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REVIEW

LIQUID MEMBRANES INVOLVED IN SOME BIOLOGICAL COMPOUNDS SEPARATION

Lucia MUTIHAC,[a*] Cristian BALTARIU,[a] Atena HOTARANU[a] and Radu MUTIHAC[b]

[a]Department of Analytical Chemistry, University of Bucharest, 4-12, Blvd. Regina Elisabeta, 030018, Bucharest, Roumania.
[b]Department of Electricity and Biophysics, University of Bucharest, PO Box MG-11, 077125 Bucharest Măgurele, Roumania

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Based on selectivity of macrocyclic hosts such as crown ethers, modified cyclodextrins, and functionalized calix[n]arenes to various guests, their ability to be used as carriers through membrane processes is reviewed. The applications of liquid membrane in separation of some biological compounds like amino acids, peptides, proteins, and saccharides is presented hereafter. Likewise, aspects and factors involved in separation through liquid membrane of biological compounds are discussed.

INTRODUCTION

The separation of molecules by membranes plays an important role in biological systems and industrial applications, too. Membrane technologies are able to produce high purity chemicals using biotechnological processes. Moreover, the importance of membrane technologies for wastewater treatment has emerged particularly during the last years.[1-10]

It is well known that the membrane processes have already been successfully applied in advanced components of analytical instruments (*i.e.*, membrane sensors), biomedical and biotechnological applications (*i.e.*, artificial organs), textile and pharmaceutical industry.[11-16] One of the most important fields in membrane operation stems from combining selective mass transfer through membrane with a specific chemical reaction in a biological process. This combination is possible using the membrane itself as a reactor. In these systems, the chemical transformation and physical separation of species from the products may take place in the same time while minimizing product inhibition phenomena. This kind of biological membrane reactor has already been used in the wastewater treatment.[14]

Membrane separation in bioreactors is one of the most attractive operations applied in biochemical processes. Enzyme membranes increase the potentiality of membrane separation in biochemical processes as well. Drioli *et al.*[17] investigated the possibility of catalytic membrane systems. Thus, membrane reactor configuration, the applications of dense and porous membranes to various reactions, their ability to work in gas or liquid phase using various driving forces (*i.e.*, transmembrane concentration gradient, transmembrane gradient pressure, and transmembrane gradient temperature) have been employed in recent applications: decomposition reactions (*e.g.*, H_2S, etc.), dehydrogenation reactions (*e.g.*, cyclohexane, ethylbenzene, etc.), and hydrogenation reactions (*e.g.*, butadiene, acetylene, etc.). The processes in a membrane bioreactor can be considered as the combination of two basic processes: (i) biological reaction and (ii) membrane separation into a single process.

The membrane fouling behavior represents the physicochemical interaction between the membrane material and certain components in the liquid that is subject of filtering. This is an important issue in membrane applications. Studies on anti-fouling were directed towards modifications of the internal as well as the external membrane surfaces. In membrane separation, highly hydrophilic membranes often exhibit better anti-fouling properties than less hydrophilic ones.[18]

* Corresponding author: mutihac@rdslink.ro

The liquid membrane processes are efficient techniques for selective separation, concentration, and purification of chemical and biological[19-26] compounds. Liquid membranes in separation of various compounds have the advantage of enhanced transport by using a selective carrier of anionic or cationic form dissolved in an organic solvent. The separation of enantiomer compounds may be carried out by using liquid membranes containing chiral transportors. The inclusion ability of cyclodextrin cavity for many kinds of compounds forming inclusion complexes was employed in liquid membranes.[27-30]

Macrocycles pre-designed to selectively interact with target inorganic or organic guests had an important impact in separations chemistry also. In this respect, crown ethers, cyclodextrins, and calixarenes were widely used as extractants or carriers through liquid membranes concerning the separation of different chemical and biological compounds. It is well known that biogenic amines, amino acids, hormones, sugars, peptides, nucleic acids, and proteins constitute the most fundamental substrates in biological and artificial processes. The understanding of specific biomolecular interactions plays a key role in modern supramolecular chemistry.

The present review focuses on recent studies on the transport through liquid membranes of some biological compounds by using crown ethers, modified cyclodextrins, and functionalized calix[n]arenes as carrier. Due to extensive and remarkable reports published on this subject, we will briefly present some of the most representative in this field.

1. Separation of amino acids from proteins by crown ether receptors

The relatively new class of chemical compounds called macrocyclic ligands[31-47], which are able to specifically recognize through controlled interactions according to size, shape, and structure of various cations, and then by means of these, various anions, has allowed the study the amino acids whose amphion type molecule may appear either as cation in acid medium or as anion in basic medium (Fig. 1). The amino acids may be considered the fundamental constituents of a wide variety of biological macromolecules. In this respect the separation of these compounds is important for both analytical point of view and biochemical as well. Moreover, the understanding of selective recognition and transport of amino acids is of fundamental interest, in part from the point of view of mimicking natural biological systems.

Mixtures of α-amino acids may be separated in cationic form through a liquid membrane of 18-crown-6 (18C6), benzo-18-crown-6 (B18C6), dibenzo-18-crown-6 (DB18C6) in 1,2-dichloroethane in the presence of picrate anion. The separation process is performed according to character (more hydrophobic or hydrophilic) of the R-chain from the amino acid molecule $\left(R-CH(NH_2)COOH\right)$. Therefore, a classification of amino acids naturally appears: (i) extractibles (*i.e.*, L-Met, L-Ile, L-Phe, L-Leu, L-α-Ala, L-Cys), and (ii) non-extractibles (*i.e.*, L-Asp Acid, L-Glu Acid, L-Ser, L-Tre, L-Pro, L-Lys, L-Tyr, L-Arg, L-Hys) from mixtures.[48-54]

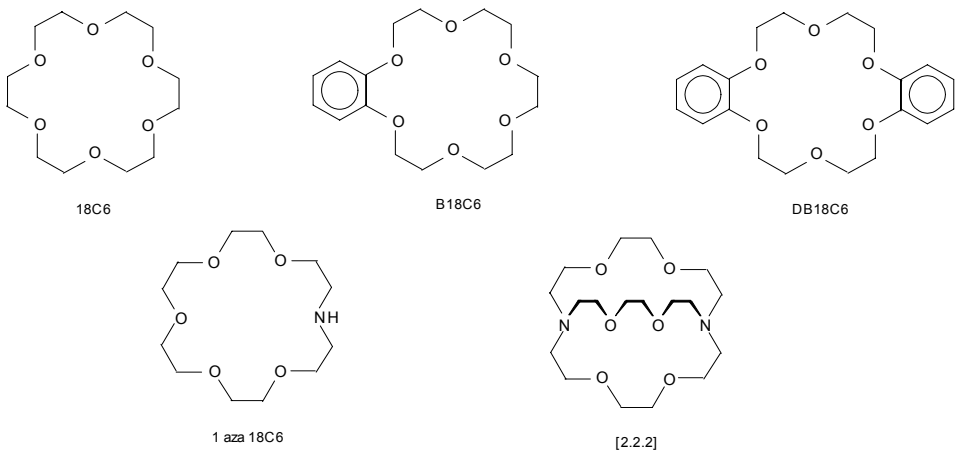


Fig. 1 – Chemical structures of some macrocyclic ligands used in bulk membrane separation.

Models which describe the macrocyclic mediated transport of cations across bulk liquid membranes indicate that the membrane solvent plays an important role in global membrane performance. There are four of the parameters mainly affecting the mass transfer which are solvent-dependent: (*i*) the equilibrium constant for cation-macrocyclic interaction in the membrane, (*ii*) the thickness of the boundary layers of the membrane, (*iii*) the partition coefficients, and (*iv*) the diffusivities of the species involved in transport. As a general rule, any solvent employed as an organic phase should be one that retains the mediating carrier to a very large extent and yet can accommodate relatively high concentrations of water to aid in the transfer of hydrated ionic species without loss of carrier within the aqueous phases. Both requirements are influenced by the polarity of the solvent, which appears to be the most important factor in determining its effectiveness as membrane medium.[55]

The overall rate of substrate transport through bulk liquid membranes can be controlled by one of a series of resistances to mass transfer in the membrane system. Specifically, in carrier mediated transport, the rate may be limited by resistances associated with mass transfer of substrate within the source or receiving phases, diffusion across the organic phase, or interfacial complexation/decomplexation reactions. Estimation of the rate-limiting process provides the possibility to optimize both the substrate transport in separation techniques (i.e., mass transfer and selectivity), as well as the design of new synthetic carrier molecules.

Generally, the bulk liquid membranes do not permit a good control of the hydrodynamic behavior of the systems involved. An important issue in their study concerns the diffusion processes, which depend on stirring rate variations, whereas interfacial processes do not. Several mathematical models have been proposed in the literature to characterize the active transport processes in bulk liquid membranes.[56-59] The conceptual model of amino acid mediated transport by macrocyclic ligands through bulk liquid membranes assisted by *pH* gradient is presented in Fig. 2.

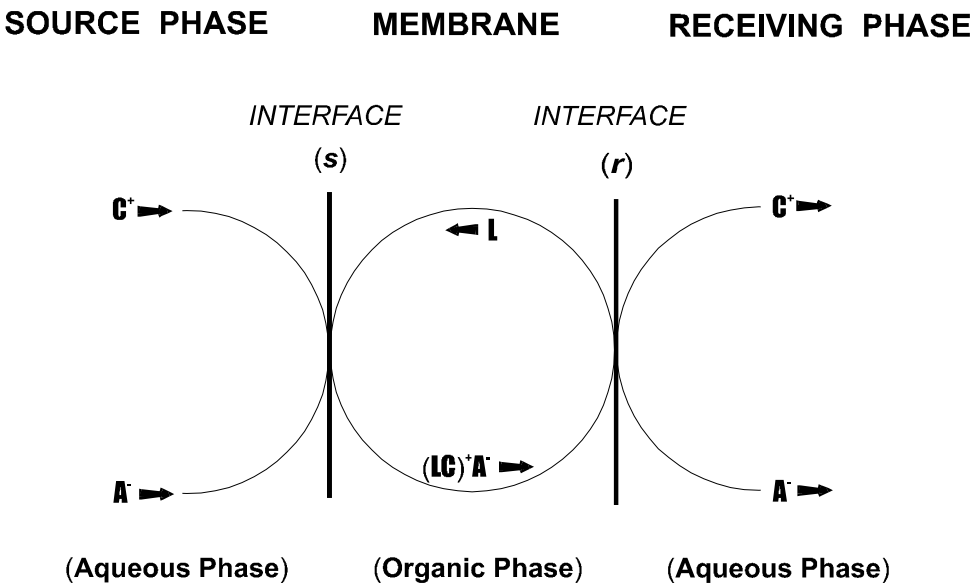


Fig. 2 – Mechanism of carrier (L) mediated active transport of ion pair (LC^+A^-) through bulk liquid membranes assisted by pH gradient.[58]

The experimental results of studies on amino acid active transport through liquid membranes were applied to acid hydrolisated samples originating in proteins from milk Casein (Table 1)[9] and Albumina Bovis (Table 2).[9] According to the experimental data showed in Table 1 and 2, the extraction yields within 50% and 81% (depending on the amino acid) for β-Casein and within 55% and 87% for Albumina Bovis are fairly high. For some amino acids like lysine, the extraction yields (6% for β-Casein and 7% for Albumina Bovis) were evidently much lower as compared with the model mixtures in the same conditions.

Table 1

Experimental results obtained in separation through liquid membrane of the amino acids from proteic hydrolysate of Casein[9]

Amino Acid	Casein				
	Initial amount	Source phase (5 mL)		Receiving phase (10 mL)	
	μg / 5 mL	μg	%	μg	%
Isoleucine	91.50	13.72	15	73.67	81
Leucine	112.50	11.97	11	88.30	79
Phenylalanine	66.00	20.79	32	39.64	60
Valine	90.00	34.65	39	47.07	52
Methionine	53.50	21.50	42	31.69	59
Alanine	41.00	18.04	44	20.83	51
Cystine	14.00	6.30	45	7.03	50
Lysine	131.50	118.61	90	8.05	6
Histidine	42.00	42.19	100	_	_
Arginine	62.50	60.75	97	_	_
Aspartic acid	121.50	115.47	95	_	_
Treonine	70.00	72.93	104	_	_
Serine	90.00	89.86	100	_	_
Glutamic acid	321.50	308.80	96	_	_
Proline	116.50	110.67	95	_	_
Glycine	36.50	35.77	98	_	_
Tyrosine	116.50	113.58	98	_	_

Source phase: amino acid; [picric acid] = 1.6×10^{-3} M; HCl, 0.05 N (*pH*=2.02);
Receiving phase: LiOH, 0.1 N (*pH*=13.01);
Membrane: [18-crown-6] = 10^{-2} M/1,2-dichloroethane;
Phase ratio: 5:50:10 (v/v/v).

Table 2

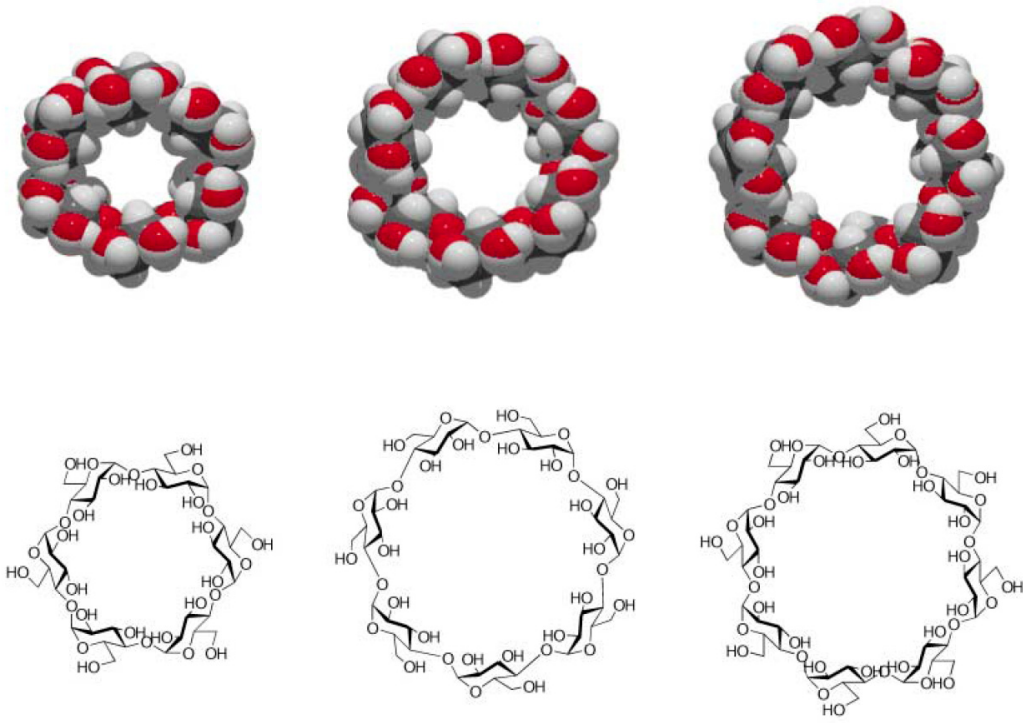
Experimental results obtained in separation through liquid membrane of the amino acids from proteic hydrolysate of Albumina Bovis[9]

Amino Acid	Albumina Bovis				
	Initial amount	Source phase (5 mL)		Receiving phase (10 mL)	
	μg / 5 mL	μg	%	μg	%
Isoleucine	43.50	5.22	12	37.63	87
Leucine	135.00	14.17	11	114.75	85
Phenylalanine	84.50	16.97	20	64.32	76
Valine	81.00	25.11	31	52.93	65
Methionine	100.50	35.24	35	63.06	63
Alanine	26.00	8.29	32	14.70	56
Cystine	182.50	73.36	40	100.37	55
Lysine	179.00	161.99	91	12.17	7
Histidine	57.00	54.76	96	_	_
Arginine	93.00	91.97	99	_	_
Aspartic acid	125.00	123.75	99	_	_
Treonine	91.50	86.19	94	_	_
Serine	66.50	63.51	96	_	_
Glutamic acid	234.50	231.45	99	_	_
Proline	66.50	64.30	97	_	_
Glycine	10.00	9.50	95	_	_
Tyrosine	86.00	84.62	98	_	_

Source phase: amino acid; [picric acid] = 1.6×10^{-3} M; HCl, 0.05 N (*pH*=2.02);
Receiving phase: LiOH, 0.1 N (*pH*=13.01);
Membrane: [18-crown-6] = 10^{-2} M/1,2-dichloroethane;
Phase ratio: 5:50:10 (v/v/v).

2. Liquid membranes containing modified cyclodextrin as carriers

Cyclodextrins are cyclic oligosaccharides composed of six (α- cyclodextrin), seven (β- cyclodextrin), eight (γ- cyclodextrin) or more glucose units per macrocycle, respectively (Fig. 3). The size of the cyclodextrin cavities, which is controlled by the number of glucose residues in the cyclodextrin ring, is characterized by an internal diameter 4.5, 7, and 8.5 Å for α-, β-, and γ-cyclodextrin, respectively. As a result of different cyclodextrin ring sizes for any given guest complexation, complexes are produced with different stabilities.[60-66] The cyclodextrins have a hydrophilic exterior and a hydrophobic cavity able to extract a large variety of compound guests as a function of size, shape, and hydrophobicity of both the cyclodextrin and the guest compound (Fig. 3).[67, 68]



α-cyclodextrin **β-cyclodextrin** **γ-cyclodextrin**

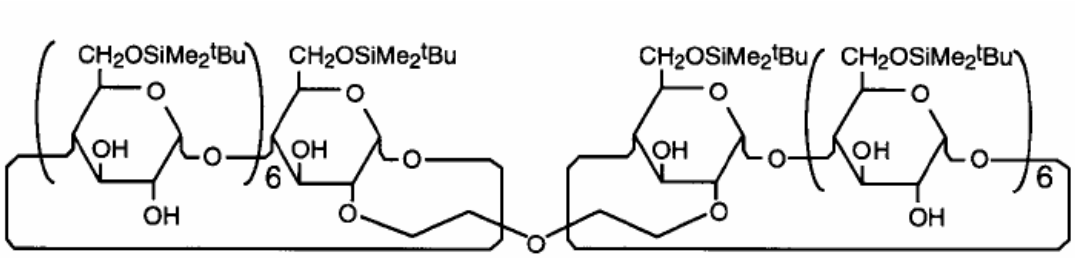
Fig. 3 – The structures of cyclodextrins

Biologically derived cyclodextrins and their synthetically derivatized analogs are used in host-guest chemistry.[69, 70] Cyclodextrins and their derivatives have also been used in membrane separation processes. They are good candidates for employing in different separation techniques such as chromatographic separations, capillary electrophoresis, and liquid membranes. As compared with their use in some other applications, relatively few reports have been published on the use of cyclodextrins in membrane separation.

By means of the transport of the aromatic hydrocarbon (*o*-, *m*-, and *p*- xylene, naphthalene, anthracene, and pyrene) from one hexane phase to another through aqueous phase with α-cyclodextrin and β-cyclodextrin, Poh *et al.*[71] determined the association constants of the cyclodextrin-aromatic hydrocarbon complexes. The values of association constants of 1:1 complexes formed are in good agreement with those determined by other methods.

The modification of cyclodextrins with various organic compounds such as spectroscopically, catalytic, or functionalized ones gives the cyclodextrins new functions different than native cyclodextrins. Hence, a host of their applications in many different fields.[72] By using a cyclodextrin dimer **1** Ikeda *et al.*[73] reported a selective transport of saccharides (Chart 1) through liquid membrane. The separation of a mixture of saccharides is difficult because most saccharides are isomers that only differ in the configuration of specific hydroxyl groups. The system of liquid membrane is useful not only for separation of a mixture of

saccharides, but for clarifying the mechanism of action of saccharide transport through a biomembrane as well. The reported system is more successful for the transport of the saccharide through the liquid membrane than the system using the cyclodextrin monomer.[73]



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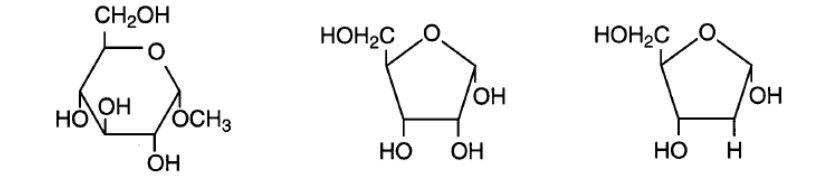


Methyl-α-D-glucoside (Me-Glu) D-ribose (Ri) D-2-deoxyribose (De)

Chart 1

3. Selective transport through liquid membrane by using calix[n]arenes as carriers

The family of calix[n]arenes is deeply involved in molecular recognition of chemical and biological compounds (Fig. 4). The calix[n]arenes have an important role in chemical separations, apart from other important applications like chromogenic and fluorogenic sensors and field effect transistors, ion selective electrodes, calixarene molecules for nonlinear optical devices, as liquid crystals or as catalysts in synthetic reactions.[74-80] By using calix[n]arene derivatives with functional groups such as ether, amide, ketonic, ester, and crown ether increases further their potential applications.[81-87]

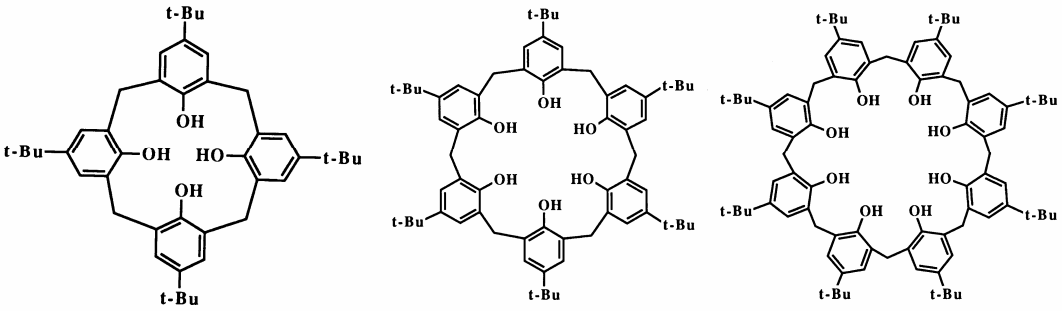


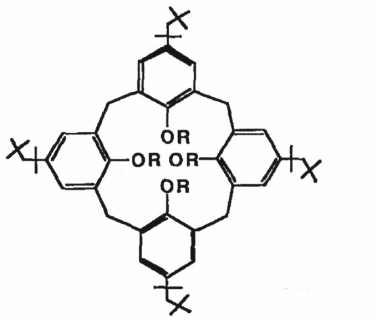
Fig.4 – Structure of *p*-tert-butylcalix[n]arenes, n = 4, 6, 8.

The molecular dynamic simulations on the structural properties of the complexes of calix[6]arene-crown-6 with ammonium ions (NM_4^+ and NH_4^+) in the presence of acetate as counterion were performed in chloroform solution establishing the location of the guest inside the host.[88]

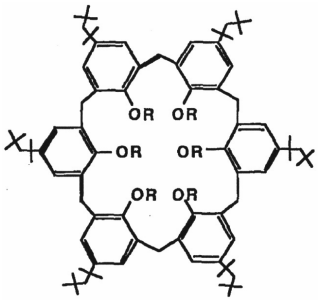
The transport of N-benzoyl amino acids through a chloroform liquid membrane by using a calix[*6*]arene ethyl ester derivative as selective carrier at 25^0C was reported by Chang *et al.*[89] The transport rate was function of hydrophobicity of the guest anions and the size of alkaline metal cations, which coexisted in the source phase. The transport rate for a given cation increased with increasing hydrophobicity

of amino acids as follows: Bz-Gly < Bz-Ala < Bz-Val ≅ Bz-Trp < Bz-Phe. In these experiments, the separation of amino acids was carried out in carboxylate form of amino acids, a common form of amino acids and proteins in physiological fluids. The experimental results obtained by the same author[90] suggested that the ethoxycarbonylmethyl derivative of *p*-tert-butylcalix[6]arene can be used as a carrier for selective recognition and separation of phenylalanine and tryptophan over glycine, alanine and 4-aminobutyric acid (GABA). A schematic mechanism concerning the interaction between the phenylalanine and tryptophan ester on one side, and the calix[*6*]arene receptor on the other side was drawn in the same time.

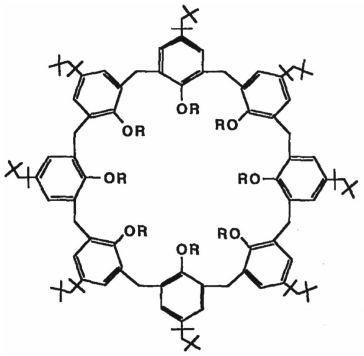
By designing a new calix[4]arene having chiral pendant groups, Okada *et al.*[91] performed the transport through liquid membrane of some amino acid ethyl and methyl esters and Z-amino acid carboxylates into CH_2Cl_2. The transport rates of amino acids esters, however, were lower than those of Z- carboxylates due to deep inclusion into cavity. The amino acid esters have higher enantioselectivity than Z-carboxylates in order to interact directly with the binding sites. These results could be explained by the pendant group readily fixed the substrate amino acid of the same chirality to form hydrogen-bonded aggregates in lipophilic media.[91] Hu *et al.*[92] synthetized (R)-cysteine-containing calix[4]arenes which might serve as good chiral macrocyclic ligands in the studies of chiral recognition and chiral catalysis. Chiral recognition of amino acids by calixarenes was also investigated also Kubo *et al*.[93]



R = CH_2COOH
Tetracarboxylic acid derivative
of *p-tert*-octylcalix[4]arene



R = CH_2COOH
Hexacarboxylic acid of
p-tert-octylcalix[6]arene



R = CH_2COOH
Octacarboxylic acid derivative of
p-tert-octylcalix[8]arene

Fig. 5 – Structures of calix[n]arene derivatives used in Oshima's experiments[95]

The studies concerning the ability of calix[6]arene hexacarboxylic acid derivatives (Fig. 5) to act as carriers through liquid membrane for transporting aromatic amino acids were reported by Oshima *et al.*.[94, 95] The calix[6]arene hexacarboxylic acid has a cyclic structure capable to include an amino acid ester and bears six ionizable carboxylic acids contributing to electrostatic interaction, as a carrier through liquid membrane. It was also demonstrated that a calix[6]arene acid derivative exhibits high extractability for amino acids compared to ordinary commercial extractants and their structural analogues.[96]

The hydrophobic amino acid esters (L,D-tryptophan methyl ester hydrochloride, L-phenylalanine methyl ester hydrochloride, and L-tyrosine methyl ester hydrochloride) and L-tryptophan were transported by the carrier above mentioned. Based on complexation characterized by a proton-exchange mechanism, the transport through membrane was controlled by changing the *pH* gradient between the source and the receiving aqueous phases. The calix[6]arene hexacarboxylic acid exhibited a high transport ability compared to the other calix[n]arene derivatives (n = 4, 8, Fig.5). By combining an enzyme reaction and a liquid membrane transport with the calix[6]arene, an optical resolution system for a racemate of tryptophan methyl ester was developed.[94] Thus, it was realized a novel liquid membrane system for the chiral separation. Shinkai *et al.*[85] showed spectroscopically that the pseudo-C_2-symmetrical homooxacalix[3]arene exibit enantiomeric recognition properties toward alanine ethyl ester and phenylalanine ethyl ester.

Along the same line, the transport of aromatic amino acids methylesters through a chloroform liquid membrane containing *p*-tert-butylcalix[6]arene, and *p*-tert-butylcalix[8]arene as carriers in the presence of picrate was reported.[97] The sequence of transport efficiency for the amino acid methylesters was found as: L-PheOMe > L-TrpOMe > L-TyrOMe when *p*-tert-butylcalix[8]arene was used as carrier (Fig. 6).

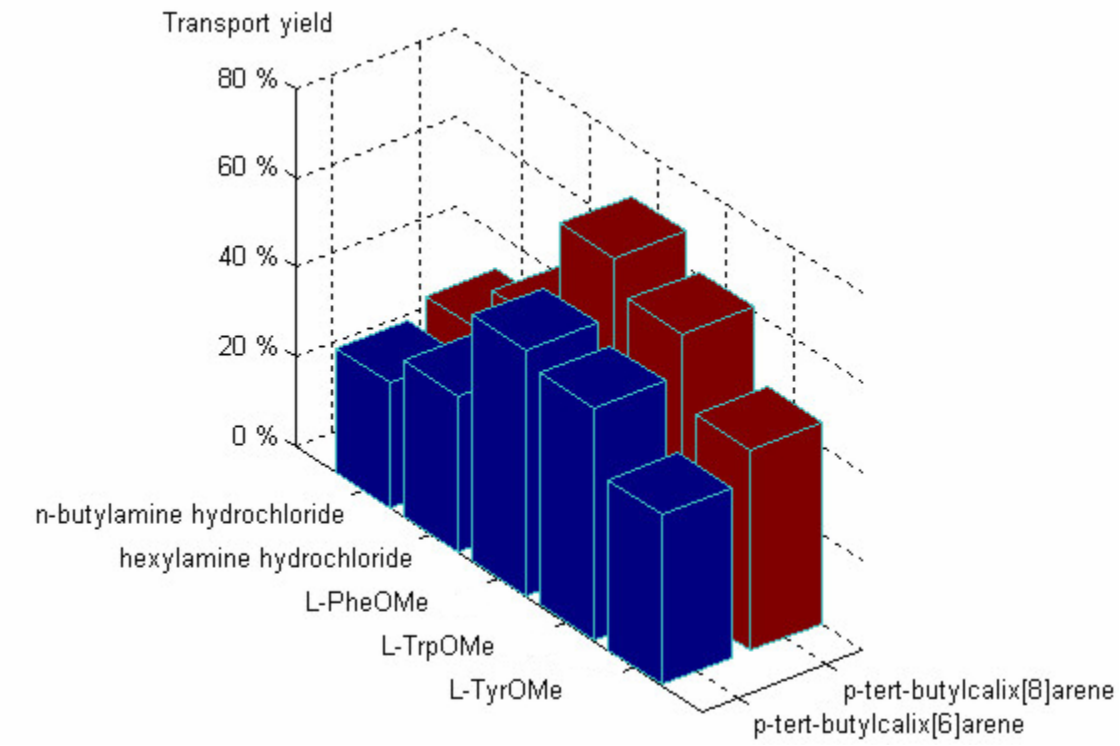


Fig. 6 – Experimental data of the transport of some amine and aromatic amino acid methylester hydrochlorides through chloroform liquid membrane by *p*-tert-butylcalix[n] arene (n = 6, 8). The transport yield is the amino acid percentage found in the receiving phase after *24* hours of stirring.[97]

Using a supported liquid membrane composed of a porous polymeric support, Antipin *et al.*[98] studied the separation of zwitterionic form of aromatic amino acids by calix[4]arene based α-aminophosphonates. The aminophosphonate groups contributed to the transport efficiency and the order of the transport rate.

The transport through chloroform liquid membrane of *p*-tert-butylcalix[n]arenes (n = 6, 8) upon some amino acid methylesters (L-leucine, L-valine, L-cysteine, L-isoleucine, L-serine, and L-phenylalanine) was investigated.[99,100] The experimental results suggested that the amino acid methylesters are transported by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00 ([*4*-(4′-anilinophenylazo)benzenesulphonic acid]) as counterion. The transport was proved to be essentially controlled by the structure of calix[n]arene and the nature of the amino acid.

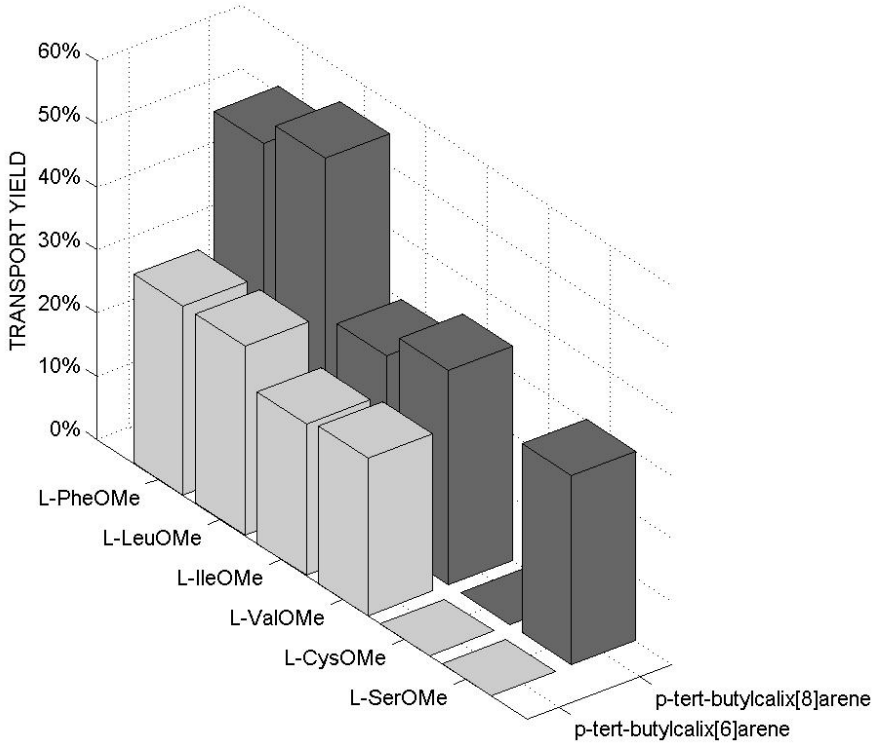


Fig. 7 – The transport yields of some amino acid methylester hydrochlorides through chloroform liquid membrane by *p*-tert-butylcalix[n]arenes (n = 6, 8) in the presence of tropaeolin 00 after *24* hours of stirring *200* rpm.[99]

The effects of the physicochemical parameters, such as the structure of calixarene, the ionic strength, the pH, the nature of solvent, and the nature of the anion used as counterion were investigated. In addition, the influence of the composition and structure of the the compounds under study upon the partition processes occurring in triphasic systems was reported.

From the results obtained, it came out that *p*-tert-butylcalix[8]arene exhibited better transport ability than *p*-tert-butylcalix[6]arene for the amino acids methylesters through chloroform liquid membrane (Fig 7). The sequence of the transport yields of amino acids using *p*-tert-butylcalix[6]arene as carrier was the following: L-PheOMe > L-LeuOMe > L-IleOMe ≅ L-ValOMe, whereas using *p*-tert-butylcalix[8]arene as carrier, the sequence of amino acid yields was the following: L-LeuOMe > L-PheOMe > L-ValOMe > L-IleOMe ≅ L-SerOMe.[99]

These results suggest further possibilities for optimal separation of amino acids derivatives and other biological species.

CONCLUSION

The use of membranes in separation and purification of biological compounds was unanimously recognized as one of the most successful novel applications developed during the last years. Future developments of membrane processes in biotechnology and artificial organs should proceed together. Mixtures of amino acids can be separated in cationic form through a liquid membrane using macrocyclic ligands as carriers. Separation is carried out accordingly to the hydrophobic or hydrophilic character of the R-chain from the amino acid structure.

The wide interest in the use of cyclodextrins and their derivatives in the separation field arises from the fact that cyclodextrins can improve the selectivity of separation because of their ability to form inclusion complexes with a large number of organic and inorganic compounds. Calix[n]arenes and their derivatives have been attracting much attention recently as interesting novel type host compounds with applications in separation science especially in liquid membrane.

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