Imprinted Polymer Membranes for the Selective Transport of Targeted Neutral Molecules

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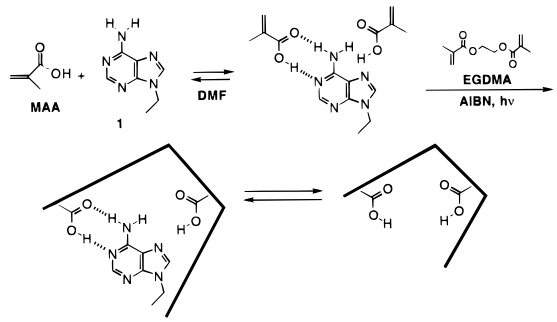
The development of artificial/synthetic membranes which rival biological membranes in selectivity is an important current goal in bioorganic and environmental chemistry.[1] Specific receptor−ligand interactions are responsible for the efficacy of biological membranes. There are a number of synthetic membranes that selectively transport solutes. In some cases, the selectivity is due to reversible complex formation between a membrane bound carrier and solute and subsequent shuttling of the solute between carrier sites in the membrane.[2−7]

The selective transport of a broad range of neutral molecules and proteins through synthetic solid polymer membranes is challenged by the difficulty of incorporating specific receptors for such molecules. Molecular imprinting is a technique in which recognition sites are created in synthetic polymer networks.[8−10] Most studies with imprinted polymers have been carried out using polymer particles either in the batch or chromatographic mode for purposes of selective binding or catalysis.[8−10] Here we report the preparation of membranes of imprinted polymers for the selective transport and separation of neutral molecules. We have shown that these membranes, which utilize specific interactions between the target molecule and the imprint site, selectively transport the target molecule from a mixture of structurally similar molecules.

Molecularly imprinted films were prepared with 9-ethyladenine (**1**) as the template and methacrylic acid as the polymerizable complementary functional monomer (Scheme 1).[11] The free-standing films of imprinted polymers were obtained by the simultaneous polymerization and film formation of a DMF solution of ethylene glycol dimethacrylate and methacrylic acid with 9-ethyladenine (**1**) as the template and AIBN as initiator on silanized glass slides at 65−70 °C under nitrogen atmosphere. Reference polymer films were prepared with the same monomer composition but in the absence of a template or with benzylamine, a generic amine template in place of **1**. The template was quantitatively extracted with methanol (25 °C, 24 h).

Scanning electron micrographs of these membranes show a homogeneous smooth surface, while the cross-sectional view

Scheme 1



shows an array of closely packed polymer domains of size varying from 50 to 100 nm (Figure 1).

Transport studies were carried out with an H-shaped two-compartment cell with constant stirring. The concentration of substrate in the receiving phase as a function of time was quantified by HPLC.

Preliminary studies established that a steady state flux was achieved after a period of 3−6 h. Under these conditions, a linear correlation between amount of substrate transported and time was observed for the duration of the experiment (maximum 3 days). The transport rates of adenine, thymine, and cytosine were measured. In all cases it was found that adenine was transported at a higher rate than other nucleic acid bases through membranes imprinted with the template **1**. Furthermore, the imprinted membranes (template **1**) transported adenosine at a higher rate compared to that of guanosine.[12] In contrast, reference polymer membranes, prepared in the absence of template **1**, transported adenine at a comparable rate to those of cytosine and thymine.

Further, to substantiate the selectivity, competitive transport studies were undertaken. In these studies, adenine was found to be transported at a higher rate than cytosine or thymine. The enhanced rate of transport is defined as the selectivity factor (flux of adenine or adenosine/flux of other substrate). For example, in a competitive transport experiment using equimolar adenine and thymine in methanol with a membrane containing 23.2 μmol sites/membrane (0.12 g/membrane), the steady state fluxes of adenine and thymine were 1.62×10^{-8} and 1.18×10^{-8} mol cm^{-2} h^{-1}, respectively. This corresponds to a selectivity factor of 1.37. A reference polymer membrane of the same monomer composition prepared without template **1**, on the other hand, transports adenine at rates comparable to those of thymine and cytosine.[13]

Transport selectivity was found to be influenced by both membrane loading and solvent. Higher selectivities were observed at lower membrane loadings. For example, the selectivity factor for adenine/thymine in methanol is 1.4 with a membrane containing 23.2 μmol sites/membrane compared with 2.2 with a membrane containing 1.4 μmol sites/membrane. It was also noted that the flux through the membrane *decreased* as the sites/membrane decreased.[14]

It was subsequently found that selectivity could be significantly improved by changes in the solvent medium. From an

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(12) A membrane imprinted with 9-ethyladenine (1.4 μmol/0.12 g membrane) transported adenosine and guanosine in methanol/chloroform (20:80 v/v) with fluxes of 2.7×10^{-10} and 1.6×10^{-10} mol cm^{-2} h^{-1}, respectively.

(13) For example, a control membrane transported adenine and cytosine in methanol solvent with fluxes of 1.4×10^{-8} and 1.2×10^{-8} mol cm^{-2} h^{-1}.

(14) A 9-ethyladenine-imprinted membrane (23.2 μmol sites/membrane) in methanol solvent using a feed concentration of adenine = thymine = 145 μmol had an adenine flux of 4.35×10^{-9} mol/(cm^2 h) and a thymine flux of 3.11×10^{-9} mol/(cm^2 h). A similar membrane with 1.4 μmol sites/membrane in methanol solvent using a feed concentration of adenine = thymine = 155 μmol had an adenine flux of 2.12×10^{-10} mol/(cm^2 h) and a thymine flux of 0.94×10^{-10} mol/(cm^2 h).

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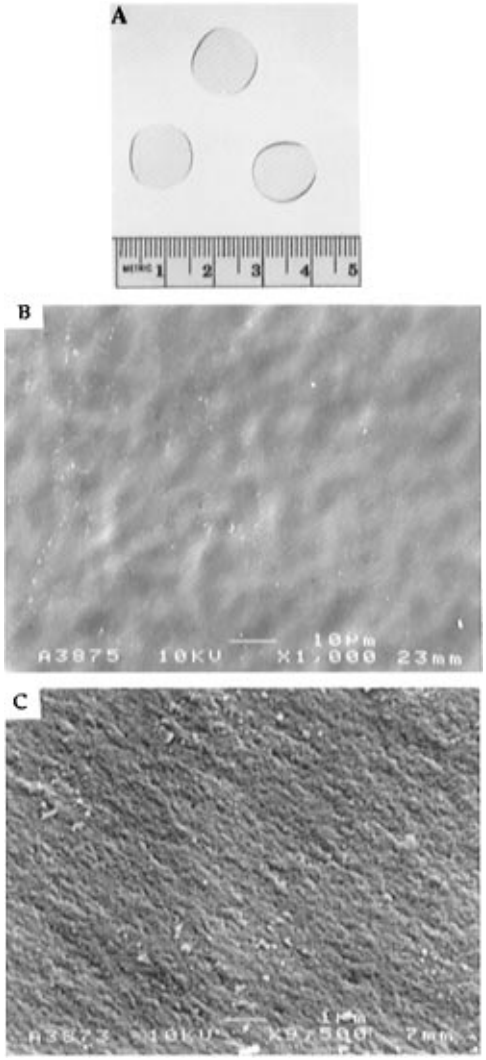

Figure 1. (a) Representative imprinted polymer membranes used in the selective transport studies. (b) Membrane surface and (c) cross-sectional view obtained by scanning electron microscopy.

Table 1. The Effect of Solvent on the Transport of Adenosine and Guanosine through Imprinted and Nonimprinted Polymer Membranes

entry	imprint molecule[a]	solvent	feed conc. (As = Gs) (μM)	flux ($\times 10^{-10}$ mol cm^{-2} h^{-1}) As	Gs	selectivity factor
1	9-EA	methanol	26	4.2	2.8	1.5
2	9-EA	methanol	75	3.8	2.2	1.7
3	none	methanol	78	3.5	3.3	1.1
4	9-EA	methanol/ chloroform (20:80 v/v)	26	0.7	0.4	1.7
5	9-EA	methanol/ chloroform (20:80 v/v)	78	4.6	2.5	1.9
6	9-EA	methanol/ chloroform (6:94 v/v)	76	1.8	0.5	3.4
7	none	methanol/ chloroform (6:94 v/v)	76	1.1	1.6	0.7

[a] Membranes imprinted with 9-ethyladenine (9-EA) contained 1.4 μmol sites/0.12 g membrane. Reference membranes were prepared in an identical manner to imprinted membranes, only without 9-EA.

equimolar mixture of adenosine and guanosine (As = Gs = 26 μM) in methanol, the former was transported at a higher rate with a selectivity factor of 1.5−1.7 (Table 1). When the solvent was changed from methanol to chloroform−methanol (80:20 v:v), the fluxes remained approximately constant while the selectivity factor increased to the range of 1.8−2.0 (Table 1).

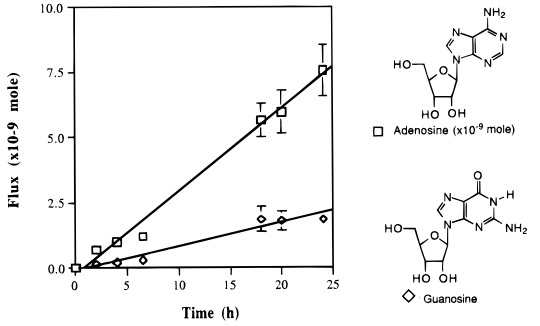

Figure 2. Plot of the facilitated transport of adenosine from an equimolar methanol/chloroform (6:94 v/v) solution (As = Gs = 26 μM) of adenosine and guanosine. The membrane was imprinted with 9-ethyladenine. The selectivity factor is 3.4.

Further decrease in methanol content (6% methanol:94% chloroform) resulted in a *decrease* in the fluxes, but the selectivity factor increased to 3.4 (Table 1, Figure 2).

Unlike the imprinted polymers, reference polymer membranes prepared without template **1** did not show any preference for adenine or adenosine (Table 1, **2** and **3**). Under otherwise identical conditions, the As/Gs selectivity in methanol for the imprinted polymer is 1.7 while the ratio of fluxes for the control is 1.1. In methanol/chloroform (6:94 v/v), the As/Gs selectivity is 3.4 while the reference membrane exhibits a slight *guanosine* preference (0.7).

The absence of adenosine selectivity was also found when a generic amine (benzylamine) was substituted for 9-ethyladenine in the polymer membrane formulation. Thus, at a membrane loading of 1.4 μmol sites/membrane in methanol/chloroform solvent (20:80 v/v), the As/Gs selectivity was 0.9 compared with the 9-ethyladenine imprinted membrane As/Gs = 1.9.

The origin of selective transport can be attributed to adenine selective binding sites in the polymer membrane created by the imprinting process.[11] The selective transport arises from a process that involves reversible complexation and exchange between adenine and sites in the membrane. Since 9-ethyladenine EGDMA/MAA imprinted polymers show little affinity for other purine and pyrimidine bases, the competing substrates do not have this pathway available for transport. This proposal is consistent with observed solvent effects on selectivity. The higher selectivity factor observed at lower methanol content can be attributed to the formation of a more stable membrane bound adenosine complex in the less polar medium. Binding studies of adenosine and guanosine to 9-ethyladenine imprinted polymers in both polar and nonpolar media have shown that the K_d and selectivity in binding of adenosine over guanosine were greater in the *less polar* medium.[15] Although the selective complexation between the target molecule and their imprints site has been well documented, the present study is the first known evidence for the selective transport of targeted molecules across imprinted membranes.

In conclusion, we have developed membranes of imprinted polymers which transport target molecules across the membrane. The stability and mechanical strength of these membranes and the ease of creating receptor sites for a variety of classes of molecules by the molecular imprinting approach provide opportunities for continuous separation processing. Chiral separation of drugs and amino acids in addition to protein transport using imprinted membranes is under current investigation.

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Supporting Information Available: Experimental details (3 pages). See any current masthead page for ordering and Internet access instructions.

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