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The Transport of Amino Acids by 18-Crown-6 Through Liquid Membranes

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Abstract. The possibility of separating in cationic form some α-amino acids (L-Methionine, L-Leucine, L-Isoleucine, L-Valine, L-Phenylalanine, L-α-Alanine and L-Cysteine) from mixtures in the presence of picrate anion has been investigated by means of active transport assisted by a pH gradient through liquid membranes. 18-Crown-6 in 1,2 dichloroethane has been used as a selective carrier. The effect of stirring rates at different volumes of the membrane, suggests a diffusional rate-limiting process of the amino acid transport.

Key words: Amino acid, liquid membrane, 18-crown-6, active transport.

1. Introduction

The techniques of separation and concentration through membranes have been progressively introduced in conventional processes of analytical chemistry and some technologies, mostly biotechnologies [1–3].

The investigations in this field have been focused on biomimetic aspects of the systems which are able to imitate 'in vitro', the biologic transport. The technique of separation through liquid membranes with macrocyclic carriers combines the selectivity of extraction with the active transport of compounds.

The new class of chemical compounds – the macrocyclic ligands [4–6], which are able to specifically recognize through controlled interactions according to size, shape and structure, various cations and then, by their means, various anions – has

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allowed the study of amino acids of which amphion type molecule may appear either as a cation in acid medium or as an anion in basic medium. The possibility of coupling two or more chemical species, according to a natural model by means of certain physico-chemical affinities, without formation of covalent bonds, is referred to as 'recognition' [7].

This study has investigated the separation of a series of α-amino acids from a mixture, making use of macrocyclic ligands of the crown polyether type and the technique of liquid membranes.

The transport mechanism is of the active type (a coupled transport of amino acids and hydrogen ions from the source phase to the receiving phase is ensured) assisted by a pH gradient.

The overall transport process is complex consisting of a mixture of diffusional and reaction (complexation/decomplexation) steps at two independent and generally different interfaces. Moreover, there may be some simultaneous steps and possible reactions in the bulk of the phases.

Modelling active transport offers means to estimate the relative significance of each step and to determine the rate-limiting process.

2. Experimental

The amino acid concentrations in the two aqueous phases have been determined by a Carlo Erba 3A 28M Amino Acid Analyzer. The crown ether 18-crown-6 (Merck) has been used without further purification. The other reagents have been of analytical grade. The pH determinations have been carried out by a digital MV – 870 pH-meter with glass electrode and saturated calomel electrode, calibrated against standard potassium diphthalate (pH = 4.01) and borax solutions (pH = 9.18). The experiments have been performed by stirring the phases (6h). The original device [8–9] employed (Figure 1) consists of two concentric tubes; the inner one which contains the source phase also acts as a stirrer. The receiving phase and the membrane phase are introduced in the outer tube.

3. Results and Discussion

The conceptual model of amino acid mediated transport by crown ethers through bulk liquid membranes assisted by a pH gradient is presented in Figure 2.

According to this system, in the source phase there is the protonated amino acid (C^+) to be transported and the picrate anion (A^-) in an aqueous solution at pH = 2, ensured by use of HCl.

At the source phase/membrane interface, the 18-crown-6 macrocyclic carrier complexes the amino acid through the protonated amino group; the so-formed complex cation is extracted into 1,2 dichloroethane membrane by ion pairing with the picrate anion, and diffuses through the membrane to the membrane/receiving phase interface.

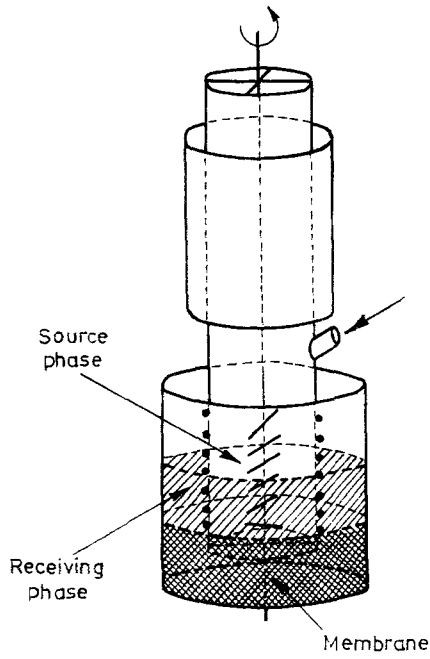

Fig. 1. The device employed in separation of some amino acids through a liquid membrane with 18-crown-6.

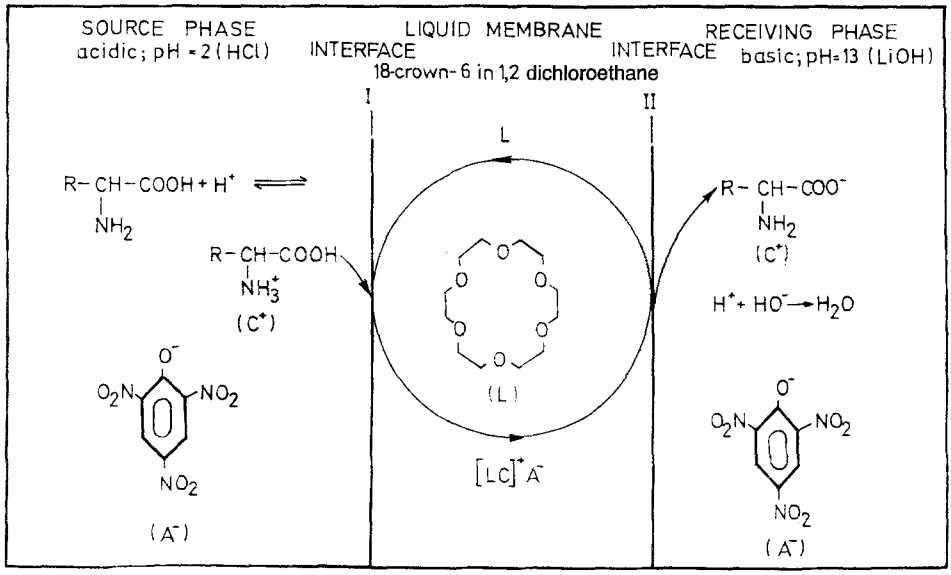

Fig. 2. Mechanism of carrier (L) mediated active transport of ion pair (LC^+A^-) through bulk liquid membranes assisted by a pH gradient.

The receiving phase at pH = 13, transforms the protonated amino group $-NH_3^+$ into $-NH_2$; the amino acid is released from the ternary complex and passes into the receiving phase as a lithium salt. The empty carrier *L* diffuses back to the source phase/membrane interface where the whole cycle starts again and again until the equilibrium between the phases of the system is achieved.

The basic pH of the receiving phase is ensured by using LiOH because the Li^+ ion, being much smaller than the crown ether ring size, is not complexed by the macrocyclic carrier and so it does not determine any additional reactions which could possibly influence the transport phenomenon.

Consequently, the acid medium of the source phase allows the formation of an ion pair complex (LC^+A^-) which diffuses through the membrane. The basic medium of the receiving phase facilitates the dissociation of the complex and thus the amino acid passes from the organic phase into the receiving phase. A coupled transport of amino acid and H^+ ions from the source phase to the receiving phase is accomplished this way. Therefore, the transport occurs according to an active transport mechanism with pH gradient.

The 16 α-amino acids (L-Methionine, L-Isoleucine, L-Phenylalanine, L-Leucine, L-Valine, L-α-Alanine, L-Cysteine, Glycine, L-Aspartic Acid, L-Glutamic Acid, L-Serine, L-Treonine, L-Proline, L-Lysine, L-Asparagine and L-Histidine) have been studied; seven of them (L-Methionine, L-Isoleucine, L-Phenylalanine, L-Leucine, L-Valine, L-α-Alanine and L-Cysteine) have been proved to be extractable in 1,2 dichloroethane, under experimental conditions imposed to the source and receiving phases.

Separation of α-amino acids by means of the membrane system has been carried out under stirring of phases in contact during 6 h; the experimental results are listed in Table I.

The above yields have been obtained during shorter intervals of time (6 h) compared to those required by the classic U-shaped devices (24 h) [10] which demonstrate the efficiency of the former device.

The results of this study have suggested the possibility of classifying the amino acids as extractable and nonextractable from an acid aqueous phase into an alkaline phase under certain conditions. This classification is based on the structural distinction between α-amino acids imposed by the more hydrophobic or hydrophilic character of the chain R (Table I).

The separation of amino acids in cationic form by 18-crown-6 in 1,2 dichloroethane in the presence of the picrate anion may be referred to as molecular recognition of the group $-NH_3^+$, recognition of the chain R and recognition of the anion. The recognition of $-NH_3^+$ is determined by the nature of the macrocyclic ligand cavity, the dimensional and the structural-functional fit of the cavity and $-NH_3^+$ group. This recognition may be influenced by changing the macrocyclic ligand (the overall number of atoms and the number of oxygen atoms in the polyether ring) and the basicity of the $-NH_2$ group in the amino acid which determines the pH of the source phase.

TABLE I. Experimental data of the study on the transport of some protonated amino acids through a liquid membrane (18-crown-6/1,2 dichloroethane)

Amino Acid	R_n		Found in the receiving phase after 6 h of stirring
	n	R	%
L-Methionine	1	$-CH_2-CH_2-S-CH_3$	83
L-Isoleucine	2	$-CH(CH_3)-CH_2-CH_3$	81
L-Phenylalanine	3	$-CH_2-C_6H_5$	81
L-Leucine	4	$-CH_2-CH(CH_3)-CH_3$	81
L-Valine	5	$-CH(CH_3)-CH_3$	79
L-α-Alanine	6	$-CH_3$	74
L-Cysteine	7	$-CH_2-SH$	50

The recognition of the R-chain is also determined by the correlation between the nature of the chain R and the solvent which makes up the membrane (the dielectric constant type, by solvent -R interactions).

Thus, this recognition may be influenced by changing the solvent. A recognition of the R-chain by the macrocyclic ligand itself must not be disregarded as long as various interactions may occur between the two entities.

The recognition of the anion as a component of the ion pair associated in the membrane phase is in its turn determined by the lipophilic character and the basicity constant of this anion and by the nature of the solvent (mainly its dielectric constant). This recognition may also be influenced by the change of solvent and the pH of the aqueous source phase.

The three types of amino acid recognition in the system of liquid membranes suggest multiple possibilities of optimizing the process of amino acid separation with respect to the increasing selectivity and the final yields.

Our experiments have been firstly focused to check the transport mass transfer dependence on the stirring rate, which has proved to be linear up to about 400 rpm (Table II). Higher rates may possibly cause mixing of the phases.

Source phase: [L-Leucine] = 1.6 mM; [picric acid] = 0.8 mM; HCl 0.05N (pH = 2), 4 mL.

Membrane: [18-crown-6] = 10 mM/1,2 dichloroethane, 40 mL.

Receiving phase: LiOH 0.1N (pH = 13); 10 mL; 25° ± 1°C.

TABLE II. Experimental data of a study on transport of amino acid as a function of stirring rate

Amino acid in receiving phase after 6h of stirring (%)	Stirring rate (rpm)
71	100
80	180
86	300
88	400

We have chosen 180 rpm stirring speed to study the transport rate dependence on the volume (thickness) of the liquid membrane [9].

Although the Nernst layers are affected by the stirring rates, our experiments have clearly proved the dependence of the transport rate on the liquid membrane thickness. If stirring rates do not affect the complexation/decomplexation reactions at the interfaces, then our results conclude that the rate-limiting process in amino acid macrocyclic mediated transport through liquid membranes is the diffusion across the bulk of the organic phase. Generally, unsupported liquid membranes are poorly defined in respect with hydrodynamic behaviour and not commercially available, yet they perform macrocycle-mediated cation separations, giving correct predictions concerning the selectivity of various macrocyclic structures.

4. Conclusions

Mixtures of α-amino acids may be separated as $-NH_3^+$ through a liquid membrane by 18-crown-6 in 1,2 dichloroethane in the presence of picrate anion.

The transport mechanism is actively assisted by the pH gradient and it is optimized by the geometry of the experimental device employed, so that an efficient extraction is ensured (yields between 50 and 83%) depending on the amino acid extending over a relatively short time (6 h).

Our results conclude that the rate-limiting process in amino acid macrocyclic mediated transport through liquid membranes is the diffusion across the bulk of the organic phase.

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