AMINO ACIDS RECUPERATION BY LIQUID-LIQUID EXTRACTION AND SUPPORTED LIQUID MEMBRANE EXTRACTION. OPTIMIZATION OF THE PROCESSES

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Received: 25/09/2008
Accepted after revision: 05/03/2009

Abstract: The amino acids constitute a significant class in biochemical sciences. Of this importance, their extraction and purification do not cease developing with the sight of research in progress.
Our work concerns a comparative study by two techniques liquid-liquid extraction (ELL) and supported liquid membrane (SLM), of phenylalanine (phe) and tyrosine (tyr). Several extractants were the subject of this study, the di 2-ethylhexyl phosphoric acid (D2EHPA), the tri-octy phosphine oxide (TOPO), the tri-butyl phosphate (TBP) and the quaternary ammonium salt Aliquat 336 and "D2EHPA+TBP" mixture. The results showed that the D2EHPA, is high performance in ELL of phe (82%) and tyr (23.4%). Mixture "D2EHPA+TBP", produced a synergistic effect on phe (73.5%) by ELL. The extraction by SLM of phe (feed solution 36.4%, stripping 100%) and of tyr (feed solution 2,4%; stripping 5.6%) by TBP, shows that this technique is powerful, to separate the mixture from these two amino acids in one extraction stage. The effects of solvents and salt were also tested; however the optimization of these processes remains to be perfected.

Keywords: *Phe, Tyr, LLE, SLM, Extractants, Effect of solvent, Effect of salt, UV-Visible Spectroscopy, Reactive extraction.*

INTRODUCTION

The liquid-liquid and liquid membrane process are known as a novel and effective method for selective separation and concentration of various species from dilute solutions, including metal ions, weak acids and bases, hydrocarbons or biologically important compounds [1], and gaseous mixtures [2, 3].
Amino acids are the main structural components of proteins and enzymes, which are very important products for human activity. Amino acids can be obtained by biosynthesis or from protein hydrolysis, but their separation from fermentation broths or from protein hydrolysates is rather difficult [4, 5]. They dissociate in the aqueous solutions, forming characteristic ionic species as a function of solution pH. Selective separation of amino acids by reactive extraction constitutes a important process in the biotechnology domain [6, 7].
The objective of our work concerns a comparative study by two techniques liquid-liquid extraction (ELL) and supported liquid membrane (SLM), of phenylalanine (phe) and tyrosine (tyr) by the extractants D2EHPA, TBP, TOPO and Aliquat 336.

EXPERIMENTAL

Reagents and methods

Phenylalanine 99% and Tyrosine 99% were purchased from BDH Chemicals Ltd Poole England, and used without further purification.
HNO_3 32%, HCl 32%, NaOH, KCl and $CHCl_3$ were provided from Fluka. TBP 98% ($C_{12}H_{27}O_4P$) (Aldrich), TOPO 97% [$(C_8H_{17})_3P{=}O$] (Fluka), D_2EHPA (Fluka) and Aliquat 336 [$(C_8H_{17})_3(CH_3)N^+Cl^-$](Aldrich) were used as extractants. Kerosene was provided from Sonatrach.
The liquid membrane support was Millipore FGLP 04700 (Fig. 1a) with an average pore size of 0.22 μm, a porosity of 75% and a total thickness of 125 μm of which about 115 μm is polyethylene backing. The membrane was placed between the two blocks of the type polytetrafluoroethylene (Fig. 2).

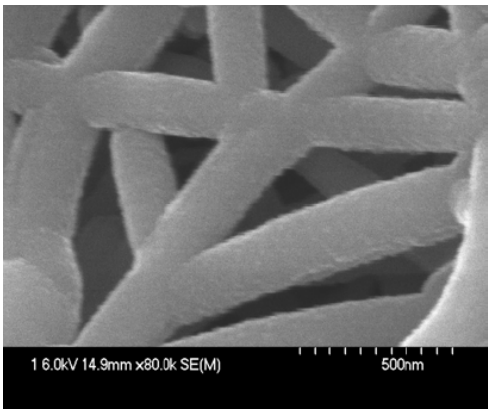

Figure 1a. *Microscopic view of the Fluoropore membrane before impregnation*

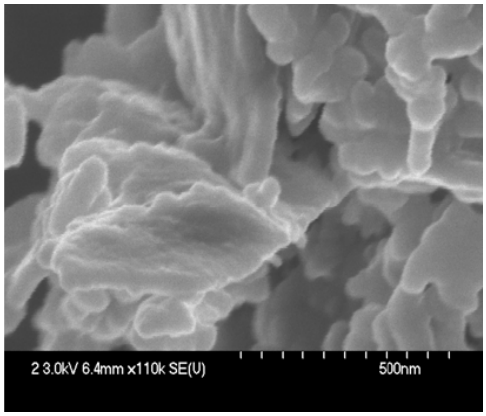

Figure 1b. *Microscopic view of the Fluoropore membrane after impregnation with D2EHPA*

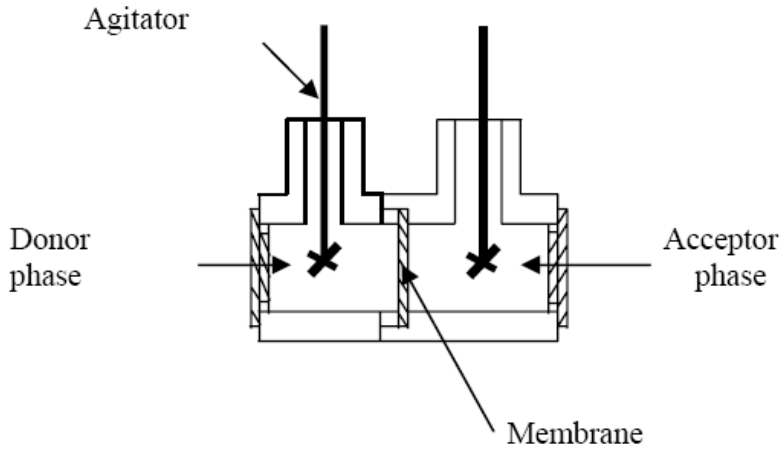

Figure 2. *System for liquid membrane extraction of amino acids*

Impregnating liquid membrane

Each liquid membrane was prepared by impregnating the support by soaking it for 12 hours in adequate extractant (D2EHPA (Fig. 1b), TOPO, TBP), diluted in chloroform. Aliquat 336 was diluted in kerosene.

Membrane equipment

The membrane unit consisted of two circular polytetrafluroethylene (PTFE) blocks (1). After impregnating, the feed solution was pumped through the feed channel. All experiments were performed at room temperature. SLM extraction was studied in discontinuous extraction mode.

Sample analysis

The concentrations of amino acids in the aqueous phase were determined by UV-Visible [8]. We have used a Perkin Elmer Lambda 800 functioning from 200 to 900 nm.
A Consort C 831 *pH*-meter with combined glass electrode was used to measure the *pH* of the aqueous solution at equilibrium, after extraction.

Extraction experiments

The extraction experiments were performed with both the extractants, D2EHPA, TOPO and TBP dissolved in chloroform. The Aliquat 336 was dissolved in kerosene.

Liquid – Liquid Extraction
The appropriate volume of aqueous solution (5 mL) containing amino acid (Phe 5.10^{-3}M or Tyr $0.88.10^{-4}$M) and 5 mL of organic solution containing 0.1 mol.L^{-1} of D2EHPA, were mixed in glass flasks and shaken in a moderate way, for different times. All the experimental studies were carried out at 20 °C. The same experiments of extraction were carried out with TBP (1.10^{-2}M), TOPO (5.10^{-2}M) and Aliquat 336 (1.10^{-2}M).

Supported Liquid Membrane Extraction

After impregnation by D2EHPA 0.1 M and installation of the membrane (see figure 1), the appropriate volume of aqueous solution (50 mL) containing amino acid (Phe 5.10^{-3}M, Phe 1.10^{-2}M, Tyr $0.88.10^{-4}$M) in the first compartment and 50 mL of HCl 0.5M in the second compartment were agitated. All the experimental studies were carried out at 20 °C. The same experiments of extraction were carried out with TBP (1.10^{-2}M and 5.10^{-2}M), TOPO (5.10^{-2}M) and Aliquat 336 (1.10^{-2}M).

RESULTS AND DISCUSSION

To study the extraction process the variables taken as response were extraction efficiency *E* (%), and extraction yield *R* (%), defined as follows [9]:

$$E(\%) = \frac{n_A}{n_I} \times 100 \quad (1)$$

$$R(\%) = \frac{m_e}{m_f} \times 100 \quad (2)$$

Solvent extraction of phenylalanine with TBP

It is known that an amino acid contains one amino group and one or more carboxylic groups [10]. Many types of dissociation reactions are involved in aqueous solutions. For the phenylalanine, we have:

$$\underset{(A)}{(C_6H_5)CH_2CH(NH_3^+)COOH} \Leftrightarrow \underset{(B)}{(C_6H_5)CH_2CH(NH_3^+)COO^-} + H^+ \quad pK_a = 1.83 \quad (1)$$

$$\underset{(B)}{(C_6H_5)CH_2CH(NH_3^+)COO^-} \Leftrightarrow \underset{(C)}{(C_6H_5)CH_2CH(NH_2)COO^-} + H^+ \quad pK_b = 9.13 \quad (2)$$

Although the three types of species may be simultaneously present, they predominate at each *pH* range, i.e. $pH < pK_a$, $pK_a < pH < pK_b$, and $pH > pK_b$ respectively.

Liquid-Liquid Extraction

We have worked in the *pH* domain from 6.67 to 7.19. TBP react with Phe as:

$$(C_6H_5)CH_2CH(NH_3^+)COO^- + TBP \Leftrightarrow (C_6H_5)CH_2CH(NH_3^+)COO^- \cdot TBP \quad (3)$$

Equilibrium time of extraction was 10 min.

In presence of KCl (1 M), Phe in the form (B) reacts with KCl as:

$$\underset{(D)}{(C_6H_5)CH_2CH(NH_3^+)COO^-K^+} \quad (4)$$

Equilibrium time of extraction was 20 min.

Extraction by SLM

Donor phase: The kinetics is slow. Indeed, equilibrium time of extraction was 42 h. The yield of extraction was 36.4%. The results obtained confirm that the extraction by SLM is more effective than the liquid-liquid extraction.

Acceptor phase: As it can be seen in figure 3, the total extraction from the membrane requires 50 h.

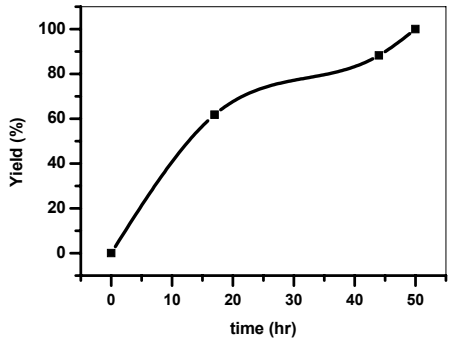

Figure 3. *Influence of the time on the extraction yield*

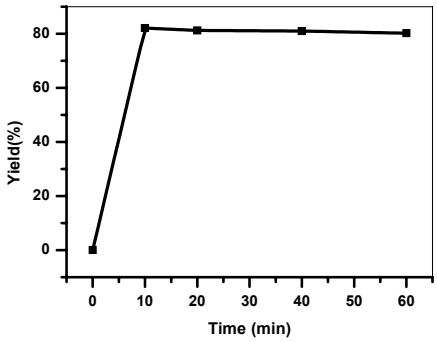

Figure 4. *Influence of the time on the extraction yield*

Solvent extraction of phenylalanine with TOPO

Liquid-Liquid Extraction

Equilibrium time of extraction was 10 min. The yield of extraction is better than with the TBP. In presence of KCl (1 M), Phe in the form (B) reacts with KCl as the reaction (4). TOPO is especially used in the mixtures of extractants, seldom only [11].

Extraction by SLM

SLM extraction is more powerful than the liquid-liquid extraction. The yield by SLM is the double of liquid-liquid extraction yield.

Solvent extraction of phenylalanine with Aliquat 336

At initial *pH* equal to 6.52, equilibrium time of extraction was 30 min, with 10% from extraction yield.

$pK_a < pH_i = 6.52 < pK_b$, Aliquat 336 reacts with Phe as :

$$(C_6H_5)CH_2CH(NH_3^+)COO^- + Aliquat^+Cl^- \Leftrightarrow Aliquat^+ \cdot (C_6H_5)CH_2CH(NH_2)COO^- + H^+Cl^- \quad (5)$$

At initial *pH* equal to 7.58, equilibrium time of extraction was 30 min, with 20% from extraction yield. At initial *pH* equal to 9.09, equilibrium time of extraction was 10 min, with 32% from extraction yield. The kinetics is very fast. At this *pH*, the Aliquat 336 is a good extractant for the phenylalanine in one extraction stage only.

Solvent extraction of phenylalanine with Aliquat 336 and 2-Octanol

Yield of extraction was 90% at equilibrium time from 20 min [12]. Initial *pH* equal to 9.05 was adjusted with NaOH.

Solvent extraction of phenylalanine with D2EHPA

Liquid-Liquid Extraction

At initial *pH* equal to 5.19, the yield of extraction reached a maximum of 82.12% corresponding at an equilibrium time of 10 min (see figure 4). The stoichiometric coefficients obtained from the plot ln*E* vs *pH* shown in figure 5 allows suggesting the probable reaction mechanism of extraction of phenylalanine. The equation of extraction equilibrium can be written as:

$$4(C_6H_5)CH_2CH(NH_3^+)COOH + 2(HR)_2 \Leftrightarrow 4[(C_6H_5)CH_2CH(NH_3^+)COOH \cdot R^-] + 4H^+ \quad (6)$$

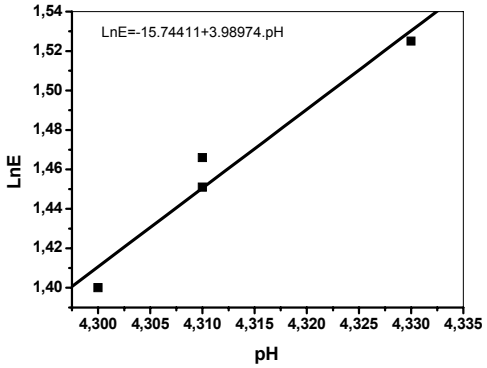

Figure 5. *Effect of pH on the distribution ratio*

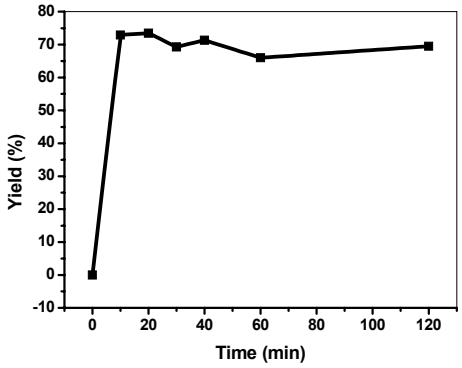

Figure 6. *Influence of the time of extraction on the yield*

Extraction by SLM

For $pH_i < pK_a$, the extraction by D2EHPA is good. The mechanism of extraction is complex because two species coexist, the neutral form $(C_6H_5)CH_2CH(NH_3^+)COO^-$ and the cation form $(C_6H_5)CH_2CH(NH_3^+)COOH$. The same function PO(OH) of D2EHPA does not allow the two species to fix themselves. The yield of extraction equal to 25.83% requires 40 min.

Solvent extraction of phenylalanine with D2EHPA (70%) + TBP (30%)

As it can be seen in figure 6, the yield of extraction equal to 75% requires 20 min.
Synergistic effect has been observed on the yield of extraction of phenylalanine [13].

Solvent extraction of tyrosine with TBP and KCl

As it can be seen in figure 7, COOH function and phenol OH function give to the tyrosine a double acid character. In presence of KCl (1 M), Tyr in the form (E) reacts with KCl giving the form G (see figure 8).
The influence of the ionic strength on the yield of extraction of tyrosine, with TBP diluted in chloroform, has bee controlled by modification of aqueous phase with the addition of the potassium chloride. The ionic force is determined by the formula of D. The yield of extraction increases.

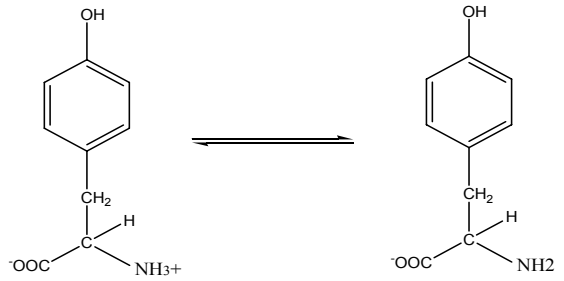

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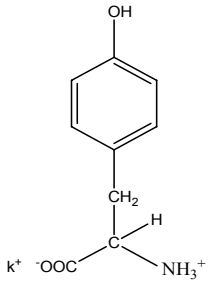
Figure 7. *Balance form of the tyrosine*



G

Figure 8. *Salt of potassium tyrosine*

Solvent extraction of tyrosine with Aliquat 336

Equilibrium time of extraction was 30 min. The yield of extraction is better than with the TBP. In more the use of the kerosene in the place of chloroform is better, because of the synergistic effect and its great availability.

Solvent extraction of tyrosine with D2EHPA

Under the conditions studied, it is found that the value of E increases with increasing pH_{eq} at D2EHPA concentration of 0.1M. Stoichiometry of extraction reactions with D2EHPA (HR) can be represented by the cation–exchange reaction and proton–transfer reaction:

$$(C_6H_4OH)CH_2CH(NH_3^+)COOH + (HR)_2 \Leftrightarrow (C_6H_4OH)CH_2CH(NH_3^+R^-)COOH \cdot HR + H^+ \quad (7)$$

$$(C_6H_4OH)CH_2CH(NH_3^+)COO^- + (HR)_2 \Leftrightarrow (C_6H_4OH)CH_2CH(NH_3^+R^-)COOH \cdot HR \quad (8)$$

where $(HR)_2$ represents the D2EHPA dimers.

Extraction by SLM of tyrosine with TOPO

In this case, TOPO, due to its neutral property, was better than TBP in the same conditions. The Topo-Tyr complex is a strong complex.

CONCLUSIONS

Liquid-liquid extraction of the phenylalanine gives good yields of extraction with D2EHPA (82.12%), D2EHPA + TBP (73.48%) and Aliquat 336 + kerosene (30.1%).
The separation yield is controlled by the *pH* value of the aqueous phase, which is due to the acidic or basic character of each amino acid.
Liquid-liquid extraction of the tyrosine by D2EHPA or Aliquat 336 gives the better yields: 23.4% and 22.44% respectively.
The extraction by SLM of phe (feed solution 36.4%, stripping 100%) and of tyr (feed solution 2,4%; stripping 5.6%) by TBP, shows that this technique is powerful, to separate the mixture from these two amino acids in one extraction stage only.
The proposed extraction methods can be developed and used for the selective separation of amino acids from fermentation broths or protein hydrolysates.

ACKNOWLEDGEMENTS

We gratefully acknowledge the ANDRS (Algeria) for their financial support.

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