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Selective separation of aminoacids mixture by reactive extraction and pertraction

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Abstract. Separation of some amino acids from their mixture obtained either by fermentation or protein hydrolysis by reactive extraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) indicated the possibility of the amino acids selective separation as a function of the pH value of aqueous solution and the acidic or basic character of each amino acid. Thus, using multistage extraction, the total separation of the following amino acids groups has been performed: neutral amino acids (l-glycine, l-alanine, l-tryptophan) at pH 5–5.5 (nine extraction stages), basic amino acids (l-lysine, l-arginine) and l-cysteine at pH 4–4.5 (ten extraction stages), l-histidine at pH 3–3.5 (five extraction stages), and acidic amino acids. Further, in order to reduce the number of stages required for an efficient separation and, therefore, the corresponding energy and material consumption, the study on facilitated pertraction of these amino acids from their mixtures using di-(2-ethylhexyl) phosphoric acid (D2EHPA) as carrier was performed.

Keywords: liquid membrane, carrier, mass flow, selectivity, amino acids

1. Introduction

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. Consequently, the interest in developing the techniques that can improve the selectivity and the yield of downstream processes for the separation and purification of amino acids increased significantly in the last decades [1], [2]. The separation techniques currently applied for removal and purification of amino acids from dilute aqueous solutions consist generally in ionic exchange, crystallization at the isoelectric point or chromatography [2]. However, these techniques are rather difficult to be transposed at larger scale, thus affecting the production of amino acids and increasing the cost of the applied technology.

Amino acids dissociate in aqueous solutions, forming characteristic ionic species depending on the solution pH-value. These properties make amino acids to be hydrophilic at any pH-value. For this reason, the amino acids solubility in organic solvents is low, their physical extraction being practically impossible. The liquid-liquid extraction of amino acids is possible only by using the reactive extraction technique with extractants of organophosphoric acid derivatives [2], high molecular weight amines [3], [4], or crown-ethers types [4]-[6].

As the application of biotechnology has progressed much in recent years, demands for more sophisticated separation methods have increased. The liquid membrane process is known as a novel and effective method for selective separations and concentration of various species from dilute solutions including biologically important compounds: organic acids, antibiotics and amino acids. Also, this technique avoids the use of multistage extraction for selective separation of the mixture [7].

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In this article, the separation of 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) by reactive extraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) is explored experimentally and the results discussed. Using the experimental data of the study on the individual reactive extraction, the selective separation of the considered amino acids from a mixture by reactive extraction and facilitated pertraction has been analyzed.

2. Materials and Methods

The experiments have been carried out in two steps. In the first step, the selective separation of amino acids from a mixture by reactive extraction has been studied. For this purpose, an extraction column with vibratory mixing has been used, this laboratory equipment being described in detail in previous papers [8]. Each amino acid had an initial concentration in the mixture of 0.015 M. The extraction degree has been calculated by means of the amino acid concentrations in the initial solution and in the raffinate.

In the second step of the experiments, the selective separation of amino acids from a mixture by facilitated pertraction has been studied. 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 [9]. The liquid membrane phase consisted of a solution of 20 –100 g L^{-1} D2EHPA (95 %, Sigma Chemie GmbH) as carrier dissolved in dichloromethane (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 (99 %, Fluka), the initial concentration of each amino acid being 0.03 mol L^{-1}. The pH of the feed phase varied from 1 to 6, being 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. The evolution of pertraction was followed by means of the amino acids initial and final mass flows, and permeability factors. 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. The amino acids concentrations have been measured by high performance liquid chromatography (HPLC) (HP 1090 liquid chromatography).

3. Results and Discussion

The studies on the reactive extraction of individual amino acids indicated that the reactive extraction of amino acids with D2EHPA is possible only if the amino acids exist in aqueous solution in their cationic forms (pH of aqueous phase has to be below the isoeletric point). Thus, the interfacial reactions can be described as follows (HP symbolizes D2EHPA) [10]:

acidic amino acids

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

neutral amino acids

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

basic amino acids

$$H_3N^+\text{-}R\text{-}CH(NH_3^+)\text{-}COOH_{(aq)} + 2HP_{(o)} \rightleftharpoons H_3N^+\text{-}R\text{-}CH(NH_3^+)\text{-}COOH.P^-_{2\ (o)} + 2H^+_{(aq)}$$

On the basis of the difference between the pH-values corresponding to their isoelectric points, the amino acids mixtures (containing amino acids of acidic character: l-aspartic acid, l-glutamic acid, basic character: l-histidine, l-lysine, l-arginine, and neutral one: l-glycine, l-tryptophan, l-cysteine, l-alanine) were selectively separated either on individual amino acids or groups of amino acids with similar physico-chemical properties [10]. Using these data, a process flow sheet for the selective separation of the amino acids by reactive extraction with D2EHPA has been elaborated and applied (Fig. 1).

However, due to the rather low extraction degrees of each group of amino acids at the required pH-values for selective separation, for total recovery of the considered amino acids a multistage extraction has been used (table 1).

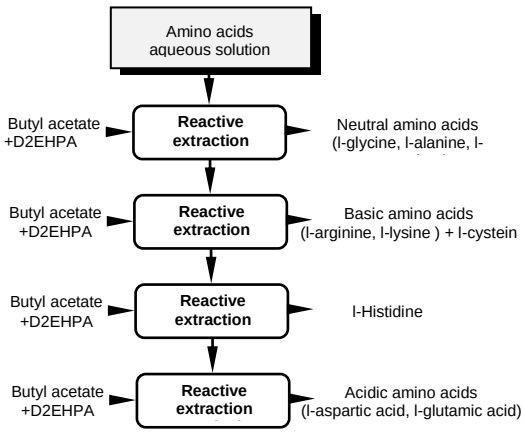

Fig. 1: Operation chart for selective extraction of amino acids with D2EHPA.

Table 1. The experimental conditions for the reactive extraction and separation of amino acids from a mixture

Extracted amino acids	pH domain	One stage extraction degree (% w/w)	Number of extraction stages
Neutral amino acids	5–5.5	20-25	9
Acidic amino acids	2–2.5	60-63	3
Basic amino acids	4–4.5	18-25	10

In order to reduce the number of extraction stages, in the second part of the study, the amino acids selective extraction by pertraction was performed. 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. The influence of the pH-gradient between the aqueous phases was amplified by the formation of the ionized forms of amino acids in the aqueous phases and controlled both the efficiency of extraction and stripping, and the transport rate through the solvent layer.

Considering the aqueous solution containing the mixture of the nine amino acids mentioned before, the shapes of the plotted dependence between their mass flows and pH of the feed phase were similar and indicated that it is possible to reach the maximum value of each amino acid mass flow at a specific pH_S (Fig. 2). For the initial mass flows, the values of the pH_F corresponding to their maximum were 2 for the acidic amino acids and 3 for the other ones. According to the interfacial mechanism of amino acids reactive extraction with D2EHPA, this influence of the pH_F is the results of two phenomena with negative effects on efficiency of extraction into the membrane phase: the protonation of carrier at strong acidic pH_F-domain, which becomes unable to react with the amino acid, and the decrease of the total amount of cationic species of amino acids at higher pH_F [11].

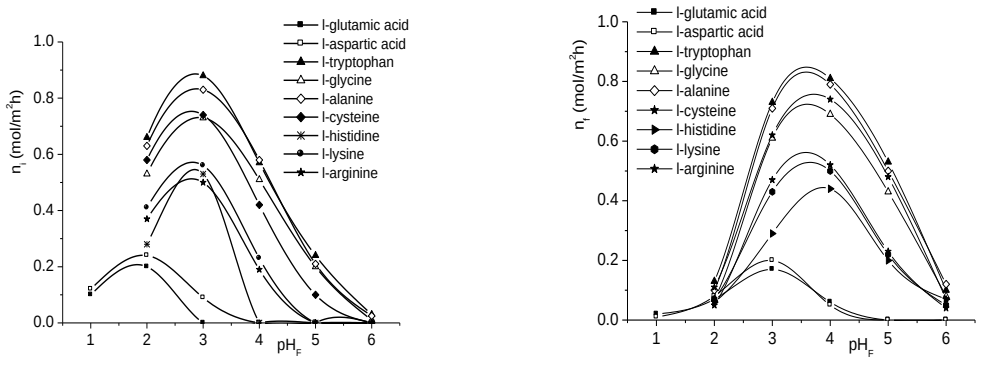

Fig. 2: Influence of pH value of feed phase on mass flows of amino acids (pH_S = 2, D2EHPA concentration = 40 g/L).

The further increase of the pH-value of feed phase promotes the formation and accumulation of the acidic and neutral amino acids zwitterions, or of the basic amino acids dication-anionic species or zwitterions,

reducing significantly their initial mass flows. Therefore, at the isoelectric point, the reactive extraction of amino acids became impossible. However, the pertraction of basic amino acids was not possible even if the pH_F-values were lower than those corresponding to their isoelectric points, due to the formation of the dication-anionic species. Similar variations were recorded for final mass flows, the pH_F-values corresponding to the maximum level of final mass flows being moved to 3, for aspartic and glutamic acids, and to 4, for the rest of amino acids, respectively (Fig. 2). Because the amino acids were extracted in the liquid membrane in various proportions, the differences between the final mass flows respected those between the initial mass flows. The further increase of pH_F to the neutral domain led to the reduction of the final mass flows, owing to the change of the sense of pH-gradient which controls the direction of amino acids transfer through the liquid membrane. The increase of pH_F exhibited a favourable effect on the amino acids permeability factors, which reached values over 1 for $pH_F \geq 3$ [11]. This result suggested that the final mass flows became superior to the initial ones, phenomenon that is without physical significance and was possible due to the stripping of the supplementary amount of amino acids accumulated into the organic layer.

The increase of the pH-value of stripping phase determined the continuous diminution of both initial and final mass flows of the amino acids, influence that was recorded also for the permeability factors (Fig. 3). Both variations indicated that by increasing the pH_S the sense of the solutes transport through liquid membrane was inverted, and, consequently, the amount of the accumulated amino acids inside the solvent layer increased significantly.

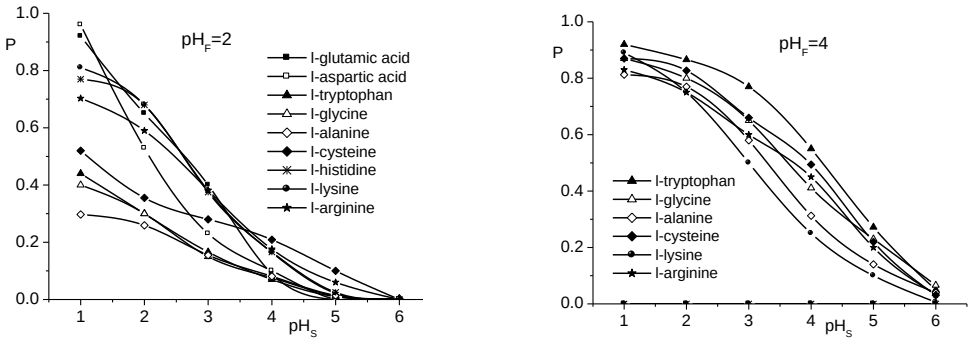

Fig. 3: Influence of pH-value of stripping phase on permeability factors (D2EHPA concentration = 40 g/L).

The mass flows of all amino acids were continuously intensified by increasing D2EHPA concentration inside the liquid membrane, as the results of the increase of the concentration of one reactant participating either at the interfacial reaction in the extraction into organic phase or at the re-extraction into stripping phase (Fig. 4).

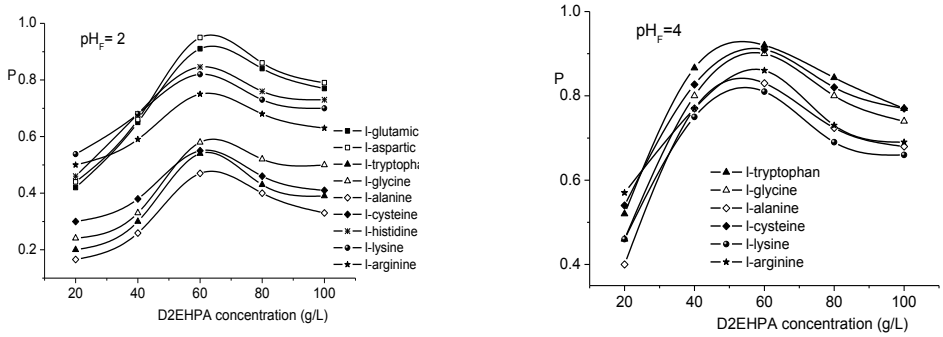

Fig. 4: Influence of D2EHPA concentration on permeability factors ($pH_S = 2$).

The dependences between the permeability factors and carrier concentration differed from those between mass flows and the same parameter. According to Fig. 4, the permeability factors initially increased with D2EHPA concentration, reached a maximum value, followed by their reduction. Indifferent of the value of pH_F and for all pertracted amino acids, the maximum level of permeability factor was recorded for the carrier concentration of 60 g/L, this value being directly correlated with the kinetic resistance to the re-extraction

process from the solvent layer to the stripping phase [11]. Thus, for D2EHPA concentration over 60 g/L, the kinetic resistance of the stripping process exceeded that corresponding to the extraction from the feed phase.

In these circumstances, the selective separation of amino acids mixtures by facilitated pertraction was possible for different groups of amino acids with similar acidic properties, 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 stripping from the liquid membrane and, consequently, their concentration gradients between the two aqueous phases, and the carrier concentration, which controls the capacity of liquid membrane to transport the solute.

4. Conclusions

The study on separation of amino acids by reactive extraction and facilitated pertraction indicated that it is possible to selectively extract amino acids from a mixture obtained by fermentation broths or protein hydrolysates. Due to the rather low extraction degrees of each group of amino acids at the required pH-values for selective separation, for total recovery of the considered amino acids a multistage extraction has to be used. Using multistage extraction, the total separation of the following amino acids groups has been performed: neutral amino acids at pH 5–5.5, basic amino acids and l-cysteine at pH 4–4.5, l-histidine at pH 3–3.5, and acidic amino acids at pH 2–2.5. The proposed extraction method was developed and used for the selective separation of amino acids by facilitated pertraction which presents the advantage of reducing the number of extraction stage and so being an economical alternative to other extraction methods.

The studies on facilitated pertraction of amino acids of acidic, basic character or neutral character from their mixtures using D2EHPA as a carrier 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.

5. Acknowledgements

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