b_0, b_1, b_2, b_3, b_{11}, b_{22} 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 (0.986) of the reduced model was significantly more than that (0.869) of the full model. Multiple linear regression analysis (Table 3) revealed that 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 OMT (X_1), reaction temperature (X_2, °C) and reaction time (X_3, h) obtained from response surface were 3, 60 °C and 3 h, respectively.

3.2. 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 OMT–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 OMT "present as a complex" in the complex obtained with predicted models were shown in Table 4. The results showed good agreement on preparation properties with theoretical predictions.

Table 4
Model-predicted and observed values of the yield (%) of OMT "present as a complex" in the OMT–PLC prepared according to the optimal parameters.

Predicted yield (%)	Observed yield (%)	Bias (%)[a]
95.70	94.65 ± 1.24	−1.10

[a] Bias was calculated according to equation: bias/% = (predicted value − observed value)/predicted value × 100%.
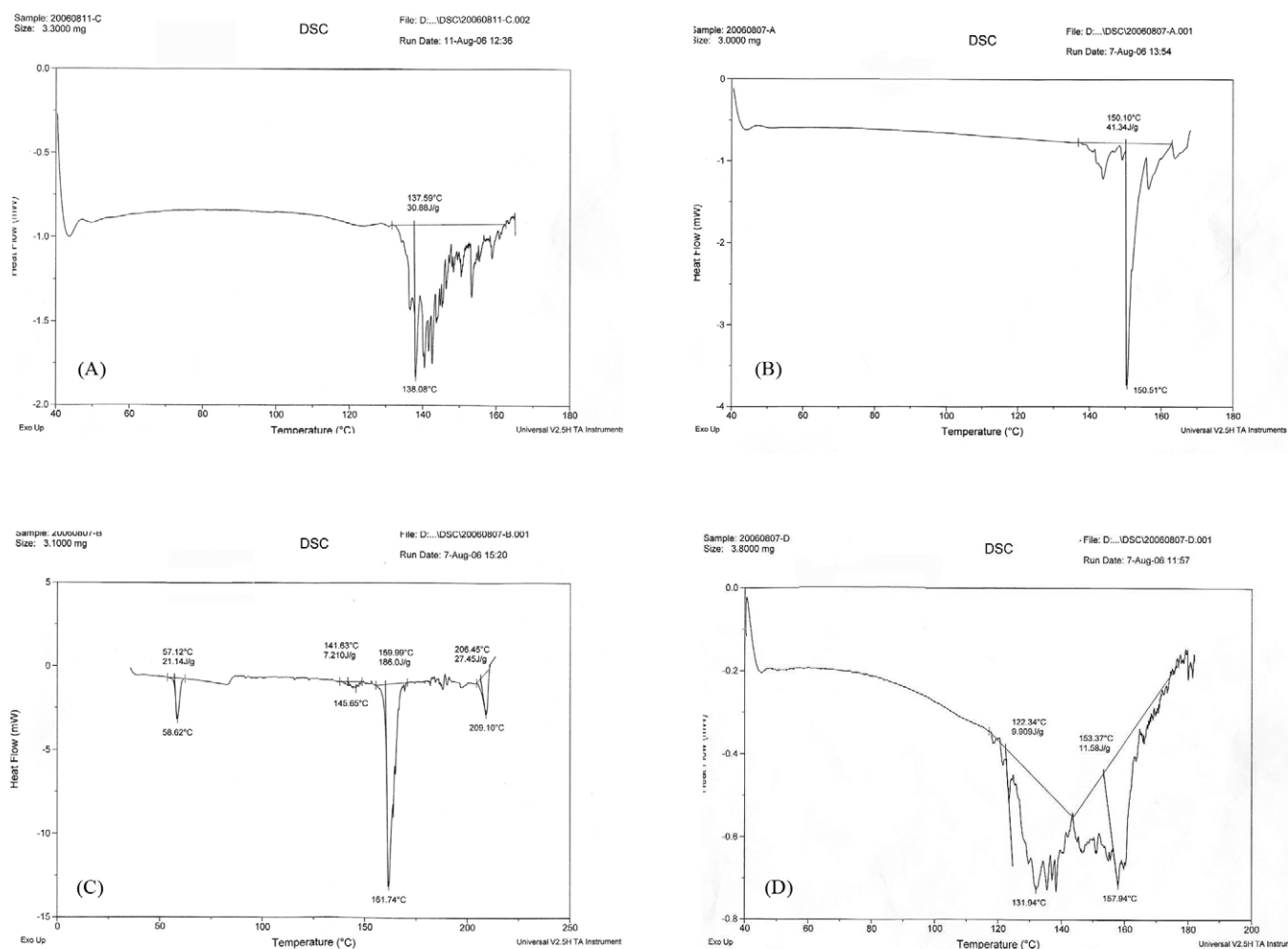
Fig. 3. DSC thermograms of phospholipids complex (A), phospholipids (B), oxymatrine (C) and physical mixture (D).

3.3. Differential scanning calorimetry

Fig. 3 showed the DSC curves of phospholipids, oxymatrine physical mixture and phospholipids complex. DSC of phospholipids complex showed the endothermal peaks of oxymatrine and phospholipid are disappeared and the phase transition temperature is lower than the phase transition temperature of phospholipids, it was considered that oxymatrine and phospholipids should have some interaction, such as the combination of hydrogen bonds or van der Waals force (Venema and Weringa, 1988; Lasonder and Weringa, 1990). After the combination of oxymatrine and the phospholipids molecule polarity parts, the carbon–hydrogen chain in phospholipids could turn freely and enwrap the phospholipids molecule polarity parts, which made the sequence decrease between phospholipids aliphatic hydrocarbon chains, made the second endothermal peak of phospholipids disappear and depressed the phase transition temperature.

3.4. X-ray diffractometry

Fig. 4 showed the powder X-ray diffraction patterns of oxymatrine, phospholipids, their physical mixture and the complex. The oxymatrine powder diffraction pattern shown in Fig. 4(c) displayed partial sharp crystalline peaks, which is the char-

acteristic of a molecule with some crystallinity. In contrast, phospholipids shown in Fig. 4(b) were amorphous lacking crystalline peaks. Compared with that of the physical mixture, the crystalline peaks had disappeared in the complex shown in Fig. 4(a). It was concluded that oxymatrine in the phospholipids lipid matrix was either molecularly dispersed or amorphous form. However, as seen in Fig. 4(d), some crystalline drug signal was still detectable in the physical mixtures of oxymatrine and phospholipids.

3.5. N-octanol/water partition coefficient (P) of OMT–PLC

Table 5 showed the n-octanol/water partition coefficient (P) of OMT, the physical mixture and OMT–PLC at different pH. The data showed that OMT–PLC significantly increased the lipophilicify of OMT, and P of OMT–PLC in n-octanol and water was about 10 multiples more than that of OMT material ($p < 0.05$), these were due to the strong dispersibility or/and amorphous form of the phospholipids complexes and polar group of OMT were masked by phospholipids. However, P of OMT in physical mixture was about 4 multiples more than that of OMT material ($p < 0.05$), because the phospholipids slightly improved the hydrophilicity and lipophilicify of OMT in physical mixture by means of its solubilization.
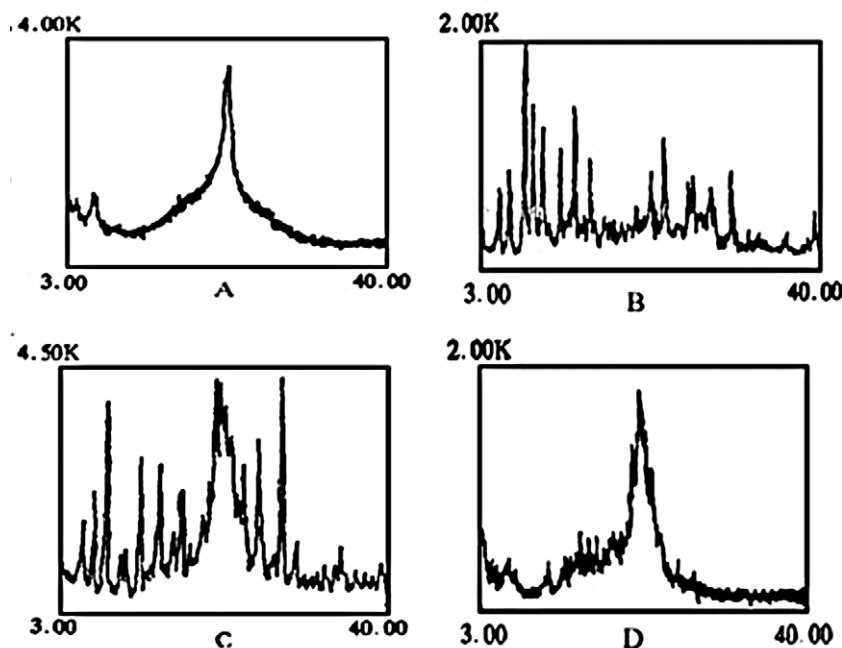
Fig. 4. X-ray diffractometry spectra of (A) complex, (B) oxymatrine, (C) phospholipids, and (D) physical mixture.

Table 5
N-octanol/water partition coefficient (P) of oxymatrine, physical mixture and OMT–PLC at different pH. Values are mean ± S.D. ($n=3$).

Samples	pH	C_w (g ml^{-1})	C_o (g ml^{-1})	P (C_o/C_w)
	1.5	0.183 ± 0.0098	0.022 ± 0.0038	0.120
	2.5	0.175 ± 0.0086	0.024 ± 0.0046	0.137
	3.5	0.173 ± 0.0034	0.023 ± 0.0073	0.133
OMT	4.5	0.165 ± 0.0007	0.034 ± 0.0057	0.206
	5.5	0.168 ± 0.0097	0.037 ± 0.0068	0.220
	6.5	0.164 ± 0.0086	0.031 ± 0.0038	0.189
	7.5	0.167 ± 0.0067	0.033 ± 0.0043	0.197
	1.5	0.136 ± 0.0076	0.066 ± 0.0075	0.485
	2.5	0.137 ± 0.0084	0.069 ± 0.0037	0.503
	3.5	0.139 ± 0.0039	0.068 ± 0.0028	0.489
Physical mixture	4.5	0.133 ± 0.0059	0.066 ± 0.0063	0.496
	5.5	0.142 ± 0.0065	0.057 ± 0.0052	0.401
	6.5	0.148 ± 0.0098	0.055 ± 0.0084	0.372
	7.5	0.147 ± 0.0056	0.047 ± 0.0057	0.320
	1.5	0.074 ± 0.0050	0.125 ± 0.0063	1.69
	2.5	0.063 ± 0.0185	0.133 ± 0.0057	2.11
	3.5	0.064 ± 0.0044	0.127 ± 0.0052	1.98
OMT–PLC	4.5	0.066 ± 0.0050	0.138 ± 0.0057	2.091
	5.5	0.065 ± 0.0015	0.129 ± 0.0036	1.984
	6.5	0.064 ± 0.0089	0.128 ± 0.0073	2.03
	7.5	0.066 ± 0.0088	0.127 ± 0.0067	1.924

3.6. Hepatocytes permeability

Table 6 showed that the hepatocytes permeability of the complex was about 10 multiples than of oxymatrine at the predetermined time-points ($p < 0.05$). The data showed that the hepatocytes permeability of oxymatrine was increased remarkably by formation of phospholipids complex. This might be achieved by meas of delivery systems, which could enhance the rate and/or the extent of drug solubilizing into hepatocytes fluids. Phospholipids played a major role in drug delivery technology. The mechanism about increasing hepatocytes permeability is being further studied.

3.7. Rats bioavailability

Oxymatrine in plasma was completely separated under analytical conditions (Fig. 5). The calibration curve was found to be linear

Table 6
Hepatocytes permeability of the complex and oxymatrine.

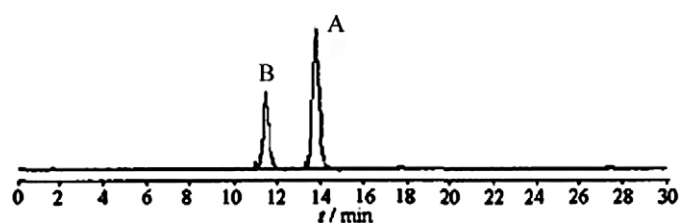| Culture time (min)	Complex (peak area)	Physical mixture	Oxymatrine (peak area)
6	1223.4 ± 54.71	245.95 ± 64.47	109.4 ± 36.33
12	2956.2 ± 63.53	547.71 ± 57.63	423.6 ± 38.69
24	7312.5 ± 132.22	756.83 ± 58.49	635.4 ± 50.37

Values are mean ± S.D. ($n=3$).

Fig. 5. Typical HPCE of oxymatrine (A) with internal standard (cimetidine, B) after oral administration of oxymatrine–phospholipids complex.
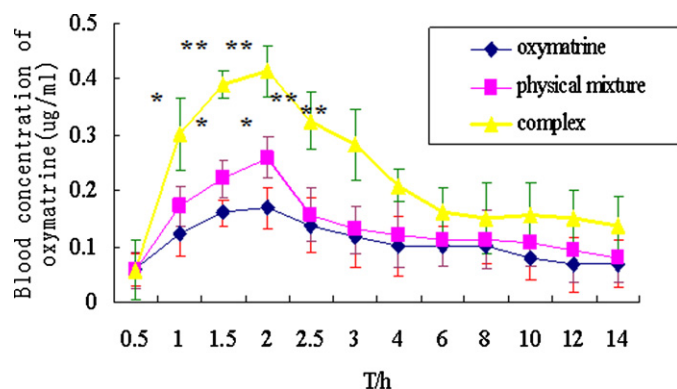

Fig. 6. Mean plasma concentration–time curve of oxymatrine in rats after oral administration of oxymatrine–phospholipid complex, oxymatrine and physical mixture equivalent to 100 mg/kg of oxymatrine ($n = 6$), respectively. Values are mean ± SD ($n = 6$/group/time point). $^*p < 0.05$ and $^{**}p < 0.01$ are statistical significances with the OMT–PLC versus OMT or physical mixture.

$13.501x - 0.046$ ($r = 0.9992$, where x is the concentration ratio of OTM to CA and y is the corresponding peak-area ratio OMT/CA) in the $0.0179–0.1790$ mg ml^{-1} range. The results attained from the method recoveries of high, middle and low concentrations were 85.15, 87.11 and 88.37%, respectively. 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 that recoveries and R.S.D. in days or intra-days were acceptable, and the lowest detection limit was 35 ng ml^{-1}.

Fig. 6 showed the sample equivalent to 100 mg/kg of oxymatrine of phospholipids complex and oxymatrine were orally administered to rats ($n = 6$), respectively. From the above profile and Table 7, it was known that the average value of C_{max} is 0.437 μg ml^{-1} after oral administration of phospholipids complex with a T_{max} of about 2.17 h. However, the average value of C_{max} was 0.164 μg ml^{-1} after oral administration of oxymatrine solution with a T_{max} of about 1.71 h, the average value of C_{max} was 0.247 μg ml^{-1} after oral administration of the physical mixture with a T_{max} of about 1.91 h. The average value of AUC$_{0-\infty}$ of oxymatrine, the physical mixture and the complex in rats were 2.87, 3.23 and 9.43 μg h ml^{-1}, respectively. And the AUC$_{0-\infty}$ of OMT–PLC was 3.29 multiples than those of oxymatrine. The relative bioavailability of OMT–PLC (AUC$_{0-\infty}$)

Table 7
The main pharmacokinetic parameters of phospholipids complex, physical mixture and oxymatrine in rats ($n = 6$).

Parameters	Oxymatrine	Physical mixture	Complex
AUC$_{0-24}$ (μg h ml^{-1})	1.97 ± 0.218	2.42 ± 0.371	6.21 ± 0.859*
AUC$_{0-\infty}$ (μg h ml^{-1})	2.87 ± 0.417	3.23 ± 0.317	9.43 ± 0.384*
CL (ml h^{-1})	4.72 ± 0.45	4.83 ± 0.76	4.97 ± ±0.85
T_{max} (h)	1.71 ± 0.54	1.91 ± 0.64	2.17 ± 0.46
C_{max} (μg ml^{-1})	0.164 ± 0.045	0.247 ± 0.075	0.437 ± 0.083*

$^*P < 0.01$ are statistical significances with the OMT-PLC versus OMT or physical mixture.

compared with OMT (AUC$_{0-\infty}$) was 329%. The increase of the relative bioavailability of OMT–PLC after oral administration might be due to the following reasons: (1) phospholipids were an important component of cell membrane, having the effects of keeping cell membrane fluidity. (2) Compared with those of OMT, the P of OMT–PLC in n-octanol—water was significantly increased. The lipophilicify of OMT–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 absorbing into intestinal mucosa. Phospholipids played a major role in drug delivery technology. (3) It was reported that the OMT could be partly metabolished as matrine by intestinal bacteria in vivo (Wang et al., 2005; Menggendalai et al., 2003). This might decrease the bioavailability and activity of the oxymatrine. But the OMT–PLC might improve the absorption of oxymatrine and decrease the metabolism of oxymatrine in vivo in order to increase the bioavailability of oxymatrine in vivo. However, the mechanism on decreasing the metabolism of OMT by this drug delivery system is still under studying.

The pharmacokinetical data were simulated by non-linear least squares. The results showed that open two-compartment model and 1st-order absorption were fitted to both phospholipids complex and oxymatrine plasma concentration–time course in vivo of rats.

4. Conclusion

In this study, the results of central composite design study confirmed that the values of phospholipid-to-drug ratio, reaction temperature and the reaction time were significantly influenced the dependent variable the yield (%) of OMT "present as a complex" in the complex. DSC and XRD curves of phospholipids complex showed that OMT and phospholipids combined and formed some kind bond, such as hydrogen bonds or van der Waals force. The N-octanol/water partition coefficient (P) of OMT–PLC studies showed OMT–PLC surprisingly increased the lipophilicify of OMT. Compared with OMT material and the physical mixture, the phospholipid complex can significantly improve the bioavailability of OMT in vivo of rats. It would be further studied about the absorbed mechanism of OMT–PLC through small intestine and therapeutic evaluation in vivo. The OMT–PLC would be more prospective preparation in future. Our study could be suitable references for the clinical application of oxymatrine phospholipid complex.

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