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Separation of Amino Acids by Simulated Moving Bed Using Competitive Langmuir Isotherm

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Abstract The separation of two amino acids, phenylalanine and tryptophan, was carried out using laboratory simulated moving bed (SMB) chromatography. The SMB process consisted of four zones, with each zone having 2 columns. The triangle theory was used to obtain the operating conditions for the SMB. The mass transfer coefficients of the two amino acids were obtained from the best-fit values by comparing simulated and experimental pulse data. The competitive adsorption isotherms of the two amino acids were obtained by single and binary frontal analyses, taking into consideration the competition between the two components. A competitive Langmuir isotherm, obtained from single-component frontal chromatography, was used in the first run, and the isotherm from binary frontal chromatography in the second, with the flow rate of zone I modified to improve the purity. Compared to the first and second runs, the competitive Langmuir isotherm from the binary frontal chromatography showed good agreement with the experimental results. Also, adjusting the flow rate in zone I increased the purity of the products. The purities of the phenylalanine in the raffinate and the tryptophan in the extract were 99.84 and 99.99%, respectively.

Keywords: simulated moving bed, phenylalanine, tryptophan, competitive Langmuir isotherm

INTRODUCTION

Simulated moving bed (SMB) chromatography is a continuous chromatographic process developed in the 1960's by UOP (United Oil Products) [1,2]. SMB chromatography has the advantages of a high purity and yield, with less consumption of the mobile phase over that of batch chromatography [3], and its large-scale applications also reduces separation costs [4-6]. It has been used in the petrochemical and sugar [7] industries for large-scale separation, and for amino acids [8] and chiral separations [9] in fine chemical industries. Nevertheless, SMB chromatography has the disadvantages of high process costs and complexity of operation. Research to reduce the cost of the SMB process has been conducted, such as the one-column SMB process [10].

In the design of complex SMB chromatography, the optimization of the operation conditions relies on the determination of accurate adsorption isotherms. The success of experiments and modeling are directly related to accurate adsorption isotherms and their parameters [11]. Most SMB chromatography is carried out under nonlinear conditions, and the nonlinear behavior should be considered properly in the equilibrium isotherms. The Langmuir model is the most popular equilibrium isotherm among the various nonlinear isotherm models. A multi-component Langmuir isotherm explains the competition of two components for available adsorption sites [12,13]. The competitive Langmuir isotherms for SMB chromatography has been studied by Juza [12,14] and Guiochon [13,15], and the multi-component competitive isotherm was shown to be better than that of the single-component isotherm [16].

The objective of this study was to obtain the optimal operating conditions for phenylalanine and tryptophan separation in an SMB run. Competitive Langmuir isotherms, from single-component frontal and binary frontal analyses, were compared in term of the SMB separation, and the results of the experimental and simulation works compared.

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THEORY

SMB Process

The SMB unit consisted of four zones, with each zone having 2 columns connected in series. The feed, desorbent, extract and raffinate ports were placed between the columns. The port between the columns allowed for the opening or closing of the inlet (feed, desorbent) and outlet streams (raffinate, extract) at a specified switching time. The counter-current movement between the stationary and mobile phases was simulated by the port

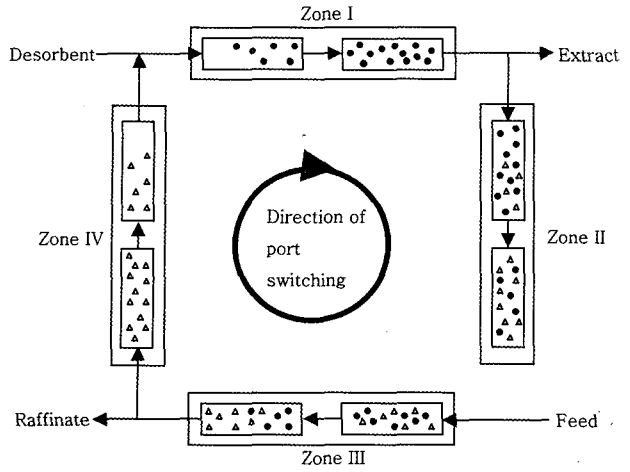

Fig. 1. Schematic diagram of a four-zone SMB system.

movements, as shown in Fig. 1. The feed was introduced between zones II and III. The low and high affinity solutes were separated and collected in the raffinate and extract, respectively. The desorbent was fed between zones I and IV. In order to achieve successful separation, for the migration velocity of the solute, i, in the zone, j, then u^j_{si} must satisfy the following conditions in Eq. (1) [17]:

$$u^I_{s2} - v > 0,\ u^{II}_{s1} - v > 0,\ u^{III}_{s2} - v < 0,\ u^{IV}_{s1} - v < 0 \qquad (1)$$

where v is the desorbent movement velocity, and subscripts 1 and 2 represent the low (phenylalanine) and high (tryptophan) affinity solutes, respectively.

Material Balance

The following material balances (Eqs. (2)-(4)) are used between the stationary and mobile phases [18]:

$$\varepsilon^* \frac{\partial C_i}{\partial t} + (1-\varepsilon^*)\frac{\partial q_i}{\partial t} + v_l \frac{\partial C_i}{\partial Z} = \varepsilon^* E_z \frac{\partial^2 C_i}{\partial Z^2} \qquad (2)$$

$$\frac{\partial q_i}{\partial t} = K_f (q_i^* - q_i) \qquad (3)$$

$$q_i^* = f_{eq}(C_i) \qquad (4)$$

where C_i is the mobile phase concentration of component i, q_i the adsorbed solid phase concentration of component i, ε^* the total porosity, v_l the superficial velocity and E_Z and K_f the axial dispersion coefficient and the lumped mass transfer coefficient, respectively. The lumped mass transfer coefficient was used for the mass transfer between the mobile and stationary phases assuming a linear driving force. q_i^* represents the adsorbed solid phase concentration in equilibrium with the mobile phase concentration, and is usually related to C_i by the multi-component Langmuir equation.

Triangle Theory

The operation of SMB chromatography depends on the flow rate within the four zones, as well as the switching time. In order to find successful operating conditions, Morbidelli *et al.* proposed the Triangle theory, which is based on the equilibrium theory [19]. The equilibrium theory neglects the axial dispersion and mass transfer resistance. The parameter m_j is the flow rate ratio, and is defined as the ratio of the net fluid flow rate over the solid phase in zone j. Based on the flow ratio, m_j, the experimental conditions for SMB can be found:

$$m_j = \frac{Q_j^{SMB} t^* - V\varepsilon^*}{V(1-\varepsilon^*)} \qquad (5)$$

where Q_j^{SMB} is the internal volumetric flow rate in zone j, t^* the switching time and V the volume of a single column

Equilibrium Adsorption Isotherm

The Langmuir isotherm for a single-component is:

$$q = \frac{aC}{1+bC} \qquad (6)$$

where q and C are the concentrations of the solute in the stationary and mobile phases at equilibrium, respectively [20], and a and b are characteristic parameters of the solute in a given system. The parameters are estimated using a single-component frontal analysis for each component. The amount adsorbed onto the stationary phase can be calculated by the Eq. (7):

$$q_{i+1} = q_i + \frac{(C_{i+1} - C_i)\{V_{F,i+1} - V(V_0 + V_D)\}}{V_{sp}} \qquad (7)$$

where V_F is the retention volume of the inflection point of the breakthrough curve, and V_0 and V_D the column void and system dead volumes, respectively. V_{sp} is the volume of adsorbent in the column. The subscript i relates to the number of step changes in the concentration.

For a multi-component system, the isotherm of component i is:

$$q_i = \frac{a_i C_i}{1 + \sum_j b_j C_j} \qquad j = 1,2,...,N \qquad (8)$$

where a_i and b_i are parameters for component i, with the subscript j being the number of components in the mixture. The binary frontal analysis was performed with different compositions of the two components [6,15,21,22]. The breakthrough curve of a binary mixture is shown in Fig. 2, and the amount adsorbed onto the stationary phase calculated by the following mass balance equation:

$$q_i = \frac{[(V_2 - V_D)(C_{i,b} - C_{i,a}) - (V_2 - V_1)(C_{i,sub} - C_{i,a})]}{V_{sp}} \qquad (9)$$

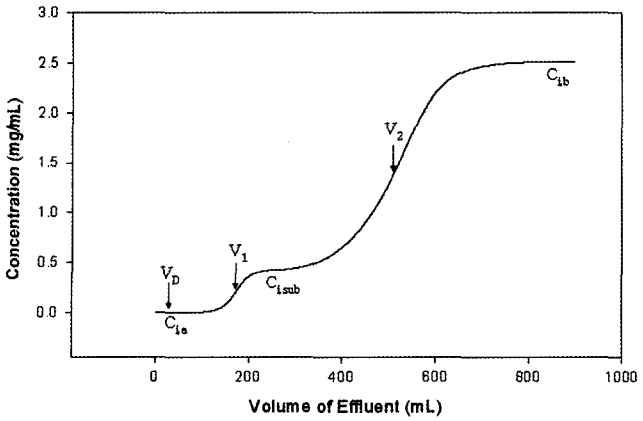

Fig. 2. Diagram of the concentration profile in frontal chromatography. $C_{i,a}$, $C_{i,b}$ and $C_{i,sub}$ are the concentrations of component i in initial plateau, final plateau and subplateau. V_1 and V_2 are the retention volumes of the inflection point of the breakthrough curve and V_D the dead volume of the system.

The concentration of the mobile phase changes from $C_{i,a}$ to $C_{i,b}$, and $C_{i,sub}$ is the concentration at the subplateau. V_1 and V_2 are the retention volumes at the inflection point of the breakthrough curve.

MATERIALS AND METHODS

Materials

The two amino acids, L-phenylalanine and L-tryptophan, were purchased from Sigma (St. Louis, MO, USA). The deionized water (DI) was obtained from Milli-Q system (Bedford, MA, USA), and filtered through 0.22 μm filters. The Blue Dextran and NaCl, used for the column porosity measurements, were purchased from Sigma (St. Louis, Mo, USA). The HPLC grade acetonitrile was purchased from Fisher Scientific (NJ, USA), and was used with deionized water as the mobile phase in the HPLC assay. The PVP resin (poly-4-vinylpyridine cross-linked, Reillex HP polymer) was purchased from Reilly Industries (Indianapolis, IN, USA). The resin was washed with 1 M NaOH, followed by 1 M HCl, and then by 50% NaOH, for 3~4 bed volumes. Between each step, the resin was washed with several bed volumes of deionized water. All solutions were degassed using He.

Equipments

The columns for the batch and SMB experiments were purchased from Alltech (Deerfield, IL, USA). A small column (12.5 × 1.5 cm ID) was used in the batch experiment, and 8 large columns (21.7 × 2.5 cm ID) in the SMB work. A fast protein liquid chromatography (FPLC) pump was used for the isotherm measurement. The concentrations of both the phenylalanine and tryptophan were analyzed by HPLC (LC-10AD, Shimadzu, Japan) using a Waters 486 tunable single wavelength detector.

Analytical Techniques

The concentrations of the extract and raffinate in the SMB experiment, and the effluent from the binary frontal chromatography, were analyzed by HPLC, with an injection volume of 20 μm. The column was packed with Metasil (5 μm ODS, 250 × 4.6 mm, Metacham, USA), with 20% acetonitrile used as the mobile phase, at a flow rate of 0.5 mL/min, and detected at 260 nm.

Measurements of Isotherm

The intraparticle and total porosities were obtained from Blue Dextran and NaCl pulse tests, respectively, with the interparticle porosity calculated from the total porosity. The single-component isotherms of the two amino acids were determined by multiple frontal analyses of each component. The isotherms were obtained by stepwise changing of the amino acids concentrations. A small column (12.5 × 1.5 cm) was used, with the FPLC pump set at 1.0 mL/min. The competitive isotherms were obtained using several frontal tests at different amino acid mixture concentrations, with a flow rate of 3 mL/min and detection at 254 nm. The samples at the subplateau were collected at the end of the column during the frontal analysis. The composition of the effluent was measured by HPLC.

Laboratory SMB Unit

A laboratory four-zone SMB was used for the experiments, which was composed of 8 columns (21.7 × 2.5 cm ID), with each zone having two columns. Two FPLC pumps controlled the feed and desorbent streams, and two FMI (Fluid Metering, NY, USA) pumps controlled the extract and recycle streams. Eight rotary valves (VICI, Switzerland), with 1 inlet and 8 outlet ports, were used. Each column had one rotary valve connected to the four streams. Each valve changed the flow path every switching time. The valves were controlled by the Labview program (National Instruments, TX, USA).

RESULTS AND DISCUSSION

SMB chromatography was used to separate the two amino acids, phenylalanine and tryptophan. The configuration of the SMB was 2-2-2-2, *i.e.* two columns in each zone. The system parameters for simulation of the SMB operation are listed in Table 1. The simulation was performed using Aspen chromatography, with the operating conditions obtained using the Triangle theory. The mass transfer coefficient was obtained by comparing the simulated with the experimental pulses of the amino acids. The axial dispersion and diffusivity were estimated from the breakthrough curves of the frontal chromatography, using the same method as for the mass transfer coefficient.

A single-component multiple frontal analysis was performed to obtain the isotherms of the two amino acids, as

Table 1. System parameters for Runs 1 and 2

System parameters	
Column length (cm)	21.70
Column internal diameter (cm)	2.50
Particle radius (μm)	211.25
Interparticle porosity (ε_i)	0.30
Intraparticle porosity (ε_p)	0.55
Mass transfer coefficients (min^{-1})	
Phenylalanine	1.42
Tryptophan	1.38
Liquid viscosity (cP)	0.89
Mass density of eluent (kg/m^3)	1000

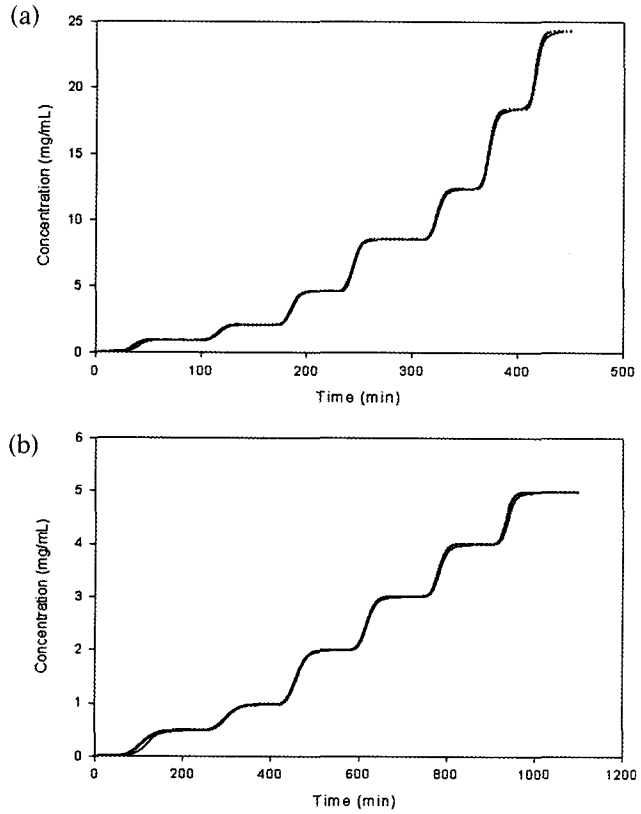

Fig. 3. Comparison of the experimental and simulated results of the multiple frontal analyses of phenylalanine (a) and tryptophan (b). The solid lines are the experimental data and the dotted lines the simulated results.

shown in Fig. 3. The experimental and simulated results of the multiple frontal analyses for phenylalanine and tryptophan showed good agreement. The Langmuir isotherm was used to fit the function of q at a given c, as presented in Fig. 4. Fig. 4 shows the single component

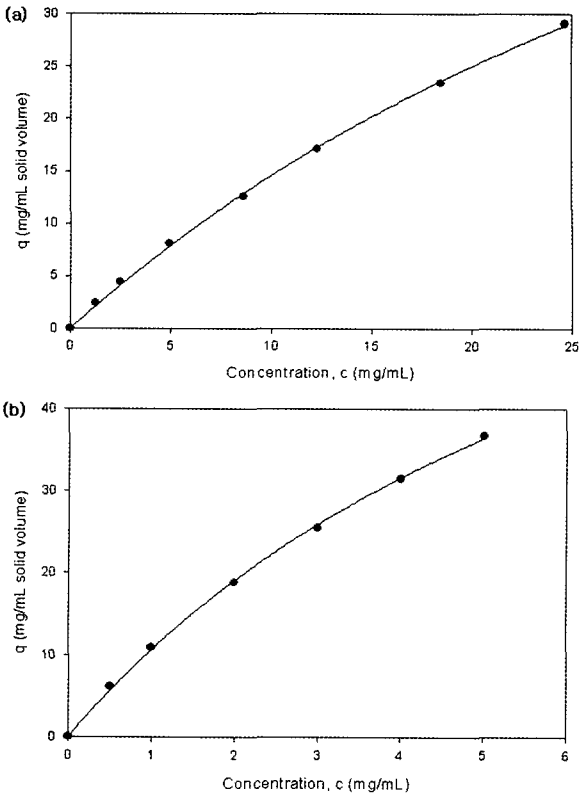

Fig. 4. Single component Langmuir isotherms of phenylalanine (a) and tryptophan (b). The circles were calculated from multiple frontal analysis and solid line fitted for the Langmuir isotherm model.

Langmuir isotherms of phenylalanine (a) and tryptophan (b). The circles were calculated from multiple frontal analysis and the solid line fitted for the Langmuir isotherm model. The single-component Langmuir isotherms for the amino acids were as follows:

$$q_{Phe} = \frac{1.7473C_{Phe}}{1+0.02C_{Phe}} \quad q_{Trp} = \frac{11.9542C_{Trp}}{1+0.1285C_{Trp}} \tag{10}$$

The competitive parameters from the single-component Langmuir isotherms were used in the first SMB run, as follows:

$$q_{Phe} = \frac{1.7473C_{Phe}}{1+0.02C_{Phe}+0.1285C_{Trp}}$$

$$q_{Trp} = \frac{11.9542C_{Trp}}{1+0.02C_{Phe}+0.1285C_{Trp}} \tag{11}$$

where C_{Phe} and C_{Trp} are the mobile phase concentrations, and q_{Phe} and q_{Trp} the adsorbed solute concentrations of the phenylalanine and tryptophan, respectively. The competitive isotherm parameters were used to obtain the operating condition in the first run, and are listed in Table 2. The simulated purities of the phenylalanine in the raffinate and the tryptophan in the extract were 99.40 and

Table 2. Operating conditions for Runs 1 and 2

		Run 1	Run 2
Flow rate (mL/min)	Feed	5.00	5.00
	Zone I	10.19	17.15
	Zone II	3.37	3.13
	Zone III	8.37	8.13
	Zone IV	2.79	2.73
	Desorbent	7.40	14.42
	Extract	6.82	14.02
	Raffinate	5.58	5.40
Switching time (min)		46.55	46.60

Table 3. Experimental and simulated results in Runs 1 and 2

		Run 1		Run 2	
		Raffinate (phe)	Extract (trp)	Raffinate (phe)	Extract (trp)
Simulated	Purity (%)	99.40	98.33	99.99	99.66
	Yield (%)	99.16	98.80	99.18	99.67
Experiment	Purity (%)	94.58	99.99	99.84	99.99
	Yield (%)	99.99	87.78	99.83	98.45

98.33%, respectively. The simulated yields of the phenylalanine in the raffinate and the tryptophan in the extract were 99.16 and 98.80%, respectively. The experimental results showed purities for the phenylalanine in the raffinate and the tryptophan in the extract of 94.58 and 99.99%, respectively. The yields of the phenylalanine in the raffinate and the tryptophan in the extract were 99.99 and 87.78%, respectively (Table 3). In Fig. 5(a), the experimental concentration of the tryptophan was lower than that from the simulated result at the mid-switching time. The tryptophan concentration in the raffinate increased with time, making the purity of the raffinate and the yield of the extract decrease, as shown in Fig. 5(b). Fig. 5 shows a comparison of experimental and simulated results in the effluent histories of the extract (a) and raffinate (b) in run 1. The dotted lines are the simulated results with the switching time, and the dashed lines the simulated results at the mid-switching time. The open and closed circles are the experimental results for the tryptophan and phenylalanine, respectively. In Fig. 6, the experimental tryptophan concentration was lower than that for the simulated results.

To reduce this discrepancy, the competition effect was considered in the equilibrium isotherm, using competition parameters. According to the Gibbs-Duhem relationship, the parameters of a single-component Langmuir isotherm can be used as the competitive isotherm parameters only when a_i/b_i is the same for all components

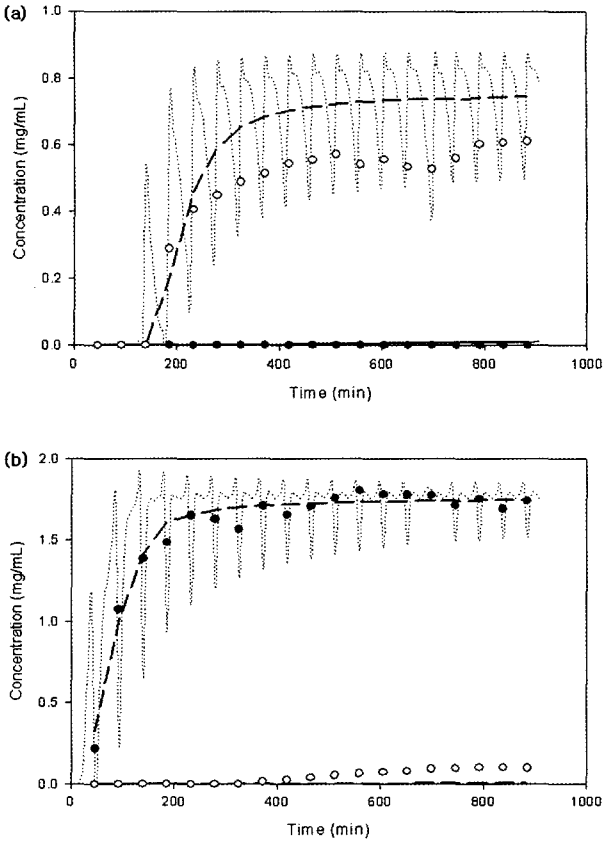

Fig. 5. Comparison of the experimental and simulated results in the effluent histories of the extract (a) and the raffinate (b) in run 1. The dotted lines are the simulated results with the switching time, and dashed lines the simulated results at the mid-switching time. The open and closed circles are the experimental results for the tryptophan and phenylalanine, respectively.

[23]. Since this condition is not satisfied in a general real system, the parameters of a single-component isotherm may not accurately represent the competition between the two components. Therefore, the determination of a_i and b_i from direct measurement is important in multicomponent systems. The breakthrough curve of a binary mixture is shown in Fig. 2, and the amount adsorbed onto the stationary phase calculated by Eq. (9). The competitive Langmuir isotherm was fitted by the function of q at a given c. The a_i and b_i were estimated by comparing calculated and fitted values.

In this work, a binary frontal analysis was performed over a wide concentration range, with mixtures of phenylalanine and tryptophan. The results were as follows;

$$q_{Phe} = \frac{1.9953C_{Phe}}{1 + 0.0585C_{Phe} + 0.1143C_{Trp}}$$
$$q_{Trp} = \frac{14.26C_{Trp}}{1 + 0.0585C_{Phe} + 0.1143C_{Trp}} \quad (12)$$

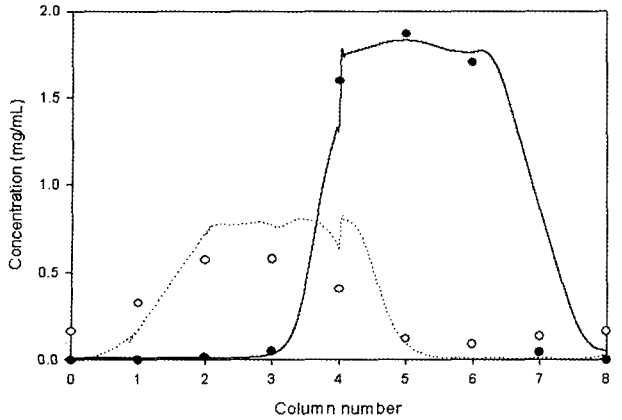

Fig. 6. Comparison of experimental and simulated results in run 1. The solid and dotted lines are the simulation profiles of phenylalanine and tryptophan, respectively. The closed and open circles are the experimental data for phenylalanine and tryptophan, respectively.

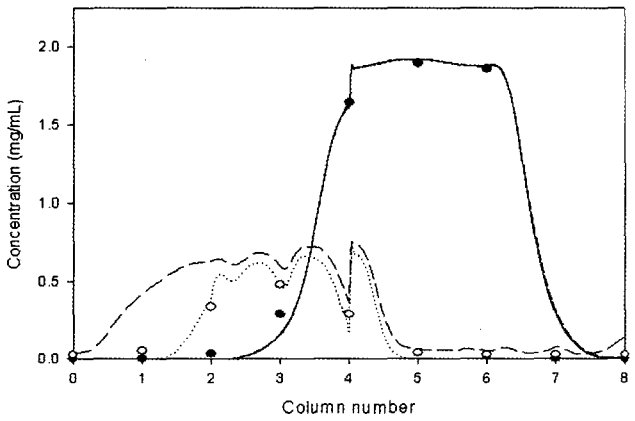

Fig. 7. Comparison of the experimental and simulated results in run 2. The solid (phenylalanine) and dotted (tryptophan) lines are the simulation profiles with increasing m_1. The closed (phenylalanine) and open (tryptophan) circles are the experimental data with increasing m_1. The dashed lines are the simulated results for a small m_1.

The operating conditions for the second run were obtained from the competitive isotherm of the binary frontal chromatography, and are presented in Table 2. The experimental column profile of the phenylalanine was a good match for that of the simulated result as shown in Fig. 7. However, the migration of tryptophan (dashed lines) was so slow that it penetrated zones IV through I, and contaminated the raffinate. In order to prevent this contamination, the flow rate in zone I was increased by increasing the value of the ratio of the net fluid flow rate over the solid phase flow rate in zone I, m_1. To maintain the mass balance in the SMB system, the amount of increased flow in zone I was compensated for by increasing the extract flow rate. The purities and yields in the raffinate and extract were increased in the simulated results,

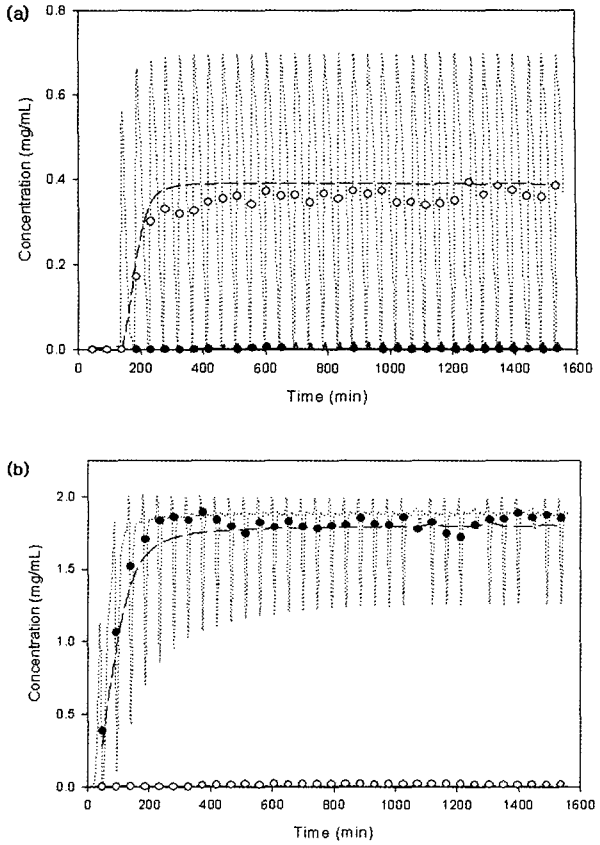

Fig. 8. Comparison of the experimental and simulated results in the effluent histories of the extract (a) and the raffinate (b) in run 2. The dotted lines are the simulated results with the switching time, and the dashed lines the simulated results at the mid-switching time. The open and closed circles are the experimental data for tryptophan and phenylalanine, respectively.

with increased m_1. Compared with the dashed lines (Run 1 condition) and the dotted lines (Run 2 condition), the simulated purity of the raffinate and the yield of the extract were both improved in Run 2, as shown in Fig. 7. The second run of the SMB experiment was carried out based on these operating conditions. The experimental purities and yields of the raffinate and the extract were 99.18 and 99.83%, and 99.67 and 98.45%, respectively, as shown in Table 3. The effluent histories of run 2 are presented in Fig. 8. The experimental results showed good agreement with the simulated data.

CONCLUSION

The separation of two amino acids, phenylalanine and tryptophan, was carried out using laboratory simulated moving bed (SMB) chromatography. The configuration of the SMB was 2-2-2-2, *i.e.* two columns in each zone. The triangle theory was used to obtain the operating conditions for the SMB. The competitive adsorption isotherms of the two amino acids were obtained by single

and binary frontal analyses. A competitive Langmuir isotherm, obtained from single-component frontal chromatography, was used in the first run, and the isotherm from binary frontal chromatography in the second, with the flow rate of zone I modified to improve the purity. A competitive adsorption isotherm was used to explain the competition between two adsorbing components in a SMB process. The competitive Langmuir isotherms from a multi-component frontal analysis gave a more adequate isotherm, as proved experimentally. To improve the purity and yield, the zone flow rates were modified. The experimental results showed better separation of the phenylalanine and tryptophan when the competition between these components was considered, with subsequent changes in the zone I flow rate. Compared to the first and second runs, the competitive Langmuir isotherm from the binary frontal chromatography showed good agreement with the experimental results. Also, adjusting the flow rate in zone I increased the purity of the products. The purities of the phenylalanine in the raffinate and the tryptophan in the extract were 99.84 and 99.99%, respectively.

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NOMENCLATURE

- C solute concentration of mobile phase (g/L)
- $C_{i,a}$ initial concentration of component i in the equilibrated column (g/L)
- $C_{i,b}$ final concentration of component i in the column (g/L)
- $C_{i,sub}$ concentration of subplateau (g/L)
- ε total porosity
- ε_p intraparticle porosity
- ε_i interparticle porosity
- v_l superficial velocity of the fluid (cm/min)
- E_z axial dispersion coefficient (cm^2/min)
- K_f film mass transfer coefficient (min^{-1})
- Q_j volumetric flow rate in zone j (mL/min)
- m_j ratio of mobile phase flow rate over stationary phase flow rate in zone j
- q_i adsorbed solid phase concentration of component i (mg/mL solid volume)
- q^* adsorbed solid phase concentration at equilibrium (mg/mL solid volume)
- t^* switching time (min)
- R particle radius (μm)
- V total column volume (mL)
- V_0 column void volume (mL)
- V_D system dead volume (mL)
- $V_{F,i+1}$ retention volume of the inflection point of $(i+1)$ front (mL)
- V_i retention volumes of fronts (mL)
- V_{sp} volume of adsorbed solid phase in the column (mL)

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