

Selective separation of *trans*-cinnamic and *p*-methoxycinnamic acids by facilitated pertraction.

Mathematical modeling of pertraction selectivity

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Abstract

Using the statistical analysis and a factorial experiment of second order, a mathematical correlation between the selectivity factor of the facilitated pertraction of cinnamic and *p*-methoxycinnamic acids and the main parameters influencing this process (*pH*-value of feed phase, *pH*-value of stripping phase, mixing intensity of the aqueous phases) have been established. For both extraction systems, the considered variables control the selectivity of separation in a 90.5% extent, the last two parameters exhibiting the most important influence.

Keywords: *trans*-cinnamic acid, *p*-methoxycinnamic acid, Amberlite LA-2, pertraction, liquid membrane, mathematical modeling, regression equation, regression coefficient, determination coefficient.

Introduction

The cinnamic acid, also known as phenylacrylic acid, is a natural compound derived from phenylalanine, the main vegetable sources being the cinnamon, the resin of *Liquidambar* tree, the storax, the balsam of tolu, and the balsam of Peru. The *trans*-cinnamic acid and its related acids (*p*-methoxy- and *p*-hydroxycinnamic acids) are used in the cosmetic, pharmaceutical, food and chemical industries [1-3].

This compound could be obtained by extraction from vegetable raw materials, by chemical synthesis or biosynthesis. New fermentation or/and enzymatic methods for *trans*-cinnamic acid and its main derivatives production, the *p*-hydroxy- and *p*-methoxycinnamic acids, have been recently developed. Therefore, *Saccharomyces cerevisiae*, *Escherichia coli*, *Pseudomonas sp.* have been cultivated on glucose, and *Cellulomonas galba* on *n*-paraffins with addition of alkylbenzenes [4,5]. The glucose, fructose, lactose, sugar, cellulose and starch can be enzymatic 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.*, *Rhodospiridium sp.*, *Sporobolomyces sp.*, *Rhizoctonia solani*, *Trichosporon cutaneum*, *Rhodobacter sp.* [6-9].

Excepting our previous works [10,11], there are no reports on the possibility of *trans*-cinnamic acid and its related acids separation by liquid-liquid extraction from fermentation broths or enzymatic media, maybe due to their low solubility into the solvents immiscible with water. Their extraction becomes possible by adding into the solvent of an extractant which could react with these acids, leading to the formation of hydrophobic compounds (reactive extraction). In this purpose, the extractant of aminic type (Amberlite LA-2) has been previously used [10,11].

The reactive extraction can be developed by solute extracting and transporting through liquid membranes, 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* which are separated by a solvent layer of various sizes. The pertraction efficiency could be significantly enhanced by adding of a carrier in liquid membrane, such as organophosphoric compounds, long chain amines or macrocyclic derivatives, the separation process being called *facilitated pertraction* [12-15].

Compared with the conventional liquid-liquid extraction, the use of pertraction reduces the loss of solvent during separation cycle, needs small quantity of solvent and carrier, owing to their continuous regeneration, and offers the possibility of solute transport against its concentration gradient, as long as the *pH*-gradient between the two aqueous phases is maintained [12-15].

The previous studies on facilitated pertraction with Amberlite LA-2 of *trans*-cinnamic and *p*-methoxycinnamic acids indicated that *trans*-cinnamic acid can be selectively separated from these acids mixture [16]. The pertraction selectivity was found to be mainly controlled by the *pH*-gradient between the feed and stripping phases and mixing intensity. For the considered experimental conditions, the maximum selectivity

factor was reached at pH=2 of feed phase, pH=8 of stripping phase, rotation speed lower than 300 rpm, carrier concentration higher than 40 g/l.

The previous experiments have been continued by mathematical modeling of *trans*-cinnamic and *p*-methoxycinnamic acids selective pertraction, using the statistical analysis, respectively the factorial experiment of second order. In this purpose, the influences of the pH-value of feed phase, pH-value of stripping phase and mixing intensity on the selectivity of pertraction will be analyzed (the influence of carrier concentration into the liquid membrane is less important [16]).

Materials and Method

The experiments have been carried out using 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 [15,16].

The two aqueous solutions are independently mixed by means of double blade stirrers with 6 mm diameter and 3 mm height, having the same rotation speed varying between 300 and 500 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 $1.59 \times 10^{-3} \text{ m}^2$. The interfaces between the phases remained flat, and hence the interfacial area constant, for 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 40 g/l Amberlite LA-2 (carrier) dissolved in dichloromethane.

Because the solubility of *trans*-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 the both 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 and selectivity factor. The initial mass flow represents the acid mass flow from the feed phase to the liquid membrane, while the final (overall) mass flow the acid mass flow from the liquid membrane to the stripping phase. The selectivity factor, *S*, has been previously defined as the ratio between the final mass flow of *trans*-cinnamic acid and that of *p*-methoxycinnamic acid [16].

The acids mass flows through liquid membrane have been calculated by determining the *trans*-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 an UV detector at 280 nm [17]. 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.

Results and Discussion

In a previous paper, we concluded that the pertraction of *trans*-cinnamic and *p*-methoxycinnamic acids is strongly influenced mainly by the pH-gradient between the aqueous phases and these phases mixing intensity [16].

The influence of 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 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, described in the earlier papers [15,16]. Thus, the initial and final mass flows of the *trans*-cinnamic acid were continuously reduced with the increase of pH-value of feed phase. On the other hand, the mass flows of *p*-methoxycinnamic acid initially increased with the pH increase, reached a maximum level at pH=4, decreasing then. This variation was found to be more pronounced for the initial mass flow [16].

The increase of stripping phase pH-value induced 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 [16].

The cumulated effects of pH-values of feed and stripping phases on cinnamic and p-methoxycinnamic initial mass flows are plotted in Figure 1.

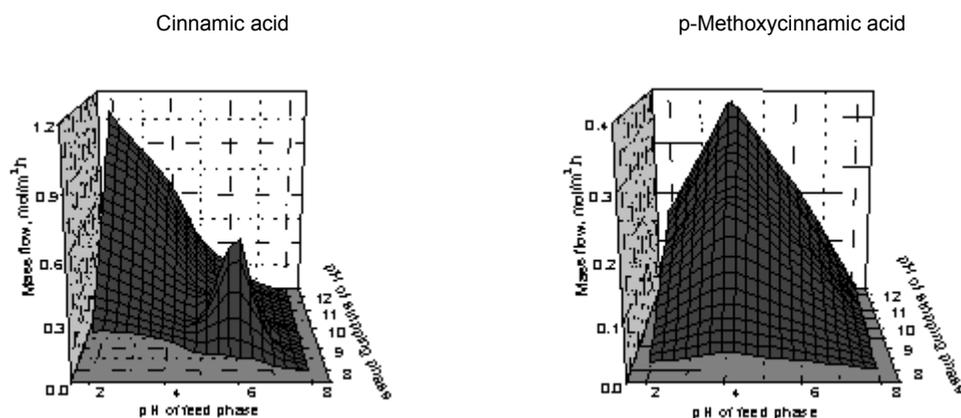


Figure 1. Cumulated influences of pH-values of feed and stripping phases on acids mass flows (Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm).

Because the diffusion is an important limiting factor, the mixing intensity of the aqueous phases represented another important factor influencing the pertraction of the studied acids. According with the previous results, the intensification of mixing led to the increase of the initial and final mass flows of the two acids [16]. But, for rotation speed below 200 rpm, only *trans*-cinnamic acid diffused from the feed phase to 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 supplementary reduced the acids diffusion rate, effect that was more important for p-methoxycinnamic acid [16].

The increase of carrier concentration into the liquid membrane generated the increase of the initial and final mass flows of both acids [16]. At lower concentration of Amberlite LA-2, the initial mass flow of p-methoxycinnamic acid was higher, due to its superior acidity compared with *trans*-cinnamic acid. The increase of Amberlite LA-2 amount in the organic phase exhibited a more pronounced effect on *trans*-cinnamic acid mass flow, because it compensated the lower acidity of this acid [16].

For describing the selectivity of pertraction, the *selectivity factor*, S , has been earlier defined [16]. For the considered experimental conditions, the maximum value of selectivity factor was 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 induced a negative effect on the selectivity of *trans*-cinnamic acid separation, the selectivity factor becoming less than 1 for pH of feed phase over 6 [16].

The increase of stripping phase pH-value determined the diminution of the selectivity factor [16]. Thus, the selectivity factor reached the highest values for $\text{pH} \leq 9$ (at $\text{pH}=8$, $S=14$), decreasing strongly for more alkaline pH-domain. This variation was due to the kinetic resistance which hinders the reextraction process, this resistance being more important in the case of the stronger acid, namely p-methoxycinnamic acid. The increase of stripping phase pH, respectively the increase of sodium hydroxide concentration, accelerated the acids reextraction rate and, therefore, their final mass flow, effect that was more accentuated for p-methoxycinnamic acid [16].

The influences of the pH-values of the two aqueous phases on the selectivity factor are indicated in the Figure 2.

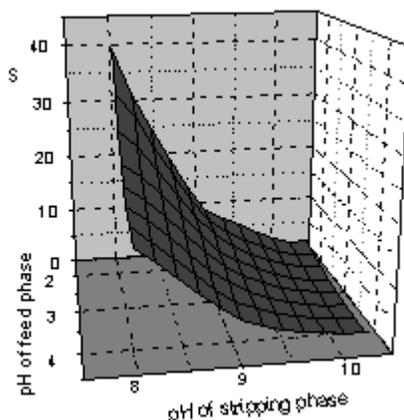


Figure 2. Cumulated influences of pH-values of feed and stripping phases on selectivity factor (Amberlite LA-2 concentration = 40 g/l, rotation speed = 500 rpm).

Due to the above mentioned reasons, the highest values of selectivity factor were recorded for poor mixing of the aqueous phases. The intensification of mixing led to the strongly decrease of selectivity factor from 16 for 300 rpm to 0.95 for 700 rpm [16].

The positive influence of the increase of carrier concentration was more important in the case of *trans*-cinnamic acid, thus the selectivity factor increased 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 remained at the constant level [16].

On the basis of these results, the previous works are continued by modeling the selective pertraction of the two acids. In this purpose a factorial experiment of second order has been used. The mathematical model quantifies the influences of the main parameters (pH-value of feed phase, pH-value of stripping phase, mixing intensity of the two aqueous phases) on the selectivity of separation, and it was established by statistical analysis. Thus, the real values of the process variables were chosen arbitrarily, their limits and coding being given in Table 1.

Table 1. The limits and coding of process variables for cinnamic and p-methoxycinnamic pertraction.

Variable	Code	Variable level			Step
		-1	0	+1	
pH of feed phase	x_1	2	5	8	3
pH of stripping phase	x_2	8	10	12	2
Rotation speed, rpm	x_3	300	400	500	100

In order to settle the correlation between the selectivity factor, Y , and the above mentioned parameters the following model of polynomial equation type has been taken into account:

$$S = b_0 + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + b_{12} \cdot x_1 \cdot x_2 + b_{13} \cdot x_1 \cdot x_3 + b_{23} \cdot x_2 \cdot x_3 + b_{11} \cdot x_1^2 + b_{22} \cdot x_2^2 + b_{33} \cdot x_3^2 \quad (1)$$

where b_0, \dots, b_{33} are the regression coefficients.

The plan of the factorial experiment of second order is given in Table 2.

Table 2. The experimental matrix and the experimental values of selectivity factor.

No. exp.	x_1	x_2	x_3	S_{exp}
1.	-1	-1	-1	446.85
2.	1	-1	-1	164.15
3.	-1	1	-1	108.98
4.	1	1	-1	6.15

5.	-1	-1	1	104.11
6.	1	-1	1	7.54
7.	-1	1	1	3.78
8.	1	1	1	0.81
9.	-1	0	0	139.45
10.	0	0	0	33.05
11.	0	-1	0	153.82
12.	0	1	0	17.02
13.	0	0	-1	155.63
14.	0	0	1	18.00
15.	0	0	0	73.19
16.	0	0	0	74.01
17.	0	0	0	72.28

By means of the obtained data, the regression coefficients have been calculated using the following relations [18]:

$$b_0 = \bar{S}_{15-17}, \quad b_j = \frac{\sum_{i=1}^{15} x_{ji} S_i}{\sum_{i=1}^{15} x_{ji}^2}, \quad b_{jk} = \frac{\sum_{i=1}^{15} x_{ji} x_{ki} S_i}{\sum_{i=1}^{15} x_{ji}^2 x_{ki}^2}, \quad b_{jj} = \frac{\sum_{i=1}^{15} x'_{ji} S_i}{\sum_{i=1}^{15} (x'_{ji})^2}$$

$$x'_{ji} = x_{ji} - \frac{1}{15} \sum_{i=1}^{15} x_{ji}^2 \quad (2)$$

$i = 1 \dots 15$ number of experiments $j = 1 \dots 3$ number of variables.

The obtained values of regression coefficients are listed in Table 3.

Table 3. The values of regression coefficients for vitamin C reactive extraction.

Regression coefficient	Value
b_0	73.16
b_1	-53.40
b_2	-68.10
b_3	-68.97
b_{12}	44.20
b_{13}	45.23
b_{23}	57.09
b_{11}	12.59
b_{22}	11.56
b_{33}	14.11

For checking the normal results obtained in the program center the Q test was applied [18]. Thus, using the values of the experimental selectivity factor, given in Table 2, the calculated Q value is:

$$Q = \frac{|a_1 - a_2|}{A} = 0.526 \quad (3)$$

where: a_1 - the uncertain value (72.28);

a_2 - the closest to the uncertain value (73.19);

A - the amplitude (difference between the most distant values, 1.73).

For a certain threshold of 0.05, $Q = 0.77$ was found in literature [18]. Since the calculated value of 0.526 is lower than the tabulated one, it could be concluded that the uncertain value of 72.28 is also a normal value.

Consequently, all of the three obtained values for the selectivity factors (experiments no. 15 - 17) were taken in calculation.

Hence, the regression equation may be written as:

$$S = 73.16 - 53.40 \cdot x_1 - 68.10 \cdot x_2 - 68.97 \cdot x_3 + 44.20 \cdot x_1 \cdot x_2 + 45.23 \cdot x_1 \cdot x_3 + 57.09 \cdot x_2 \cdot x_3 + 12.59 \cdot x_1^2 + 11.56 \cdot x_2^2 + 14.11 \cdot x_3^2 \quad (4)$$

The experimental and calculated with eqn. (4) values of selectivity factor are tabulated in Table 4.

Table 4. The experimental, S_{exp} , and calculated values, S_{calc} , for selectivity factor.

No. exp.	S_{exp}	S_{calc}
1.	446.85	448.41
2.	164.15	163.75
3.	108.98	109.63
4.	6.15	3.21
5.	104.11	105.83
6.	7.54	5.53
7.	3.78	2.08
8.	0.81	0.67
9.	139.45	139.15
10.	33.05	34.35
11.	154.97	155.82
12.	17.02	16.62
13.	155.63	156.24
14.	18.00	18.30
15.	73.19	73.16
16.	74.01	
17.	72.28	

The maximum limits between which these values, calculated with the regression equation, oscillate around the experimental values are determined with the relation [18]:

$$S_{\text{calc}_i} = S_{\text{exp}_i} \pm t \cdot E_{Y_X} \quad (5)$$

The standard deviation $E_{Y_X}^2$ was calculated using the following relationship [18]:

$$E_{Y_X}^2 = \frac{\sum_{i=1}^8 (S_{\text{exp}_i} - S_{\text{calc}_i})^2}{n - (k + 1)} = 5.473 \quad (6)$$

where n is the number of experiments and k the number of the considered variables.

The t values are to be found in the tables for Student distribution [18], for a confidence threshold of 0.05 and 15 experiments, namely:

$$t = 2.131 \quad \text{and} \quad S_{\text{calc}_i} = S_{\text{exp}_i} \pm 11.663 \quad (7)$$

The individual influence of the factors under consideration is estimated by means of the value of the correlation coefficient, r_{S_X} [18]:

$$r_{S_X} = \frac{\sum_{i=1}^8 [x_i \cdot (S_i - \bar{S})]}{\sqrt{\sum_{i=1}^8 x_i^2 \cdot \sum_{i=1}^8 (S_i - \bar{S})^2}} \quad (8)$$

which describes the nature of dependence between the process variables and the pertraction selectivity. The determination coefficient, which represents the square of correlation coefficient, indicates the fraction of the selectivity factor that can be explained by variable x_i variation. In this case, the calculated values of determination coefficients are:

$$r_{S_1}^2 = 0.223 \quad r_{W_2}^2 = 0.323 \quad r_{W_3}^2 = 0.359$$

These values suggest that the considered parameters influence the selectivity of *trans*-cinnamic and p-methoxycinnamic acids pertraction in a 90.5% extent, the pH of stripping phase and mixing intensity being the most important factors. The rest of 9.5% can be attributed to the effects of other factors, namely: carrier concentration, volumetric flow, mixing intensity of the organic layer etc.

Conclusions

The experimental results of the study on *trans*-cinnamic and p-methoxycinnamic acids facilitated pertraction with Amberlite LA-2 dissolved in dichloromethane indicated that *trans*-cinnamic acid can be selectively separated from the acids mixture. The efficiency and selectivity of pertraction are mainly controlled by the pH-values of the feed and stripping phases and mixing intensity of the two aqueous phases, being less influenced by the carrier concentration.

By means of the statistical analysis and using a factorial experiment of second order, the selectivity of facilitated pertraction has been modeled. Thus, a mathematical correlation between the selectivity factor and the mentioned parameters has been established. For the studied extraction system, the considered variables determine the extraction process in a 90.5% extent, the pH-value of stripping phase and mixing intensity exhibiting the most important influences.

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