Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 798 (2003) 323–331
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Separation and purification of phosphatidylcholine and phosphatidylethanolamine from soybean degummed oil residues by using solvent extraction and column chromatography

Weinong Zhang [a,b], Haibo He [a], Yuqi Feng [a,1], Shilu Da [a,*]

[a] College of Chemical and Molecular Science, Wuhan University, Wuhan 430072, PR China
[b] Department of Food Science, Wuhan Polytechnic University, Wuhan 430023, PR China

Received 26 June 2003; received in revised form 3 October 2003; accepted 6 October 2003

Abstract

Natural phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were separated and purified from soybean degummed oil residues in this work. Crude PC and PE were first separated from degummed oil residues by extraction with 95% ethanol, and then the crude PC and PE were used as raw materials to prepare high purity PC and PE by using column chromatography of silica gel (100–200 mesh) with different eluents and elution modes. The high purity PC (content >90%) was obtained from the crude PC by using isocratic elution with methanol as eluent. Compared with the methods reported by using isocratic elution with mixed solvents as eluent or gradient elution, the procedure proposed exhibits low cost and industry potentialities because of some advantages, such as operation simplicity, cheap equipment and solvent to be recovered easily. The purity of the PE product prepared from the crude PE was more than 75%. The gradient elution was preferable to isocratic elution for reducing the elution time and eluent consumption when to prepare PE from the crude PE. The effects of loading amount and the flow-rate on separation efficiency were also investigated. For obtaining high separation efficiency, the loading amount should be less than 2.0 g crude PC or PE/100 g silica gel, and the flow-rate should be controlled under 4 ml/min for crude PC and 3 ml/min for crude PE, respectively.

Keywords: Phosphatidylcholine; Phosphatidylethanolamine

1. Introduction

Natural phospholipids, main constituents of cell membranes, have a lot of important biological functions to all living organisms. Since 1970s, with the development of molecular biology it is discovered that phospholipids are the precursors of many informational macromolecules and biological activated substance for message transmissions and lipoprotein metabolisms, are also chemical mediators to prevent varieties of diseases, such as heart, brain, blood cycle, breath system and reproductive physiology diseases. It is known that a number of diseases correlate with disturbance in phospholipid metabolism [1–3]. The phospholipids are complex mixtures that include various classes of compounds based on differences in the polar groups of molecular structures such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphoric acid (PA), etc. Each class is also a mixture that contains many molecular species with different fatty acid chains [3,4].

Two main classes among the phospholipids, PC and PE, were extensively applied as emulsification, stabilization and wetting agents in the fields of health products, foods, industrial manufacture of various products, etc. [5–7]. The PC and PE used in these industries were usually crude products (30–60% content) prepared from different natural materials. With the increase in application of PC and PE in pharmaceuticals, nourishment and cosmetics, the demand for high purity PC and PE, the purities of which are superior 80%, are rapidly increasing. However, the high purity PC and PE

* Corresponding author. Tel.: +86-27-87684066; fax: +86-27-87647617.
E-mail addresses: yqfeng@public.wh.hb.cn (Y. Feng), dashilu@public.wh.hb.cn (S. Da).
[1] Co-corresponding author. Tel.: +86-27-87880776.

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.10.005

do not meet the needs of various fields because of the limit to their high prices at present. Consequently, a new low-cost technology for the purification of PC and PE is worth of great importance for extending applications.

Soybean is one of important sources for preparation of natural phospholipids. The phospholipids are extracted from soybean together with oils during extraction procedure, and then separated from oils as residues during degumming process in oil factory [8,9]. The degummed oil residues, considered as waste, contain rich phospholipids. Hence, it is a desirable source for the preparation of cheap pure PC and PE products.

Separations and purifications of the phospholipid classes have been extensively studied by a number of researchers in different academic fields [10–21]. Pure PC and PE are usually purified by using various chromatographies, such as TLC [10,11], high performance liquid chromatography (HPLC) [12–16] and low-pressure column chromatography (LPCC) [17–21], etc. Amongst these methods, separation efficiency of HPLC is the best since small diameter packing can be used, however, the equipment is expensive and the costs of operation and maintenance are also high. The disadvantages of TLC are difficult to detect on line and recover the products. Therefore, TLC and HPLC were not appropriate for the purification of the phospholipid classes as industry processes with low cost. Compared with HPLC and TLC methods, LPCC exhibits a great potentiality for industrial production owing to lots of advantages: operation simplicity; gradient elution to be used conveniently; low cost and high yield.

Many researchers used mixed solvents in LPCC for separation of the classes of phospholipids [18–21]. In these processes, recovery of mixed solvents and reutilization in certain ratio are very difficult.

The soybean phospholipids were usually separated by TLC, HPLC or LPCC without pre-separation in previous literatures [10,13–16]. The phospholipid classes could not be separated by using single solvent due to low resolution. For improving the resolution, ternary or even more mixed solvents were usually used as developer, mobile phase or eluent, which is hindrance to reducing the cost of production.

An important factor to develop an economic LPCC process is to use a simple solvent system as eluent. In this paper, the procedure with simple solvent system was proposed, which is worth to decrease separation cost. The industrial raw product of phospholipids, namely powder phospholipids (acetone-insoluble content >90%), was first separated into crude PC and PE by using ethanol extraction, which were used as raw materials to prepare pure PC and PE by using silica gel column chromatography, respectively. The separation conditions were optimized by using various single solvents and binary mixed solvents as eluents, and isocratic and gradient elution modes to be performed. The effects of sample loading on the column and the flow-rate of eluent on separation efficiency were also studied.

2. Experimental

2.1. Chemicals

Soybean degummed oil residues were provided by Yonggu oil factory (GuangZhou, China). Silica gel (100–200 mesh, irregular) was obtained from Haiyang Chemical Group (Qingdao, China). Standards PC, PE, PI, PA and PS were all purchased from Sigma (Superior Chemicals and Instrument Co. Ltd., Beijing, China).

Chloroform, methanol, ethanol, acetone, acetonitrile, and phosphoric acid were of analytical grade, obtained from Shanghai Medicine Company (Shanghai, China).

Water used in this work was re-distilled.

2.2. Preparation of crude PC and PE from degummed oil residues

The industrial degummed oil residues contained a lot of impurities such as oil and water besides the phospholipids. The powder phospholipids were obtained from the degummed oil residues by washing with acetone, and then removing solvent and drying under vacuum below 80 °C. The powder phospholipids were dispersed in 95% aqueous ethanol (solid to liquid ratio 1:10, g/ml) in a round-bottomed flask, stirred and refluxed 30 min. The ethanol extracts (soluble substances) and ethanol-insoluble substances as residues were separated by decantation. The ethanol extracts were concentrated under vacuum, precipitated by addition of acetone and then separated by pouring out solvent to obtain crude PC with removing solvent under vacuum at 60 °C. The ethanol-insoluble substances contain mainly PE, and then was removed ethanol under vacuum at 60 °C to obtain crude PE. The crude PC and PE were used as raw materials to prepare pure PC and PE by using LPCC method, respectively.

2.3. Analytical methods

2.3.1. HPLC

The HPLC apparatus was consisted of a LC-10A pump (Shimadzu, Tokyo, Japan), a Rhecdyne 7725i injector with 20 ul sample loop (Cotati, CA, USA) and an SPD-10A ultraviolet detector (Shimadzu, Tokyo, Japan). The chromatographic data was acquired by chromatography working station Echrom98 (Elite, Dalian, China). The separation was performed on a 150 mm × 4.6 mm column packed with 5 μm home-made silica.

The mixture of acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v) was chosen as mobile phase. The mobile phase was filtrated and degassed before use. The flow-rate of mobile phase was set at 0.5 ml/min. All experiments were carried out at ambient temperature between 15 and 20 °C. The detection wavelength of UV was 203 nm.

The quantitative analyses were performed by using external standard based on the height of peak. The method was calibrated by using a series of phospholipid standards

of known concentrations. The standards and samples were dissolved by mixed solvents of chloroform–methanol (2:1, v/v). Each sample was analyzed triplicate.

2.3.2. FTIR analysis

The FTIR instrument was AVTAR-360 (Thermo Nicolet, Madison, USA). Sample about 2 mg was mixed with 300 mg KBr, pressed into a thin disc for infrared analysis.

2.4. Preparative low-pressure column chromatography

A 600 mm × 30 mm glass tube was used as the preparative chromatography column with a valve to control the flow-rate of eluent. Silica gel about 100 g (100–200 mesh) was activated 40–60 min at 120 °C, and then slurry packed into the column. Various single solvent and binary mixed solvents were used as eluents, and different elution modes were tried. The effects of chromatography conditions on separation efficiency were studied by change in sample loading from 1.5 to 3.0 g and the flow-rate from 2 to 5 ml/min.

2.5. Isocratic elution

2.0 g crude PC was dissolved in a little eluent, loaded and eluted at a flow-rate of 3 ml/min. The effluents were collected, respectively, according to 25 fractions, each of which was 30 ml. All fractions were analyzed by using HPLC. The fractions containing PE or PC were combined together, respectively, and removed solvent under vacuum at 50 °C. Purities and recoveries of these PE and PC products were determined by using HPLC. The purity of a given product was defined for this study as the PE or PC percentage of the product. Recovery of a given product was evaluated by dividing the quantity of PE or PC in the product obtained by that in loading sample. Ethanol, methanol, chloroform–methanol (1:2, v/v), chloroform–methanol (1:1, v/v), chloroform–methanol (2:1, v/v) were used as eluents, respectively. The column was packed again and the above procedure was repeated when the eluent changed.

1.5 g crude PE was dissolved in a little eluent. The procedure was the same as the above except using different eluents. Chloroform–methanol (2:1, v/v), chloroform–methanol (1:1, v/v), chloroform–methanol (1:2, v/v), and chloroform–methanol (1:3, v/v) were used as eluents for separation when the crude PE was used as raw material.

2.6. Gradient elution

2.0 g crude PC or 1.5 g crude PE were dissolved in a little chloroform–methanol (2:1, v/v) respectively, loaded and eluted with three different gradient modes. The gradient elution were performed by A and B solvent systems. The eluent A, chloroform–methanol (2:1, v/v), was the same in three modes. The eluent B were different in various elution modes, which is chloroform–methanol (1:1, v/v) in Mode 1, chloroform–methanol (1:2, v/v) in Mode 2 and methanol in Mode 3, respectively. The eluent A was used at first, and replaced by the eluent B after 120 min. The flow-rate was controlled at 3 ml/min. Twenty-five fraction elutes were collected and each fraction was 30 ml. The column was packed again with the changes in gradient modes. The fractions were treated as those in isocratic elution.

3. Results and discussions

3.1. High performance liquid chromatography

The mixture of acetonitrile–methanol–85% phosphoric acid was used as mobile phase for phospholipids analysis by a lot of researchers [22–24]. The proportions of the mobile phase components were different in these literatures because of different HPLC systems to be used. We found that the resolution of phospholipids was unsatisfactory when using these reported mobile phases in our HPLC system. Hence, the proportions of the solvents in mobile phase were changed in order to find an appropriate proportion for our phospholipids analysis. We found that better resolution was obtained with acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v) as mobile phase.

Soybean phospholipids mainly included PC, PE, PI, PA and PS [25]. There were almost no sphingomyelin and cardiolipin in the soybean. Thus, the mixture of five standard phospholipids was separated by HPLC. Fig. 1 shows the HPLC chromatogram of five standard phospholipids. Repeatability of the retention time and the height of peak were estimated and the results are presented in Table 1.

The coefficient of variation of the height of PA peak was great (8.99%), see Table 1. The result would be inaccurate

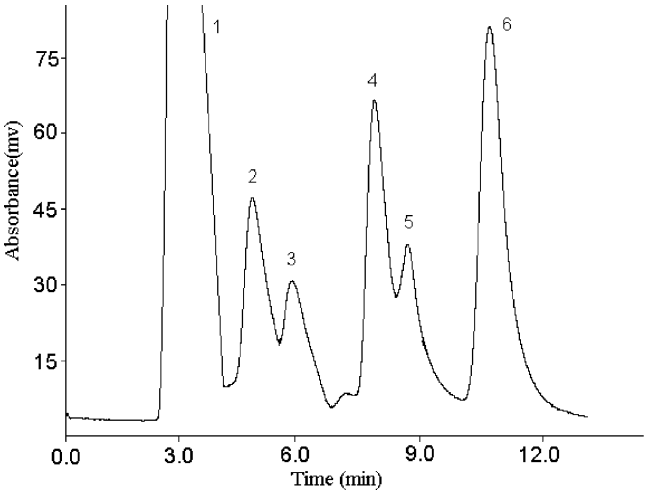

Fig. 1. Chromatogram of five standard phospholipids. Column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min. Peaks: (1) solvents; (2) PI; (3) PS; (4) PE; (5) PA; (6) PC.

Table 1
The repeatability of the retention time and the height of peak for phospholipids[a]

Phospholipids	Retention time (min)	Retention time CV (%)[b]	Height of peak	Height of peak CV (%)[b]
Phosphatidylinositol	4.86 ± 0.02	0.43	117.93 ± 5.61	4.76
Phosphatidylserine	5.52 ± 0.02	0.36	85.50 ± 3.69	4.31
Phosphatidylethanolamine	7.84 ± 0.06	0.94	201.66 ± 2.32	1.15
Phosphatidic acid	8.49 ± 0.06	0.85	20.40 ± 1.83	8.99
Phosphatidylcholine	10.53 ± 0.08	0.92	221.62 ± 4.52	2.04

Conditions: column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min.

[a] The concentrations of standards for analyses were 0.55 mg/ml for phosphatidylcholine and phosphatidylethanolamine, 0.5 mg/ml for phosphatidylserine and phosphatidylinositol, 1.0 mg/ml for phosphatidic acid. Each sample was determined triplicate, and each determination was interval 1 h. Values given are means (±S.D.).

[b] CV is coefficient of variation.

Table 2
Parameter of calibration lines of the phospholipids ($Y = A + BX$)[a]

Phospholipids	A	B	Correlation coefficient
Phosphatidylcholine	0.892	411.703	0.99643
Phosphatidylethanolamine	2.146	482.429	0.99753
Phosphatidylinositol	0.438	270.602	0.99915

Conditions: column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min.

[a] Based on five concentrations for each phospholipid standard.

if quantitative determination was based on the height of peak. Therefore, the quantitative determination of PA was not performed in our work. In addition, PS was not quantitatively determined either because the content of it was little in soybean phospholipids. Only three phospholipids (PC, PE and PI) were quantitatively determined by external standard method based on the height of peak. The parameters of calibration lines are given in Table 2.

Recovery was estimated by addition of known amounts of standards, which was 89.5% for PC and 92.1% for PE, respectively.

The main components of the powder phospholipids, crude PC and crude PE are presented in Table 3.

Figs. 2 and 3 show the HPLC chromatograms of crude PC and PE.

Table 3
The main components of powder phospholipids, crude PC and PE (wt.%)[a]

	PC	PE	PI
Powder phospholipids	23.1 ± 0.2	19.8 ± 0.1	13.9 ± 0.4
Crude PC	56.5 ± 0.3	14.7 ± 0.1	6.5 ± 0.2
Crude PE	8.9 ± 0.1	51.8 ± 0.2	34.5 ± 0.5

Conditions: the HPLC conditions were the same as the Table 2. Five milligram samples were dissolved in 5 ml chloroform–methanol (2:1, v/v). PC, PE and PI contents of each sample were calculated by the calibration formula (see Table 2).

[a] Values shown are means (±S.D.) of the triplicate determinations for each sample.

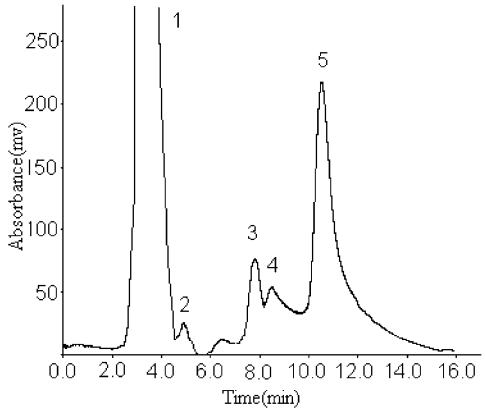

Fig. 2. Chromatogram of crude PC. Column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min; sample concentration, 1 mg/ml (in chloroform–methanol, 2:1, v/v). Peaks: (1) solvents; (2) PI; (3) PE; (4) PA; (5) PC.

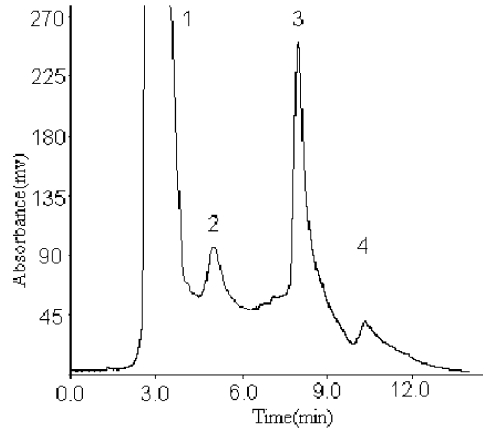

Fig. 3. Chromatogram of crude PE. Column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min; sample concentration, 1 mg/ml (in chloroform–methanol, 2:1, v/v). Peaks: (1) solvents; (2) PI; (3) PE; (4) PC.

Table 4
The results of separation with isocratic elution for the crude PC as raw material

Eluent	PE			PC		
	Collection number[a]	Purity[b] (%)	Recovery[c] (%)	Collection number[a]	Purity[b] (%)	Recovery[c] (%)
Ethanol	ND[d]	ND	ND	ND	ND	ND
Methanol	6–7	72.1 ± 0.1	92.2	12–15	91.4 ± 0.1	94.1
Chloroform–methanol (1:2, v/v)	6–8	72.3 ± 0.1	92.8	14–18	91.4 ± 0.2	94.3
Chloroform–methanol (1:1, v/v)	7–8	73.2 ± 0.2	92.7	16–20	92.2 ± 0.1	95.7
Chloroform–methanol (2:1, v/v)	8–10	79.0 ± 70.2	93.1	21–24	93.6 ± 0.1	98.9

Conditions: column, 600 mm × 30 mm glass column packed with 100 g silica gel (100–200 mesh); loading amount, 2.0 g crude PC dissolved in the eluent; flow-rate, 3 ml/min.

[a] Numbers shown are the ranges of fractions containing PE or PC, respectively.

[b] The fractions containing PE or PC were combined together, respectively, removed solvent under vacuum at 50 °C. About 5 mg PE or PC product was weight exactly and dissolved by 25 ml chloroform–methanol (2:1, v/v), and then the concentration of which was determined triplicate. Purity was calculated by the following formula: (25 × concentration of PE or PC)/product weight.

[c] Recovery was evaluated by the following formula: (25 × concentration of PE or PC)/(crude PC weight × content of PE or PC in crude PC).

[d] ND refers the phospholipids to be not detected.

3.2. Selection of eluents in isocratic elution

It was vital for reducing the cost of production to use a simple solvent system as eluent. Here, single solvent and binary mixed solvents were used as eluents for the preparation of pure PC from the crude PC. Since the crude PE is alcohol-insoluble, pure alcohol could not be used as eluents. Otherwise, PE would block the column during elution. Therefore, only binary mixed solvents were used as eluents when the crude PE was used for separation as raw materials.

Recoveries of all PE and PC products were more than 90% (see Tables 4–7), which illustrated that the fractions combined for recovering PE or PC were collected completely. Therefore, the purities of the products were valuable to evaluate the separation efficiency.

The results of separation with isocratic elution for the crude PC as raw material are given in Table 4.

The solvent strength of ethanol was so weak that PE and PC were not eluted and determined after eluting 750 ml. PE and PC could be all eluted out of the column by using other eluents at less than 750 ml. With the decrease of methanol content in the mixed solvents, the purities of the PC and PE products increased, but the elution time and eluent consumption also increased. The elution time and consumption of eluent decreased to the least when methanol was used as eluent, and the purities of the products were slightly less than those eluted by mixed solvents (see Table 4). Consequently, methanol as single solvent was a desirable eluent for reducing the cost in isocratic elution. When we obtained this result, we found that Yoon also used methanol as mobile phase for separation of egg PC with HPLC in order to reduce the cost [12].

The results of isocratic elution for the crude PE as raw material are presented in Table 5.

The results in Table 5 showed that the purities of the PC products were slightly changed (the range of changing from 94.2 to 92.5%) with different mixed solvents as eluents. With the increase of methanol content in the mixed solvents, the purities of the PE products decreased sharply (from 76.1 to 53.3%), and elution time and consumption of eluent also decreased in a great degree (from 230 to 160 min and from 690 to 480 ml, respectively) in isocratic elution (see Table 5).

Table 5
The results of separation with isocratic elution for the crude PE as raw material

Eluent	PE			PC		
	Collection number[a]	Purity[b] (%)	Recovery[c] (%)	Collection number[a]	Purity[b] (%)	Recovery[c] (%)
Chloroform–methanol (2:1, v/v)	8–12	76.1 ± 0.3	91.9	22–23	94.2 ± 0.1	97.7
Chloroform–methanol (1:1, v/v)	7–10	65.4 ± 0.3	92.3	17–18	93.8 ± 0.1	96.9
Chloroform–methanol (1:2, v/v)	7–9	55.8 ± 0.2	92.1	16–17	92.9 ± 0.1	96.8
Chloroform–methanol (1:3, v/v)	6–8	53.3 ± 0.3	90.4	15–16	92.5 ± 0.1	95.4

Conditions: column, 600 mm × 30 mm glass column packed with 100 g silica gel (100–200 mesh); loading amount, 1.5 g crude PE dissolved in the eluent; flow-rate, 3 ml/min.

[a] Numbers shown are the ranges of fractions containing PE or PC, respectively.

[b] The fractions containing PE or PC were combined together, respectively, removed solvent under vacuum at 50 °C. About 5 mg PE or PC product was weight exactly and dissolved by 25 ml chloroform–methanol (2:1, v/v), and then the concentration of which was determined triplicate. Purity was calculated by the following formula: (25 × concentration of PE or PC)/product weight.

[c] Recovery was evaluated by the following formula: (25 × concentration of PE or PC)/(crude PE weight × content of PE or PC in crude PE).

Table 6
The results of separation with gradient elution for the crude PC as raw material

Gradient elution mode	PE			PC		
	Collection number[a]	Purity[b] (%)	Recovery[c] (%)	Collection number[a]	Purity[b] (%)	Recovery[c] (%)
Mode 1[d]	8–10	78.9 ± 0.1	92.9	18–22	92.4 ± 0.1	94.5
Mode 2[d]	8–10	79.3 ± 0.2	92.7	17–20	91.9 ± 0.2	94.1
Mode 3[d]	8–10	79.9 ± 0.2	92.6	15–18	91.5 ± 0.1	94.1

Conditions: column, 600 mm × 30 mm glass column packed with 100 g silica gel (100–200 mesh); loading amount, 2.0 g crude PC dissolved in a little chloroform–methanol (2:1, v/v); flow-rate, 3 ml/min.

[a] Numbers shown are the ranges of fractions containing PE or PC, respectively.

[b] The fractions containing PE or PC were combined together, respectively, removed solvent under vacuum at 50 °C. About 5 mg PE or PC product was weight exactly and dissolved by 25 ml chloroform–methanol (2:1, v/v), and then the concentration of which was determined triplicate. Purity was calculated by the following formula: (25 × concentration of PE or PC)/product weight.

[c] Recovery was evaluated by the following formula: (25 × concentration of PE or PC)/(crude PC weight × content of PE or PC in crude PC).

[d] Gradient elutions were performed by A and B solvent systems. A solvent systems, chloroform–methanol (2:1, v/v), were the same in three modes. B solvent systems were different, which was chloroform–methanol (1:1, v/v) for Mode 1, chloroform–methanol (1:2, v/v) for Mode 2 and methanol for Mode 3.

Hence, it was difficult to select any mixed solvents as eluent while to obtain high purity PE with low cost.

3.3. Selection of eluents in gradient elution

The results of separation with gradient elution for the crude PC and PE as raw materials are listed in Tables 6 and 7, respectively.

The purities of the PC and PE products were slightly different in three gradient elution modes when the crude PC was used as raw material. With the increase of methanol content in B solvent system, the elution time was shortened and volume of eluent was consumed less. When single methanol was used as B solvent, the elution time and consumption of eluent decreased to the least (from 220 to 180 min and from 660 to 540 ml, respectively, see Table 6). The same trend for the crude PE as raw material was summarized in Table 7. Consequently, the Mode 3, in which single methanol was used as B solvent, should be selected in gradient elution for reducing the cost.

3.4. Selection of elution mode

As compared with the results between isocratic elution and gradient elution when the crude PC was used as raw material, it was seen that the purity of the PE product (72.1%) prepared by using isocratic elution with methanol as eluent was less than that by using gradient elution with Mode 3 (79.9%). However, the purities of the PC products obtained by two elution modes were not obviously different. Owing to PC to be the main component in the crude PC, the purity of the PC product was basis to select the elution mode. Moreover, the elution time and consumption of eluent were less in isocratic elution with methanol as eluent (150 min and 450 ml) than those in gradient elution (180 min and 540 ml for Mode 3, see Tables 4 and 6). So that, isocratic elution with methanol as eluent was preferable to gradient elution when the crude PC used as raw material.

Fig. 4 shows the HPLC chromatogram of the PC product prepared by using isocratic elution with methanol as eluent.

Table 7
The results of separation with gradient elution for the crude PE as raw material

Gradient elution mode	PE			PC		
	Collection number[a]	Purity[b] (%)	Recovery[c] (%)	Collection number[a]	Purity[b] (%)	Recovery[c] (%)
Mode 1[d]	8–12	77.8 ± 0.3	91.7	21–22	93.9 ± 0.1	97.3
Mode 2[d]	8–12	77.2 ± 0.2	92.0	19–20	93.4 ± 0.1	98.1
Mode 3[d]	8–12	76.3 ± 0.3	91.5	16–17	92.9 ± 0.1	98.0

Conditions: column, 600 mm × 30 mm glass column packed with 100 g silica gel (100–200 mesh); loading amount, 1.5 g crude PE dissolved in a little chloroform–methanol (2:1, v/v); flow-rate, 3 ml/min.

[a] Numbers shown are the ranges of fractions containing PE or PC, respectively.

[b] The fractions containing PE or PC were combined together, respectively, removed solvent under vacuum at 50 °C. About 5 mg PE or PC product was weight exactly and dissolved by 25 ml chloroform–methanol (2:1, v/v), and then the concentration of which was determined triplicate. Purity was calculated by the following formula: (25 × concentration of PE or PC)/product weight.

[c] Recovery was evaluated by the following formula: (25 × concentration of PE or PC)/(crude PE weight × content of PE or PC in crude PE).

[d] Gradient elutions were performed by A and B solvent systems. A solvent systems, chloroform–methanol (2:1, v/v), were the same in three modes. B solvent systems were different, which was chloroform–methanol (1:1, v/v) for Mode 1, chloroform–methanol (1:2, v/v) for Mode 2 and methanol for Mode 3.

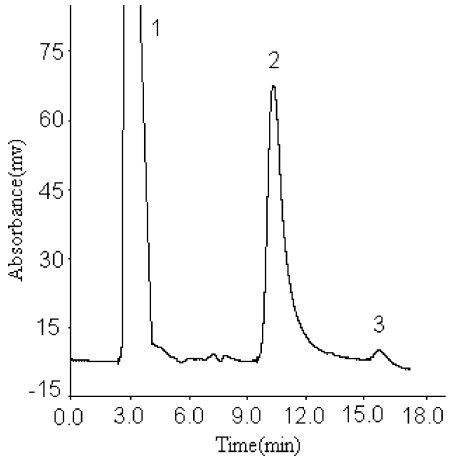

Fig. 4. Chromatogram of the PC product purified from the crude PC by using isocratic elution with methanol as eluent. Column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min; sample concentration, 0.2 mg/ml (in chloroform–methanol, 2:1, v/v). Peaks: (1) solvents; (2) PC; (3) unknown peak.

Figs. 5 and 6 give the spectra of infrared analyses for standard PC and the PC product, respectively.

According to the IR spectrum and HPLC analysis of authentic standard, it was indicated that the PC separated from the column chromatography was basically pure PC to contain very small amounts of impurities (see Table 1, Figs. 4–6).

Since PE was the main component in the crude PE, the purity of the PE product was used to evaluate the separation efficiency when the crude PE used as raw material. The results in Table 5 show that the purities of the PE products prepared by using the other eluents were low (purity <70%) except using chloroform–methanol (2:1, v/v) as eluent (purity 76.1%) in isocratic elution. However, the elution time was long (230 min) and the eluent was consumed much (690 ml) when this mixed solvents was used as eluent. This

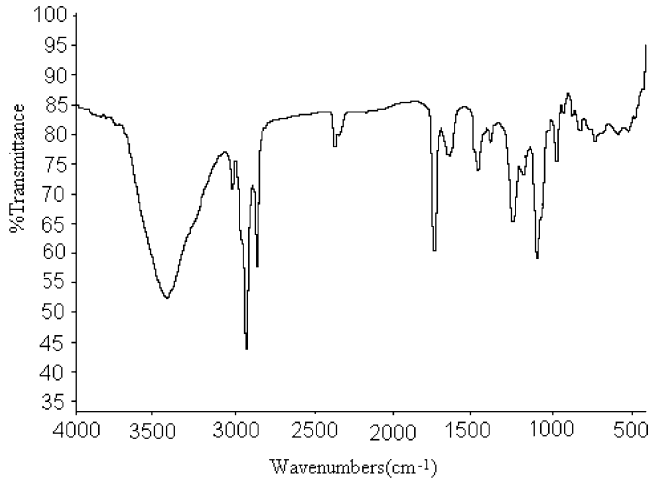

Fig. 5. IR spectrum of standard PC.

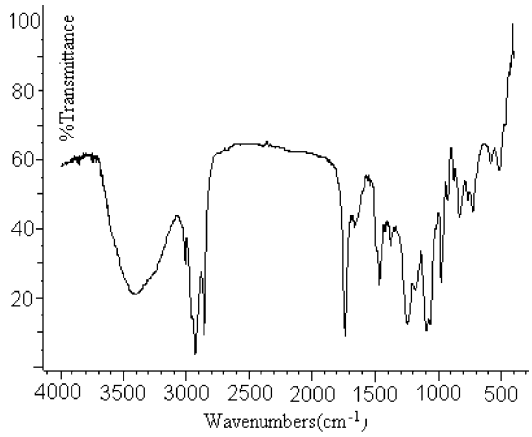

Fig. 6. IR spectrum of the PC product purified from the crude PC by using isocratic elution with methanol as eluent.

problem could be overcome by using gradient elution. In the gradient elution, PE was usually eluted out of the column before the B solvent to be changed. Thus, the purity and recovery of the PE product were greatly affected by the composition of A solvent. Because the A solvent systems, chloroform–methanol (2:1, v/v), were the same in three gradient elution modes, the purity and recovery of the PE products in three gradient elution and those in isocratic elution using chloroform–methanol (2:1, v/v) as eluent were slightly different (see Tables 5 and 7). However, the elution time of the PC products were great different with different strength of the solvents to be used as B solvent systems in gradient elution. With the increase of the strength of the B solvents, the total separation time and solvent consumption decreased. The B solvent was single methanol in gradient Mode 3, the strength of which was the strongest. Consequently, although the purity of the PE product was slightly different (purity 76.3 and 76.1%), the elution time and consumption of eluent decreased much (only 170 min and 510 ml to be used) by using gradient Mode 3 (see Table 7) compared with those by using chloroform–methanol (2:1, v/v) as eluent for isocratic elution (230 min and 690 ml, see Table 5).

As a whole, gradient elution was preferable to isocratic elution for separation and purification of PE when crude PE used as raw material, and gradient Mode 3 should be used for saving the separation time and consumption of eluent.

Fig. 7 shows the HPLC chromatogram of the PE product purified from the crude PE by using gradient Mode 3. The purity of the PE product was not high (<80%) owing to being always accompanied by some PI (see Fig. 7). The resolution of PI and PE was not good when using irregular silica as packing and simple binary solvents as eluent in our work. Unless the higher efficient silica or more complex solvent system was used, the PE and PI would not be separated completely. However, it was not our aim because of the increase of the production cost. Some impurities usually together with PI were eluted out of the column earlier than PE, so that the purity of the PI was low. Furthermore, the recovery of the PI was also low because PI was difficult to

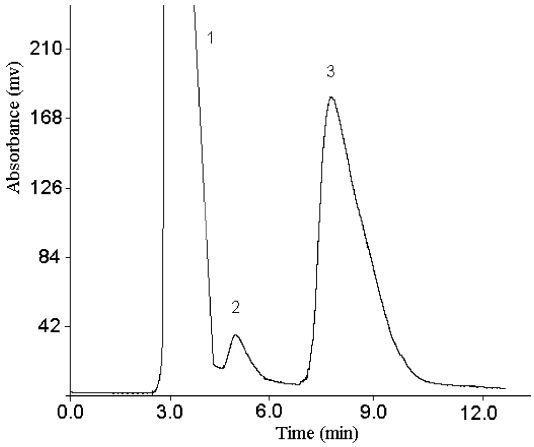

Fig. 7. Chromatogram of the PE product purified from the crude PE by using gradient Mode 3. Column, 150 mm × 4.6 mm silica column; mobile phase, acetonitrile–methanol–85% phosphoric acid (180:3:1, v/v); injection volume, 20 ul; flow-rate, 0.5 ml/min; sample concentration, 0.5 mg/ml (in chloroform–methanol, 2:1, v/v). Peaks: (1) solvents; (2) PI; (3) PE.

be separated from PE thoroughly. Hence, the recovery of PI was not considered in our work. It was needed to be further researched that the high purity PI and PE were separated from the crude PE.

3.5. Effect of loading amount on separation of column chromatography

The crude PC was separated by methanol as eluent and the crude PE was separated by using gradient Mode 3 with the flow-rate to be controlled at 3 ml/min. The fractions containing PC or PE should be combined together for guaranteeing the recoveries of the products to be more than 90%. The other conditions and procedures were the same as the above. The effect of loading amount on separation efficiency was evaluated by changing the sample loading from 1.5 to 3.0 g.

The plots in Fig. 8 demonstrated that the purities of the PE and PC products separated from the crude PC and PE, respectively, changed with the increase in the loading amount from 1.5 to 3.0 g.

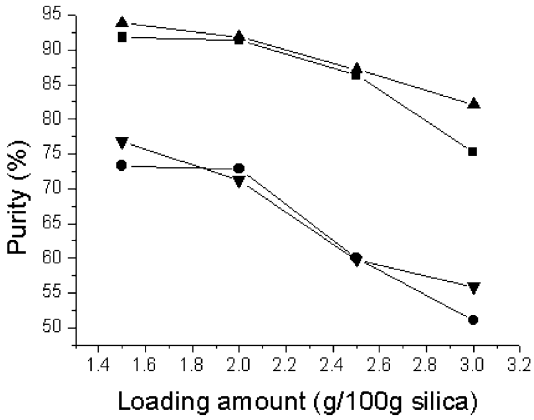

Fig. 8. The purities of the PC and PE products as functions to loading amount. (■) PC from the crude PC; (●) PE from the crude PC; (▲) PC from the crude PE; (▼) PE from the crude PE.

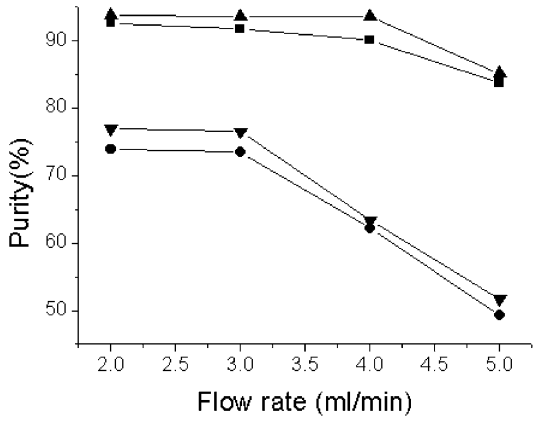

Fig. 9. The purities of the PC and PE products as functions to flow-rate. (■) PC from the crude PC; (●) PE from the crude PC; (▲) PC from the crude PE; (▼) PE from the crude PE.

The purities of the products prepared from the crude PC and PE were all high when sample loading was less than 2.0 g (>90% for PC and >70% for PE). Once the loading amounts were more than 2.0 g, the purities of the products decreased sharply (see Fig. 8). Consequently, the loading amount should be limited to less than 2.0 g/100 g silica gel.

3.6. Effect of the flow-rate on separation of column chromatography

1.5 g crude PC and PE were used for preparation of the pure PC and PE products, respectively. The elution modes and procedures were the same as the above except the flow-rate to be changed from 2 to 5 ml/min.

The purities of the PE and PC products as functions to the flow-rate were plotted in Fig. 9. The purities of the PC products prepared from the crude PC or PE were not obvious differences when the flow-rate of eluent was less than 4 ml/min (purities of all PC products >90%). When the flow-rate surpassed 4 ml/min, the purities of the PC products would decrease rapidly. The purities of the PE products were high (>70%) when the flow-rate was under 3 ml/min. Once the flow-rate was more than 3 ml/min, the purities of the PE products dropped sharply.

Therefore, the appropriate flow-rate was controlled under 4 ml/min for preparation of the pure PC product from the crude PC and under 3 ml/min for obtaining the PE product from the crude PE.

4. Conclusion

The low-cost process proposed in this work to separate and purify the high purity PC and PE from soybean degummed oil residues is feasible. The degummed oil residues were removed from impurities firstly, and then separated

by extraction into the crude PC and PE to be used as raw materials, which was a vital step to total separation process, for further purification with column chromatography.

The high purity PC (purity >90%) was obtained successfully from the crude PC by using isocratic elution with methanol as eluent. Compared with those methods reported by using ternary mixed solvents as eluent or gradient elution mode, the process proposed exhibits short elution time, low cost and industry potentialities due to some advantages, such as operation simplicity, cheap equipment and solvent to be recovered easily.

The purer PE (purity >75%) could be prepared from the crude PE by using binary mixed solvents as eluents, and the gradient elution was preferable to isocratic elution for shorting the elution time and decreasing the consumption of eluent.

Acknowledgements

The authors would to acknowledge the support of the Science and Technique Bureau of Panyu District at GuangZhou City.

References

[1] C.A. Demopoulos, S. Antonopoulou, N.K. Andrikopoulos, V.M. Kapoulas, J. Lip. Chromatogr. Rel. Technol. 521 (1996) 19.
[2] S.L. Abidi, T.L. Mounts, J. Chromatogr. 93 (1997) 773.
[3] S. Uran, A. Larsen, P.B. Jacoben, T. Skotland, J. Chromatogr. 265 (2001) 758.
[4] N.U. Olsson, N. Salen Jr., J. Chromatogr. 245 (1997) 692.
[5] B.F. Szuhaj, Lecithins: Sources, Manufacture and Uses, American Oil Chemists' Society, Champaign, 1989.
[6] H. Pardun, Fat Sci. Technol. 45 (1989) 91.
[7] P.R. Eckard, L.T. Taylor, G.C. Sleck, J. Chromatogr. 241 (1998) 826.
[8] D. Swern, Bailey's Industrial Oil and Fat Products, Wiley/Interscience, New York, 1982.
[9] C. Hanras, J.L. Perrin, J. Am. Oil Chem. Soc. 804 (1991) 68.
[10] G. Lendrath, A. Nasner, L. Kraus, J. Chromatogr. 385 (1990) 502.
[11] J. Touchstone, J. Chromatogr. 169 (1995) 671.
[12] T.H. Yoon, I.H. Kim, J. Chromatogr. 209 (2002) 949.
[13] J.J. Myher, A. Kuksis, J. Chromatogr. 3 (1995) 671.
[14] B.D. Meulenaer, P.V. Meeren, J. Vanderdeelen, L. Baert, Chromatographia 41 (1995) 527.
[15] W.J. Hurst, R.A. Martin, R.M. Sheeley, J. Liq. Chromatogr. 2969 (1986) 9.
[16] P.E. Balaza, P.L. Schmit, B.F. Szuhaj, J. Am. Oil Chem. Soc. 193 (1996) 73.
[17] D.J. Hanahan, A Guide to Phospholipid Chemistry, Oxford University Press, New York, 1997.
[18] W. Li, Y.Y. Shao, G.D. Huang, Chin. Oils Fats 6 (2001) 26.
[19] X.F. Du, C.X. Liu, Chin. Acta Nutr. Sinica 50 (1995) 17.
[20] Y.H. Wang, B. Yang, S.Z. Liang, Y.J. Wang, Chin. Oils Fats 36 (2000) 25.
[21] R.S. Zhao, X.P. Hou, B.X. Yan, Chin. J. Modern Appl. Pharm. 39 (2001) 18.
[22] S.S.-H. Chen, A.Y. Kou, J. Chromatogr. 25 (1982) 227.
[23] S.U. Rehman, J. Chromatogr. 29 (1991) 567.
[24] A. R-B de Quiros, J. Lopez-Hernandez, J. Simal-lozano, J. Chromatogr. 71 (2002) 770.
[25] J.F. Brouwers, et al., J. Lip. Res. 344 (1998) 39.