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INVESTIGATION OF REVERSE PHASE SMB-CHROMATOGRAPHIC BIOSEPARATIONS OF AMINO ACID AQUEOUS SOLUTIONS

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The authors investigated amino acid aqueous solutions as model system for the purpose of studying reverse phase chromatographic bioseparation. Desalting of DL-β-phenylalanine was studied on a small-laboratory scale simulated moving bed (SMB) preparative liquid chromatograph (number of columns=6, column length=125mm, column I.D.=13mm). DIAION HP20 polymeric adsorbent resin was used for the reverse phase chromatography. The feed (sample) of the SMB equipment is 3.5g DL-β-phenylalanine/dm^3 and 58.5g sodium-chloride/dm^3 aqueous solution. With the three zones opened loop SMB with 2-2-2 column configuration can amino acid product be achieved with less than 50ppm NaCl in crystal form after evaporation. Later a large laboratory scale (number of columns=4, column length=500mm, column ID=50mm) automatized SMB equipment was constructed. The applied model system for bioseparation contains glycine (1.5g/dm^3), L-phenylalanine (3.3g/dm^3) in water and SEPABEADS SP825 adsorbent. Both L-phenylalanine and glycine were produced in more than 99.9% m/m purity and 99% yield at productivity 3.7-9.5mg/(g adsorbent h) in case of three zones open loop 2-1-1 column configuration. The SMB experiments were simulated with the help of equilibrium cascade model. The measured and calculated data agreed well.

Keywords: simulated moving bed (SMB), column liquid chromatography, polymeric adsorbent resin, amino acid aqueous solution

Introduction

It is typical in biotechnology and pharmaceutical industry that water phase mixture for processing contains end-product or active ingredient in small concentration beside the contaminant or polluting components. Simulated moving bed (SMB) preparative chromatography can be advantageous among the end-product recovery methods for continuous processing of high purity products, or products being difficult to isolate.

Basically the SMB chromatograph works similarly to the true moving bed (hereafter abbreviated as TMB). The TMB works in the following way by Figure 1a: in a column the mobile phase moves upwards through the adsorbent that moves downwards simultaneously. The column is fitted with a feed inlet in the middle of the side-wall, a raffinate outlet on the upper part and an extract outlet on the lower part. Choosing the appropriate adsorbent moving velocity value and suitable eluent, feed, extract and raffinate flow rates, a stationary state can be obtained in the column with constant concentration profiles. The more binding component occurs in the lower (I-II) part of the column and the less binding one is located on the upper part (III-IV) of the column. This way two pure components can be obtained simultaneously in the extract and in the raffinate. The chromatographic quality of TMB is difficult to realize technically, therefore an SMB was employed in the preparative chromatography.

SMB liquid chromatograph (hereafter-abbreviated SMB-LC) (Fig. 1b) is a multi-column system with two inputs and outputs (products), in

which liquid phase moves in counter-current of adsorbent phase. The counter-current stream is not real, but simulated, since the packed chromatographic stationary phase moves periodically after each switching time. The shorter the switching time and the more the number of columns are in the SMB-LC, the better it converges to the TMB-LC.

The SMB technique is basically a two product preparative chromatographic operation. It is suitable for mixtures to be separated having two components or can be produced two product fractions.

In case of continuous system the two input streams are the fresh eluent and feed, the two outputs are the extract and raffinate. Above all in basic case regenerated eluent of re-circulating stream is added to the fresh eluent.

Similarly to the TMB the lower part (I-II) of the column is rich in the more binding component and at the upper part (III-IV) of the column contains the less binding component. The inlet and the outlet fluid flow streams divide the column system into four zones *(Fig.1.)*

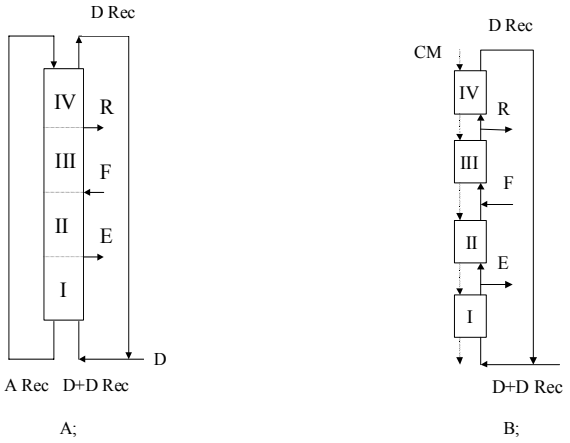


Figure 1. The scheme of a) true moving bed (TMB) and b) simulated moving bed (SMB). True moving bed (TMB) adsorber: I, II, III, IV – zones; D – desorbent (solvent, eluent); D Rec – recirculated eluent; A Rec – adsorbent recirculation; E – extract stream with the better adsorbed component A; F – feed stream with the components A and B; R – raffinate stream with the less adsorbed component B. Simulated moving bed (SMB) liquid chromatograph – I, II, III, IV – zones, respectively HPLC columns; CM – direction of simulated moving of HPLC columns.

The inlet liquid stream of the first zone is the mixture of the fresh and recirculated eluent. The first column of the first zone has to be regenerated till the end of each switching time period to protect carrying strongly adsorbed components by adsorbent phase. The inlet liquid stream of the second zone is the mobile phase from the first zone minus the flow stream of the extract. This flow stream must be determined so that the less binding component could leave the first column of the second zone till the end of the switching period avoiding to get into the extract. The inlet liquid stream of the third zone is the mobile phase from the second zone plus the feed stream to be separated. The function of this zone is holding the more binding component in the adsorbent phase, since the less binding component is taken away as a raffinate at the end of the zone. Four-zone SMB is favourable when retarding the less binding component – regenerating the solvent. The regenerated solvent can be recycled and added to the fresh eluent. In case of recycled solvent the system is called closed loop SMB. This version is more favourable compared to the open loop system from economic and environmental point of view. Three-zone SMB is preferred in systems with high selectivity factor, when the less binding component has low capacity factor flowing nearly together with the mobile phase [1,2].

According to the above facts the operational parameters of the process are the switching time, the flow rates of the mobile phase in each zones determined by the external flow rates (fresh and recycled eluent, extract, feed, raffinate).

Summing up the possibilities for amino acid preparative separation the following chromatographic methods were applied in practice: ion-exchange column liquid chromatography, ion-exchange parametric pumping, size-exclusion chromatography, reverse phase adsorption chromatography.

In case of reverse phase adsorption chromatography for separation of amino acids solved in water: the styrene-divynilbenzene copolymers with non-polar surface and the polymethacrylate resins with weakly polar surface can be used in the presence of electrolytes or polar solvent. In such systems the adsorption equilibrium depends on the temperature, the solvent strength, the pH [3,4] and on the electrolyte concentration of aqueous solution [2].

The design of industrial scale SMB-LC process requires numerous preliminary experiments. At the selection of the packing we can reduce the number of the possible alternatives if we consider the chemical character of the adsorbents. The most frequently used technique is the determination of adsorption selectivity with an analytical HPLC instrument with a given adsorbent by injection method. The adsorbent is giving the best selectivity to be examined further on within small-scale or large-scale laboratory circumstances. We examined the model samples by frontal adsorption-desorption

method on the small scale lab size column packed with polymer adsorbent. The advantage of this method is that frontal adsorption and desorption processes of the SMB-LC can be investigated.

After determining the equilibrium data of the selected systems and the column packing characteristics the initial operating parameters of the SMB can be calculated. The initial operating parameters for a three zone open loop SMB was calculated by the method of *Morbidelli* et al. [5]. The first zone regeneration is appropriate when:

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

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 retarding of the better-adsorbed component, namely this component must not break through the third zone:

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

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

In a two component-system a m_{II}-m_{III} area can be determined from the geometric data of the SMB equipment, the equilibrium parameters of Langmuir-type isotherms and the concentrations of the mixture to be separated.

The given operating conditions determined a point in the m_{II}-m_{III} diagram. This point must be placed in the Morbidelli-area (or Morbidelli-triangle), then both products stream purity are theoretically 100%. When the optimal chromatographic packing is selected, the following operating variable can have influence on the SMB-LC operation: fresh eluent, recirculated eluent, feed, extract and raffinate flow rates, column switching time. During calculation the influence of a given parameter can be investigated while the others must be considered as constants.

Better productivity, product purity, yield, eluent consumption can be achieved by optimizing operating conditions by computer.

The Morbidelli-area changes with the change of the following parameters during the planning: geometrics of SMB equipment, temperature and exchange of adsorbent, composition of fresh eluent and feed.

During the planning of a preparative chromatography it must be considered, that the models of linear chromatography described is not appropriate the system. Many research schools are dealing with the mathematical modeling of the nonlinear chromatography [6,7].

Experiments

The examined model systems during research are the aqueous solution of DL-β-phenylalanine and sodium-chloride, and the aqueous solution of glycine and DL-β-phenylalanine.

Select reversed phase packing

In case of L-phenylalanine and glycine amino acids separation in water the next reversed phase non-polar polymer adsorbents were studied by frontal adsorption-desorption methods: DIAION HP20, SEPABEADS SP825. For desalting DL-β-phenylalanine DIAION HP2MG, Amberlite XAD7 polymethacrylate, Amberlite XAD1180, DIAION HP20 styrene-divynilbenzene copolymer resins were investigated.

Parameters of research: measurement went on glass column in which resin bed length was 122 mm, I.D. 13 mm. L-phenylalanine concentration 0.005 mol L-phenylalanine/dm^3 aqueous solution, glycine concentration 0.0025 mol glycine/dm^3 aqueous solution, volumetric stream 2.9 ml/min. L-phenylalanine was detected on-line by GILSON116 UV-spectrophotometer at 271 nm wavelength. Glycine and phenylalanine analyses went on afterwards, samples fractionally taken in each 4 ml by OE914 amino acid analyzer. While desalting 0.02 mol/dm^3 DL-β-phenylalanine aqueous solution and 1mol sodium-chloride/dm^3 aqueous solution, the next detection was used during adsorbent selection. The phenylalanine was detected on-line UV at 271nm, NaCl by conductivity meter with flowing through cuvette. Ion exchanged water was used for all experiments as desorbents.

Measurement of packing properties

Frontal adsorption–desorption method for adsorbent selection gave the best packing filled by slurry technique into SMB-LC column. After packing, the number of theoretical plate (NTP) was measured in function of volume stream for L=500mm I.D.=50mm preparative SMB-column in 30-180 cm^3/min range using Na_2SO_4 detecting compound. In case of L=125mm ID=13mm small-

laboratory SMB column NTP and column porosity (ε) were determined in 3.0-22 cm^3/min range by injection method with 0.25 mol Na_2SO_4/dm^3 water solution at 20°C. The answer function of the injection was detected by a RADELKIS OK102/1 conductivity meter with flow-through cuvette. This date is necessary for modeling, too. Measurement of bulk density: resin being packed in a column was quantitatively removed and placed in max 5 mm thin layer on a plate in known mass and dried at 85-90°C till constant mass, then re-measured.

Measurement of equilibrium isotherms

Multi-step frontal adsorption method was used in a glass column (L=122 mm, ID=13 mm) packed with DIAION HP20 respectively 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, cc. 2 cm^3/min volumetric stream. The 60°C isotherm measurement went on jacketed glass column equipped with plunger with 147 mm packing length and ID 12 mm similarly to the measurement at 20°C. L-phenylalanine was detected at 271 nm, glycine at 230 nm by UV-spectrophotometer. The equilibrium isotherms of DL-β-phenylalanine were measured in ion-exchanged water, further in 0.25 and 1 mol NaCl / dm^3 aqueous solutions. Equilibrium data were used for planning processing parameters and mathematical models.

Description of production-scale SMB-LC and small-laboratory SMB-LC equipments

There are geometric and other parameters of the 4-column production scale SMB-LC equipment being constructed by the Central Workshop of University of Veszprém: column number is four and made of stainless steel. Useful column length: 500 mm, ID 50 mm. Special hole-channel system and 50 μm stainless steel fritts assure liquid distribution and collection on the full cross-section. Columns are supplied with stainless steel thermostable jacket. Joining pipes are 1/8'' made of stainless steel. For switching time controlling of input and output sites 4 pieces of 4-way 5 set and 4 pieces of 2 way 3 set cocks were built in. Above all, there are 2 pieces of 2 ways 3 set deairation cocks built in for removing air from pump outputs. The two input streams are assured by membrane pumps in 0-250 ml/min region; output streams are forwarded by two plunger type pumps. Pumps are placed on separate scaffolds. The third output point, where regenerated solvent is taken away in the four-zone running, is open to the atmosphere without recirculation. There are the working parameters of the 4-column SMB-LC: a four-zone 1-1-1-1-column configuration and a 3-zone 1-1-2-0, 2-1-1-0 and 1-2-1-0 column configuration. The listed working methods have been built in the program of the PLC controlled automation.

The six-column, three-zone small-laboratory SMB-LC was built of six glass columns of equal geometry, with packing length of 127 mm and with inner diameter of 13 mm, a six-position low pressure multifunction valve, a time actuator and Teflon pipes. The upper part of the valve is fixed through which the inlet of the sample and the eluent is carried out. The lower part is the rotating one which revolves along with the columns by a column position in every given time interval *(Fig. 2.)*

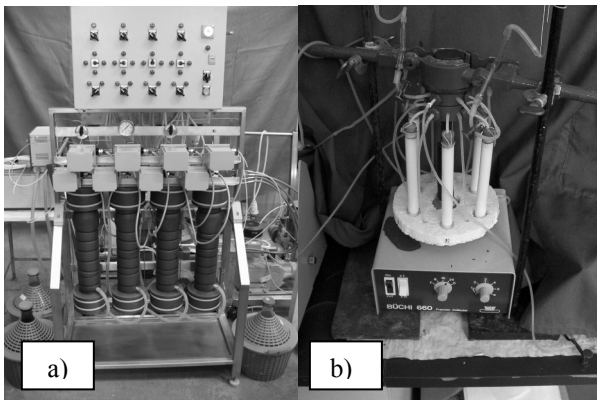


Figure 2. Photography of a) production scale SMB and b) small-lab SMB equipment.

Parameters of SMB-LC measurements

The L-phenylalanine – glycine – water separation was carried out on the production scale SMB equipment (AA03, AA06). The initial experiment at 20°C, later on at 60°C was improved with the help of the equilibrium cascade model. The desalinization process of DL-β-phenylalanine was carried out on the small-lab scale SMB (SMB 3, 4, 5) unit. We varied the flow streams of eluent, feed, raffinate, extract and the switching time *(Table I.)*

Table I. Operating conditions of desalting of phenylalanine with the small-laboratory scale six-column SMB (SMB3, SMB4, SMB5), and of phenylalanine-glycine separation with the preparative four-column SMB (AA03, AA06).

Small lab SMB	c_F (g/dm^3)		Column configuration	Temperature	T	D	E	F	R
	Phe	NaCl			(min)	(cm^3/min)	(cm^3/min)	(cm^3/min)	(cm^3/min)
SMB3	3.5	58.5	2:2:2	20°C	26	2.86	1.23	0.33	1.96
SMB4	3.5	58.5	2:2:2	20°C	15	5.55	2.45	0.83	3.93
SMB5	3.5	58.5	2:2:2	20°C	12.5	6.95	3.06	1.21	5.11
Prep. SMB	c_F (g/dm^3)		Column configuration	Temperature	T	D	E	F	LROUT
	Phe	NaCl			(min)	(cm^3/min)	(cm^3/min)	(cm^3/min)	(cm^3/min)
AA03	3.3	1.5	2:1:1:0	20°C	45	241.9	201.6	20.3	60.6
AA06	3.3	1.5	2:1:1:0	60°C	30	237.4	202.1	43.5	78.8

SMB product stream analysis

Production scale SMB unit – phenylalanine – glycine separation: Both extract and raffinate were collected in separate reservoir by switching times. After shaking it, sample was taken out of the reservoirs and analyzed by AminoChromII OE914 amino acid analyzer. Over the average sample, part sample was taken in the last switching time by a given periodicity to examine concentration transient within cycle [quasi-stationary state]. Half a minute sample was taken in every 7 minutes at the 20°C measurement, at 60°C first in the second minute, then by 5 minutes.

Summarizing the amino acid analysis: glycine and L-phenylalanine were separated on high efficiency Durrum DC-4A cation exchange column at constant pH and ion strength. Components leaving the column were mixed with ninhydrine reagent and reacted in capillary pipe reactor at 80 °C for ca. 10 min, the forming colorful products were detected by spectrophotometer at 570 nm. After proper dilution the samples were injected by automatic injector from the 30 µl loop (extract was not diluted, raffinate and feed in 5 times dilution). Used eluent pH is 4.25, ion strength is 0.2, volume stream is 20 ml/hour. Ninhydrine volume stream is 10 ml/hour. Amino acid content of the sample is proportional to the area beneath the chromatographic peak. Amino acid mixture was used as calibrating standard with 0.0005 mol/l amino acid concentration. The analysis time was ca. 25 min/sample.

Small-lab SMB unit – phenylalanine desalting: both in extract and raffinate stream on-line UV detection of amino acid at 271 nm wavelength. Salt detection: on-line by conductivity meter with flowing-through cuvette.

Results

Packing selection

Frontal measurements were evaluated by the next capacity relation:

$$k' = \frac{V_{aminoacid} - V_{NaCl}}{V_{NaCl}} \quad (4)$$

Dead volume (V_{NaCl}) was defined by 1 mol NaCl/dm^3 water injection (V_{NaCl}). The following capacity factors were given at 20°C for the selected resins:

HP20 for desalinization: DL-phenylalanine k'=4.20

SP825 for glycine-phenylalanine separation: L-phenylalanine k'=12.99; glycine k'=0.73
(Table II.)

Table II. Capacity factors of the investigated resins.

Adsorbent for Phe desalinization	Sample composition [g dm^{-3}]		Volume/breaktrough curve inflection point [cm^3]		Capacity factor k'
	NaCl	Phe	NaCl	Phe	
XAD7	123.00	3.00	13.80	29.30	1.12
	58.40	3.00	13.80	23.40	0.70
	58.40	3.00	13.80	23.80	0.72
HP2MG	58.50	2.70	12.10	33.20	1.74
	58.50	3.22	12.10	21.00	0.74
XAD1180	58.50	2.70	14.00	62.90	3.49
	58.50	3.22	14.00	56.00	3.00
HP20	58.50	2.70	12.50	75.30	5.02
	58.50	3.22	12.50	65.00	4.20
Adsorbent for Gly-Phe separation	Sample composition [g dm^{-3}]		Volume/breaktrough curve inflection point [cm^3]		Capacity factor k' Gly Phe
	Gly	Phe	Gly	Phe	
HP20	1.50	3.30	14.85	62.70	0.19 4.03
SP825	1.50	3.30	16.50	133.65	0.73 12.99

Equilibrium measurement

L-phenylalanine and glycine adsorption on SP825 resin can be written by Langmuir equilibrium isotherms. The amino acid isotherms at 20°C *(Fig. 3.a):*

$$q_{Gly} = \frac{0.5344 c_{Gly}}{1 + 0.9291 c_{Gly}} \quad \text{and}$$

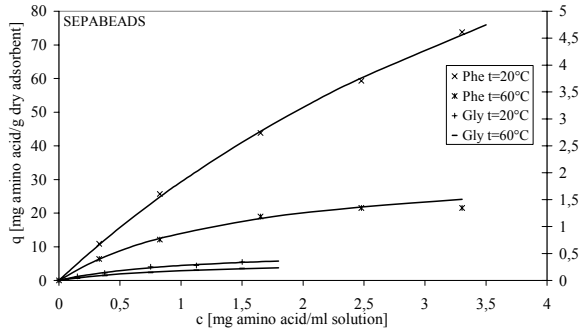
$$q_{Phe} = \frac{34.01 c_{Phe}}{1 + 0.1621 c_{Phe}} \quad (5)$$

At 60°C:

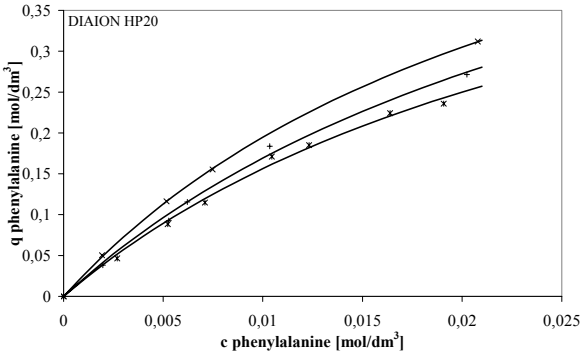
$$q_{Gly} = \frac{0.353 c_{Gly}}{1 + 0.9535 c_{Gly}} \quad \text{and}$$

$$q_{Phe} = \frac{23.47 c_{Phe}}{1 + 0.671 c_{Phe}} \quad (6)$$

The unit of liquid phase concentrations (c_{Phe}, c_{Gly}) is mg amino acid/cm^3 solution, the solid phase concentrations (q_{Phe}, q_{Gly}) are in mg amino acid/ g dry resin. Rising temperature solid phase amino acid quantity decreases.



(a)



(b)

Figure 3. a) Adsorption isotherms of glycine and of L-Phenylalanine in aqueous solution at temperature 20°C and 60°C on SP825 resin. 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. b) Isotherms of DL-β-phenylalanine on HP20 resin. Langmuir parameters depends on the NaCl concentration of the liquid phase: in water (marker *): a=20.86, b=33.5; in 0.25 M NaCl solution (marker +): a=22.37, b=32.1; in 1 M NaCl solution (marker x): a=26.94, b= 32.8. The dimension of Langmuir-b parameter: cm^3 mmol^{-1}. Langmuir-a is dimensionless.

The investigated adsorption of the DL-β-phenylalanine in aqueous phase and in salt solution on HP20 adsorbent can be characterized also by Langmuir isotherms *(Fig. 3.b)*. Increase of the concentration of the salt solution means the DL-β-phenylalanine concentration increase in the resin phase. Taking into consideration the dependence of the "a" parameter of the Langmuir equation on the salt concentration it can be written as:

$$q_{Phe} = \frac{6.08 c_{NaCl}\, c_{Phe} + 20.86 c_{Phe}}{1 + 32.8 c_{Phe}} \quad (7)$$

The concentration units are in mmol amino acid/ ml solution (c_{NaCl}, c_{Phe}), respectively in mmol amino acid/ ml packing total volume (q_{Phe}).

Packing characteristics

SMB-LC columns were characterized by Na_2SO_4 – water solution injection at different volumetric streams. The production-scale SMB-columns I.D.=50mm L=500mm were packed with 0.3 mm particle diameter SP825 resin by water slurry technique. Each column was filled with 265.0 g SP825 adsorbent air dried till constant mass value. For example the NTP of the column No.2. can be determined with the following equation:

$$\mathrm{NTP} = \frac{50}{0.0043u + 1.2035} \quad (8)$$

Unit of "u" : cm^3/min mobile phase flow rate. The NTP is defined with this equation for 500 mm packing length. Overall porosity of columns were determined by salt-solution injection and ε=0.59 value was received for all the four columns. The bulk density value is 0.27 g dry resin/cm^3 column volume.

The NTP of the small-lab scale SMB packed with 16.0 g HP20 resin per column can be determined:

$$\mathrm{NTP} = \frac{125}{0.00225v_0 + 0.0291} \quad (9)$$

Unit of „v_0" : mm/min mobile phase velocity. Overall porosity: ε=0.71 .

Results of SMB-LC measurements

Results of measurement at 20°C on 2-1-1-0 column configuration of the production-scale four-columns SMB unit: more than 99.9% m/m amino acid purity in 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, glycine productivity is 1.72 mg/g SP825/h (AA03 measurement).

In case of measurement at 60°C (AA06 measurement) on 2-1-1-0 column configuration the feed volumetric velocity was increased from 25 to 45 ml/min, so productivity was increased 1.8 times and less diluted product output was given. Values of purity are over 99.9% m/m and the yields over 99% m/m. Desalting DL-β-phenylalanine (SMB 3, 4, 5 measurements): productivity for phenylalanine was 0.67-2.3 mg/g HP20/h, yield 89-96% and amino acid purity 98.5% m/m *(Table III.)*

Discussion

Planning of SMB-LC processing parameters

The task of the first zone in the 4 zone recirculation SMB-LC is the regeneration of adsorbent, namely the washing down and desorption of the better binding component, till the end of switching time. The second and third zones are the separation zones. 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 retarding of the better binding component on the stationary phase. In the fourth zone a portion of the eluent is recovered purely, so here the less adsorbing component is to be adsorbed, retained. Because of the very weak glycine respectively NaCl adsorption in this water phase system, very low recirculation is possible – not much more than empty volume liquid quantity calculated from column total porosity. Thus, the fourth zone was neglected and the work was continued in the open three-zone SMB-LC system.

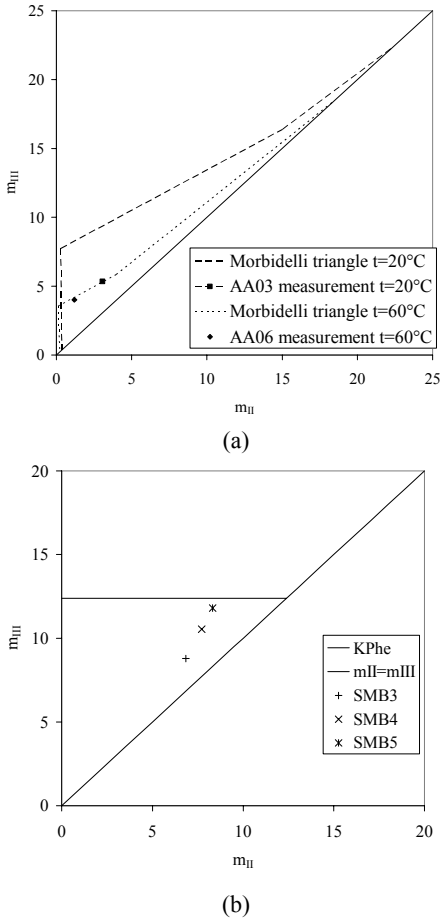


*Figure 4.*The measurements points placed in the Morbidelli triangle: a) phenylalanine-glycine separation at temperature 20°C (AA03) and 60°C (AA06) b) desalting of phenylalanine (SMB 3, 4, 5).

Table III. Operation parameters of the SMB separations.

Small lab SMB	Productivity [mg/gh] Phenylalanine		Purity [%] Phenylalanine		Yield [%] Phenylalanine	
SMB3	0.667		98.34		95.58	
SMB4	1.645		98.5		93	
SMB5	2.302		98.21		89.07	
Preparative SMB	Productivity [mg/gh]		Purity [%]		Yield [%]	
	Phenylalanine	Glycine	Phenylalanine	Glycine	Phenylalanine	Glycine
AA03, t=20°C	3.760	1.724	>99.9	>99.9	>99.9	>99.9
AA06, t=60°C	8.130	3.697	>99.9	>99.9	>99.9	>99.9

Beside the prescribed purity and yield in industrial production the productivity must be the highest, the solvent use the less and adsorbent utility is the best. The initial operating conditions were planned with the help of Morbidelli's equilibrium method (*Fig. 4.*). The first parameters can be improved, while increasing the feed value or concentration. An obvious possibility is the increase of all flow rates (eluent, feed, extract, raffinate) proportionally and decrease the switching time. We used this method for desalting of DL-β-phenylalanine on the small-lab SMB. There are kinetic limits of the flow rate increase (*Fig. 5.*). Other possibility is to improve the regeneration of the first zone for example by increasing temperature. With this method less fresh eluent is necessary, thus we can increase the feed flow rate and so the productivity improves. By the phenylalanine–glycine separation lower selectivity was measured at higher temperature. The initial steep of adsorption equilibrium isotherm decreased, therefore L-phenylalanine desorption went on easier. Thus the switching time could be reduced from 45 min to 30 min, so feed stream was increased from 20.3 ml/min to max. 43.5 ml/min.

Conclusions

We planned initial parameters with the help of Morbidelli's equilibrium method for SMB separation in water of phenylalanine-glycine, respectively phenylalanine–sodium-chloride model systems on polymer adsorbents. We investigated two ways to improve the productivity. The increase of all flow streams and the decrease of period time in the desalting of DL-β-phenylalanine is limited by adsorption and desorption kinetics of the amino acid: the productivity was increased 3 times, but the yield decreased from 95.6% to 89% (SMB 3, 4, 5 measurements).

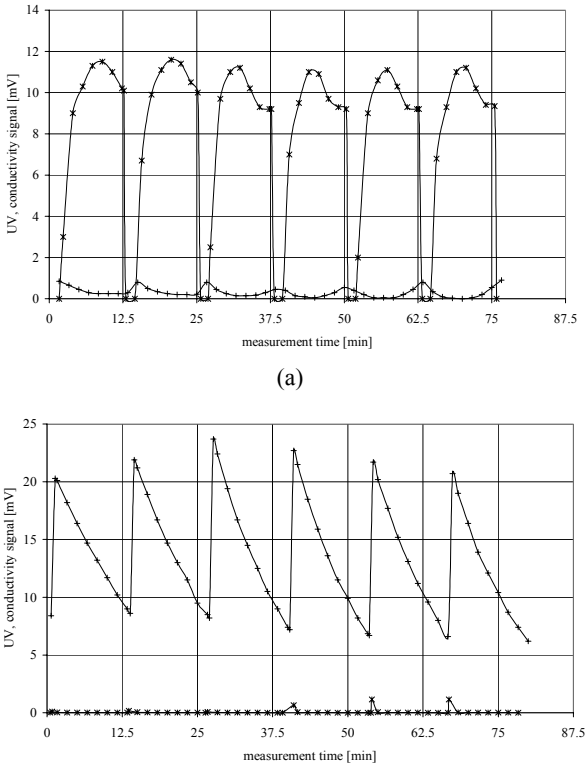


Figure 5. Measured concentration commensurable signals by SMB5 experiment in a) raffinate and b) extract. Markers: signal of phenylalanine (+) and signal of NaCl (*).

The phenylalanine–glycine SMB-LC system temperature was risen from 20°C to 60°C (AA03, AA06 measurements), thus productivity was increased 1.8 times. Rising temperature gives solution only for an optimal value because application of ventiles, cocks, fittings, etc. is limited by temperature.

SYMBOLS

D – flow rate of eluent (cm^3/min)

E – flow rate of extract (cm^3/min)

F – flow rate of feed to be separated (cm^3/min)

T – period time or column switching time (min)

A – cross-section of the SMB-column (cm^2)

L – column length (cm)

ε – overall porosity

m_I, m_{II}, m_{III}, m_{IV} – Morbidelli's parameters

K_A, K_B – equilibrium distribution coefficient

k' – capacity factor

$V_{aminoacid}$, V_{NaCl} – inflection point of the breakthrough curve (cm^3)

q_{Phe} – phenylalanine concentration in stationary phase (mg/g)

c_{Phe} – phenylalanine concentration in mobile phase (mg/cm^3)

c_{NaCl} – NaCl concentration in mobile phase (mg/cm^3)

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