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Microelectrochemical enzyme transistors

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Conducting polymers, such as poly(aniline) or poly(3-methylthiophene), change their conductivity by many orders of magnitude upon oxidation or reduction. This modulation of the conductivity of the polymer by redox reactions can be utilised to fabricate microelectrochemical transistors—electrochemical devices that behave in many ways as analogues of solid state junction field effect transistors. When combined with suitable redox enzymes these devices can be developed as miniature biosensors which offer a number of interesting advantages, particularly for use with small sample volumes or at low analyte concentrations.

Introduction

Electrochemical biosensors have been extremely successful as disposable devices for the measurement of blood glucose for use by diabetics world-wide. This success is based on ease of manufacture at low cost but with high control of quality and reproducibility combined with ease of use. The clinical range for glucose in blood lies between 1 and 20 mM. In this concentration range direct amperometric measurement at millimetre sized electrodes yields readily measured currents in the microamp range. Future applications will require much smaller devices so that more assays can be carried out in a single drop of blood and assays for analytes such as hormones, drugs or enzymes present at much lower concentrations (typically nano- and micro-molar). Taken individually and together these requirements will be difficult to meet with present types of amperometric biosensor because as the size of the electrode and/or the concentration of the analyte decreases the current measured also decreases. It is therefore appropriate to look for other types of electrochemical biosensor which, while retaining the simplicity of use and low cost of the amperometric devices, also provide the potential for greater sensitivity and small size.

The majority of electrochemical biosensors are based on either potentiometric or amperometric detection (Fig. 1). In the case of a potentiometric enzyme electrode the electrode senses the steady state concentration of some species, usually a product of the enzyme catalysed reaction (such as H^+, NH_4^+ *etc.*), at the electrode surface. This steady state concentration is determined by the kinetics of the enzyme catalysed reaction and by the mass transport of reactants to, and products away from, the electrode. Under suitable circumstances this steady state concentration is directly related to the concentration of the analyte in the solution. The operation of potentiometric enzyme electrodes suffers from a number of problems which limit their sensitivity for many applications.[1] In addition an accurate and stable reference electrode is essential and this is not easy to fabricate as a disposable structure. Consequently amperometric approaches have generally been favoured, although this is not always possible (for example in measurement of urea or penicillin). In an amperometric enzyme electrode the current is a direct measure of the rate of the enzyme catalysed reaction and therefore, under suitable circumstances, is directly related to the concentration of analyte. The great majority of redox enzymes or coenzymes such as NADH and NADPH do not undergo rapid, direct electrochemical reaction at electrode surfaces and

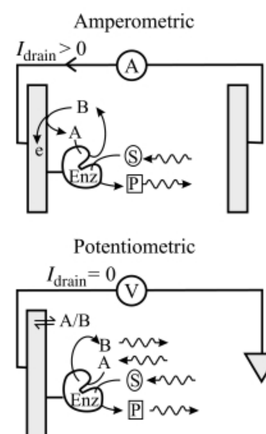

Fig. 1 Schematic representations of an amperometric enzyme electrode and a potentiometric enzyme electrode. In the amperometric enzyme electrode the conversion of substrate, S, to product, P, by the enzyme is linked to the electrode through the mediator couple A/B. The current for the conversion of B to A is then related to the rate of the enzyme catalysed reaction and thus to the substrate concentration. In the potentiometric enzyme electrode the electrode senses the concentrations of A and B at the electrode surface. These concentrations depend on the rate of the enzyme catalysed reactions and the rates of mass transport of the A/B couple and the substrate in solution.

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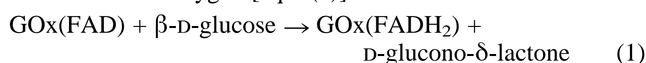
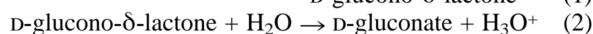
consequently it is necessary to provide some means to couple the enzyme catalysed reaction to oxidation or reduction at the electrode surface. In the first amperometric enzyme electrodes, such as the Clark glucose electrode,[2] this was done by oxidising or reducing one of the co-reactants or products of the enzyme catalysed reaction at a metal electrode. The enzyme glucose oxidase (E.C. 1.1.3.4) catalyses the reaction of β-D-glucose with oxygen to give D-glucono-δ-lactone and hydrogen peroxide. This reaction occurs in two stages. In the first step [eqn. (1)] the oxidised flavin prosthetic group, FAD, bound in the enzyme active site is reduced by reaction with glucose to give the bound reduced flavin, $FADH_2$, and the product gluconolactone, which undergoes hydrolysis to give gluconic acid [eqn. (2)]. In a subsequent step the reduced flavin is reoxidised by reaction with molecular oxygen [eqn. (3)].



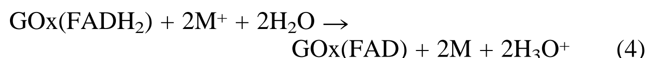
$$GOx(FAD) + \beta\text{-D-glucose} \rightarrow GOx(FADH_2) + \text{D-glucono-}\delta\text{-lactone} \quad (1)$$



$$\text{D-glucono-}\delta\text{-lactone} + H_2O \rightarrow \text{D-gluconate} + H_3O^+ \quad (2)$$

$$GOx(FADH_2) + O_2 \rightarrow GOx(FAD) + H_2O_2 \quad (3)$$

These reactions can be followed electrochemically by either measuring the rate of consumption of molecular oxygen or by measuring the rate of production of hydrogen peroxide. Although both approaches have been adopted they suffer from a number of problems. At high glucose concentrations, or in situations where the supply of oxygen is restricted, the response can be limited by the oxygen supply rather than by the concentration of glucose. In addition both oxygen and hydrogen peroxide are difficult species to measure electrochemically because of their poor electrode kinetics. Consequently measurements in whole blood, serum or other biological media are often difficult because of interference from other, readily oxidised compounds such as uric acid or ascorbate present in the sample. For these reasons it is preferable to avoid the use of oxygen and replace the second reaction by one in which a redox mediator is used to reoxidise the enzyme [eqn. (4)].



$$GOx(FADH_2) + 2M^+ + 2H_2O \rightarrow GOx(FAD) + 2M + 2H_3O^+ \quad (4)$$

Using this approach the mediator can be chosen so that it has both a fast reaction with the reduced enzyme, good stability and fast electrode kinetics at a potential where the interferences from other species present in the sample are minimal. The most successful example of this approach has been the use of ferrocene derivatives to mediate oxidation of glucose oxidase first suggested by Cass *et al.*[3] and subsequently the basis of a very successful commercial disposable glucose sensor.

For glucose oxidase, direct electrochemical oxidation of the enzyme is slow because the active site is buried deep within the enzyme core.[4] Homogeneous solution mediators, such as the ferrocene derivatives, are presumably able to diffuse into and out of the enzyme's active site and thus to act as a shuttle carrying electrons from the active site to the electrode. Although the use of homogeneous, freely diffusing mediators has proved a very successful and flexible approach to the problem it is not ideal for all applications because the mediator can diffuse away from the electrode surface and be lost. For this reason other approaches have been investigated including the co-immobilisation of the enzyme and mediator in carbon paste electrodes and sol-gel electrode structures, the covalent modification of the enzyme by attachment of the redox mediator to amino acid residues of the protein,[5,6] and the immobilisation of the enzyme in redox polymer films containing the covalently attached mediator. This last approach, pioneered by Heller,[7] has been very successful in linking the re-oxidation of several flavoproteins, including glucose oxidase, to electrodes for use in amperometric biosensors.

Despite these successes in designing amperometric enzyme electrodes there are important limitations to their performance if we wish to go towards either smaller sensors or analytes at lower concentrations. In an amperometric enzyme electrode the current is directly related to the rate of turnover of the enzyme. For example for glucose oxidase the maximum rate of turnover of the enzyme is 800 s^{-1}. For a monolayer coverage of the enzyme (1.6×10^{-12} mol cm^{-2})[8,9] on a 1 cm^2 electrode this corresponds to a maximum current of 240 μA. As the size of the electrode is decreased the current will also decrease because the number of enzyme molecules decreases. For a 70 micron diameter electrode (the diameter of a human hair, 3.85×10^{-5} cm^2) the maximum current for a monolayer coverage of glucose oxidase will be 9.2 nA; for a 1 micron diameter electrode (7.85×10^{-9} cm^2, a realistic size using present fabrication techniques) the current will be reduced to 1.9 pA. Clearly, in principle, this current can be increased if the enzyme coverage is increased from a monolayer to multilayer. However the increase in current will be less than linear with increasing enzyme coverage because of the restriction on diffusion of the substrate through the enzyme layer at the electrode surface. Thus, realistically we can probably only expect an increase of one or two orders of magnitude in the current if we use multilayer enzyme coverage on the electrode.

These currents are for high concentrations of the analyte (>20 mM) and for an enzyme with fast kinetics. At lower concentrations, or for many other enzymes, the currents would be less. This also becomes a problem when we try to develop amperometric biosensors for analytes such as hormones or drugs which are biologically active at much lower concentrations.

To address these problems it is appropriate that we investigate other electrochemical sensing strategies which maintain the advantages of electrochemical biosensors in terms of low cost and ease of use and manufacture, but which offer other mechanisms to allow enhanced sensitivity at low analyte concentrations for devices of small size. One such approach is provided by the microelectrochemical transistor or switch devices first described by Wrighton and colleagues.[10] These devices make use of the ability to switch the conductivity of thin conducting polymer films by five or more orders of magnitude on oxidation or reduction. This large change in conductivity of the polymer leads to amplification of the signal and can be used as the basis of a chemical sensor or biosensor which acts as a electrochemical transistor analogous to a junction field effect transistor in its operation and characteristics. In addition these devices can be made very small so that they may be suitable for use in small sample volumes.

Conducting polymers

The microelectrochemical transistors and diodes described by Wrighton make use of the very large changes in the conductivity of electronically conducting polymers which accompany oxidation or reduction of the polymer backbone. Conducting polymers, such as poly(pyrrole) or poly(3-methylthiophene) are formed by oxidative coupling of the respective monomers. This coupling can be carried out chemically or electrochemically. The latter approach has the advantage that the polymerisation process is localised at the electrode surface and that the amount of polymer deposited can be controlled through the total amount of charge passed. On cycling the potential of the conducting polymer film in a solution of background electrolyte the polymer can be repeatedly oxidised and reduced (Fig. 2). The amount of charge passed in this process depends on the amount of polymer present. For polymers such as poly(pyrrole) and poly(3-methylthiophene) the oxidised form is conducting and the reduced form is insulating. Conduction in the oxidised form of the polymer is through the mobile bipolarons[11] (Fig. 2).

The oxidation and reduction of the conducting polymer is also accompanied by the ingress and egress of anions, cations and solvent and by polymer chain relaxation processes. Thus the dynamics of the redox transformation of these films is a complex process and one which we are only now beginning to

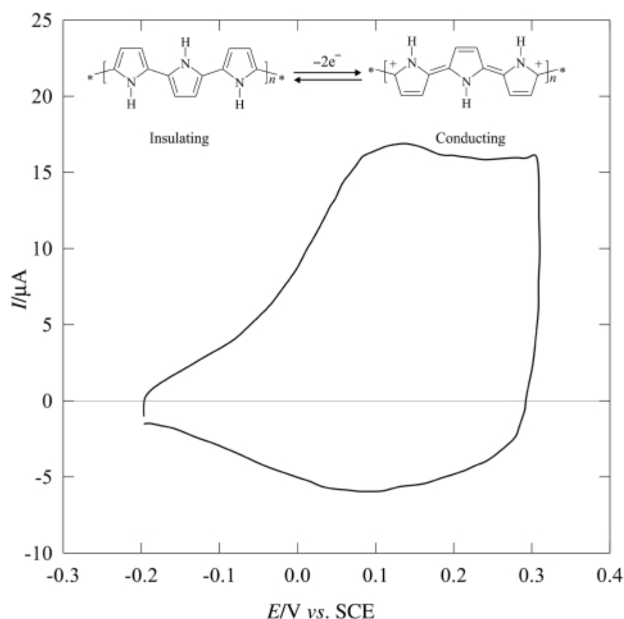


Fig. 2 A cyclic voltammogram of a poly(pyrrole) film recorded at 200 mV s^{-1} in 2 mol dm^{-3} H_2SO_4. The film was grown on a 0.5 mm diameter platinum electrode at 0.65 V *vs.* SCE from a solution of 0.5 mol dm^{-3} pyrrole in 1 mol dm^{-3} KCl. The inset shows the schematic structures for the insulating and conducting forms of the polymer. Note that in the fully oxidised polymer there is typically one charge for every four monomer units.

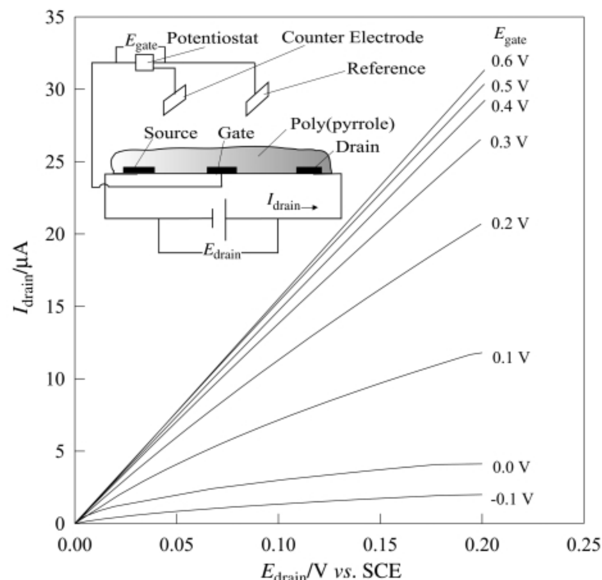


Fig. 3 Plots of the drain current, I_{drain}, as a function of the drain voltage, E_{drain}, for a poly(pyrrole) based microelectrochemical transistor operated in MeCN containing 0.1 mol dm^{-3} Bu_4NClO_4. Each curve corresponds to a different value of the gate voltage, E_{gate}. As the gate voltage increases from –0.1 V *vs.* SCE to 0.6 V the resistance of the poly(pyrrole) film decreases. The inset shows the arrangement used to make the measurements. (Adapted with permission from White *et al.*[10] Copyright 1984 American Chemical Society.)

fully comprehend through the use of a variety of *in situ* techniques.[12,13] This movement of ions is essential to maintain the electroneutrality of the polymer film. This requirement for the motion of ions to accompany the oxidation and reduction of the polymer constrains the maximum rate at which these films can switch between conducting and insulating states.

Microelectrochemical transistors and diodes

The first microelectrochemical transistor, described by Wrighton and colleagues in 1984,[10,14] comprised a poly(pyrrole) film deposited across the gaps between three independent gold microband electrodes 1.4 μm apart on a oxidised silicon substrate (Fig. 3). By analogy with a junction field effect transistor the three gold electrodes are referred to as the source, gate and drain. By using the central gate electrode Wrighton and colleagues were able to alter the redox state of the poly(pyrrole) film. With the gate at potentials, E_{gate}, negative of -0.2 V *vs.* SCE the polymer is insulating and no current flows through the polymer film between the outer source and drain electrodes when a voltage, E_{drain}, is applied between them—the device is in the 'off' state. When the gate potential, E_{gate}, is changed to more positive values the poly(pyrrole) film becomes oxidised and changes to its conducting state. Now when a voltage is applied between source and drain a significant drain current, I_{drain}, flows between the source and drain electrodes through the poly(pyrrole) film—the device is in the 'on' state. Fig. 3 shows the current–voltage characteristics of the device at different gate voltages. In this device a small signal applied to the gate electrode leads to a large change in the drain current flowing through the polymer; thus the device amplifies the signal in the same way that a solid state transistor can be use to amplify a signal.

A significant difference between the type of microelectrochemical transistor described here and conventional solid-state devices lies in their response times. In a solid-state semiconductor device the switching is achieved by movement of electrons or holes within the semiconductor. In the microelectrochemical enzyme transistor the switching is accompanied by the movement of ions and solvent within the film (see above). Consequently the process is much slower; switching for Wrighton's first poly(pyrrole) device took on the order of 10 s.[10] In subsequent studies Wrighton and colleagues demonstrated that other conducting polymer such as poly(3-methylthiophene) and poly(aniline) could be used to make microelectrochemical transistors[15,16] and that these devices could be made to operate faster by careful choice of the polymer film and by reducing the size of the inter-electrode gap. In this way they were able to demonstrate power amplification up to frequencies of 10 kHz.[17] Nevertheless this is still considerably slower than for solid-state devices.

However although switching in microelectrochemical transistors is slow they have other properties which solid state devices do not possess, notably that the devices can be switched by chemical reactions and therefore can, in principle, be used as chemical sensors. Thus, instead of using a potentiostat and external circuit the redox state of the polymer film can be changed by oxidation or reduction by a species in solution and this change can again be sensed by a change in the drain current flowing through the device—the chemical reaction switches the device between 'on' and 'off' states. The first examples of this used simple outer sphere redox couples such as $[Fe(CN)_6]^{3-/4-}$ or $[Ru(NH_3)_6]^{3+/2+}$ to turn devices based on poly(aniline) films 'on' and 'off' respectively.[16] The amount of redox reagent which can be detected with this type of device is very small. For example for a poly(3-methylthiophene) based transistor Wrighton *et al.* were able to detect the injection of 1×10^{-9} mol of $[IrCl_6]^{2-}$ corresponding to the reaction of 8×10^{-16} mol of the reagent at the polymer surface.[18] Subsequently responses to molecular hydrogen and oxygen were demonstrated for microelectrochemical transistors based on poly(3-methylthiophene) films modified with platinum particles to catalyse the reactions of hydrogen and oxygen[19] (Fig. 4). Despite the use of the platinum particles to catalyse the reduction of oxygen the response of the devices was still rather slow, taking around 2 min to respond to 1 atm of dissolved molecular oxygen. In these devices the microelectrochemical transistor acts as a sensor for the redox potential of the solution without any particular chemical selectivity for the species present.

Whilst these initial studies by Wrighton and colleagues demonstrated the concept of using a microelectrochemical

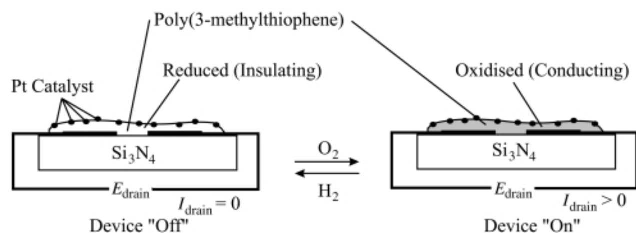


Fig. 4 A two terminal poly(3-methylthiophene) based microelectrochemical enzyme transistor. The device can be turned 'off' by reduction with hydrogen or 'on' by oxidation with oxygen. The platinum particles are necessary to catalyse the reactions of the gases at the surface of the polymer. (Adapted with permission from Thackeray *et al.*[19] Copyright 1986 American Chemical Society.)

transistor as a chemical sensor they did not go on to demonstrate selective devices or to look at the possible use of other types of catalyst to provide selectivity in the device response.

Microelectrochemical enzyme transistors

An obvious way to impart chemical selectivity to a microelectrochemical transistor is to use an immobilised enzyme as the catalyst.[20] However this has proved to be difficult because of the problems of coupling the enzyme electrochemistry to the conducting polymer film and finding the right conducting polymer with the correct properties for the application. The obvious starting point is to use poly(pyrrole) or one of its derivatives since these polymers can be deposited from neutral aqueous solutions and can be used to entrap enzymes such as glucose oxidase during deposition of the polymer.[21–23] This was the approach adopted by Matsue to make the first microelectrochemical enzyme transistor, an NADH responsive device based on diaphorase.[24] They used an interdigitated platinum electrode structure fabricated on glass with 10 μm wide bands separated by 10 μm wide gaps. These were coated with a thin electropolymerised film of co-polymerised pyrrole and *N*-methylpyrrole containing diaphorase (E.C. 1.6.99.- purified from *Bacillus stearothermophilus*). In order to couple the enzyme catalysed reaction to the reduction of the polymer 1 mM anthraquinone-2-sulfonate was added to the solution as a mediator. On addition of NADH the oxidation of NADH, catalysed by the diaphorase, led to reduction of the pyrrole–*N*-methylpyrrole copolymer film causing the device to switch from 'on' to 'off'. This change in the conductivity of the polymer was observed by recording the change in the drain current, I_{drain}, flowing through the device as a function of time after exposure to NADH (Fig. 5). After exposure to NADH the device is in its 'off' state and must be reset by reoxidising the polymer before it can be used to make another measurement of NADH. This was achieved by electrochemical oxidation of the polymer film.

Despite proving to be a useful way of immobilising enzymes at electrode surfaces for amperometric enzyme electrodes,[21–23] poly(pyrrole) and its derivatives are not the most suitable polymers for the fabrication of microelectrochemical enzyme transistors because the stability of the polymer in solution at neutral pH is not good, it is hard to reproducibly deposit the films from neutral aqueous solutions, and the electrical conductivity of the polymer is irreversibly destroyed by reaction with hydrogen peroxide.[25,26] This is a particularly serious problem if one wants to use flavoproteins, such as glucose oxidase, which inevitably generate some peroxide even when a redox mediator is incorporated into the film with the enzyme.[27]

These problems can be overcome by using poly(aniline) which is stable, does not react with hydrogen peroxide and which can be deposited very reproducibly by electrochemical methods. The electrochemistry of poly(aniline) is more complex that that of poly(pyrrole) because there are three accessible oxidation states: the leucoemeraldine, emeraldine, and pernigraniline forms[28,29] (Fig. 6). Of these three forms only the protonated emeraldine form is electronically conducting. The emeraldine base (Fig. 6) is an insulator. Thus poly(aniline) generally only exists in its conducting form in acidic solution and the polymer is normally electrochemically deposited from

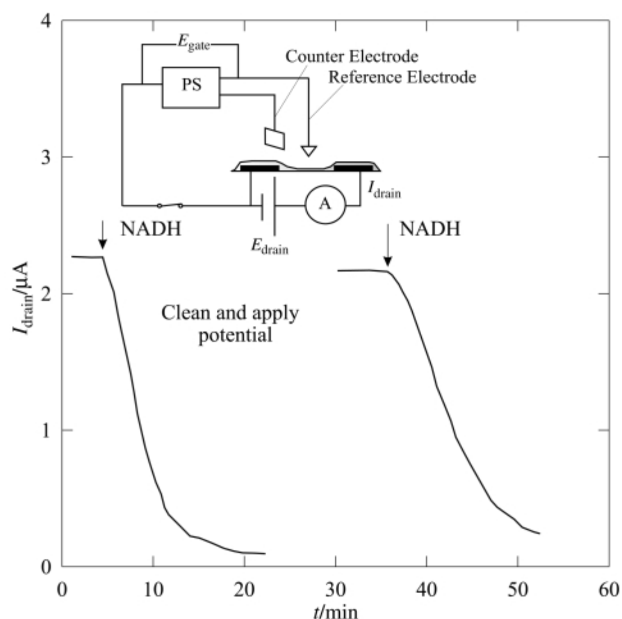


Fig. 5 Response of the drain current, I_{drain}, to the addition of 1 mmol dm^{-3} NADH for a microelectrochemical enzyme transistor based on a copolymer of pyrrole and *N*-methylpyrrole and incorporating diaphorase and anthraquinone-2-sulfonate. The device was operated at a drain voltage of 20 mV in 0.1 mol dm^{-3} KNO_3 in 20 mmol dm^{-3} phosphate buffer at pH 6.8. The inset shows the experimental arrangement for the measurement. (Adapted from Matsue *et al.*[24])

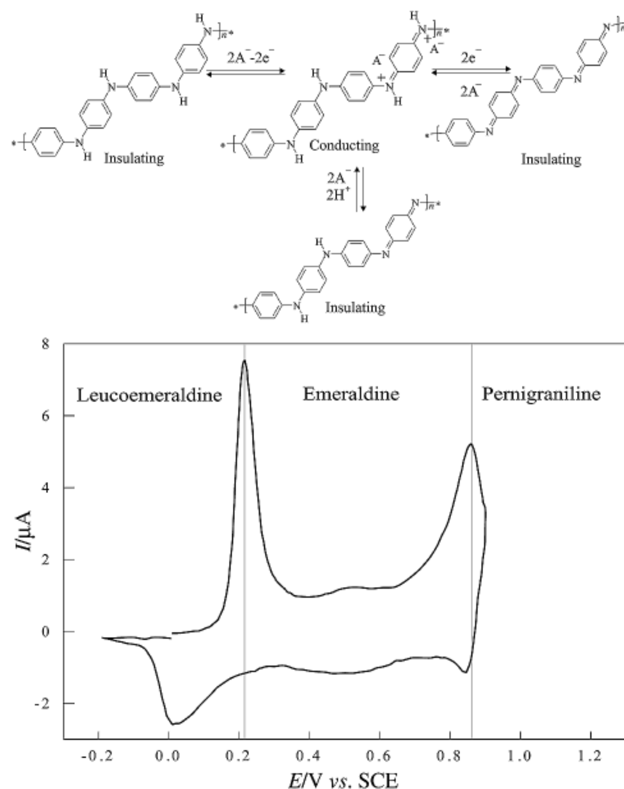


Fig. 6 Cyclic voltammogram for a poly(aniline) film recorded at 20 mV s^{-1} in 2 mol dm^{-3} H_2SO_4. The film was grown on a 0.5 mm diameter platinum electrode at 0.9 V *vs*. SCE from a solution of 0.4 mol dm^{-3} aniline in 2 mol dm^{-3} H_2SO_4. The three different forms of the material are indicated. Of the three only the protonated emeraldine form is electronically conducting. The diagram also shows schematic representations of the structures of the different forms.

acidic solutions (such as 1 M H_2SO_4). Poly(aniline) films can be cycled between the three redox states electrochemically in acidic solutions (Fig. 6) but at low pH the pernigraniline form is unstable and undergoes hydrolysis leading to degradation of the film.

In order to use poly(aniline) films with flavoproteins it is essential to work in neutral, or at best weakly acidic, solutions to avoid the destruction of the enzyme. The workable pH range for poly(aniline) films grown from sulfuric acid solution is determined by the pK_a of the emeraldine form of the polymer. This is around 5.5, so that these films can be operated in buffered solutions at pH 5. Microelectrochemical enzyme transistors responsive to glucose were fabricated by electrochemically depositing a thin film of poly(aniline) from sulfuric acid solution across a 20–25 μm gap between two screen printed carbon microbands.[30,31] Glucose oxidase was then immobilised on to the surface of the poly(aniline) in a second step carried out in buffered solution at pH 5. The glucose oxidase was immobilised on the poly(aniline) surface in an electropolymerised film of 1,2-diaminobenzene. 1,2-Diaminobenzene forms a thin insulating film under these conditions which is permselective and can be used to give selectivity against interferents in solution.[32] Thus the film serves the dual purpose of immobilising the enzyme onto the surface of the poly(aniline) and providing some selectivity against interference from solution species. The fact that the film is deposited electrochemically also means that it coats the surface of the device evenly. The construction of the device is shown in Fig. 7. To

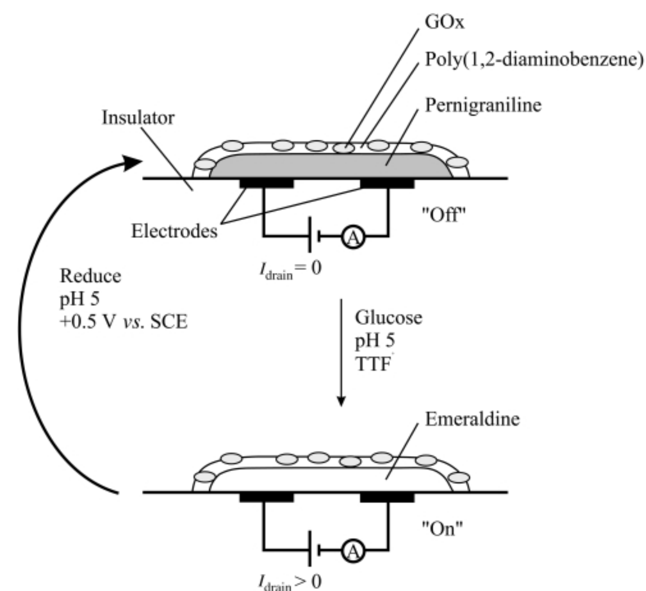
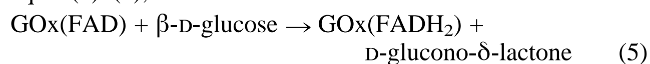
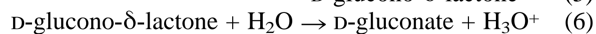


Fig. 7 The construction and operation of a microelectrochemical enzyme transistor responsive to glucose. The device is based on a poly(aniline) film deposited across the gap between two electrodes. On top of the film the enzyme glucose oxidase (GOx) is immobilised in a electropolymerised film of poly(1,2-diaminobenzene). The device is switched from 'off' to 'on' on exposure to glucose. The device can be reset by electrochemical oxidation of the poly(aniline) film at +0.5 V *vs*. SCE at pH 5.0 to the insulating pernigraniline state.

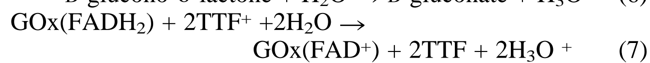
couple the enzyme catalysed oxidation of glucose to the reduction of the poly(aniline) film tetrathiafulvalene (TTF) was used as a mediator. The overall reaction scheme is then given by eqns. (5)–(8),



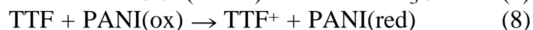
$$\mathrm{GOx(FAD)} + \beta\text{-D-glucose} \rightarrow \mathrm{GOx(FADH_2)} + \text{D-glucono-}\delta\text{-lactone} \quad (5)$$



$$\text{D-glucono-}\delta\text{-lactone} + H_2O \rightarrow \text{D-gluconate} + H_3O^+ \quad (6)$$



$$\mathrm{GOx(FADH_2)} + 2\mathrm{TTF}^+ + 2H_2O \rightarrow \mathrm{GOx(FAD^+)} + 2\mathrm{TTF} + 2H_3O^+ \quad (7)$$



$$\mathrm{TTF} + \mathrm{PANI(ox)} \rightarrow \mathrm{TTF}^+ + \mathrm{PANI(red)} \quad (8)$$

where PANI(ox) represents the fully oxidised, insulating, pernigraniline form of poly(aniline) and PANI(red) the partially oxidised, conducting, emeraldine form. Thus the enzyme catalysed reaction turns the device from 'off' to 'on' (Fig. 7). Following exposure to glucose the device can be reset by electrochemical oxidation of the poly(aniline) to the pernigraniline form at 0.5 V *vs*. SCE.

For a device of this type the rate at which the drain current increases upon exposure to the analyte and the time taken for it to switch between states depend on the concentration of analyte. Fig. 8 shows a set of response curves for a single device exposed

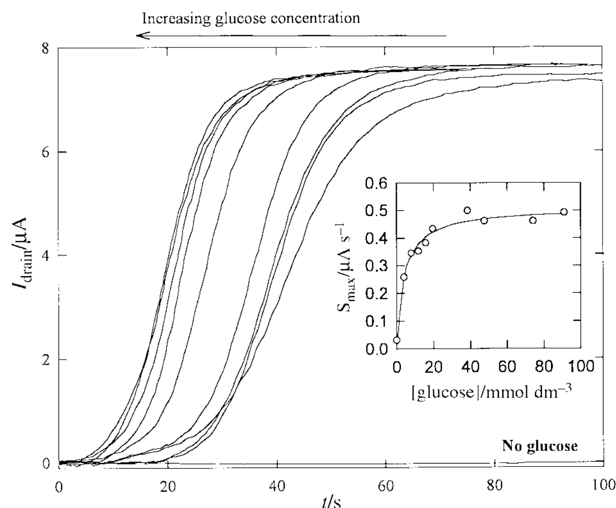


Fig. 8 A set of response curves for a glucose oxidase based microelectrochemical enzyme transistor. The structure of the transistor is shown in Fig. 7. Between each measurement the device was reset electrochemically to its insulating state at 0.5 V vs. SCE. Measurements were made with a drain voltage, E_{drain}, of 20 mV in pH 5 phosphate/citrate buffer containing 0.5 mol dm^{-2} Na_2SO_4 and 0.5 vol % Triton X100 saturated with tetrathiafulvalene. In each case aliquots of sample solution were added at time zero. In the absence of glucose the drain current, I_{drain}, flowing through the device does not change. When an aliquot of glucose is added the drain current increases with time following the addition until it eventually reaches a plateau when the device is in the 'on' state. The time taken for the device to switch on and the maximum switching rate, S_{max} (defined as the maximum slope of the plot of drain current against time), depends on the concentration of glucose. The inset shows the calibration curve for this device. (Reprinted from Bartlett and Birkin.[30] Copyright 1993 American Chemical Society.)

to different concentrations of glucose. First it is clear that the device can be reused at least nine times with no apparent loss in function, in fact many more than nine consecutive operations are possible for this device under these conditions. It is also clear that the response of the device depends on the concentration of glucose added to the solution. Without added glucose there is no change in the drain current over the course of the experiment. When glucose is added there is initially no change in the drain current flowing through the poly(aniline) film but then, as the enzyme catalysed reaction begins to reduce the polymer it is converted to its conducting, emeraldine state and the drain current increases, eventually reaching a plateau when the polymer is highly conducting and the drain current is limited by the base resistance of the carbon microband structure (in this case about 3 kΩ). Note that on switching from 'off' to 'on' the resistance of this device changes by around 3 orders of magnitude. We can show that the device does indeed operate through changes in the oxidation state of the poly(aniline) film by following the potential of the film during switching [Fig. 9(*a*)]. Before addition of glucose the potential of the polymer is around +0.28 V vs. SCE, where the poly(aniline) film is insulating. On addition of glucose the potential immediately begins to shift cathodic as the poly(aniline) is reduced and then the drain current increases as the polymer becomes more conducting. Fig. 9(*b*) shows the variation of the polymer resistance with potential.

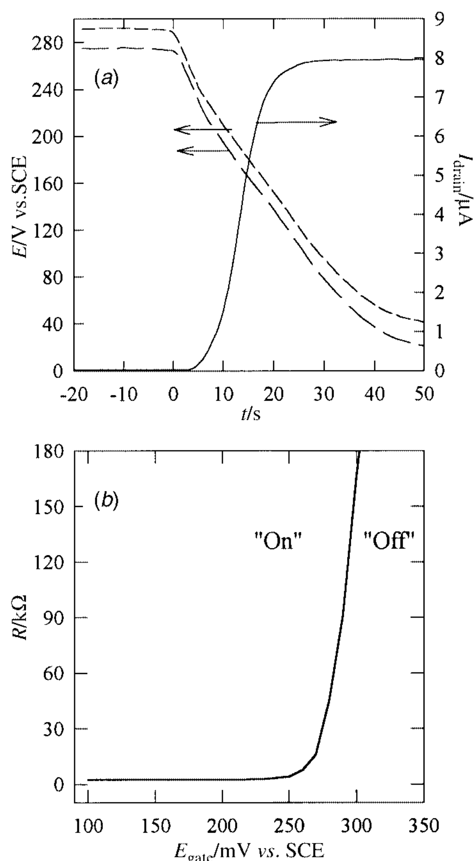


Fig. 9 (*a*) Simultaneous measurement of the drain current, I_{drain}, and the potentials of the two microband electrodes as a function of time after addition of glucose. (*b*) resistance of a poly(aniline) film as a function of the redox potential at pH 5. (Reprinted from Bartlett and Birkin.[31] Copyright 1994 American Chemical Society.)

Studies of the effect of varying the amount of polymer deposited onto the device and the enzyme loading allow us to optimise the speed of response of these devices to glucose and support our model of the operation of these devices.[31] By optimising the amount of poly(aniline) deposited across the 20 μm gap a switching time of less than 10 s can be achieved. This is significantly faster than the best switching times reported by Matsue *et al.* for their NADH responsive device.[24] The selectivity of this type of device for glucose over other sugars is largely determined by the selectivity of the enzyme itself, with D-(+)-glucose giving much faster switching rates than D-(+)-mannose or D-(+)-galactose (Table 1).

Table 1 Comparison of the relative homogeneous rates, amperometric response, and switching rate recorded for a single device in the presence of 0.15 mol dm^{-3} substrate and 1.4 mmol dm^{-3} TTF^{+} (from ref. 31)

Substrate	Relative homogeneous rate[a]	Amperometric response/μA	Switching rate/s^{-1}
D-(+)-Glucose	100	63	0.209
2-Deoxy-D-glucose	25	42.5	0.218
D-(+)-Mannose	0.98	8.8	0.081
D-(+)-Galactose	0.14	3.3	0.034

[a] Ref. 53.

Advantages of microelectrochemical enzyme transistors

Microelectrochemical enzyme transistors have a number of potential advantages as biosensor devices which could be beneficial in some applications. First, the devices do not require a potentiostat or a reference electrode to operate. Measurement of the drain current can be achieved with very simple instrumentation and, at least for devices operating in the 'off' to 'on' direction (see below), the instrumentation required to re-set the device after each measurement can be very simple. Second, the devices can be made very small without loss of sensitivity. As the gap between the electrodes is decreased and the polymer film is made thinner the speed of response will increase and the magnitude of the drain current will increase. Third, these devices can be thought of as counting the number of molecules which have reacted with the enzyme. Thus it will take a certain number of glucose molecules to switch the device from its 'off' state to its 'on' state. This number of molecules depends on the amount of poly(aniline) in the device—for a device 50 μm by 50 μm we estimate that 60 fmol of glucose is required to reach the halfway point where I_{drain} is one half its maximum value. As the size of the device decreases the number of moles of glucose will also decrease. Fourth, the device integrates the analytical signal. Thus if the device is removed from the solution containing glucose the drain current does not fall back to zero but stays at the value reached. If the device is returned to the glucose solution the drain current increases once more. This is because the enzyme catalysed reaction causes the reduction of the polymer so that each molecule of glucose which reacts injects one charge carrier into the polymer and these charge carriers accumulate there leading to the increase in conductivity. This is in direct contrast of the operation of superficially similar devices also based on conducting polymer films and enzymes which operate by sensing a local change in pH caused by the enzyme catalysed reaction.[33–35] In these devices the response returns to the baseline value when the analyte is removed and in their operation they resemble potentiometric enzyme electrodes and suffer from the same problems as these devices in terms of sensitivity and effect of solution buffer concentration.[1]

We have made use of this ability of the microelectrochemical enzyme transistor to integrate the analyte signal in the measurement of low glucose concentrations using a device of the type described above.[36] In this work we were able to show that by the choice of suitable deposition and fabrication conditions we can make microelectrochemical enzyme transistors responsive to glucose with good reproducibility and that they can be used with good repeatability to make measurements of glucose concentration down to 2 μM in air-saturated buffer at pH 5. This was 40 times better than the performance of the corresponding amperometric device.

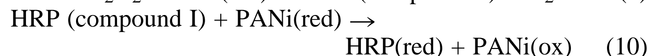
Despite the success in fabricating working devices responsive to glucose some problems and disadvantages remain. First it is important to realise that devices of this type will suffer from similar problems of interference, enzyme kinetics and selectivity as amperometric enzyme electrodes. Indeed if a device works as a microelectrochemical enzyme transistor then it will generally also function as an amperometric sensor simply by holding it at the appropriate potential and measuring the current. The advantages of the microelectrochemical enzyme transistor over the amperometric enzyme electrode lie in its sensitivity and simplicity of measurement. Second, for the devices described above freely diffusing mediator species have been necessary to couple the enzyme reaction to the reduction or oxidation of the polymer. Third, the use of poly(aniline) with bisulfate counterion is restricted to pH 5 and below and this is not compatible with all enzymes or enzyme assays. In the next section we address some of these challenges.

Further developments

Not all enzymes require the use of redox mediators to achieve oxidation or reduction at electrode surfaces. For glucose oxidase the flavin redox active site is buried deep within the core of the protein, making direct oxidation at an electrode very difficult, but this is not the case for all enzymes. For horseradish

peroxidase (E.C. 1.11.1.7) the haem redox site is located at the periphery of the enzyme and is solvent accessible. In this case direct reduction of the haem at electrode surfaces is possible.[37–39] Horseradish peroxidase can also be directly reduced at poly(aniline) coated electrodes[40] and so it is possible to make microelectrochemical enzyme transistors responsive to hydrogen peroxide using horseradish peroxidase as the catalyst.[41] In this case the reactions are those in eqns. (9) and (10).

$$H_2O_2 + HRP(red) \rightarrow HRP(compound\ I) + H_2O \quad (9)$$



$$HRP\ (compound\ I) + PANi(red) \rightarrow HRP(red) + PANi(ox) \quad (10)$$

In this case the poly(aniline) is converted from its reduced, conducting, emeraldine form to its oxidised, insulating, pernigraniline form so that the device operates in the 'on' to 'off' direction [see Fig. 9(*b*)], rather that the other way around, as was the case for the glucose responsive device. This has an important consequence. Because the device starts in the conducting state the time taken to switch the device in any given concentration of analyte is much more sensitive to the choice of setting potential for the poly(aniline) film (the initial value of E_{gate}). This is because the more cathodic the initial gate potential the greater the amount of charge that must be removed from the polymer before its conductivity will change significantly, switching the device 'off'. In contrast, for the glucose responsive device the poly(aniline) starts in the insulating state where the capacitance of the polymer is orders of magnitude lower. As a result small differences in the initial gate voltage make no difference in the switching time for the device and for the same reason the switching times are significantly faster.[41] The clear conclusion from this comparison is that, if possible, it is better to configure devices so that they operate in the 'off' to 'on' direction than *vice versa*.

The work described above for both glucose and hydrogen peroxide responsive devices was all carried out using poly(aniline) at pH 5. It is obviously desirable to remove this constraint and to be able to work at neutral pH. For poly(aniline) the problem is that the emeraldine form of the polymer deprotonates above about pH 5 (see Fig. 6 above) and that the deprotonated form is not an electronic conductor. For a poly(aniline) film the deprotonation of the emeraldine form is associated with the egress from the film of both the protons and the associated counter anions, [Fig. 10(*a*)]. This is only possible if the counter anions are small and mobile, for example chloride or bisulfate anions. If these mobile counter anions are replaced by long chain polymeric counter anions these become trapped within the poly(aniline) film and the overall process changes [Fig. 10(*b*)]. Now if the protons leave the film they must be replaced by cations from the solution in order to maintain electroneutrality. As a consequence of this change the conductivity of the poly(aniline) can be maintained to much higher pH[42] [in effect there is a Donnan type potential established across the poly(aniline) solution interface which alters the electrochemical potential of the protons within the film]. We have made use of this effect to deposit films of poly(aniline) with polymer counterions such as poly(vinylsulfonate) or poly(styrenesulfonate) which remain electrochemically active and conducting at neutral pH. These composite films can be used to make microelectrochemical enzyme transistors responsive to glucose[43] and to make devices responsive to NADH operating at pH 7.[44] In the latter case no enzyme is required because poly(aniline) is an excellent catalytic surface for the oxidation of NADH to NAD^+.[45]

Thus for glucose we have shown that we can make microelectrochemical enzyme transistors which respond rapidly and reproducibly,[25,30,31] that such devices show sensitivity to low glucose concentrations,[36] and that we can make them operate at pH 7.[43] However in all cases it has been necessary to use a freely diffusing redox mediator to couple the oxidation of the reduced flavin to the reduction of the poly(aniline). There

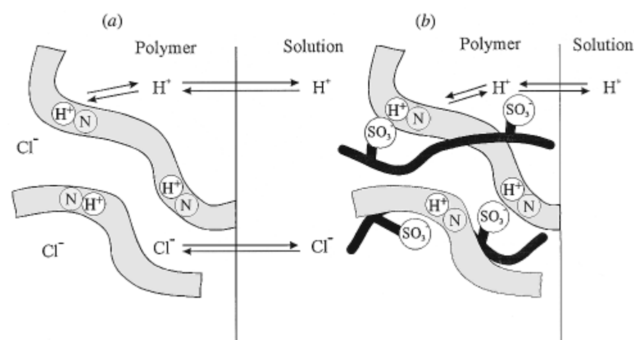


Fig. 10 Protonation equilibria for poly(aniline) films. For poly(aniline) grown with a mobile anion (*a*), such as chloride, the emeraldine state of the polymer can deprotonate on contact with neutral solutions by simultaneous expulsion of protons and chloride ions to leave the insulating emeraldine base. In contrast, for poly(aniline) films grown with long chain polymeric anions (*b*), such as poly(styrenesulfonate), the polymer can only deprotonate if it exchanges protons for cations from the solution. As a result the emeraldine form of the polymer remains protonated even when placed in contact with neural solution.

are two potential strategies to overcome this problem. The first, following the work of Heller on redox hydrogels,[7] is to use a redox polymer deposited on top of the poly(aniline) to establish electrochemical communication between the enzyme and the conducting polymer. Another alternative is to covalently attach the redox mediator to the enzyme.[5,6,46] Both approaches have been demonstrated in amperometric measurements and should work with microelectrochemical enzyme transistors if the redox potentials and rate constants for mediation are suitable.

Conclusions

Microelectrochemical enzyme transistors are good examples of integrated chemical systems as described by Wrighton[47] and Bard.[48] They are constructed by combining together, in a spatially organised manner, a number of distinct chemical components. These components (conducting polymer, insulating polymer, redox enzyme, redox mediator *etc*.) are each individually selected to perform a particular role in the final device and each possess different specific properties. By combining these discrete components in the proper way we are able to make functioning molecular devices. An important aspect of this approach is in the methods used to construct the final devices. These methods need to allow spatial control over the localisation of the different components. In constructing our microelectrochemical enzyme transistors we have used electrochemical polymerisation and adsorption to localise and immobilise the different components onto structures made using screen printing or photolithographic methods.

We believe that the prospects for the exploitation of microelectrochemical enzyme transistors and related devices are good. Their construction and operation is compatible with the current developments of disposable microsensor structures, sensor arrays[49–51] and conducting polymer based electronic circuits.[52] Microelectrochemical enzyme transistors offer significant advantages for the detection of analytes at low concentrations as a result of the inherent integration of the analyte signal and amplification that they provide. These features could be of significant benefit in developing small, disposable devices for immunoassay and DNA assay applications. Finally we note that the output of a microelectrochemical enzyme transistor can be treated digitally, that is it is possible to use such devices to make digital, as opposed to the conventional analogue, chemical sensors. In such devices the concentration of the analyte is encoded as a frequency of switching rather than as an analogue voltage or current. This is an as yet unexplored area. In addition, by combining arrays of microelectrochemical

enzyme transistors responsive to different analytes it is, in principle, possible to make devices which perform simple logical operations, again opening up a new and potentially useful field of study.

Notes and references

1 M. J. Eddowes, D. G. Pedley and B. Webb, *Sens. Actuators*, 1985, **7**, 233.
2 L. C. Clark, *Biosens. Bioelectron.*, 1993, **8**, iv.
3 A. E. G. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. J. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott and A. P. F. Turner, *Anal. Chem.*, 1984, **56**, 667.
4 H. J. Hecht, H. M. Kalisz, J. Hendle, R. D. Schmid and D. Schomburg, *J. Mol. Biol.*, 1993, **229**.
5 P. N. Bartlett, R. G. Whitaker, M. J. Green and J. Frew, *J. Chem. Soc., Chem. Commun.*, 1987, 1603.
6 Y. Degani and A. Heller, *J. Phys. Chem.*, 1987, **91**, 1285.
7 A. Heller, *J. Phys. Chem.*, 1992, **96**, 3579.
8 C. Sun, P.-H. Ho-Si and D. J. Harrison, *Langmuir*, 1991, **7**, 727.
9 A. Szucs, G. D. Hitchens and J. O. M. Bockris, *J. Electrochem. Soc.*, 1989, **136**, 3748.
10 H. S. White, G. P. Kittlesen and M. S. Wrighton, *J. Am. Chem. Soc.*, 1984, **106**, 5375.
11 J. Heinze, *Top. Curr. Chem.*, 1990, **152**, 3.
12 E. J. Calvo, R. Etchenique, P. N. Bartlett, K. Singhal and C. Santamaria, *J. Chem. Soc., Faraday Discuss.*, 1997, **107**, 141.
13 A. R. Hillman, D. C. Loveday, M. J. Swann, S. Bruckenstein and C. P. Wilde, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2047.
14 G. P. Kittlesen, H. S. White and M. S. Wrighton, *J. Am. Chem. Soc.*, 1984, **106**, 7389.
15 E. P. Lofton, J. W. Thackeray and M. S. Wrighton, *J. Phys. Chem.*, 1986, **90**, 6080.
16 E. W. Paul, A. J. Ricco and M. S. Wrighton, *J. Phys. Chem.*, 1985, **89**, 1441.
17 T. E. Turner Jones, O. M. Chyan and M. S. Wrighton, *J. Am. Chem. Soc.*, 1987, **109**, 5526.
18 J. W. Thackeray, H. S. White and M. S. Wrighton, *J. Phys. Chem.*, 1985, **89**, 5133.
19 J. W. Thackeray and M. S. Wrighton, *J. Phys. Chem.*, 1986, **90**, 6674.
20 M. S. Wrighton, J. W. Thackeray, M. J. Natan, D. K. Smith, G. A. Lane and D. Belanger, *Phil. Trans. R. Soc. Lond. B*, 1987, **316**, 13.
21 M. Umãna and J. Waller, *Anal. Chem.*, 1986, **58**, 2979.
22 N. C. Foulds and C. R. Lowe, *J. Chem. Soc., Faraday Trans. 1*, 1986, **82**, 1259.
23 P. N. Bartlett and J. Cooper, *J. Electroanal. Chem.*, 1993, **362**, 1.
24 T. Matsue, M. Nishizawa, T. Sawaguchi and I. Uchida, *J. Chem. Soc., Chem. Commun.*, 1991, 1029.
25 P. N. Bartlett and P. R. Birkin, *Synth. Met.*, 1993, **61**, 15.
26 D. Belanger, J. Nadreau and G. J. Fortier, *J. Electroanal. Chem.*, 1989, **274**, 143.
27 P. N. Bartlett, Z. Ali and V. Eastwick-Field, *J. Chem. Soc., Faraday Trans.*, 1992, **88**, 2677.
28 G. Inzelt and G. Horanyi, *Electrochim. Acta*, 1990, **35**, 27.
29 W. S. Huang, B. D. Humphrey and A. G. MacDiarmid, *J. Chem. Soc., Faraday Trans. 1*, 1986, **82**, 2385.
30 P. N. Bartlett and P. R. Birkin, *Anal. Chem.*, 1993, **65**, 1118.
31 P. N. Bartlett and P. R. Birkin, *Anal. Chem.*, 1994, **66**, 1552.
32 C. Malitesta, F. Palmisano, L. Torsi and P. G. Zambonin, *Anal. Chem.*, 1990, **62**, 2735.
33 M. Nishizawa, T. Matsue and I. Uchida, *Anal. Chem.*, 1992, **64**, 2641.
34 M. Nishizawa, T. Matsue and I. Uchida, *Sens. Actuators, B*, 1993, **13-14**, 53.
35 D. T. Hoa, T. N. Suresh Kumar, N. S. Punekar, R. S. Srinivasa and R. Lal, *Anal. Chem.*, 1992, **64**, 2645.
36 P. N. Bartlett, J. H. Wang and W. James, *Analyst*, 1998, **123**, 387.
37 L. Gorton, G. Jonsson-Peterson, E. Csoregi, K. Johansson, E. Dominguez and G. Marko-Varga, *Analyst*, 1992, **117**, 1235.
38 U. Wollenberger, J. Wang, M. Ozsos, E. Gonzalez-Romero and F. Scheller, *Bioelectrochem. Bioenerg.*, 1991, **26**, 287.
39 G. Jonsson and L. Gorton, *Electroanalysis*, 1989, **1**, 465.
40 P. N. Bartlett, P. R. Birkin, F. Palmisano and G. De Benedetto, *J. Chem. Soc., Faraday Trans.*, 1996, **92**, 3123.
41 P. N. Bartlett, P. R. Birkin, J. H. Wang, F. Palmisano and G. De Benedetto, *Anal. Chem.*, 1998, **70**, 3685.
42 G. E. Asturias, G. W. Jang, A. G. MacDiarmid, Z. Doblhofer and C. Zhong, *Ber. Bunsenges. Phys. Chem.*, 1991, **95**, 1381.
43 P. N. Bartlett and J. H. Wang, *J. Chem. Soc., Faraday Trans.*, 1996, **92**, 4137.
44 P. N. Bartlett, J. H. Wang and E. N. K. Wallace, *Chem. Commun.*, 1996, 359.
45 P. N. Bartlett, P. R. Birkin and E. N. K. Wallace, *J. Chem. Soc., Faraday Trans.*, 1997, **91**, 1951.
46 P. N. Bartlett, S. Booth, D. J. Caruana, J. D. Kilburn and C. Santamaria, *Anal. Chem.*, 1997, **69**, 734.
47 M. S. Wrighton, *Comments Inorg. Chem.*, 1985, **4**, 269.
48 A. J. Bard, *Integrated Chemical Systems. A Chemical Approach to Nanotechnology*, Wiley, New York, 1994.
49 M. B. Madaras, I. C. Popescu, S. Ufer and R. P. Buck, *Anal. Chim. Acta*, 1996, **319**, 335.
50 M. B. Madaras and R. P. Buck, *Anal. Chem.*, 1996, **68**, 3832.
51 G. Nagy, X. X. Clarke and R. P. Buck, *Anal. Chem.*, 1998, **70**, 2156.
52 C. J. Drury, C. M. J. Mutsaers, C. M. Hart, M. Matters and D. M. de Leeuw, *Appl. Phys. Lett.*, 1998, **73**, 108.
53 M. Dixon and E. C. Webb, *Enzymes*, Longman, London, 1979.

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