

Purification of Soybean Phosphatidylcholine Using D113-III Ion Exchange Macroporous Resin Packed Column Chromatography

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Abstract Ten ion-exchange resins were compared for their abilities to separate soybean phosphatidylcholine (PC). D113-III resin was selected for PC purification among ten resins tested. The optimum PC adsorption conditions were: concentration of PC solution, 3 mg/mL; pH of PC solution, 7.5; and adsorption flow rate, 1 mL/min. The optimum PC desorption conditions were: eluent, 95% ethanol aqueous solution; and elution flow rate, 1 mL/min. Under these conditions, the PC content in the product and the amount of product were 94 and 80% (wt.%), respectively, and the D113-III resin was able to be used at least six times for purifying PC. The adsorption and desorption characteristics were also studied.

Keywords Ion-exchange resin · Phosphatidylcholine · Phospholipid separation

Introduction

Soybean phosphatidylcholine (PC) is extensively used as emulsification, stabilization and wetting agents in manufacturing foods, cosmetics, and pharmaceuticals [1–3]. However, a large amount of soybean PC, although preferred with high purity (>80%) [4], is used currently with low purity (30–60%) due to limitations of purification technology and costs [5–8]. Various methods have been

attempted for purifying soybean PC, including solvent extraction [9, 10], supercritical fluid extraction (SFE) [11], HPLC [12, 13], and column chromatography [14]. In these methods, solvent extraction and SFE have an unsatisfactory separation efficiency; HPLC, although having the highest separation efficiency, possesses the least industrial feasibility so far due to its high costs; and column chromatography is considered more appropriate for industrial purification of phospholipids, yet its potential has not been fully tapped for large-scale purification of PC due to the poor availability of cost-effective chromatographic media that give satisfactory yields and purities of the products. Alumina packed column chromatography of PC has been used in industry to prepare PC of high purity and yield, but the price of these matrices is still very high [15].

Macroporous resin adsorption chromatography, in general, is an efficient separation method used for industrial refining and purifying of bioactive substances from natural resources [16, 17]. This method has major advantages over conventional matrices-based chromatography including a more than tenfold increase in sample loading capacity, higher concentration of fractions eluted, higher adsorption specificities, more facile desorption, better mechanical strength and reusability, and lower fluid resistance [18]; and the continuing development of novel macroporous resins adds to the versatility of this technology in purifying natural products. However, a search of the literature by the authors failed to find reports regarding the purification of soybean PC by macroporous resin adsorption chromatography.

The aim of the present work was to develop a cost-effective process for preparing high-purity soybean PC from crude soybean PC by macroporous ion-exchange resin packed-column chromatography. Commonly used

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macroporous ion-exchange resins were to be screened by using static adsorption and desorption tests; the chromatography conditions pertaining to the selected resin for separating soybean PC optimized by using dynamic column adsorption and desorption tests; and the reusability of the selected resin was also evaluated.

Materials and Methods

Materials

Alcohol-soluble phospholipids (ASP) (PC content ~40%), which were prepared by extracting food-grade lecithin powder with 95% (v/v) alcohol, were provided by the East Ocean Oils & Grains Industries Co. Ltd (Zhangjiagang, PR China). Isopropanol, hexane, and methanol were all of HPLC purity. All other reagents were of analytical grade. Ion-exchange resins were provided by Suqing Group (Jiangyin, PR China). The structural and functional properties of these ion-exchange resins are listed in Table 1.

Pretreatment of Ion-exchange Resins

Each cation-exchange resin was treated consecutively with the treating medium discharged on completion of each step: soaking in ~2 volumes of saturated sodium chloride (NaCl) solution for 18–20 h; washing with fresh water until no yellow color was observed in the water; washing in ~2 volumes of 4% sodium hydroxide (NaOH) solution for 2–4 h; re-washing with fresh water until neutrality was reached; soaking in ~2 volumes of 5% hydrochloric acid (HCl) solution for 2–4 h; and a final washing with fresh water until neutrality was attained. Each anion-type resin was treated following the steps similar to that for cation-

type resins except that the order of the alkali and acid treatments was reversed.

Static Adsorption Tests

Static adsorption tests were performed as follows: 2 g of each pretreated ion-exchange resin (dry weight) was placed in a flask followed by adding 30 mL alcohol-soluble ASP solutions of PC (1.63 mg/mL). Each flask was capped and shaken at 180 rpm for 6 h in a water bath maintained at 25 °C. The solutions after adsorption were analyzed by HPLC.

Static Desorption Tests

Each adsorbate-laden resin was first washed with deionized water and then desorbed with 30 mL aqueous ethanol at different volumetric ratios (20:80, 40:60, 60:40, 80:20, 95:5). The flasks were then shaken at 180 rpm for 6 h in a 25 °C water bath. Effluents were analyzed by HPLC.

Dynamic Adsorption and Desorption Tests

According to the results of the preceding tests, D113-III resin was determined to be the resin most suitable for further testing. Dynamic adsorption and desorption experiments were carried out in glass columns (Φ 1.6 × 50 cm) wet-packed with 4 g D113-III resin (dry weight). PC solution (2.23 mg/mL) was applied onto the bed at 1 mL/min and the concentrations of PC in the effluent were monitored by HPLC. On adsorption equilibrium, the adsorbate-laden column was eluted successively with deionized water and 80% aqueous ethanol, both at a flow rate of 1 mL/min. The PC concentrations in the effluent were also determined by HPLC.

HPLC Analysis

Samples were analyzed by HPLC using a Waters 1525 liquid chromatographic system equipped with a Lichrospher Si column (25 × 0.46 cm I.D., 5 μ m) maintained at 30 °C. PC was eluted using a binary gradient of solvent A (1:1, v/v, hexane/isopropanol) and solvent B (4:4:1, v/v/v, hexane/isopropanol/1% aqueous acetic acid) at 1 mL/min. Elution was conducted using a linear gradient from 100% (v/v) A to 100% B in 20 min followed by an isocratic flow of 100% B for 5 min at 35 °C. The effluent was monitored at 205 nm.

Working calibration curves, as constructed using PC and phosphatidylethanolamine (PE) standard solutions, showed good linearity over the range of 5–55 and 5–200 μ g/mL for PC and PE, respectively. Regressions for PC and PE were: $y = 21088x + 23296$ ($R^2 = 0.9968$) and $y = 18905x +$

Table 1 Structural and functional properties of ten ion-exchange resins tested

Model	Functional group	Ion form	Quantity exchange volume (mmol/g)	Volume exchange capacity (mmol/mL)
D001	–SO ₃ [–]	Na	≥4.35	≥1.8
001 × 7			≥4.5	≥1.9
SQD-61			≥4.35	≥1.8
201 × 4	–N ⁺ (CH ₃) ₃	Cl	≥3.8	≥1.1
201 × 7			≥4.0	≥0.75
D296			≥3.6	≥1.1
D113-III	–COOH	H	≥11.0	≥4.3
SQD-88			≥10.0	≥4.0
D301-III	–N(CH ₃) ₂	OH	≥4.8	≥1.45
D318	–NH(CH ₂) ₃ [–]		≥7.0	≥2.2

7894.8 ($R^2 = 0.9927$), respectively, where y is the peak area of the analyte and x the injected quantity (μg) of pure PC and PE corrected for their respective purity in the standards.

Calculation of Adsorption Capacity, Adsorption Ratio, Desorption Ratio, and Leakage Ratio

Adsorption capacity and adsorption ratio were calculated using the following equations:

$$Q = \frac{(C_0 - C_e) \times V}{W} \quad (1)$$

$$X(\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

where Q is the saturated adsorption capacity (mg/g resin); X the adsorption ratio defined as the percentage of quantity adsorbed at equilibrium relative to the initial quantity; C_0 and C_e are initial and equilibrium PC concentrations of the solutions (mg/mL), respectively; V the volume of the initial sample solution (mL); and W the weight of the dry resin (g).

The desorption ratio was calculated using the following equation:

$$B(\%) = \frac{C_1 \times V_1}{(C_0 - C_e)} \times 100 \quad (3)$$

where B is the desorption ratio (%); C_1 the concentration of PC in the effluent (mg/mL); V_1 the volume of effluent (mL); and C_0 and C_e are the same parameters as in Eqs. 1 and 2.

The leakage ratio was calculated using the following equation:

$$\eta(\%) = \frac{C_t \times V_t}{C_0 \times V_0} \times 100 \quad (4)$$

where η is the leakage ratio (%); C_t the PC concentration of the effluent (mg/mL); V_t the volume of the effluent (mL); C_0 the PC concentration of initial solution (mg/mL); and V_0 the volume of the initial solution (mL).

Statistical Analysis

The data obtained were analyzed using the ANOVA ($P < 0.05$) package of Statistica TM 6.0 data analysis software (Statsoft, Inc., USA).

Results and Discussion

Static Adsorption and Desorption Tests of Different Resins

Among ten resins tested in static adsorption tests, resins coded 201 \times 7, 201 \times 4 and D296 showed considerably

higher adsorption capacities and adsorption ratios towards PC, and that coded D113-III, 001 \times 7, and SQD-88 showed intermediate adsorption capacities and adsorption ratios (Table 2). Thus, only these six resins were subjected to subsequent static desorption tests. D113-III and SQD-88 resins showed the highest desorption ratios among the six resins tested (Table 2), which is attributable to the interactions of weak acidic groups on the micropore surface and the strong acidic quaternary ammonium structure present in PC molecules. D113-III, thanks to its overall benefits in terms of weak acidity, specific surface area and pore sizes suitable for adsorption and desorption, excellent resistance to physical breakage, osmotic shock fracture, and high resistance to organic fouling, in addition to its lower cost, was therefore considered to be the matrix of choice to establish a packed column chromatographic process for purifying soybean PC.

Effect of the Concentration of Aqueous Ethanol Solution on the Desorption Ratio of D113-III Resin

The eluent was selected according to PC solubility and the polarity of the resin. PC dissolves readily in methanol, ethanol, benzene and other organic solvents. We selected aqueous ethanol as the eluent because of its low cost and safety. Aqueous ethanol (60 and 80%, v/v) had higher desorption ratios, with the highest desorption ratio (87%) observed when 60% aqueous ethanol concentration was used (Fig. 1).

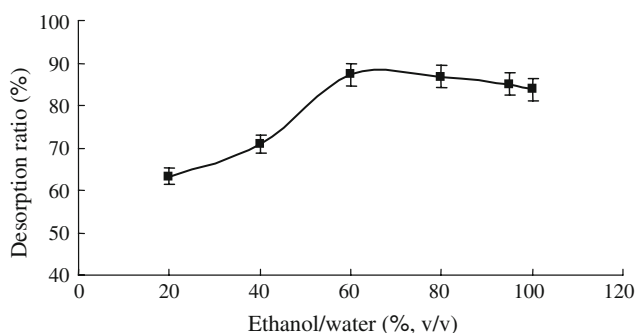
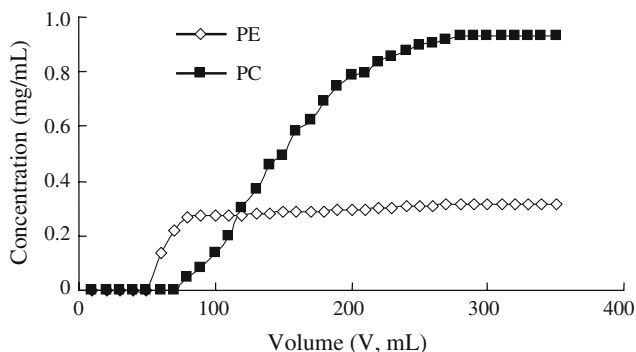
Dynamic Leakage Curve for D113-III Resin

The mere use of static evaluation is not enough for assessing the separating performance of an adsorbent. The adsorption effect of a macroporous resin is determined by its surface adsorption and sieve classification, surface electrical property, hydrogen bonding, etc. Leakage of solutes from the resin, which occurs after equilibrium of adsorption, should be evaluated by setting up relevant leakage curves in order to establish the appropriate volume of crude PC solution relative to the quantity of resin. The concentration of PC in the effluent increased with an increasing amount of crude PC solution volume applied. Adsorption equilibrium was reached when the effluent combined amounted to 270 mL, which indicated resin saturation by absorbed PC and PE. The saturated adsorption capacity was determined to be 38 mg PC/g resin and 4 mg PE/g resin (Fig. 2).

The dynamic desorption curves were obtained based on the volumes of effluent versus the concentrations of eluents. Aqueous ethanol (95%) had the highest PC desorption capacity among ethanol concentrations tested. Approximately, 400 mL solution completely desorbed PC from the

Table 2 Adsorption ratio, adsorption capacity and desorption ratio of PC on different resins

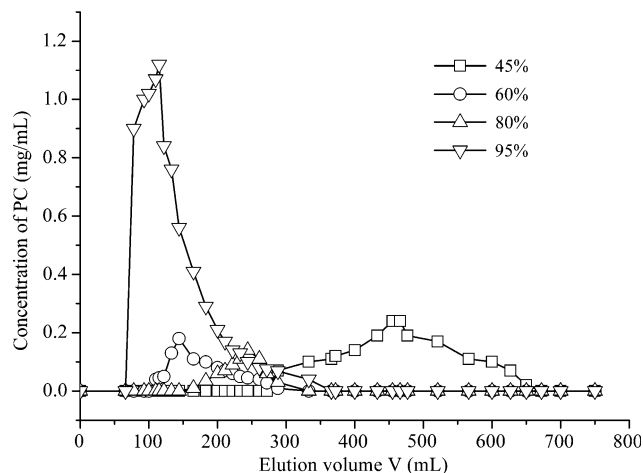
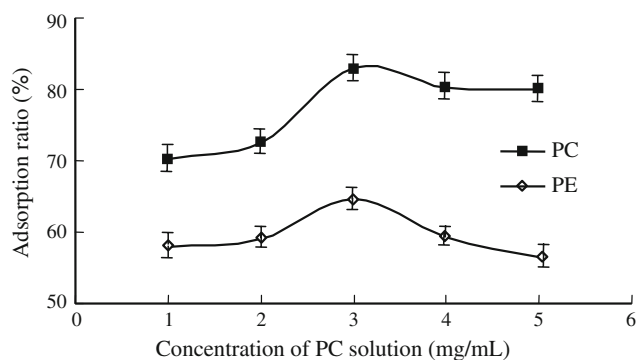
Resins	201 × 7	D301-III	D001	201 × 4	D113-III	001 × 7	SQD-61	SQD-88	D296	D318
Adsorption ratio (%)	98.0 ± 1.8	17.9 ± 2.0	41.3 ± 1.5	91.8 ± 2.0	63.9 ± 2.0	59.3 ± 2.1	39.1 ± 1.8	57.6 ± 2.2	86.7 ± 2.4	16.1 ± 1.3
Adsorption capacity (mg/g)	107.2 ± 1.5	19.3 ± 0.9	45.8 ± 1.3	100.8 ± 1.6	71.2 ± 1.8	67.9 ± 1.6	43.2 ± 1.0	62.7 ± 1.3	100.9 ± 2.1	16.1 ± 0.8
Desorption ratio (%)	2.1 ± 0.2			6.8 ± 0.4	88.1 ± 2.2	46.5 ± 1.5		80.1 ± 2.1	50.1 ± 1.4	

**Fig. 1** Effect of ethanol concentration on the desorption ratio of D113-III resin**Fig. 2** Dynamic leakage curve of D113-III resin

D113-III resin. Approximately, fourfold packed-bed volumes of 95% aqueous ethanol was the minimum for sufficient PC desorption (Fig. 3), and was thus considered the appropriate amount of eluent to attain both sufficient desorption and the lowest PC dilution in the effluent.

Effect of PC Concentration on the Adsorption Ratio

PC adsorption ratio showed increasing–decreasing patterns with increasing crude PC concentrations, with the maximum absorption ratio (83%) obtained when the PC concentration was 3 mg/mL (Fig. 4). Consequently, considering both separation efficiency and adsorption ratio, a crude PC concentration of 3 mg/mL was used in the following experiments.

**Fig. 3** Effects of different concentrations of ethanol aqueous solutions on PC elution**Fig. 4** Effect of PC concentration on the adsorption ratio of D113-III resin

Effect of pH of Crude PC Solution on the Adsorption Ratio

The pH of the crude PC solution determines the extent of ionization and the adsorption affinity of PC molecules towards the resin. As observed in this work, the adsorption ratio of PC reached its maximum at pH 7.5, and then decreased with any further increase in the pH of the crude PC solution (Fig. 5). This is understandable since D113-III resin is a low-acidic cation-type resin, which has higher

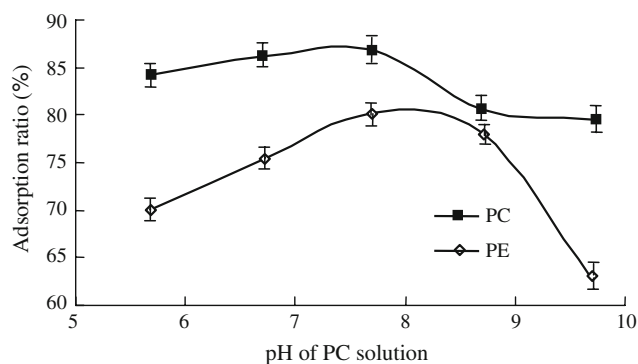


Fig. 5 The effect of pH of PC solution on the adsorption ratios of D113-III resin

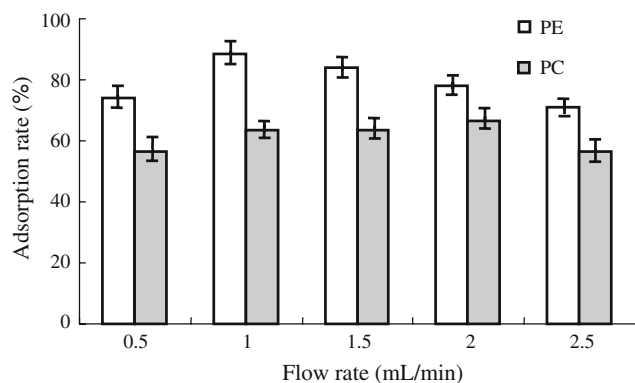


Fig. 6 Effect of adsorption flow rate on adsorption rate of D113-III resin

ion-exchange capacities for PC and PE in low-acidic environments. When the pH of the crude PC solution was >7.5 , excessive Na^+ may compete with PC and PE for adsorption, and the alkaline environment might also cause damage to the resin. Considering both the adsorption ratio and cost, the crude PC solution with a pH of 7.5 was not adjusted in pH prior to chromatographic adsorption.

Effect of Flow Rate on Adsorption Rate

Adsorption flow rate mainly affects the diffusion rate of solute in the bed. The PC and PE adsorption rates as functions of the flow rate were plotted. PC adsorption rates had an increasing–decreasing pattern with increasing flow rate, showing its maximum (89%) at a flow rate of 1 mL/min (Fig. 6). Thus, for favorable PC enrichment, an adsorption flow rate of 1 mL/min was used in subsequent experiments.

Effect of Elution Flow Rate on Desorption Ratio

Appropriate elution flow rate is a key parameter for attaining higher product purity and elution capacity. PC

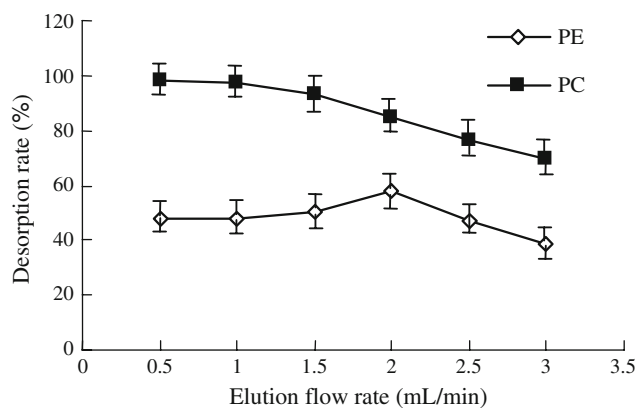


Fig. 7 Effect of eluent flow rate on desorption rate of D113-III resin

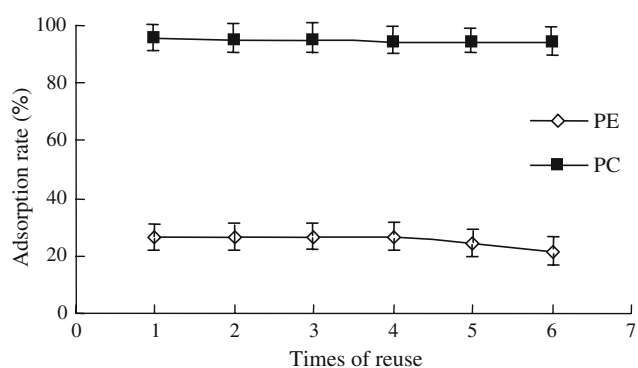


Fig. 8 Effect of reusing resin on adsorption rate

concentration in the effluent was relatively high (0.5–1 mL/min), compared to those at higher elution flow rates (Fig. 7). The desorption rate decreased sharply when the elution flow rate was increased above 1.5 mL/min (Fig. 7). On full consideration of the elution time and desorption ratio, the appropriate elution flow rate was selected as 1 mL/min.

Reusability of Resin

The recovered resin was able to be used six and four times without significantly decreasing the PC and PE adsorption ratios, respectively (Fig. 8). These results indicate that D113-III resin could be used at least six times for PC purification, which is encouraging for industrial production of high-purity soybean PC.

Verification of Optimized Parameters for PC Purification

A PC content of 94% (wt%) in the product at a recovery of 80% (wt%) was obtained in confirmative experiments using the optimized D113-III resin packed-column chromatographic process, which was comparable with the

performance of Al₂O₃ column chromatography [15] and preparative chromatography [19]. The method established here, however, has lower costs and higher loading amounts.

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