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Selective Separation of Cinnamic and p-Methoxycinnamic Acids by Facilitated Pertraction

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Abstract: Cinnamic acid can be selectively separated from p-methoxycinnamic acid by facilitated pertraction with Amberlite LA-2 dissolved in dichloromethane, using a U-shaped pertraction cell which allows obtaining the free liquid membrane. The pertraction has been analyzed by means of initial and final mass flows, permeability and selectivity factors. The main factors which control the pertraction selectivity were identified to be the pH-gradient between the feed and stripping phase and the mixing intensity of the aqueous phases. The maximum selectivity factor has been recorded for pH = 2 of feed phase, pH = 8 of stripping phase, rotation speed lower than 300 rpm, and carrier concentration higher than 40 g/l.

Keywords: Cinnamic acid, p-methoxycinnamic acid, Amberlite LA-2, D2EHPA, pertraction, mass flow, permeability factor, selectivity factor

INTRODUCTION

The cinnamic acid, also known as phenylacrylic acid, is a natural compound derived from phenylalanine, its main vegetable sources being the cinnamon,

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the resin of *Liquidambar* tree, the storax, the balsam of tolu, and the balsam of Peru (Fig. 1).

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 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 have different pharmaceutical applications, for pulmonary affections, cancer, lupus, infectious diseases (diarrhea, dysentery) possessing antibacterial and antifungal activity (1–3). It is used in food, or for the synthetic ink, resins, elastomers, liquid crystalline polymers and adhesives production.

Among the two isomers of the cinnamic acid, the isomer *trans* is the most encountered and exhibits the highest biological activity (Fig. 1). 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 (4).

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 (5–8). The cinnamic acid is synthesized from styrene and carbon tetrachloride, by oxidation of cinnamic aldehyde, or from benzyl dichloride and sodium acetate (9, 10). However, the chemical methods are expensive due to the costs of the starting materials, the high number of required stages for product purification, and the generation of 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. To this purpose, Saccharomyces cerevisiae, Escherichia coli, Pseudomonas sp. have been cultivated on glucose, and Cellulomonas galba on n-paraffins with addition of alkylbenzenes (11, 12). 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

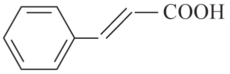

Figure 1. Chemical structure of *trans*-cinnamic acid (3-phenyl-2-propenoic acid, phenylacrylic acid).

into the media by the mutant strains of E. coli, Rhodotorula sp., Rhodosporidium sp., Sporobolomyces sp., Rhizoctonia solani, Trichosporon cutaneum, Rhodobacter sp. (10, 13–15).

Except from our previous work (16–18), 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 into the solvent, this compound reacting with the cinnamic acids and leading to the formation of hydrophobic derivatives (reactive extraction). According to the earlier results, the reactive extraction of the cinnamic acids becomes efficient if an extractant of aminic type (Amberlite LA-2) is used (16, 17).

The experimental data previously obtained for the reactive extraction of cinnamic and p-methoxycinamic acids indicated the possibility to selectively separate the cinnamic acid from their mixture at the aqueous phase pH-value of 2–3, domain corresponding to the maximum difference between the extraction yields of the two acids (17).

The reactive extraction can be developed by solute extracting and transporting through liquid membranes, a technique called pertraction or permeation through liquid membranes. The principle of this separation method consists in the transfer of a solute between two aqueous phases of different pH, phases that are separated by a solvent layer of various sizes. Commonly, liquid membranes can be obtained either by emulsification, when their stability is poor, or by including the solvent in a hydrophobic porous polymer matrix (18, 19). Moreover, the liquid membranes could be obtained using pertraction equipments of special construction, which allow to separate and easily maintain the three phases without adding surfactants (free liquid membranes) (19, 20).

The pertraction efficiency and selectivity could be significantly enhanced by adding a carrier in liquid membrane, such as organophosphoric compounds, long chain amines or crown-ethers, the separation process being called facilitated pertraction (18–21).

Compared with the conventional liquid-liquid extraction, the use of pertraction reduces the loss of solvent during the separation cycle, needs a 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 (18–21).

On the basis of the previous investigations of the mechanism and influencing factors of reactive extraction of cinnamic and p-methoxycinnamic acids, the aim of this paper is to study the possibility to selectively separate the two compounds from their mixture by facilitated pertraction with Amberlite LA-2. In this purpose, the influences of the pH-gradient between the aqueous phases, the carrier concentration in the liquid membrane, and the mixing intensity on the efficiency and the selectivity of pertraction will be analyzed.

MATERIALS AND METHOD

The following materials have been used: dichloromethane (≥99%) (Aldrich) as solvent, Amberlite LA-2 (Sigma Chemie GmbH) as carrier, cinnamic acid (≥99%) (Merck) and p-methoxycinnamic acid (≥98%) (Alfa Aesar).

The experiments have been carried out using a pertraction equipment that allows obtaining and easily maintaining the free liquid membrane. The pertraction cell has been described in the previous papers and consists on a U-shaped glass pipe having an inner diameter of 45 mm and a total volume of 400 ml, the volume of each compartment being equal (21, 22).

The aqueous solutions are independently mixed by means of double blade stirrers with 6 mm diameter and 3 mm height, having a rotation speed between 0 and 800 rpm. In order to reach high diffusional rates through the solvent layer, the organic phase has been mixed with a stirrer of the same design, at a constant rotation speed of 500 rpm. The area of mass transfer surface, both for extraction and for re-extraction, was of 1.59×10^{-3} m^2. The interfaces between the phases remained flat, and hence the interfacial area constant, for the entire rotation speed domain used.

The experiments were carried out in a continuous system, at the steady state conditions related to the aqueous phases. The aqueous solutions have been separately fed with a volumetric flow of 2.9 l/h.

The liquid membrane phase consists of a solution of 0–80 g/l Amberlite LA-2 (carrier) dissolved in dichloromethane.

Because the solubility of cinnamic acid in water is very low and p-methoxycinnamic acid is insoluble in water, the feed phase was a hydro-alcoholic solution (50% vol. ethanol) of the two acids. The initial concentration of each acid was of 0.2 g/l. The pH of the initial phase varied between 2 and 8, being adjusted with solutions of 3% sulfuric acid or 3% sodium hydroxide, depending on the prescribed pH-value.

The stripping phase consists of sodium hydroxide solutions with pH = 8–12.

The pH-values of both the aqueous phases were determined using a digital pH-meter of HI 213 (Hanna Instruments) type and have been recorded throughout each experiment. Any pH change was noted during the pertraction experiments.

The evolution of pertraction was followed by means of the acids initial and final mass flows, the permeability, and the selectivity factors. The initial mass flow, n_i, represents the acid mass flow from the feed phase to the liquid membrane, while the final (overall) mass flow, n_f, the acid mass flow from the liquid membrane to the stripping phase. The permeability factor, P, conveys the capacity of a solute transfer through the liquid membrane, and has been defined as the ratio between the final mass flow and the initial mass flow of solute (22).

The acids mass flows through the liquid membrane have been calculated by determining the cinnamic and p-methoxycinnamic acids concentrations in the feed and stripping phases and using the acids mass balance for the pertraction system. The acids concentrations in the aqueous phases have been measured by HPLC analysis using a LiChrospher RP-18 column and a UV detector at 280 nm (23). The mobile phase was a mixture of acetic acid-methanol-acetonitrile-water (0.1:5:34:61 vol.), its flow rate being of 1 ml/min, at 25°C. Samples removals were carried out from the aqueous phases evacuation outlets.

Each experiment has been carried out for three or four times under identical conditions. The average value of the measurements was used in the calculations. The maximum experimental error was of ± 4.06%.

RESULTS AND DISCUSSION

Generally, the pertraction is strongly influenced by the pH-gradient between the aqueous phases, the carrier concentration in the liquid membrane, and the mixing intensity of the phases.

In the case of separation of cinnamic and p-methoxycinnamic acids, the influence of the pH-gradient is amplified by the ionization-protonation of these two compounds in the aqueous phases, these processes controlling the efficiency of extraction and re-extraction, as well as the rate of the transport through the liquid membrane.

Due to the methoxy group which differentiates the studied acids, the influence of the feed phase pH is based on two different mechanisms. Thus, from Fig. 2, plotted for the pH of the stripping phase of 10, it can be observed that the initial and final mass flows of the cinnamic acid are

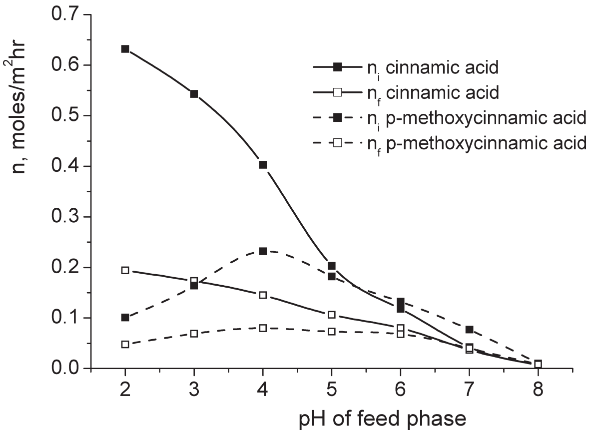

Figure 2. Influence of pH-value of feed phase on mass flows of cinnamic and p-methoxycinnamic acids (pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm).

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 the reactive extraction of the two acids, which is based on the formation of strong hydrophobic compounds at the interface between the aqueous and organic phases. According to the previous studies, 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 the hydrogen bonding type with the undissociated carboxylic groups, or of ionic type, if the acids dissociate in the aqueous solution:

$$\text{R-COOH}_{(aq)} + n\text{E}_{(o)} \rightleftarrows \text{R-COOH} \cdot \text{E}_{n(o)}$$

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 (17).

For the pH-values below 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 ($pK_a = 4.44$ for cinnamic acid, $pK_a = 4.28$ for p-methoxycinnamic acid (24)). 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 (17). 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 with 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 the liquid membrane, due to the diminution of the amount of acid accumulated into the organic phase (Fig. 3). Thus, the maximum value of permeability factor for the considered experimental conditions was of 0.93, being reached at pH = 8.

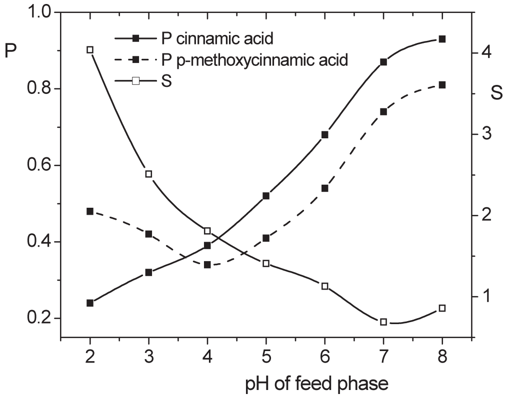

Figure 3. Influence of pH-value of feed phase on permeability and selectivity factors (pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm).

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 from the 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 compared 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 Fig. 3 it can be seen that the maximum value of the 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 the 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 the higher final mass flow compared with those of cinnamic acid (Fig. 2).

The increase of the 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 (Fig. 4). The variations of the corresponding permeability factors are similar with those of mass flows, thereby suggesting that by increasing the pH of the stripping phase the

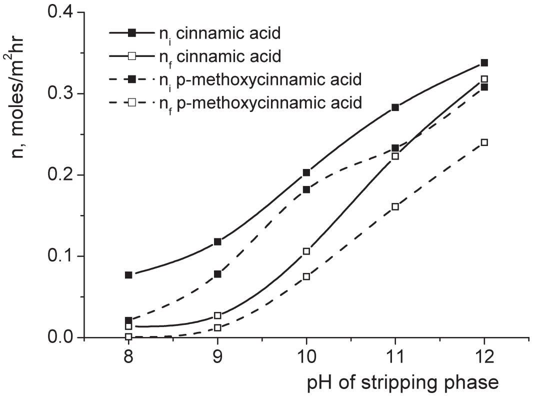

Figure 4. Influence of pH-value of stripping phase on mass flows of cinnamic and p-methoxycinnamic acids (pH of feed phase = 5, Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm).

acceleration of the reextraction rates becomes more important than that of the extraction rates (Fig. 5).

The selectivity factor reaches the highest values for pH $\leq$ 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 the organic phase is more stable than that formed by cinnamic acid and reacts slowly with sodium hydroxide from the stripping phase. The increase of the stripping phase pH, respectively the increase of sodium hydroxide

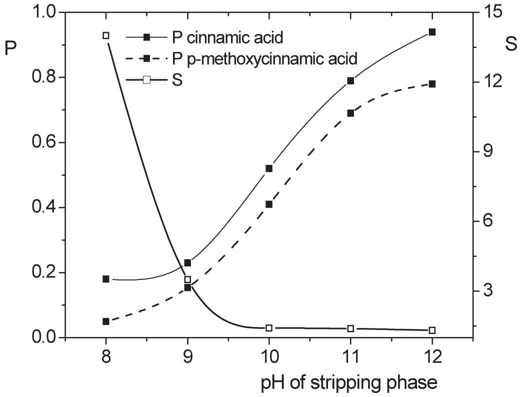

Figure 5. Influence of pH-value of stripping phase on permeability and selectivity factors (pH of feed phase = 5, Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm).

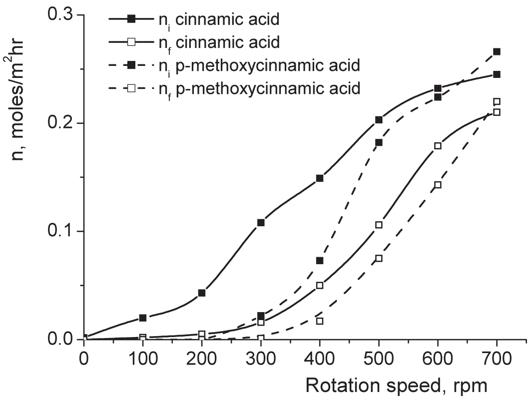

Figure 6. Influence of mixing intensity on mass flows of cinnamic and p-methoxy-cinnamic acids (pH of feed phase = 5, pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/l).

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 a 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 the pertraction of the two acids in a different manner.

Figure 6 indicates that the intensification of mixing leads to the increase of the initial and final mass flows of the two acids. But, for the rotation speed below 200 rpm, only cinnamic acid diffuses from the feed phase to the liquid membrane, owing to its lower resistance to diffusion compared with p-methoxycinnamic acid. The formation of sodium salts at the interface between the organic and the stripping phases supplementarily reduces the acids' diffusion rate, an effect that is more pronounced for p-methoxycinnamic acid. For this reason, the value of the final mass flow of cinnamic acid is 0 for the rotation lower than 100 rpm, and that of p-methoxycinnamic acid for the rotation speed lower than 300 rpm.

The increase of the rotation speed over the above-mentioned level exhibits a favorable effect, because the mixing intensification of the two aqueous phases determines the transfer acceleration for both studied carboxylic acids, as the result of the diminution of resistance to the diffusion through the boundary layers formed at the two separation interfaces. The magnitude of this effect is more pronounced for p-methoxycinnamic acid, its mass flows becoming higher than those of cinnamic acid for the rotation speed over 600 rpm. Moreover, the mass flows of cinnamic acid reach

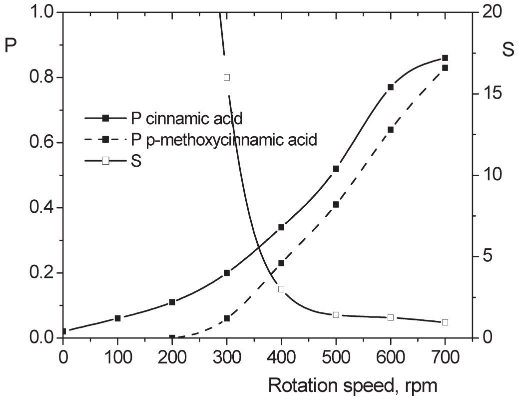

Figure 7. Influence of mixing intensity on permeability and selectivity factors (pH of feed phase = 5, pH of stripping phase = 10, Amberlite LA-2 concentration = 40 g/l).

a constant level for the rotation speed over 600 rpm, this variation suggesting that its pertraction is controlled by diffusion only for the rotation speed below 600 rpm, over this value the limiting factor being of the kinetic type.

The positive influence of the rotation speed on the permeability factors is plotted in Fig. 7. The increase of the permeability factors with rotation speed intensification indicates a stronger influence of mixing on the final mass flows, due to the more accentuated resistance to the diffusion through the stripping phase, as the result of the sizes of sodium salt molecules from this phase which is larger compared with free acids molecules from the feed phase. From the above-mentioned reasons, the final mass flow of p-methoxycinnamic acid is 0 for rotation speed lower than 200 rpm, over this value the influence of mixing intensification being more significant for this acid pertraction than for cinnamic acid.

The highest values of the 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 (Fig. 7).

The increase of carrier concentration into the liquid membrane induces the increase of the initial and final mass flows of both acids. According to Fig. 8, 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.

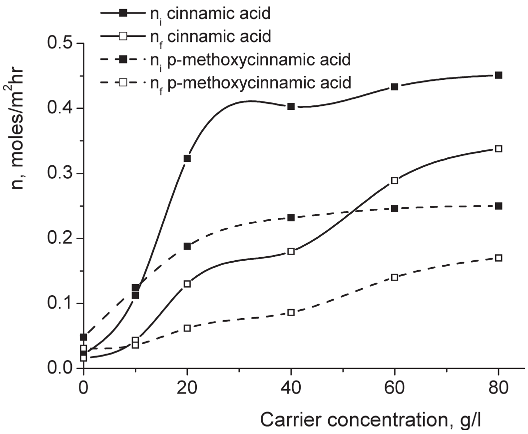

Figure 8. Influence of carrier concentration on mass flows of cinnamic and p-methoxycinnamic acids (pH of feed phase = 5, pH of stripping phase = 10, rotation speed = 500 rpm).

The 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.

The evolution of the acids permeability factors are different. Thus, 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 (Fig. 9).

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),

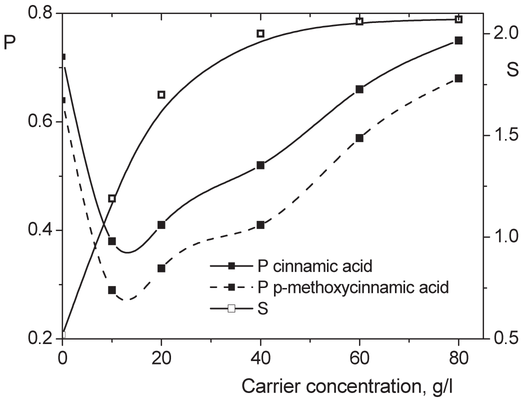

Figure 9. Influence of carrier concentration on permeability and selectivity factors (pH of feed phase = 5, pH of stripping phase = 10, rotation speed = 500 rpm).

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 the acid and the 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 the 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, in comparison with the free pertraction, the final mass flow will be initially smaller.

The positive influence of the increase of the carrier concentration is more important in the case of cinnamic acid, thus 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 a constant level.

CONCLUSIONS

The studies on facilitated pertraction with Amberlite LA-2 of cinnamic and p-methoxycinnamic acids indicated that cinnamic acid can be selectively separated from these acids mixture. The pertraction selectivity is mainly controlled by the pH-gradient between the feed and stripping phases and mixing intensity.

For obtaining the maximum selectivity factor, the pertraction has to be carried out at $pH = 2$ of the feed phase, $pH = 8$ of the stripping phase, a rotation speed lower than 300 rpm, and a carrier concentration higher than 40 g/l.

Because the proposed pH-value of the stripping phase and mixing intensity do not allow to reaching concomitantly the higher mass flows or the permeability factors, the purpose of future studies is to optimize the pertraction conditions for obtaining simultaneously the desired values of mass flows, the permeability factors, and selectivity.

NOTATIONS

n	Mass flow of acid (moles/m^2hr)
n_i	Initial mass flow of acid (moles/m^2hr)
n_f	Final (overall) mass flow of acid (moles/m^2hr)
P	Permeability factor (−)
S	Selectivity factor (−)

Subscript

aq	Aqueous phase
o	Organic phase.

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