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Separation of amino acids with simulated moving bed chromatography☆

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Abstract

Authors have constructed an automatized four-column large laboratory scale (I.D. = 50 mm, L = 500 mm) simulated moving bed (SMB) equipment. The applied model system for separation of biomolecules is glycine, L-phenylalanine, water and Sepabeads SP825 adsorbent. The authors determined the adsorption equilibrium data and the packing characteristics. The operating conditions of SMB equipment were calculated with the help of the Morbidelli variables. During the SMB experiments, glycine and L-phenylalanine were separated in water on Sepabeads SP825 with an average particle size 0.3 mm at temperatures 20 °C and 60 °C. The measurement series were carried out on a four-column three-zone open loop SMB. Both L-phenylalanine and glycine were produced with more than 99.9% (m/m) purity and 99% yield at productivity 1.7–3.7, with productivity 3.7–8.1 mg/(g adsorbent h) in case of 2-1-1-0 column configuration. The measured and the calculated data agreed well.

Keywords: Column liquid chromatography; Three-zone simulated moving bed; Reversed phase polymeric resin; Effect of temperature

1. Introduction

It is typical in biotechnology and pharmaceutical industry that water-phase mixture for processing contains an end-product or an active ingredient in a small concentration besides the contaminant or polluting components. SMB preparative chromatography can be advantageous among the end-product recovery methods for continuous processing of high purity products. The SMB applicability in the separation of diluted aqueous amino acid solution was investigated and presented in this publication. The diluted solution of amino acids can arise, e.g. from protein hydrolysis.

SMB liquid chromatography (hereafter-abbreviated SMB-LC) (Fig. 1) is a multi-column system with two inputs (fresh eluent and feed to be separated) and outputs (products: extract and raffinate), in which liquid phase moves in counter-current of adsorbent phase. Above all, in the basic case regenerated eluent of recirculation stream is added to the fresh eluent. The counter-current stream is not real, but simulated, since the packed chromatographic stationary phase moves periodically after each switching time.

The inlet and the outlet fluid flow streams divide the column system into four zones (I–II–III–IV). The sections I–II of the SMB are rich in the more binding component and sections III–IV contain the less binding component.

Three-zone open loop SMB (Fig. 2) is preferred in systems with a high selectivity coefficient, when the less binding component has a low capacity factor (k) almost running together with the mobile phase [1,2].

Summarizing the column liquid chromatographic methods, which are feasible for SMB chromatographic separation of amino acids, the following were applied in practice:

(1) Ion-exchange column liquid chromatography.
The continuous version is the SMB packed with ion-exchange resin chromatographic quality [3].
(2) Size exclusion chromatography.

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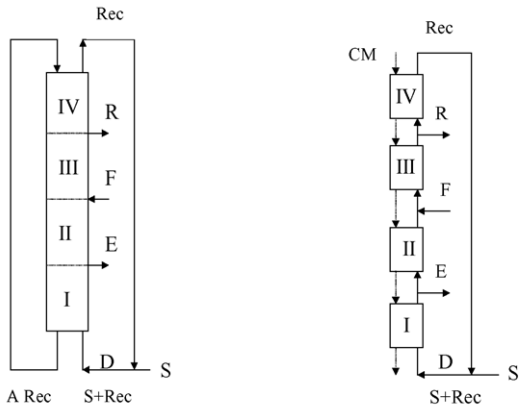

Fig. 1. (A) True moving bed (TMB) adsorber: I, II, III, IV—zones; S—desorbent (solvent, eluent); Rec—recirculated eluent; A Rec—adsorbent recirculation; E—extract stream with the better adsorbed component A; F—feed stream with the components A and B; R—raffinate (liquid outlet) stream with the less adsorbed component B. (B) Simulated moving bed (SMB) adsorber: I, II, III, IV—zones, respectively HPLC columns; CM—direction of simulated (relative) moving of HPLC columns.

Protein hydrolizate separation and fractioning on chromatographic gel column [4].

(3) Reversed-phase adsorption chromatography.

The styrene–divynilbenzene copolymers with nonpolar surface are favorable for the separation of amino acids dissolved in water, electrolyte solutions or any polar solvent. In such systems, the adsorption equilibrium depends on the temperature, the solvent strength, the pH value [5–7] and also the electrolyte concentration in aqueous solution.

The examined model system during research is the diluted aqueous solution of glycine and L-phenylalanine, which must continuously be recovered. For this purpose, the reversed-phase adsorption chromatography is probably the most favorable solution, as the ion-exchange characteristics of both

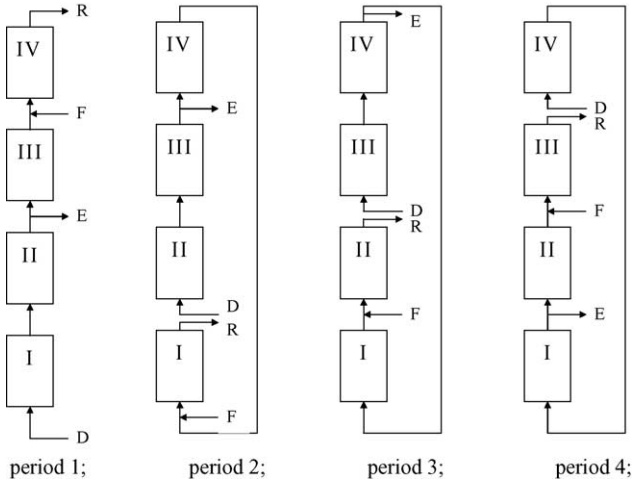

Fig. 2. Fluid streams in the four period (total cycle) of a four columns three zones open loop SMB with 2-1-1-0 column/zone configuration.

the L-phenylalanine and glycine are similar. The size exclusion is not applicable because of the small molecule size of both the examined amino acids.

The initial step of designing an SMB-LC operation is to choose the most advantageous mobile phase–stationary phase combination namely selectivity and capacity factors. For this purpose, the Diaion HP20 and Sepabeads SP825 reversed-phase adsorbent resins were compared with frontal chromatography. Both the examined resins are styrene–divynilbenzene copolymers with large specific surface area (600–1000 m^2/g). On the bases of selectivity factors, application of both adsorbents' seems to be advantageous for chromatographic separations of the better-adsorbed L-phenylalanine and less-adsorbed glycine. In this publication, experiments carried out with SP825 resin are described, however the results of HP20 resins used successfully were published elsewhere [8]. The first experiments with SP825 verified whether the high capacity factor of the phenylalanine is not advantageous for the SMB separation, namely the regeneration of the adsorbent in the SMB zone I. Therefore, capacity-reducing investigations were carried out, e.g. increasing temperature. Further, higher temperature can be advantageous to fluid dynamics, because of smaller viscosity and faster adsorption–desorption kinetics.

After determining the equilibrium data of the selected systems and the column packing characteristics, the initial operating parameters of the SMB can be calculated. Through the small capacity factor of glycine, a three-zone open loop SMB is advisable for the separation. The initial operating parameters for a three-zone open loop SMB were calculated by the triangle method of Morbidelli and co-workers [9].

The equation for the total regeneration of the first column of the first zone is:

$$K_A < m_I = \frac{(D/A)T - L\varepsilon}{L(1-\varepsilon)} \tag{1}$$

where K_A = equilibrium distribution coefficient of better adsorbed component, m_I = Morbidelli's velocity ratio for the first SMB-zone, D = eluent flow rate (mobile phase volumetric velocity in the first zone (cm^3/min)), T = switching time (min), A = cross-section of one SMB-column (cm^2), L = SMB column length (cm), and ε = total porosity of the SMB column.

In the second and third zone, the components must be separated by their distribution coefficient. The less-binding component must be removed from the second zone till the end of the switching time. The function of the third zone is the retardation of the better-adsorbed component, that is, this component must not break through the third zone. Obviously, the Morbidelli conditions for the second and third SMB-zones are:

$$K_B < m_{II} = \frac{((D-E)/A)T - L\varepsilon}{L(1-\varepsilon)} < K_A \tag{2}$$

$$K_B < m_{III} = \frac{((D-E+F)/A)T - L\varepsilon}{L(1-\varepsilon)} < K_A \tag{3}$$

where K_B = equilibrium distribution coefficient of less adsorbed component, m_{II} = Morbidelli's velocity ratio for the second SMB-zone, m_{III} = Morbidelli's velocity ratio for the third SMB-zone, $D-E$ = mobile phase volumetric velocity in the second zone (cm^3/min), and $D-E+F$ = mobile phase volumetric velocity in the third zone (cm^3/min).

In a two component-system, a m_{II}–m_{III} diagram can be determined. To make both product stream purities theoretically 100%, the operating conditions must be determined in order to place the measurement point (with coordinates of Morbidelli's velocity ratios [m_{II}; m_{III}]) on the so called Morbidelli's area. In the linear case, it is a rectangular triangle with the next coordinates (K_B; K_B), (K_B; K_A), (K_A; K_A). The use of the above presented simple planning method for SMB parameters is advantageous when the system is characterized by linear equilibrium conditions (e.g. by small feed concentration). In the preparative practice, the most frequently applied isotherm is the Langmuir or multi-component Langmuir type isotherm:

$$q_i = \frac{a_i c_i}{1 + \sum b_i c_i} \tag{4}$$

where q_i = concentration in solid (stationary) phase (mg/g), c_i = concentration in liquid phase (mg/cm^3), a_i, b_i = Langmuir parameters, and i = index of components.

The Langmuir parameter a_i can be nominated as K_i, namely when all of "b" parameters are ignorably small, then $\sum b_i c_i \approx 0$, and the Eq. (4) approaches to the linear equilibrium equation. The Langmuir parameter "a" is corresponds to equilibrium distribution coefficient "K".

When we have a system with non-linear Langmuir or multi-component Langmuir equilibrium, then the Morbidelli's area (area of pure extract and pure raffinate in m_{II}–m_{III} diagram) is bound practically to the following sections: "w to b", "w to r", "r to a", "b to a". The coordinates of point "a" are (K_A; K_A), point "b" are (K_B; K_B). The definitions of points "r" and "w" are:

$$r\left[\frac{\omega_G^2}{K_A}; \frac{\omega_G[(K_A-\omega_G)(K_A-K_B)+K_B\omega_G(K_A-\omega_G)]}{K_A K_B(K_A-\omega_F)}\right] \tag{5}$$

$$w\left[\frac{K_B\omega_G}{K_A}; \frac{\omega_G[\omega_F(K_A-K_B)+K_B(K_B-\omega_G)]}{K_B(K_A-\omega_F)}\right] \tag{6}$$

The ω_G and ω_F parameters are the roots of the following equation:

$$(1+b_A c_A^F + b_B c_B^F)\omega^2 - [K_A(1+b_B c_B^F) + K_B(1+b_A c_A^F)]\omega + K_A K_B = 0, \quad \omega_G > \omega_F > 0 \tag{7}$$

where C_A^F, C_B^F = feed concentrations (mg/cm^3), A and B = indices of components in a two-component system.

From the above equations, it can be seen that the pure extract—pure raffinate area is smaller by higher feed concentration than the rectangular triangle, consequently we have less possibility by parameter variation. The Morbidelli's area changes, when the following possibilities of intervention vary during the planning: column geometrics of SMB equipment, temperature, changing adsorbent, and composition of the solvent (eluent).

When we have already selected the chromatographic packing for the separation, the next task is to choose operating variables: fresh eluent, re-circulated eluent, feed, and extract, raffinate flow rates, switching time. During calculation, the influence of a given parameter is to be investigated, the others must be considered as constants.

Better productivity, product purity, yield, and eluent consumption can be achieved by optimizing operating conditions by computer with the exact mathematical model of the SMB. The basic equation of the mathematical model can be deduced from the component balance of the solid–liquid equilibrium system [10,11]. The equal balance for the component "k" is:

$$v_0\left(\frac{\partial c_k}{\partial z}\right)_t + (1-\varepsilon)\left(\frac{\partial q_k}{\partial t}\right)_z + \varepsilon\left(\frac{\partial c_k}{\partial t}\right)_z = 0 \tag{8}$$

where v_0 = velocity of fluid phase (cm/min), dc_k/dz = place derivative of liquid concentration of component k, dc_k/dt = time derivative of liquid concentration of component k, and dq_k/dt = time derivative of solid concentration of component k.

When we discuss a two-component equilibrium system the competitive multi-Langmuir type isotherm can be written as:

$$q_1 = \frac{a_1 c_1}{1 + b_1 c_1 + b_2 c_2} \quad \text{and} \quad q_2 = \frac{a_2 c_2}{1 + b_1 c_1 + b_2 c_2} \tag{9}$$

The denominator of isotherm (9) was replaced by "N":

$$N = 1 + b_1 c_1 + b_2 c_2 \tag{10}$$

Considering the equal balance (8) and concentration (c_1, c_2) derivatives of Eq. (9) can be written:

$$-\frac{v_0}{1-\varepsilon}N^2\frac{\partial c_1}{\partial z} = \frac{\partial c_1}{\partial t}\left(\frac{a_1 N - a_1 b_1 c_1}{N^2} + \frac{\varepsilon}{1-\varepsilon}\right) \times \frac{a_1 b_2 c_1}{N^2}\frac{\partial c_2}{\partial t} \tag{11}$$

The derivatives were substituted with difference quotients:

$$-\frac{v_0}{1-\varepsilon}N^2\frac{\Delta c_1}{\Delta z} = \frac{\Delta c_1}{\Delta t}\left(a_1 N + \frac{\varepsilon}{1-\varepsilon}N^2\right) - a_1 c_1\left(b_1\frac{\Delta c_1}{\Delta t} + b_2\frac{\Delta c_2}{\Delta t}\right) \tag{12}$$

Difference Eq. (12) was rearranged and written for component "k":

$$-\frac{v_0}{1-\varepsilon}N^2\frac{\Delta c_k}{\Delta z}+a_k c_k\sum_{k=1}^{n}b_k\frac{\Delta c_k}{\Delta t}=\frac{\Delta c_k}{\Delta t}\left(a_k N+\frac{\varepsilon}{1-\varepsilon}N^2\right)-a_k b_k c_k \quad (13)$$

Eq. (13) is the base of the numeric simulation software. The component transfer is calculated with Eq. (13) between the so-called equilibrium cascades. In our model, the number of equilibrium cascades are compliant with the number of theoretical plates. Based on the mathematical model, a computer program was written in Turbo Pascal computer language [12]. Frontal adsorption experiments and SMB-LC measurements can be simulated by the computer program. At low NTP (about 35/column) the calculation time on a 1 GHz personal computer is 5% of the measurement time. The input data of the software: number of components, feed concentration, adsorption equilibrium properties (Langmuir parameters), column geometrical data (average) NTP/column, total porosity, bulk density, volumetric velocities (eluent, feed, extract, raffinate, recirculation).

The initial SMB operating conditions were calculated with the equilibrium triangle method. We considered the maximal flow rate of eluent pump, and minimal column switching time was chosen. After the first measurement, we compared software simulations of 1-1-2-0 column/SMB zone configuration with 2-1-1-0 configuration. Though slow desorption kinetics is favorable in the first SMB zone, it has to apply to more columns. Further, the regeneration will be faster, when the capacity factor of better-adsorbed component is reduced. We investigated the increase in the system temperature, in the isotherm case (every SMB zones with the same temperature). The varied equilibrium parameters on a single column were measured with multi-step frontal chromatography. Increase in temperature in the new SMB operating conditions was calculated with the help of the simulation software with changing the measured Langmuir parameters.

2. Experiments

2.1. Select reversed-phase packing

In case of L-phenylalanine and glycine amino acid separation in water, the next reversed phase apolar polymer adsorbents were studied by frontal adsorption–desorption methods: Diaion HP20, Sepabeads SP825. Parameters of research: measurement was carried on glass column in which resin bed length was 122 mm, I.D. 13 mm. The glass column was made at the Department of Glass Technique, University of Veszprém and designed for low pressure (max 0.5 MPa) with polypropylene connection and rubber gasket. L-Phenylalanine concentration was 0.005 mol L-phenylalanine/dm^3 aqueous solution, glycine concentration 0.0025 mol glycine/dm^3 aqueous solution, volumetric stream 2.9 cm^3/min, temperature 20 °C. L-Phenylalanine was detected on-line by Gilson 116 UV spectrophotometer at 271 nm wavelength. Glycine and L-phenylalanine analyses were conducted thereafter, samples fractionally taken in each 4 cm^3 by an OE914 amino acid analyzer. The details of the analysis are given in a further chapter (SMB product stream analysis).

2.2. Measurement of packing properties

SP825 adsorbent was packed into the SMB-LC 500 mm long and I.D. 50 mm column by slurry technique. After packing, the number of theoretical plate (NTP) was measured in function of volume stream in 30–180 cm^3/min range by injection method with 0.25 mol Na_2SO_4/dm^3 water solution at 20 °C acting as one of the basic input data for mathematical modeling. Column total porosity (ε) was determined with 0.25 mol Na_2SO_4/dm^3 water solution injection. The salt concentration was detected by a Radelkis OK102/1 type flow through cuvette conductivity meter. Measurement of bulk density: resin being packed in a column was quantitatively removed and placed in a thin layer of max 5 mm on a plate with known mass and dried at 85–90 °C to constant mass. After the measurements, the given dried resin mass was divided by column volume.

2.3. Measurement of equilibrium isotherms on SP825 packing

Multi-step frontal adsorption method was used in a glass column (L = 122 mm, I.D. = 13 mm) packed with Diaion HP20 or Sepabeads SP825 at 20 °C in 0.002 mol/dm^3 ··· 0.02 mol/dm^3 aqueous solution concentration range with 0.002, 0.005, 0.01, 0.015, and 0.02 mol amino acid/dm^3 water solutions, ca. 2 cm^3/min volumetric stream. The 60 °C isotherm measurement went on jacketed glass column equipped of plunger with 147 mm packing length and I.D. 12 mm similar to the measurement at 20 °C. L-Phenylalanine was detected at 271 nm, and glycine at 230 nm by a UV spectrophotometer.

2.4. Investigation of adsorption temperature dependence and resin aging

For determining the packing, frontal adsorption–desorption experiments were carried out with SP825 resin from 30 °C to 70 °C in 5 °C steps. The feed was aqueous 0.02 mol/dm^3 L-phenylalanine solution with a rate of 2.5 cm^3/min. The first and the last experiments of the series were measured at 60 °C. We investigated the similarity of breakthrough-curves of both the 60 °C measurements.

2.5. Description of production scale SMB-LC equipment

The SMB instrument was designed basically for a four-zone open loop operation. In this manner, the number of

columns was divided into zones marked as follows: 1-1-1-1. The pumps work outside the SMB loop. If the raffinate pump does not work, the instrument is used as a three-zone open loop 1-1-2-0 column configuration unit. The automation of the equipment provides the possibility for 2-1-1-0 column configuration to work.

The four-column production scale SMB-LC equipment has been constructed by the Central Workshop of University of Veszprém: column number is four and is made of stainless steel, useful column length is 500 mm, I.D. 50 mm. Special hole-channel system and 50 μm stainless steel frits assure liquid distribution and collection on the full cross-section. Columns are supplied with stainless steel thermostation jacket. Joining pipes are 1/8 in., made of stainless steel. For switching time controlling of input and output sites four pieces of four-way five-port and four pieces of two-way three-port cocks were built in. Above all, there are two pieces of two-way three-port degasser cocks built in for removing air from output pumps. The two input streams are assured by Lewa and Prominent membrane pumps in 0–250 cm^3/min region; two plunger type pumps (Lewa EK-16 type, extract, raffinate) work against 2.5 MPa check valve. The pumps are placed on separate scaffolds. The third output point, where regenerated solvent is taken away in the four-zone open loop, is the atmosphere. Flow rate of this output is determined by the other four flow rates. The working variations of the four-column SMB-LC are as follows: a four-zone 1-1-1-1-column configuration, a three-zone 1-1-2-0, 2-1-1-0 and 1-2-1-0 column configuration. The listed working variations are built in the program of the PLC controlled automation (Fig. 3).

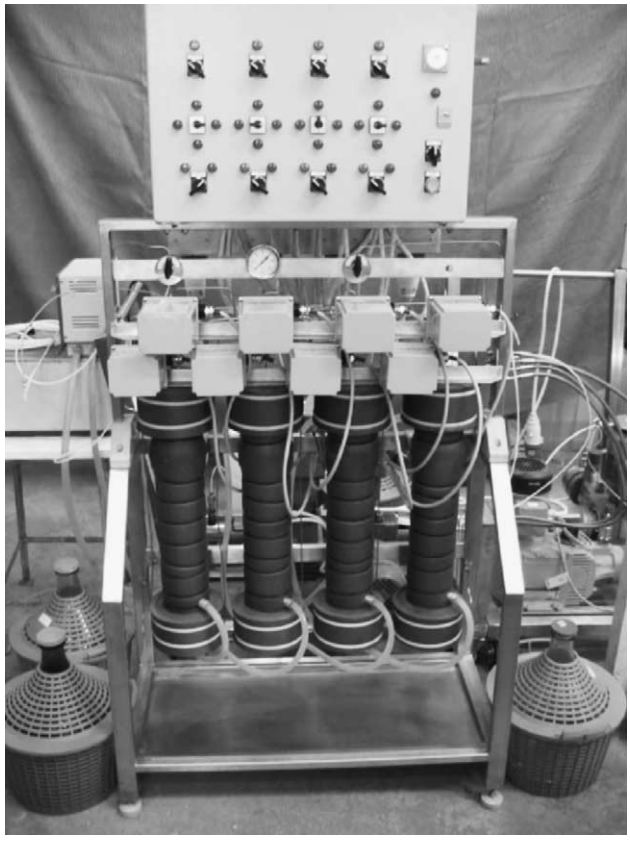

Fig. 3. The photography of the SMB equipment. The four jacketed stainless steel columns are in thermal isolation. Four pieces two position cocks and four pieces four position cocks are actuated by a PLC. Two pieces of two position manual cocks given for degassing.

2.6. Parameters of SMB-LC measurements

Four-zone SMB is used when the weakly adsorbing component can be retarded by the packing to get the proper amount of solvent recirculated. If an open loop SMB is used, then no recirculation exists, but solution is collected and regenerated on a different way (indirect recycle). The glycine weakly adsorbs on the studied polymer resins (Diaion HP20 or Sepabeads SP825), so a three-zone open loop SMB has been designed for the separation. The composition of feed was 0.02 mol L-phenylalanine/dm^3 water, that is 3.3 g L-phenylalanine/dm^3 water, and 0.02 mol glycine/dm^3 water, that is 1.5 g glycine/dm^3 water. Measurements were done at 20 °C with the following experimental parameters at 1-1-2-0 and 2-1-1-0 column configuration. In the following delineated liquid volume streams are the designed parameters. Because of the pumping fluctuation, the experimental parameters can be deviated from these. Fresh eluent was 240 cm^3/min, L-phenylalanine was extracted with 200 cm^3/min, feed was 25 cm^3/min, the outlet liquid was 65 cm^3/min and the switching time was 45 min. Process parameters were determined by Morbidelli method (Fig. 4), minimal switching time was chosen according to the maximal eluent pump capacity being necessary in the first zone

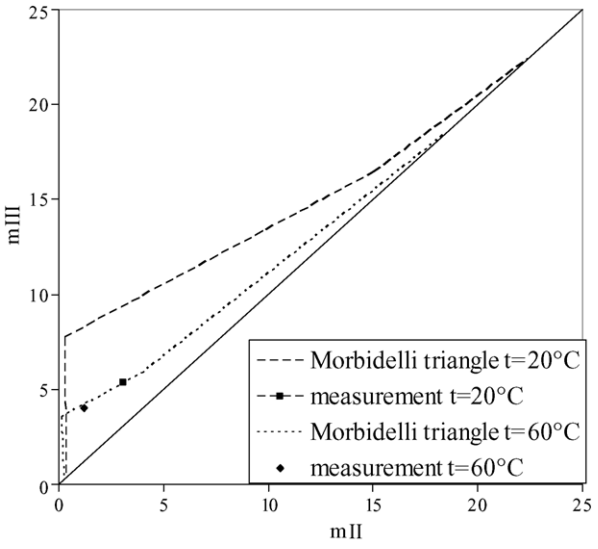

Fig. 4. The measurements points placed in the m_{II}–m_{III} diagram, at temperature 20 °C and 60 °C. The Morbidelli's area was calculated for the SMB separation at 20 °C and 60 °C at the same concentration.

Table 1
The operating conditions of the SMB at 20 and 60 °C by 1-1-2-0 and 2-1-1-0 column configurations

Feed concentration (g/dm^3)	C_{Gly}	1.5	1.5	1.5	1.5
	C_{Phe}	3.3	3.3	3.3	3.3
Column/zone configuration		1-1-2-0	2-1-1-0	1-1-2-0	2-1-1-0
Temperature (°C)		20	20	60	60
Eluent flow rate (cm^3/min)	D	239.4	241.9	239.8	237.4
Extract flow rate (cm^3/min)	E	207.9	201.6	178.7	202.1
Feed flow rate (cm^3/min)	F	25.7	20.3	23.4	43.5
Raffinate (liquid outlet) flow rate (cm^3/min)	R	57.2	60.6	84.5	78.8
Switching time (min)	T	45	45	30	30
Morbidelli criterias		$m_I > K_A = 22.4$		$m_I > K_A = 15.5$	
		$0.352 = K_B < m_{II} < K_A = 22.4$		$0.232 = K_B < m_{II} < K_A = 15.5$	
		$0.352 = K_B < m_{III} < K_A = 22.4$		$0.352 = K_B < m_{III} < K_A = 15.5$	
Morbidelli parameters	m_I	25.34	25.62	16.44	16.26
	m_{II}	2.08	3.07	3.12	1.19
	m_{III}	4.96	5.34	4.86	4.44

for the regeneration of adsorbent. Feed value was optimized by a mathematical model.

The 60 °C measurement was also done using the above concentrations by 1-1-2-0 and 2-1-1-0 column configuration. Columns were thermostated by ion-exchanged water through a jacket, the eluent through pipe spiral and the equipment was insulated outside. The first zone was easily regenerated at 60 °C from L-phenylalanine, so assuring 240 cm^3/min fresh eluent volume stream being the same as in the previous measurement, 30 min switching time was enough. Extract volume stream was 200 cm^3/min. Due to the shortest switching time, sample feed could be larger and the maximal feed was 50 cm^3/min according to the model. Our measurement went on with 45 cm^3/min feed at 2-1-1-0 column configuration, so the quantity of the outlet liquid was 85 cm^3/min. We summarized the experimental parameters in Table 1.

Due to the relatively long switching time, the experiments were carried out for two total cycles, i.e. eight switching times. Both the mathematical model and the experiments verified that in case of 0.02 mol/dm^3 L-phenylalanine and 0.02 mol/dm^3 glycine feed, even the first cycle was sufficient to establish the quasi-stationary state.

2.7. SMB product stream analysis

Both extract and raffinate were collected in separate reservoirs by switching times. After shaking it, sample was taken out of the reservoirs and analyzed by AminoChromII OE914 amino acid analyzer. Several samples were taken during the last switching time to examine the concentration transient within the cycle [quasi-stationary state]. Half a minute sample was taken in every 7 min at the 20 °C measurement, at 60 °C first in the second minute, then in 5 min.

The amino acid analysis in brief: glycine and L-phenylalanine were separated on high efficiency Durrum DC-4A cation-exchange column at constant pH value and ionic strength. Amino acids leaving the column were mixed with ninhydrine reagent and reacted in capillary pipe reactor at 80 °C for 10 min and formed colorful products detected by spectrophotometer at 570 nm. The initial amino acid samples after proper dilution were injected by automatic injector from the 30 μl loop (extract was not diluted, raffinate and feed in five times dilution). Used eluent pH value was 4.25, ionic strength 0.2, volume stream 20 cm^3/h. Ninhydrine volume stream was 10 cm^3/h. Amino acid mixture was used as calibrating standard with 0.0005 mol/dm^3 amino acid concentration. According to the above parameters of the analysis, the retention time of glycine was 4 min, and the retention time of L-phenylalanine was 13 min.

3. Results

3.1. Packing selection

On the bases of previous experiments, the selection of examined resins was restricted to the following two ones: Diaion HP20 white color styrene–divinylbenzene copolymer resin with particle diameter ca. 0.3–0.5 mm; Sepabeads SP825 can be similarly characterized and its dry color is light brown, in water or ethanol it turns dark brown.

Frontal measurements were evaluated by the next capacity relation:

$$k = \frac{V_{\text{aminoacid}} - V_{\text{NaCl}}}{V_{\text{NaCl}}} \tag{14}$$

Respectively by the selectivity coefficient:

$$\alpha = \frac{V_{\text{Phe}} - V_{\text{NaCl}}}{V_{\text{Gly}} - V_{\text{NaCl}}} = \frac{k_{\text{Phe}}}{k_{\text{Gly}}} \tag{15}$$

where $V_{\text{aminoacid}}$ = retention volume of the amino acid (glycine or phenylalanine) (cm^3), V_{Phe} = retention volume of phenylalanine (cm^3), V_{Gly} = retention volume of glycine (cm^3), and V_{NaCl} = dead volume, determined with water soluted NaCl injection (cm^3).

Dead volume was defined as 1 mol NaCl/dm^3 water injection (V_{NaCl}). Next capacity factors were given at 20 °C for the two examined resins:

HP20: L-phenylalanine, $k = 4.03$; glycine, $k = 0.19$.
SP825: L-phenylalanine, $k = 12.99$; glycine, $k = 0.73$.

The selectivity factors for the phenylalanine–glycine aqueous system are:

HP20: $\alpha = 21.2$.
SP825: $\alpha = 17.8$.

3.2. Adsorption equilibrium measurement

The isotherm measurement was done at 20 °C, and later at 60 °C. L-Phenylalanine and glycine adsorption on SP825 resin can be written by Langmuir equilibrium isotherms. Aromatic amino acid L-phenylalanine adsorbs stronger than glycine does. At a higher liquid concentrations solid phase amino acid concentration changes according to the Langmuir equilibrium isotherm.

The amino acid isotherms at 20 °C are:

$$q_{Gly} = \frac{0.5344 c_{Gly}}{1 + 0.9291 c_{Gly}} \quad \text{and} \quad q_{Phe} = \frac{34.01 c_{Phe}}{1 + 0.1621 c_{Phe}} \tag{16}$$

At 60 °C:

$$q_{Gly} = \frac{0.353 c_{Gly}}{1 + 0.9535 c_{Gly}} \quad \text{and} \quad q_{Phe} = \frac{23.47 c_{Phe}}{1 + 0.671 c_{Phe}} \tag{17}$$

(Fig. 5). While increasing the temperature to 60 °C, the selectivity factor in the 3.3 g/dm^3 phenylalanine and 1.5 g/dm^3 glycine aqueous system decreases to about 50%, while capacity factor for phenylalanine decreases by about 33%.

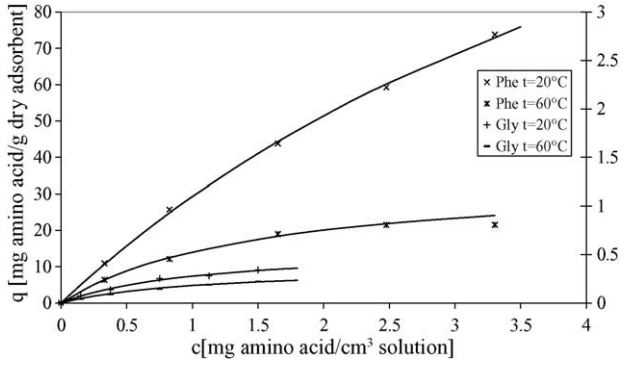

Fig. 5. Adsorption isotherms of glycine and of L-phenylalanine in aqueous solution at temperature 20 °C and 60 °C. *c*: liquid phase amino acid concentration in mg amino acid/cm^3 liquid, *q*: solid phase amino acid concentration in mg amino acid/g dry adsorbent. The Langmuir parameters of glycine: $a = 0.5344$ cm^3 liquid/g dry adsorbent, $b = 0.9291$ cm^3 liquid/mg glycine at temperature 20 °C; $a = 0.353$ cm^3 liquid/g dry adsorbent, $b = 0.9535$ cm^3 liquid/mg glycine at temperature 60 °C. Isotherm parameters of L-phenylalanine: $a = 34.01$ cm^3 liquid/g dry adsorbent, $b = 0.1621$ cm^3 liquid/mg L-phenylalanine at temperature 20 °C; $a = 23.47$ cm^3 liquid/g dry adsorbent, $b = 0.671$ cm^3 liquid/mg L-phenylalanine at temperature 60 °C.

3.3. Packing characteristics

SMB-LC columns were characterized by Na_2SO_4 aqueous solution injection at different volumetric ratios. The columns (I.D. = 50 mm, $L = 500$ mm) were packed with 0.3 mm particle diameter SP825 resin by aqueous slurry technique. Each column was filled with 265.0 g SP825 air-dried adsorbent. For example the theoretical number of plates of column number 2 can be written as:

$$\text{NTP} = \frac{50}{0.0043u + 1.2035} \tag{18}$$

Liquid flow rate (u) is given as cm^3/min and the NTP corresponds to 50 cm packing length. For example the NTP value at 35 cm^3/min volumetric ratio is measured for four columns and $\text{NTP}_{average} = 36.92$, $\sigma = 0.35$. Overall porosity of columns were determined by aqueous salt solution injection and $\varepsilon = 0.59$ value was received for all the four columns. The bulk density value was 0.27 g dry resin/cm^3 column volume.

3.4. Resin aging on higher temperature

In order to compare the breakthrough and desorption curves, the series started as well as ended at 60 °C. Significant difference was not found between the first and the last 60 °C frontal experiments.

3.5. Results of SMB-LC measurement

The 1-1-2-0 configuration is probably disadvantageous in the studied system, since the L-phenylalanine can only be removed with difficulty from the first zone. To compare the 2-1-1-0 and the 1-1-2-0 configurations, experiments were carried out within the same conditions.

In case of 1-1-2-0 column configuration at 20 °C the L-phenylalanine yield was only 91.8%, L-phenylalanine productivity 4.40 mg/h g SP825, and L-phenylalanine purity >99.9% (m/m). The glycine yield and productivity cannot be defined because of the 85% (m/m) glycine purity.

When investigating the outlet liquid samples of the last 45 min period of the measurement, it was found that L-phenylalanine concentration was very high besides glycine directly after the column switching. The L-phenylalanine contamination in the outlet liquid can be explained by the non-complete regeneration of the first zone. In case of 1-1-2-0 column configuration, the operational parameters of the experiment at 60 °C were improved compared to the experiment at 20 °C. The investigations were carried out with the same flow rates, but the applied switching time was 30 min instead of 45 min. This way 4.11 mg/h g SP825 L-phenylalanine productivity could be achieved with >99.9% (m/m) extract purity

Table 2
The operation parameters—productivities, purities and yields of both phenylalanine and glycine in the presented measurements

Feed concentration (g/l)	C_{Gly}	1.5	1.5	1.5	1.5
	C_{Phe}	3.3	3.3	3.3	3.3
Column/zone configuration		1-1-2-0	2-1-1-0	1-1-2-0	2-1-1-0
Temperature (°C)		20	20	60	60
Switching time (min)		45	45	30	30
Productivity of Gly (mg aminoacid/g adsorbent h)	P_{Gly}	2.26	1.75	2.06	3.70
Purity of Gly (raffinate) (%)	m/m%$_{Gly}$	85.0	>99.9	99.3	>99.9
Yield of Gly (raffinate) (%)	Y%$_{Gly}$	>99.9	>99.9	>99.9	>99.9
Productivity of Phe (mg aminoacid/g adsorbent h)	P_{Phe}	4.40	3.76	4.11	8.13
Purity of Phe (extract) (%)	m/m%$_{Phe}$	>99.9	>99.9	>99.9	>99.9
Yield of Phe (extract) (%)	Y%$_{Phe}$	91.8	>99.9	99.8	>99.9

and >99.9% L-phenylalanine yield, and the glycine purity was >99.9% (m/m) in the raffinate.

Results of the measurement at 20 °C on 2-1-1-0 column configuration: more than 99.9% (m/m) amino acid purity in the extract for L-phenylalanine and in raffinate for glycine. The yields are over 99% for both amino acids. Productivity depending on the feed volumetric velocity and concentration is relatively low. L-Phenylalanine productivity is 3.76 mg/g SP825/h, and glycine productivity is 1.75 mg/g SP825/h.

In the case of measurement at 60 °C on 2-1-1-0 column configuration, the feed volumetric velocity was increased from 25 cm^3/min to 45 cm^3/min, so productivity was increased 1.8 times and less diluted product output was given. Values of purity were over 99.9% (m/m) and the yields over 99% (m/m) (Table 2).

The operational parameters were calculated according to the last four periods of the experiments.

3.6. Mathematical modeling

The computer program was first used during the frontal adsorption–desorption measurement. Most important data can be seen in Section 2.1. After the isotherm measurement, the given equilibrium data was applied in the computer program, so the calculated and the measured data were compared to each other (Fig. 6). The calculated and measured break-through and elution curves show very good agreement, which means that the measured equilibrium data can be used for the SMB simulation. Before SMB-LC measurements, several previous simulations were done at 20 °C and at 60 °C.

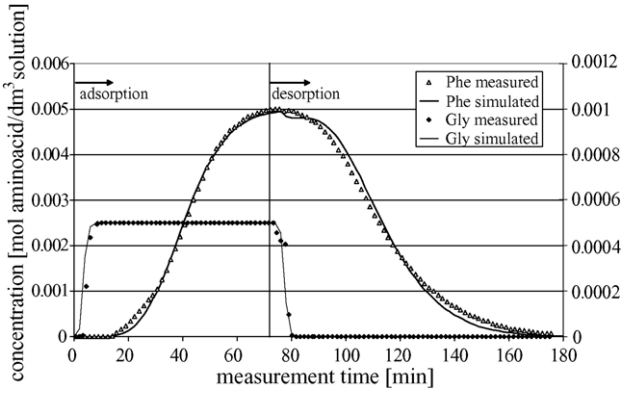

Fig. 6. Frontal adsorption–desorption measurement on SP825 adsorbent, at temperature 20 °C. Mathematical modelling compared to experimental data points. Volumetric stream was 2.9 cm^3/min on a L = 122 mm I.D. = 13 mm glass column.

4. Discussion

4.1. Planning of initial processing parameters

Because of the very week glycine adsorption on SP825 adsorbent in water very low recirculation is possible—not much more than empty volume liquid quantity calculated from column total porosity. Thus, the fourth SMB zone was neglected and the work was continued in the open loop three-zone SMB-LC system. Parameters of the open loop three-zone SMB-LC process: switching time, eluent-, feed-, extract-, raffinate (outlet liquid) volume stream, column configuration, solvent strength and temperature. By linear Morbidelli method, minimal switching time, feed, extract and raffinate streams were determined according to the fresh eluent-pump maximal flow rate and the geometrical parameters of production scale laboratory SMB equipment.

4.2. Parameter optimizing

Object of optimization is to achieve the prescribed purity and yield in industrial production at maximal productivity and minimal solvent use. First, we tried to improve the productivity of measurements at 20 °C. It can be realized by increasing feed flow rate or feed concentration.

There is high selectivity, and large k values in the examined L-phenylalanine–SP825 resin system. Heavy desorption of L-phenylalanine may cause problems in adsorbent regeneration in the first zone, so the next task was to rise the temperature of the system. Lower selectivity, and smaller k were measured at higher temperature, that is better regeneration in L-phenylalanine–glycine–water–SP825 sys-

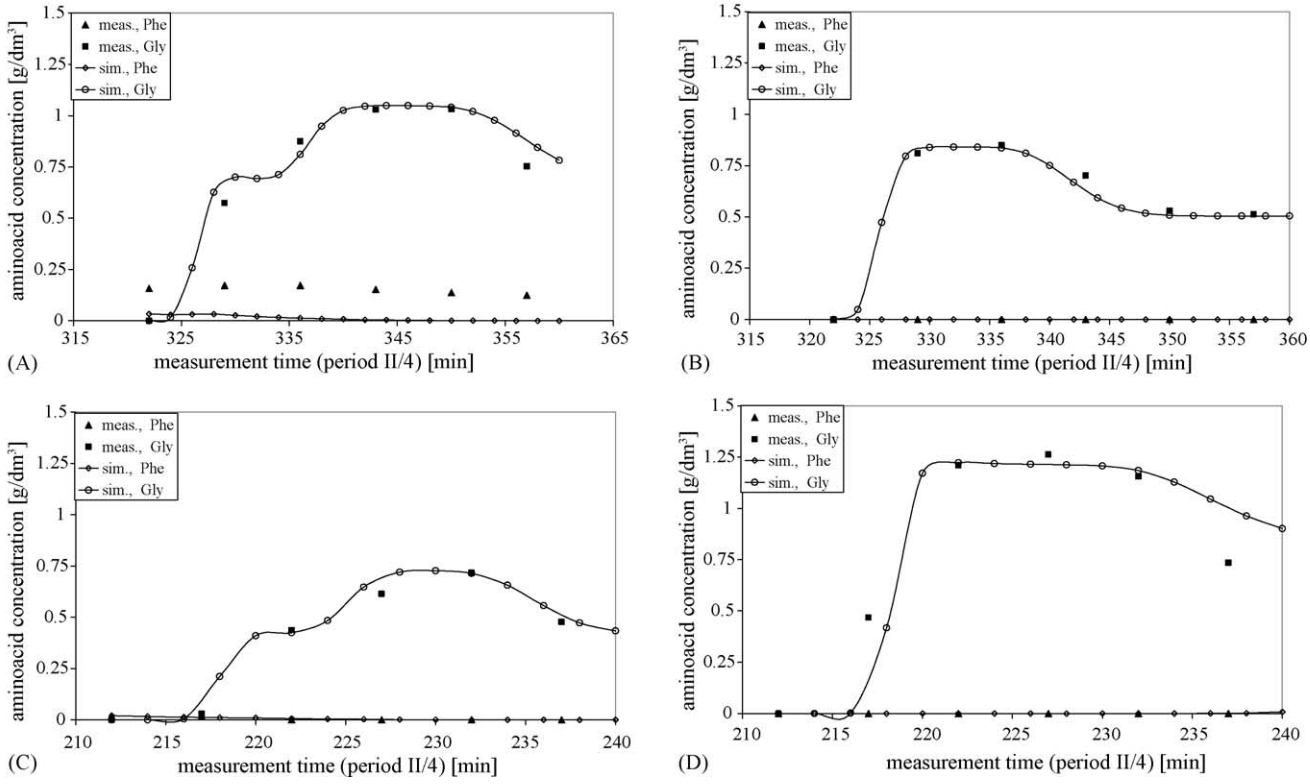

Fig. 7. (A, B) The amino acid concentrations vs. time in the period II/4 of the SMB measurements at 20 °C by 1-1-2-0 column/zone configuration (A) and 2-1-1-0 column/zone configuration (B). (C, D) The amino acid concentrations vs. time in the period II/4 of the SMB measurements at 60 °C by 1-1-2-0 column/zone configuration (C) and 2-1-1-0 column/zone configuration (D).

tem. Switching time could be reduced from 45 min to 30 min, so feed stream was increased from 25 cm^3/min to a maximum 45 cm^3/min.

Further advantage of SMB-LC planned for 60 °C is that the system can be heated by cheap industrial waste heat, so running costs do not rise; the same spare parts can be used as in the case of 20 °C.

In the case of 1-1-2-0 column configuration increasing the temperature from 20 °C to 60 °C, higher regeneration degree could be achieved in the first zone (and higher outlet liquid, raffinate purity) than in the previous case. Insufficient regeneration can be seen from the L-phenylalanine concentration versus time diagram of the outlet liquid, raffinate stream (Fig. 7). The better-adsorbed amino acid appears already in the beginning of period in the raffinate, consequently it comes from the first SMB zone. Changing the column configuration (1-1-2-0 → 2-1-1-0) is advantageous to consider the kinetics and, to increase the feed flow rate without a decline in the obtained purity and yield.

In principle, the temperature increase can be disadvantageous since it promotes the destruction of the structure of the co-polymer resin. Considering the results of the resin ageing studies, no change was observed in quality; no change in equilibrium and kinetic properties. In the case of long-term industrial SMB operation resin SP825 co-polymer packing can be easily exchanged due to cheap price, when its quality diminishes.

5. Conclusion

The SMB-LC system temperature was increased from 20 °C to 60 °C. Temperature rise is limited and allowed to a given value because ventiles, cocks, fittings, etc. applications depend on temperature. It can be concluded that the most advantageous column configuration is the 2-1-1-0 at 60 °C. With this column configuration and temperature, the prescribed purities and yields can be achieved and 1.8 times higher productivity can be observed compared to 1-1-2-0 configuration at 20 °C, with the other parameters remaining constants.

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