Synthetic Polymer Membranes with Molecular Recognition

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The synthesis is described of a macroporous electrostatically-spun poly(ether urethane) membrane, functionalised to show molecular recognition of uracil derivatives.

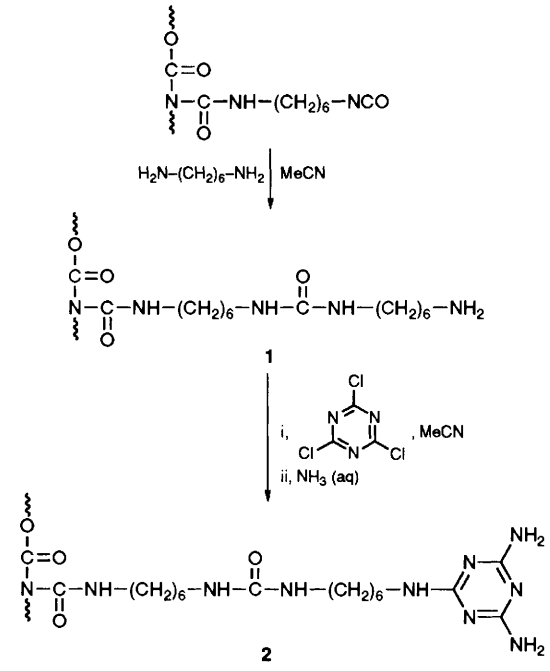
Membranes coupled to systems exhibiting molecular recognition play a vital part in biology and have potential applications in industrial processes. We have recently described the synthesis of an electrostatically-spun poly(ether urethane) membrane with covalently-attached protein A and demonstrated molecular recognition of the latter by immunoglobulin G (IgG).[1] The resulting binding of these two proteins is reversible by appropriate changes in pH. This work was extended into the membrane immunodiagnosis field; *e.g.* mouse monoclonal antibody when coupled to the membrane retained its activity to recognise and bind to tumour necrosis factor (TNFα), thus providing the basis of an ELISA (enzyme linked immunosorbent assay) technique.[2]

In this communication we describe the construction of a fully synthetic membrane-recognition system comprising an electrostatically-spun poly(ether urethane) membrane carrying moieties of diaminotriazine. This derivative is related to melamine, which has been shown to crystallise from chloroform with compounds such as cyanuric acid to form molecular complexes linked by networks of hydrogen bonds.[3]

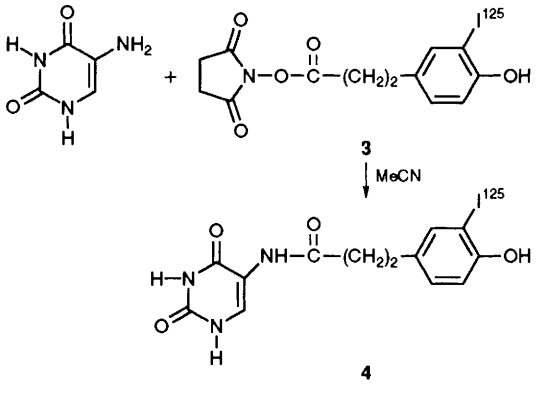
Membranes produced by electrostatic spinning are composed of very thin fibres [diameter (d) ~1 μm] melded at many points to enclose irregular pores with a typical dimension 10 μm. The surface area is consequently large, of the order 4 m^2 g^{-1}. Commercial poly(ether urethanes) Biomer (aromatic) and Tecoflex (aliphatic) were used. After spinning, the membrane was reacted with an excess of 1,6-diisocyanatohexane in dry acetonitrile (40% v/v) at 40 °C for 48 h. The resulting isocyanated membrane was then treated with an excess of 1,6-hexanediamine solution to obtain an aminated membrane **1**, which was coupled with 2,4,6-trichloro-1,3,5-triazine in acetonitrile (16% m/v) at 4 °C for 4 h. Under these conditions only one chlorine per molecule reacts.[4] The product was washed and allowed to react with aqueous ammonia (30% m/v) at 40 °C for 2 h. These processes are shown in Scheme 1, which depicts a single urethane group in a polymer chain. Adducts **1** and **2** typify the control and the final functionalised membranes, respectively.

IR observations were consistent with the proposed scheme. A film of poly(ether urethane) prepared by casting was

aminated and reacted with 2,4,6-trichloro-1,3,5-triazine according to the procedures described to give the dichlorotriazine derivative. After extensive washing the film showed IR bands at 1616, 1621 and 1627 cm^{-1}, which we attribute to C=N stretching vibrations; a window in the poly(ether urethane) absorption permits these measurements to be made. The literature gives bands in the region 1615–1625 cm^{-1}, for comparable structures. A band at 787 cm^{-1}, corresponding to a C–Cl stretching frequency, also appeared. After amination the C=N bands were slightly modified to 1614, 1621 and 1626 cm^{-1}; this absorption is consistent with literature data. The final film (containing structures such as **2**), unlike its dichlorotriazine precursor, was found to dye strongly with Eosin Y.



Scheme 1



Scheme 2

The binding of a uracil derivative to the membrane **2** through hydrogen bonding was examined in three different media, namely water, borate buffer (pH 8.9) and chloroform, with the aid of radiolabelling with ^{125}I. An appropriate uracil derivative was synthesised by reacting 5-aminouracil with ^{125}I-labelled Bolton-Hunter reagent **3** to form the required compound **4**, (Scheme 2). Discs (*d* 2.54 cm) cut from membranes **1** and **2**, were incubated in suspensions of **4** (4 mg per ml) in the various media for 2 h at room temperature. **4** is partially soluble in these media, having the highest solubility in chloroform. Each disc was then washed copiously with the pure solvent before counting. Results presented in Table 1 show that significant coupling occurs in all liquids, especially in water solution. A smaller amount of binding ('subsidiary binding') is found with structure **1** (the control). We believe that the major portion of the coupling is attributable to the formation of the hydrogen-bonded structure **5**. The hydrogen-bonded complex has a structure similar to that between uracil and diaminopurine derivatives identified by Simundza *et al*[5] and subsequently studied by many other workers, notably Lehn,[6] Mathias[3] and their colleagues.

Uptake of a solute from solution by a macroporous membrane is dependent on the liquid/solid contact, and our previous work[2] has shown that from this point of view simple immersion is much inferior to perfusion. Consequently we think that the couplings given in Table 1 are unlikely to represent maximum values. Nevertheless they compare very satisfactorily with estimates made on the basis of the number of active (coupling) sites in the membrane deduced from data in ref. 1, which allow for the fact that some sites are below the fibre surface and may be inaccessible.

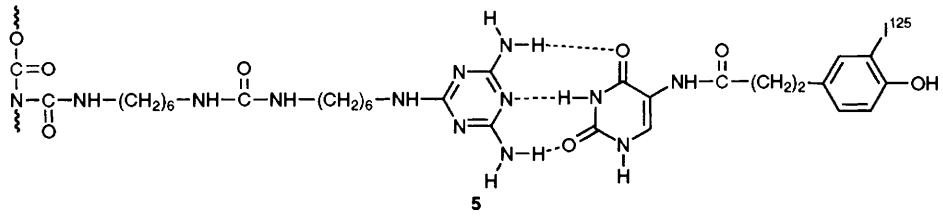
In these earlier experiments[1] a functionalised poly(ether urethane) membrane **1** was prepared using similar (although

Table 1 Binding of uracil derivative **4** to functionalised membrane **2**

Membrane	Medium	Mass of **4** bound to membrane/mg of **4** per g of membrane
2	Distilled water	24.96
Control	Distilled water	1.67
2	Borate buffer	5.40
Control	Borate buffer	0.89
2	Chloroform	13.65
Control	Chloroform	3.23

Table 2 Release of uracil moiety **4** from adduct **5**

Membrane	Eluent	% **4** released
5	Distilled water	0.77
5	Urea (aq), 8.3 mol dm^{-3}	23.62
5	Guanidine hydrochloride (aq), 10 mol dm^{-3}	84.71
1 with subsidiary binding of **4**	Distilled water	8.89



5

not identical) procedures to those described above and accessible amino groups were estimated by coupling to ^{125}I-labelled Bolton-Hunter reagent. The value obtained was approximately 10^{-4} mol g^{-1}. In the first experiment in Table 1 the binding corresponds to 6.5×10^{-5} mol g^{-1}, suggesting that under the prevailing conditions (distilled water medium) most of the triazine residues become bonded to the uracil derivative. Obviously this is only an order-of-magnitude comparison. It is of interest that in coupling to IgG only about 1/3000 of the sites appear accessible,[1] a result not surprising in view of the size of the protein molecule.

Release of the uracil moiety from the complex formed in aqueous media was examined in three eluents, at ambient temperatures in a period of 2 h, with results presented in Table 2. These findings recall the familiar disruption of hydrogen bonds by urea and guanidine and are therefore consistent with structure **5**. They also indicate the relative weakness of the subsidiary binding between the uracil moiety and membrane **1**.

We have also found that membrane **2** binds the nucleoside deoxythymidine 5′-triphosphate (dTTP) in aqueous solution and believe a similar mechanism is involved. We intend to publish full details after further study.

The authors thank Kodak Limited, Harrow for financial support, and Drs M. P. Purbrick and T. Wear for their interest in the work.

Received, 28th May 1993; Com. 3/03059H

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