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Facilitated Transport of Carbohydrates, Catecholamines, and Amino Acids Through Liquid and Plasticized Organic Membranes

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Abstract. A number of methods are described to facilitate the transport of monosaccharides, catecholamines, and amino acids through bulk liquid membranes, supported liquid membranes and plasticized cellulose triacetate membranes. Transport is mediated by carrier compounds, such as boronic acids, quaternary ammonium salts and crown ethers, that are dissolved within the lipophilic membranes. Two types of transport mechanisms are described, carrier diffusion and fixed-site jumping.

Key words: facilitated transport, liquid membranes, plasticized membranes, carbohydrate recognition.

1. Separations Using Liquid Membranes

Purification using liquid membranes has a number of features that make it an attractive industrial technology [1]. The process is inherently low-energy, continuous, and can be made highly-automated. The amounts of organic solvent required are generally very small, and thus the technology is environmentally benign. Facilitated liquid membrane transport is related to liquid extraction, since passage through the membrane involves a similar sequence of chemical reactions. Carrier compounds are dissolved in the liquid membrane and act as transport catalysts. Even if these carriers are expensive fine chemicals, their catalytic role ensures the associated expense is minimized. The major advantages with facilitated transport are high transport fluxes and exceptional solute selectivities. The major technical difficulty is long-term membrane instability. Although research on facilitated transport has been underway for more than twenty years, there has been limited commercial application to date, due primarily to the problem of membrane instability. While the potential of liquid membranes to separate gases and metal cations is

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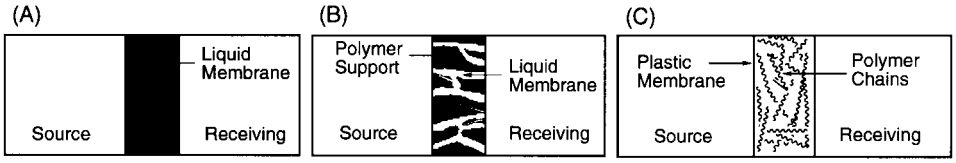


Figure 1. (A) Bulk liquid membrane, BLM; (B) supported liquid membrane, SLM; (C) plasticized cellulose triacetate membrane.

well-recognized, there are very few examples of membranes devised for molecular separations [2].

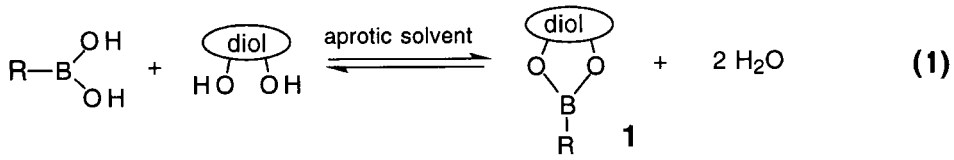
This review summarizes our recent efforts to develop lipophilic membranes that are selectively permeable to small, hydrophilic biomolecules such as carbohydrates, catecholamines, and amino acids. Our approach is to use the principles of molecular recognition to design and evaluate transport carriers that selectively bind a specific analyte and facilitate its passage through the membrane. There are a variety of potential applications for such membranes including small and large scale separations, electrode sensing, drug delivery, and controlled-release technology.

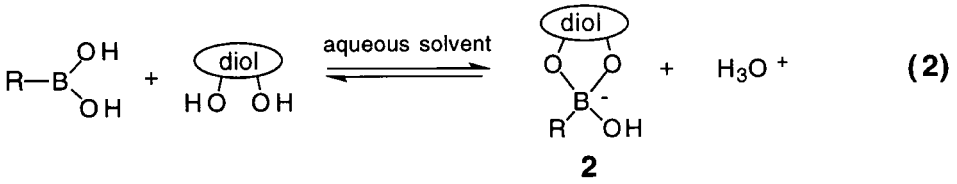
A range of related membrane compositions has been investigated (Figure 1) [3]. Much of the early work used Bulk Liquid Membranes (BLMs), where an aqueous source layer in one arm of a U-tube apparatus is separated from an aqueous receiving layer in the other arm by a liquid organic phase (Figure 1A). Subsequent studies used Supported Liquid Membranes (SLMs), where the liquid membrane is held within the pores of a thin, porous polymer sheet (Figure 1B). Our preference is to use 2-nitrophenyl octyl ether (2-NPOE) supported by porous polypropylene (Accurel or Celgard 2500). 2-NPOE is an excellent liquid membrane because it has the remarkable property of being as polar as acetone yet it is very hydrophobic. Although the flat sheet design is less efficient than other geometries, such as hollow fibers, the flat sheet is preferred for laboratory studies as its simple geometry makes mechanistic interpretation of the transport results more straightforward. Our most recent efforts have used flat sheets of plasticized cellulose triacetate (Figure 1C). Although plasticized membranes are used extensively in ion-selective electrodes they have received only minor attention as materials for separations. The general expectation with plasticized membranes is that the gel-like nature of the membrane increases viscosity which inhibits leaching. On the other hand, the increased viscosity slows diffusion and decreases fluxes for transport systems that use a carrier-diffusion mechanism

2. Introduction to Molecular Recognition with Boronic Acids

In anhydrous aprotic solvents, boronic acids readily condense with diol-containing compounds to form trigonal boronate esters, **1** (Equation (1)). In aqueous solution, the trigonal boronates are unstable and either hydrolyze back to starting compounds or ionize to form anionic tetrahedral boronates, **2** (Equation (2)). A salient point

is that although covalent bonds are formed, the equilibrium that produces **2** is rapid and reversible. Since **1** is more acidic than its parent boronic acid, significant amounts of the boronate **2** are observed at pH 7, even with a weakly acidic boronic acid such as phenylboronic acid (pK_a = 8.9). The amount of **2** increases with pH until highly alkaline conditions are reached and hydroxide ions begin to displace the diol. The equilibrium that forms **2** is strongly dependent on the structure of both the boronic acid and the diol.





3. Carbohydrate Transport Through Bulk Liquid Membranes (BLMs) Using Boronic Acid Carriers

Lipophilic boronic acids are currently the only carrier compounds known to selectively facilitate the transport of hydrophilic carbohydrates through liquid membranes [4]. Our research efforts in this area were inspired by two communications in the literature describing facilitated transport of monosaccharides and ribonucleosides through BLMs using a carrier mixture of phenylboronic acid and lipophilic quaternary ammonium salt [5, 6].

The goal of our earliest work was to properly understand the mechanisms behind the transport processes. A detailed study was made of the chemical and physical factors that controlled the transport of nucleosides, glycosides, and catecholamines through BLMs [4]. The results of this work allowed a unified transport model to be proposed that encompasses two parallel complexation pathways (Equations (1) and (2), above), whose transport kinetics are controlled by diffusion through the unstirred layers of the three-phase system. This model is an extension of the accepted interpretation for ionophore mediated transport of metal cations through BLMs.

Since a potential application of this work is in sugar separation, particular attention was paid to the sugar transport selectivity, and the conditions needed to achieve active transport. The trigonal boronate pathway (Equation (1)) was found to have a selectivity for cyclic, diol-containing compounds in the order: *cis*-1,3-diol $>$ *cis*-1,2-diol $\approx$ *trans*-1,3-diol $\gg$ *trans*-1,2-diol; whereas the tetrahedral boronate

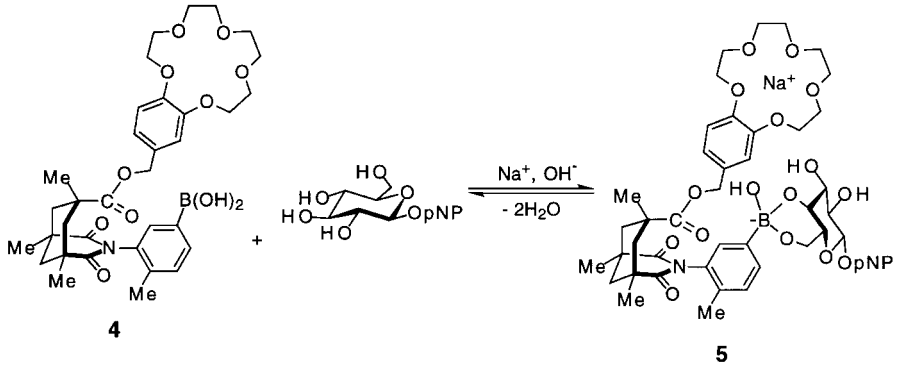
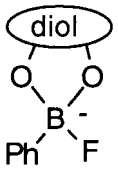


Figure 2. Glycoside/metal cation co-transport.

pathway (Equation (2) in the presence of lipophilic quaternary ammonium cation had a slightly different selectivity of *cis*-1,2-diol > *cis*-1,3-diol > *trans*-1,3-diol ≫ *trans*-1,2-diol. Active transport driven by a pH gradient was demonstrated for the tetrahedral boronate pathway (Equation (2)). Other ways of driving active transport were investigated using alternative electrochemical gradients. The first approach used a fluoride ion gradient. Since the ability of F^- to form dative bonds with trigonal boron acids is similar to that of OH^-, it was hypothesized that F^- ions could substitute for OH^- and induce transport via fluoroboronates such as **3**. This was indeed the case. Active nucleoside transport through a BLM, was achieved at neutral pH in the direction of a F^- concentration gradient [7].



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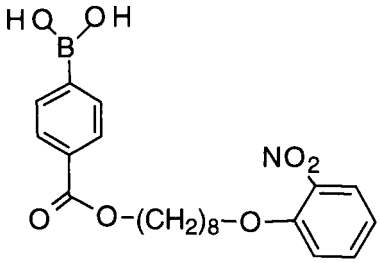
Active transport systems were developed that could be driven by metal cation gradients. We reasoned that an alternative way of producing a lipophilic cation to ion-pair with the sugar-boronate anion in Equation (2), was to complex a metal cation inside a lipophilic ionophore. This idea lead to the design and synthesis of compound **4** as a functionally biomimetic sodium-saccharide cotransporter [8]. The putative binding equilibrium in Figure 2 indicated that glycoside transport should be sensitive to Na^+ ion concentrations in the aqueous phases. Moreover, active transport in the direction of a Na^+ ion gradient was predicted to occur and subsequently found to be the case.

4. Carbohydrate Transport Through Supported Liquid Membranes (SLMs) Using Boronic Acid Carriers. A New Way to Produce High Fructose Syrup

In 1967 the production of High Fructose Syrup (HFS) became commercially viable. Since then its favorable properties and low price have resulted in a phenomenal rate of acceptance. HFS is used extensively in beverages, processed food, canned fruit, and dairy products. The current annual world production of HFS is currently around 6×10^9 kg [9]. The most common process for HFS production involves four major steps: (i) wet milling corn to extract the starch; (ii) enzymatic or acid hydrolysis of the starch to produce a feed stream that is about 94% glucose; (iii) enzymatic isomerization of the glucose to fructose; since the enzymatic equilibrium constant for this process is $\sim$1 maximum achievable fructose concentration is around 42%; (iv) in many applications (e.g., beverages) it is necessary to increase the sweetness of glucose/fructose syrup to the level of sucrose. This means the fraction of fructose in the syrup has to be increased to 55%, which is achieved by chromatographic enrichment. The glucose/fructose mixture is passed through the calcium form of an ion-exchange column. The fructose is retained longer on the column and thus the later fractions are fructose enriched.

One of the goals of our work with sugar permeable membranes is to improve the last two steps in the industrial HFS process, that is the glucose–fructose isomerization and fructose enrichment steps. By developing a fructose selective membrane it may be possible to combine these two steps into a single process. The pioneering work of Shinbo and coworkers showed that a BLM containing a carrier mixture of phenylboronic acid and trioctylmethylammonium chloride (TOMAC) transports fructose faster than glucose. Our aim was to build on this knowledge and develop a more industrially feasible SLM system. Moving to SLMs requires the carrier to be highly lipophilic, so as to prevent leaching of the carrier into the aqueous phases. To counter this we prepared the lipophilic boronic acid **6** and tested its ability to transport fructose and glucose through SLMs containing 2-NPOE [10].



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Competitive transport experiments were conducted using a source phase containing equal amounts (150 mM) of glucose and fructose (Table I). Under these competitive transport conditions, boronic acid carrier **6** at pH 7.3 exhibited an

Table I. Competitive transport of fructose and glucose through SLMs containing different carriers[a]

Carrier[c]	Aqueous phase pH[d]	Flux (10^{-8} mol/m^2 s)[b]	
		Glucose	Fructose
6	7.3	2.9 (2.6)[e]	55 (32)[e]
6 + TOMAC	7.3	4.6	57
6 + TOMAC	pH gradient[f]	17 (2.4)[e]	91 (31)[e]

[a] Source phase: sodium phosphate (100 mM), fructose (150 mM), glucose (150 mM); Liquid membrane: carrier(s) dissolved in 2-NPOE supported by a flat sheet of Accurel (16 cm^2); Receiving phase: sodium phosphate (100 mM); Temperature: 298 °K.
[b] Initial flux extrapolated to $t = 0$, $\pm$ 10%.
[c] Each carrier component was 250 mM in 2-NPOE.
[d] pH in source and receiving phases.
[e] Repeated run with the same membrane after it had been washed for 20 h.
[f] Source phase pH 10, receiving phase pH 6.

eighteen-fold transport selectivity in favor of fructose. Mechanistic studies showed that transport mediated by **6** alone occurs via the trigonal boronate pathway (Equation (1)). A carrier mixture of **6** and TOMAC at pH 7.3 transported fructose twelve times faster than glucose. When this carrier mixture was tested using a pH gradient, both fluxes increased; however, the transport selectivity decreased to five-fold in favor of fructose. Under these conditions transport is mediated by tetrahedral boronate which is formed according to Equation (2).

With a fructose-permeable membrane in hand, it was a logical progression to combine this separation step with the enzymatic glucose isomerization. Immobilized glucose isomerase was added to a solution of glucose in the source compartment (300 mM, pH 7.3) of a transport cell operating at 50 °C, and the fructose levels in both aqueous phases were monitored over time. After five hours with an SLM containing carrier **6** the source phase had reached an equilibrium position of 50% fructose, whereas the fraction of fructose in the receiving phase was greater than 85% [10]. Thus, glucose isomerization and fructose enrichment have been converted from two discrete steps into a single operation (Figure 3).

Most recently we have prepared and evaluated the transport ability of the covalently linked carrier **7** [11]. Preliminary results show that this carrier is able to transport monosaccharides through SLMs more than twenty times better than the carrier mixture of **6** and TOMAC. Moreover, membranes containing **7** are significantly more stable than those containing **6** and TOMAC. Unfortunately, **7** appears

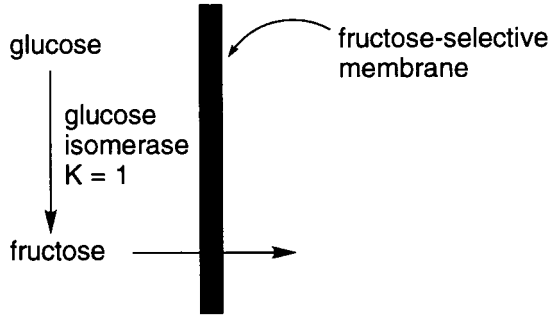
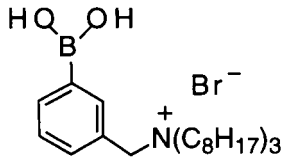


Figure 3. HFS production using fructose-selective membranes.

to be less selective than **6**, as competition experiments show a transport selectivity for fructose over glucose of only three.



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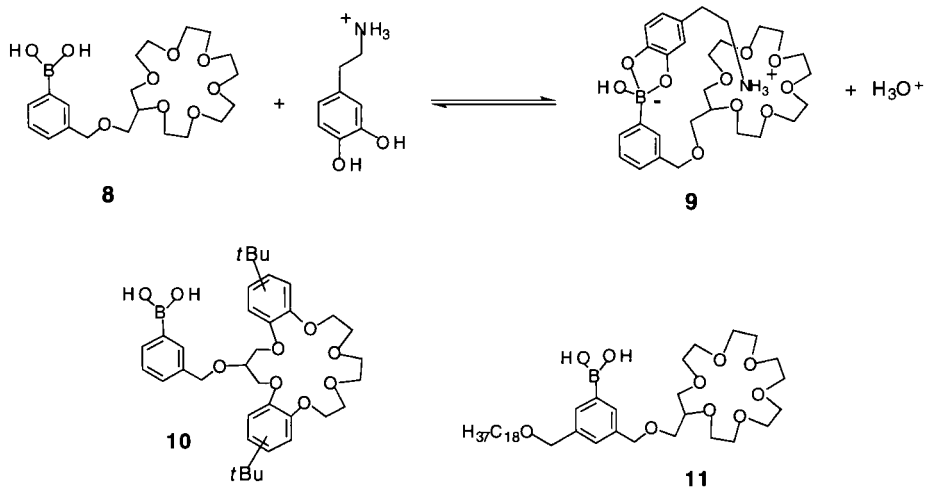
5. Catecholamine Separation Using SLMs

Catecholamines play an important role in health, and are indicators of a range of disease states. Numerous methods have been proposed for the determination of catecholamines and their metabolites in biological fluids such as urine and plasma [12]. The most widely used are spectrometric, fluorometric, radioenzymatic, and HPLC with electrochemical detection. Because of their higher concentrations and greater stability, the metabolites are often the primary assay target. However, in certain cases the concentrations of the parent catecholamines are more diagnostic. This means that highly sensitive, specific, and reliable methods are required for measuring the normally very low concentrations of dopamine, norepinephrine, and epinephrine. In most cases, a preliminary extraction and purification of the biological sample is necessary. The most common pretreatments are labor-intensive, and require a fair amount of technical expertise to ensure reliable results. A simpler method of purifying and concentrating clinical samples for catecholamine analysis would be useful. As an ongoing project, we are attempting to develop a membrane-based purification system for catecholamines [13].

Our approach is to design crowned boronic acids that can selectively and actively transport catecholamines through liquid membranes. For example, the crowned boronic acid **8** was designed as a selective carrier for dopamine transport. A BLM containing 1 mM of **8** transported dopamine 160 times faster than

background diffusion. A combination of evidence obtained by kinetic analysis, as well as NMR and mass spectroscopy indicates that the transported complex is the 1 : 1 cyclic structure, **9**. Since the association of dopamine and carrier **8** is an acid-producing equilibrium, it is possible to use a pH gradient to actively drive the dopamine into an acidic receiving phase.

Carriers **10** and **11** were synthesized as second-generation dopamine transporters to be used in SLMs (Figure 1B). In the absence of carrier, the dopamine flux at pH 7.4 was negligible. When the liquid membrane contained carrier **10** (2% wt), a respectable dopamine flux of 5×10^{-7} mol/m^2 s was observed. The carrier appeared to be quite stable as repeated runs with the same membrane produced no change in flux. With carrier **11**, the flux was an impressive ten times higher. This is close to the diffusion controlled limit for transport through this liquid membrane system. Unfortunately carrier **11** did not display long-term stability, as repeated runs with the same membrane showed a decline in observed fluxes. Nonetheless, these results are highly encouraging, and suggest a high-flux dopamine transport device can be developed using SLM technology.



6. Carbohydrate Transport Through Plasticized Membranes

Although liquid membranes have many promising attributes there has been little industrial application [1]. The major problem is membrane instability due to leaching of the membrane components into the aqueous phases [14]. Most attempts to overcome this problem have tried to increase the membrane partition ratio by covalently attaching the transport carriers to highly lipophilic or polymeric chains [3]. An alternative approach is to incorporate the transport carriers in plasticized polymeric membranes (Figure 1C) [15–17].

We have discovered that plasticized cellulose triacetate membranes containing large amounts of trioctylmethylammonium chloride (TOMAC) are selectively

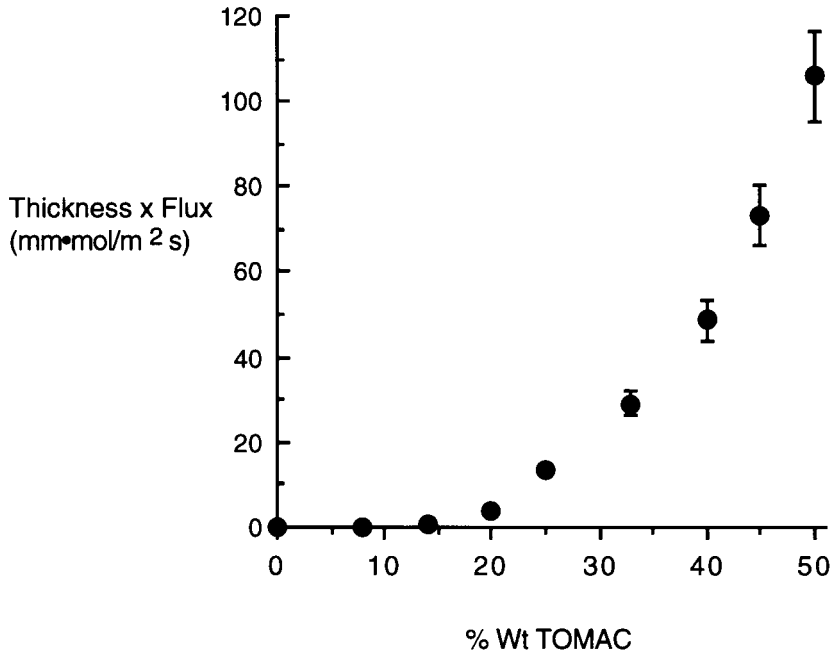


Figure 4. Membrane thickness × fructose flux versus % wt of TOMAC in plasticized membranes that also include cellulose triacetate (0.10 g) and 2-NPOE (0.20 g).

permeable to small, neutral carbohydrates [18]. The plastic films, which were originally designed as anion exchange membranes [17], exhibit high saccharide fluxes and high membrane stabilities. Two of the most likely mechanistic possibilities are carrier diffusion and fixed-site jumping. The kinetic profiles for these two transport processes are generally quite similar. One major difference is the dependency of flux on transporter concentration [19]. In the case of transport by carrier-diffusion, the expected profile is a linear plot passing through the origin. In the case of fixed-site jumping a percolation threshold is predicted if transport does not occur when the distance between fixed-sites becomes too great to allow solute jumping. A plot of carbohydrate flux as a function of membrane carrier concentration produced strong evidence for a percolation threshold (Figure 4), which is suggestive of a fixed-site jumping mechanism with chloride ion acting as a fixed-site sugar receptor (Figure 5) [18].

7. Amino Acid Transport Through Plasticized Membranes

These findings raise the possibility that facilitated transport through plasticized membranes by fixed-site jumping is a general way of improving membrane stability while still retaining high and selective permeability. As a consequence we have investigated the facilitated transport of other hydrophilic solutes through plasticized cellulose triacetate membranes containing TOMAC. Most recently we have

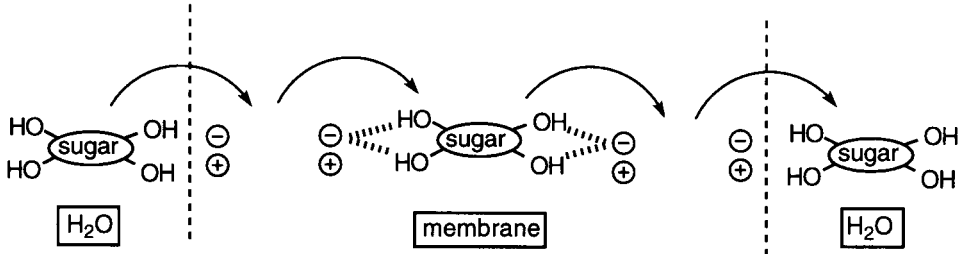


Figure 5. Postulated sugar fixed-site jumping transport mechanism.

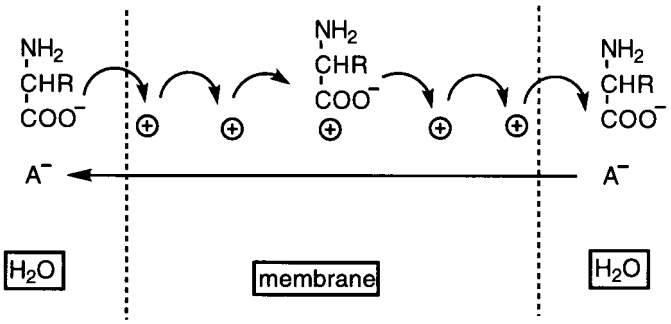


Figure 6. Postulated amino acid fixed-site jumping transport mechanism.

found that these membranes are highly permeable to amino acids at neutral pH [20]. There is clear evidence of a percolation threshold, in agreement with an anion counter-transport process that uses fixed-site jumping (Figure 6). Conversely, in other cases concerning cation transport, we and others [16] do not observe the onset of fixed-site jumping at high carrier concentrations. Thus, it remains to be seen if this remarkable transport effect, which we attribute to fixed-site jumping, can be duplicated with membranes containing carriers other than quaternary alkylammonium salts.

Acknowledgements

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