spectra of the two zinc complexes in solution indicate the presence of two kinds of carbamates, in a 2 : 1 ratio, exactly as required by the solid state structure of **1**.⁸ The cadmium complex is insoluble in common solvents, but the solid has a remarkably similar IR spectrum to those of the zinc analogues; the structure may be similar but involving some degree of polymerization between tetrameric units.

There are a number of structures known for compounds in which both an alkyl group and oxygen (or other group δ) atoms coordinate to zinc. Coordinative saturation at zinc is often attained by some degree of association,⁶ and common structural units include dimers such as the fused tetrahedra in $K_2[Et_2Zn(OBu^t)_2ZnEt_2]$ ⁷ and $[MeZn(S_2CNEt_2)]_2$.¹ Another common geometry is based on a tetrahedral arrangement of zinc atoms as in basic zinc acetate $[Zn_4O(O_2CMe)_6]$ ⁹ and the cubane structure found for neutral alkoxy species such as $[MeZn(OMe)]_4$.¹⁰ The present structure conforms to none of these established structural types.

The compounds **1–3** are involatile and do not sublime on heating on a vacuum line (10^{-2} Torr; $<200^\circ\text{C}$). Attempts to pyrolyse small quantities of compound **1** *in vacuo* have, on one occasion, led to the explosion of the tube, behaviour which is quite different from that of the parent dimeric seleno- and thio-carbamates. These compounds are not likely to be useful precursors for the metal oxides. However, the structure of **1** is, to our knowledge, unique in containing a planar tetramer of zinc atoms involving the fusion of tetrahedral and trigonal pyramidal zinc sites.

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Amperometric Biosensor based on Direct Communication between Glucose Oxidase and a Conducting Polymer inside the Pores of a Filtration Membrane

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A biosensor, based on direct communication between glucose oxidase and polypyrrole, incorporated in the pores of track-etch membranes, is described; with this sensor, glucose in the concentration range $1\text{--}25\text{ mmol dm}^{-3}$ can be measured.

Biosensors based on the principle of direct electrical communication between redox enzymes and bare metal electrodes are rare.¹ The reason is that the active centres of such enzymes often are prevented from transferring electrons to the electrode due to an insulating protein shell. Indirect communication, however, is possible using low-molecular-weight mediators, which diffuse into and out the enzyme. Amperometric glucose sensors have been developed according to this principle.² The disadvantage of these systems is that they suffer from low stability because of leakage of the mediator.³

We describe here a very robust biosensor in which the selector (glucose oxidase, [E.C. 1.1.3.4]) directly transfers electrons to a conducting polymer which is incorporated into the pores of a filtration membrane (Fig. 1).[†]

Polypyrrole, poly(3-methylthiophene) and poly(3-hexylthiophene) were synthesized within the linear, cylindrical pores of Nucleopore® or Cyclopore® membranes (pore diameters 600, 800 and 1000 nm) according to a procedure described in the literature.⁴ By allowing the polymerization reaction to take place for the appropriate period of time, we were able to obtain membranes which contained conducting polymer and which were still porous. The polymerization

agent was removed by extensive washing with distilled water. The pores, partially filled with conducting polymer, showed good electrical conductivities (e.g. polypyrrole in 1000 nm pores; $1\text{--}2\text{ S cm}^{-1}$) and were found to immobilize glucose oxidase when they were brought into contact with an aqueous solution containing the enzyme. The best results were obtained with Cyclopore® filters with 600 nm pores, treated for 1 min with polypyrrole.

A biosensor [Fig. 1(b)] was constructed by coating one side of a membrane, containing conductive polymer microcylinders, with 100 nm platinum (argon plasma, 7 mbar, 50 nA sputter current). Attachment of the enzyme to the conducting polymer was realised by adsorption. To this end the membrane was soaked into a solution of glucose oxidase in 10 mmol dm^{-3} phosphate buffer (pH 7) and dried overnight at 4°C . By using fluorescein-labelled glucose oxidase, we were able to confirm that the enzyme was located inside the pores and not on the membrane surface. Independent assay of glucose oxidase activity, by a method described in literature⁵ gave an enzyme activity of $25 (\pm 5)$ milli-units for 1 cm^2 of membrane. This value was found for all membranes tested. Representative results obtained with this sensor are given in Fig. 2. Shown in Fig. 2(a) is the amperometric response to the addition of 10 mmol dm^{-3} glucose (the apparent K_m of our immobilised glucose oxidase is 15 mmol dm^{-3} , the K_m of the

[†] Abbreviations used: FADH₂ = flavin adenine dinucleotide.

Fig. 1 (a) Electron shuttle, showing how electrons are transported from the FADH₂ redox centres of glucose oxidase to an electrode *via* a conducting polymer. (b) Schematic representation of a microporous membrane/conducting polymer based biosensor.

native enzyme is 33 mmol dm⁻³).⁶ The working potential is 0.350 V *vs.* a Ag/AgCl reference electrode. The response time is less than a minute, which is fast considering the high diffusion resistance of the microporous filter-membranes. Measurements of glucose in the concentration range 1–25 mmol dm⁻³ were conducted in a continuous-flow system for a period of two weeks without loss of sensitivity. Glucose oxidase remained highly selective; the biosensor did not respond to fructose, citrate, lactate or pyruvate, nor to urea or uric acid at mmol dm⁻³ concentrations.

It was shown earlier that communication between glucose oxidase and polypyrrole is possible,⁷ although no current values were reported and it said that the direct interaction of glucose oxidase and polypyrrole weak.⁸ We observed a distinct current response (100–1000 nA). Evidence for direct electron transfer in our system came from the following. First, an equal response was observed in both the presence and absence of oxygen. In these experiments the response to glucose was compared for the same sensor, either under oxygen or under argon, the latter in combination with 180 IU ml⁻¹ catalase in solution. Second, a decrease in current response was measured when salt (NaCl) in various concentrations was added to the glucose solution [Fig. 2(b)]. The response, however, fully recovered when the initial salt concentration was restored. This suggests that the interaction between enzyme and conducting polymer is electrostatic in nature. It also indicates that the enzyme is very strongly bound within the pores of the filtration membrane.[‡] We presume

Fig. 2 Response of a membrane/conducting polymer biosensor to 10 mmol dm⁻³ glucose, obtained at 0.35 V (*vs.* Ag/AgCl). Phosphate buffered solution (pH 7), 180 units ml⁻¹ catalase. Measured under an argon atmosphere. * Addition of glucose.

that the salt screens the charges of the enzyme and the conducting polymer, resulting in suppression of the transfer of electrons. Third, we measured glucose continuously for several days under oxygen atmosphere without catalase present. This caused no significant loss of membrane sensitivity. The formation of H₂O₂, due to oxygen mediation, would have led to denaturation of the enzyme. We also measured the response of the sensor to H₂O₂ separately. At a potential of 100 mV *vs.* Ag/AgCl the current due to the addition of glucose was still positive, whereas even small amounts of purposely added H₂O₂ (10⁻⁴ wt. % in phosphate buffer, pH 7.4) gave a strongly negative response.⁹ Taken together, we may conclude that oxygen mediation does not take place in our system and, as no other mediators are present,[§] the response to glucose must come from electron transfer between enzyme and conducting polymer.

The present biosensor is simple in construction, stable and selective. The basic idea can be translated to many other combinations of redox enzymes and conducting polymers and we are currently investigating this.

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[‡] The lower response at increasing salt concentration could also be due to enzyme denaturation. As in our case the decrease in response is reversible, this is not likely to be the case.

[§] Measurements are performed in a flow system. Therefore, low-molecular-weight mediators, *e.g.* released FAD, are immediately washed out and cannot accidentally mediate the electron transfer.