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SELECTIVE SEPARATION OF BIOSYNTHETIC PRODUCTS BY PERTRACTION - CHALLENGE FOR THE "WHITE BIOTECHNOLOGY"

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Abstract: This review presents our original results on selective separation of some biosynthetic products (antibiotics, carboxylic acids, amino acids) by free or facilitated pertraction (extraction and transport through liquid membranes). Selecting the optimum conditions, for all studied cases these pertraction technique simplify the technologies applied at industrial scale for separation and purification, allows to reaching high selectivity and reducing the overall cost of the products.

Keywords: *liquid membrane, pertraction, antibiotic, carboxylic acid, amino acid*.

INTRODUCTION

The industrial biotechnology has been considerably developed in Europe, especially for the fine chemicals and food technologies (for examples, more than 70% of the overall enzymes production is located in Europe) [1]. This evolution of the biotechnology at large-scale is supported by favorable political and social sentiments and leads to the gradually replace of the chemical technologies by sustainable biochemical technologies with significant benefits.

According to the Lisbon strategy, the improvement of the current technologies until 2010 is the major European objective and it is also an economic, technological and social challenge [2]. This objective can be reached by defining a unitary vision concerning the European industrial biotechnology, by ensuring a feasible program for developing biotechnology within EU Framework Program 7 or future ones, by increasing through knowledge and transparent information the public interest and support on industrial biotechnology, by establishing the partnerships between the public and private institutions.

Thus, the new concept of "white biotechnology" was born in Europe; it is considered to be the "New Era" of biotechnology and joins all the initiatives dedicated to producing goods or services by sustainable biotechnologies.

Being directed to the identification and utilization of the natural renewable sources of raw materials for biosynthesizing valuable bioactive compounds, by means of clean processes which will improve the classical chemical technologies and cut the waste generation and high energy consumption, the driving force of the white biotechnology is the sustainability by carefully managing of the finite resources. Therefore, according with the definition given by Gro Harlem Brundtland, the former Chair of the World Commission on Environment and Development, in its report *Our common future* (April 1987), the sustainable development imposes the equilibrium of three equally important requirements, of economic, ecologic and social types. This idea has been also underlined by Thomas Rachel, German Presidency of the Council of the European Union at the opening ceremony of the International Conference *European BioPerspectives - "En Route to a Knowledge-Based Bio-Economy"*(31 May - 1 June 2007, Cologne) [3].

It is very important to think about the "white biotechnology" not only in terms of its potential economic benefits, but also in terms of environmental protection or of the starting-point for new business. The industrial biotechnology has became a hot topic especially among the manufacturers and companies using chemical synthesis technologies, because the biotechnology possesses the potential to improve and, then, to maintain the level of products competitiveness.

In this context, the actual trend to implement "the white biotechnology", defined as "the third wave of the biotechnology" too, is also dedicated to the design, optimization and application at macro-scale of new techniques for separation and purification. Compared to the chemical methods, the biosynthesis represents a very advantageous alternative for production of many compounds with biological activity, because of the reduction of the overall process stages number and of the advanced utilization of the raw materials. However, the separation of the biosynthetic products is complicate, this being a particularity of the industrial biotechnologies, owing to their high dilution in fermentation broth, to their chemical and thermal lability and to the presence of

secondary products in the fermentation broth. Therefore, the purification of these products requires a laborious succession of separation stages with high material and energy consumption, the contribution of these stages to the final cost being of 20 - 60%, or even more [4].
For these reasons, new techniques have been developed or adapted for the separation of the biosynthetic products. Among them, some new extraction techniques, namely as: reactive extraction, extraction and transport through liquid membranes, supercritical fluid extraction, aqueous two phases extraction, extraction by reverse micelles, direct extraction from broths, have been experimented and applied at laboratory or at industrial scale for bioseparations [4].
Pertraction, defined as the extraction and transport through liquid membranes, is rather new separation technique and consists in the transfer of a solute between two aqueous phases of different pH-values, phases that are separated by a solvent layer of various sizes [5 – 7]. 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 etc., the separation process being called facilitated pertraction and facilitated transport [8 – 13].
The liquid membranes can be obtained either by emulsification, but their stability is poor, by including the solvent in a hydrophobic porous polymer matrix, or using pertraction equipments of special construction, which allow to separate and easily maintain the three phases without adding surfactants (free liquid membranes) [14].
Compared to the physical or reactive liquid-liquid extraction, the use of pertraction reduces the loss of solvent during the separation cycle, needs 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 [14, 15].
Beside the separation conditions and the physical properties of the liquid membrane, the pertraction mechanism and, implicitly, its performance are controlled by the solute and carrier characteristics, respectively by their ability to form products soluble in the liquid membrane. Among the mentioned factors, the pH-difference between the feed and stripping phase exhibits the most significant influence, this parameter controlling the yields and selectivity of the extraction and reextraction processes, on the one hand, and the rate of the solute transfer through the solvent layer, on the other hand.
In this context, this review presents the results of our experiments on selective separation of some biosynthetic products (antibiotics, carboxylic acids, amino acids) by free or facilitated pertraction, using carriers of long chain amines or organophosphoric acids types and original pertraction equipment.

SELECTIVE PERTRACTION OF BETA-LACTAM ANTIBIOTICS

The biosyntehsis of beta-lactam antibiotics (Penicillins G and V) by *Penicillium sp.* or *Aspergillus sp.* requires the use of precursors (phenylacetic acid, or phenoxyacetic acid, respectively). Due to their toxicity, the precursors are added in portions during the fermentation, their concentration being maintained at a constant level. Therefore, the acids final concentrations in the fermentation broth vary between 0.2 and 0.6 g/L, depending on the strain and biosynthesis conditions.

For this reason, the selective separation is required for obtaining beta-lactams antibiotics with high purity. Although this operation is difficult by using conventional separation techniques because of the similarities in the physical and chemical characteristics, the antibiotics can be selectively separated from their precursors by facilitated pertraction with Amberlite LA-2 in 1,2-dichloroethane [16].
Figures 1 and 2 demonstrate the major role of pH on the performance of selective separation of these compounds. It could be observed that maximum values of selectivity factor (S) are obtained in the case of minimum difference between pH-values of the aqueous phases. Thus, at a constant level of stripping phase pH of 10 and for a pH value of 6 for feed phase, S = 80.4 is obtained and, respectively, at a pH value of feed phase maintained at 3 and a pH value of stripping phase of 7 leads to S = 24.2.

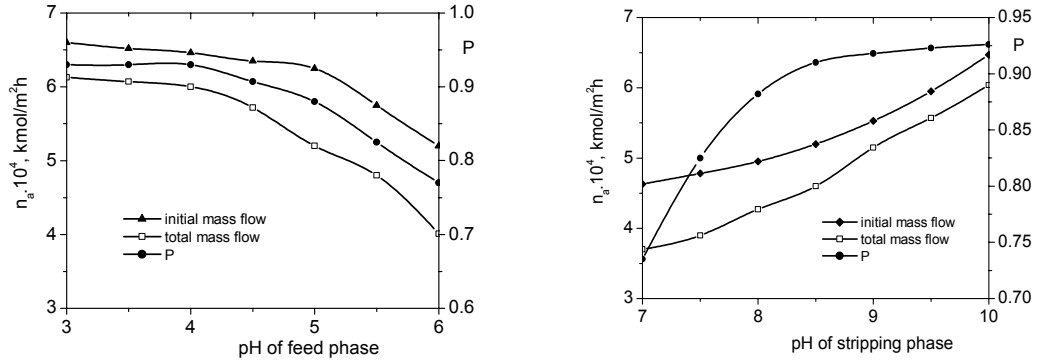

Figure 1. *Variation of the Penicillin V mass flows and permeability factor in function of the pH-values of feed and stripping phases (carrier concentration = 80 g/L, rotation speed = 500 rpm)*

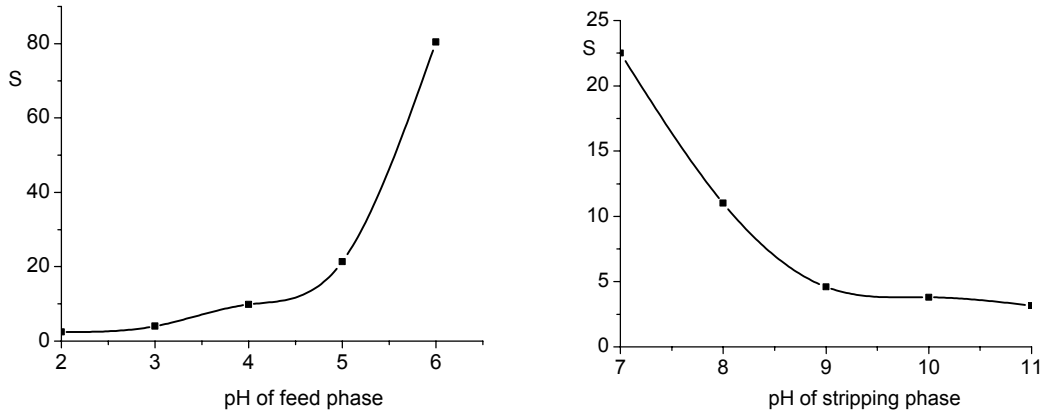

Figure 2. *Effect of feed phase and stripping phase pH-values on the selectivity factor (rotation speed = 500 rpm, carrier concentration = 80 g/L)*

Another important factor is the concentration of Amberlite LA-2 inside the liquid membrane. Even if the effect of carrier concentration is quite similar for the two components of mixture, the initial decrease of permeability factor of phenoxyacetic acid is more significant. Increasing the Amberlite LA-2 concentration inside the liquid

membrane, an approaching of the permeability factors of the two compounds can be observed. This phenomenon, indicated in Figure 3 by the ratio of evolution of permeability factors, suggests that at its low concentrations the carrier preferentially reacted with the compound of higher acidity, namely Penicillin V. At high Amberlite LA-2 concentrations, additional amounts of carrier will exist nearly at the interface, meaning that the carrier will react even with the weaker acid, namely phenoxyacetic acid. These results have been found in the variation of selectivity factor, which, in the considered experimental conditions, has a maximum of 6.5 for 10 g/L Amberlite LA-2 in 1,2-dichloroethane.

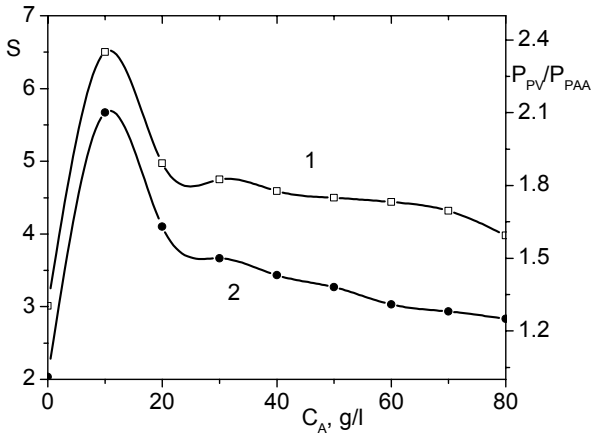

Figure 3. *Effect of carrier concentration on the ratio between permeability factors of Penicillin V and phenoxyacetic acid and on the selectivity factor (pH-value of feed phase = 3, pH-value of stripping phase = 10, rotation speed = 500 rpm; 1 - P_{PV}/P_{PAA}, 2 - selectivity factor S)*

These experimental results underlined the possibility for selectively separating Penicillin V from phenoxyacetic acid by means of extraction and transport through a liquid membrane consisting of 1,2-dichloroethane and Amberlite LA-2 as carrier, on the base of the difference in acidity and hydrophobicity of the two compounds. The efficiency of selective separation could be enhanced by diminishing the pH gradient between the feed and stripping phase and by using low concentrations of carrier inside the liquid membrane, in the conditions of a strong mixing of the aqueous solutions.

SELECTIVE PERTRACTION OF GENTAMICINS

Gentamicin is an aminoglycoside antibiotic, isolated in 1963 by Weinstein from the *Micromonospora purpurea* cultures. It was introduced in therapeutic practice in 1969 in USA [17]. Gentamicin has a broad spectrum against the aerobic Gram positive and Gram negative bacteria, including the strains resistant to tetracycline, chloramphenicol, kanamycin, and colistin, namely *Pseudomonas, Proteus, Staphylococcus, Streptococcus, Klebsiella, Haemophilus, Aerobacter, Moraxella* and *Neisseria*. It was the first antibiotic efficient against *Pseudomonas*, being one of the most important members of the aminoglycoside antibiotics family [18, 19].

This antibiotic is industrially obtained by *Micromonospora purpurea* or *echinospora* biosynthesis, the product being a complex mixture of some components of very similar structures. Among them, three are the most important: Gentamicins C_1, C_{1a} and C_2 (Gentamicin C_{2a} is considered also to be Gentamicin C_2, because it is its stereoisomer) [19, 20]. The biosynthetic complex contains also the active Gentamicin C_{2b}, but its concentration is very low [19, 20]. The chemical structures of the major Gentamicins are indicated in Figure 4 [21, 22].

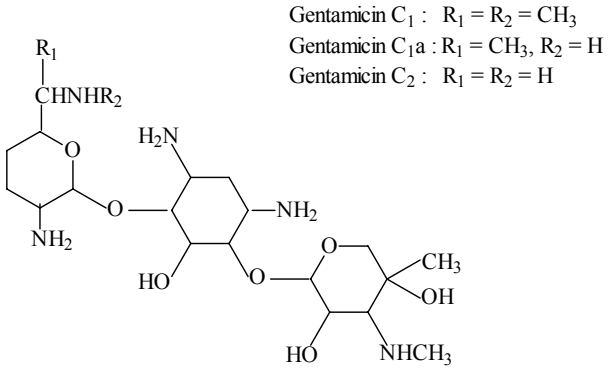

Figure 4. *Chemical structure of biosynthetic Gentamicins*

The ratio of these components in the mixture varies from one biosynthetic product to another, the average values of their concentrations being: Gentamicin C_1 35%, Gentamicin C_{1a} 25%, Gentamicin C_2 (including Gentamicin C_{2a}) 40% [23].

The antibacterial activity of the Gentamicins, respectively their affinity for the bacterial ribosomes, is different. Thus, the most efficient is Gentamicin C_{1a}, its activity being slightly higher than that of Gentamicin C_2. Gentamicin C_1 binds the ribosomal subunits with the lowest efficiency compared with the other two Gentamicins (there are no reports concerning the specific affinity of Gentamicin C_{2a}, probably due to its assimilation with Gentamicin C_2) [24].

The separation of Gentamicin from the fermentation broths at industrial scale is achieved by sorption by cation-exchangers, followed by it's desorption with a solution of 4 - 5% sulfuric acid. After the neutralization, the solution is purified and concentrated under vacuum, the antibiotic being precipitated as sulfate salt by acetone addition [25]. But, this technique doesn't allow the fractionation of the complex mixture of Gentamicins, the use only of Gentamicins C_{1a} and C_2 increasing the specific biological activity per weight unit of antibiotic.

On the basis of the previous investigations on the mechanism of Gentamicins reactive extraction and on the factors influencing the efficiency and selectivity of separation, it was studied the possibility to fractionate the biosynthetic mixture of Gentamicins by facilitated pertraction with D2EHPA dissolved in dichloromethane [26]. In this purpose, the original pertraction cell [27] has been used and the influences of the pH-gradient between the aqueous phases, carrier concentration in the liquid membrane and mixing intensity on the efficiency and selectivity of pertraction have been analyzed.

In the case of separation of Gentamicins, the influence of pH-gradient is amplified by the ionization-protonation of these antibiotics in the two aqueous phases, these processes controlling the efficiency of extraction and re-extraction, as well as the rate of the transport through liquid membrane. Thus, from Figure 5 it can be observed that the

initial and final mass flows of Gentamicins are continuously increased with the increase of pH-value.

This variation is the result of the mechanism of reactive extraction of the Gentamicins. According to the previous studies, the reactive extraction with D2EHPA occurs by means of the formation of a strong hydrophobic compound by the following ionic exchange reaction [26]:

$$\text{Gentamicin}^{n+}_{(aq)} + n\,HP_{(o)} \rightleftharpoons \text{Gentamicin.nHP}_{(o)} + n\,H^{+}_{(aq)}$$

where Gentamicin^{n+} represents the antibiotic with protonated aminic groups, and HP the carrier, respectively (n = 1 - 5). The aminic groups of Gentamicins are involved in the reactive extraction, the interactions between the antibiotic and extractant being of ionic type. Gentamicins possess five aminic groups, which could react with the extractant, similar to the reaction with sulfuric acid in the desorption process from the cation-exchangers [25]. But, due to the voluminous molecules of the antibiotic and extractant, the steric hindrances appear, thus limiting the number of the aminic groups that can react. Furthermore, the basic character of the aminic groups is different and induces the competition between them in the reaction with D2EHPA. The substitutes, which differentiate the Gentamicins, control the basicity of the specific aminic groups and induce their different reactivity, respectively their different mass flows [26].

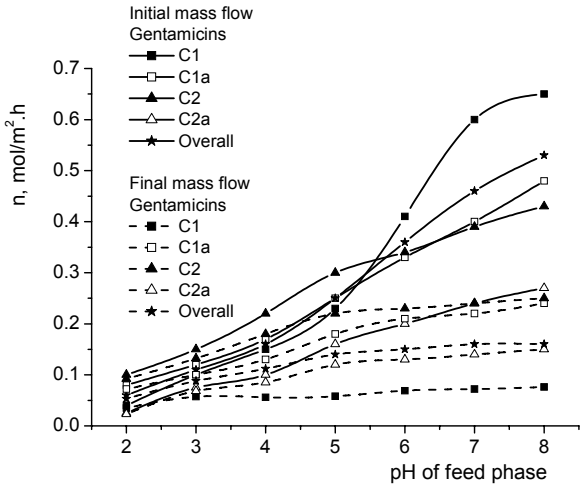

***Figure 5**. Influence of pH-value of feed phase on mass flows of Gentamicins (pH of stripping phase = 1.5, D2EHPA concentration = 20 g/L, rotation speed = 500 rpm)*

Although the effect of the pH-value of feed phase is similar for all Gentamicins, for the neutral pH domain there were recorded important modifications of the relative extraction rate of the four Gentamicins in the membrane phase. For pH-value below 5 the order of the increase of initial mass flows is due to the decrease of dissociation degree from Gentamicin C_1 to Gentamicin C_2, being as follows:

gentamicin C_2 > gentamicin C_{1a} > gentamicin C_1 > gentamicin C_{2a}.

This order also indicated significant difference between the initial mass flows of the Gentamicins C_2 and C_{2a}, both compounds having the same chemical structure and molecular weight. This phenomenon, also observed in the case of simple reactive extraction [26], could be the result of the molecular conformation of Gentamicin C_{2a},

which alters the strength of the interactions of solvation type with the solvent molecules, and, consequently, its solubility in dichloromethane.
For pH-values of feed phase over 5, the initial mass flows of Gentamicins C_1 and C_{1a} become superior to those of the other two Gentamicins (Figure 5). The reactive extraction with D2EHPA needs the protonation of Gentamicins in aqueous solution, this process being affected by the pH increase. Due to the different basicity of Gentamicins specific substituted aminic groups, the relative magnitude of the pH influence on initial mass flows is different. Thus, the increase of the mass flow for pH-value over 5 becomes more pronounced for the Gentamicin containing aminic groups with higher basicity, namely Gentamicin C_1. For the reasons above considered, the lowest mass flow was recorded for Gentamicin C_{2a}.
For describing the selectivity of pertraction, the selectivity factor (S) has been used, being defined as the ratio between the permeability factor of all Gentamicins and that of Gentamicin C_1. According with the above results, from Figure 6 it can be seen that the variation of pH of feed phase from 2 to 8 exhibits a favorable influence of the selectivity factor, this parameter increasing from 1 to 3.1 in the considered domain of pH.

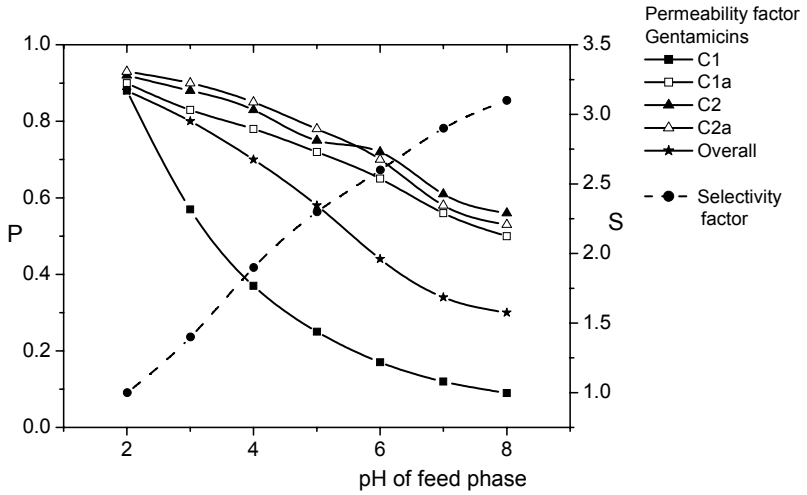

***Figure 6**. Influence of pH-value of feed phase on permeability and selectivity factors (pH of stripping phase = 1.5, D2EHPA concentration = 20 g/L, rotation speed = 500 rpm)*

The increase of stripping phase pH-value induces the significant reduction of initial and final mass flows of all Gentamicins (Figure 7). This variation is controlled by the re-extraction mechanism. Therefore, the re-extraction is based on the interfacial reaction between the Gentamicins-D2EHPA salts and five equivalents of sulfuric acid for each mole of Gentamicin [25, 26]:

$$\text{Gentamicin.HP}_{(o)} + 5/2\ H_2SO_{4(aq)} \rightleftharpoons \text{Gentamicin}^{5+}.5/2SO_4^{2-}{}_{(aq)} + HP_{(o)}$$

The reactivity of Gentamicins in the reaction with sulfuric acid is determined also by the basicity of their specific aminic groups, because they control the strength of the ionic interactions between the antibiotic and the carrier, and therefore the easiness of the antibiotic release from the membrane phase [26].

From Figure 7 it can be observed that at higher acidic pH-domain of stripping phase the highest initial mass flow corresponds to Gentamicin C_1. The decrease of the sulfuric acid concentration, respectively the increase of stripping phase pH-value, leads to the decrease of all Gentamicins initial mass flows, this variation being more pronounced for Gentamicin C_1. Therefore, for pH-value over 2 the initial mass flow of Gentamicin C_1 becomes lower than those of the other Gentamicins.

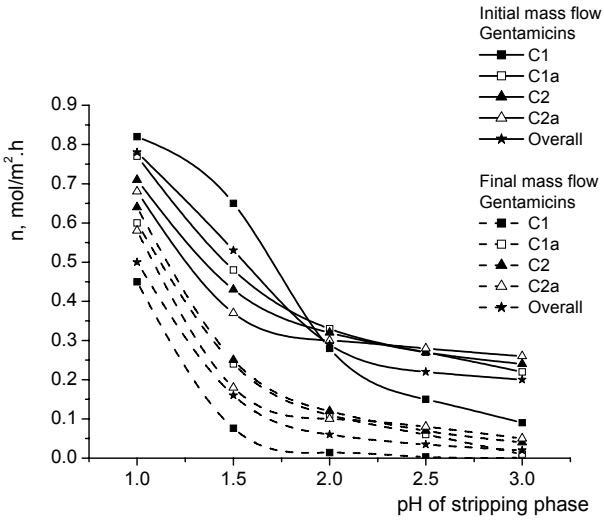

***Figure 7**. Influence of pH-value of stripping phase on mass flows of Gentamicins (pH of feed phase = 8, D2EHPA concentration = 20 g/L, rotation speed = 500 rpm)*

This evolution is due to the different basicity of the specific aminic groups of Gentamicins, which induces different rates of re-extraction in the stripping phase, consequently different concentration gradients of Gentamicins between the two aqueous phases. At lower pH-value, the concentration gradients are maximum and, therefore, the extracted mass flows of all Gentamicins are high. At higher pH-values of stripping phase, owing to the significant increase of the re-extraction efficiency of Gentamicins C_{1a}, C_2 and C_{2a} compared to Gentamicin C_1, the order of the decrease of the initial mass flows is changed, the Gentamicin C_1 becoming the poorer extracted compound.

The selectivity factor increases with the increase of pH of stripping phase and reaches the highest values for pH = 3 (S = 10.8). This variation is in concordance with the above results and indicates that the lowest permeability through liquid membrane and the most significant negative influence of stripping phase pH-value correspond to the pertraction of Gentamicin C_1, due to the above presented reasons.

The highest values of selectivity factor have been recorded for less intense mixing of the two aqueous phases, below 200 rpm, due to the lowest value of Gentamicin C_1 permeability factor. The intensification of mixing leads to the strongly decrease of selectivity factor from 13 for 100 rpm to 1.6 for 700 rpm (Figure 7).

The increase of carrier concentration into the liquid membrane induces the increase of the initial and final mass flows of both acids, but the basicity of the specific aminic groups controls the magnitude of this influence. According to the results obtained for reactive extraction of Gentamicins [26], if the carrier exists in a stoechiometric deficit related to the complete reaction with all Gentamicins, it will firstly reacts with

Gentamicin having the characteristic aminic group with the highest basicity, consequently with Gentamicin C_1. For this reason, the maximum difference between the initial mass flow of Gentamicin C_1 and those of the other Gentamicins is reached for the D2EHPA concentration below 20 g/L. Moreover, contrary to the variation of Gentamicin C_1 initial mass flow, the mass flows of Gentamicins C_{1a}, C_2 and C_{2a} continuously increase without reaching any evident constant level in the domain 0 - 60 g/L D2EHPA.

The variation of the selectivity factor with carrier concentration is opposite to that of the permeability factors (Figure 8). The maximum value of selectivity factor (S = 5) corresponds to the minimum of permeability factors, thus suggesting that at lower carrier concentration Gentamicin C_1 is less efficiently pertracted. The selectivity of pertraction is dimished for about 2.5 times by increasing the carrier concentration in the liquid membrane from 10 to 60 g/L, due to the reasons above discussed.

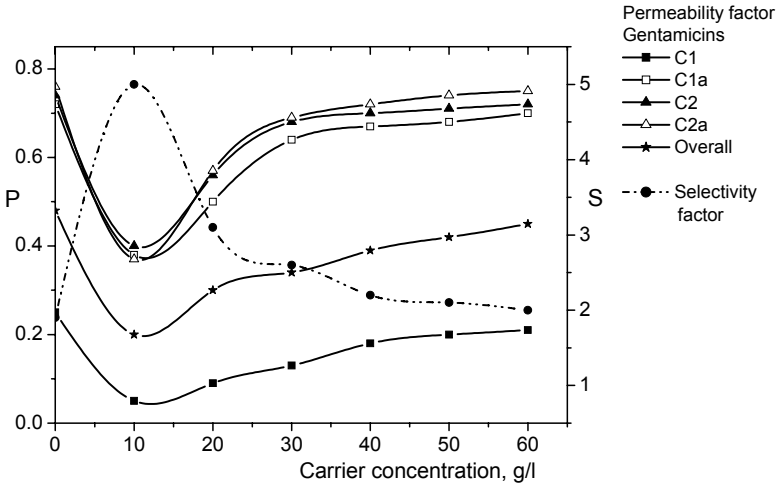

Figure 8. *Influence of carrier concentration on permeability and selectivity factors (pH of feed phase = 8, pH of stripping phase = 1.5, rotation speed = 500 rpm)*

In conclusion, these studies indicated that Gentamicin C_1 can be selectively removed from the mixture obtained by biosynthesis. Using the proper levels of the factors influencing the separation process (pH of feed phase of 8, pH of stripping phase of 3, rotation speed of the feed and stripping phases below 100 rpm and carrier concentration of 10 g/L), the most active Gentamicins (Gentamicins C_{1a}, C_2 and C_{2a}) can be selectively pertracted from the initial mixture. Therefore, the removal by facilitated pertraction of Gentamicin C_1, which has the lowest activity against the infections, from the biosynthetic mixture increases the therapeutic activity of the antibiotic.

SELECTIVE PERTRACTION OF CARBOXYLIC ACIDS PRODUCED BY CITRIC FERMENTATION

Citric acid is one of the widely used carboxylic acids, having multiple applications in chemical, pharmaceutical, food and cosmetic industries. This compound is mainly obtained through a fermentation process by *Aspergillus niger* cultivated on molasses

[28, 29]. Due to the presence in the final broth of other carboxylic acids as secondary metabolic products, especially malic and succinic acids, the separation and purification technology of citric acid is quite complicated. Thus, the citric acid represents about 80 - 95% from the total amount of organic acids in the broth at the end of fermentation, its concentration being of 50 g/L. The rest are secondary acids, their concentration reaching 4 g/L [28].

At industrial scale, the separation and purification of citric acid consist on carboxylic acids precipitation as calcium salts, solubilization of calcium citrate by heating the solution and citric acid release by treating with sulfuric acid [28]. This technology needs high amount of raw materials and energy consumption and produces large amounts of calcium sulphate as the by-product, without leading to high purity of citric acid.

Owing to the differences between the extraction mechanisms, acidity of these carboxylic acids and hydrophobicity of the compounds formed with the carrier, the selective removal of the malic and succinic acids from the final fermentation broth by pertraction with Amberlite LA-2 has been performed [30].

In the case of these acids pertraction from a mixture, the dependence of their mass flows on the pH gradient has to be correlated with their acidity, because the acidity controls the rate of interfacial reactions between solute and carrier. Thus, the obtained order of the pertraction efficiency, given as follows:

succinic acid < citric acid < malic acid,

is the result of the higher acidity of citric and malic acids, on the one hand, and of the superior hydrophobicity of malic acid – Amberlite LA-2 complex .

Figure 9 indicates that the permeability factor tends to 1 with the increase of pH-gradient, underlining the approach between the acid extraction and re-extraction yields. Moreover, the values of permeability factors suggest an inverse proportionality between the transport capacity of liquid membrane and the acidity of transferred solute, the order of permeability factors decrease being:

succinic acid > malic acid > citric acid.

This order could be explain by the similar variation of the rate of interfacial reaction between acid - carrier compound and sodium hydroxide, the increase of acidity leading to the appearance of a kinetic resistance to the re-extraction process.

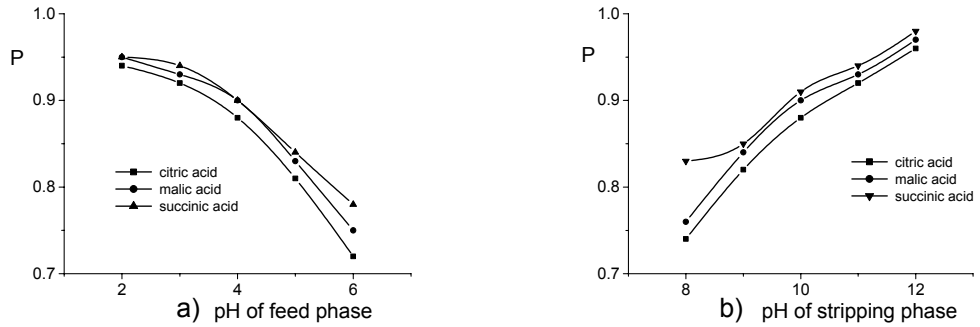

Figure 9. *Influence of pH values of feed and stripping phases on citric, malic and succinic acids permeability factors (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, carrier concentration = 0.3 M, rotation speed = 500 rpm; a – pH of stripping phase = 11, b – pH of feed phase = 3)*

Concentration of Amberlite LA-2 inside of the liquid membrane induces a different influence on pertraction efficiency of the carboxylic acids. The difference on carrier effects is due to the difference on acids extraction mechanisms, as well as to the difference on solutes acidity and on hydrophobicity of the extracted compounds. As it can be seen from Figure 10, by increasing the carrier concentration the malic acid, succinic acid and citric acid are successively pertracted.

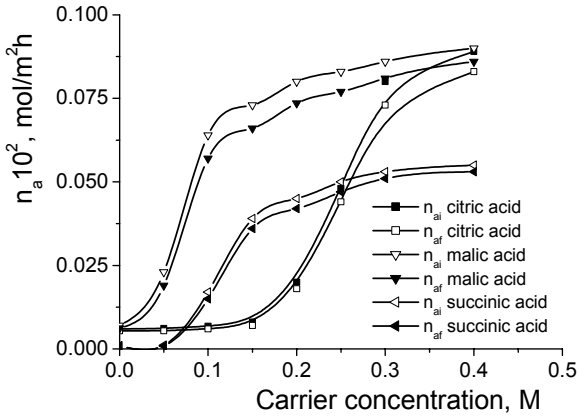

Figure 10. *Influence of carrier concentration on citric, malic and succinic acids mass flows (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, rotation speed = 500 rpm, pH of feed phase = 3, pH of stripping phase = 11)*

The succinic acid is extracted after the Amberlite LA-2 concentration exceeds the value stoichiometric needed for interfacial reaction with malic acid, respectively after it exceeds the molar ratio between carrier and malic acid of 1. The citric acid is extracted for carrier concentration level higher than that corresponding to an equimolecular ratio with malic and succinic acids. Below the carrier concentrations that allow the reactive extraction of succinic and citric acids, their pertraction is possible only by physical solubilization in 1,2-dichloroethane, but the acids mass flows are very low. These results demonstrate the major influence of the Amberlite LA-2 concentration inside the liquid membrane on pertraction selectivity.

The above discussed results suggest the possibility to selective pertracted the malic and succinic acids, the citric acid remaining in the raffinate phase. In order to confirm this hypothesis and to establish the required conditions for reaching a high selectivity of separation, the influences of pH gradient between the aqueous phases, carrier concentration and mixing intensity on pertraction selectivity have been studied.

The selectivity of pertraction was described by means of the selectivity factor, defined in the case of separation of malic and succinic acids from citric acid as:

$$S = \frac{n_{a_{f.malic.acid}} + n_{a_{f.succinic.acid}}}{n_{a_{f.citric.acid}}} \quad (1)$$

and for the separation of malic acid from succinic acid as:

$$S_1 = \frac{n_{a_{f.malic.acid}}}{n_{a_{f.succinic.acid}}} \quad (2)$$

As it can be observed from Figure 11, the reduction of pH gradient leads to the increase of selectivity factors S and S_1, but this effect magnitude is rather different. The modification of pH value of feed phase induces a stronger effect on separation selectivity of secondary carboxylic acids from citric acid, while the modification of stripping phase pH exhibits a more pronounced effect on separation selectivity of malic acid from succinic acid.

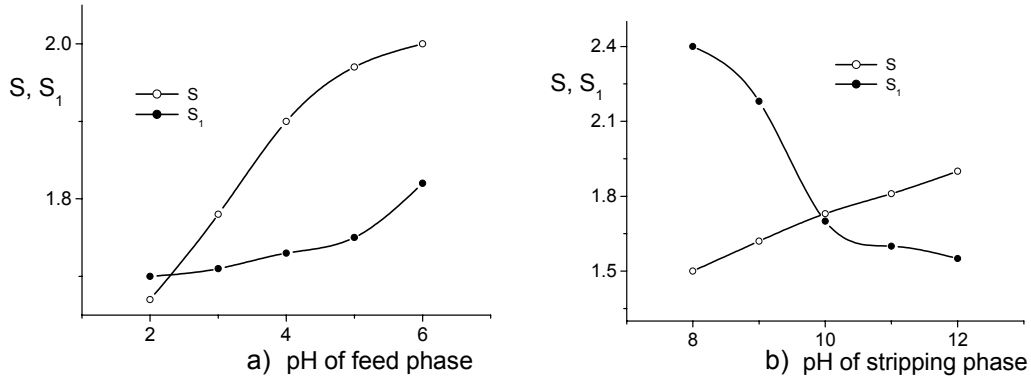

Figure 11. *Influence of pH values of feed and stripping phases on selectivity factors (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, carrier concentration = 0.3 M, rotation speed = 500 rpm; a – pH of stripping phase = 11, b – pH of feed phase = 3)*

These variations are due to the different ionization of the carboxylic acids by modifying of aqueous solutions pH, the efficiency of extraction and transport of corresponding ionic species through liquid membrane being different. Thus, it can be concluded that the selectivity of separation of malic and succinic acids from citric acid could be enhanced by increasing the pH values of both aqueous phases, and that of separation of malic acid from succinic acid by carrying out the pertraction at neutral domain of pH.
The decisive influence of carrier concentration on pertraction selectivity is underlined by the dependence between the selectivity factors and this parameter (Figure 12).
Similar to the variation of acids mass flows with carrier concentration, the experimental data show that the maximum selectivity both for separation of secondary carboxylic acids from citric acid, and for separation of malic acid from succinic acid is reached for an equimolecular ratio between Amberlite LA-2 and the pertracted acids. Furthermore, an about sevenfold increase in the selectivity factors can be achieved by optimizing of the carrier concentration in comparison to the optimization by modifying the aqueous phase pH.
The effect of mixing intensity on selectivity factors S and S_1 is different. From Figure 13 it can be seen that the selectivity of separation of malic and succinic acids from citric acid is not influenced by rotation speed value. However, the selectivity of malic acid from succinic acid separation is amplified by mixing intensification. These variations confirm the previous results indicating a diffusional resistance more accentuated in the case of malic acid pertraction compared to that of succinic acid.

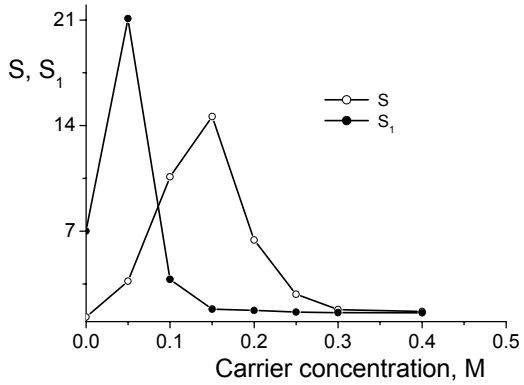

Figure 12. *Influence of carrier concentration on selectivity factors (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, rotation speed = 500 rpm, pH of feed phase = 3, pH of stripping phase = 11)*

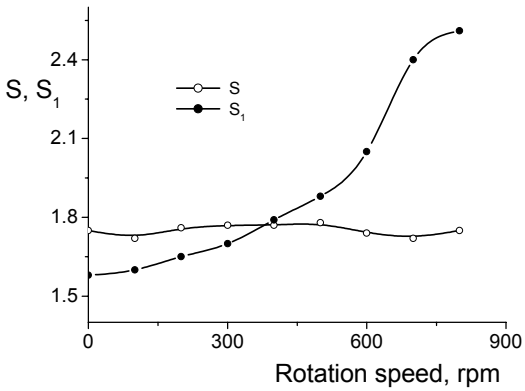

Figure 13. *Influence of rotation speed on selectivity factors (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, carrier concentration = 0.3 M, pH of feed phase = 3, pH of stripping phase = 11)*

Therefore, although the separation of malic and succinic acids from citric acid is not influenced by mixing intensity, the increase of rotation speed leads to the increase of acids mass flows through liquid membrane and to the enhancement of separation selectivity of malic acid from succinic acid.

In order to verify these conclusions, in the first step the pertraction of citric, malic and succinic acids from a mixture similar to that obtained by citric fermentation was performed. The concentrations of the carboxylic acids in the feed solution were as follows: 50 g/L (0.26 M) citric acid, 2.5 g/L (2.1×10^{-2} M) malic acid, respectively 2.5 g/L (1.9×10^{-2} M) succinic acid. In the second step, the malic acid was pertracted from a mixture containing 2.5 g/L (2.1×10^{-2} M) malic acid and 2.5 g/L (1.9×10^{-2} M) succinic acid. In both cases, the pertraction was carried out using the separation conditions that offer maximum selectivity and high rate of transport through liquid membrane. Thus, the selective pertraction of the secondary acids from mixture of the three acids was

performed at a carrier concentration of 0.04 M, rotation speed of 500 rpm, pH of feed phase of 4 and pH of stripping phase of 11. Then, the selective pertraction of malic acid from the mixture with succinic acid was performed at a carrier concentration of 0.018 M, rotation speed of 700 rpm, pH of feed phase of 4, pH of stripping phase of 8. The obtained results are given in Tables 1 and 2.

Table 1. *Selective separation of malic and succinic acids from citric acid*

Citric acid		Malic acid		Succinic acid		S
$n_{ai}\times10^2$, mol/(m^{2}h)	$n_{af}\times10^2$, mol/(m^{2}h)	$n_{ai}\times10^2$, mol/(m^{2}h)	$n_{af}\times10^2$, mol/(m^{2}h)	$n_{ai}\times10^2$, mol/(m^{2}h)	$n_{af}\times10^2$, mol/(m^{2}h)	
0.45	0.29	5.8	4.8	2.7	2.3	24.5

Table 2. *Selective separation of malic acid from succinic acid*

Malic acid		Succinic acid		S_1
$n_{ai}\times10^2$, mol/(m^{2}h)	$n_{af}\times10^2$, mol/(m^{2}h)	$n_{ai}\times10^2$, mol/(m^{2}h)	$n_{af}\times10^2$, mol/(m^{2}h)	
5.2	3.7	0.1	0.078	47.5

It can be observed that, by combining the favorable effects of pertraction parameters, superior values of selectivity factors have been obtained. Consequently, the facilitated pertraction of carboxylic acids obtained by citric fermentation allows reaching high selectivity of separation and constitutes an advantageous alternative to the technique industrially applied for citric acid separation from fermentation broths.

SELECTIVE PERTRACTION OF CINNAMIC ACIDS

Cinnamic acid, also known as phenylacrylic acid, is a natural compound derived from phenylalanine, its main vegetable sources being the cinnamon, the resin of *Liquidambar* tree, the storax, the balsam of tolu, and the balsam of Peru. This acid, as well as its derivatives, constitutes important metabolic blocks in the formation of lignins from superior plants. It is also the intermediary for biosynthesis of some vegetable secondary metabolites (pigments, compounds having pungent taste that deter the herbivores etc.).
The main utilization of cinnamic acid is in the cosmetic industry, in the perfumery production, especially as methyl, ethyl or benzyl esters (the cinnamic acid and its volatile benzylic ester are responsible for the cinnamon flavor). The cinnamic acid itself, or the *p*-hydroxy- and *p*-methoxycinnamic acids, has different pharmaceutical applications, for pulmonary affections, cancer, lupus, infectious diseases (diarrhea, dysentery), possessing antibacterial and antifungal activity [31 – 33]. It is used in food, or for the synthetic ink, resins, elastomers, liquid crystalline polymers and adhesives production.
From the two isomers of the cinnamic acid, the *trans* isomer is the most encountered and exhibits the highest biological activity. For example, the *trans*-cinnamic acid is a competitive inhibitor for all isomers of phenylalanine ammonia lyase, the enzyme that induces the conversion of phenylalanine to cinnamic acid, unlike the *cis*-cinnamic acid which inhibits only one isomer of this enzyme [34].
This compound could be obtained by extraction from vegetable materials, by chemical synthesis or biosynthesis. New methods have been recently developed for cinnamic acid

extraction (supercritical fluid extraction, vapor phase extraction, pressurized fluid extraction), but their applications are rather limited for high quantities of vegetable materials [35 – 38]. The cinnamic acid is synthesized from styrene and carbon tetrachloride, by oxidation of cinnamic aldehyde, or from benzyl dichloride and sodium acetate [39, 40]. However, the chemical methods are expensive due to the costs of the starting materials, the high number of required stages for product purification, and they generate large amounts of unwanted secondary products.

For these reasons, the production by fermentation or/and enzymatic methods of cinnamic acid and its main derivatives, the *p*-hydroxy- and *p*-methoxycinnamic acids, have been developed. For this purpose, *Saccharomyces cerevisiae*, *Escherichia coli*, *Pseudomonas sp.* have been cultivated on glucose, and *Cellulomonas galba* on n-paraffins with addition of alkylbenzenes [41, 42]. The glucose, fructose, lactose, sugar, cellulose and starch can be enzymatically transformed by phenylalanine ammonia lyase or tyrosine ammonia lyase in alkaline media. These enzymes are synthesized directly into the media by the mutant strains of *E. coli*, *Rhodotorula sp.*, *Rhodosporidium sp.*, *Sporobolomyces sp.*, *Rhizoctonia solani*, *Trichosporon cutaneum*, *Rhodobacter sp.* [40, 43 – 45].

Except from our work, there are no reports on the possibility of separating cinnamic acid and its related acids from fermentation broths or enzymatic media by liquid-liquid extraction. This is probably due to the low solubility of these compounds in solvents immiscible with water. Their extraction could become possible by adding an extractant of aminic type into the solvent, this compound reacting with the cinnamic acids and leading to the formation of hydrophobic derivatives.

The reactive extraction has been developed by cinnamic and *p*-methoxycinnamic acids pertraction with Amberlite LA-2 as carrier and dichloromethane as liquid phase, using the same experimental pertraction equipment [46].

Due to the methoxy group which differentiates the two studied acids, the influence of the feed phase pH is based on two different mechanisms. Thus, from Figure 14, plotted for pH of stripping phase of 10, it can be observed that the initial and final mass flows of the cinnamic acid are continuously reduced with the increase of pH-value. On the other hand, the mass flows of *p*-methoxycinnamic acid initially increase with the pH increase, reach a maximum level at pH = 4, decreasing then. This variation is more pronounced for the initial mass flow.

These variations are the result of the mechanism of reactive extraction of the two acids. The reactive extraction occurs by means of the interfacial interactions between the carboxylic groups of the cinnamic and *p*-methoxycinnamic acids, R-COOH, and Amberlite LA-2, E. These interactions could be of hydrogen bonding type with the undissociated carboxylic groups, or of ionic type, if the acids dissociate in the aqueous solution [46]:

$$\text{R-COOH(aq)} + \text{n E(o)} \rightleftharpoons \text{R-COOH.En (o)}$$

The initial mass flow of cinnamic acid continuously decreases with the pH increase due to its dissociation at higher pH-values. The existence of the maximum level of the initial mass flow of *p*-methoxycinnamic acid is the result of two opposite phenomena occurring with the pH increase: the diminution of the methoxy group protonation, this promoting the extraction, and the dissociation of the carboxylic group, with negative effect on extraction.

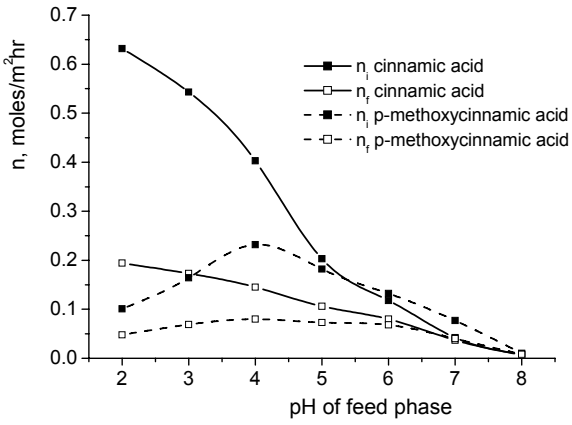

Figure 14. *Influence of pH-value of feed phase on mass flows of cinnamic and p-methoxycinnamic acids (pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/L, rotation speed = 500 rpm)*

For pH-values bellow 5, the initial mass flow of cinnamic acid exceeds that of *p*-methoxycinnamic acid. Over pH = 5, due to the superior hydrophobicity and acidity of *p*-methoxycinnamic acid, its initial mass flow becomes higher than that of cinnamic acid (pKa = 4.44 for cinnamic acid, pKa = 4.28 for *p*-methoxycinnamic acid [47]). But, for the pertraction process, the differences between the mass flows of the two acids recorded for pH > 5 are less pronounced than in the case of reactive extraction [48]. This result is the consequence of the less intense mixing in the pertraction system, and, therefore, of the resistance to the diffusion through the boundary layers from liquid membrane interfaces, which is more important than that induced for the reactive extraction process, especially for the compounds with higher molecular weight. Among the two acids, the resistance to the diffusion of p-methoxycinnamic acid is higher, due to its more voluminous molecule.

The variations of the two acids final mass flows are similar to those of the initial mass flows, owing to their direct dependence to the acids concentrations in the organic layer.

The permeability factor of cinnamic acid increases with the pH increase, this variation suggesting that the reduction of its initial mass flow exhibits a positive effect on the permeability through liquid membrane, due to the diminution of the amount of acid accumulated into the organic phase (Figure 15). Thus, the maximum value of permeability factor for the considered experimental conditions was of 0.93, being reached at pH = 8.

The permeability factor of *p*-methoxycinnamic acid has a particular evolution with the pH increase. This parameter initially decreases and reaches a minimum level at pH = 4, then increasing similarly as for cinnamic acid. For pH < 4, the increase of the amount of *p*-methoxycinnamic acid extracted in organic layer exceeds the increase of its final mass flow, due to the high initial mass flow. Because the initial mass flow of *p*-methoxycinnamic acid is lower comparatively with cinnamic acid, its permeability factor is superior to that of cinnamic acid in this domain of pH.

For higher pH-values, due to the resistance to the diffusion from the liquid membrane to the stripping phase, which is more important for *p*-methoxycinnamic acid, the permeability factor of this acid becomes lower than that of cinnamic acid.

For describing the selectivity of pertraction, the selectivity factor (S) has been defined as the ratio between the final mass flow of cinnamic acid and that of *p*-methoxycinnamic acid. From Figure 15 it can be seen that the maximum value of selectivity factor is reached at the pH of feed phase of 2, as the result of the highest difference between the acids extraction degree and, consequently, between their concentrations in the liquid membrane. The increase of pH induces a negative effect on the selectivity of cinnamic acid separation. Thus, for pH > 6, the selectivity factor is less than 1, owing to the higher amount of *p*-methoxycinnamic acid in the liquid membrane and higher final mass flow compared to those of cinnamic acid.

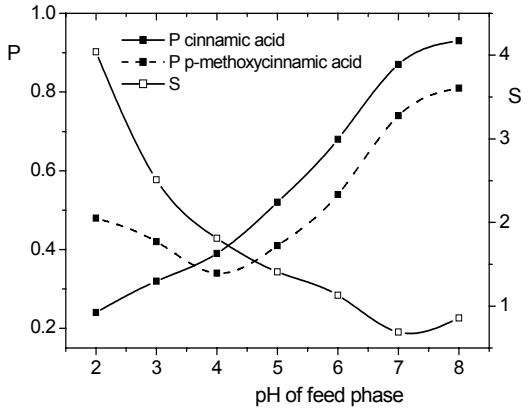

***Figure 15**. Influence of pH-value of feed phase on permeability and selectivity factors (pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/L, rotation speed = 500 rpm)*

The increase of stripping phase pH-value induces the significant increase of initial and final mass flows of the two acids, due to the increase of the pH-gradient between the aqueous phases (Figure 16). The variations of the corresponding permeability factors are similar to those of mass flows, this suggesting that by increasing the pH of stripping phase the acceleration of reextraction rates becomes more important than that of extraction rates (Figure 17).

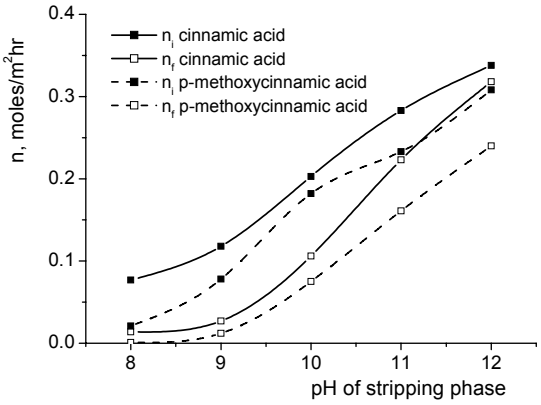

***Figure 16**. Influence of pH-value of stripping phase on mass flows of cinnamic and p-methoxycinnamic acids (pH of feed phase = 5, Amberlite LA-2 concentration = 40 g/L, rotation speed = 500 rpm)*

The selectivity factor reaches the highest values for pH ≤ 9 (at pH = 8, S = 14), decreasing strongly for more alkaline pH-domain. This variation is due to the kinetic resistance which hinders the reextraction process. This resistance is more pronounced in the case of the stronger acid, namely *p*-methoxycinnamic acid, because its compound formed with Amberlite LA-2 in organic phase is more stable than that formed by cinnamic acid and reacts slowly with sodium hydroxide from the stripping phase. The increase of stripping phase pH, implicitly the increase of sodium hydroxide concentration, increases the reextraction rate and, therefore, the final mass flow, effect that is more evident for *p*-methoxycinnamic acid.

The mixing intensity of the aqueous phases represents another important factor influencing the pertraction of the studied acids. For avoiding the appearance of the waves at the two interfaces, the pertraction is carried out at lower rotation speed of the stirrers. Therefore, the diffusion becomes an important limiting factor. Because the magnitude of the resistance to diffusion is enhanced for the solute with voluminous molecule, the mixing intensity is expected to influence in a different manner the pertraction of the two acids. The highest values of selectivity factor have been recorded for poor mixing of the aqueous phases, up to 300 rpm, due to the lowest value of the final mass flow of *p*-methoxycinnamic acid. The intensification of mixing leads to the strongly decrease of selectivity factor from 16 for 300 rpm to 0.95 for 700 rpm.

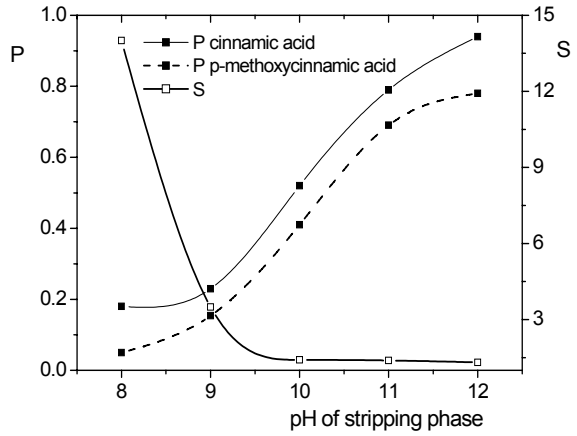

***Figure 17**. Influence of pH-value of stripping phase on permeability and selectivity factors (pH of feed phase = 5, Amberlite LA-2 concentration = 40 g/L, rotation speed = 500 rpm)*

The increase of carrier concentration into the liquid membrane induces the increase of the initial and final mass flows of both acids. According to Figure 18, at concentration of Amberlite LA-2 below 10 g/L, the initial mass flow of *p*-methoxycinnamic acid is higher, due to its superior acidity compared with cinnamic acid. The increase of Amberlite LA-2 amount in the organic phase exhibits a more pronounced effect on cinnamic acid mass flow, because it compensates the lower acidity of this acid. This phenomenon cumulated with the slower diffusion of *p*-methoxycinnamic acid generates significant differences between the values of acids mass flows for carrier concentrations over 10 g/L.

The initial mass flows of the acids reach a rather constant level at 40 g/L Amberlite LA-2. The variation of the final mass flows is similar, the constant level being reached for Amberlite LA-2 concentration of 60 g/L.

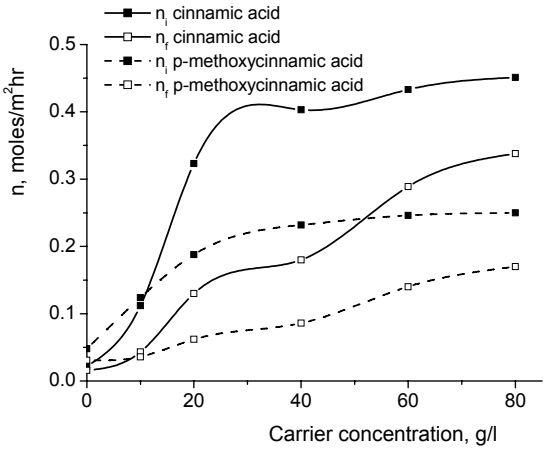

Figure 18. *Influence of carrier concentration on mass flows of cinnamic and p-methoxycinnamic acids (pH of feed phase = 5, pH of stripping phase = 10, rotation speed = 500 rpm)*

The evolution of the acids permeability factors are different. Similar to the pertraction of carboxylic acids obtained by citric fermentation, they initially decrease from a value corresponding to the absence of Amberlite LA-2 in the organic solvent to a minimum value for a concentration of 10 g/L Amberlite LA-2 and finally increase concomitantly with the carrier concentration.

As in the case of pertraction of other carboxylic acids, this variation could be the result of the changes in the relative rate of the interfacial chemical reactions. In the absence of the carrier (free pertraction), the extraction and transport of the acids through the liquid membrane occur by physical process of solubilization, the limiting steps of the overall separation process being only of diffusional type. The addition of Amberlite LA-2 in dichloromethane leads to the change of separation mechanism. Due to the chemical reaction between acid and carrier at the feed phase - liquid membrane interface, as well to the chemical reaction between acid - carrier compound and sodium hydroxide at the liquid membrane - stripping phase interface, the additional limiting steps of kinetic type appeared. Moreover, because the acids do not participate in free acid form to the reextraction process (they are combined with the carrier), the rate of sodium salt formation is diminished. Consequently, comparing with the free pertraction, the final mass flow will be initially smaller.

The positive influence of the increase of carrier concentration is more important in the case of cinnamic acid, this leading to the increase of the selectivity factor from 0.6 for free pertraction to 2 for facilitated pertraction with 40 g/L Amberlite LA-2. For higher carrier concentration, the selectivity factor remains at the constant level.

Therefore, the cinnamic acid can be selectively separated from the mixture with the *p*-methoxycinnamic acid, the pertraction having to be carried out at pH = 2 of feed phase, pH = 8 of stripping phase, rotation speed lower than 300 rpm, and carrier concentration higher than 40 g/L for reaching the maximum selectivity factor.

MODELING OF *p*-AMINO AND *p*-HYDROXYBENZOIC ACIDS FACILITATED PERTRACTION

p-Aminobenzoic acid, also called vitamin B_{10} or factor R, was found to be the part of the folic acids. Being component of the pteroylglutamate, it is considered to act as provitamin for some bacteria and growth factor for some superior animals, in the human body possessing the capacity to synthesize folates [49].
p-Aminobenzoic acid (PABA) is used in cosmetic as additive in sunscreen lotions. The medical applications are for skin protection against vitiligo, sclerodermy and male infertility treatment. It is also used in diagnostic tests for the state of the gastrointestinal tract. It was established that PABA is an inducer of endogenous interferon and immunomodulator, displays a virucidal, synergistic antiviral effect when combined with chemical drugs and possesses the properties of a direct anticoagulant [50]. Although this compound is not considered an essential nutrient, it is included in vitamin B or multivitamins supplements [49].
The most recent methods for PABA production are the chemical synthesis using methyl-4-formylbenzoate as a starting material [51] or biosynthesis by mutant strains of *E. coli* [52]. In both cases the separation stages are complex and require a large energy and material consumption. Due to the insolubility of PABA in organic solvents immiscible with water, its separation by physical extraction is impossible. Because the chemical structure of PABA contains an acidic group, -COOH, and a basic one, $-NH_2$, its separation by facilitated pertraction was possible by using carriers of aminic or organophosphoric acid types [14]. For emphasizing the influences of the separation conditions and physical and chemical characteristics of acids and carriers on pertraction, the facilitated pertraction of *p*-aminobenzoic acid (PABA) and *p*-hydroxybenzoic acid (PHBA) with two carriers, Amberlite LA-2 and di-(2-ethylhexyl)phosphoric acid (D2EHPA) respectively, has been comparatively analyzed and modeled for the pseudosteady-state regime.
The concentration profile of the solute transferred through the liquid membrane is given in Figure 19. According to this, the solute is reactively extracted by the carrier at the interface between the feed phase (F) and membrane phase (M), the formed compound is transferred across the liquid membrane, and the solute is finally re-extracted through the interface between the membrane phase and stripping phase (S).
In the case of pseudosteady-state regime, the aqueous phases are assumed to be perfectly stirred in the steady-state mode, while the membrane phase is perfectly stirred in the unsteady-state mode.
The solutes (AcH) dissociate in the feed phase, either at –COOH groups, or at $-NH_3^+$ group in the case of PABA, with the equilibrium constant:

$$K_a = \frac{C_{F^-} \cdot C_{H^+}}{C_F} \tag{3}$$

Taking into account the distribution coefficients of solutes between the feed phase and membrane phase and between the stripping phase and membrane one, the acids overall concentration and their concentrations in the two aqueous boundary layers on both sides of the liquid membranes, the following expression for the calculation of the permeability factor has been established:

$$P=\frac{C_M-C_S\cdot\left[D_S\cdot\left(1+\frac{Q}{k_W\cdot A}\right)+\frac{Q}{k_W\cdot A}\right]}{D_F\cdot C_F-C_M+\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W}\cdot(1-D_F)}=\frac{C_M-C_S\cdot\left[D_S\cdot\left(1+\frac{Q}{k_W\cdot A}\right)+\frac{Q}{k_W\cdot A}\right]}{\frac{C_M}{1+\frac{K_a}{C_{H^+}}}-C_M+\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W}\cdot\left[1-\frac{C_M}{C_F\cdot\left(1+\frac{K_a}{C_{H^+}}\right)}\right]} \quad (4)$$

Equation (4) represents the model that describes the capacity of liquid membrane to transport the solute between the feed and stripping phases.
Owing to the pseudosteady-state operation regime of the pertraction equipment, the solute is accumulated inside the membrane phase. Thus, the accumulated mass flow can be defined by the expression:

$$n_M=\frac{V}{A}\cdot\frac{\frac{C_o-C_F}{k_W\cdot Q\cdot A}+C_S\cdot\left[D_S\cdot\left(1+\frac{1}{k_W\cdot Q\cdot A}\right)+\frac{1}{k_W\cdot Q\cdot A}\right]\cdot\left[\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{C_o-C_F}{k_W\cdot Q\cdot A}\right)-1\right]}{\left\{1-\left[\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{C_o-C_F}{k_W\cdot Q\cdot A\cdot C_F}\right)-1\right]\cdot P\right\}^2}\cdot\frac{dP}{dt} \quad (5)$$

Equation (5) describes the kinetics of solute accumulation in the liquid membrane.
Finally, by solving Equation (5) for the following initial conditions: for t = 0 P = 0, respectively for t = t P = P, the following mathematical model is obtained:

$$P=\left[\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W\cdot C_F}\right)-1\right]^2\cdot\left[\frac{n_M\cdot\frac{A}{V}\cdot\left[\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W\cdot C_F}\right)-1\right]}{\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W}+C_S\cdot\left[D_S\cdot\left(1+\frac{Q}{k_W\cdot A}\right)+\frac{Q}{k_W\cdot A}\right]\cdot\left[\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W}\right)-1\right]}\cdot t+\frac{1+\frac{K_a}{C_{H^+}}}{1-\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W}}\right]^{-1}-$$

$$\frac{\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W\cdot C_F}\right)-2}{\left[\frac{1}{1+\frac{K_a}{C_{H^+}}}\cdot\left(1-\frac{Q}{A}\cdot\frac{C_o-C_F}{k_W\cdot C_F}\right)-1\right]^2} \quad (6)$$

which indicates the reduction of the permeability through liquid membrane and, consequently, the solute accumulation inside the liquid membrane during the pertraction. This model is the first model reported in literature which describes the kinetics of the solute accumulation inside the liquid membrane for the pseudosteady-state mode.

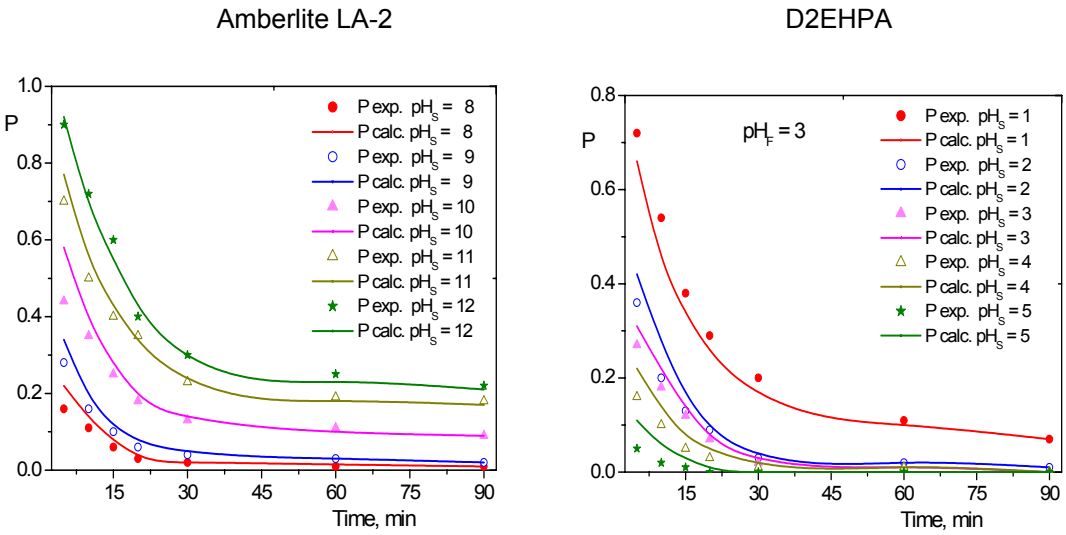

***Figure 20**. Comparison between the experimental and calculated values of PABA permeability factors (carriers concentration = 20 g/L)*

Similar difference has been recorded for the facilitated pertraction with D2EHPA, but its magnitude was less significant.
The correlations between the proposed model and the experimental data are indicated in the Figures 20 and 21.

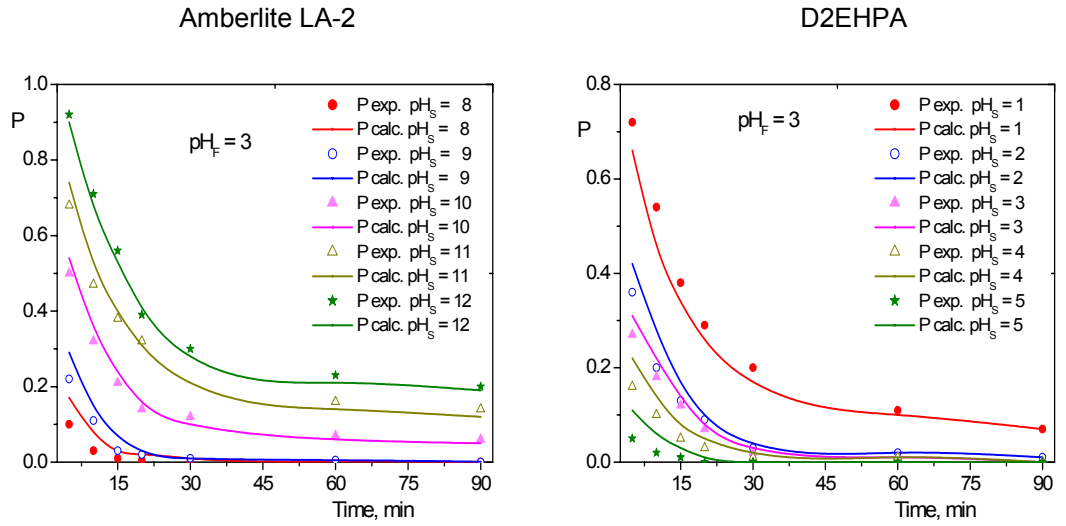

***Figure 21**. Comparison between the experimental and calculated values of PHBA permeability factors (carriers concentration = 20 g/L)*

Therefore, it can be seen the good concordance between the calculated and the experimental values of the permeability factor. However, for the facilitated pertraction with Amberlite LA-2 of both acids, at pH_F below 4 the experimental values of permeability factors are lower than the calculated ones. This difference can be attributed to the supplementary amounts of undissociated acid molecules which are co-extracted by physical solubilization at lower pH_F-values.

But, if the validity of the mathematical model is analyzed at different values of pH_S, it can be observed that at $pH_S = 1$ the experimental data for the permeability factor are higher than the calculated ones. In this case, the difference is the result of the simultaneously re-extraction of the supplementary amounts of PABA and PHBA physically solubilized in the liquid membrane.

FRACTIONATION OF AMINO ACIDS MIXTURE BY PERTRACTION

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 [53]. The separation techniques currently applied for removal and purification of amino acids from dilute aqueous solutions typically employ the ion exchange, crystallization at the isoelectric point or chromatography [11]. 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 reactive extraction became 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 [11, 53 – 59], high molecular weight amines [60 – 62] or some types of crown-ethers [11, 63].

The 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). In this context, the aim of our experiments was to analyze 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) from their mixtures obtained either by fermentation or protein hydrolysis using the facilitated pertraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) in dichloromethane [64]. 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.

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/reextraction and the transport rate through the solvent layer. Thus, from Figure 22 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 obtained results by studying the amino acids reactive extraction [64], 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 $pH_{isoelectric}$). Thus, the interfacial reactions can be described as follows:

- 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

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

where HP is the carrier.

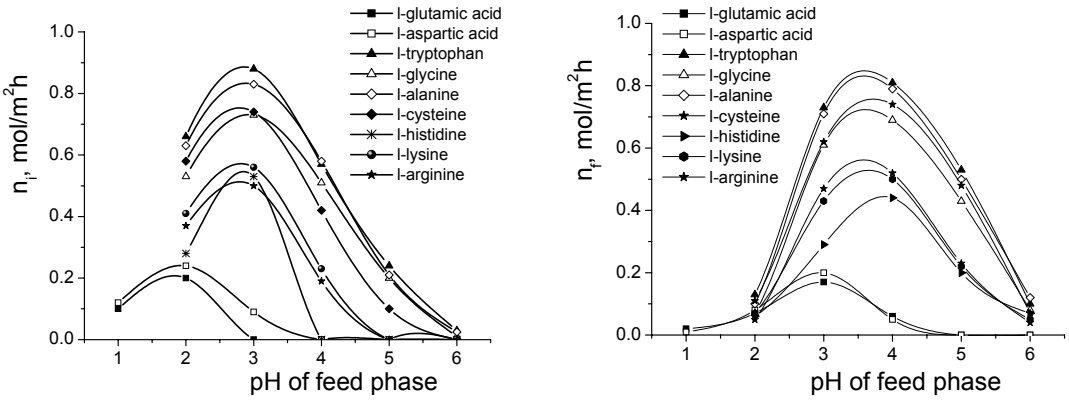

Figure 22. *Influence of pH value of feed phase on mass flows of amino acids (pH of stripping phase = 2, carrier concentration = 40 g/L, rotation speed = 500 rpm)*

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 domain 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 [56]).

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: $n_i = 0$ for $pH_i \geq 4$, L-lysine: $n_i = 0$ for $pH_i \geq 5$, L-arginine: $n_i = 0$ for $pH_i \geq 5$).

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 [56].
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 $pH_i = 3$ for aspartic and glutamic acids, and respectively, at $pH_i = 4$ 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 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 which 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 $pH \geq 3$. 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 Figure 23. For example, although at $pH_i = 2$ all the amino acids are extracted, at $pH_i = 4$ the initial mass flows of L-aspartic acid, L-glutamic acid and L-histidine are 0, for the above presented reasons.

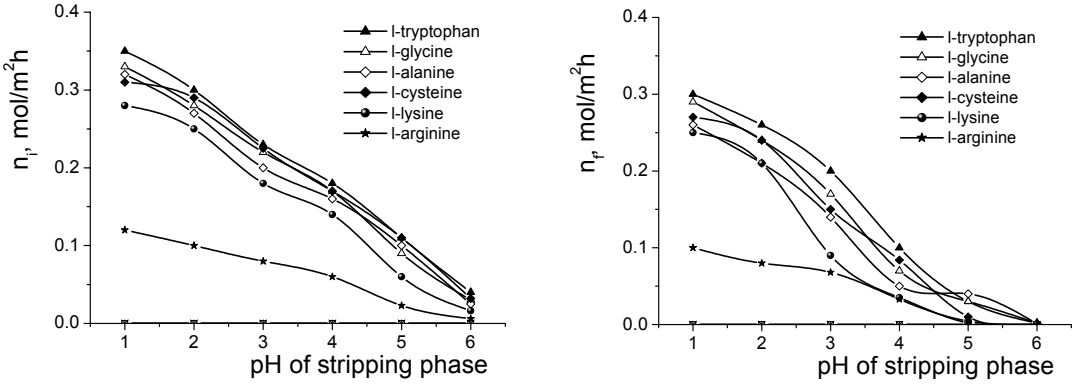

Figure 23. *Influence of pH-value of stripping phase on mass flows of amino acids (pH of feed phase = 4, carrier concentration = 40 g/L, rotation speed = 500 rpm)*

A similar variation has been recorded also for the permeability factors as a function of the pH value of stripping phase (Figure 24). 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 Figure 23, indicates that by increasing the pH_f, 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 Figure 24, 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 Figure 25, obtained at pH_i = 4, and from the similar results for pH_i = 2, it can be observed that the initial and final mass flows of all the amino acids extracted are continuously increased with the increase of D2EHPA concentration in the liquid membrane.

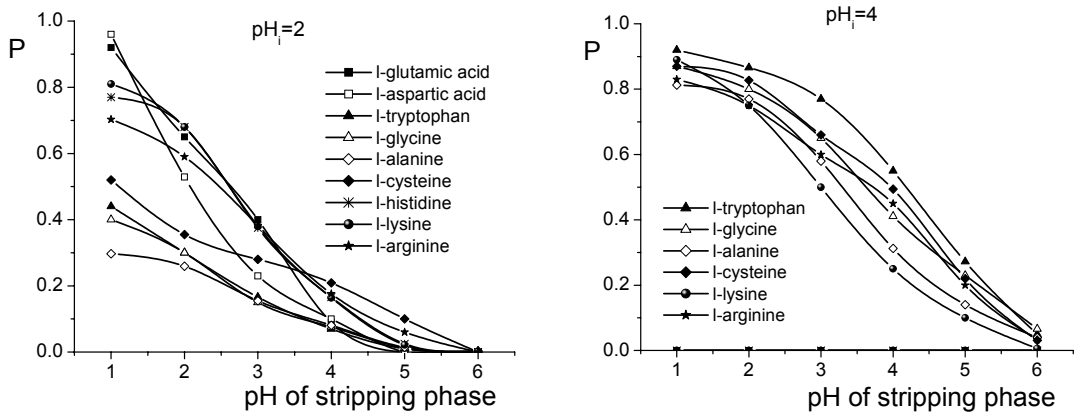

Figure 24. *Influence of pH-value of stripping phase on permeability factors (carrier concentration = 40 g/L, rotation speed = 500 rpm)*

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.

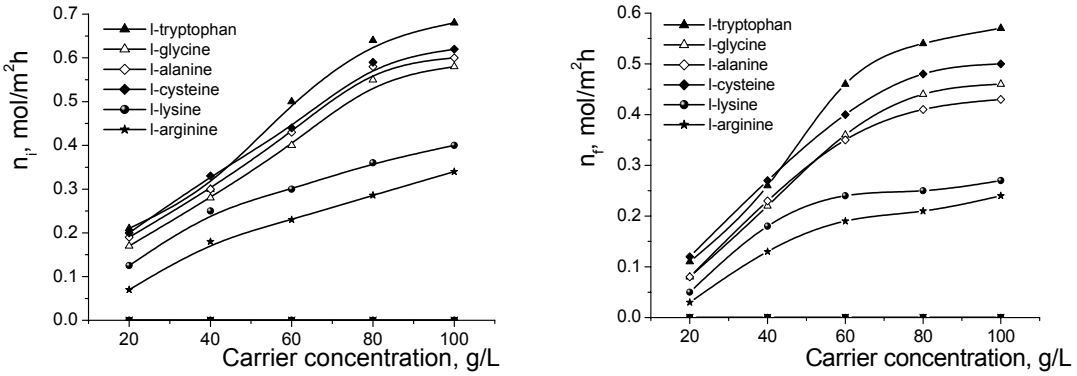

Figure 25. *Influence of D2EHPA concentration on mass flows of amino acids (pH of feed phase = 4, pH of stripping phase = 2, rotation speed = 500 rpm)*

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 permeability factors (Figure 26). 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 500 rpm, 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.

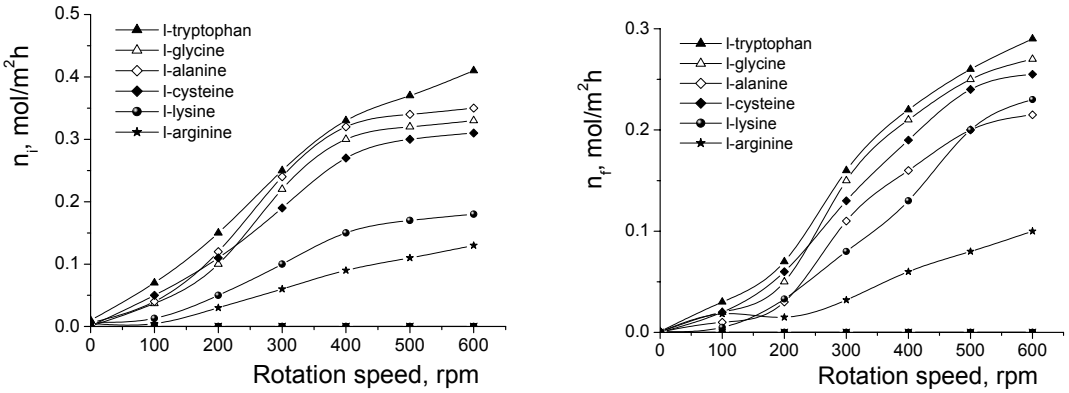

Figure 26. *Influence of mixing intensity on mass flows of amino acids (pH of feed phase = 4, pH of stripping phase = 2, carrier concentration = 40 g/L)*

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

Extraction and transport through liquid membranes, also called pertraction, constitutes advantageous alternative to the conventional separation methods, because it reduces the number of stages required for an efficient separation and, therefore, the corresponding energy and material consumption. For these reasons, this technique has a considerable potential for biosynthetic products separation and purification, being required for further

development of many biotechnologies, and represents very attractive research domain for chemical and biochemical engineers. In the actual context of the "white biotechnology", the studies are dedicated to extending the application area of pertraction for including the separation of other biosynthetic or natural products and to scaling-up this technique at industrial level.

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NOTATIONS

C_o - initial solute concentration in feed phase (mol/L)
C_F - solute concentration in feed phase (mol/L)
$\overline{C_F}$ - overall solute concentration in feed phase (mol/L)
C_{F^-} - dissociated solute concentration in feed phase (mol/L)
C_{H^+} - proton concentration in feed phase (mol/L)
C_{FM} - solute concentration in feed phase at the interface between the feed phase and membrane phase (mol/L)
C_M - solute concentration in membrane phase (mol/L)
C_{MF} - solute concentration in membrane phase at the interface between the feed phase and membrane phase (mol/L)
C_{MS} - solute concentration in membrane phase at the interface between the stripping phase and membrane phase (mol/L)
C_S - solute concentration in stripping phase (mol/L)
C_{SM} - solute concentration in stripping phase at the interface between the stripping phase and membrane phase (mol/L)
D_F - distribution coefficient between feed phase and membrane phase (-)
D_S - distribution coefficient between stripping phase and membrane phase (-)
k_F - mass transfer coefficient in feed phase (m/s)
k_{MF} - mass transfer coefficient in membrane phase at interface between feed phase and the membrane phase (m/s)
k_{MS} - mass transfer coefficient in membrane phase at interface between stripping phase and the membrane phase (m/s)
k_S - mass transfer coefficient in stripping phase (m/s)
k_W - mass transfer coefficients in aqueous phases (m/s)
K_a - acidity index (mol/L)
Q - volumetric flow (L/s)
n - mass flow (mol/(m^{2}s))
n_F - initial mass flow (mol/(m^{2}s))
n_M - accumulated mass flow (mol/(m^{2}s))
n_S - final (overall) mass flow (mol/(m^{2}s))
P - permeability factor (-)

pH_F - pH-value of feed phase (-)
pH_S - pH-value of stripping phase (-)
S - selectivity factor (-)
t - time (s)

subscript
aq - aqueous phase
o - organic phase

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