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Separation of Biosynthetic Products by Pertraction

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1. Introduction

The industrial biotechnology has been considerably developed in the last years, especially for the fine chemicals production and food technologies (Caşcaval & Galaction, 2007). 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 was the major objective until 2010 and remains an economic, technological and social challenge (Daugherty, 2006). This objective can be reached by defining an unitary vision concerning the world industrial biotechnology, by ensuring feasible framework programs for developing biotechnology, 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" 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 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 to 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) (Caşcaval & Galaction, 2007). 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 low-cost raw materials. However, the undesirable particularity of industrial biotechnologies is the complexity of the separation from fermentation broths of the obtained products, especially due to their high dilution in broth, chemical and thermal lability and to the presence of secondary products. Therefore, the purification of biosynthetic compounds requires a laborious succession of separation stages with high material and energy consumption, the contribution of these stages to the overall cost being of 20 - 60%, or even more (Baird, 1991; Schugerl, 1994).

For these reasons, modern techniques have been developed or adapted for the separation of the biosynthetic products. Derived from the "classical" solvent extraction method, some new extraction techniques, namely as: reactive extraction, extraction and transport through liquid membranes, supercritical fluid extraction, two aqueous phases extraction, extraction by reverse micelles, have been experimented and applied at laboratory or industrial scale for bioseparations. One of the most attractive techniques is pertraction, defined as the extraction and transport through liquid membranes. Pertraction consists in the transfer of a solute between two aqueous phases of different pH or other chemical properties value, phases that are separated by a solvent layer of various sizes (Noble & Stern, 1995; Yordanov & Boyadzhiev, 2004; Kislik, 2010). The pertraction efficiency and selectivity could be significantly enhanced by adding a carrier, such as organophosphoric compounds, long chain amines or crown-ethers etc., into the liquid membrane, the separation process being called *facilitated pertraction* or *facilitated transport* (Li, 1978; Teramoto et al., 1990; Juang et al., 1998; Scovazzo et al., 2002; Luangrujiwong et al., 2007; Caşcaval et al., 2009).

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 by using pertraction equipments of special construction, which allow to separate and easily maintain the three phases without adding surfactants (free liquid membranes) (Caşcaval et al., 2009).

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 allows the solute transport against its concentration gradient, as long as the pH-gradient between the two aqueous phases is maintained (Baird, 1991; Schugerl, 1994; Fortunato et al., 2004; Kislik, 2010).

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 selectivities 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.

Because of its generous offer in the field of biosynthetic compounds separation, pertraction represents a continuous challenge for bioengineering and biotechnology. Thus, this Chapter

presents the main results of our experiments on individual or selective separation of some biosynthetic products (antibiotics, carboxylic acids, amino acids, vitamins) by free or facilitated pertraction, using carriers of long chain amines or organophosphoric acids types.

2. Selective pertraction of Penicillin V

The biosynthesis of beta-lactamic 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-lactamic antibiotics with high purity. Although this operation is difficult by using conventional separation techniques due to the similar physical and chemical characteristics, the antibiotics can be selectively separated from their precursors by facilitated pertraction with Amberlite LA-2 in 1,2-dichloroethane (Caşcaval et al., 2000).

For Penicillin V, the experiments emphasized the major role of pH on the permeability through liquid membrane and selectivity of separation of this antibiotic from phenoxyacetic acid. Thus, the permeability factor, P, is positively influenced by increasing the pH-gradient between the two aqueous phases (the permeability factor 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).

Contrary, Figure 1 indicates that the maximum values of selectivity factor, S, correspond to the minimum difference between the pH-values of the aqueous phases (the selectivity factor has been defined as the ratio between the final mass flow of antibiotic and the final mass flow of precursor). Thus, at a constant level of stripping phase pH of 10 and for a pH-value for feed phase of 6, S was 80.4. If the pH-value of feed phase is maintained at 3 and the pH-value of stripping phase is of 7, the value S = 24.2 was obtained.
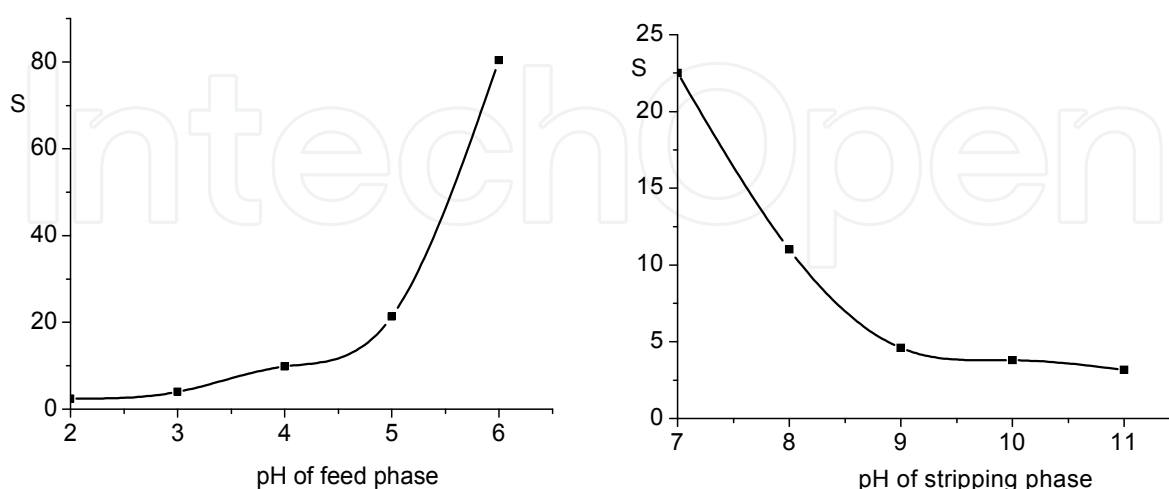

Fig. 1. Effect of feed phase and stripping phase pH-values on the selectivity factor (rotation speed = 500 rpm, carrier concentration = 80 g/l)

Another important factor is the concentration of Amberlite LA-2 inside the liquid membrane. Although the effect of this factor is quite similar for the two components of mixture, at lower carrier concentration the decrease of permeability factor of phenoxyacetic acid is more significant. By increasing the Amberlite LA-2 concentration inside the liquid membrane, the approaching of the permeability factors of the two compounds can be observed. This phenomenon, indicated in Figure 2 by the ratio of permeability factors, suggests that at low carrier concentrations it preferentially reacts with the compound of higher acidity, namely Penicillin V.
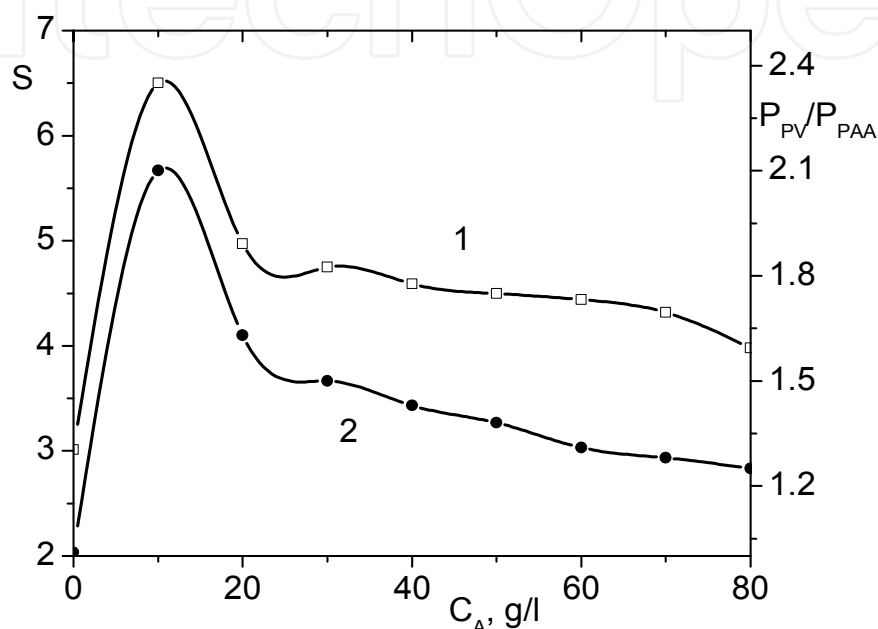

Fig. 2. Effect of carrier concentration on the ratio between permeability factors of Penicillin V and phenoxyacetic acid and on the selectivity factor (pH-value of feed phase = 3, pH-value of stripping phase = 10, rotation speed = 500 rpm;   1 - selectivity factor S, 2 - P_{PV}/P_{PAA})

At high Amberlite LA-2 concentrations, additional amounts of carrier will be available nearly the interface, means that the carrier will react also with the weaker acid, namely phenoxyacetic acid. These results have been found in the variation of selectivity factor, which reached the maximum value of 6.5 for 10 g/l Amberlite LA-2 inside the liquid membrane.

3. Direct pertraction of Erythromycin

Erythromycin is a macrolide antibiotic biosynthesized by *Streptomyces erythreus* on glucose substrate, being very active against the infections produced by *staphylococcus*, gram-positive *bacterium*, etc. Erythromycin exhibits a significant inhibitory effect, this leading to the diminution of microbial activity or cells lysis with the antibiotic accumulation in the broth (Galaction & Caşcaval, 2006). The phenomenon can be avoided by direct removal of antibiotic during the fermentation process.

At industrial scale, the antibiotic separation from fermentation broths is carried out by physical extraction with butyl acetate, with or without preliminary filtration of biomass, followed by its reextraction with diluted solutions of hydrochloric acid. Due to

Erythromycin dissociation and to the low polarity of butyl acetate, the physical extraction is possible only in alkaline pH-domain, the maximum extraction yields being reached for the pH-value of aqueous phase greater than 9 (Galaction & Caşcaval, 2006). In these conditions, some other components of fermentation broths, which are non-dissociated at the extraction pH-value, can be supplementary extracted, the ulterior purification of the antibiotic becoming more difficult.

For the above mentioned reason, a new separation method of Erythromycin from aqueous solutions by reactive extraction with di-(2-ethylhexyl) phosphoric acid (D2EHPA) has been investigated (Caşcaval & Galaction, 2004). This method has been developed and applied as facilitated pertraction, the addition of D2EHPA inside the liquid membrane allowing to increasing the antibiotic mass flow compared to the free pertraction without any carrier (Kawasaki et al., 1996; Caşcaval et al. 2007). But, in the case of media possessing rheological behavior and apparent viscosity similar to the *S. erythreus* broths, the efficiency of pertraction was strongly affected. The increase of apparent viscosity of feed phase from 1 to 30 cP led to the maximum decrease of antibiotic mass flows by the factors of 42.5 and 7.5 for free and facilitated pertraction, respectively (Galaction et al., 2009).

Similar to the direct extraction of other biosynthetic compounds from the fermentation broths (Katikaneni & Cheryan, 2002; Monteiro et al., 2005; Vijayakumar et al., 2008; Kang & Sim, 2008), the presence of biomass could supplementary affect the Erythromycin pertraction, owing to the following phenomena: the appearance of supplementary resistance to the antibiotic transfer from the feed phase to the liquid membrane due to the physical barrier induced by the cell adsorption to the interface; the increase of the apparent viscosity of the feed phase, and, consequently, the amplification of antibiotic diffusional resistance; the mechanical lysis of cells, as the result of the shear stress promoted by the impellers, with the release of the cytoplasmatic compounds which can be co-extracted (amino acids) or can precipitate (proteins).

The study on Erythromycin pertraction from aqueous solutions or simulated broths indicated that the free pertraction is not possible for the pH-value of feed phase, pH_f, lower than 4, due to the pronounced antibiotic ionization (Caşcaval et al. 2007; Galaction et al., 2009). By increasing the pH_f above this level, both the initial and final mass flows are strongly increased, as the result of the increase of physical extraction efficiency. This dependence between the mass flows and the pH of feed phase is respected also in the case of Erythromycin free pertraction from *S. erythreus* suspensions (Figure 3).

But, the accumulation of biomass led to the significant decrease of the initial mass flow (by increasing the biomass concentration from 0 to 20 g/l d.w., the initial mass flow has been reduced for about 7 times).

The increase of the stripping phase pH-value, pH_s, leads to the significant reduction of the antibiotic initial mass flow, this effect becoming more pronounced with the microorganism accumulation in the feed phase. In this case, the negative influence of the biomass is amplified by increasing pH_s, as the result of the supplementary effect of the neutral domain of pH_s (by increasing the biomass concentration from 0 to 20 g/l d.w., the initial mass flow of Erythromycin decreasing for about 5.8 and 19.2 times at pH_s of 2 and 7, respectively). The decreasing of the final mass flow is more important, this parameter reaching the value 0 for pH_s over 7 (at this value of pH_s the pH-gradient between the feed and stripping phases becomes 0). The increase of *S. erythreus* concentration induces the supplementary decrease of antibiotic final mass flow. Thus, comparatively with the pertraction from water, at 20 g/l

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d.w. *S. erythreus* the final mass flow of Erythromycin is reduced for about 2.8-5.8 times, this effect being amplified at lower values of pH_s.
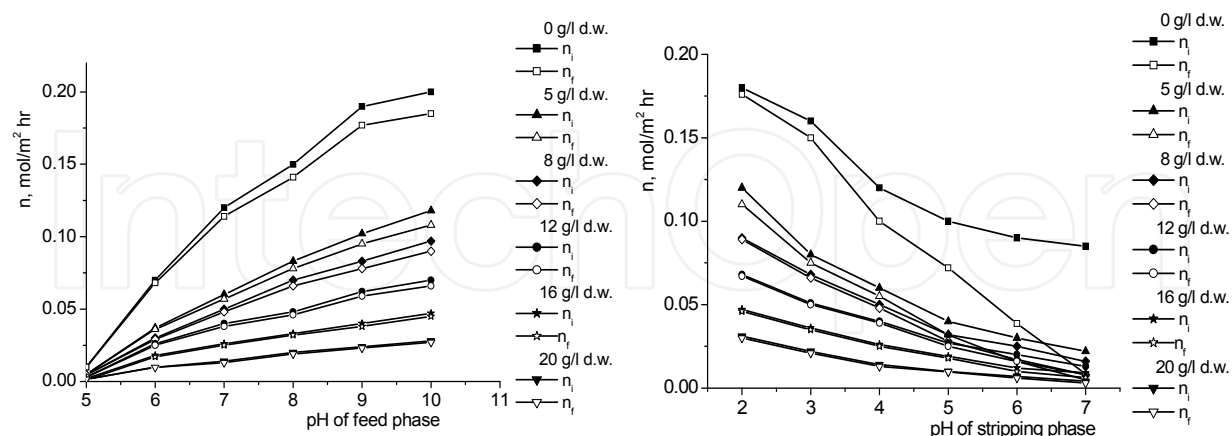

Fig. 3. Influence of pH-values of feed and stripping phases on initial, n_i, and final, n_f, mass flows of Erythromycin for free pertraction from *S. erythreus* broths (rotation speed = 500 rpm)

Independently on the biomass concentration in the feed phase, the permeability factor is continuously reduced by the increase of pH_f over 4, this suggesting that pH_f exhibits a more important influence on the initial mass flow than on the final one (Figure 4).
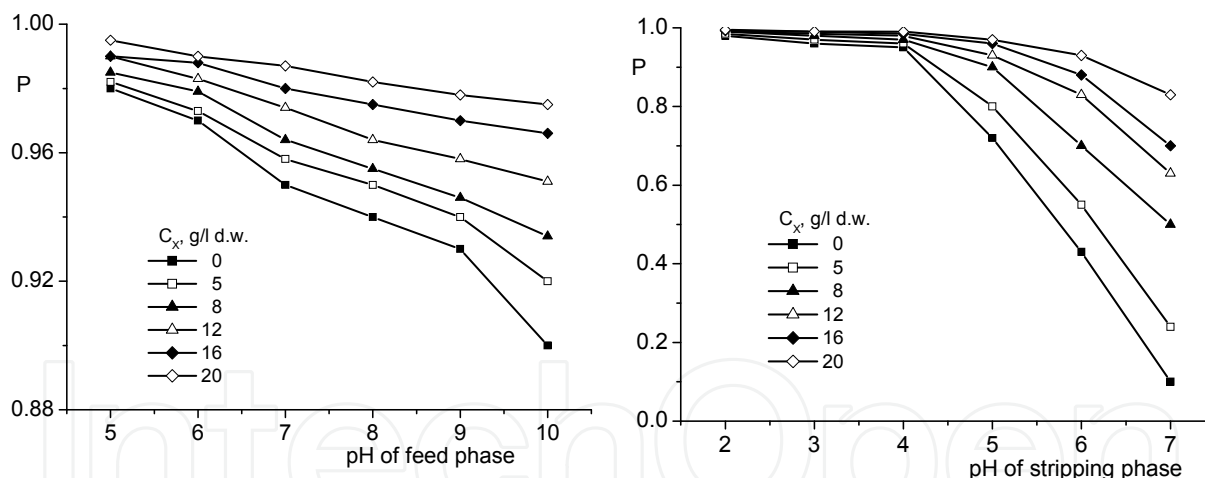

Fig. 4. Influence of pH-values of feed and stripping phases on permeability factor for free pertraction from *S. erythreus* broths (rotation speed = 500 rpm)

The magnitude of the effect of pH_f is diminished with the biomass accumulation, because the amount of antibiotic extracted in the liquid membrane becomes lower, thus facilitating its almost complete reextraction in the stripping phase.

For quantifying the effect of biomass presence, the *reduction factor*, F, has been defined as the ratio between the initial mass flows corresponding to the pertraction from *S. erythreus* broths and simulated ones (without biomass), F_i, respectively between the final mass flows recorded for the same two cases, F_f. The influence of the biomass is clearly underlined in the Figure 5, being recorded the reduction of over 3 times of the factors F_i and F_f with the accumulation of biomass to 20 g/l d.w. This effect is stronger for *S. erythreus* concentration

increase up to 5 g/l d.w., thus emphasizing the important role of solid phase in hindering the pertraction.

The addition of the carrier, di-(2-ethylhexyl) phosphoric acid, D2EHPA, in the liquid membrane offers the possibility to carry out the pertraction also at pH-values lower than 4, due to the modification of the mechanism of Erythromycin extraction in the dichloromethane phase. Thus, the previously proposed and verified mechanism of antibiotic reactive extraction with D2EHPA occurs by means of an interfacial reaction of ionic exchange type controlled by the pH of aqueous phase (Caşcaval & Galaction, 2004):

$$Er - N^+H\left(CH_3\right)_{2\,(aq)} + HP_{(o)} \rightleftharpoons Er - N^+H\left(CH_3\right)_2 P^-_{(o)} + H^+_{(aq)}$$
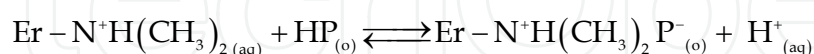
where HP is the carrier. According to the extraction mechanism, the carrier reacts only if Erythromycin exists in aqueous solution in its cationic forms, therefore at an acidic pH-value of the solution ($pH_f < 4$).
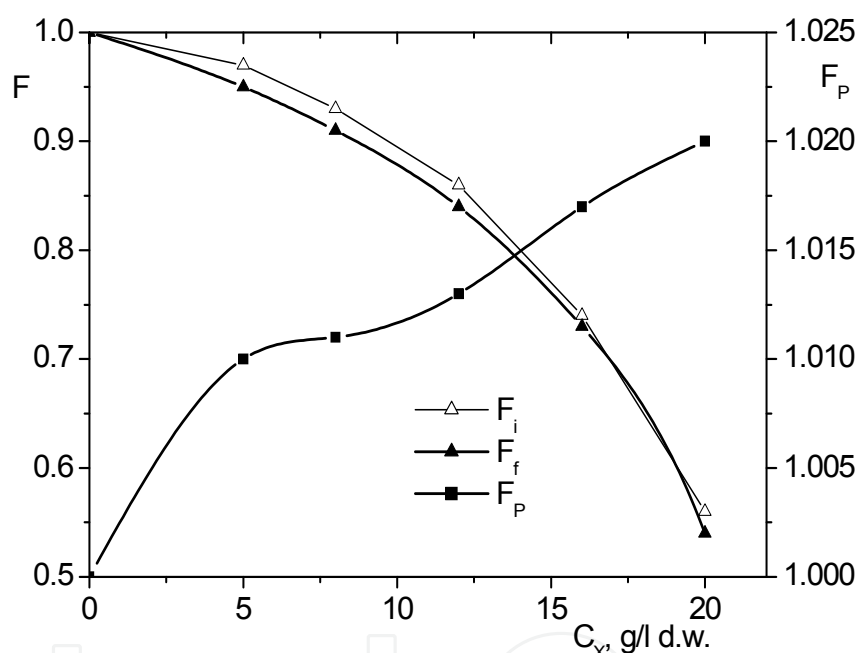

Fig. 5. Influence of biomass concentration, C_X, on factors F and F_P for facilitated pertraction from *S. erythreus* broths (pH of feed phase = 4, pH of stripping phase = 2, carrier concentration = 40 g/l, rotation speed = 500 rpm)

In the case of facilitated pertraction, the accumulation of biomass from 0 to 20 g/l d.w. led to the reduction for about 1.8 times of the factors F_i and F_f. Comparatively to the free pertraction, the magnitude of this effect is attenuated by D2EHPA addition, which increases the initial mass flows of Erythromycin. The dependence between the factor F_P and biomass concentration is opposite to those describing the variation of mass flows ratios. According to those concluded for the free pertraction, the accumulation of *S. erythreus* induces the equalization of the final and initial mass flows. For this reason, the permeability factors are greater for the facilitated pertraction from *S. erythreus* broths than those for the facilitated pertraction from simulated broths, thus leading to the increase of the factor F_P with the biomass concentration.

4. 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. 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 (Korzybski, 1978; Williams & Lemke, 2002). 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) (Isoherranen & Soback, 2000; Silverman, 2004). The biosynthetic complex contains also the active Gentamicin C_{2b}, but its concentration is very low. The chemical structures of the major Gentamicins are indicated in Figure 6.

Gentamicin C_1 : $R_1 = R_2 = CH_3$
Gentamicin C_1a : $R_1 = CH_3$, $R_2 = H$
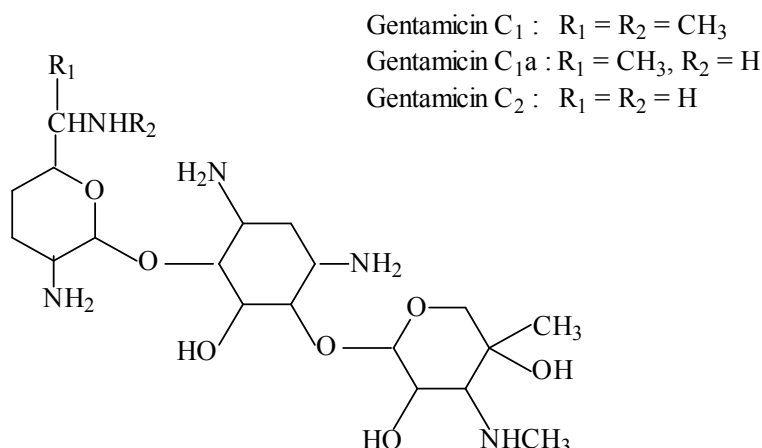Gentamicin C_2 : $R_1 = R_2 = H$

Fig. 6. 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% (Yoshizawa et al., 1998). The antibacterial activities of the Gentamicins, respectively their affinities for the bacterial ribosomes, are 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) (Rosenkrantz et al., 1980).

The separation of Gentamicin from the fermentation broths at industrial scale is achieved by sorption by cation-exchangers, followed by its 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 (Savitskaya et al., 1982). But, this technique doesn't allow the fractionation of the complex mixture of Gentamicins, the use only of Gentamicins C_{1a} and C_2 improving the specific biological activity per weight unit of antibiotic.
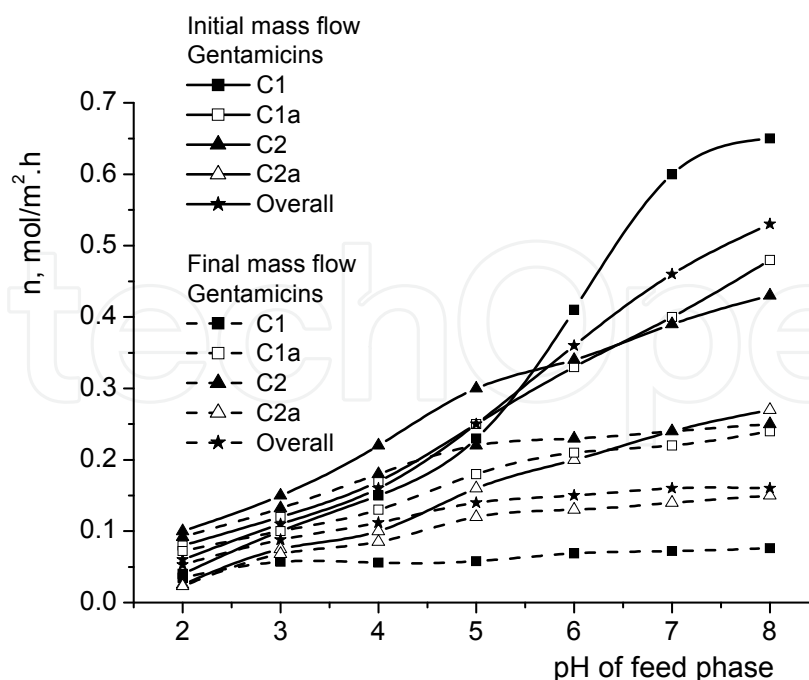
Fig. 7. Influence of pH-value of feed phase on mass flows of Gentamicins (pH of stripping phase = 1.5, D2EHPA concentration = 20 g/l, rotation speed = 500 rpm)

The investigations on the reactive extraction of Gentamicins (Caşcaval et al., 2007) have been developed by studying the possibility to fractionate the biosynthetic mixture of Gentamicins by facilitated pertraction with D2EHPA dissolved in dichloromethane as liquid membrane (Galaction et., 2008). The influence of pH-gradient on the Gentamicins pertraction 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 7 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 (Caşcaval et al., 2007):

$$\text{Gentamicin}^{n+}_{(aq)} + n\,HP_{(o)} \rightleftharpoons \text{Gentamicin}.nHP_{(o)} + n\,H^{+}_{(aq)}$$
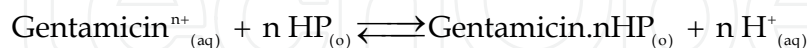
where Gentamicin^{n+} represents the antibiotic with protonated aminic groups, and HP the carrier, respectively (n = 1 - 5). The aminic groups of Gentamicins are involved in the reactive extraction, the interactions between the antibiotic and extractant being of ionic type. Gentamicins possess five aminic groups, which could react with the extractant, similar to the reaction with sulfuric acid in the desorption process from the cation-exchangers (Savitskaya et al., 1982). 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.

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:

$$\text{gentamicin } C_2 > \text{gentamicin } C_{1a} > \text{gentamicin } C_1 > \text{gentamicin } C_{2a}.$$

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 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. 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 to the above results, from Figure 8 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.

The increase of stripping phase pH-value induces the significant reduction of initial and final mass flows of all Gentamicins (Figure 9). This variation is controlled by the re-extraction mechanism, which is based on the interfacial reaction between the Gentamicins-D2EHPA salts and five equivalents of sulfuric acid for each mole of Gentamicin (Savitskaya et al., 1982; Caşcaval et al., 2007):

$$\text{Gentamicin.HP}_{(o)} + 5/2\,H_2SO_{4(aq)} \rightleftharpoons \text{Gentamicin}^{5+}.5/2\,SO_4^{2-}{}_{(aq)} + HP_{(o)}$$
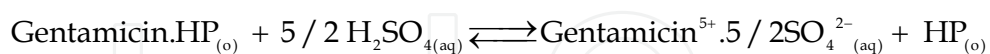
The reactivity of Gentamicins in the reaction with sulfuric acid is determined also by the basicity of their specific aminic groups, because they control the strength of the ionic interactions between the antibiotic and the carrier, and therefore the easiness of the antibiotic release from the membrane phase. Figure 9 indicates 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. 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.
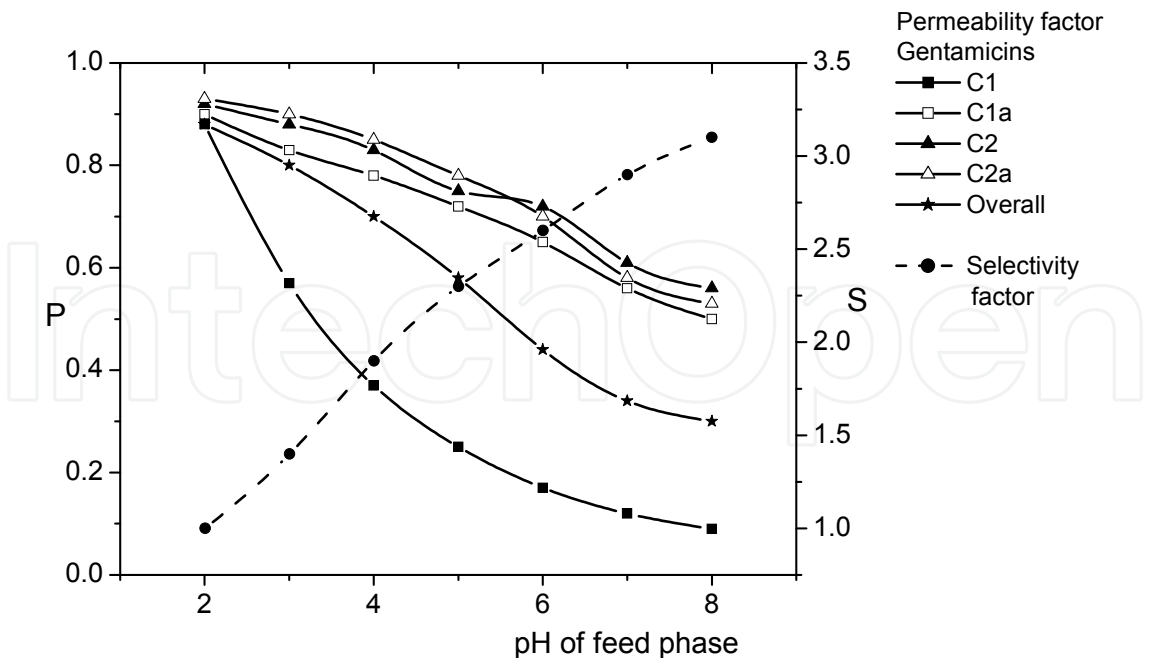
Fig. 8. Influence of pH-value of feed phase on permeability and selectivity factors (pH of stripping phase = 1.5, D2EHPA concentration = 20 g/l, rotation speed = 500 rpm)
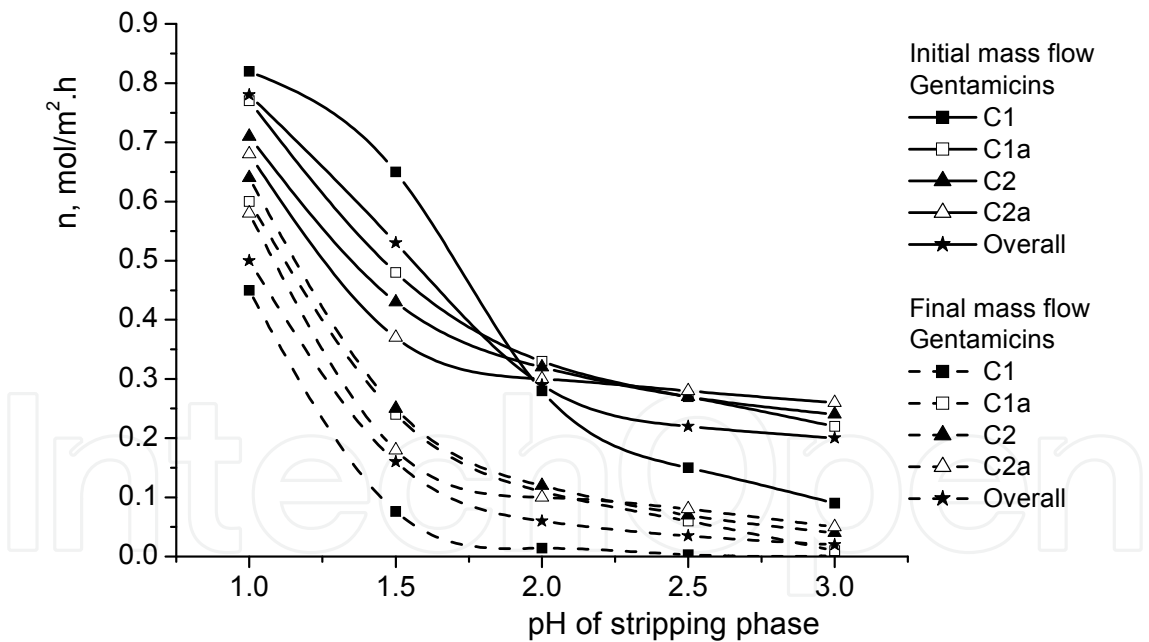

Fig. 9. Influence of pH-value of stripping phase on mass flows of Gentamicins (pH of feed phase = 8, D2EHPA concentration = 20 g/l, rotation speed = 500 rpm)

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 discussed reasons.

The increase of carrier concentration into the liquid membrane induces the increase of the initial and final mass flows of all Gentamicins, but the basicity of the specific aminic groups controls the magnitude of this influence. According to the results obtained for reactive extraction of Gentamicins (Caşcaval et al., 2007), if the carrier exists in a stoichiometric 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.
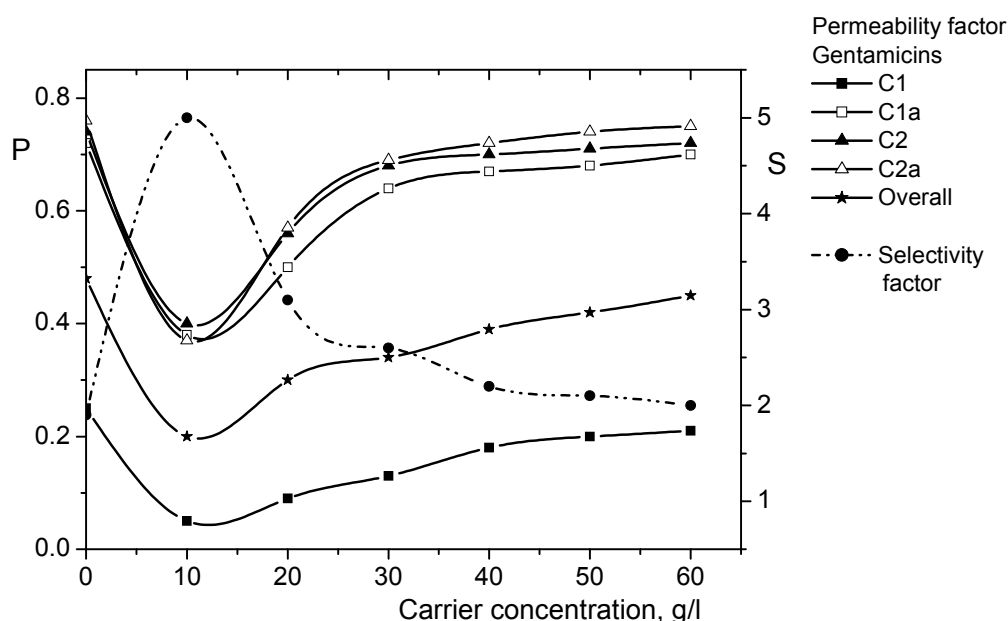

Fig. 10. Influence of carrier concentration on permeability and selectivity factors (pH of feed phase = 8, pH of stripping phase = 1.5, rotation speed = 500 rpm)

The variation of the selectivity factor with carrier concentration is opposite to that of the permeability factors (Figure 10). 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 diminished for about 2.5 times by increasing the carrier concentration in the liquid membrane from 10 to 60 g/l.

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. By removing Gentamicin C_1 from the biosynthetic mixture the biological activity of the antibiotic is enhanced and the therapeutic dose is reduced, its secondary effects being diminished.

5. Selective pertraction of carboxylic acids obtained 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 (Moo-Young et al., 1985). 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. 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. 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 (Moo-Young et al., 1985). This technology needs high amount of raw materials and energy consumption and produces large amounts of calcium sulfate as the by-product, without leading to high purity of citric acid.
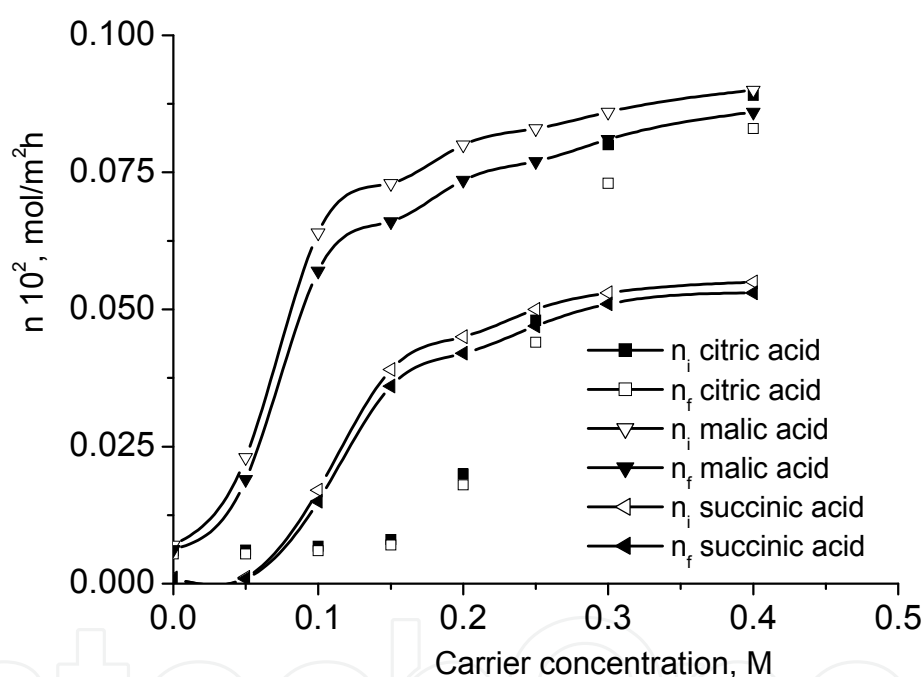

Fig. 11. Influence of carrier concentration on citric, malic and succinic acids mass flows (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, rotation speed = 500 rpm, pH of feed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

Based on 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 (Caşcaval et al., 2004a). In the case of these acids pertraction from a mixture, the dependence of their mass flows on the pH gradient has been 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, was 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, on the other hand.

The permeability factors of all studied acids tended 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 diminution 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.

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 hydrophobicity. As it can be seen from Figure 11, by increasing the carrier concentration the malic acid, succinic acid and citric acid are successively pertracted.
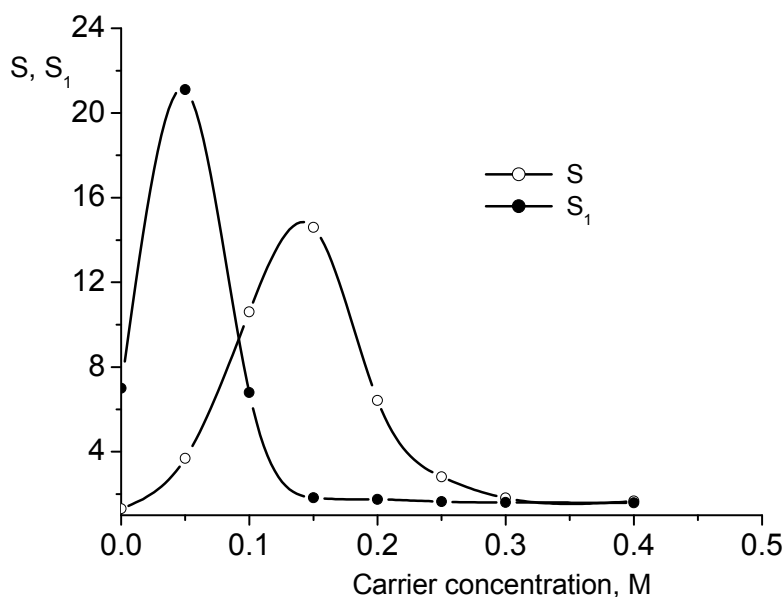

Fig. 12. Influence of carrier concentration on selectivity factors (citric acid concentration in feed phase = 7.8×10^{-2} M, malic acid concentration in feed phase = 7.8×10^{-2} M, succinic acid concentration in feed phase = 7.8×10^{-2} M, rotation speed = 500 rpm)

The succinic acid is extracted after the Amberlite LA-2 concentration exceeds the value stoechiometrically needed for the 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 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 on pertraction selectivity.

The above discussed results suggested the possibility to selectively remove the malic and succinic acids, the citric acid remaining in the raffinate phase. For confirming this hypothesis and establishing 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 factors S and S_1. The selectivity factor S was introduced for the separation of malic and succinic acids from citric acid, and it was defined as the ratio between the sum of the final mass flows of malic and succinic acids and the final mass flow of citric acid. The factor S_1 was used for the separation of malic acid from succinic acid, being the ratio between the final mass flow of malic acid and that of citric acid.

The reduction of pH gradient leads to the increase of selectivity factors S and S_1, but the magnitude of this effect 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.

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.

In order to verify the above conclusions, initially 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, and 2.5 g/l (1.9×10^{-2} M) succinic acid, respectively. 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: carrier concentration of 0.04 M, rotation speed of 500 rpm, pH of feed phase of 4 and pH of stripping phase of 11. The obtained results indicated that, by combining the favorable effects of pertraction parameters, superior values of selectivity factors can be obtained: S = 24.5, S_1 = 47.5.

6. Synergetic pertraction of p-aminobenzoic acid

p-Aminobenzoic acid (PABA), also called vitamin B_{10} or factor R, was found to be part of the folic acids. Because it is 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. The most recent methods for PABA production are the chemical synthesis using methyl-4-formylbenzoate as the starting material or biosynthesis by mutant strains of *E. coli* (Amaratunga et al., 2000; Park et al., 2003). In both cases the separation stages are complex and require the consumption of a large amount of energy and materials.

Due to the insolubility of PABA in organic solvents immiscible with water, its separation by physical extraction is impossible. But, owing to the chemical structure of PABA which contains an acidic group, -COOH, and a basic one, $-NH_2$, the reactive extraction has been taken into consideration and has been performed using extractants of aminic and organophosphoric acid types, namely Amberlite LA-2 and (D2EHPA), respectively (Galaction et al., 2010). Because the formation of the third phase has been observed during the reactive extraction of PABA, the mechanisms and, consequently, the factors which

control the mechanisms of acid extraction with the two extractants in presence of 1-octanol as phase modifier have been also investigated. On the basis of the experiments on the synergetic reactive extraction of PABA, the facilitated pertraction of this acid using a liquid membrane without and with 1-octanol has been comparatively analyzed from the viewpoint of the influences of the process parameters (pH gradient between the feed and stripping phases, carrier concentration, mixing intensity) (Kloetzer et al., 2010).
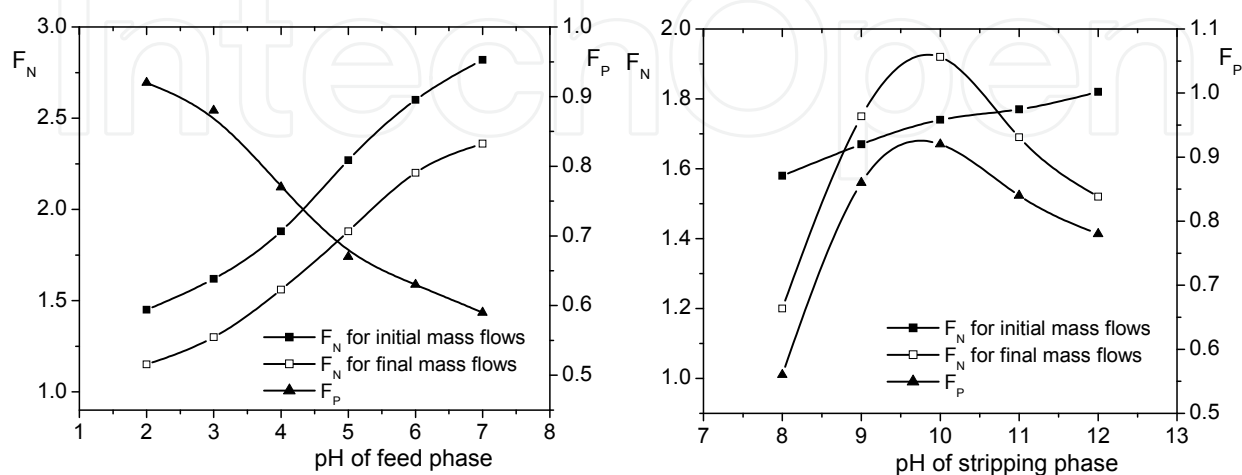

Fig. 13. Influence of pH-values of feed and stripping phases on factors F_N and F_P (Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm, 1-octanol concentration = 10% vol.)

In order to quantify the effect of 1-octanol addition inside the liquid membrane on the initial and final mass flows, the factor F_N has been considered and defined as the ratio between the mass flows recorded in presence and in absence of alcohol. Similarly, the factor F_P has been calculated as the ratio between the permeability factors reached for liquid membrane with and without 1-octanol.

The dependence of the values of factors F_N and F_P on the feed phase pH, plotted in Figure 13, suggests that the addition of 1-octanol exhibits two different effects. The factor F_N, calculated either for the initial mass flows or for the final ones, is greater than the unit for the entire considered pH_F-domain and increases with the increase of pH_F. Thus, for pH_F variation from 2 to 7, F_N increased from 1.5 to 2.9 for the initial mass flows, respectively from 1.2 to 2.4 for the final mass flows. These results are the consequence of the favorable effect of 1-octanol on the solubilization of PABA molecules, free or bounded to the carrier molecules, on the membrane phase. The increase of pH_F induces the dissociation of PABA in the feed phase, the presence of 1-octanol allowing also the extraction of the dissociated molecules of acid. The relative magnitude of the positive effect of alcohol addition is superior in the case of the initial mass flow, due to the supplementary kinetic resistance to the acid reextraction process from the membrane phase to the stripping solution.

Contrary, the values of factor F_P are lower than 1 for the entire experimented domain of the feed phase pH, the increase of pH_F inducing the reduction of this factor. In this case, the significant increase of the initial mass flow of PABA by adding 1-octanol inside the liquid membrane exceeds the membrane capacity to transport the acid and to release it into the stripping phase.

Similar to the influence of pH_F, the factors F_N are superior to 1 for all the considered pH_S-values, but the influence of pH_S has to be distinctly analyzed for the initial and final mass

flows ratios. Thus, the factor F_N, calculated as the ratio between the initial mass flows, increased slowly with the increase of stripping phase pH, from 1.6 to 1.8. The variation of factor F_N related to the final mass flows with pH_S indicated its increase to a maximum level, corresponding to $pH_S = 10$, followed by its decrease. The maximum F_N (1.9) exceeded that obtained for the initial mass flows indifferent of pH_S-value, due to the more important influence of pH_S on the PABA reextraction step from the membrane phase.

The variation of F_P follows that of F_N calculated for the final mass flows, the two factors being directly correlated. Moreover, for the entire investigated domain of stripping phase pH, the value of F_P was lower than 1 (maximum F_P was 0.92).

Due to its favorable effect on PABA extraction from the feed phase into the liquid membrane, the addition of 1-octanol in dichloromethane induces the increase of the initial and final mass flows of the acid. Thus, for 40 g/l Amberlite LA-2 and alcohol concentration variation from 5 to 20% vol., the initial mass flow was amplified for about 1.4-2.2 times and the final mass flow for about 1.1-1.6 times compared with the corresponding mass flows in absence of 1-octanol (Caşcaval et al., 2009). This effect is more significant for the initial mass flow, because the reextraction rate tended to its maximum level for the given experimental conditions. For the same reason, the permeability factor was increased slowly from 0.4 to 0.7 by increasing the alcohol concentration inside the membrane phase.

7. 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. The main utilization of cinnamic acid is in the cosmetic/perfumery industry, 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 (Saraf & Simonyan, 1992; Tawata et al., 1996; Lee et al., 2004). It is also used in food, or for the synthetic ink, resins, elastomers, liquid crystalline polymers and adhesives production.

The cinnamic acids 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 (Bartova et al., 2002; Palma et al., 2002; Smelz et al., 2004; Naczk & Shahidi, 2004). The cinnamic acid is synthesized from styrene and carbon tetrachloride, by oxidation of cinnamic aldehyde, or from benzyl dichloride and sodium acetate. The chemical methods are expensive due to the costs of the starting materials, the high number of required stages for product purification, and generated 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. Thus, *Saccharomyces cerevisiae*, *Escherichia coli*, *Pseudomonas sp.* have been cultivated on glucose, and *Cellulomonas galba* on n-paraffins with addition of alkylbenzenes (Parales et al., 2002). 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.* (Hanson & Havir, 1981; Naczk & Shahidi, 2004).

Excepting from our works, 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 became 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 (Camarut et al., 2006). This technique has been considered for developing the cinnamic and p-methoxycinnamic acids selective pertraction with Amberlite LA-2 (Galaction et al., 2007). 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.
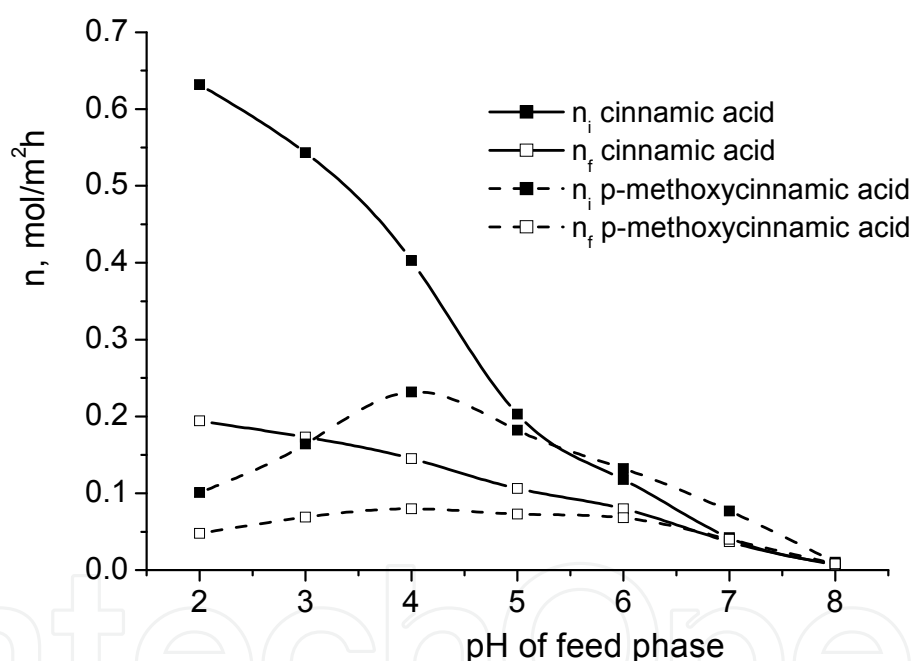

Fig. 14. Influence of pH-value of feed phase on mass flows of cinnamic and p-methoxy-cinnamic acids (pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm)

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 and Amberlite LA-2. 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. The initial mass flow of cinnamic acid continuously decreased 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.

For pH-values bellow 5, the initial mass flow of cinnamic acid exceeded 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 (Weast, 1974)). 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. 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.
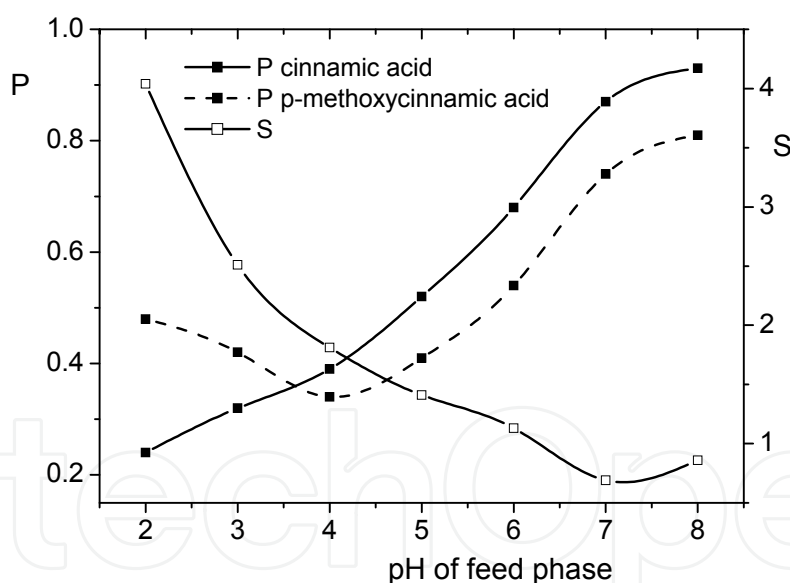

Fig. 15. Influence of pH-value of feed phase on permeability and selectivity factors (pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm)

The 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 to 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.

The increase of carrier concentration into the liquid membrane induces the increase of the initial and final mass flows of both acids. At the concentration of Amberlite LA-2 bellow 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 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.

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.

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.

8. 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 (Liu & Dai, 2003). 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 (Caşcaval et al., 2004b). 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 used technology.

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 (Kelly et al., 1998; Liu et al., 1999; Caşcaval et al., 2001; Juong & Wang, 2002; Lin et al., 2006; Lin & Chen, 2006), high molecular weight amines (Rehm & Reed, 1993; Schugerl, 1994; Tan et al., 2007) or some types of crown-ethers (Deblay et al., 1990).

The pertraction could be also used for amino acids separation, the proper carrier being chosen from the above listed extractants (organophosphoric acid, high molecular weight amines or crown-ethers). In this context, 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 D2EHPA in dichloromethane has been analyzed (Blaga et al., 2008).

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 16 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.
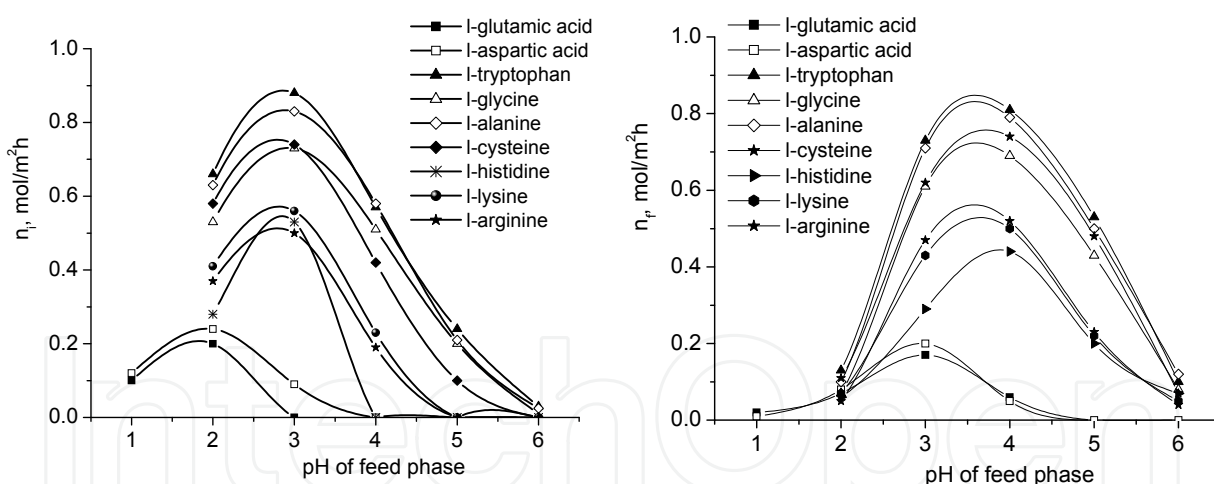

Fig. 16. Influence of pH value of feed phase on mass flows of amino acids (pH of stripping phase = 2, carrier concentration = 40 g/l, rotation speed = 500 rpm)

The 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 (Caşcaval et al., 2001), 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 $pH_{isoelectric}$). 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 (Caşcaval et al., 2001)). 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 (Caşcaval et al., 2001).

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 at $pH_i = 4$ for the rest of amino acids, respectively. 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, 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 caused the reducing of both initial and final mass flows of the amino acids that can be extracted at the prescribed pH of feed phase. 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.

A similar variation has been recorded also for the permeability factors as a function of the pH value of stripping phase (Figure 17). 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, indicated 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 17, 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 hydrophobicity, which promote the reextraction in the stripping phase.

Therefore, 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.
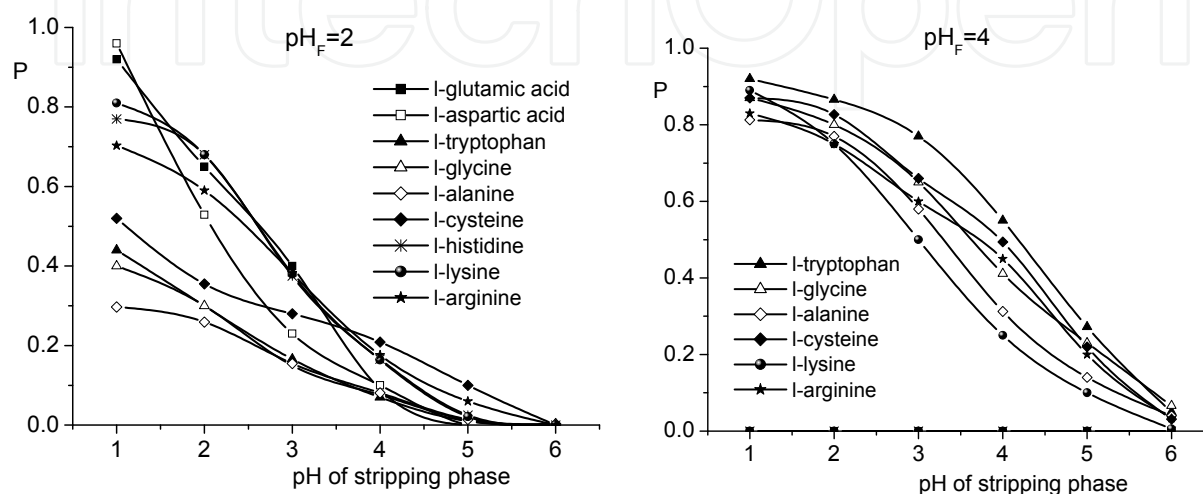

Fig. 17. Influence of pH-value of stripping phase on permeability factors (carrier concentration = 40 g/l, rotation speed = 500 rpm)

9. 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 in the field of bioseparations 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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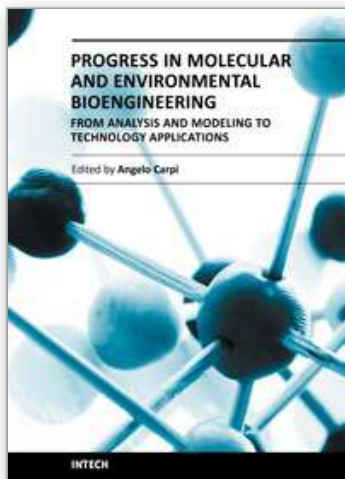
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