

## Separation of Amino Acids from Their Mixture by Facilitated Pertraction with D2EHPA

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Original scientific paper  
Received: March 12, 2008  
Accepted: September 16, 2008

The study on facilitated pertraction of some amino acids with D2EHPA as carrier underlined the significant influence of the pH gradient between the aqueous phases, carrier concentration and mixing intensity. The amino acids can be separated selectively by varying the pH value of feed phase, due to the formation of the corresponding ionic species. The increase of the carrier concentration inside the liquid membrane leads to the intensification of the initial and final mass flows. The amplitude of this effect depends on the molecular size of the pertracted amino acid. The permeability factors are positively influenced by the increase of the pH value of feed phase and rotation speed of the stirrers in the aqueous phases, but are negatively affected by the increase of the stripping phase pH. For all studied amino acids, the pertraction system reaches its maximum capacity of solute transfer at  $\gamma = 60 \text{ g L}^{-1}$  carrier concentration.

### Key words:

Amino acid, D2EHPA, liquid membrane, pertraction, mass flow, permeability factor

## Introduction

Biotechnology has progressed much in recent years, representing the most advantageous method for obtaining some very important products for human activity. In this context, the production of amino acids, which are the main structural components of proteins and enzymes, constitutes one of the major directions for the development of the biotechnological processes.

The amino acids can be obtained by biosynthesis, by protein hydrolysis or by extraction from natural sources. The most efficient methods are the first two, but the separation of amino acids from fermentation broths or protein hydrolysates is rather difficult. In the last decades, a continuous and increasing interest has been observed in developing the techniques that can improve the selectivity and the yield of downstream processes for the separation and purification of amino acids.<sup>1</sup> The separation techniques currently applied for removal and purification of amino acids from dilute aqueous solutions typically employ ion exchange, crystallization at the isoelectric point or chromatography.<sup>2</sup> But, these techniques are rather difficult to be transposed to the industrial scale, thus affecting the production of amino acids and increasing the cost of the technology used.

The liquid-liquid extraction has become a very attractive method for amino acids separation, because it offers an advantageous alternative to the above-mentioned separation techniques. Amino acids dissociate in aqueous solutions, forming characteristic ionic species as a function of the solution pH value. This property makes amino acids hydrophilic at all pH-values and, thus, complicates their recovery by solvent extraction. For this reason, the amino acids solubility in conventional organic solvents is lower, their physical extraction being practically impossible. The liquid-liquid extraction of amino acids becomes possible only by adding extractants into the organic phase, namely derivatives of phosphoric acid,<sup>2–8</sup> high molecular weight amines<sup>9–11</sup> or some types of crown-ethers<sup>2,12,13</sup> (reactive extraction).

The extraction and transport through liquid membrane technique, also called pertraction or permeation through liquid membranes, represents the development of the liquid-liquid extraction, and can be applied for separating a very large palette of biosynthetic compounds (antibiotics, carboxylic acids, vitamins etc.). The principle of this separation method consists of the transfer of a solute between two aqueous phases of different pH, phases that are separated by a solvent layer of various sizes. Commonly, liquid membranes can be obtained either by emulsification,<sup>14–16</sup> when their stability is poor, or by including the solvent in a hydrophobic porous

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polymer matrix.<sup>17–19</sup> Moreover, the liquid membranes could be obtained using pertraction equipment of special construction, which allows for the separation and easy maintenance of the three phases without adding surfactants (*free liquid membranes*).<sup>20,21</sup> The pertraction efficiency and selectivity could be significantly enhanced by adding a carrier in the liquid membrane, such as organophosphoric compounds, long chain amines or crown-ethers, the separation process being called *facilitated pertraction*.<sup>22,23</sup>

Compared with the conventional liquid-liquid extraction, the use of pertraction reduces the loss of solvent during the separation cycle, needs relatively small quantity of solvent and carrier, owing to their continuous regeneration, and offers the possibility of solute transport against its concentration gradient, as long as the pH gradient between the two aqueous phases is maintained.<sup>20–23</sup>

Pertraction could be also used for amino acids separation, the proper carrier being chosen from the above-mentioned extractants (organophosphoric acid, high molecular weight amines or crown-ethers).

Therefore, the aim of these experiments was to analyze the possibility of separating some amino acids of acidic character (L-aspartic acid, L-glutamic acid), basic character (L-histidine, L-lysine, L-arginine) or neutral character (L-glycine, L-tryptophan, L-cysteine, L-alanine) from their mixtures obtained either by fermentation or protein hydrolysis using facilitated pertraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA). For this purpose, the influence of the pH gradient between the aqueous phases, carrier concentration in the liquid membrane and mixing intensity on the efficiency and selectivity of pertraction has been analyzed.

## Materials and method

The experiments have been carried out using pertraction equipment that allows for the creation and easy maintenance of the free liquid membrane. The pertraction cell has been described in previous papers and consists of a U-shaped glass pipe having an inner diameter of 45 mm and a total volume of 400 mL, the volume of each compartment being equal.<sup>20,21</sup>

The aqueous solutions are independently mixed by means of double blade stirrers with 6 mm diameter and 3 mm height, having a rotation speed between  $n = 0$  and  $600 \text{ min}^{-1}$ . In order to reach high diffusional rates through the solvent layer, the organic phase has been mixed with a stirrer of the same design, at a constant rotation speed of  $n = 500 \text{ min}^{-1}$ . The area of mass transfer surface, both for extraction and for re-extraction, was  $1.59 \cdot 10^{-3} \text{ m}^2$ .

The interfaces between the phases remained flat, and hence the interfacial area constant, for the entire rotation speed domain used.

The experiments were carried out in a continuous system, at steady-state conditions relative only to the aqueous phases, and at  $25 \text{ }^\circ\text{C}$ . The aqueous solutions have been separately fed with a volumetric flow rate  $Q = 1.9 \text{ L h}^{-1}$ .

The liquid membrane phase consisted of a solution of  $\gamma = 20 - 100 \text{ g L}^{-1}$  ( $C_C = 0.06 - 0.30 \text{ mol L}^{-1}$ ) D2EHPA (di-(2-ethylhexyl)phosphoric acid) ( $\geq 95 \%$ , Sigma Chemie GmbH) as carrier dissolved in dichloromethane ( $\geq 99 \%$ , Aldrich).

The feed phase contained a mixture solution of L-aspartic acid, L-glutamic acid, L-histidine, L-lysine, L-arginine, L-glycine, L-tryptophan, L-cysteine and L-alanine ( $\geq 99 \%$ , Fluka), the initial concentration of each amino acid being  $c_{i0}^{\text{FI}} = 0.03 \text{ mol L}^{-1}$ . The pK values of the studied amino acids are given in Table 1.<sup>24</sup>

pH of the feed phase varied from 1 to 6, being

Table 1 – pK and  $pH_{\text{isoelectric}}$  values of the studied amino acids at  $25 \text{ }^\circ\text{C}$ <sup>24</sup>

Amino acid	pK <sub>1</sub> (COOH)	pK <sub>2</sub> ( $\alpha\text{-NH}_3^+$ )	pK <sub>3</sub> (R)	$pH_{\text{isoelectric}}$
L-aspartic acid	2.09	9.82	3.87	3.0
L-glutamic acid	2.19	9.66	4.28	3.2
L-cysteine	1.96	8.18	10.30	5.1
L-tryptophan	2.38	9.39	-	5.9
L-glycine	2.35	9.78	-	6.1
L-alanine	2.34	9.87	-	6.1
L-histidine	1.77	9.20	6.20	7.6
L-lysine	2.18	8.95	10.50	9.7
L-arginine	2.02	9.04	12.50	10.8

adjusted at the prescribed values with a solution of 3 % sulfuric acid (Sigma Chemie GmbH). The pH of stripping solution has been adjusted with 3 % hydrochloric acid (Sigma Chemie GmbH) solution in the pH-domain of 1 to 5. The pH-values of the both aqueous phases were determined using two digital pH-meters of HI 213 (Hanna Instruments) type and have been recorded throughout each experiment. Any pH change was noted during the pertraction experiments.

The evolution of pertraction was followed by means of the amino acids initial and final mass flows, and permeability factors. The initial mass flow,  $J_i$ , represents the acid mass flow from the feed

phase to the liquid membrane, while the final (overall) mass flow,  $J_f$ , the acid mass flow from the liquid membrane to the stripping phase. The permeability factor,  $P$ , conveys the capacity of a solute transfer through liquid membrane, and has been defined as the ratio between the final mass flow and the initial mass flow of solute.<sup>20</sup>

For calculating these parameters, the amino acids concentrations in the feed and stripping solutions have been measured by high performance liquid chromatography technique (HPLC) with a HP 1090 liquid chromatography, then the mass balance being used. Therefore, the mass flows have been calculated with the following relationships:

$$J_i = \frac{Q_i^F \cdot (c_{i0}^F - c_i^F)}{A} \quad (1)$$

$$J_f = \frac{Q_i^S \cdot c_i^S}{A} \quad (2)$$

Each experiment has been carried out three or four times under identical conditions. The average value of the measurements was used in the calculations. The maximum experimental error was  $\pm 3.88\%$ .

## Results and discussion

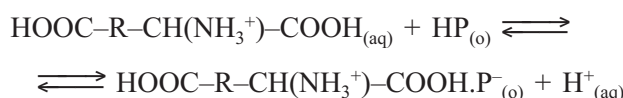
Extraction and transport through liquid membranes is strongly influenced by the pH-gradient between the feed and stripping phases, carrier concentration in liquid membrane, and mixing intensity of the phases. In the case of amino acids pertraction, the influence of the pH gradient between the phases is enhanced by the formation of the ionized forms of amino acids in the aqueous phases and controls both the efficiency of extraction/re-

extraction and the transport rate through the solvent layer.

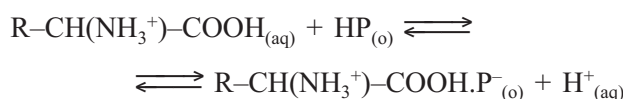
Thus, from Fig. 1 it can be observed that for all studied amino acids the initial mass flows increase with the increase of feed phase pH, reach a maximum value followed by their strong decrease.

The value of the pH corresponding to the maximum initial mass flows is 2 for the acidic amino acids, and 3 for the other amino acids. This influence of the pH value on amino acids mass flows is the consequence of the reactive extraction mechanism of amino acids with D2EHPA, which occurs by means of an interfacial chemical reaction of the ion exchange type controlled by the pH of aqueous phase. According to the previous results,<sup>20</sup> the carrier, D2EHPA, reacts only if the amino acids exist in aqueous solution in their cationic forms (pH of aqueous phase has to be below  $pH_{\text{isoelectric}}$ ). Thus, the interfacial reactions can be described as follows:

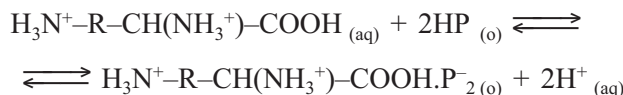
– acidic amino acids



– neutral amino acids



– basic amino acids



where HP is the carrier.

The maximum of mass flow is the result of two opposite phenomena which occur with the pH increase: the increase of the concentration of extractant active form (in the strong acidic pH do-

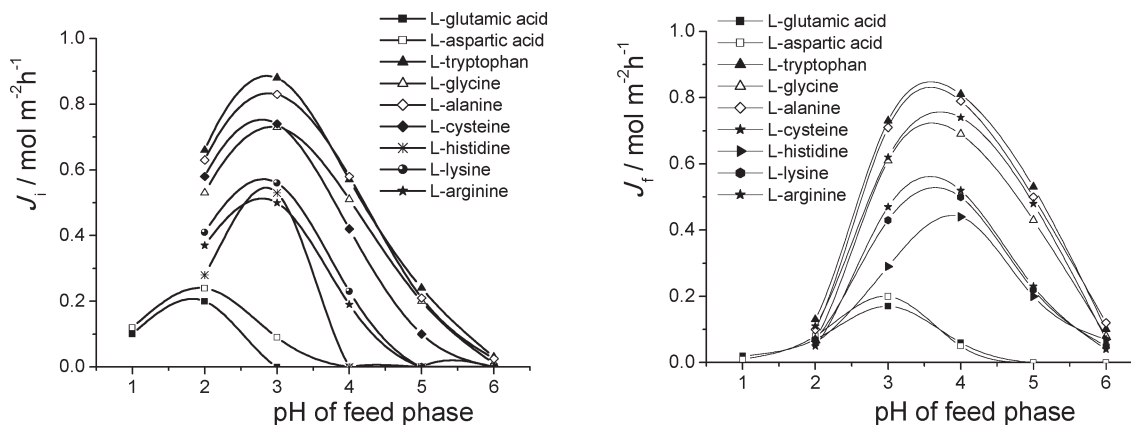


Fig. 1 – Influence of pH value of feed phase on mass flows of amino acids (pH of stripping phase = 2, carrier concentration  $\gamma = 40 \text{ g L}^{-1}$ , rotation speed  $n = 500 \text{ min}^{-1}$ )

main the extractant is protonated and, consequently, becomes unable to react with the amino acid), and the decrease of the total amount of amino acid existing in cationic form. The further increase of the pH value of feed phase leads to the increase of the concentration of the acidic and neutral amino acids zwitterions, and respectively, of the basic amino acids dication-anionic species or zwitterions, thus reducing significantly the initial mass flows of the amino acids (at the isoelectric point the reactive extraction of amino acids becomes impossible<sup>20</sup>).

Unlike the acidic or neutral amino acids, the pertraction of basic amino acids is not possible even if the pH values are lower than those corresponding to their isoelectric points, due to the formation of the dication-anionic species (L-histidine:  $J_i = 0$  for  $\text{pH}_i \geq 4$ , L-lysine:  $J_i = 0$  for  $\text{pH}_i \geq 5$ , L-arginine:  $J_i = 0$  for  $\text{pH}_i \geq 5$ ).

The recorded differences between the initial mass flows of the solutes are probably the result of the different hydrophobicity of the radicals R from the amino acids structures, this being in concordance with the previous conclusions regarding the reactive extraction yields of the same amino acids with D2EHPA.<sup>20</sup>

The final mass flows of amino acids initially increase with pH of the feed phase, owing to their accumulation in the liquid membrane, reaching the maximum values at  $\text{pH}_i$  3 for aspartic and glutamic acids, and respectively, at  $\text{pH}_i$  4 for the rest of amino acids. Because the amino acids are accumulated in the liquid membrane in different proportions, the differences between the final mass flows are rather similar to those between the initial mass flows. The further increase of  $\text{pH}_i$  to the neutral pH domain leads to the decrease of the final mass flows, owing to the change of the direction of pH gradient that controls the direction of solute transfer through the liquid membrane.

For all considered amino acids, the permeability factors strongly increase with the pH increase, becoming higher than 1 for  $\text{pH} \geq 3$ . This variation indicates that the final mass flows become larger than the initial ones, a phenomenon that is possible due to the reextraction of the additional amount of amino acids accumulated into the organic layer.

The increase of the pH-value of the stripping phase causes the reducing of both initial and final mass flows of the amino acids that can be extracted at the prescribed pH of feed phase, as it can be seen from Fig. 2. For example, although at  $\text{pH}_i$  2 all the amino acids are extracted, at  $\text{pH}_i$  4 the initial mass flows of L-aspartic acid, L-glutamic acid and L-histidine are 0 (Fig. 2), for the above presented reasons.

A similar variation has been recorded also for the permeability factors as a function of the pH value of stripping phase (Fig. 3). The maximum values of the permeability factors are reached for the pH value of stripping phase of 1. This result, together with the variations of mass flows discussed above and given in Fig. 2, indicates that by increasing the  $\text{pH}_b$ , the direction of the solutes transport through liquid membrane is inverted, and consequently the amount of the accumulated amino acids inside the solvent layer increases significantly.

According to the Fig. 3, the maximum values of the permeability factors are higher for L-aspartic and L-glutamic acids, owing both to their lower initial mass flows, and to their lower hydrophobicities, which promote the reextraction in the stripping phase.

Another important factor influencing the amino acids pertraction is the carrier concentration inside the liquid membrane. From Fig. 4, obtained at  $\text{pH}_i$  4, and from the similar results for  $\text{pH}_i$  2, it can be observed that the initial and final mass flows of all the amino acids extracted are continuously in-

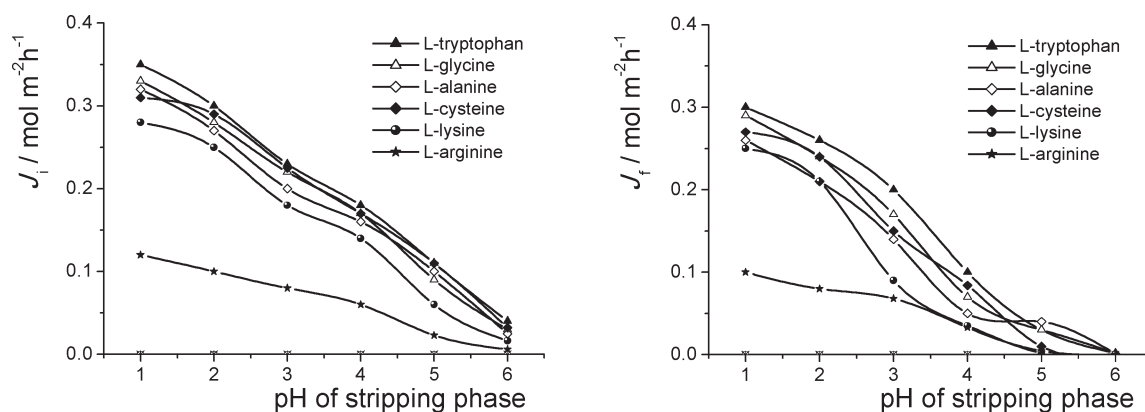


Fig. 2 – Influence of pH-value of stripping phase on mass flows of amino acids ( $\text{pH}$  of feed phase = 4, carrier concentration  $\gamma = 40 \text{ g L}^{-1}$ , rotation speed  $n = 500 \text{ min}^{-1}$ )



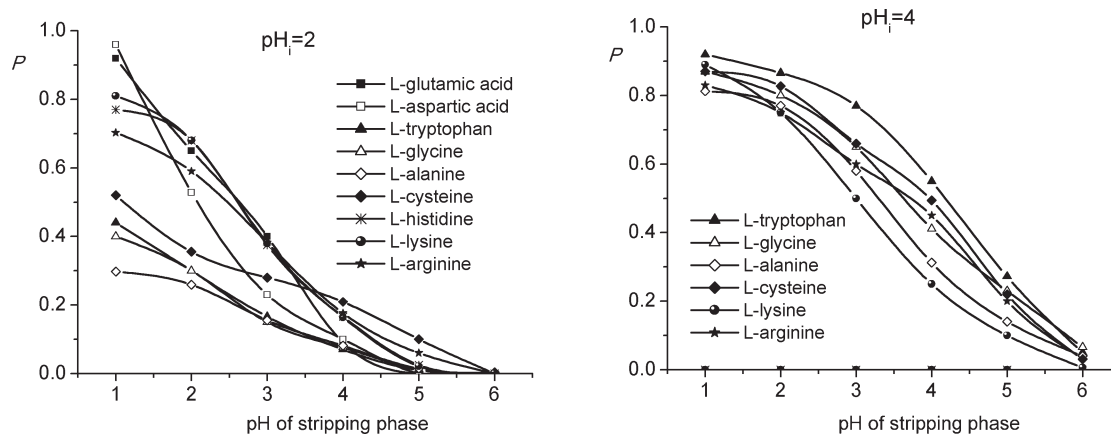


Fig. 3 – Influence of pH-value of stripping phase on permeability factors (carrier concentration  $\gamma = 40 \text{ g L}^{-1}$ , rotation speed  $n = 500 \text{ min}^{-1}$ )

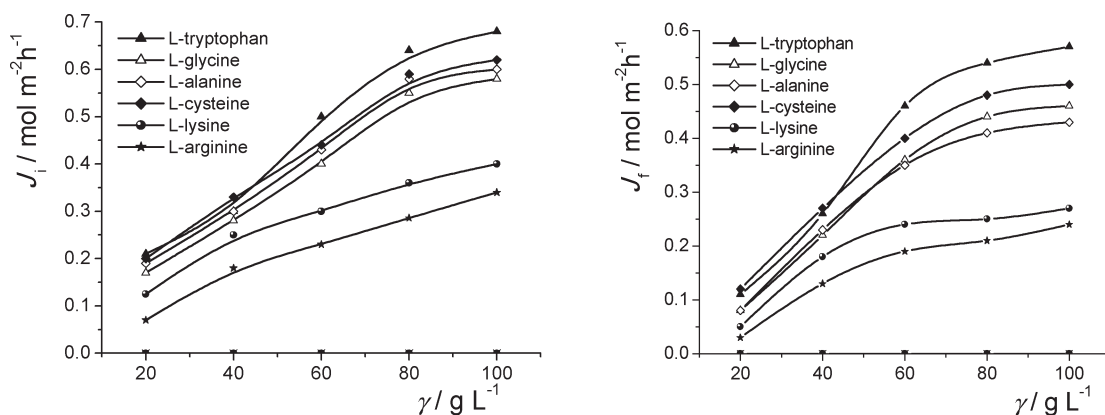


Fig. 4 – Influence of D2EHPA concentration on mass flows of amino acids (pH of feed phase = 4, pH of stripping phase = 2, rotation speed  $n = 500 \text{ min}^{-1}$ )

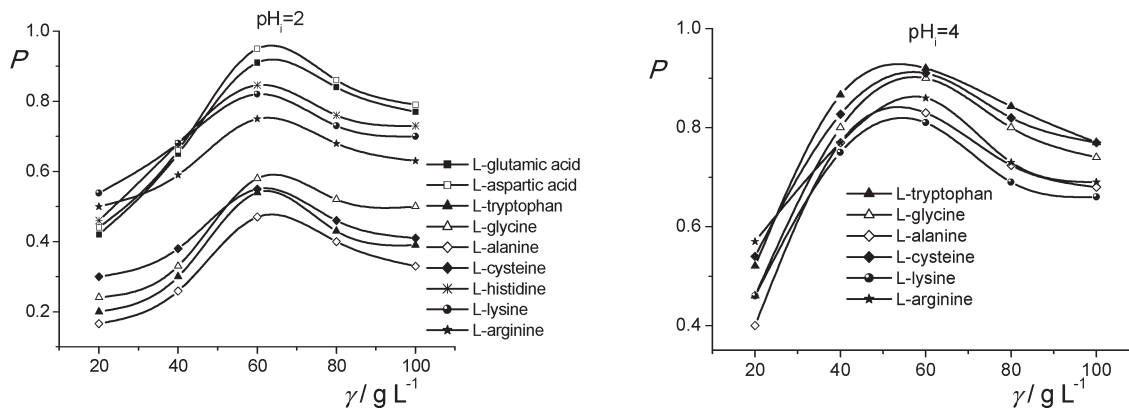


Fig. 5 – Influence of D2EHPA concentration on permeability factors (pH of stripping phase = 2, rotation speed  $n = 500 \text{ min}^{-1}$ )

creased with the increase of D2EHPA concentration in the liquid membrane.

The increase of the amino acids mass flows is the results of the increase of the concentration of one of the reactants which participates at the interfacial reaction in the extraction process, and of the accumulation of the interfacial compounds into the organic layer. The highest values of initial mass

flows have been again recorded for the more hydrophobic amino acids.

The dependences of the permeability factors on the carrier concentration are plotted in Fig. 5 and their shape are different from the ones discussed above. According to these results, the permeability factors initially increase with D2EHPA concentration, reach a maximum value, and then decrease. For

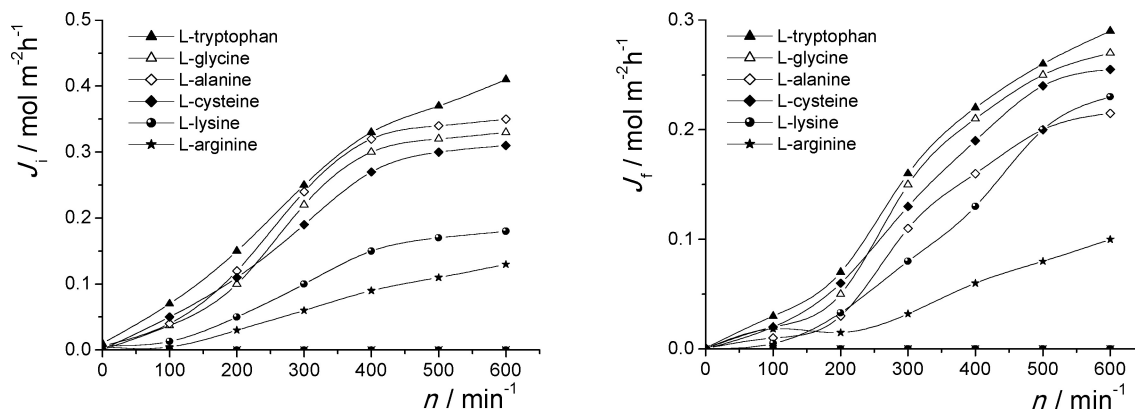


Fig. 6 – Influence of mixing intensity on mass flows of amino acids ( $pH$  of feed phase = 4,  $pH$  of stripping phase = 2, carrier concentration  $\gamma = 40 \text{ g L}^{-1}$ )

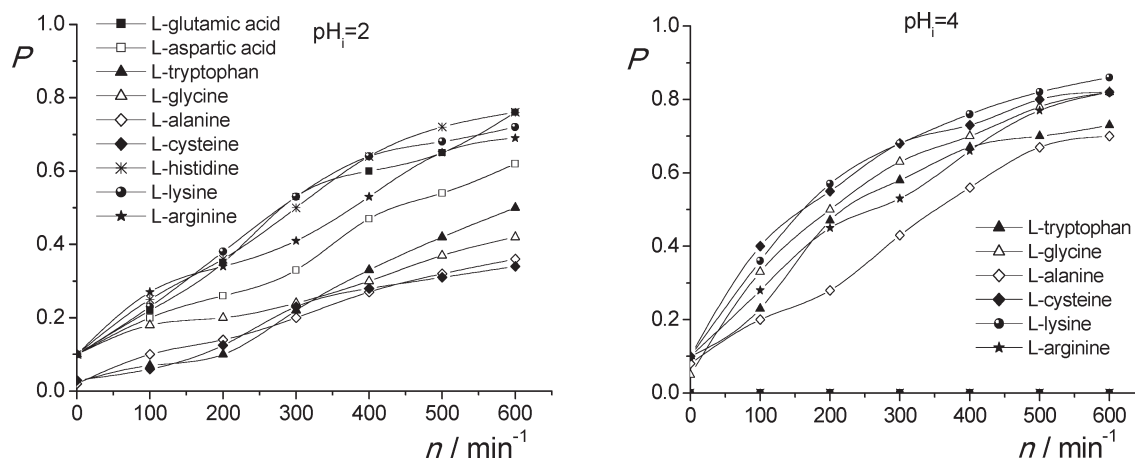


Fig. 7 – Influence of mixing intensity on permeability factors ( $pH$  of stripping phase = 2, carrier concentration  $\gamma = 40 \text{ g L}^{-1}$ )

all the amino acids considered, the maximum of permeability factor corresponds to a carrier concentration of  $\gamma = 60 \text{ g L}^{-1}$ . Because the amino acids do not participate in their free acid form to the reextraction process (they are combined with D2EHPA), the recorded variation could be the result of the kinetic resistance to the reextraction process from the solvent layer to the stripping phase. At carrier concentration over  $\gamma = 60 \text{ g L}^{-1}$ , this resistance becomes superior to that for the extraction process from the feed phase. Therefore, it can be concluded that the pertraction system reaches its maximum capacity of solute transfer at  $\gamma = 60 \text{ g L}^{-1}$  carrier concentration, for the given experimental conditions.

For the above discussed reasons, the highest values of the permeability factors have been reached at  $pH_i$  2 for L-aspartic and L-glutamic acids. If the pertraction is carried out at  $pH_i$  4, the acidic amino acids and L-histidine cannot be extracted from the feed phase.

In all cases, the intensification of the aqueous phases mixing leads to the acceleration of the amino acids mass flows and to the increase of per-

meability factors (Fig. 6). The dependences of amino acids mass flows on rotation speed suggest that the overall separation process could be controlled by the diffusional processes or by the interfacial chemical reactions.

The relative magnitude of these resistances depends on the size of the amino acids molecules. Thus, for the solutes with smaller molecules (L-glycine, L-alanine, L-cysteine, L-aspartic acid, L-lysine, L-glutamic acid), the increase of the mass flows with mixing intensification is more important for rotation speed up to  $n = 500 \text{ min}^{-1}$ , over this level the influence of mixing being diminished due to the amplification of the kinetic resistance. For more voluminous amino acids (L-histidine, L-arginine, L-tryptophan), the mass flows continuously increase with mixing intensification for the entire domain of the rotation speed.

The increase of permeability factors with the rotation speed indicates a stronger influence of mixing on final mass flows, due to the more important resistance to the diffusion through the boundary layer on the stripping phase side (Fig. 7).

According to the above results, by combining the feed phase pH-value, which strongly limits the amino acids transfer to the membrane phase, the pH-value of stripping phase, which controls the rate of the amino acids re-extraction from the liquid membrane and, consequently, their concentration gradients between the two aqueous phases, the carrier concentration, which controls the capacity of liquid membrane to transport the solute, and the mixing intensity, which can selectively diminish the resistance to the diffusion, the selective separation by facilitated pertraction becomes possible for different groups of amino acids with similar acidic properties. Therefore, for pH of feed phase over 5 only L-glycine, L-alanine, L-tryptophan and L-cysteine are pertracted, for pH of feed phase between 4 and 5 these amino acids and L-lysine and L-arginine, for pH of feed phase between 3 and 4 L-histidine can be added to the previous list of pertracted amino acids, and below pH of 3 L-aspartic acid and L-glutamic acid can be also separated. Beside the selectivity, the relative pertraction efficiency of the amino acids from a given group can be enhanced by using the proper experimental conditions, taking into account the studied influences.

## Conclusions

The studies on facilitated pertraction of amino acids of acidic character (L-aspartic acid, L-glutamic acid), basic character (L-histidine, L-lysine, L-arginine) or neutral character (L-glycine, L-tryptophan, L-cysteine, L-alanine) from their mixtures by facilitated pertraction with D2EHPA underlined the major influence of pH gradient between the feed and stripping phases, carrier concentration in organic layer and mixing intensity of aqueous phases.

Therefore, the amino acids can be selectively separated depending on the pH value of feed phase. Due to the formation of the corresponding ionic species, L-aspartic acid, L-glutamic acid and L-histidine cannot be pertracted at pH value over 4, while L-lysine and L-arginine are not separated at pH over 5.

The increase of the carrier concentration inside the liquid membrane leads to the intensification of the initial and final mass flows, the amplitude of this variation depending on the size of the pertracted amino acid molecule.

The permeability factors are positively influenced by the increase of the pH value of feed phase and rotation speed of the stirrers from the aqueous phases, but are negatively affected by the increase of the stripping phase pH. The analysis of the effect of the carrier mass concentration indicated that the permeability factors reached a maximum level for  $\gamma = 60 \text{ g L}^{-1}$  D2EHPA.

## Notations

- $A$  – surface area,  $\text{m}^2$   
 $C_C$  – carrier concentration,  $\text{mol L}^{-1}$   
 $c_{i0}^{\text{FI}}$  – input concentration of amino acids on the feed side,  $\text{mol L}^{-1}$   
 $c_i^{\text{F}}$  – measured concentration of amino acids on the feed side,  $\text{mol L}^{-1}$   
 $c_i^{\text{S}}$  – measured concentration of amino acids on the stripping side,  $\text{mol L}^{-1}$   
 $J_i$  – initial mass flow of amino acid,  $\text{mol m}^2 \text{h}^{-1}$   
 $J_f$  – final (overall) mass flow of amino acid,  $\text{mol m}^2 \text{h}^{-1}$   
 $n$  – rotation speed,  $\text{min}^{-1}$   
 $P$  – permeability factor, –  
 $\text{pH}_i$  – pH value of the feed phase, –  
 $\text{pH}_f$  – pH value of the stripping phase, –  
 $Q_i^{\text{F}}$  – volumetric flow rate of the feed phase,  $\text{L h}^{-1}$   
 $Q_i^{\text{S}}$  – volumetric flow rate on the stripping phase,  $\text{L h}^{-1}$   
 $\gamma$  – carrier mass concentration,  $\text{g L}^{-1}$

## Subscript

- aq – aqueous phase  
 o – organic phase

## References

- Liu, Y. S., Dai, Y. Y., *Sep. Sci. Technol.* **38** (2003) 1217.
- Caşcaval, D., Oniscu, C., Galaction, A. I., *Biochemical Engineering and Biotechnology. 3. Bioseparations, Performantica, Iasi*, 2004, pp. 131–134.
- Kelly, N. A., Lukhezo, M., Reuben, B. G., Dunne, L. J., Verrall, M. S., *J. Chem. Technol. Biotechnol.* **72** (1998) 347.
- Liu, Y. S., Dai, Y. Y., Wang, H. D., *Sep. Sci. Technol.* **34** (1999) 2165.
- Caşcaval, D., Oniscu, C., Galaction, A. I., *Biochem. Eng. J.* **7** (2001) 171.
- Juon, R.-S., Wang, Y.-Y., *J. Membr. Sci.* **207** (2002) 241.
- Lin, S. H., Chen, C. N., Juang, R. S., *J. Chem. Technol. Biotechnol.* **81** (2006) 406.
- Lin, S.-H., Chen, C.-N., *J. Membr. Sci.* **280** (2006) 771.
- Rehm, H. J., Reed, G., *Biotechnology. 3. VCH, Weinheim*, 1993, pp. 87.
- Schuegerl, K., *Solvent Extraction in Biotechnology*. Springer, Berlin, 1994, pp. 101–103.
- Tan, B., Luo, G., Wang, J., *Sep. Purif. Technol.* **53** (2007) 330.
- Deblay, P., Minier, M., Renon, H., *Biotechnol. Bioeng.* **35** (1990) 123.
- Mutihac, L., Mutihac, R., Constantinescu, T., Luca, C., *J. Incl. Phenom. Macro. Chem.* **17** (1993) 45.
- Teramoto, M., Yamashiro, T., Inoue, A., Yamamoto, A., Matsuyama, H., Miyake, Y., *J. Membr. Sci.* **58** (1991) 11.
- Hong, S. A., Choi, H. J., Nam, S. W., *J. Membr. Sci.* **70** (1992) 225.
- Eyal, A. M., Bressler, E., *Biotechnol. Bioeng.* **41** (1993) 287.
- Cardoso, M. M., Viegas, R. M. C., Crespo, J. P. S. G., *J. Membr. Sci.* **156** (1999) 303.

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18. Saikia, B., Dutta, N. N., Dass, N. N., *J. Membr. Sci.* **225** (2003) 1.
19. Hossain, M. M., *Sep. Purif. Technol.* **42** (2005) 227.
20. Cascaval, D., Oniscu, C., Cascaval, C., *Biochem. Eng. J.* **5** (2000) 45.
21. Cascaval, D., Galaction, A. I., Oniscu, C., *Sep. Sci. Technol.* **39** (2004) 1907.
22. Noble, R. D., Stern, S. A. (Eds.). *Membrane Separations Technology. Principles and Applications*, Elsevier, London, 1995, pp. 104.
23. Belafti-Bako, K., Gubicza, L., Mulder, M. (Eds.), *Integration of Membrane Processes into Bioconversions*, Kluwer Academic, New York, 2000, pp. 118–121.
24. Lehninger, A. L., *Biochemistry*, vol. 1, Worth, New York, 1975, pp. 156.