Pure & Appl. Chem., Vol. 70, No. 6, pp. 1259–1262, 1998.
Printed in Great Britain.

Conducting polymers for biosensors: Rationale based on models

K.S.V.Santhanam

Chemical Physics Group, Tata Institute of Fundamental Research ,Colaba, Mumbai 400 005, India.

Abstract : With conducting polymers acting as three dimensional matrix for the immobilised enzymes where reactants are converted to the products, several biosensors have been developed in the last decade. The biosensors are modelled here based on the reactions to focus on their advantage and limitations caused by over oxidation of the polymer matrix. A proposal to overcome these limitations has been modelled. The role of conducting polymers in developing neurotransmitter sensors is discussed.

The electronic conducting polymers have an organised molecular structure on metal substrates, which permit them to function as a three dimensional matrix for the immobilisation of active catalysts and preserve the activity for long duration. This property of the conducting polymer together with its functionality as a membrane has provided opportunities to investigate the development of sensors. The polymer matrix is considered as not oxidisable/reducable in the potential range of interest and hence is inert for the envisaged biosensor activity. The following three possible models are proposed.

Model I :

Consider a biosensor reaction [1]

$$S + E_{ox} + O_2 \rightarrow P + E_{red} + H_2O_2 \qquad (1)$$

where S is biological substrate, E_{ox} is the oxidised enzyme, P is the product and E_{red} is the reduced form of the enzyme. This reaction is carried out in solutions where pH 7.4 (biological pH) is maintained. Since S is of interest to be estimated, it is desirable to have E_{ox} immobilised in a three dimensional matrix such as a conducting polymer so that the product, E_{red}, formed by reaction (1) also remains immobilised. By a subsequent conversion procedure it can be converted to E_{ox}. For the estimation of S, the immobilised enzyme can be placed into a solution of S and O_2. Reaction (1) occurs and the product and hydrogen peroxide will be localised in the conducting polymer. Either hydrogen peroxide can be determined colorimetrically or by electrochemical method. The latter provides an advantage in that the measurement is fast and simple. This would require the conducting polymer electrode be used as the working electrode with immobilised enzyme along with a counter electrode and a reference electrode in a three electrode configuration biosensor as shown in Figure 1.

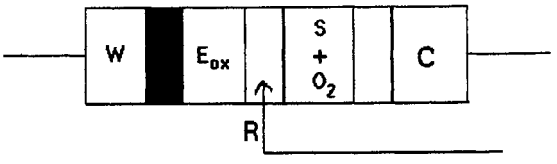

Fig.1 A typical biosensor

Since hydrogen peroxide is oxidised at 0.90 V vs SCE, by stepping the potential of the electrode from its equilibrium value to 0.90V, a diffusion current will start to flow in the electrochemical cell. By integration of the current it is possible to estimate the moles of peroxide formed in reaction (1).

$$2\,H_2O_2 \text{ ------> } H_2O + O_2 \qquad \text{at the polymer electrode at 0.90 V} \qquad (2)$$

$$E_{red} + O_2 \text{ -------> } E_{ox} + H_2O \qquad (3)$$

In the polymer matrix the regeneration of the enzyme will occur spontaneously. For this model to be operative three important conditions should be fulfilled. (i) The polymer matrix should not undergo changes during the reactions (1), (2) and (3). The natural tendency of conducting polymers would be to undergo overoxidation with a loos of conductivity. (ii) The reactions occurring within the polymer matrix should not disturb the polymer redox state. The diffusion of S and O_2 into polymer matrix should be fast for the sustained reaction. In situations where the potential at which peroxide is oxidised, the conducting polymer is electrochemically active, Model II described below would be an alternative.

Model II.

Consider a mediated electron transfer reaction in the polymer matrix. An oxidised mediator, M_{ox} is attached to the substrate electrode by covalent linking and the conducting polymer matrix is formed over this mediating film. This model uses bilayer films in comparison to Model I which uses a monolayer film. The enzyme is immobilised in the polymer matrix and the reaction described by (1) occurs in this phase. The mediator reacts with the peroxide as described by reaction (4)

$$M_{ox} + H_2O_2 \text{ ------> } M_{red} \qquad (4)$$

The amount of M_{red} is proportional to the peroxide generated in the reaction (1) and hence by determining M_{red}, the concentration of S can be estimated. The M_{red} is electrochemically oxidisable to M_{ox} and hence the charge required will provide the information on the concentration of S. The three steps are depicted in Fig.2.

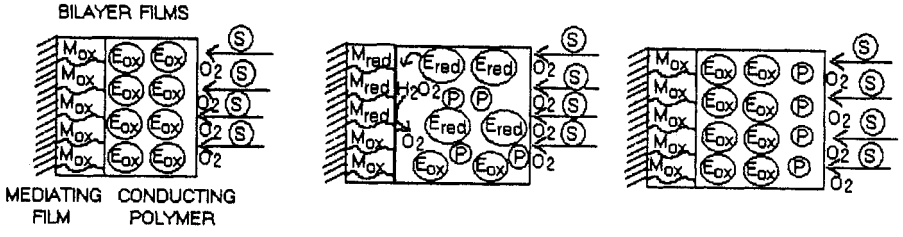

Fig.2 Three stages in the working of biosensor

To overcome the limitation of the Model I, it is necessary to have the redox potential of M_{ox} / M_{red} situated below the potential at which overoxidation of the polymer occurs. Furthermore, the mediating film should be capable of sustained turnovers in the estimation of S without getting disintegrated in the potential cycling. When this poses a limitation, the following Model III will serve as an alternative.

Model III :

This model is a refinement of Model II, where the mediating film has a metal ion active centre, whose redox activity can be monitored. The mediating film is not disturbed in the process of repeated measurements. The reaction occurs between E_{red} and the metal ion center rather than with oxygen in the regeneration of enzyme.

Model IV :

This model utilises a new feature of selectivity between biomolecules. The conducting polymer selectively sequesters into it the biomolecule of interest and then it is oxidised within the polymer. This model is especially important for neurotransmitter studies.

The above models are adaptable to the representative systems (ref.1-13), confirming the assumptions and requirements. The following illustrations are provided for the biosensor pathways. The immobilisation of glucose oxidase (GOD) in polypyrrole matrix for glucose estimation follows Model I. The reactions occuring are given by

$$\text{Glucose} + G_{ox} \text{-----> Gluconolactone-}G_{red} \quad (5)$$

$$\text{Gluconolactone-}G_{red} \xrightarrow{O_2} G_{ox} + H_2O_2 + \text{Gluconolactone} \quad (6)$$

Matrix Polypyrrole - but it undergoes overoxidation at 0.90V vs SCE

Model II can be depicted by

$$\text{Glucose} + O_2 + G_{ox} \text{-----> Gluconolactone} + H_2O_2\,(\text{polymer}) \quad (7)$$

$$PMePc(f)_{ox} + H_2O_2\ (\text{Polymer}) \text{------>} O_2 + PMePc(f)_{red} + 2\,H^+ + 2e \quad (8)$$

$$PMePc(f)_{red} \text{----->} PMePc(f)_{ox} + e \quad \text{Electrode reaction} \quad (9)$$

Where PMePc(f) is polymetallpthalocyanine film attached to the electrode. The redox potential of this couple is 0.20 V as compared with hydrogen peroxide at 0.90 V.

Model III is a modification of Model II since the organic film which is a mediating film has been replaced by either metal or its complex is covalently linked to the electrode surface which brings out the mediating reaction. Representing metal as M, the glucose sensing can be rationalised as

$$\text{Glucose} + G_{ox}\,(\text{FAD}) \text{------>Gluconate-}G_{ox}\,(FADH_2) \quad (10)$$

$$G_{ox}\,(FADH_2) + M^+ \text{--------> } G_{ox}\,(\text{FAD}) + 2\,M \quad (11)$$

In this model no peroxide formation is involved. Model IV typifies the selective performance of a biosensor especially for the sensing of dopamine or dopa. A conducting polymer under selected conditions acts as a membrane for the transport of charged species in the medium (ref. 14-15). This property has been studied for a variety of purposes, such as ion transport, sequestering through the membrane, permeability etc. As a result of the membrane-like structure existing with conducting polymers, the normal electroactivity of some of the electroactive organics and inorganics has not been observed at these electrodes. Polycarbazole is one typical example of this category and has been examined for electrochemical oxidation and reduction processes (ref. 16,17). The electrochemical oxidation of dopamine has been studied for different polymer film thicknesses; the observed cyclic voltammetric patterns are shown in Figure 5. The excellent reversibility observed for its oxidation surpasses the reported results at other electrodes such as glassy carbon or Pt (ref.18). At the latter electrodes the product of oxidation undergoes internal cyclisation. Hence, the complementary cathodic peak current is reduced or negligible. The cyclised product shows a cathodic peak at more negative potentials than the oxidised product. We tend to observe this behaviour at thinner polycarbazole films (<1 micrometer). The response of polycarbazole electrode to different sweep rates as a function of polymer film has also been investigated to optimise the response factor for the sensing action of L-dopa or dopamine (ref. 16). Kawde and Santhanam (ref. 17) have analysed a solution of dopamine in the presence of 200 mM ascorbic acid by differential pulse voltammetric technique and found negligible interference of ascorbic acid. The linearity of the differential current with concentration of dopamine suggests the utility of this electrode for analytical determination. A model has been developed by the same authors for the selective sensing of L-dopa or dopamine at Polycarbazole electrode which is depicted in Figure 6. Acting as an ion selective membrane, it permits the entry of dopamine or dopa into it but not ascorbic acid even at high concentrations. Two situations are visualised here, based on a) sequestering of the neurotransmitter (dopa or dopamine) into the polymer layer and direct interaction between polycarbazole and the neurotransmitter and b) membrane structure and electron transfer. In the first model the polymer film of thickness d, is considered to sequester the neurotransmitter (NT) with S=1 C_{NT} (PCZ)/C_{NT} (soln.). The anodic current for the elctrode reaction is given by $i_a = 2\,FA\,k_{ox} \int o^d C_{NT}(x).C_{PCZ}(X)dx$, where n is the number of electrons transferred, A is the area of the electrode, F is Faraday and K_{ox} is the rate constant for the electron transfer between PCZ and the neurotransmitter, and C_{NT} (PCZ) refers to the polycarbazole phase. If the charge transfer is occurring in thin layer near the polymer-solution interface, the distance dependence of CNT may be neglected and $i_a = 2FA\,k_{ox}\,C_{NT}(PCZ)\,C_{PCZ}.m$,where m is the thin reaction layer. This expression can be rewritten as $i_a = 2FA\,SC_{PCZ}\,C_{NT}$ (soln). m. A hydrophobic membrane will be less permeable to ions. For this model to be effective it is necessary to have $NT + PC^+ \text{--->} NT^+ + PC$ with further reactions to form NT^{++}. In the present situation with dopamine it is endergonic. The second model assumes the electron transfer occurs at the substrate and PCZ. The polymer film is

assumed to act as a barrier for the transport. Using Fick's second law of diffusion and solving for the concentration of the neurotransmitter, $C_{NT}(x=0) = C_{NT}[1- erf(d/2)] D_{NT} t$ where d=0.5 mm, D_{NT} (PCZ)=10^{-10} cm^2 / s, t=20 h to reach C_{NT} (x=0)/ C_{NT} / (X=d)=0.99. The time required is long in comparison to the charge transfer reaction with m= 10^{-7} - 10^{-8} cm (molecular dimensions), the anodic current can be written as i_a= 2 FAS C_{NT} (soln). D_{NT} (PCZ)d^{-1} with S considered as a result of the specific chemical interaction and electrical interaction. This model appears to conform to the observed results.

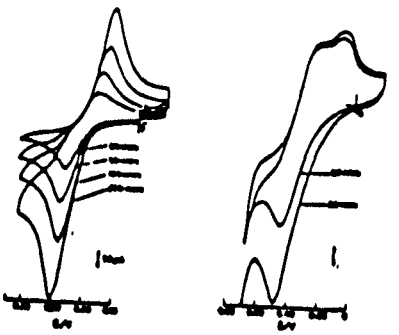

Fig.3 Cyclic volatammetric curves for dopamineat 1 μm film (left) and 0.1μm polycarbazole films.Sweep rates (1) 20mV/s (2) 50 mV/s (3) 100 mV/s and (4) 200 mV/s

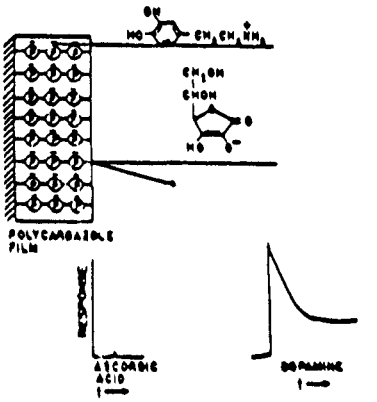

Fig. 4 Selectivity model with currents due to ascorbic and dopamine are shown in the lower part.

The above models demonstrate a few pathways by which biosensors could be developed.

REFERENCES

1. C.R.Lowe et. al., *J.Chem.Soc., Faraday Trans.*, **1,** 82 (1986)
2. J.M.Cooper and D.Bloor, **5**,883 (1993)
3. T.Tatsuma et al. *Anal. Chem.*, **64,** 1183 (1993)
4. T.Tastsuma et al.*J.Electronal. Chem.* 356, 245 (1993)
5. P.N.Barlet et al., *J.Chem.Soc., Faraday Trans.*, **88**, 2677 (1992)
6. T.S.Sun and H.Tachikawa, *Anal. Chem.*, **64**, 1112 (1992)
7. S.Kuwabata and C.R.Martin, *Anal. Chem.*, **66**, 2757 (1994)
8. C.G.Kooal and J.M.Nolte, *J.Chem.Soc., Chem.Commun.*, 1691 (1991)
9. B.F.Yonhin, M.Smolander, T.Crompton, and C.R.Lowe, *Anal. Chem.*, **65**, 2067 (1993)
10. W.J.Albery, P.N.Bartlet, B.J.Driscoll and R.B.Lennox, *J.Electroanal. Che.*, **323**, 77 (1992)
11. M.Marchesiello and E.Genies, *J.Electroanal. Chem.* **358,** 35 (1993)
12. T.Tatsuma and T.Watanabe, *Anal.Chem.*, **65**, 3129 (1993)
13. P.N.Bartlet and P.R.Birkin, *Anal.Chem.*, **64,** 1118 (1992)
14. K.Maksymiuk and K.Doblhofer, *Electrochimica Acta*, **39**, 217 (1994)
15. P.N.Bartlet, Z.Ali and V.Eastwickfield, *J.Chem.Soc.Faraday Trans.*, **88**, 2677 (1992)
16. R.B. Kawde, N.B.Laxmeshwar and KS.V.Santhanam, *J.Sensors and Actuators*, **23**, 35 (1995)
17. R.B.Kawde and K.S.V.Santhanam, *J.Bioelectrochem, Bioenergetics*, **38**, 405 (1995)
18. F.Zhang and G.Dryhurst, *J.Med.Chem.*, **37**, 1084 (1994)