Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Carrier-Mediated Transport of Amino Acids Through Bulk Liquid Membranes

R. Bucci[a]; S. Canepari[a]; E. Cardarelli[a]; A. M. Girelli[a]; A. Pietrodangelo[a]; M. Valiente[b]
[a] Department of Chemistry, University of Rome 'La Sapienza, Rome, Italy [b] Quimica Analitica Universitat Autònoma de Barcelona, Bellaterra, Spain

Online publication date: 08 July 2010

To cite this Article Bucci, R. , Canepari, S. , Cardarelli, E. , Girelli, A. M. , Pietrodangelo, A. and Valiente, M.(2004) 'Carrier-Mediated Transport of Amino Acids Through Bulk Liquid Membranes', Separation Science and Technology, 39: 16, 3821 — 3838
To link to this Article: DOI: 10.1081/SS-200041143
URL: http://dx.doi.org/10.1081/SS-200041143

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 39, No. 16, pp. 3821–3838, 2004

Carrier-Mediated Transport of Amino Acids Through Bulk Liquid Membranes

R. Bucci,[1] S. Canepari,[1,*] E. Cardarelli,[1] A. M. Girelli,[1] A. Pietrodangelo,[1] and M. Valiente[2]

[1]Department of Chemistry, University of Rome 'La Sapienza,' Rome, Italy
[2]Quimica Analitica Universitat Autònoma de Barcelona, Bellaterra, Spain

ABSTRACT

A novel carrier, the N-methyl, N-dodecyl-ephedrinium ion, was studied for the selective transport of some aromatic amino acids [phenylalanine (Phe), histidine (His) and tryptophan (Trp)] through bulk liquid membranes. Investigations of the main parameters limiting the aided transport of the amino acids were carried out. Transport rates that resulted were affected by the amino acid lipophilicity, the pH variations of feed and receiving solutions, the concentration, and the nature of countertransport ions. Further results indicate that the carrier can interact with amino acids by ion pairing and nonionic interactions. Membrane selectivity showed to be strictly related to kinetics of the amino acid release at the

*Correspondence: S. Canepari, Department of Chemistry, University of Rome 'La Sapienza,' P.le Aldo Moro, 5, Rome, Italy; E-mail: silvia.canepari@uniroma1.it.

DOI: 10.1081/SS-200041143 0149-6395 (Print); 1520-5754 (Online)

membrane/receiving interface, and its results were satisfactory when the receiving solution was kept in acidic conditions. In these conditions, when a mixture of the three amino acids was used as the feed solution, the transport rate of phenylalanine was over three times higher with respect to tryptophan, while the transport of histidine was almost completely damped down.

Key Words: Bulk liquid membranes; N-methyl, N-dodecyl-ephedrinium ion; Aromatic amino acids; Carrier-assisted transport.

INTRODUCTION

The use of separation techniques based on liquid membranes (LMs) is growing rapidly, as a consequence of their selectiveness efficiency, flexibility on selecting strategies, and cost-saving advantages with respect to traditional separation processes. The Two Module Hollow Fiber Liquid Membranes systems[1–4] particularly exhibit excellent multistage performances, being able to solve some of the relevant problems (i.e., membrane instability) encountered in industrial applications with emulsion and supported liquid membranes (ELMs and SLMs). As the Two Module Hollow Fiber Liquid Membranes share with bulk liquid membranes (BLMs) strong analogies in the transport mechanism,[5,6] a BLM would be the proper system to test the performances of new carriers prior to their application to a Two Module Hollow Fiber membrane.

The separation of amino acids represents an important and not yet solved industrial problem. Many recent patented industrial methods adopt LM technologies,[7–9] using various transport mechanisms and different carriers. Amino acids pertraction by LMs usually involves neutral,[10–14] metallic,[15–18] anionic,[19–22] and cationic carriers,[23–26] the latter appearing particularly successful for the transport of amino acids in their anionic form by ion pairing. The need of further efforts in this field is related to the high costs of taylor-made synthetic carriers (like crown ethers or calixarene derivatives,[27]) which present good selectivities, or to the low selectivity of ionic commercial low-costs ones (like aliquat 336 or phosphoric acid derivatives).[21]

In this work the performances of the N-methyl, N-dodecyl-ephedrinium ion has been investigated as a cationic carrier for the selective separation of aromatic amino acids. The carrier studied in this work is commercially available and relatively unexpensive. Its structure allows combination of the ion-pair formation, characteristic of ionic carriers, with nonionic $\pi-\pi$ and hydrogen bonding interactions, usually coresponsible for the selectivity of

the synthtetic carriers. The hydroxyl group and the benzene ring of this quaternary ammonium salt are, in fact, expected to interact with the aromatic amino acids, providing, in this event, further selectivity criteria.

EXPERIMENTAL

Reagents

N-methyl, N-dodecyl-ephedrinium ion (Fluka, Switzerland), $CHCl_3$ (Carlo Erba, Italia), (±)-phenylalanine, (±)-tryptophan and (±)-histidine (Sigma, Germany), and all other commercial products and solvents were pure for analysis and ISO grade. They were used without further purification. Methanol and acetonitrile used for the eluents were pure for HPLC grade (Carlo Erba) and the water was ultrapure from a MilliQ–RG Millipore Water Purification System.

Membrane Transport Experiments

A BLM system was used. Feed solutions were aqueous amino acids solutions in phosphate buffer, 0.1 M at different pHs; receiving solutions were phosphate buffer 0.1 M at different pHs, and the liquid membrane was a solution of N-methyl, N-dodecyl-ephedrinium ion in $CHCl_3$. A 20 mL volume of membrane solution was introduced in a home-made U-tube cell (i.d. 25 mm; height of each arm 6 cm) and covered filling each arm of the tube with 8 mL of aqueous receiving and feed solutions, respectively. The membrane phase was stirred at a constant rate by a magnetic 15-place multistirrer (VELP scientifica, Italy).

Experiments related to the membrane selectivity were run out at $21 \pm 0.5^{\circ}C$ in a properly home-made recirculation tank connected to an electric thermostat Crioterm 10–80 (Isco, Italy) and placed on the multiplace magnetic stirrer. Furthermore pH of the aqueous phases feed and receiving was periodically controlled during all membrane performances by a glass-combined electrode connected to a Crison micro pH 2001 potentiometer. Each set of membrane experiments was run in parallel using a series of 10 membrane cells. Feed and receiving solutions were sampled at fixed times by 60 μL withdrawals. Amino acids concentrations were then analyzed by HPLC injecting samples amounts of 20 μL.

Chromatographic Analysis

A column RP-18 (5.0 μm; i.d. 4.6 mm; L 25 cm), Supelcosil, and a quaternary system, Waters 600 with a photodiode array detector, Waters 996, were used. The Phe and Trp solutions were eluted with phosphate buffer 0.01 M (pH 5.6) : methanol = 75 : 25 (v/v) and respectively detected at λ 254 and 242 nm, with retention times (t_r) of 4.5 min and 6.5 min. The His was eluted with a phosphate buffer, 0.01 M at pH 7.6 ($t_r = 4.0$ min), and detected at λ 218 or 225 nm depending on the expected concentrations (218 nm for concentrations <1 mM and 225 nm for higher concentrations). Ternary Phe, His, and Trp solutions were eluted with a trifluoroacetic acid 0.1% in H_2O/acetonitrile linear gradient: a) up to 2.5 min the eluent was 100% trifluoroacetic acid 0.1% in H_2O; b) from 2.5 up to 10.0 min the eluent composition was taken to 30% trifluoroacetic acid 0.1% in H_2O : 70% acetonitrile; c) from 10.0 up to 12.0 min the composition linearly turned back to 100% trifluoroacetic acid 0.1% in H_2O, this condition was kept up to 16.0 min. Retention times of His, Phe, and Trp were respectively 3.7, 11.8, and 12.4 min, and the detection was respectively at 218 nm, 254 nm, and 242 nm.

RESULTS AND DISCUSSION

Preliminary Studies and Investigation on Experimental Conditions

The choice of membrane solvent was made taking into account both carrier and analytes solubilities, which strongly influence aided and not-aided pertraction rates, and the surface tension, which regulates the interface stability. Chloroform was particularly suitable, as it allows a fast aided amino acids transport and sufficiently stable interface layers, even when membrane stirring is applied.

To the aim of weighting the contribution of not-aided passive transport, the flux of amino acids has been evaluated in the absence of carrier at various feed and receiving pH conditions.

In the case of His, no detectable amount was found in the receiving solution within the first 24 hours in any conditions. On the other hand Phe and Trp showed a low passive transport rate. The highest values (pH_{feed} 5–6 and $pH_{receiving}$ <5 or >9) were respectively 0.2 and 0.7 $\mu mol\,L^{-1}\,h^{-1}\,cm^{-2}$. The observed fluxes agree with the solubility of amino acids in chloroform and results were negligible if compared with those observed in the presence

of the carrier, clearly indicating the active role of the carrier in the transmembrane transport.

Preliminary experiments were performed to fix amino acids (aa) and carrier transport concentrations, in which both concentrations of the carrier N-methyl, N-dodecyl-ephedrinium ion in chloroform ($[efe]_{membrane}$) and of aa in the feed solution ($[aa]_{feed}$) were varied from 5 to 40 mM. In these trials pH of feed and receiving solutions was 11.0 and 4.0, respectively. Results showed that the transport rate of each tested aa is independent from the carrier concentration when the latter is >25 mM, and $[aa]_{feed} < 30$ mM. These conditions seem to indicate the saturation at the feed/membrane interface.

Therefore, all membrane transport experiments were then performed, unless otherwise noted, at $[efe]_{membrane} = 30$ mM and $[aa]_{feed} = 10$ mM. These conditions allowed us to maximize the transport rate of each aa and to isolate its dependence on pH conditions.

The influence of pH on membrane transport rates was studied by ranging the pH of feed or receiving solutions between 2.0 and 11.0 reciprocally and keeping the pH of the other solution fixed. It is worth noting that, although the phosphate buffer is not effective over the whole considered pH range, it's buffering power was strong enough to effectively control pH variations. Indeed, spontaneous pH variations were established to be lower than 0.2 pH unities within 250 minutes at any pH and, when longer periods were investigated, they were adjusted by adding small (order of μL) volumes of acid or base. Transport rates exhibited a constant value, at any pH investigated, during the first 200 min, after which a progressive decrease was observed.

Then, in order to make transport data independent from time influence, all results of the present study were discussed in terms of initial (up to 200 min) transport.

When the influence of pH_{feed} on amino acids fluxes was investigated, the $pH_{receiving}$ was kept at the value corresponding to the lowest amino acid concentration in the membrane phase. This concentration was determined at any time by calculating the mass balance from the $[aa]_{feed}$ and $[aa]_{receiving}$. The lowest aa concentration in the membrane solution was reached at different pHs of the receiver, depending on the amino acid under transport and it resulted to be 4.0, 7.0, and 10.5 respectively for Phe, Trp, and His. Finally, the overall experimental conditions employed in the following sections of the study were as reported in Table 1.

The reproducibility of data results was strongly affected by the stirring mode. To the aim of assuring homogeneous stirring of the feed and receiving solutions a taylor-made immersion stirrer was developed. It consists of two coaxial teflon disks, one suspended in the feed and membrane solutions inside one arm of the glass U-tube, and the other in the receiving and

Table 1. Experimental conditions for the study of feed and receiving pH influence on amino-acids transport rate. Samplings of 60 μL of both fired and receiving solutions were made and analyzed by HPLC at 20 min intervals.

		Phe	Trp	His
Feed/membrane interface	pH_{feed}	4 ÷ 10	4 ÷ 10	4 ÷ 11
	$pH_{receiving}$	4.0	7.0	10
Membrane/ receiving interface	pH_{feed}	11	11	11
	$pH_{receiving}$	4 ÷ 10	4 ÷ 10	4 ÷ 10

membrane solutions inside the other arm. However this stirring device caused a marked irreproducibility (RSD $> 25\%$), so that the sole membrane was magnetically stirred. The RSD calculated by running 10 replicates ([carrier] $= 30$ mM, $(aa)_{feed} = 10$ mM, $pH_{feed} = 11$, $pH_{receiving} = 3.5$) was of about 5% and was still mainly due to the irreproducibility of the stirring conditions.

Influence of pH_{feed} on Transport Rates

The influence of pH_{feed} was studied by measuring the aa concentrations both in the feed and in the receiving solutions and the obtained results did not show significant differences. Considering that the analytical error due to HPLC measurements is much lower when the concentration increase in the receiving phase is monitored instead of its decrease in the feed phase, all amino acid fluxes through the membrane were referred to the concentration variations in the receiving solution. In Fig. 1 the Phe flux (J) in the receiving solution is reported as a function of the pH_{feed} at different initial concentrations of the aa. The same behavior also was obtaind for Trp. It can be noted that the transport rate increases with pH_{feed} up to a maximum that is independent of the initial concentration of aa in the feed, indicating the saturation at feed/membrane interface.[25] The observed behavior is in agreement with an extractive mechanism of exchange diffusion of two substrates, as shown in the Scheme 1, in which the formation of carrier—aa (C^+aa^-) ion pair shifts the protonation equilibrium of the amino acids toward dissociation. It can also be noted that the saturation that occurs at lower pH_{feed} is higher then is the aa analytical concentration in the feed solution. This behavior again agrees with the mechanism showed in Scheme 1, as an increase of aa concentration aids the ion pair formation

Nevertheless, some further considerations can be made. According to the literature,[25] conditions for membrane saturation occur at a constant value of

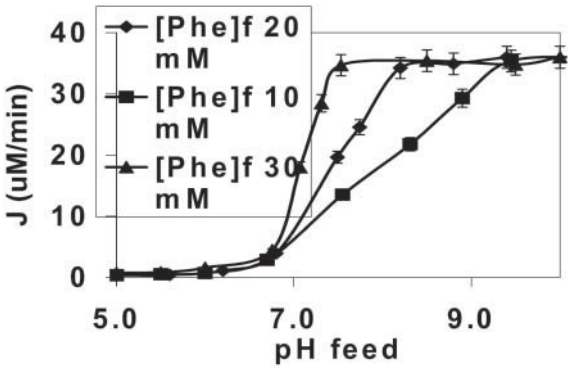


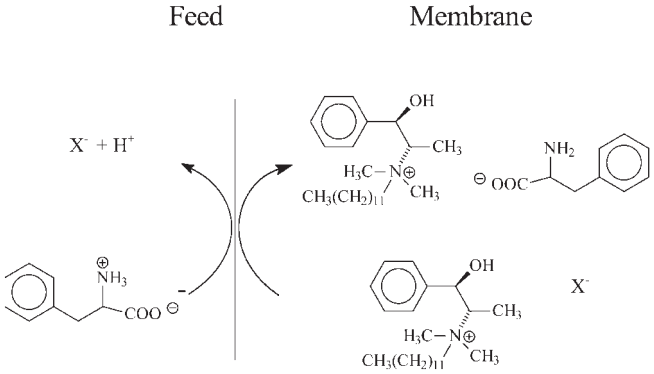
Figure 1. Initial fluxes (μM/min) vs. pH_{feed} at different $[Phe]_{feed}$; $[carrier]_{membrane} = 30$ mM; $pH_{receiving}$ 4.0.

the ratio $[C^+aa^-]_{membrane}/[C^+X^-]_{membrane}$, where X^- represents the counter ion. Hypothesizing a diffusion-limited process at the feed/membrane interface,[28,29] the constant of the extraction equilibrium K_{ex} may be expressed as

$$K_{ex} = [C^+aa^-]_{membrane}[X^-]_{aq}/[aa^-]_{aq}[C^+X^-]_{membrane}$$

and, considering the acidic dissociation of the aa,

$$1/K_{ex}([C^+aa^-]_{membrane}/[C^+X^-]_{membrane}) = (1/[X^-]_{aq})/[aa]_{feed}(K_a/(K_a + [H^+]_{aq})) \quad (1)$$



Scheme 1. Scheme of the exchange diffusion/competitive extraction mechanism of amino acids (Phe) at feed/membrane interface.

where K_a is the acidic constant of the aminogroup–protonation equilibrium of aa and $[aa^-]_{aq}$ is the aqueous concentration of the totally deprotonated fraction of aa.

Eq. (1) relates the $[C^+aa^-]_{membrane}/[C^+X^-]_{membrane}$ ratio to the aa analytical concentration and to pH. If the contribute of $[X^-]_{aq}$ to transport is considered as a constant[1] and if the values of $[aa]_{feed}$ and pH corresponding to the achievement of the maximum transport rate are assigned in Eq. (1), a decrease of $1/K_{ex}[(C^+aa^-)_{membrane}/(C^+X^-)_{membrane}]$ with the $[aa]_{feed}$ increasing is found. This fact indicates that when the $[aa]_{feed}$ is increased the saturation at feed/membrane interface occurs at milder conditions. This result could be explained considering a certain degree of nonionic interactions between the carrier and the aa, with an extraction mechanism of the kind:

$$(aaH)_{aq} + (C^+X^-)_{membrane} \leftrightarrow (C^+aaH)_{membrane} + (X^-)_{aq} \leftrightarrow (H^+)_{aq} + (C^+aa^-)_{membrane} + (X^-)_{aq} \qquad (2)$$

In Fig. 2 the curves of transport vs. pH_{feed} related to Phe, Trp, and His are reported, to make comparable each series of data in terms of initial fluxes. These were normalized as $J/J_{max} = m/m_{max}$, where m is the linear part slope of $[aa]_{rec}$ vs. time and m_{max} is the maximum value for each examined aa. The relative position of curves on the pH axis reflects the position of Eq. (2), which is determined by the strenght of C–aa interactions and by the lipophilicity of aa.

It is worth noting that the curve of His fits within the experimental error with its anionic distribution curve, showing a flex point closed to its pK_{a3} ($pK_{a3} = 9.12$). For His the transport rate follows, thus the $[aa^-]_{aq}$ and the saturation at interface doesn't seem to occur at given conditions. On the other hand, for Trp (pK_{a2} 9.32) the saturation conditions are reached at lower pH than for Phe (pK_{a2} 9.11), despite its higher pK_{a2} value. Thus, the observed results are in agreement with the lipophilicity order of amino-acids,[21,30,31] the low lipophicicity of His probably being the reason for its low extraction from the feed solution.

[1] In each experiment of Fig. 1 the membrane and receiving solutions have the same concentrations and pH. Furthermore, as yet evidened, in conditions of membrane saturation the aa transport rate is constant at any initial concentration of aa. Then the contribution of counter ion may be reasonably considered constant too.

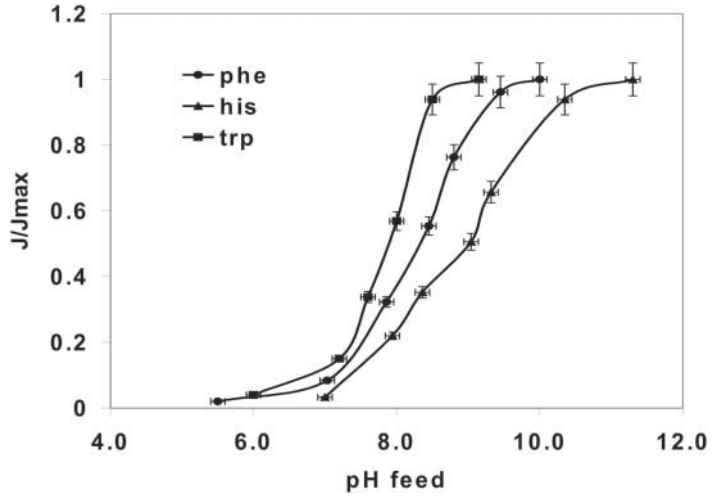


Figure 2. Normalized initial flux (J/J_{max}) vs. pH_{feed}: of Phe, Trp and His. $[aa]_{feed} = 10\,mM$; $[carrier]_{membrane} = 30\,mM$; $pH_{receiving}$: 4.0 (Phe), 7.0 (Trp) and 10.5 (His).

Influence of $pH_{receiving}$ on Transport Rates

The influence on transport rates of $pH_{receiving}$ was studied fixing pH_{feed} at 11. The J/J_{max} vs. $pH_{receiving}$ curves are reported in Fig. 3. These curves differ from the trends of the transport curves vs. pH_{feed}, which are similar for all the examined amino acids, significant differences have been observed in this case.

For Phe and Trp the minimum transport rate is observed at basic pH. In the range 7–11 of $pH_{receiving}$ the trends result nearly reversed with respect to those related to the dependence on pH_{feed} variations of Fig. 2, while at pH values <7 the decrease of $pH_{receiving}$ leads to a lowering of the transport rate. On the other side His shows a quite opposite behavior, with a flux increasing with $pH_{receiving}$ up to a maximum in correspondence to pH 8.

In order to explain these results, the mechanism of release of the amino acids at the membrane/receiving (m/r) interface has to be considered. This process requires the ion-pairing breakage and may be favored by a countertransport mechanism,[6] which is characteristic of ion transport, or by the protonation of aa. The latter event maintains a sufficiently low concentration of active species (aa^-) in the receiving solution and avoids the establishing of saturation at interface.[25] The protonation of aa, thus, may account for the increase of transport rate observed in the range 7 ± 11 of $pH_{receiving}$, while it is not consistent with the decrease at acidic pH. This behavior

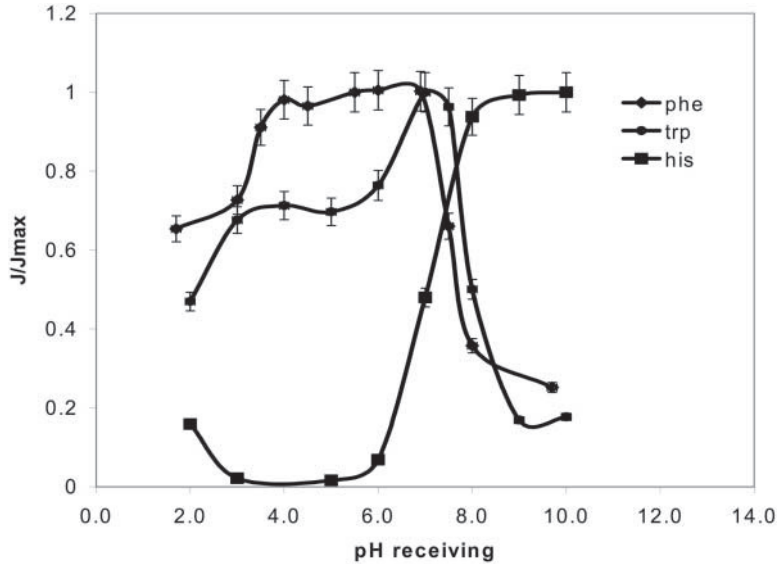


Figure 3. Normalized initial flux (J/J_{max}) vs. $pH_{receiving}$: of Phe, Trp and His; $[aa]_{feed} = 10\,mM$; pH_{feed} 11.0; $[carrier]_{membrane} = 30\,mM$.

could be interpreted on the basis of the efficiency of the counter transport mechanism. In these experiments, the counter transport is expected to be assisted by the phosphate anions present in the receiving solutions (HPO_4^{2-} and $H_2PO_4^-$), whose relative concentrations are dependent on the pH value. Particularly, taking into account the phosphate distribution diagram, the HPO_4^{2-} relative concentration decreases in the pH range 6–8, with a trend similar to the J/J_{max} curves. The HPO_4^{2-} species seems then to be the principal responsible of counter transport.

It should be noticed, however, that the counter transport due to phosphate anions is difficult to investigate since, as already mentioned, it depends on $pH_{receiving}$ variations. On the other hand, investigation on counter transport by mean of an anion whose concentration is not affected by pH changes, like Cl^- for istance, is not possible if no phosphate buffer is provided to this BLM process, because of the significant pH increases with the increasing aa concentration in the receiver. Some investigations were then carried out by adding different amounts of NaCl to the receiver buffered with 0.1 M phosphate at various pHs. The results are reported in Fig. 4. Again, the fluxes were normalized with respect to the maximum flux observed for each amino acid in order to put on evidence the sole contribution of $[Cl^-]_{receiving}$.

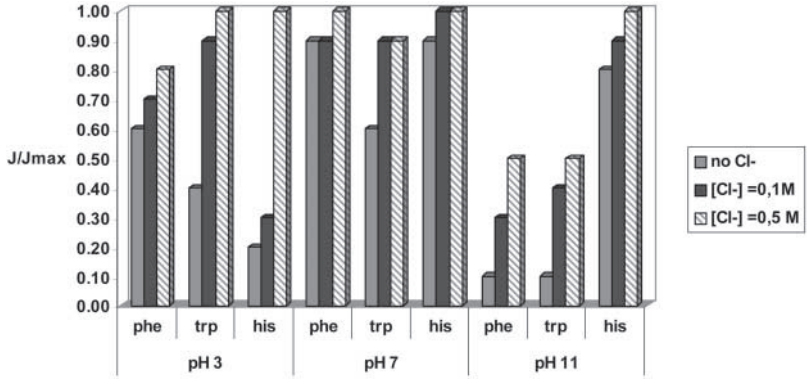


Figure 4. Fluxes normalized with respect to the maximum value related to each amino acid at different conditions of $[Cl^-]$ and pH in the receiving solution. $pH_{feed} = 11$; $[aa]_{i,feed} = 10\,mM$; $[carrier]_{membrane} = 30\,mM$.

Figure 4 shows that increasing $[Cl^-]_{receiving}$ involves an overall increase of transport rates, confirming the effectiveness of the counter transport mechanism. However responses to the variations of $[Cl^-]_{receiving}$ differed widely, depending on the amino acid and on pH.

Particularly, at $pH_{receiving}$ 11 the transport rate of Phe and Trp showed a relevant increase, however, keeping a lower value than the respective rates at pH 3 and 7. For these amino acids the protonation mechanism seems then to be necessary for an efficient release at the membrane/receiving interface and high concentrations of counter transport ion seem to be necessary to promote the process in the absence of aa protonation. At $pH_{receiving}$ 3 the behavior of Cl^- compensates for the rate decrease observed in its absence, confirming the hypothesis of a kinetic effect due to a decrease of $[HPO_4^{2-}]$. On the other hand, the trend of His at $pH_{receiving}$ 11 and 7 is scarcely sensitive to the $[Cl^-]$, indicating the sufficiency of phosphate ions concentration to the release of this amino acid, while at acidic pH, a strong increase of its transport rate with $[Cl^-]_{receiving}$ was noted. The protonation mechanism thus seems not to influence the transport trend of His in any conditions.

The anomalous behavior of His, which deserves further investigations, could depend on nonionic interactions with the carrier, other than those given by Phe and Trp, or on the different properties of His at the membrane/receiving interface. At this purpose, it is worth noting that the transport of His is very slow and it is scarcely extracted by the membrane, as yet noted in the discussion about the uptake of aas at feed/membrane interface. In these conditions, again no saturation is expected at the release interface.[25]

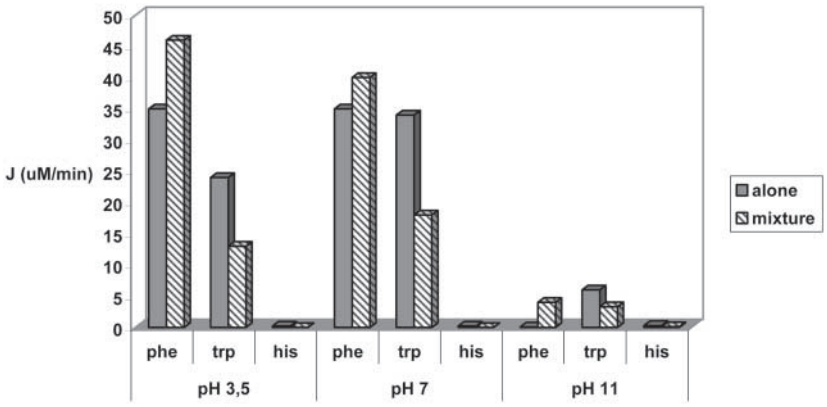


Figure 5. Tryptophan amounts (μmol) in the feed, membrane, and receiving solutions vs. time (min) at pH a) 3, b) 7, and c) 11. $pH_{feed} = 11.0$; $[carrier]_{membrane} = 30\,mM$.

The influence of the dissociation kinetics at the membrane/receiving interface may be better highlighted by the comparison of feed, membrane, and receiving [aa] variations with time at different $pH_{receiving}$. In Fig. 5, as an example, the results obtained for Trp are reported, in which it is evident that fluxes in the receiving depend on the release process at the membrane/receiving interface. In fact, the decrease of [aa] in the feed solution, at least for the initial portion of the curves, is nearly independent on the $pH_{receiving}$, while the build up of aa in the membrane and receiving solutions are complementary and strongly dependent on $pH_{receiving}$. In particular, at $pH_{receiving}$ 3 (Fig. 5a) a fast initial build up in the membrane phase followed by a slow quantitative release in the receiving solution is noted, which is consistent with the mentioned low efficiency of the counter transport mechanism. A similar trend is observed at $pH_{receiving}$ 11 (Fig. 5c), at which the protonation mechanism doesn't occur. Also in this case a quantitative transport is not expected, as feed and receiving solutions are in the same pH conditions. On the other hand, at $pH_{receiving}$ 7 (Fig. 5b) both mechanisms are efficient and the release is fast and quantitative, showing a low and transient build up of the aa in the membrane phase.

Selectivity of the N-methyl, N-dodecyl-ephedrinium Ion

In order to investigate the selectivity of the carrier, membrane experiments were performed using solutions of a single amino acid or ternary mixtures of Phe, Trp, and His as the feed phase. In these experiments the

feed solutions were maintained at pH 11, to maximize the extraction process at the feed/membrane interface, while the $pH_{receiving}$ was varied to show the influence on membrane selectivity of the release kinetics at the membrane/receiving interface. The initial transport rates (as mean from three replicates) of each amino acid alone or in a mixture were then compared and the results are reported in Fig. 6.

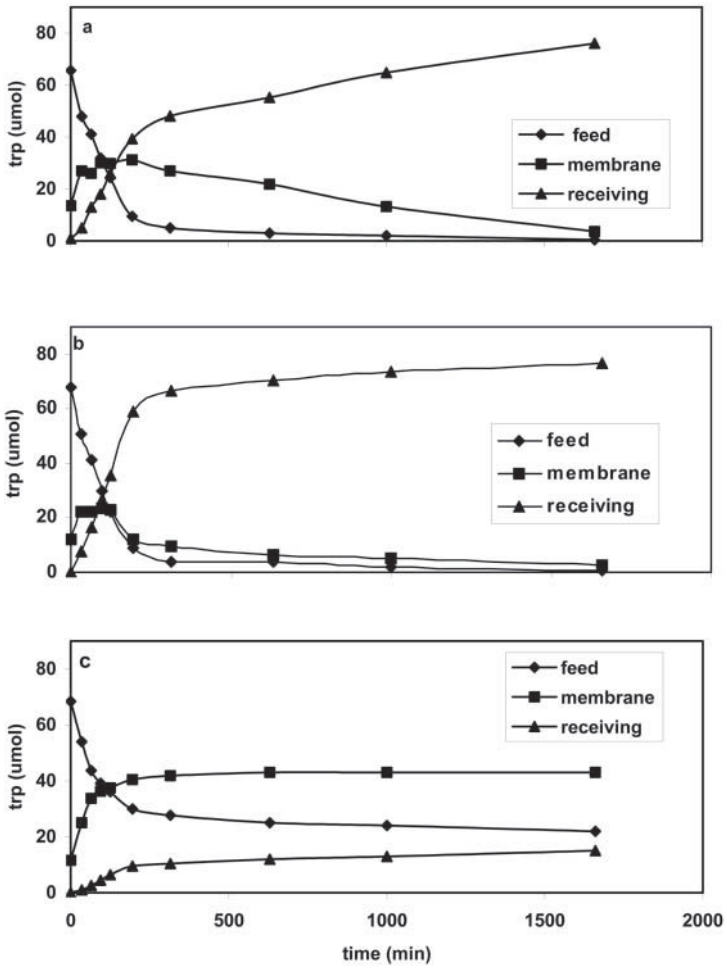


Figure 6. Initial transport rates (μM/min in the receiving phase) of amino acids alone or in mixture. $T = 21.0 \pm 0.5°C$; $[aa]_{feed} = 10\,mM$; $pH_{feed} = 11.0$; $[carrier]_{membrane} = 30\,mM$.

Referring to the single amino acid transport experiments, the comparison between Phe and Trp behavior is particularly interesting: e.g., Phe shows transport rates higher than does Trp at $pH_{receiving}$ 3.5, while at $pH_{receiving}$ 11 the opposite order is observed, and at $pH_{receiving}$ 7 the two rates are comparable. As is well known, the whole transport rate results from the contributions of amino acids lipophilicity,[21,30,31] of the strength of interactions with the carrier, and of the exchanges kinetics at interfaces. As all the other conditions are kept constant and considering the results reported in Fig. 5, the differences observed as a function of $pH_{receiving}$ are mainly related to the kinetics of the release process at the m/r interface. As previously discussed, the efficiencies of protonation and counter transport mechanisms are different depending on the amino acid. The highest efficiency of protonation mechanism of Phe could account for its highest transport rate at acidic pH, while the more rapid transport observed for Trp at $pH_{receiving}$ 11 could be explained by considering both the lipophilicity order and the contribution of counter transport mechanism, which is prevalent at these conditions and showed to be more efficient for Trp.

About His, its transport rates remain lower than those of Phe and Trp at a 10^2 term both at $pH_{receiving}$ 3.5 and 7, while at $pH_{receiving}$ 11 they are comparable with the Phe rate. This behavior is in agreement with the low lipophilicity of His, whose interactions with the carrier are likely not strong enough to overcome its scarce affinity for the membrane phase at the feed/membrane interface. As well, the inefficiency of protonation mechanism in the release at the second interface doesn't effectively aid His to transfer from the membrane to the receiving solution at low $pH_{receiving}$.

When a mixture of the three amino acids is used as feed solution, the differences in transport rates were enhanced. Particularly, at any $pH_{receiving}$ the transport rate of Phe increases, while the Trp and His rates become lower. This result should mainly derive from a competition effect in the extraction process at the feed/membrane interface, and should then account for a higher affinity of Phe for the membrane phase. Since the lipophilicity could explain the His behavior, but not that of Phe and Trp, this result could suggest that a stronger interaction of Phe with the carrier occurs with respect to Trp. This consideration is confirmed by the distribution coefficients, given as the ratio $[aa]_{membrane}/[aa]_{aq}$, whose values are respectively $D_{Phe} = 0.26$, $D_{Trp} = 0.08$, $D_{His} < 0.02$.[2] These coefficients were obtained by debeating aqueous solutions 10 mM in aa at pH 11 with a membrane

[2] D_{His} was not calculated, as the fraction extrated by the membrane solution was too low as compared with the analytical error in the determintion of $[aa]_{aq}$.

solution 30 mM in N-methyl, N-dodecyl-ephedrinium ion. The $[aa]_{membrane}$ were calculated by mass balance (three replicates of each measurement were run), subtracting the $[aa]_{aq}$ at the equilibrium to the initial analytical concentration. As the ion pair interactions should be of comparable strength, the less lipophilic Phe seems to give the carrier additional nonionic interactions.

The membrane selectivity is influenced by the kinetics of the release process at the m/r interface too, and results are enhanced at acidic $pH_{receiving}$, where the release of Phe is favored by the protonation mechanism. In these conditions, the ratio between Phe and Trp rates is 3.6, therefore, showing a very satisfactory membrane selectivity for a BLM process. This result are better evidenced in Fig. 7, where the increase of amino acids concentration with time in the receiving solution at pH 3.5 is reported. Differences in transport rates between the three amino acids are evident, with more than 80% of Phe transported in the receiving phase after ca. 300 min, while Trp percentage remains at about 30% and His is under detection limits. It is worth noting that at longer times a nearly quantitative transport of Phe and Trp can be obtained.

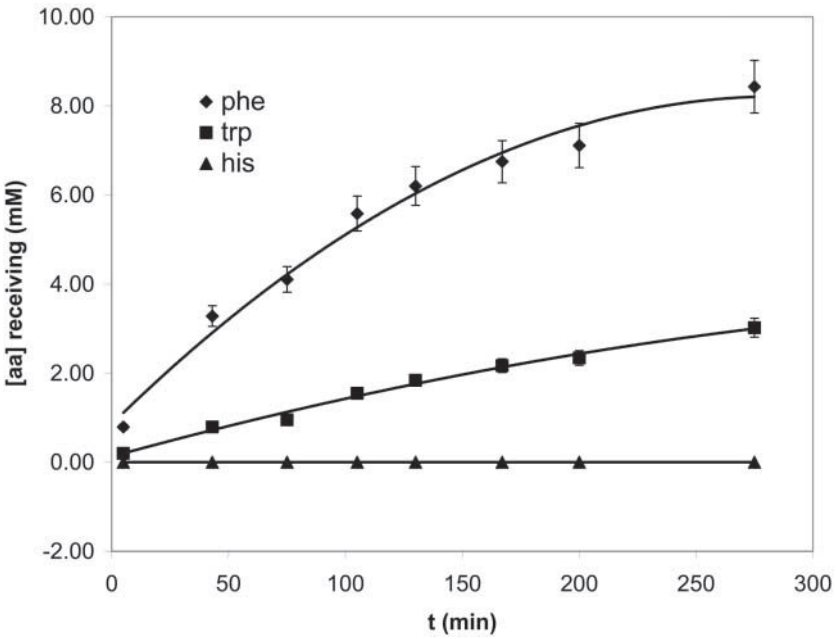


Figure 7. Amino acids concentration in the receiving solution with time at $pH_{receiving}$ 3.5 when a mixture of Phe, Trp and His is used as feed solution. $[aa]_{feed} = 10$ mM in each aa; pH_{feed} 11.0; $[carrier]_{membrane} = 30$ mM; $pH_{receiving}$ 3.5.

CONCLUSIONS

The work has demonstrated the good capability of N-methyl, N-dodecyl-ephedrinium ion as a carrier for aromatic amino acids aided transport through a bulk choloroform membrane. This carrier, which is commercially available and inexpensive, may establish $\pi-\pi$ interactions and hydrogen bonding with amino acid side chains, respectively, dependent on the aromatic ring and on the hydroxyl group. Although the main interaction is the ion-pairing between the carrier and the deprotonated form of the amino acid, these interactions appear to be co-responsible for the selectivity exhibited by the membrane, influencing both the extraction process at the feed/membrane interface and the release kinetic at the membrane/receiving one.

The selectivity show by the membrane is appreciable for BLM, and gives hopeful signs of improvement by applying the results to flow membrane systems based on the use of hollow fibers.

ACKNOWLEDGMENTS

Thanks are due to Dr. J.A. Calzado for the precious scientific support and to Mr. Silvano Ghighi for technical assistance.

REFERENCES

1. Sengupta, A.; Basu, R.; Prasad, R.; Sirkar, K.K. Separation of liquid solutions by contained liquid membranes. Sep. Sci. Technol. **1988**, *23* (12–13), 1735.
2. Pirkle, W.H.; Doherty, E.M. Enantioselective transport through a silicone-supported liquid membrane. J. Am. Chem. Soc. **1989**, *111*, 4113.
3. Pirkle, W.H.; Bowen, W.E. Preparative separation of enantiomers using hollow-fiber membrane technology. Tetrahedr. Asymm. **1994**, *5* (5), 773.
4. Schlosser, S.; Rothova, I.; Frianova, H. Hollow-fiber pertractor with bulk liquid membrane. J. Membr. Sci. **1993**, *80*, 99.
5. Keurentjes, J.T.F.; Voermans, F.J.M. In *Chirality in Industry, Part II*; Collins, A.N., Sheldrake, G.N., Crosby, J., Eds.; John Wiley and Sons; 1997; 157–180, Chapter 8.
6. Brice, L.J.; Pirkle, W.H. In *Chiral Separation: Application and Technology*; Ahuja Satinder, Ed.; American Chemical Society: Washington, DC, 1993; Chapter 11.

7. Matsuyama, H.; Boku, J.; Teramoto, M. Separation and concentration of heavy metal ions by a spiral-type flowing liquid membrane module. Water Treatment **1990**, *5*, 237.
8. Teramoto, M.; Matsuyama, H.; Yamashiro, T.; Okamoto, S. Separation of ethylene from ethane by a flowing liquid membrane using silver nitrate as a carrier. J. Membr. Sci. **1989**, *45*, 115.
9. Izatt, R.M.; Bradshow, J.S.; Lamb, J.D.; Bruening, R.L. In *Liquid Membranes: Chemical Applications*; Araki, T., Tsukube, H., Eds.; CRC Press: Boca Raton, FL, 1990; Chapter 7.1.
10. Bryjak, M.; Wieczorek, P.; Kafarsky, P.; Lejczak, B. Transport of amino acids and their phosphonic acid analogs through supported liquid membranes containing macrocyclic carriers. Experimental parameters. J. Membrane Sci. **1991**, *56*, 167.
11. Chang, S.; Hwang, H.; Son, H.; Youk, J.; Kang, Y.S. Selective transport of amino acid esters through a chloroform liquid membrane by a calix[6]-arene-based ester carrier. J. Chem. Soc. Chem. Comm. **1991**, *4*, 217–218.
12. Inokuma, S.; Sakai, S.; Yamamoto, T.; Nishimura, J. Synthesis of double-looped crownophanes and their ability of amino acid transport through liquid membranes. J. Membrane Sci. **1994**, *97*, 175.
13. Buschmann, H.J.; Mutihac, L. Complexation, liquid-liquid extraction, and transport through a liquid membrane of protonated peptides using crown ether. Anal. Chim. Acta. **2002**, *466* (1), 101–108.
14. Breccia, P.; Van Gool, M.; Perez-Fernandez, R.; Martin-Santamaria, S.; Gago, F. et al. Guanidinium receptors as enantioselective amino acid membrane carrier. J. Am. Chem. Soc. **2003**, *125* (27), 8270–8284.
15. Calzado, J.A.; Palet, C.; Jonsson, J.A.; Valiente, M. Metal affinity liquid membrane: II. Facilitated transport of tryptophan. Anal. Chim. Acta **2000**, *417* (2), 159–167.
16. Diez, S.; Zhou, P.; Daunert, S.; Valiente, M. Immobilized soft-metal affinity system for amino acids based on an 8-hydroxyquinoline-Pd(II) complex; characterization using glycine as a model. Anal. Chim. Acta **1995**, *315*, 339–345.
17. Calzado, J.A.; Palet, C.; Valiente, M. Determination of structural and electrical parameters for activated composite membranes containing di-(2-thylhexyl)dithiophosphoric acid as carrier. Anal. Chim. Acta **2000**, *403*, 101–115.
18. Tsukube, H.; Uenishi, J.; Kanatani, T.; Itoh, H.; Yonemitsu, O. Enantioselective binding and extraction of zwitterionic amino acids by chiral lanthanide complexes. Chemical Communications (Cambridge) **1996**, *4*, 477–478.
19. Takeshima, S.; Wada, S.; Sakurai, H. Transport behaviour of basic aminoacids through inorganic liquid membrane system acid using a supported liquid membrane process. Sep. Sci. Tech. **1994**, *29*, 2117–2129.

20. Hossain, M.D.M.; Stanley, R.A. Selective transport of a peptide from its mixture with an aminoacid. Sep. Sci. Tech. **1995**, *30*, 3801–3819.
21. Ma, M.; Chen, B.; Luo, B.; Tan, H.; He, D.; Xie, Q.; Yao, S. Study on the transport selectivity and kinetics of amino acids through di(2-ethylhexyl) phosphoric acid-kerosene bulk liquid membrane. Journal of Membrane Science **2004**, *234* (1–2), 101–109.
22. Calzado, J.A.; Palet, C.; Valiente, M. Facilitated transport and separation of aromatic amino acids through activated composite membrane. Anal. Chim. Acta **2001**, *431*, 59–67.
23. Kamo, N. In *Liquid Membranes: Chemical Applications*; Araki, T., Tsukube, H., Eds.; CRC Press: Boca Raton, FL, 1990; Chapter 2.
24. Hidalgo, M.; Masana, A.; Salvado, V.; Freiser, H.; Al-Bazi, S.J.; Valiente, M. Accelerated mass transfer of palladium(II) through a selective solid-supported liquid membrane containing Cyanex 471. Anal. Chim. Acta **1991**, *251*, 233–239.
25. Behr, J.P.; Lehn, J.M. Transport of amino acids through organic liquid membranes. J. Am. Chem. Soc. **1973**, *95*, 6108–6110.
26. Molinari, R.; De Bartolo, L.; Drioli, E. Coupled transport of amino acids through a supported liquid membrane. I. Experimental optimisation. J. Membr. Sci. **1992**, *73*, 203.
27. Oshima, T.; Inoue, K.; Furusaki, S.; Goto, M. Liquid membrane transport of amino acids by a calix[6]arene carboxylic acid derivative. J. Membr. Sci. **2003**, *217* (1–2), 87–97.
28. Behr, J.-P.; Kirch, M.; Lehn, J.-M. Carrier-mediated transport through bulk liquid membranes: dependence of transport rates and selectivity on carrier properties in a diffusion-limited process. J. Am. Chem. Soc. **1985**, *107*, 241–246.
29. Chrisstoffels et al. In *Chemical Separations with Liquid Membranes*; Bartsch, R.A., Way, J.D., Eds.; ACS Symposium Series 642, American Chemical Society: Washington, DC, 1996; Chapter 3.
30. Radzicka, A.; Wolfenden, R. Comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. Biochem. **1988**, *27*, 1663.
31. Eltayar, N.; Tsai, R.-S.; Carrupt, P.-A.; Testa, B. Octan-1-ol-water partition coefficients zwitterionic α-aminoacids. Determination by centrifugal partition chromatography and factorization into steric/hydrophobic and polar components. J. Chem. Soc. Perkin Trans. II **1992**, 79–84.

Received March 2, 2004
Accepted August 17, 2004