AAPS PharmSciTech, Vol. 19, No. 8, November 2018 (© 2018)
DOI: 10.1208/s12249-018-1157-2

Research Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
Guest Editor: Sanyog Jain

Preparation and Evaluation of Probucol-Phospholipid Complex with Enhanced Bioavailability and No Food Effect

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Received 22 April 2018; accepted 18 August 2018; published online 20 September 2018

Abstract. To enhance the oral bioavailability and eliminate the food effect of probucol. Probucol-phospholipid complex was prepared using solvent-evaporation method in this research. Several methods were used to validate the formation of complexes, such as FT-IR, SEM, DSC and PXRD, and the solubility of PRO and PRO-PLC was detected by HPLC. Pharmacokinetic testing was conducted in the fasted and fed state. FTIR, SEM, DSC and PXRD validated the existence of PRO-PLC. The solubility of PRO in complexes was 15.05 μg/mL, which was 215-fold of the PRO-API. The dissolution rate was increased by preparing PRO-PLC. Compared with commercial tablets, the PRO-PLC complexes exhibited higher peak plasma concentration (1.69 ± 0.44 μg/mL), increased $AUC_{0-24\ h}$ (6.8 ± 1.3 μg/mL h), which mean the bioavailability of PRO was increased. In addition, the absorption of PRO was not interfered with food. In conclusion, an improved solubility and bioavailability was achieved with the preparation of PRO-PLC. Additionally, the dissolution behaviour was good and the food effect was eliminated.

KEY WORDS: solubility; bioavailability; food effect; phospholipid complex; probucol.

INTRODUCTION

Probucol (PRO) is an anti-hyper lipidemic drug initially developed in the treatment of coronary artery disease, which lowers the level of cholesterol in the bloodstream (1). Furthermore, the inhibition effects of PRO can be reflected for reducing the absorption rate of cholesterol (2) and inhibiting the pro-oxidation effect of LDLs (3).

The water solubility of PRO is only 2–5 μg/L, which has restricted its absorption in gastrointestinal tract (4). However, the absorption of PRO can be increased if taking it immediately after a meal. In previous researches, researchers studied the pharmacokinetics of PRO in the fed state, and the result indicated that the bioavailability was higher than it in the fasted state (5). As a result, it is certainly worth developing a new formulation of PRO, which can increase its oral absorption and eliminate the food effect.

In order to improve the solubility of indissolvable drug, many technologies can be adopted, such as solid dispersion, nanoparticles (6), lipid and surfactant based dispersions (7), and self-emulsified drug delivery system (8). However, these technologies may be harmful to human, for the drug concentration in plasma may be closed to toxic level. As a result, it is necessary to design a suitable formulation of PRO to improve its bioavailability and eliminate the food effect (9).

Phospholipid is a kind of biofunctional surfactants which can improve the solubility and bioavailability of poor water-soluble drug (10–13). In addition, it can easily assimilate into the body with no rejection effect, for it is one part of airframe structures (14). The mechanism of the formation of phospholipid complex is related to the gain and lose of electrons between drug and phospholipid (15). This activity is manifested even against the background of significant changes in the structure of substituents, in particular in cases where the substituents create steric hindrance, preventing the molecules from approaching a target object to a distance necessary for hydrogen bond formation. This feature of the interaction indicates that phospholipid interact with targets by a mechanism that is weakly dependent on the structure and position of substituents.

The study aimed to prepare PRO-phospholipid complex (PRO-PLC), which can increase bioavailability and eliminate the food effect. Solvent-evaporation method was used in the study. Several characterization techniques were used to validate the formation of PRO-PLC, such as Fourier transform infrared spectroscopy (FT-IR), differential thermal scanning (DSC), powder X-ray diffraction (PXRD) and scanning electron microscopy (SEM). The *in vitro* dissolution

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and *in vivo* bioavailability were also studied between PRO-PLC and commercial tablets.

MATERIALS AND METHODS

Materials

PRO was provided by Jiangsu TianQing Pharmaceutical Co., Ltd. (Nanjing, China). The commercial tablets were attained from Wuhan LaNaBai Pharmaceutical Co., Ltd. (125 mg). Phospholipid (soy lecithin) was a gift of YongHua Biology Technique Ltd. (Shanghai, China) and a 0.45 μm filter was purchased from PALL (New York, USA).

Preparation of Probucol-Phospholipid Complex

Investigation of Solvent

The solubility of PRO will affect its absorption *in vivo*, so a suitable solvent is necessary. To investigate the best solvent, several solvents such as tetrahydrofuran, dichloromethane, acetone, ethyl acetate, ethyl alcohol and methanol were studied. One mole PRO and one mole phospholipid were put into a 100-mL eggplant-shaped bottle containing the solvent mentioned above, respectively. Then, the mixed sample was transferred to the rotary evaporator to wipe off the solvent at 40°C for 3 h. The dried residues were desiccated for 24 h. The product achieved was divided into several packs with antioxidant. The drug entrapment efficiency was calculated to select the best solvent.

The drug entrapment efficiency (Y) was used as an index; the calculation formula was as follows:

$$Y = \frac{A}{B} \times 100\%$$

where A represents the drug amount detected in the PRO-PLC and B represents the drug amount added in the process of preparing the PRO-PLC.

In other words, the meaning of "the drug entrapment efficiency" represented the binding efficiency of PRO and phospholipid.

Investigation of Molar Ratio of PRO and Phospholipid

In order to conduct an efficient and economy preparation of PRO-PLC, the best molar ratio of PRO and phospholipid was selected. The PRO and phospholipid at the molar ratio of 1:0.5; 1:1; 1:2 and 1:3 were put into a 100-mL eggplant-shaped bottle containing the best solvent, respectively. Then, the mixed sample was transferred to the rotary evaporator to wipe off the solvent at 40°C for 3 h. The dried residues were desiccated for 24 h. The product achieved was divided into several packs with antioxidant.

The drug entrapment efficiency was calculated to select the best molar ratio of PRO and PLC.

Table I. Independent Variables and Response Variable in CCD for the PRO-PLC

No.	Coded values of independent variables			Actual values of independent variables			Drug entrapment efficiency
	X_1	X_2	X_3	X_1 (h)	X_2 (°C)	X_3 (μg/mL)	
1	−1	1	1	0.50	60	20	62.15
2	0	0	0	1.75	45	10.5	88.40
3	1	1	−1	3.00	60	1	56.98
4	0	0	1	1.75	45	10.5	88.90
5	−1	−1	1	0.50	30	20	56.63
6	1	−1	1	3.00	30	20	59.50
7	−1	−1	−1	0.50	30	1	58.58
8	0	1	0	1.75	60	10.5	86.43
9	0	−1	0	1.75	30	10.5	80.90
10	0	0	−1	1.75	45	1	86.08
11	0	0	0	1.75	45	10.5	88.60
12	−1	1	−1	0.50	60	1	64.10
13	1	0	0	3.00	45	10.5	91.28
14	0	0	0	1.75	45	10.5	87.90
15	0	0	1	1.75	45	20	84.12
16	0	0	0	1.75	45	10.5	88.60
17	1	−1	−1	3.00	30	1	61.45
18	−1	0	0	0.50	45	10.5	68.40
19	0	0	0	1.75	45	10.5	88.30
20	1	1	1	3.00	60	20	55.02
	Levels						
Independent variables	−1	0	1				
X_1	0.5	1.75	3				
X_2	30	45	60				
X_3	1	10.5	20				

Table II. Effect of Solvent on PRO-PLC Preparation

Solvent	Loading ratio *Y* (%)
Methanol	55.8
Absolute ethyl alcohol	80.8
Tetrahydrofuran	66.4
Dichloromethane	35.8
Acetone	69.5
Ethyl acetate	60.5

Central Composite Design and Optimization Formulation

Besides the solvent and the molar ratio of PRO and PLC, the reaction time, temperature and the concentration of PRO were also the important factors which affected the formulation of PRO-PLC. The changes of these factors can affect the drug entrapment efficiency of PRO.

Central composite design (CCD) was used in this study (16). Use response surface methodology to select the optimal formulation. Three factors with three levels were made into 20 test design scheme, which was shown in Table I. X_1, X_2, and X_3 represented the reaction time, temperature and the concentration of PRO, respectively. Prior to the central composite design, a single-factor investigation of the reaction time, temperature and the concentration of PRO was conducted. The range of the three factors above was determined with the drug entrapment efficiency > 50% as an indicator. Using the test results as a reference, the range of X_1 was 0.5~3 h; the range of X_2 was 30~60°C; and the range of X_3 was 1~20 μg/mL. The main effects, interaction effects and quadratic effects between each factor and response variable were evaluated, and the optimal numerical of each factor was gained. The drug entrapment efficiency of PRO was taken as response variable. All data in the scheme was calculated using Design-Expert.V8.0.6.1.

After analysing the data, a polynomial regression equations model was gained which demonstrated the relations between factors (X_1, X_2, X_3) and response variable.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 {X_1}^2 + \beta_8 {X_2}^2 + \beta_9 {X_3}^2$$

where Y was the response variable; β_0 was the intercept representing the arithmetic average of all quantitative outcomes of 20 runs; β_1–β_9 were the coefficients computed from the observed experimental values of Y; X_1, X_2 and X_3 were the coded levels of independent variables; X_1X_2, X_1X_3 and X_2X_3 were the factor interaction; and X_{12}, X_{22} and X_{32} were the quadratic term.

On the basis of polynomial regression equations, the contour and 3D surface plots were attained by using Design-Expert.V8.0.6.1.

The optimized technological conditions of PRO-PLC were selected by Response Surface Methodology. Then, three samples prepared with optimized conditions were validated for drug entrapment efficiency.

Table III. Effect of Molar Ratio of PRO and Phospholipid on the Preparation of PRO-PLC

Molar ratio (PRO: phospholipid)	1:0.5	1:1	1:2	1:3
Y (%)	45.6	80.8	85.2	90.6

Preparation of Probucol-Phospholipid Physical Mixture (PRO-PPM)

The PRO-PPM was prepared by grinding PRO with phospholipid in the mortar at the molar ratio of 1:1 for 10 min, and no other inactive ingredients or reagents were needed.

The residues were desiccated by 60 mesh sieve.

Validation of Probucol-Phospholipid Complex

The Drug Content in PRO-PLC

High-performance liquid chromatography (HPLC) was used to determine the content of PRO in the PRO-PLC. PRO-PLC (containing 125 mg PRO) was dissolved in 50 mL methanol. The solution was filtered by 0.45-μm filter and detected by HPLC. Three batch samples were detected. The HPLC (Waters 1525; Waters Corporation., USA) using a C_8 column (Sepax GP-C_8, 5 μm, 4.6 × 250 mm, USA) with the temperature of 30°C, the flow rate of mobile phase was 1.0 mL/min and the injection volume was 20 μL, the detective wavelength was 242 nm. The mobile phase was the mixture of 85% acetonitrile and 15% water. The linear regression equation was $A = 20029C - 29837$ ($r = 0.999$), where A represented the peak area drug and C represented the concentration of PRO (μg/mL).

Solubility Studies

Excess PRO, PRO-PPM and PRO-PLC were added into sealed glass vials, which contained 10 mL distilled water and *n*-octanol, respectively. Each experiment was performed in triplicate. The solutions were shaken in the shaker for 12 h, the speed was 100 rpm and the temperature was 37°C. After that, the solution was centrifuged at 10,000 rpm for 5 min. Then, the supernatants were filtered by 0.45-μm filter and analysed by HPLC.

Fourier Transform Infrared Spectroscopy (FT-IR)

PRO, phospholipid, PRO-PPM and PRO-PLC were detected using FT-IR spectrophotometer (Thermo Nicolet, Madison, WI, USA) to validate the formation of PRO-PLC. The samples were prepared by KBr disks with a hydrostatic press. The scan range was from 400 to 4000/cm.

Differential Scanning Calorimetry (DSC)

PRO, phospholipid, PRO-PPM and PRO-PLC were detected using ATA449-C instrument (NETZSCH, Bavaria,

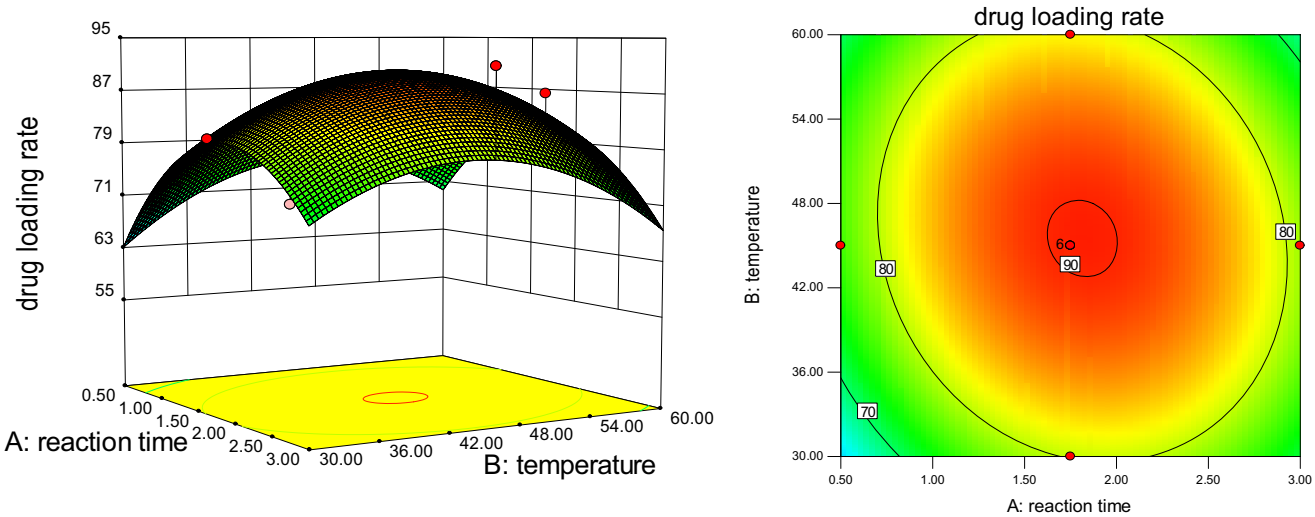

Fig. 1. Response surface plot (3D) and contour plot showing the effect of reaction time (X_1) and temperature (X_2) added on the drug entrapment efficiency (Y)

Germany) to validate the thermal characteristics of them. Samples were put into an open aluminium pan. The flow rate of nitrogen was 10 mL/min. The weight need for detection was 3~8 mg. The temperature was from 40 to 400°C at the rate of 10°C/min.

Powder X-Ray Diffraction (PXRD)

PRO, phospholipid, PRO-PPM and PRO-PLC were detected using D/MAX 2500-PC X-ray powder diffraction meter (Rigaku, Japan) to validate the diffraction patterns of them. The voltage was 50 kV and the current was 40 mA. Before the measurement, aluminium was used to verify alignment. Samples were placed in a zero background holder made of quartz. The scan range was from 3° to 40°, the speed was 1°/s.

Scanning Electron Microscopy (SEM)

PRO, phospholipid, PRO-PPM and PRO-PLC were detected using a scanning electron microscope (S-4100; Hitachi, Tokyo, Japan) to validate the surface morphology of them. Double-sided adhesive tape was used to fix samples on a brass stub. Using Hitachi Ion Sputter (E-1030) to coat the brass stub in a vacuum (6 Pa) with platinum, which made it electrically conductive, the coating time was 240 s and the current was 15 mA.

In vitro Drug Release

The dissolution study was performed according to the paddle method in Appendix XC of the second edition of the Chinese Pharmacopoeia 2015 (17). The release profiles of PRO-PLC and the commercial tablets (all those formulations contained 125 mg probucol) were studied on a dissolution apparatus (Tianjing TianDaTianFA Technology Co., China), the rotation speed of paddle was set to 100 rpm, and 900 mL dissolution medium was maintained at 37 ± 0.5°C. PRO-PLC was weighted and filled in hard gelatin capsules, and then put into the vessels directly. At 5, 10, 20, 30, 45, 60 and 90 min, 5 mL dissolution sample was gained with a disposable syringe. Five-millilitre fresh dissolution media with the

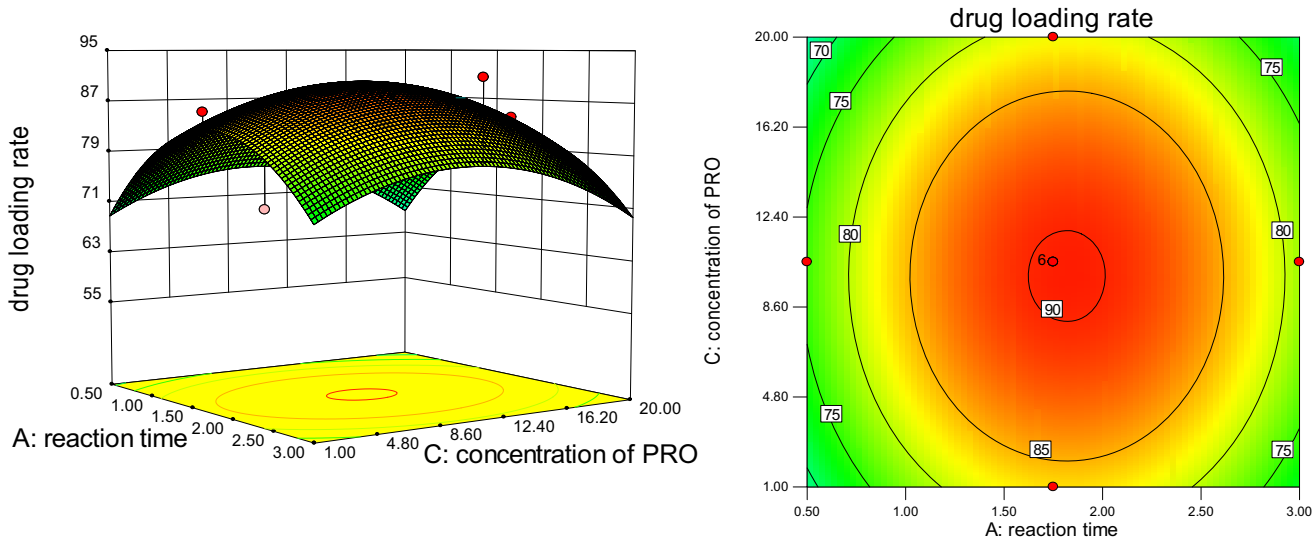

Fig. 2. Response surface plot (3D) and contour plot showing the effect of reaction time (X_1) and concentration of PRO (X_3) added on the drug entrapment efficiency (Y)

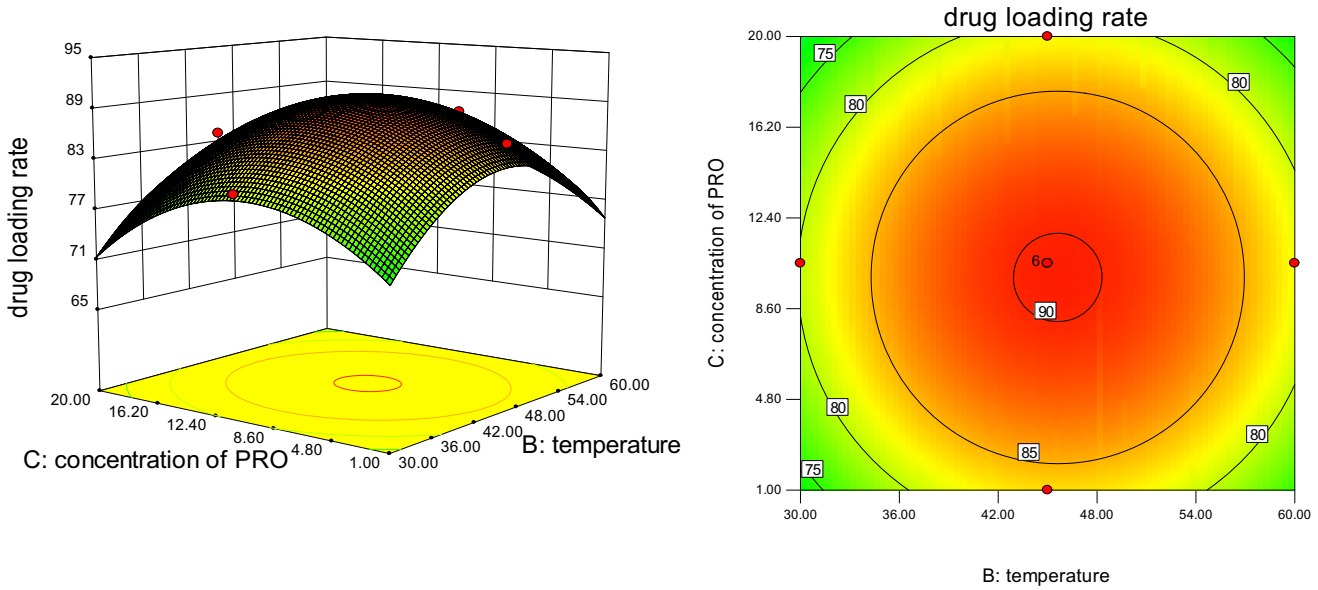

Fig. 3. Response surface plot (3D) and contour plot showing the effect of temperature (X_2) and concentration of PRO (X_3) added on the drug entrapment efficiency (Y)

temperature of 37 ± 0.5°C was added in the big vessel for keeping the media volume constant after the sampling time. The sample solution was filtered by 0.45-μm filter and analysed by HPLC. All the experiments were performed in triplicate.

Similarity factor (f_2) is the evaluation standard to find the similarity of release profiles between different dissolution mediums. It is a logarithmic transformation of the sum-squared error of differences between the test preparation and reference preparation, and the calculation equation is as follows:

$$f_2 = 50\log\frac{100}{\sqrt{1+\left(\left(\sum_{i=1}^{n}(R_t-T_t)^2\right)/n\right)}}$$

where R_t and T_t represents the cumulative drug release (%) of the reference preparation and test preparation at the sampling time, separately, and n represents the number of the time points. The value of similarity factor is in the range of 0 to 100. The higher similarity is in consistence with the larger value. If the $f_2 > 50$, the release profiles in two different dissolution medium are considered to be similar.

Pharmacokinetic Studies in Dogs

The animal experiment was conducted with the approval of Nanjing Medical University Institutional Animal Care and Use Committee (No. 20161115), and all operations were performed in accordance with the 'Principles of Laboratory Animal Care'. In this study, eight beagle dogs were randomly divided into two groups. The commercial tablets and PRO-PLC (containing 125 mg PRO) were tested under fasted and fed state.

No food was given to the dogs for 12 h before the experiment in the fasted study. Two hundred grams dry dog food was given to the dogs in the fed study after a 12-h hungry state. Drugs were orally administrated to dogs, followed with 50 mL water. Then, dogs were sent back to the cages with ample water. After the final sampling time, normal amount of food was given to them.

Five-milliliter disposable syringes with 20-gauge needles were used to take the blood from the forelimb vein of the dogs. The amount of blood taken was 5 mL. The time points were 0, 1, 2, 4, 5, 6, 7, 8, 10, 12, 18, 24, 48 and 72 h. Samples were immediately transferred into

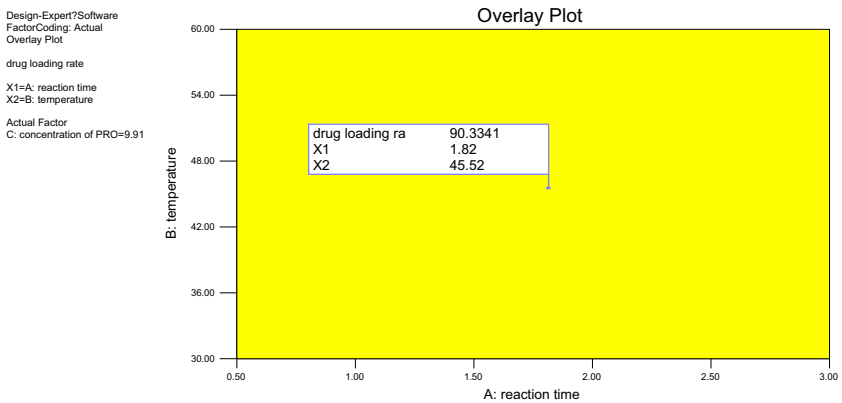

Fig. 4. The overlay plot of the response variable

Table IV. Validation of Optimized PRO-PLC

No.	Drug content (%)	Mean content (%)	Drug entrapment efficiency (%)	Mean loading ratio (%)
1	35.51		91.12	
2	36.16	35.79	89.65	90.34
3	35.69		90.25	

sodium heparin tubes to protect it from condensation. The blood samples in sodium heparin tubes were centrifuged for 10 min, the speed was 5000 rpm. After that, 1 mL plasma drawn from the supernatant was transferred into the 10-mL centrifuge tube.

Before used, the plasma was placed in the refrigerator (−20°C). The internal standard was the amiodarone methanol solution with the concentration of 1 μg/mL. The extracting agent was the mixture of 8 volume isooctane and 2 volume isopropanol.

The extraction procedure was as follows: 500 μL plasma was taken into the 10-mL centrifuge tube, and then 50 μL internal standard was added into it. After a 10-s vortex, 5 mL extracting agent was added to precipitate the proteins, followed by a 2-min vortex.

Then, the mixture was centrifuged for 10 min at the speed of 4000 rpm. The supernatant was taken into a new 10-mL centrifuge tube and evaporated by nitrogen in the nitrogen blowing instrument. The residue was reconstituted in 50 μL methanol, followed by centrifugation, and the supernatant was transferred into the intubation tubes and analysed using Waters 1525 HPLC. The column was Phenomenex C_{18} column (150 mm × 4.6 mm, 5 μm); the column temperature was 35°C; the mobile phase was a mixture of acetonitrile, water, 10% phosphoric acid and 10% *n*-butylamine (94:6:0.1:0.1; *v*/*v*), the flow rate was 1.0 mL/min, the injection volume was 20 μL and the UV detection was performed at $\lambda = 242$ nm. The linear regression equation was $As/Ai = 4.12C + 0.07$ ($r = 0.999$), where As/Ai represented the peak area ratio of drug and internal standard and C represented the plasma concentration of PRO (μg/mL).

Analysis of Data

Data for the all experiments were calculated. WINNONLINR 5.2 was used to analyse the pharmacokinetic parameters. Significant difference was confirmed if $p < 0.05$.

Table V. The Solubility of PRO, PRO-PPM and PRO-PLC in Water and *n*-Octanol at 37°C ($n = 3$)

Sample	Water solubility (μg/mL)	n-Octanol solubility (μg/mL)
PRO	0.07 ± 0.03	124.56 ± 9.55
PRO-PLC	15.05 ± 0.93	145.65 ± 11.21
PRO-PPM	0.24 ± 0.05	136.66 ± 8.12

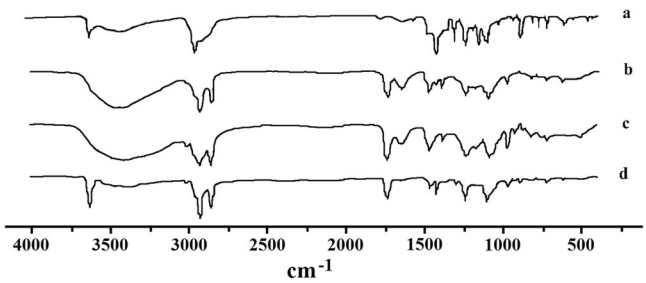

Fig. 5. Fourier transform infrared spectroscopy of probucol (**a**), probucol- phospholipid complex (**b**), phospholipid (**c**) and probucol-phospholipid physical mixture (**d**)

RESULTS AND DISCUSSION

Preparation of PRO-PLC

Investigation of Solvent

Probucol belongs to BCS II, which has the characteristics of low solubility and high permeability (18). Based on the literature (19), no suitable solvent can judge the combination rate between drugs and phospholipid. In the research, different solvent was studied for their effect on the drug entrapment efficiency of probucol and phospholipid, and the results can be seen in Table II.

The drug entrapment efficiency was highest when probucol reacted with absolute ethyl alcohol, so absolute ethyl alcohol was selected as the reaction solvent.

Investigation of Molar Ratio of Drug and Phospholipid

PRO-PLC of different ratios was prepared and the drug entrapment efficiency was measured in Table III. As the result showed, the drug entrapment efficiency of PRO-PLC was > 80.8% (*w*/*w*) when the ratio was more than 1:1, but the product was too viscous, which was conducive to the later test. The drug entrapment efficiency was < 50% (*w*/*w*) when the ratio was 1:0.5. This was probably because there was not enough phospholipid to react with PRO (20). Thus, the molar ratio of PRO and phospholipid was determined to be 1:1, and the drug entrapment efficiency was 80.8% (*w*/*w*).

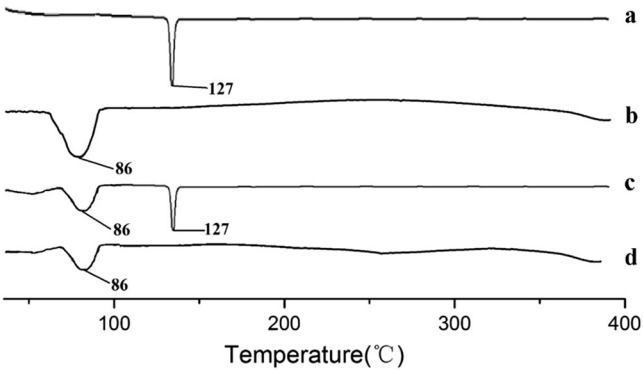

Fig. 6. Differential scanning calorimetry thermographs of probucol (**a**), phospholipid (**b**), probucol-phospholipid physical mixture (**c**) and probucol-phospholipid complex (**d**)

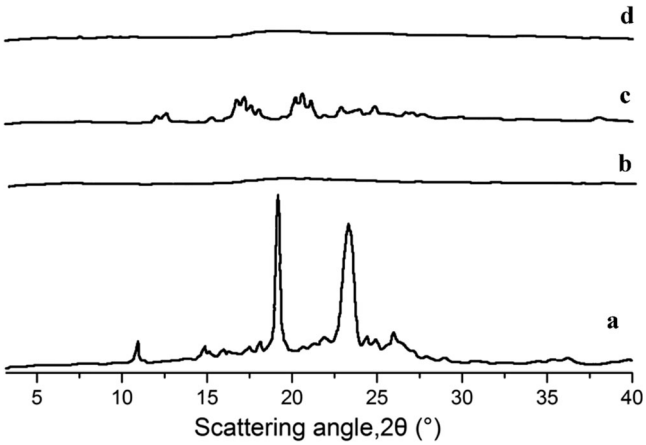

Fig. 7. Powder X-ray diffraction patterns of probucol (**a**), phospholipid (**b**), probucol-phospholipid physical mixture (**c**) and probucol-phospholipid complex (**d**)

Central Composite Design

Twenty designed tests were schemed, and the relationship between the drug entrapment efficiency and three factors was investigated. The values of the drug entrapment efficiency are filled in Table I.

Three models were established based on the data in Table I. ANOVA was used to analyse them. The correlation coefficient was close to 1, which indicated that the model was good. The p value of drug entrapment efficiency was < 0.05, indicating a significant difference between factors and the drug entrapment efficiency. The polynomial regression equations of X_1, X_2 and X_3 on the reaction time, temperature and the concentration of PRO were represented as follows:

$$Y = 90.25 + 1.44X_1 + 0.76X_2 - 0.98X_3 - 2.5X_1X_2 - 0.00125X_1X_3 - 0.00125X_2X_3 - 13.12{X_1}^2 - 9.29{X_2}^2 - 7.86{X_3}^2$$

The contour and 3D surface plots of factors and drug entrapment efficiency are drawn in Figs. 1, 2, and 3. The drug entrapment efficiency was affected with the change of the three factors. In Fig. 1, when the reaction time was kept stable, with the temperature increased, the drug entrapment efficiency increased first and subsequently decreased. In Figs. 2 and 3, when the concentration of PRO was kept stable, and the reaction time or the temperature increased, the drug entrapment efficiency increased first and subsequently decreased. The plots in figures reflected the interaction effect between the three factors. To maximize drug entrapment efficiency, Design-Expert.V8.0.6.1 was used to select the optimal conditions. The optimized formulation was obtained from the overlay plot (Fig. 4), in which the reaction time was 1.81 h, temperature was 45.5°C and the concentration of PRO was 9.91 μg/mL. Three samples prepared with the optimal conditions were detected for the drug entrapment efficiency.

The drug entrapment efficiency of optimized PRO-PLC is shown in Table IV, and the mean value was 90.34% (w/w).

Validation of Probucol-Phospholipid Complex

The Drug Content in PRO-Phospholipid Complex

The content of probucol in the PRO-PLC which was prepared by the optimal conditions was determined. The results can be seen in Table IV. The mean drug content of three batch samples was $35.79 \pm 0.34\%$ (w/w), and the RSD was 0.94%. The results showed that the samples meet the requirement of drug content.

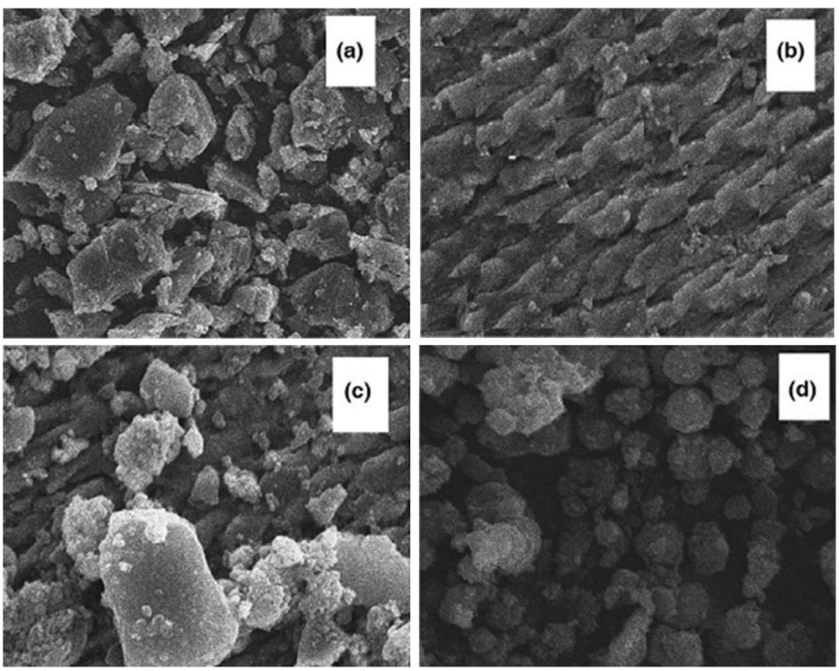

Fig. 8. Scanning electron microscopy photomicrographs of probucol (**a**), phospholipid (**b**), probucol-phospholipid physical mixture (**c**) and probucol-phospholipid complex (**d**)

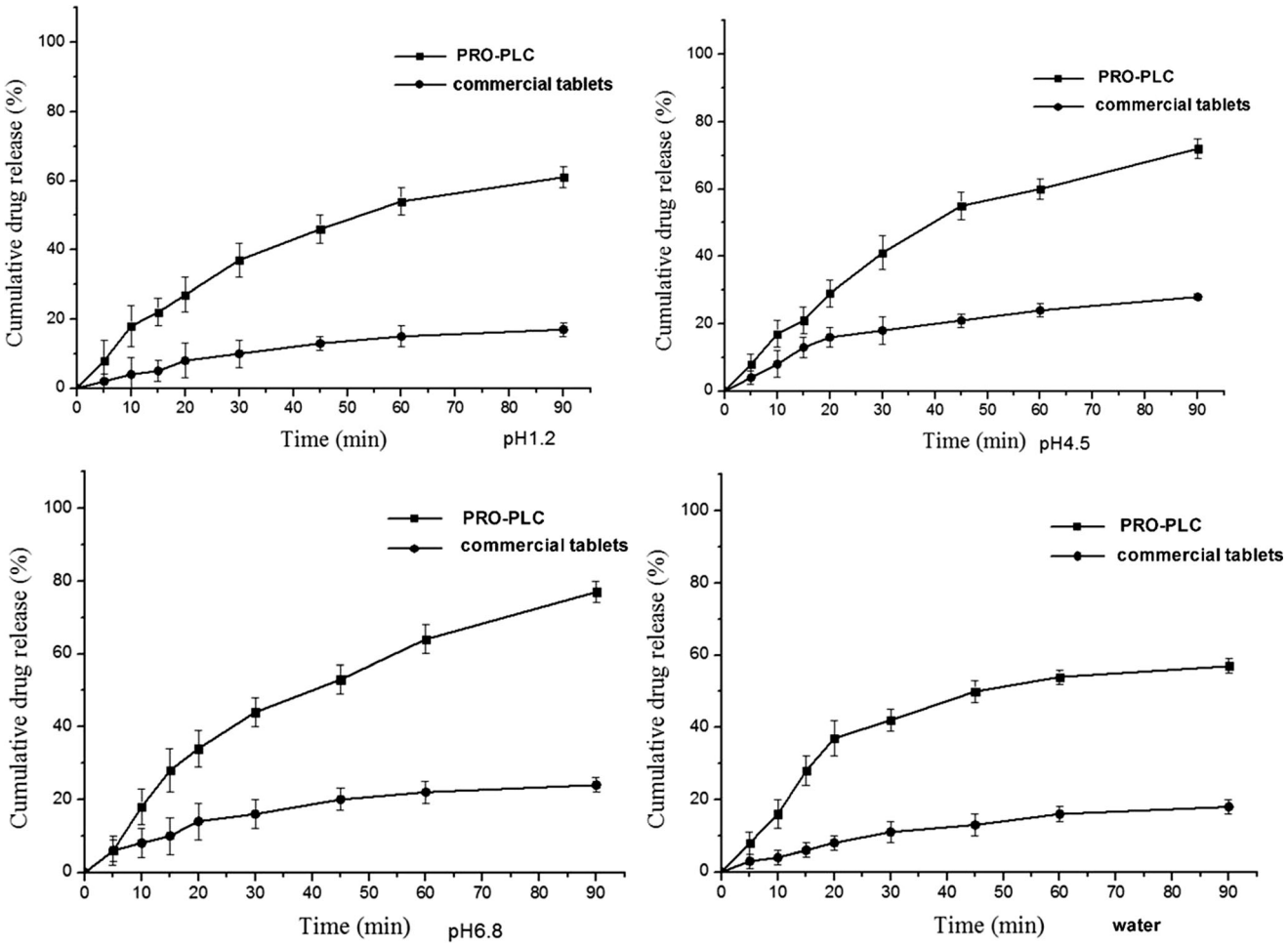

Fig. 9. Drug release profiles of PRO-PLC and commercial tablets in four dissolution medium ($n = 6$)

Solubility Studies

Water-insoluble drugs are difficult to be absorbed quickly. If a drug wants to take part in the circulation of the system, it should well dissolve in the gastric or intestinal fluids.

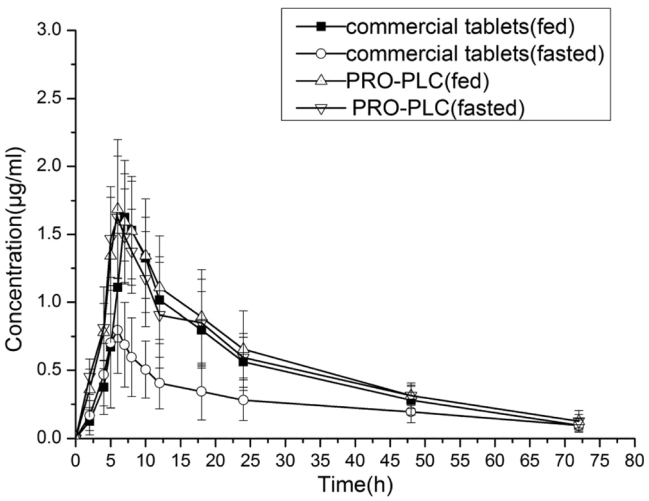

Fig. 10. Mean dose-normalized probucol concentration *versus* time profiles after administration of PRO-PLC and commercial tablets in fasted and fed dogs. Data are expressed as mean ± SD ($n = 8$)

So, it is essential to improve the solubility of insoluble drugs. The solubility of PRO, PRO-PPM and PRO-PLC was detected, the solvent were water and n-octanol, and the temperature was 37°C. As the result showed (Table V), the solubility of PRO-PLC was higher than PRO, which was approximately 215-fold of the PRO in water. This could be considered that phospholipid had the ability to improve the solubility of drugs (21).

FTIR

The FTIR spectra of PRO, phospholipid, PRO-PPM and PRO-PLC can be seen in Fig. 5. The specific peaks of PRO were ascribed to a C–H out-of-plane bending vibration (667 cm^{-1}, 746 cm^{-1}, 912 cm^{-1}), a C–H in-plane bending vibration (1384 cm^{-1}, 1416 cm^{-1}), a C–O stretching vibration (1256 cm^{-1}) and a O–H stretching vibration (3641 cm^{-1}). The spectra of PRO-PPM was significantly different from it of PRO-PLC.

The specific peaks of C–O and O–H disappeared in PRO-PLC, which indicated that some chemical bonds of PRO had reacted with phospholipid.

DSC

The DSC characteristics of PRO, phospholipid, PRO-PPM and PRO-PLC are presented in Fig. 6. PRO showed a

Table VI. Pharmacokinetic Parameters of Probucol in Beagle Dogs After Oral Administration of PRO-PLC and Commercial Tablets ($n = 8$)

	Commercial tablets (fed)	Commercial tablets (fasted)	PRO-PLC (fed)	PRO-PLC (fasted)
C_{max} (μg/mL)	1.63 ± 0.58	0.79 ± 0.47#	1.69 ± 0.44*	1.64 ± 0.57*
T_{max} (h)	7.2 ± 1.7	6.5 ± 1.2	6.8 ± 1.3	6.0 ± 0.8
$t_{1/2}$ (h)	17.48	28.59	19.05	21.09
$AUC_{(0-t)}$ (μg/mL/h)	35.8 ± 14.3	18.5 ± 13.0#	37.7 ± 21.9*	35.2 ± 15.6*
$AUC_{(0-\infty)}$ (μg/mL/h)	38.2 ± 17.8	24.0 ± 17.3#	38.4 ± 18.3*	37.0 ± 17.0*
Elimination rate constant (/h)	0.04	0.02	0.04	0.03
Volume of distribution (L/kg)	13.73	36.37	12.58	14.58
MRT (h)	28.36	41.23	29.18	30.69
Relative bioavailability (%)	100	62.8	100.5	96.8

The relative bioavailability is based on the $AUC_{(0-\infty)}$ of commercial tablets
# $p < 0.05$ *versus* commercial tablets (fed)
* $p > 0.05$ *versus* commercial tablets (fed)

sharp endothermic peaks at 127°C, which was the melting point of PRO. The endothermic behaviour of phospholipid near 86°C was probably due to the occurrence of phase transition. The endothermic peaks of PRO and phospholipid appeared in PRO-PPM, but the endothermic peak disappeared in PRO-PLC, which indicated that a new phase had been formed between PRO and phospholipid. This result confirmed the formation of PRO-PLC (22).

PXRD

The powder X-ray diffraction characteristics of PRO, phospholipid, PRO-PPM and PRO-PLC could be seen in Fig. 7. Sharp crystalline peaks were apparent in PRO, what was to say, it was a crystalline drug. In the curve of PRO-PPM, the peaks were the overlay of PRO and phospholipid. However, no crystalline peaks of PRO could be seen in PRO-PLC. The results indicated that the PRO-PLC was no longer a crystal form, which testified the formation of PRO-PLC (21).

SEM

Figure 8 presents the appearance surface of PRO, phospholipid, PRO-PPM and PRO-PLC. The surface of PRO was smooth with a crystal form. In the picture of the physical mixture, the PRO could be distinguished from phospholipid. However, the surface and shape of PRO-PLC were different from PRO and phospholipid. The shape was like spherical and the surface was smooth, this was because PRO was mixed totally with phospholipid.

In vitro Dissolution Profiles of PRO-PLC

The optimized PRO-PLC and commercial tablets were compared about the dissolution profiles. The results are shown in Fig. 9. In PRO-PLC, about 60% of drug was dissolved in the medium in 90 min, whether the cumulative drug release of commercial tablets was less than 20%. The dissolution profiles of PRO-PLC in pH 1.2 HCl solution were made as a reference. The similarity factors between pH 1.2 HCl solution and three other dissolution medium were 62.78, 55.64 and 62.06, respectively. It revealed that the dissolution medium of different pH did not interfere the dissolution profiles of PRO-PLC.

Pharmacokinetic Studies in Dogs

The relationship between time and PRO concentration in plasma of PRO-PLC and commercial tablets could be seen in Fig. 10. The pharmacokinetic parameters calculated by software are displayed in Table VI.

As the results showed, the T_{max} of commercial tablets in the fasted and fed state was 6.5 ± 1.2 h and 7.2 ± 1.7 h, respectively. In contrary, the T_{max} of PRO-PLC in the fasted and fed state was 6.0 ± 0.8 h and 6.8 ± 1.3 h, respectively. The C_{max} of PRO-PLC was about 1.64 ± 0.57 μg/mL and 1.69 ± 0.44 μg/mL in the fasted and fed state, which were close to the C_{max} of commercial tablets in the fed state.

The bioavailability of commercial tablets in the fed state was two times higher than in the fasted state. However, C_{max}, T_{max}, $t_{1/2}$, volume of distribution, MRT and $AUC_{(0-\infty)}$ of PRO-PLC showed similarity in the two state, which revealed that the absorption of PRO in PRO-PLC was not affected by food. Compared to the $AUC_{(0-\infty)}$ of the commercial tablets in the fed state, the relative bioavailability of PRO-PLC in the fasted and fed state were 96.8% and 100.5%, respectively. To conclude, the preparation of PRO-PLC could improve the bioavailability *in vivo* and there was no interference induced by food.

CONCLUSION

In the research, solvent-evaporation was used to prepare PRO-PLC. The method was easy to conduct and the product was stable. Central composite design method had been used to optimize the formulation. Solubility studies indicated that PRO-PLC was easier to dissolve in water compared to PRO. The dissolution profiles of PRO-PLC prepared by the optimized conditions were studied. The *in vitro* release rate of PRO from the PRO-PLC exhibited almost 2-fold release characteristics than commercial tablets in 60 min. Improved and similar bioavailability in the fasted and fed state was obtained from PRO-PLC, which indicated that phospholipid complex technique was a promising technology for improving oral bioavailability of the poorly water-soluble drug.

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