Roumanian Biotechnological Letters *Vol. 11, No. 4, 2006, pp. 2791-2797*

ORIGINAL PAPER

Extraction and transport of aspartic and glutamic acids through liquid membranes

Received for publication, May 20, 2006
Accepted, July 10, 2006

ALEXANDRA CRISTINA BLAGA[1)], ANCA-IRINA GALACTION[2)], DAN CAŞCAVAL[1)*]

[1)] Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering, Dept. of Biochemical Engineering, 71 D. Mangeron Avenue, 700050 Iasi, Romania, email: dancasca@ch.tuiasi.ro

[2)] University of Medicine and Pharmacy, Faculty of Medical Bioengineering, Dept. of Biotechnology, 16 University Street, 700115 Iasi, Romania, email: galact@from.ro

** the corresponding author*

Abstract

Pertraction (extraction and transport through liquid membranes) can be a high-capacity and high-selective technique for separating the polar organic compounds from dilute solutions. In this context, the study on facilitated pertraction of aspartic and glutamic acids with D2EHPA as carrier underlined the significant influences of the pH-gradient between the aqueous phases, carrier concentration and mixing intensity. The maximum mass flows of the two amino acids through liquid membrane can be reached at the pH-value of the feed phase of 2 for aspartic acid and 2.5 for glutamic acid, pH-value of the final aqueous solution of 1, D2EHPA concentration in solvent layer of 60 g/l, using an intense mixing of the aqueous phases.

Keywords: amino acids, aspartic acid, glutamic acid, D2EHPA, pertraction, liquid membrane, carrier, mass flow, permeability factor.

Introduction

The idea of performing separations in three phase-systems with a liquid membrane (LM) is relatively new, the first paper on liquid membrane being published in 1967. Pertraction or separation of substances through liquid membrane represents a combination in time and space of two separation processes: extraction and re-extraction, both using the same organic phase as an intermediary carrier for selective transfer between the feed and stripping solutions [1]. If two miscible fluids are separated by a liquid immiscible with them, but which allows the solute mass transport between the fluids, a liquid membrane is formed. The invention of new methods of contacting of three liquid phases and the new types of liquid membranes led to a significant progress of this domain in the last thirty years [2].

The general term for mass transport through nonporous membranes under the driving force of a chemical gradient type is *permeation*. If the permeates from the liquid feed are desorbed on the downstream side of the membrane into the vapour phase, this operation is called *pervaporation*. If the permeates from the liquid feed (donor solution) are transported through a nonporous membrane (polymeric or liquid) and are finally dissolved into another liquid phase (striping solution or receiving solution), this operation is called *pertraction*. The term has been derived from the Latin *per-trahere* and was introduced by Schlosser and Kossaczky in 1975 [3].

Due to its obvious advantages comparative to the solid membranes and liquid-liquid extraction, liquid pertraction attracted the attention of many scientist and engineers. At present there are more than 200 important research teams around the world exploring this new separation technique [4].

The methods used for obtaining and maintaining these membranes are difficult. Commonly, liquid membranes can be obtained either by emulsification (*liquid membrane extraction*) when its stability is poor, by including the solvent in a hydrophobic porous polymer matrix (*supported liquid membrane extraction*), or by means of special equipments (*free liquid membrane*) [2,5,6].

By comparing the extraction using liquid membranes with conventional liquid-liquid extraction, the advantages are as follows [2,4,5]:

- the quantity of solvent used is small, because of its continuous regeneration
- the loss of solvent during extraction and transport process is reduced
- as long as the pH gradient between the two aqueous phases is maintained, there is the possibility of solute transport against its concentration gradient
- higher diffusion coefficient of permeates in liquid membranes when compared with polymeric membranes
- energy consumption is very low.

Pertraction has been proposed as a convenient, economical, and effective method for the selective separation and concentration of various species such as phenolic derivatives, metal ions, amino acids, antibiotics etc. [7-9]. Liquid membranes have begun to be applied also at industrial scale [9].

Amino acids are valuable chemical products. Their principal commercial applications are in human foods, as veterinary additives, in the pharmaceutical fields, and cosmetics. Recent advances in fermentation technology led to more affordable production of amino acids. Therefore, it has been recorded an increasing interest to extending the use of amino acids as raw materials in the production of various industrial chemicals. Some of the the newest examples of the chemicals that can be derived from amino acids include amino acid-based surfactants used as oil gelating agents to recover effluent oil in seas and rivers, and poly(amino acids), used as biodegradable plastics manufacture [10,11].

The amino acids can be obtained by fermentation or by protein hydrolysis, but their separation from broths or protein hydrolysates is rather difficult [12]. In the last decades there has been observed the continuous and increasing interest in developing the techniques that can improve the selectivity and the yield of downstream processing steps for the separation and purification of amino acids [11]. The separation techniques currently applied for removal and purification of amino acids from dilute aqueous solutions typically employ the ionic exchange, crystallization at the isoelectric point or chromatography [12,13].

Aspartic and glutamic acid are acidic amino acids with important applications in medicine, food and cosmetics. Their chemical structures contain one aminic group and two carboxylic groups. The degree of ionization of the three functional groups on these amino acids is governed by the association-dissociation equilibrium in solution controlled by the pH-value. The values of equilibrium constants, k_1, k_2, and k_3 correspond to the ionization of the carboxylic and aminic groups, respectively, being given in Table 1 in logarithm form.

Table 1. The pK values of extracted amino acids [12, 13].

Amino acid	**pK_1**	**pK_2**	**pK_3**	**pH_i**
Aspartic acid	2.09	3.87	9.82	2.77
Glutamic acid	2.19	4.28	9.66	3.2

These properties make amino acids to be hydrophilic at all pH-values and, thus, complicate their recovery. Generally, the amino acids have lower solubility in conventional organic solvents, their physical extraction being impossible [5,8,11-13]. The liquid-liquid extraction of amino acids becomes possible only by adding extractants into the organic phase, namely derivatives of phosphoric acid, high molecular weight amines or crown-ethers types [12-18]. These extractants recover the amino acids by means of an interfacial reaction with the formation of an ion pair complex that is solubilized into the organic phase. As it was above mentioned, a development of reactive extraction is the *facilitated pertraction* or extraction and transport through liquid membranes. This technique uses a carrier dissolved in the solvent layer that separates the two aqueous solutions: the feed and the striping solutions. In the case of amino acids pertraction, the proper carrier could be of phosphoric acid, high molecular weight amines or crown-ethers types.

In this context, the aim of the experiments is to analyze the possibility to separation the amino acids from mixtures obtained either by fermentation or protein hydrolysis using the facilitated pertraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA). The initial studies are focused on the individual and selective pertraction of aspartic and glutamic acids.

In this paper the results obtained by studying the individual pertraction of aspartic and glutamic acids are presented.

Materials and method

The experiments have been carried out using a patented pertraction equipment of U-type cell, which allows obtaining and easily maintaining the free liquid membrane [19]. The pertraction cell has an inner diameter of 45 mm and a total volume of 400 ml, the volume of each compartment being equal.

The liquid membrane phase consists of a solution of 20 - 100 g/l D2EHPA as carrier dissolved in dichloromethane. The feed phase contains a initial solution of 3 g/l aspartic or glutamic acid.

The pH of the feed phase varied from 1 to 4, being adjusted at the prescribed values with a solution of 4% H_2SO_4. The pH of stripping solution has been adjusted with 4% HCl solution in the pH-domain of 1 to 5. The pH values were determined using a digital pH-meter of Consort C831 type.

In the experiments, the following materials have been used: dichloromethane as solvent, D2EHPA (di-(2-ethylhexyl) phosphoric acid) ≥ 95% (Sigma Chemie GmbH) as carrier, glutamic acid (≥99%) (Fluka), aspartic acid, (≥99%) (Fluka), H_2SO_4 and HCl (Sigma Chemie GmbH).

The aqueous solutions are independently mixed by means of double blades stirrers with 6 mm diameter and 3 mm height, having a rotation speed between 0 and 600 rpm. In order to reach high diffusional rates through the solvent layer, the organic phase has been mixed with one stirrer of the same design, at a constant rotation speed of 500 rpm. The area of mass transfer surface, both for extraction and for re-extraction, was of 1.59×10^{-3} m^2. At this mixing intensity level, the interfaces between the phases remained flat, and, consequently, the interfacial area constant [20].

The experiments have been carried out in a continuous system, at the steady state conditions for aqueous solutions, these solutions being separately fed with a volumetric flow of 1.875 l/h. The evolution of pertraction was followed by means of the amino acid mass flows and permeability factors through liquid membrane. The initial mass flows, n_i, represents the flux of amino acid transferred from the feed phase to the solver layer, and the final mass

flow, n_f, also called overall mass flow, represents the flux of amino acid transferred from the organic solvent to the striping phase. The permeability factor, P, is a measure of the capacity to transport the solute through the liquid membrane, being defined as the ratio between the final mass flow and the initial mass flow of amino acid:

$$P = \frac{n_f}{n_i}$$

For calculating these parameters, the amino acids concentration 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.

Results and discussion

Generally, pertraction is strongly influenced by the pH-gradient between the aqueous phases, carrier concentration in liquid membrane, and phases mixing intensity. For the pertraction of amino acid, the pH gradient between the feed and striping solutions induces a significant influence both on the efficiency of extraction and re-extraction and on the transport rate through the solvent layer.

As it was indicated in Figure 1, for both amino acids, the initial mass flows increase with the increase of feed phase pH, reach a maximum value at pH = 2.5 for glutamic acid and at pH = 2 for aspartic acid, followed by their strong decrease.

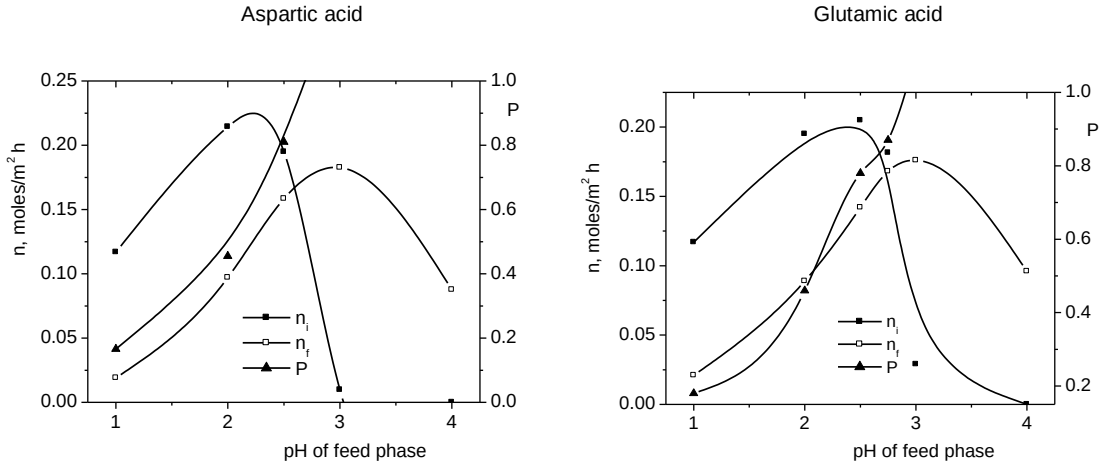

Figure 1. Variation of the amino acids mass flows and permeability factor in function of the pH-values of the feed phase (pH of stripping phase = 2, D2EHPA concentration = 40 g/l, rotation speed = 500 rpm).

This evolution is due to the reactive extraction mechanism of amino acids with D2EHPA, which occurs by means of an interfacial chemical reaction of ionic exchange type controlled by the pH of aqueous phase. The carrier, D2EHPA, reacts only if the amino acids exist in aqueous solution in their cationic form (pH of aqueous phase has to be below pK_i), the general expression of the chemical reaction being as follows:

$HOOC\text{-}R\text{-}CH(NH_3^+)\text{-}COOH_{(aq)} + HP_{(o)} \rightleftharpoons HOOC\text{-}R\text{-}CH(NH_3^+)\text{-}COOH.P^-_{(o)} + H^+_{(aq)}$

where HP is the carrier [13].

The maximum of mass flows is the result of two opposite phenomena: the increase of the concentration of extractant active form, which is able to react with the amino acid, and the decrease of the total amount of amino acid existing in cationic form. The supplementary increase of pH-value of feed phase to the isoelectric point determines the increase of

zwitterions concentration, that significantly reducing the initial mass flows of the amino acids (at the isoelectric point the reactive extraction of amino acids becomes impossible [13]). Due to its lower isoelectric point, this effect is more pronounced for aspartic acid comparatively with glutamic acid.

The final mass flows of amino acids initially increase with pH, because of their accumulation in the liquid membrane. The final mass flows reach a maximum value at pH = 3 for both amino acids. The further increase of pH leads to the decrease of the final mass flows, owing to the change of the sense of pH-gradient which controls the direction of solute transfer through liquid membrane.

The permeability factor strongly increases with the pH increase, becoming higher than 1 for pH ≥ 3. This variation indicates that the final mass flows become superior to the initial ones, phenomena that is possible due to the re-extraction of the supplementary amount of amino acids accumulated into the organic layer.

The increase of the pH-value of the stripping phase determines the reducing of both initial and final mass flows, the same evolution being registered for the permeability factor, as it can be seen from Figure 2. The maximum values of the permeability factors, which tend to 1, are reached for the pH-value of stripping phase of 1 and optimum value of pH for the feed phase (2 for the pertraction of aspartic acid, respectively 2.5 for the pertraction of glutamic acid).

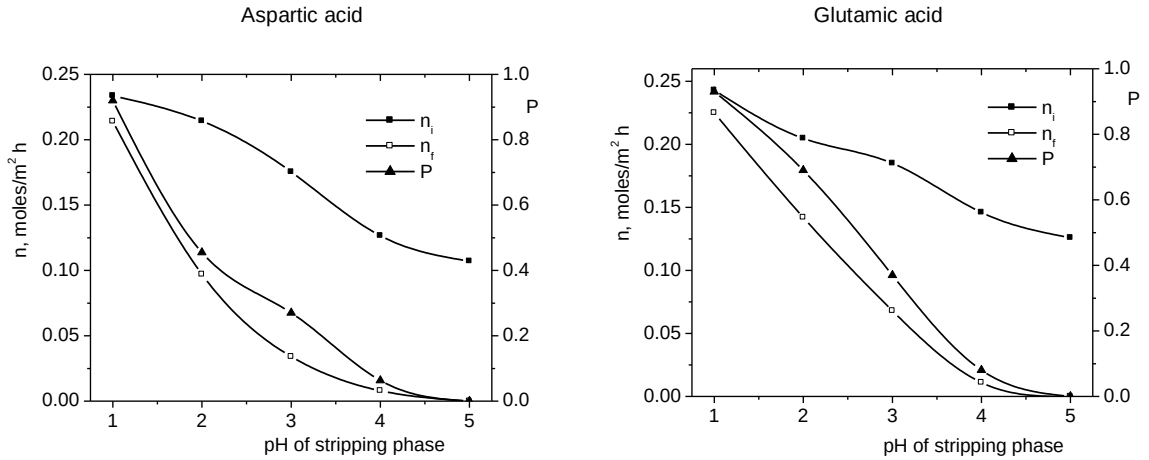

Figure 2. Variation of the amino acids mass flows and permeability factor in function of the pH-values of the stripping phase Variation of the amino acids mass flows and permeability factor in function of the pH-values of the feed phase (pH of feed phase = 2 for aspartic acid, respectively 2.5 for glutamic acid, D2EHPA concentration = 40 g/l, rotation speed = 500 rpm).

Another important factor influencing the amino acids pertraction is the carrier concentration inside the liquid membrane. From Figure 3 it can be observed that the initial and final mass flows of amino acids are continuously intensified with the increase of D2EHPA concentration to 60 g/l, due to the increase of the concentration of one of the reactants and, consequently, to the accumulation of the interfacial compound into the organic layer. Over this level, the increase of mass flows becomes slower, this variation being more accentuated for the final mass flows.

According to these results, the permeability factors initially increase with D2EHPA concentration, reach a maximum value, decreasing then. For both amino acids, the maximum level of permeability factor corresponds to a carrier concentration of 60 g/l. Therefore, it can

be concluded that the pertraction system reaches its maximum capacity of solute transfer at 60 g/l carrier concentration, for the given experimental conditions.

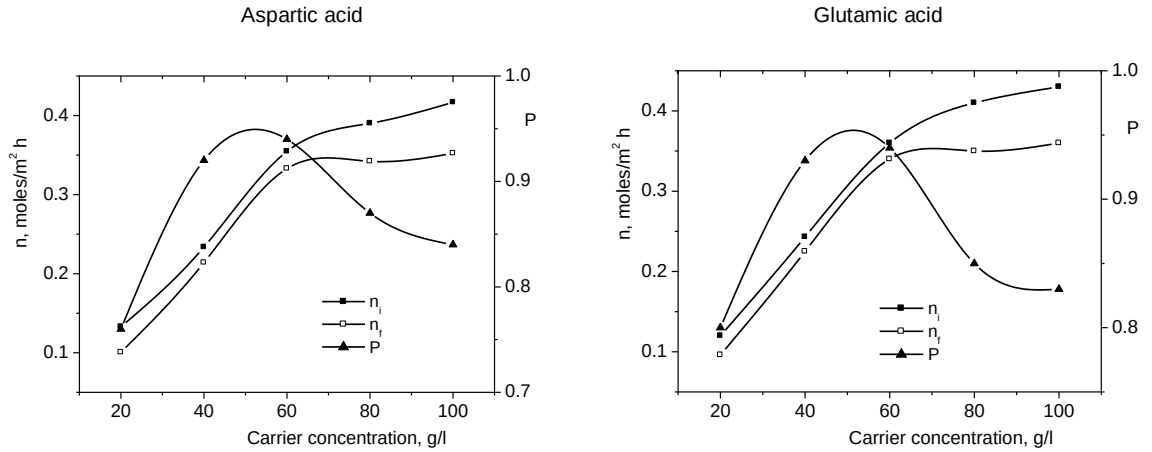

Figure 3. Variation of the amino acids mass flows and permeability factor in function of the carrier concentration (pH of feed phase = 2 for aspartic acid, respectively 2.5 for glutamic acid, pH of stripping phase = 1, rotation speed = 500 rpm).

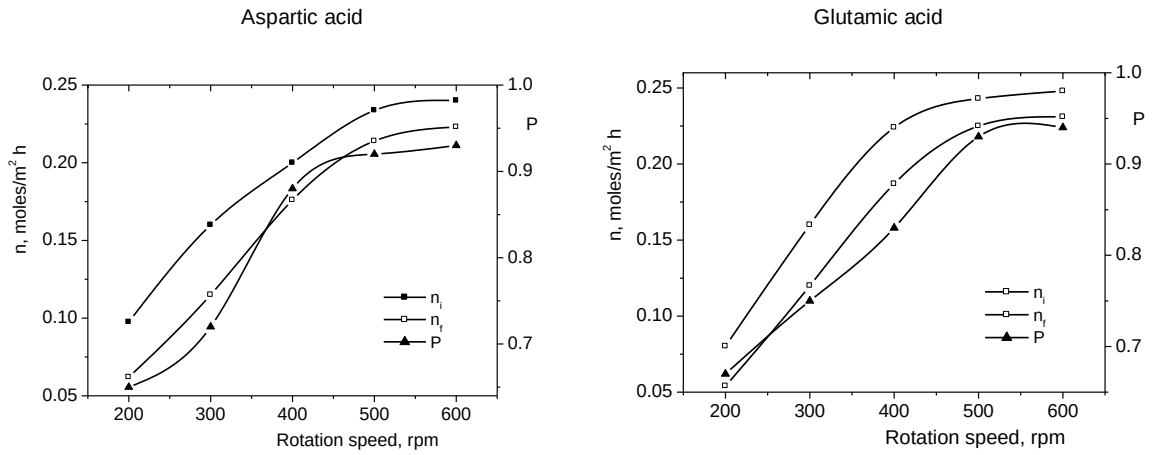

Figure 4. Variation of the amino acids mass flows and permeability factor in function of the rotation speed (pH of feed phase = 2 for aspartic acid, respectively 2.5 for glutamic acid, pH of stripping phase = 1, D2EHPA concentration = 40 g/l).

The dependences of amino acids mass flows on rotation speed, plotted in Figure 4, suggest that the overall separation process could be controlled by the diffusional processes or interfacial chemical reactions. The mixing intensification of the two aqueous phases induces the enhancement of the initial and final mass flows for both amino acids, owing to the diminution of resistance to the diffusion through the interfacial boundary layers, this evolution being recorded for the permeability factor too. The recorded influence is more important for rotation speed values below 500 rpm, over this level the kinetic resistance becoming the limiting step.

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

Conclusions

The studies on facilitated pertraction of two acidic amino acids (aspartic acid, glutamic acid) through a liquid membrane of dichloromethane and D2EHPA as a carrier underlined the major influence of pH-gradient between the feed and striping phases, carrier concentration in organic layer and mixing intensity of aqueous phases.

For reaching the maximum values of amino acids mass flows, the separation must be carried out at the pH of feed phase of 2 for aspartic acid and 2.5 for glutamic acid, pH of stripping phase of 1, D2EHPA concentrations in the liquid membrane of 60 g/l, and at an intense mixing of the two aqueous phases.

References

1. L. BOYADZHIEV, Z. LAZAROVA, *Membrane Separations Technology. Principles and Applications*, Elsevier, Amsterdam , 1995, pp 283
2. S. SCHLOSSER, *Integration of membrane processes into bioconversions*, Kluwer Academic, Plenum Publishers, New York, 2000, pp 73
3. S. SCHLOSSER, E. KOSSACZKY, *Proceed. CHISA Congress*, Prague, 1975
4. L. BOYADZHIEV, *Sep. Sci. Technol.*, **25**, 187-205 (1990)
5. D. CASCAVAL, C. ONISCU, ANCA-IRINA GALACTION, *Inginerie biochimica si biotehnologie. 3. Procese de separare*, Performantica, Iasi, 2004, pp 100
6. J. D. CLARK, B. HAN, A. S. BROWN, S. R. WICKRAMASINGHE, *Sep. Purif. Technol.*, **42**, 201-211 (2004)
7. M. MA, A.-H. SHEN, X.-B. LUO, X.-L. ZHU, B. CHEN, S.-Z. YAO, *Solv. Extr. Ion Exch.*, **22**, 321-330 (2004)
8. K. SCHUGERL, *Adv. Biochem. Eng. Biotechnol.*, **92**, 1-48 (2005)
9. R. KLAASSEN, P.H. FERON, A.E. JANSEN, *Chem. Eng. Res. Des.*, **83**, 234-246 (2005)
10. K. ARAKI, T. OZEKI, *Kirk-Othmer Encyclopedia of Chemical Technology*, Wiley, New York, 1992, pp. 504
11. Y.-S. LIU, Y.-Y. DAI, *Sep. Sci. Technol.*, **38**, 1217-1228 (2003)
12. A. SAMADI, S. M. HUSSON, *Sep. Sci. Technol.*, **38**, 2837-2850 (2003)
13. D. CASCAVAL, C. ONISCU, A.I. GALACTION, *Biochem. Eng. J.*, **7,** 171-176 (2001)
14. C. TRAVIS, S. M. HUSSON, *Sep. Sci. Technol.*, **36**, 849-866 (2001)
15. M. TERAMOTO, T. YAMASHIRO, A. INOUE, A. YAMAMOTO, H. MATSUYAMA, Y. MIYAKE, *J. Membr. Sci.*, **58,** 11-32 (1991)
16. N. A. KELLY, M. LUKHEZO, B.G. REUBEN, L.J. DUNNE, M.S. VERRALL, *J. Chem. Technol. Biotechnol.*, **72**, 347-355 (1998)
17. T. NATO, T. OHTAKE, M. MATSUMOTO, D. KITAYAMA, F. HORI, F. NAKASHIO, *J. Chem. Eng. Japan*, **24,** 20-24 (1991)
18. M. KOZBIAL, M. PIETRASZKIEWICZ, O. PIETRASZKIEWICZ, *J. Incl. Mol. Recogn. Chem.*, **30**, 69-77 (1998)
19. D. CASCAVAL, C. ONISCU, *Patent RO 119690 B1*, 2005.
20. A.I. GALACTION, A.C. BLAGA, DAN CASCAVAL, E. FOLESCU, *Rev. Med. Chirurg.*, **4**, 895-898 (2005)