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Separation of phosphatidylcholine from soybean phospholipids by simulated moving bed*

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Abstract: A simulated moving bed (SMB), equipped with eight silica-gel columns, was used to separate phosphatidylcholine (PC) from soybean phospholipids. The effects of flow rate in Sections 2 (Q_2) and 3 (Q_3), switching time, feed flow rate and feed concentration on the operating performance parameters: purity, recovery, productivity and desorbent consumption were studied. Operating conditions leading to more than 90% purity in both outlet streams have been identified, together with those achieving optimal performance. Regions leading to complete separation are observed and explained theoretically. As the mass-transfer effect was not considered, the triangle theory only gives initial guesses for the optimal operating conditions.

Key words: Simulated moving bed (SMB), Separation, Soybean phosphatidylcholine (PC)
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INTRODUCTION

Natural phospholipids, main constituents of cell membranes, have many important biological functions for all living organisms. High-purity phosphatidylcholine (PC), a fraction of phospholipids, has been extensively applied as emulsification, stabilization and wetting agents in the fields of health products, pharmaceuticals, foods and industrial manufacture of various products, etc.

High-purity PC prepared by preparative chromatography had been reported in (Li, 2004; Amari and Brown, 1990). Its inherent shortcomings are low loading amount and high desorbent consumption, which lead to high investment and production cost.

Simulated moving bed (SMB) is a continuous chromatographic countercurrent process and has been widely used on large scale in the past few decades (Ruthven, 1984; Ruthven and Ching, 1989). Compared to preparative chromatography, the adsorbent inventory and the desorbent consumption are much less. In addition, high performance can be achieved even when the adsorbent selectivity components is rather low (Jupke *et al*., 2002; Bruce, 1998).

It is known that the affinity of PC to the stationary phase (silica gel) is higher than that of all the other phospholipids in the soybean crude lipids (Meeren *et al*., 1990). Therefore, SMB is expected to be a feasible process for separation/purification of PC from crude lipids.

This work was aimed at evaluating the SMB performance in preparation of high purity PC and at studying the effect of operation parameters (flow rates in each section of SMB, switching time, feed flow rate and feed concentration) on purity, recovery of PC, productivity, and desorbent consumption. It was expected that optimal operation conditions could be obtained from this study and could be useful for scaling up of a separation process of PC by SMB.

EXPERIMENTAL DETAILS

Apparatus

The SMB unit used was a CSEP 9116 from

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Knauer, Berlin (Fig.1). It consisted of a carousel of 8 stainless steel columns (100 mm×20 mm i.d.) connected to a single multi-function valve. Two HPLC pumps (K-501, Knauer) pumped the feed and the eluent and another two pumps partially recycled the inner currents from Section 1 into Section 2 and from Section 3 into Section 4. The temperature of the columns was maintained at 30 °C by enclosing the entire rotating system within a thermostatically controlled system. The stationary phase was silica gel (20~30 μm), purchased from Meigao Chemical Group (Qingdao, China), where Brunauer-Emmett-Teller (BET) surface is: 312 m^2/g, average pore diameter is 11.8 nm. The mobile phase was *n*-hexane-isopropanol-water (1:2.1:0.5, v/v/v).

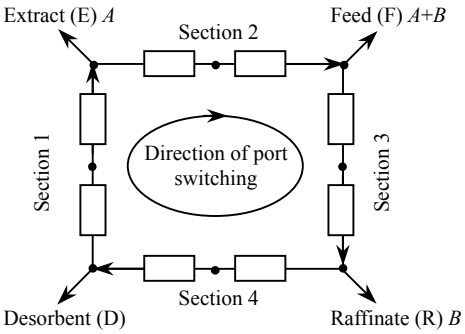

Fig.1 Scheme of a four-section SMB unit

The composition of the samples from SMB was determined by HPLC with column of 100 mm×4.6 mm i.d., packed with silica gel (5 μm). The mobile phase was ethanol-water (80:20, v/v), at flow rate of 1.0 ml/min. The wavelength of the UV detector was set as 205 nm. Phosphatidylcholine purchased from Lipoid (purity>98%, Germany) was used as standard for quantitative determination.

Column preparation and characterization

A slurry-packing technique was used for packing the columns. The total porosity of the column was determined by the retention volume of a pulse of 1,3,5-tri-tert-butyl-benzene. The bed voidage was calculated from Table 1 showing the parameters of the eight columns and their performance.

Adsorption isotherms

Since the concentration of soybean lipids in the SMB feed was quite low, linear adsorption isotherm was applied. A pulse of diluted mixture was injected into a column and the retention times of PC peaks and other components $V_{R,i}$ were determined. The Henry constant K_i was calculated using the following equation:

$$K_i=\left(\frac{V_{R,i}-V_0}{V_0}\right)\frac{\varepsilon_t}{1-\varepsilon_t},$$

where V_0 is the dead volume of the column; ε_t is the total porosity of the column.

The adsorption isotherms obtained are as follows:

$$q_A=1.42c_A \text{ and } q_B=0.19c_B,$$

where q is concentration of component in the solid phase and c is concentration of component in the liquid phase; subscripts A and B denote PC and other components respectively.

Table 1 Summary of results of column characterization (flow rate=5.0 ml/min)

No.	Dead time (min)	Total porosity	Deviation (%)
1	3.640	0.580	0.49
2	3.603	0.574	0.52
3	3.628	0.578	0.17
4	3.654	0.582	0.88
5	3.604	0.574	0.50
6	3.679	0.586	1.57
7	3.539	0.564	2.30
8	3.629	0.578	0.19
Average	3.622	0.577	

SMB experimental runs

Five series of experimental runs were performed with operating conditions in terms of flow rate (Q_j), switch time (t_s), as reported in Table 2, together with the measured separation performance in terms of product purity, recovery, desorbent consumption and productivity.

RESULTS AND DISCUSSION

The following criteria were used to evaluate the separation performance: purity of PC in the extract

$100C_E^A/(C_E^A+C_E^B)$; recovery of PC $100Q_EC_E^A/Q_FC_F^A$; desorbent consumption $Q_D/C_E^AQ_E$; and productivity of stationary phase $C_E^AQ_E/V_S$.

These criteria were affected by flow rate in each section, switching time, feed flow rate and feed concentration. Five series of experiments were carried out. In each series only one factor was changed to study its effect on the performance.

Effect of flow rate of Q_2 and Q_3

The effect of flow rate of Sections 2 (Q_2) and 3 (Q_3) are listed in series A (Table 2), and shown in Fig.2. With other flow rates constant, Q_2 and Q_3 related to each other. In other words, only one of these two flow rates could change independently. The results of series A showed that PC purity increases from 71.9% to 90.2% as Q_2 (and Q_3) increases. Since the feed flow rate was constant, increasing Q_2 (and Q_3) implied that the desorbent flow rate was increasing. In consequence, the concentration front of PC and other lipids would move toward the raffinate outlet. As a result, the PC concentration in both the extract and the raffinate became higher, i.e., PC purity became higher and the "purity" of other lipids lower (from 98.0% to 91.8%). On the other hand, since more PC moved into the raffinate, PC recovery became lower (from 96.3% to 85.5%). The productivity based on the feed should not change with the flow rate in the bed, but the productivity based on produced PC changed with recovery by the same ratio, i.e., decreased from 1.039 g/(h·L) to 0.922 g/(h·L). The desorbent consumption based on unit weight of PC in the extract became greater (from 0.782 L/g to 0.881 L/g) because the recovery of PC decreased.

Effect of switching time

The effect of switching time are listed in series B,

Table 2 Operation conditions and performance of the experimental runs

Run	Flow rates (ml/min)					t_s (min)	Purity (%)		Recovery (%)	Desorbent consumption (L/g)	Productivity (g/(h·L))
	Q_1	Q_2	Q_3	Q_4	Q_F		P_R	P_E			
A1	6.5	3.6	4.1	3.1	0.5	6	98.0	71.9	96.3	0.782	1.039
A2	6.5	3.9	4.4	3.1	0.5	6	97.2	73.4	96.2	0.783	1.037
A3	6.5	4.2	4.7	3.1	0.5	6	96.9	81.5	95.3	0.791	1.027
A4	6.5	4.5	5.0	3.1	0.5	6	95.1	89.4	93.5	0.806	1.008
A5	6.5	4.8	5.3	3.1	0.5	6	91.8	90.2	85.5	0.881	0.922
B1	6.5	4.5	5.0	3.1	0.5	4	70.1	50.2	47.4	1.589	0.511
B2	6.5	4.5	5.0	3.1	0.5	5	92.6	81.8	88.2	0.854	0.951
B3	6.5	4.5	5.0	3.1	0.5	6	95.1	89.4	93.5	0.806	1.008
B4	6.5	4.5	5.0	3.1	0.5	8	93.5	83.6	89.5	0.842	0.965
B5	6.5	4.5	5.0	3.1	0.5	10	90.0	75.7	84.2	0.895	0.908
C1	6.8	4.5	5.5	3.4	1.0	4	66.5	40.8	54.0	0.697	1.165
C2	6.8	4.5	5.5	3.4	1.0	5	91.1	78.3	85.3	0.441	1.841
C3	6.8	4.5	5.5	3.4	1.0	6	94.0	83.0	90.2	0.418	1.945
C4	6.8	4.5	5.5	3.4	1.0	8	89.8	75.2	83.8	0.449	1.808
C5	6.8	4.5	5.5	3.4	1.0	10	88.1	67.4	82.3	0.458	1.775
D1	7.0	4.5	6.0	3.6	1.5	4	65.3	39.3	53.0	0.474	1.714
D2	7.0	4.5	6.0	3.6	1.5	5	82.7	71.1	70.3	0.357	2.276
D3	7.0	4.5	6.0	3.6	1.5	6	88.7	75.6	81.8	0.307	2.646
D4	7.0	4.5	6.0	3.6	1.5	8	84.5	72.8	74.4	0.337	2.407
D5	7.0	4.5	6.0	3.6	1.5	10	78.5	68.0	61.3	0.410	1.983
E1	6.8	4.5	5.5	3.4	1.0	4	64.8	38.0	51.0	0.354	2.297
E2	6.8	4.5	5.5	3.4	1.0	5	89.9	68.4	85.6	0.293	2.769
E3	6.8	4.5	5.5	3.4	1.0	6	90.6	78.5	84.7	0.296	2.742
E4	6.8	4.5	5.5	3.4	1.0	8	87.2	73.5	78.6	0.319	2.544
E5	6.8	4.5	5.5	3.4	1.0	10	85.3	65.2	78.1	0.321	2.528

Note: Feed concentration of series A, B, C, D is C_0 (9.0 g/L), and that of series E is $1.5C_0$ (13.5 g/L); Q_F is the feed flow rate; P_R and P_E are the purities of the raffinate and the extract

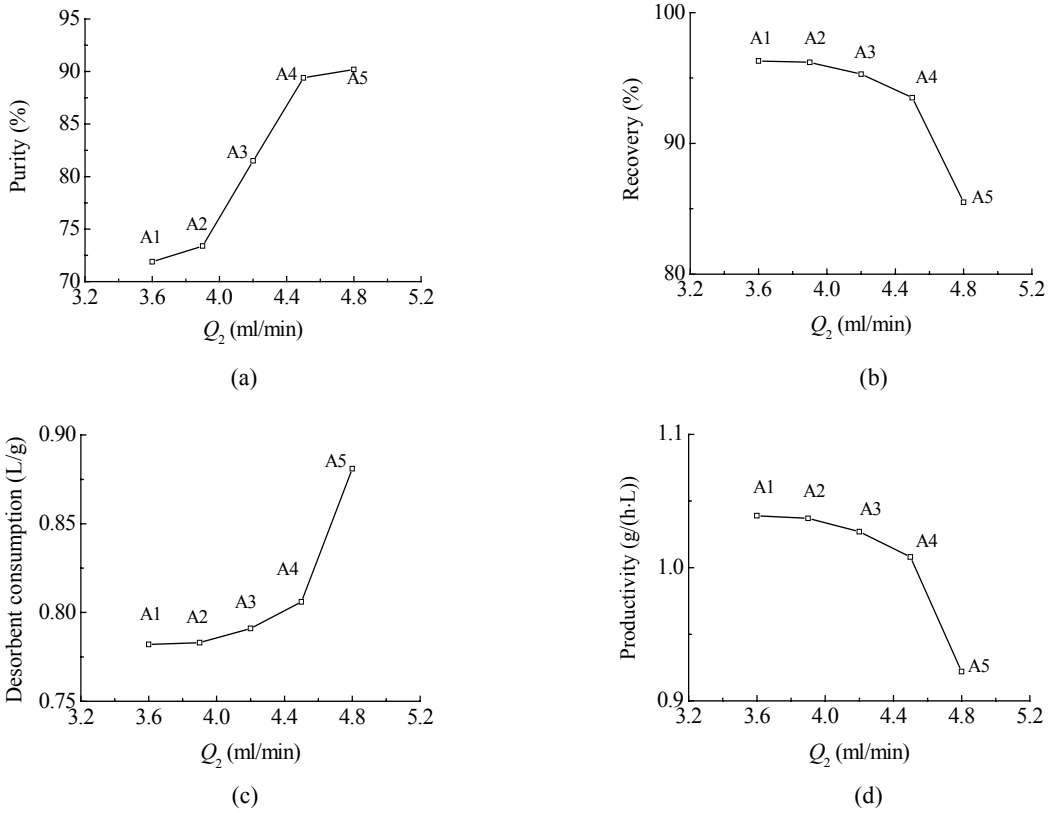

Fig.2 Effect of flow rate in Sections 2 and 3 on PC: (a) purity, (b) recovery, (c) desorbent consumption and (d) productivity

C, and D (Table 2), and shown in Fig.3 showing a peak in the curve of purity versus switching time t_s corresponding to purity peak was 6 min, and almost did not change with either the flow rate or the feed concentration. In the case of t_s greater than 6 min, and as the solvent in Section 4 between the raffinate and the desorbent port had not been cleaned before the solvent was recycled to Section 1, the extract was contaminated by the other components. This should result in worse separation so that the purity decreased. And at t_s down to 4 min, the purity of both the extract and the raffinate decreased drastically too. This could be explained by the fact that stationary phase in Section 1 between the desorbent and the extract port had not been fully desorbed before it was shifted to Section 4 so the raffinate was contaminated by PC. The relation of the recovery and productivity to t_s had the same trend as purity. In the meantime, the relation of desorbent consumption to t_s had reverse trend, i.e. had a minimum.

Effect of feed flow rate

The effect of the feed flow rate can be seen in Fig.3 too. In all these runs the desorbent flow rate remained at the same value of 3.4 ml/min, and Q_2 at 4.5 ml/min. As feed flow rate increased, Q_3 increased with the same increment. As a result, the separation in Section 3 became worse. The results showed that increasing feed flow increased productivity but that the purity and recovery of the extract (PC) and raffinate decreased.

Effect of feed concentration

The effect of feed concentration can be seen by comparing the results of series C and E (Table 2), and shown in Fig.4. As the feed concentration increased, the crude phospholipids loading increased, which caused purities and PC recovery in the extract to get worse while productivity increased.

According to the triangle method (Storti *et al*., 1993; Mazzotti *et al*., 1997; Migliorini *et al*., 1998),

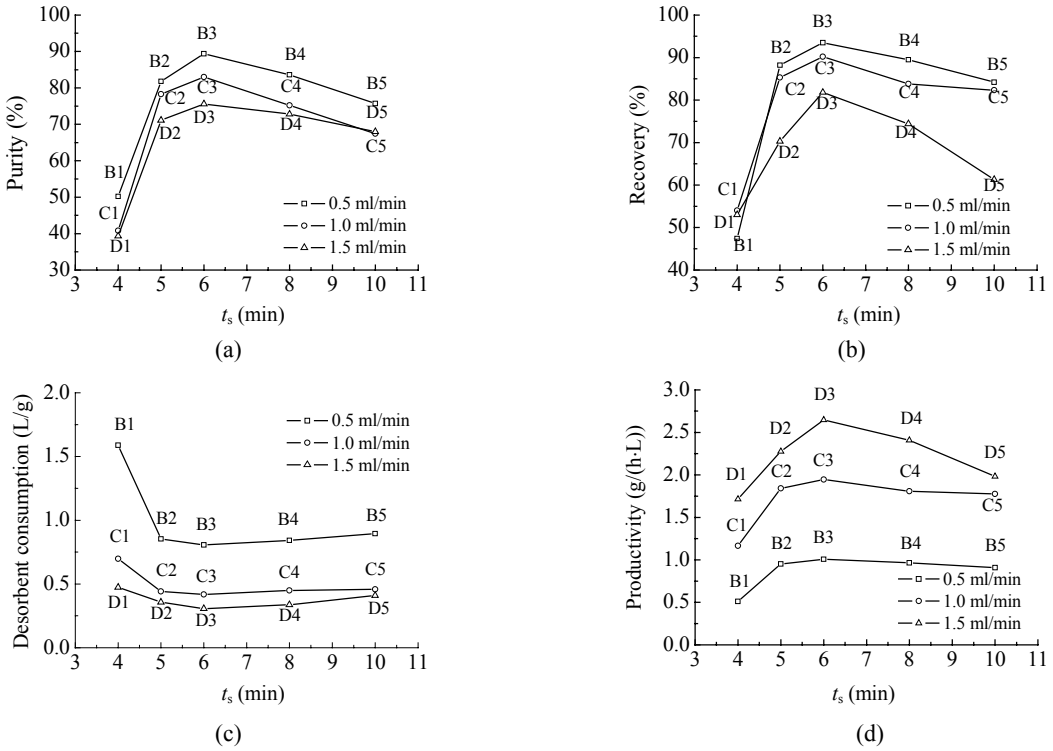

Fig.3 Effect of feed flow rate on PC: (a) purity, (b) recovery, (c) desorbent consumption and (d) productivity

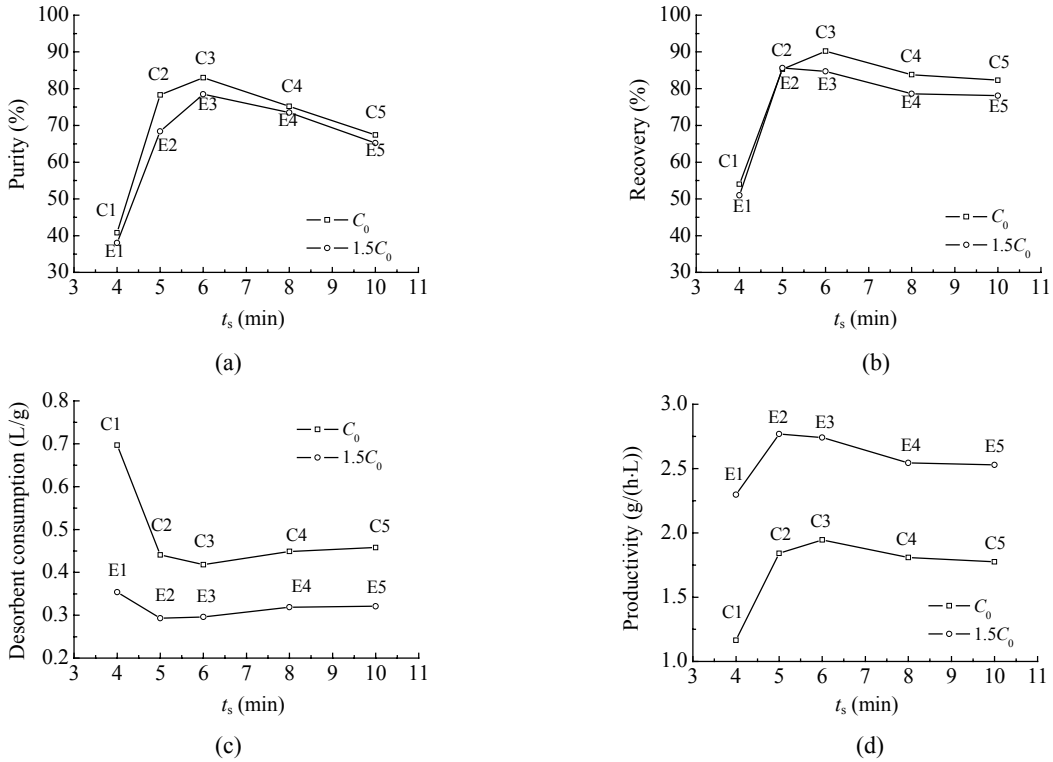

Fig.4 Effect of feed concentration on PC: (a) purity, (b) recovery, (c) desorbent consumption and (d) productivity

for complete separation in SMB, the following conditions should be fulfilled:

$$\text{Section 1: } m_1 \geq q_A^1/c_A^1;\ m_1 \geq q_B^1/c_B^1,$$

$$\text{Section 2: } q_B^2/c_B^2 \leq m_2 \leq q_A^2/c_A^2,$$

$$\text{Section 3: } q_B^3/c_B^3 \leq m_3 \leq q_A^3/c_A^3,$$

$$\text{Section 4: } m_4 \leq q_A^4/c_A^4;\ m_4 \leq q_B^4/c_B^4,$$

where $m_j = \dfrac{\text{net fluid flow rate}}{\text{adsorbed-phase flow rate}} = \dfrac{Q_j t_s - V\varepsilon_t}{V(1-\varepsilon_t)}$.

The ideal complete separation region in the m_2~m_3 plane (solid triangle) determined by triangle method and the experimental plots are shown in Fig.5 showing that the plot of best separation (A4) locates at m_2=0.67 and m_3=0.89. It is in the vicinity of the triangle base middle where the performances of the plots of other conditions get worse depart from the best condition. This fact shows that the triangle method is useful for getting initial guesses of the optimal operating conditions. On the other hand, the performances of the conditions within the triangle other than A4 are not as satisfactory as expected by the method because the method is based on the equilibrium theory and the mass-transfer effect was not considered. Mass transfer resistance would lower the column efficiency so that the range of optimal operating conditions would be much less than the ideal one.

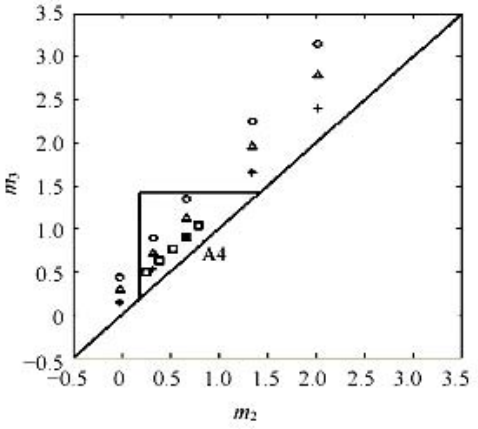

Fig.5 Points representing the operating conditions of the experiment runs in the plane (m_2, m_3)

□ represents series A; + represents series B; △ represents series C and E; ○ represents series D; For each series, from lower-left to upper-right, represents 1 to 5 respectively. The solid line indicates the linear complete separation region according to triangle method

CONCLUSION

High-purity PC was obtained in the extract of SMB. The separation performance of the unit and the effect of operating parameters, such as flow rates, switching time, feed flow rate and feed concentration, were studied under various operating conditions. Regions leading to complete separation were observed and explained theoretically. And the triangle theory only gives initial guesses for the optimal operating conditions.

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