PERIODICO di MINERALOGIA Volume 90, No. 4, 2021

Separation of *trans*-cinnamic acid by reactive extraction with Amberlite LA-2 in low-polar solvent. 1. Mechanism of separation process

MARIA CĂMĂRUŢ[1)], ANCA-IRINA GALACTION[2)], DAN CAŞCAVAL[1)]*

[1)] Technical University "Gh. Asachi" of Iasi, Faculty of Chemical Engineering, Dept. of Biochemical Engineering, 71 D. Mangeron Avenue, 700050 Iasi, Romania,

[2)] University of Medicine and Pharmacy "Gr.T. Popa" of Iasi, Faculty of Medical Bioengineering, Dept. of Biotechnology, 16 University Street, 700115 Iasi, Romania,

** the corresponding author*

Abstract

In this paper we analyze the mechanism of reactive extraction of trans-cinnamic acid from aqueous solutions with Amberlite LA-2 dissolved in n-heptane. The results indicated that the extraction occurs through a chemical reaction between one mole of each reactant, either at interface or in the organic phase near the interface. The formation of aminic associations in organic phase enhances the hydrophobicity of the reaction product and, consequently, the extraction efficiency.

Keywords: cinnamic acid, Amberlite LA-2, n-heptane, reactive extraction, extraction constant, distribution coefficient.

Introduction

The cinnamic acid, also known as phenylacrylic acid, is a natural compound derived from phenylalanine, the main vegetable sources being cinnamon, resin of *Liquidambar* tree, storax, balsam of tolu, balsam of Peru (Figure 1).

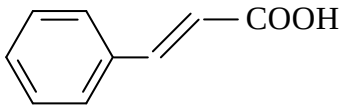

Figure 1. Chemical structure of cinnamic acid (3-phenyl-2-propenoic acid, phenylacrylic acid).

This acid, as well as its derivatives, constitutes important metabolic blocks in the formation of lignins from higher plants. It is also the intermediary for biosynthesis of some vegetable secondary metabolites (pigments, pungent taste compounds that deter the herbivores etc.).

The main utilization of cinnamic acid is in the cosmetic industry, in the perfumery production, especially as methyl, ethyl or benzyl esters (the cinnamic acid and its volatile benzylic ester are responsible for the cinnamon flavor). The cinnamic acid itself or the p-hydroxy- and p-methoxycinnamic acids have different pharmaceutical applications, for pulmonary affections, cancer, lupus, infectious diseases (diarrhea, dysentery), possessing antibacterial and antifungal activity [1-3]. It is also 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. 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 raw 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 amount 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 and of the required stages for product purification and generate large amounts of unwanted secondary products.

For these reasons, the production of cinnamic acid, especially *trans*, and its main derivatives, the p-hydroxy- and p-methoxycinnamic acids, by fermentation or/and enzymatic methods have been developed. In 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 enzymatic transformed by phenylalanine ammonia lyase or tyrosine ammonia lyase in alkaline media. These enzymes are synthesized directly into the media by mutant strains of *E. coli*, *Rhodotorula sp.*, *Rhodosporidium sp.*, *Sporobolomyces sp.*, *Rhizoctonia solani*, *Trichosporon cutaneum*, *Rhodobacter sp.* [10,13-15].

There are no reports on the possibility of cinnamic acid separation by liquid-liquid extraction from fermentation broths or enzymatic media, maybe due to its low solubility into the solvents immiscible with water. Its extraction could become possible by adding into the solvent of an extractant which could react with the cinnamic acid, leading to the formation of a hydrophobic compound (reactive extraction). Because of its chemical structure, the reactive extraction of cinnamic acid could be possible using extractants of aminic type, as in the case of other organic acids extraction [16-21].

Therefore, this paper presents the studies on the mechanism of reactive extraction of cinnamic acid with lauryl-trialkyl-methylamine (Amberlite LA-2) dissolved in n-heptane. This solvent has been selected because it doesn't exhibit any inhibitory effect on the cultivated microorganisms and possesses low capacity to solve the *trans*-cinnamic acid, thus the mechanism of reactive extraction could be better analyzed.

Materials and method

The experiments have been carried out using an extraction column with vibratory mixing, which offers high interfacial area and the possibility to reach rapidly the equilibrium state. The phase mixing was made by mean of a perforated disk with 45 mm diameter and 20% free section. The vibrations had a frequency of 50 s^{-1} and 5 mm amplitude. The perforated disk position was maintained at the initial contact interface between the aqueous and organic phases. The extraction time varied between 5 and 120 s, at a constant temperature of 25^{o}C. The resulted emulsion was broken in a centrifugal separator at 5000 rpm.

The initial concentration of *trans*-cinnamic acid (Merck) in aqueous solution was of 0.2 g l^{-1} ($1.35x10^{-3}$ M). The organic phase was a solution of Amberlite LA-2 in n-heptane, the extractant concentration varying between 0 and 60 g l^{-1} (0.16 M).

The extraction process was analyzed by means of the extraction degree and distribution coefficient. For calculating these parameters, the *trans*-cinnamic acid

concentrations in the initial solution and refined were determined by titration with a solution of $9.6x10^{-2}$ N NaOH. For calculating the *trans*-cinnamic acid concentration in organic phase the mass balance has been used.

Results and discussion

The reactive extraction of *trans*-cinnamic acid occurs by means of the formation of a strong hydrophobic compound at the interface between the aqueous and organic phases. In this case, the carboxylic group of the *trans*-cinnamic acid, R-COOH, is involved in the reactive extraction process with Amberlite LA-2, E. The interfacial interactions between the acid and the extractant could be of hydrogen bonding type with the undissociated carboxylic groups, or of ionic type, if the acid dissociates in the aqueous solution.

Furthermore, in function of the structures of system components and solvent polarity, the acidic or aminic adducts could be formed at the interface [16,22]. But, as it was observed for reactive extraction with Amberlite LA-2 of other compounds having voluminous molecules and due to the initial concentration of *trans*-cinnamic acid, which is lower than that of Amberlite LA-2, it could be assumed that the formation of acidic adducts is steric and technical hindered. Therefore, the interfacial compounds could be of ammonium salt type, formed by neutralization of the carboxylic group with one extractant molecule, or by aminic adducts type, where $n \geq 2$ [16,19,22]:

$$R\text{-}COOH_{(aq)} + n\,E_{(o)} \rightleftharpoons R\text{-}COOH.E_{n\,(o)}$$

The formation of these molecular associations is more pronounced in low-polar solvents and increases the hydrophobicity of the interfacial compound [16].

Because the solubility of *trans*-cinnamic acid in n-heptane is low and the extractant is not soluble in aqueous phase, the chemical reaction between the two compounds occurs either at interface or in the organic phase at the interface vicinity, being followed by the product diffusion into the bulk of solvent phase.

In these circumstances, the concentration of Amberlite LA-2 exhibits a significant influence on reactive extraction efficiency, as it can be observed from Figure 2.

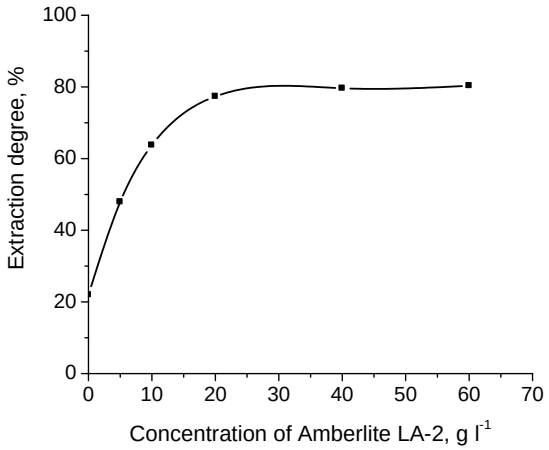

Figure 2. Influence of extractant concentration of reactive extraction yield (extraction duration = 60 s).

Therefore, the increase of extractant concentration in organic phase to 20 g l^{-1} leads to the strong increase of extraction efficiency, the extraction degree remaining at a constant level for higher Amberlite LA-2 concentrations. These results indicate that for high efficiency of

extraction is required higher Amberlite LA-2 concentration than the value needed for the equimolecular reaction with *trans*-cinnamic acid. This behavior of extraction system is due to one of the following reasons:

(a) owing to the low acidity of *trans*-cinnamic acid (K_a = 3.63x10^{-5} mole/l, at 25^{o}C [23]), the interfacial reaction occurs slowly, the accumulation of one reactant at the interface (Amberlite LA-2) increasing the rate of chemical reaction, consequently increasing the amount of extracted *trans*-cinnamic acid for a given duration of extraction process.

(b) high molecular weight amines, like Amberlite LA-2, can form molecular aggregates (20 - 40 monomers) of micelles type, especially in low-polar solvents, like n-heptane [16,22]. These amines could also formed adducts, through hydrogen bonds. The formation of these aminic aggregates induces the increase of interfacial product hydrophobicity, by its solvation or by its entrapping into the micelles.

For establishing the mechanism responsible for the extractant concentration influence on extraction efficiency, the effect of extraction duration on *trans*-cinnamic acid extraction degree has been analyzed. From Figure 3 it can be seen that the duration doesn't exhibit an important influence on extraction yield, the significant increase of extraction yield being recorded only for the duration below 5 s. The obtained variation suggests that the rate of interfacial reaction is high and the limiting step of extraction process is not of kinetic type, this excluding the hypothesis (a).

According to the hypothesis (b), the extraction mechanism was verified assuming that n molecules of extractant and one acid molecule participate to the formation of the interfacial compound through ionic and hydrogen bonds. Thus, the corresponding distribution coefficient, D, is calculated with the following relationship:

$$D = \frac{\left[R-COOHE_{n(o)}\right]}{\left[R-COOH_{(aq)}\right]} \quad (1)$$

where [R-COOH$_{(aq)}$] and [R-COOHE$_{n\ (o)}$] symbolize the overall concentrations of *trans*-cinnamic acid and extracted compound at the equilibrium state.

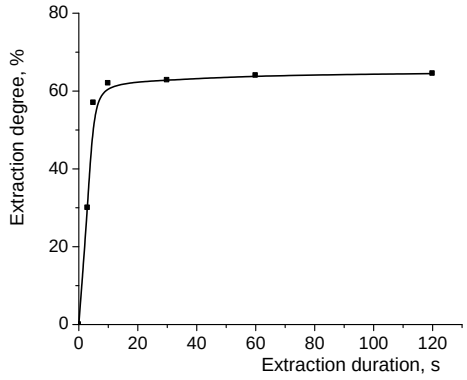

Figure 3. Influence of duration of extraction on reactive extraction efficiency (Amberlite LA-2 concentration = 10 g l^{-1}).

According to the interfacial equilibrium, the extraction constant, K_E, can be calculated with the expression:

$$K_E = \frac{\left[R-COOHE_{n(o)}\right]}{\left[\overline{R-COOH_{(aq)}}\right]\cdot\left[E_{(o)}\right]^n} \quad (2)$$

$$\Rightarrow \left[R-COOHE_{n(o)}\right] = K_E \cdot \left[\overline{R-COOH_{(aq)}}\right]\cdot\left[E_{(o)}\right]^n \quad (3)$$

The concentration of undissociated *trans*-cinnamic acid from aqueous phase, $\left[R-COOH_{(aq)}\right]$, is calculated by means of its overall concentration in aqueous phase, $\left[\overline{R-COOH_{(aq)}}\right]$, and the dissociated acid concentration, $\left[R-COO^{-}{}_{(aq)}\right]$. The dissociation constant, K_a, corresponds to the following dissociation equilibrium:

$$\text{R-COOH} \rightleftharpoons \text{R-COO}^{-} + \text{H}^{+}$$

and is determined with the relationship:

$$K_a = \frac{\left[R-COO^{-}{}_{(aq)}\right]\cdot\left[H^{+}\right]}{\left[R-COOH_{(aq)}\right]} \tag{4}$$

Thus, the concentration of undissociated *trans*-cinnamic acid is:

$$\left[R-COOH_{(aq)}\right] = \left[\overline{R-COOH_{(aq)}}\right] - \left[R-COO^{-}{}_{(aq)}\right] \tag{5}$$

$$\left[R-COO^{-}{}_{(aq)}\right] = K_a \frac{\left[R-COOH_{(aq)}\right]}{\left[H^{+}\right]} \tag{6}$$

$$\Rightarrow \left[R-COOH_{(aq)}\right] = \frac{\left[\overline{R-COOH_{(aq)}}\right]}{1+\frac{K_a}{\left[H^{+}\right]}} \tag{7}$$

Therefore, by combining the eqs. (1), (3) and (7), the following expression for the distribution coefficient, D, is obtained:

$$D = K_E \cdot \left[E_{(o)}\right]^{n} \cdot \left(1+\frac{K_a}{\left[H^{+}\right]}\right) \tag{8}$$

The correlation (8) represents in logarithmic form the equation of a straight line:

$$\ln D - \ln\left(1+\frac{K_a}{\left[H^{+}\right]}\right) = \ln K_E + n\cdot\ln\left[E_{(o)}\right] \tag{9}$$

Because the initial concentration of extractant is higher than the initial concentration of *trans*-cinnamic acid, $[E_{(o)}]$ could be assumed to be the initial concentration of Amberlite LA-2 in organic phase. Consequently, from the slope of the straight line given by eq. (9) it is possible to determine the number of extractant molecules, n, which participate to the formation of the interfacial compound, and from the intercept the value of extraction constant, K_E.

By means of the experimental data from Figure 2 and by plotting the eq. (9) the straight line from Figure 4 has been obtained.

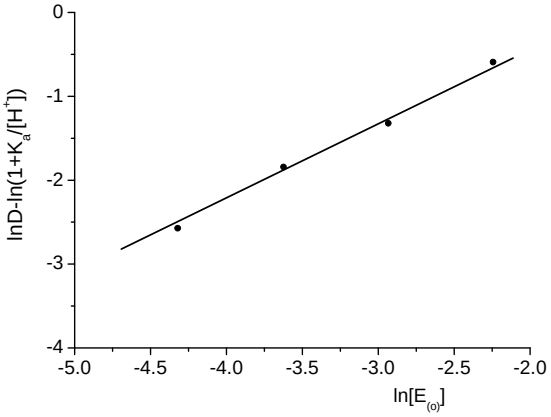

Figure 4. Graphical representation of the straight line given by eq. (9).

The value of the straight line slope is 0.94, thus indicating that one molecule of each reactant participates to the interfacial product formation.

The high interfacial reaction between the *trans*-cinnamic acid and Amberlite LA-2, the participation of one molecule of each reactant to the chemical reaction, both cumulated with the significant influence of the extractant concentration increase on reactive extraction degree suggest that the extractant forms aminic associations in organic phase, possible of micelles type, which induce the solvation of interfacial product.

The value of extraction constant was graphically determined from the intercept of the straight line with the 0y coordinate: $K_E = 3.034$ l mole^{-1}.

Conclusions

This study indicated the possibility to separate the *trans*-cinnamic acid from aqueous solutions by reactive extraction with Amberlite LA-2 dissolved in n-heptane. The extraction mechanism is based on the chemical reaction between one mole of organic acid and one mole of extractant either at interface, or in the organic phase near the interface. The hydrophobicity of the reaction product and, therefore, the extraction efficiency are enhanced by product solvation by aminic associations formed in low-polar solvents.

References

1. A.S. SARAF, A.V. SIMONYAN, Pharm. Chem. J. 1992, 26(7-8), 598-602.
2. S. TAWATA, S. TAIRA, N. KOBAMOTO, J. ZHU, M. ISHIHARA, S. TOYAMA, *Biosci. Biotechnol. Biochem.*, **60**, 909-1010 (1996).
3. S. LEE, J.M. HAN, H. KIM, E. KIM, T.S. JEONG, W.S. LEE, K.H. CHO, *Bioorg. Med. Chem. Lett.*, **14**, 4677-4681 (2004).
4. M.J. CHEN, V. VIJAYKUMAR, B.W. LU, B. XIA, N. LI, *J. Integr. Plant Biol.*, **47**, 67-72 (2005).
5. M. BARTOVA, L. OPLETAL, V. CHOBOT, H. SOVOVA, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, **770**, 283-289 (2002).
6. M. PALMA, Z. PINEIRO, C. BARROSO, *J. Cromatogr. A*, **968**, 1-6 (2002).
7. E.A. SMELZ, J. ENGELBERTH, J.H. TUMLINSON, A. BLOCK, H.T. ALBORN, *Plant J.*, **39**, 790-808 (2004).
8. M. NACZK, F. SHAHIDI, *J. Cromatogr. A*, **1054**, 95-111 (2004).

9. *** *The British Pharmaceutical Codex*, Council of the Pharmaceutical Society of Great Britain, Edn. 2001-2006.
10. A. BEN-BASSAT, F.S. SARIASLANI, L.L. HUANG, R. PATNAIK, D.J. LOWE, Patent US 60/563,633, 2004.
11. J.D. DOUROS, J.W. FRANKENFELD, *Appl. Microbiol.*, **16**, 320-325 (1968).
12. R.E. PARALES, N.C. BRUCE, A. SCHMID, L.P. WACKETT, *Appl. Environ. Microbiol.*, **68**, 4699-4709 (2002).
13. K.R. HANSON, E.A. HAVIR, *The Biochemistry of Plants*, Vol. 7, Academic Press, New York, 1981, pp 577.
14. C. APPERT, E. LOGEMANN, K. HALBROCK, J. SCHMID, N. AMRHEIN, *Eur. J. Biochem.*, **225**, 491-499 (1994).
15. E. KOOPMANN, E. LOGEMANN, K. HALBROCK, *Plant Physiol.*, **119**, 49-56 (1999).
16. K. SCHUEGERL, *Solvent extraction in biotechnology*, Springer-Verlag, Berlin, 1994.
17. M. JUNG, B. SCHIERBAUM, H. VOGEL, *Chem. Eng. Technol.*, **23**, 70-77 (2000).
18. A.F MORALES., J. ALBERT, G. KYUCHOUKOV, G. MALMARY, J. MOLINIER, *J. Chem. Eng. Data*, **48**, 876-882 (2003).
19. D. CASCAVAL, A.I. GALACTION, C. ONISCU, *Sep. Sci. Technol.* **39**, 1907-1925 (2004).
20. D. YANKOV, J. MOLINIER, J. ALBERT, G. MALMARY, G. KYUCHOUKOV, *Biochem. Eng. J.*, **21**, 63-69 (2004).
21. D. CASCAVAL, A.I. GALACTION, *Chem. Ind. J.*, **58**, 375-386 (2004).
22. D. CASCAVAL, C. ONISCU, A.I. GALACTION, *Inginerie biochimica si biotehnologie. 3. Procese de separare*, Performantica, Iasi, 2004, pp. 105.
23. R.C. WEAST (Ed.), *Handbook of Chemistry and Physics*, 54th Edition, CRC Press, Cleveland, 1974, pp. D131.