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The demonstration of an enhanced microelectrochemical transistor for measurements in neutral solution at low analyte concentration

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Abstract

We report a new, sensitive detection method for the measurement of analytes based on the combination of conducting polymer technology and redox enzyme electrochemistry. This method provides a simple measurement method potentially enabling point of care testing on disposable electrodes in under 60 s. The measurement does not require a reference or counter electrode and the analytical signal is recorded by an ammeter measuring milliampere currents through a polymer transistor. Even at very low analyte concentration, the signal is not subject to significant noise.

Keywords: Microelectrochemical transistor; Neutral solution; Analyte

1. Introduction

There is an increasing demand for point of care testing devices. Their portable format combined with simple testing procedure means that monitoring can be achieved rapidly in any situation, for example at an accident scene by a paramedic or on a contaminated site by an environment surveyor. The maintenance of this equipment is very simple because the sample is applied to a disposable unit that makes up part of the device. As a result, the disposable component of the testing device needs to be as low in cost as possible. This may introduce some limitations in the detection limit capabilities of the device. Because of the need for such devices to be light and easy to carry, low power electronics and minimal instrumentation are required to carry out measurements of this sort. Electrochemical methods have advantages for operating with micro-volumes of sample, and require simple, low power electronics. Commercial point of care devices have already proved very useful for monitoring glucose, lactate, or cholesterol.

However, since the point of care glucose test first appeared 20 years ago (MediSense glucose sensor), very few similar commercial devices have followed. One possible reason could be the difficulty of making such portable devices perfectly reliable. There is a need to improve the efficiency of these tests; for instance, the amperometric glucose test is variable below 1 mM glucose concentration. Much lower detection limits are required for immunoassays, or DNA assays, which require nanomolar-range detection limits. Such tests need to function with very small sample volumes (forensic science) and require fast result analysis (stroke detection). For a number of applications, direct amperometric detection on a portable device is either not sensitive enough, or at lower currents greatly affected by noise. These inconveniences have led us to consider the use of microelectrochemical transistors as a means to amplify very small signals, and improve the signal-to-noise ratio in the measurement.

The first microelectrochemical transistor, described by Wrighton and colleagues in 1984 [1,2], comprised a poly(pyrrole) film deposited across the gaps between three independent gold microband electrodes 1.4 μm apart on a oxidised silicon substrate. By analogy with a junction field effect transistor, the three gold electrodes are referred to as the source, gate and drain. 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

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amplifies the signal in the same way that a solid-state transistor can be used to amplify a signal [3–7].

This technology was later adapted to specific enzyme detection of NADH by Matsue et al. [8], and glucose, lactate and peroxide by Bartlett et al. [3,9,10]. These transistors operate as two electrodes connected together by the conducting polymer. The conducting polymer imposes the potential, which varies when the redox reaction takes place. Therefore, the transistor acts as a floating reference. This simplifies the electrode design because no reference electrode is required. In this configuration, a simple ammeter is able to measure the current through the transistor with very little noise. Such simple electronics make the size of the device potentially very small and inexpensive since even the microelectrochemical transistor can form part of the disposable unit.

Microelectrochemical transistors require only a small volume of analyte to operate. They are also potentially more sensitive to low analyte concentrations; Bartlett et al. [11] reported detection limits under 20 μM for a glucose transistor, although the measurement time was very slow (16 min). In these measurements the polymer itself is the collecting electrode and at low concentration a large contact area with the solution to be tested is preferable. A larger volume of polymer requires a larger charge to change its redox state; therefore, a low concentration of analyte takes longer to achieve the redox change of the polymer. Ideally, a small polymer film with a large contact area is needed [9,12].

The glucose-detecting transistor described in our earlier work [3] suffers the major disadvantage of having to operate at pH 5. This is due to a necessary compromise between the demands of the polymer electrochemistry and the enzyme activity. The emeraldine form of poly(aniline) is only conducting in its protonated form and, consequently, it requires an acidic pH to operate the microelectrochemical transistor. On the other hand, most proteins denature at low pH; as a result, pH 5 was chosen in order to retain both the transistor properties of poly(aniline) and the activity of the enzyme. To extend the conductivity of poly(aniline) to higher pH, one approach is to incorporate polyanions within the polymer structure [13]. The polyanion is entrapped within the polymer film and, as a consequence, deprotonation of the poly(aniline) can only occur if cations are incorporated into the film to maintain electroneutrality. Using this approach, the electroactivity of poly(aniline) can be extended to neutral pH.

In a microelectrochemical enzyme transistor, the electrochemistry of the redox mediator used to couple the oxidation or reduction of the enzyme to the redox reaction of the polymer may also restrict the operating conditions. The redox potential of the mediator may vary with pH. This implies that at the operating pH satisfying both the polymer and the enzyme, the redox potential of the mediator has to be in the right potential window to react with the enzyme in one redox state and react with the polymer in another.

In this paper, we report a new approach designed to overcome some of these problems while still retaining the advantages of using a microelectrochemical transistor. In our new approach, we have separated the sensing reaction and polymer switching into separate compartments so as to avoid the restrictions on the choice of solution pH and to combine a micropolymer film with a large surface area collecting electrode. This was done by using an arrangement similar to that described by Morita et al. [14]. In this way, alkaline phosphatase operating in alkaline conditions [15] can be detected by our new microelectrochemical transistor. This leads to a large increase in the sensitivity of the device [16].

The detection of *p*-aminophenol is the basis of many electrochemical immunoassays based upon the use of alkaline phosphatase [17–19]. Here we demonstrate proof of principle for the detection of *p*-aminophenol using glucose oxidase recycling and a poly(aniline) microelectrochemical transistor.

2. Experimental

2.1. Reagents

All aqueous solutions were freshly prepared using water purified by a Whatman RO 50 and a Whatman 'still plus' system. Sulfuric acid (Aldrich 97–99%), HCl (BDH, AnalaR), citric acid (Aldrich, Ultrapure grade), sodium dihydrogen orthophosphate (BDH, AnalaR), disodium hydrogen orthophosphate 12 hydrate (BDH, AnalaR), TRISMA Base (Sigma, 99.9%), PBS-Tween 20, pH 7.4 (Sigma) and KCl (BDH, AnalaR) were used as received. Poly(vinylsulfonic acid) solution was supplied as a sodium salt solution in water (25% estimated molecular weight 980–1100 g; m_n 800–995 g, Aldrich). *p*-Aminophenol (Aldrich, 98%) was recrystallised and stored under argon before use. Aniline (Sigma) was distilled and stored under argon before use. Argon (99% from BOC) was used to sparge solutions to remove dissolved oxygen. Glucose oxidase (E.C. 1.2.4.4, type VII from *Aspergillus niger*, MW 186 000, 100 units mg^{-1} (pH 5), 271.2 mg ml^{-1}) stock solution was a gift; glucose (BDH, AnalaR) solutions were always prepared 24 h before use and stored at 4 °C. KCl (BDH, AnalaR) was used as background electrolyte. Epoxy resin was Araldite CY1300 GB with hardener HY 1300 GB both supplied by Ciba Geigy Plastics used in ratio of 100 parts of resin to 39 ± 1 parts of hardener by volume.

2.2. Apparatus and procedures

Electrochemical experiments were carried out using either a Ministat potentiostat (Thompson Electrochem) or an Oxford Electrodes portable potentiostat (Oxford Electronics, model PP2) used in conjunction with an home made voltage follower, and recorded with an XY/t chart recorder

(Gould, series 60000), a Pharmacia LKB REC 102 Y/t recorder, and Keithley 175A digital voltmeter. When required, a large area platinum gauze was used as the counter electrode. All potentials are reported with respect to saturated calomel (SCE) reference electrode. All pH measurements were carried out using a 145 pH ion selective electrode probe, supplied by Corning Science Products.

2.3. Device fabrication

The base of the transistor is a double gold foil electrode. The double gold foil electrodes are designed to provide two identical conducting areas separated by a small insulating gap. Here the gold electrodes are 300-nm wide and 3-mm long; the gap can either be 6- or 12.5-μm wide between them. This precision is achieved by coating both sides of an insulating Mylar sheet (Goodfellow) with a thin gold film using vacuum evaporation. The gold deposits uniformly on the Mylar and the thickness of the gold deposition can be controlled down to 300 nm; beyond this the homogeneity of the coated layer is uncertain. The thickness of the Mylar determines the size of the insulating gap between the electrodes.

The Mylar film was coated with gold on both sides, cut to the desired dimension, and electric connections were made to the two sides using silver paint (Aldrich). Once dry, the connections were reinforced with epoxy glue. The contacted film was then introduced into a glass tube which was then filled with epoxy resin and cured for 24 h in an oven at 40 °C. Finally, the structure was polished until the edge of the gold-coated Mylar was exposed at the surface.

2.4. Polymer deposition

In this study, we used electrochemically deposited poly (aniline)–poly(vinylsulfonate) composite films to make the microelectrochemical transistors. Before polymer deposition, the dual gold microband electrodes were polished with 1- and 0.3-μm alumina, rinsed with deionised water and checked for short circuits. The polymer was deposited from 2 M H_2SO_4 containing 0.4 M aniline and 22% by weight poly(vinylsulfonate) using a three-electrode system with the two gold microbands connected together as the working electrode and a platinum mesh counter and SCE reference electrode. The polymer films were deposited at 0.9 V with a polymerisation charge of 0.7 mC. Note that below this polymerisation charge, reliable contact was not always made by the polymer between the two gold electrodes.

2.5. p-Aminophenol measurement

Measurements of *p*-aminophenol were carried out in the two-cell arrangement shown in Fig. 1. The salt bridge was a U-shaped 3-mm-diameter glass tube with a glass frit at each end containing a saturated KCl solution. The content of the salt bridge was periodically renewed by removing the stopper in the middle of the salt bridge and refilling. The collecting electrode was a 1-cm^2 basal plane graphite electrode. The top graphite layer was removed before each measurement, providing a freshly cleaved basal plane surface. Cell A is a 10-ml pyrex cell filled with 2 M sulphuric acid and contains the microelectrochemical transistor. Cell B is a 100-μl drop of buffer solution deposited directly onto the collecting electrode.

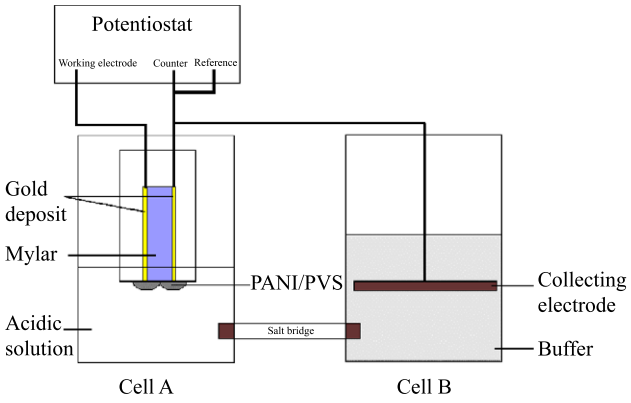

Fig. 1. The two compartment arrangement used in these experiments. Cell A contains acid. Cell B contains the buffer that best suits the bioelectrochemical reaction. The polymer is connected to the collecting electrode and the charge transfer is enabled between the two cells by a salt bridge. The potentiostat is used to apply a potential difference, the drain voltage, between the two electrodes of the polymer transistor and record the resulting drain current.

Before each measurement, the microelectrochemical transistor was successively set at 0 V vs. SCE (for 1 min), 0.35 V (for 1 min), and 0.2 V (for 1 min) in 2 M sulfuric acid (cell A). Meanwhile, 100 μl of 0.1 M pH 9.5 Tris–HCl buffer containing 100 mM glucose, 38 μM glucose oxidase (solution B) was water jacketed at 37 °C in a multiwell plate. Dilutions of *p*-aminophenol are prepared in phosphate/citrate pH 5 buffer. When the transistor was set at 0.2 V vs. SCE, solution B is placed on a 1-cm^2 graphite electrode connected to the polymer transistor and the salt bridge used to makes contact between solution B and the sulfuric acid solution covering the polymer transistor. At this point, a 30-mV drain potential was applied to the transistor and the drain current recorded. *p*-Aminophenol was then added in a 1-μl fraction to solution B and the drain current recorded as a function of time.

3. Results and discussion

Poly(aniline) is conductive in its oxidised, emeraldine, form and insulating in its reduced, leucoemeraldine, form. When poly(aniline) is at 0.31 V vs. SCE in acid solution, a potential difference applied between the two connections of the microelectrochemical transistor will result in a current flowing through the polymer. This drain current is measured by an ammeter. The larger the current flowing through the transistor, the easier it is to measure. For typical devices,

when the poly(aniline) is in the conducting emeraldine form, the drain current is in the milliampere range. A very small reduction charge is required to reduce the small volume of polymer deposited onto the device. This reduction reaction will cause the poly(aniline) to become insulating, and consequently the drain current, measured by the ammeter, decreases significantly. The reduction of the polymer can be brought about either electrochemically or by a chemical reaction with species in solution. If a redox compound in solution reduces the polymer, it causes a change in the conductivity of the poly(aniline) film and hence switches the conductivity of the microelectrochemical transistor. Measuring the current through the transistor as the redox reaction takes place is an indirect way of detecting this redox compound.

At very small concentrations of analyte, the size of the polymer film needs to be proportionally small. If a large microelectrochemical transistor is used, the total reduction charge required to switch it off is large; therefore, a small concentration of reductive material will not achieve the reduction of the polymer transistor in an acceptable time. On the other hand, a very small polymer transistor will have a very small surface area on which the compound can react, in turn, making the detection of the redox compound very slow.

By using a collecting electrode, we can have a micro-transistor and a large area to collect the charge from the redox compound. In this arrangement, the collecting electrode at which the analyte reacts is polarised by the polymer, and reflects the potential of the polymer itself. The only limitation on the size of the collecting electrode is that the polarisation charge of the collecting electrode must be less than the redox charge of the polymer. This allows the use of a large area collecting electrode leading to a significant increase in sensitivity, for example if a 0.1-mm^2 polymer is connected to a 1-cm^2 electrode, there is a 1000-fold increase in the reactive area.

In general, as described above, the emeraldine form of poly(aniline) is only conducting in acidic solution. When working with biological systems, acidic solutions are often inappropriate. The pH required to operate the transistor and the pH required for the enzyme catalysed reaction are very different and often completely incompatible. To overcome this problem, we have the microelectrochemical enzyme transistor and the collecting electrode in separate solutions connected together by a salt bridge, as shown in Fig. 1.

Fig. 2 shows the cyclic voltammogram of a poly(aniline) film in 2 M sulphuric acid. As a poly(aniline) film is cycled in acid, starting from 0 V up to 0.9 V, we observe a first oxidation peak assigned to the oxidation of leucoemeraldine into emeraldine. Near 0.9 V, a second oxidation from emeraldine into pernigraniline is observed. On the reverse sweep, the reduction of pernigraniline produces a sharp reduction peak; as the potential reaches 0 V, a large reduction signal is observed.

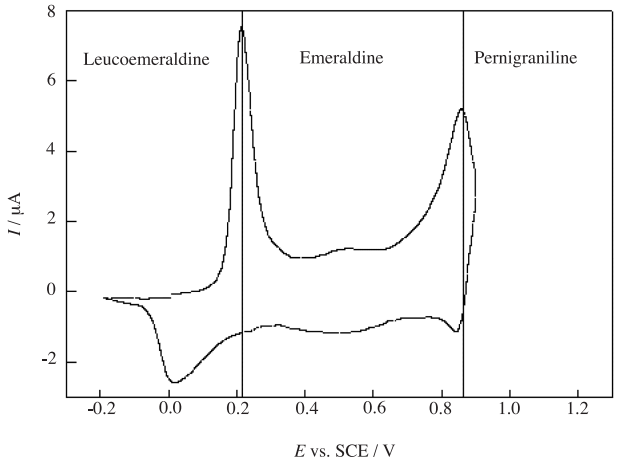

Fig. 2. A cyclic voltammogram for a poly(aniline) film deposited on a 500 μm diameter platinum electrode recorded in 2 M sulphuric acid at 20 mV/s. The polymerisation charge was 1.5 mC.

The different redox states of poly(aniline) are shown in Fig. 3 [20]. Three redox states are represented: leucoemeraldine, emeraldine, and pernigraniline. Two of these are known to be protonated under acidic conditions (the emeraldine and leucoemeraldine forms). The emeraldine salt is the conductive form of poly(aniline). When our microelectrochemical transistor is set at the potential of the emeraldine form, protons from the solution move into the polymer to form the salt. Reduction of the emeraldine salt produces the insulating leucoemeraldine state and this transition is the basis of the microelectrochemical transistor response. Fig. 4 shows the effect of the redox change on the drain current flowing through the transistor. On cycling from emeraldine to leucoemeraldine, the drain current temporarily rises and then drops sharply to zero. On the return sweep, there is significant hysteresis in the drain current which now only increases at around 0.2 V vs. SCE. A similar result has been reported by Ofer et al. [5] and Paul et al. [21] and arises because of kinetically limitations on the redox reactions of the film.

The thin polymer film can support a large drain current, but for successful application in a microelectrochemical transistor the drain current should also change rapidly with changes in the charge, i.e. it should have a high switching efficiency. Fig. 5 shows a plot of the resistance of the polymer film as a function of the total reduction change when the gate potential is decreased from 0.31 V (corresponding to the conductive emeraldine form) to 0 V vs. SCE (corresponding to the nonconductive leucoemeraldine form) at 50 mV/s. The figure shows that the resistance of the film changes rapidly with the reduction charge beyond 20 μC. Experimentally the potential of the polymer film connecting the two electrodes can be adjusted to retain a very low resistance while still being on the threshold of the region where the resistance rises rapidly with reduction charge. This makes the microelectrochemical transistor extremely sensitive with a 5-μC reduction charge (corresponding to 50

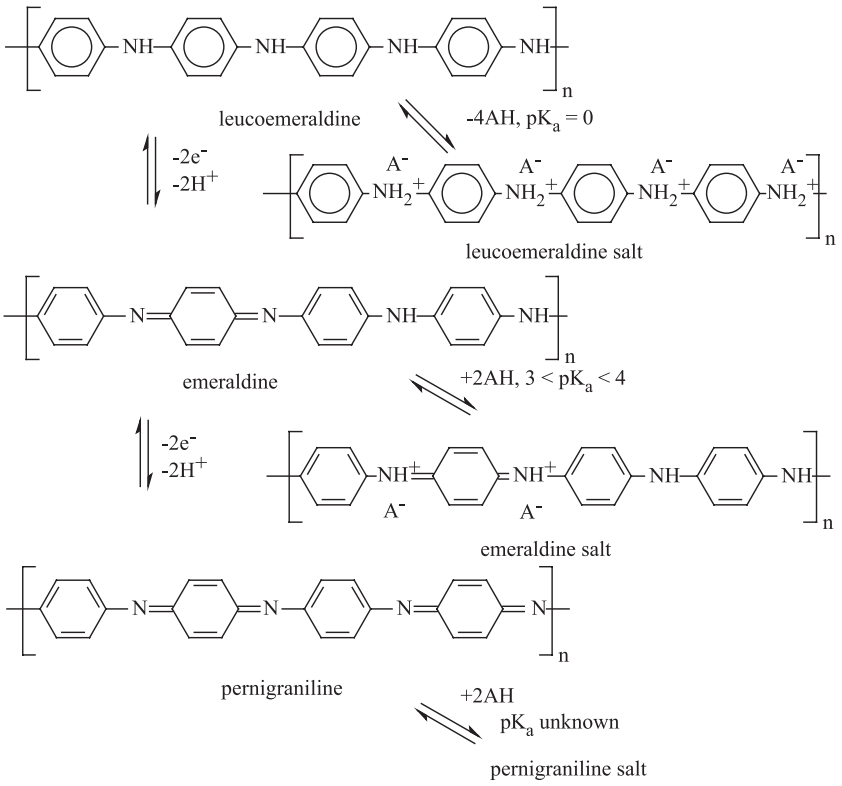

Fig. 3. The different redox and protonation states of poly(aniline) based on data from the literature [20].

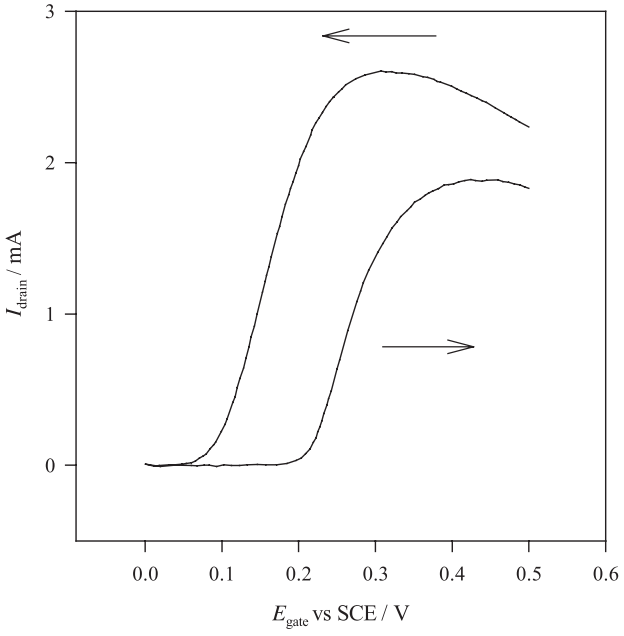

Fig. 4. Plot of the drain current as the gate potential was changed from 0.5 to 0 V vs. SCE and back at 50 mV/s in 2 M H_2SO_4. The drain voltage was maintained at 200 mV. The electrodes are 2-mm long, 300-nm wide and separated by a 12.5-μm insulating Mylar sheet. The film was deposited at 0.9 V vs. SCE in 2 M sulfuric acid containing 0.4 M aniline and 22% by weight poly(vinylsulfonate) with a deposition charge of 0.7 mC.

pmol of a one-electron reductant) leading to a 10^5-Ω increase in resistance.

The reduction charge required to switch the polymer from conductive to insulating depends on the film thickness. The film thickness is determined by the electropolymerisation charge as well as dimensions (width, length and separation) of the two contacting electrodes. The threshold in the reduction charge before the rapid increase in the polymer resistance corresponds to an applied (gate) potential of 0.2 V vs. SCE (data not shown) and is independent of film thickness.

Having demonstrated the sensitivity of the poly(aniline) microelectrochemical transistor in the acid solution, it is now necessary to show that this can be driven by a redox reaction at the collector electrode in the second solution. Fig. 6 shows the change in the potential of poly(aniline) film, measured with respect to a reference electrode in the acidic solution when *p*-aminophenol was added to the collecting electrode compartment. Data for buffer solutions of three different pH in the collecting electrode compartment are shown. In each experiment, the polymer was initially oxidised potentiostatically at between 0.3 and 0.4 V vs. SCE. From the figure we can see that in each case, upon addition of the *p*-aminophenol (marked by the arrow), the potential of the poly(aniline) immediately starts to decrease and then reaches a plateau, although the plateau potential differs for the different pH buffers. The reduction

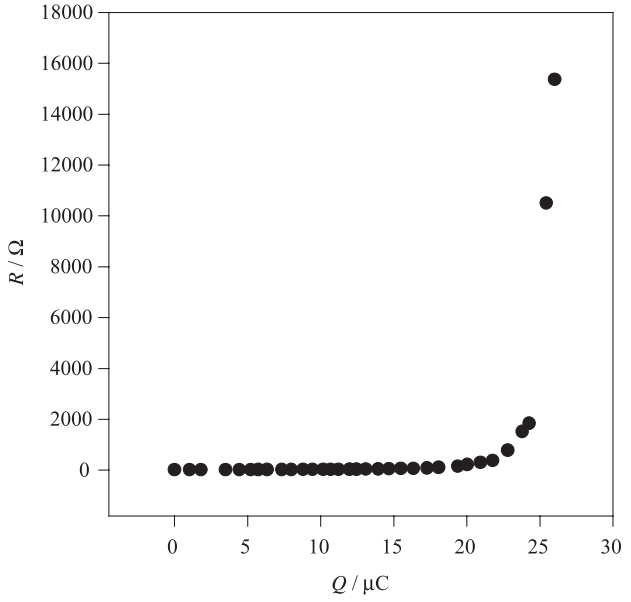

Fig. 5. Plot of the resistance, calculated from the drain current, of a poly(aniline)–poly(vinylsulfonate) film deposited across a 12.5-μm insulating Mylar gap between two electrodes as a function of the total reduction charge passed as the film was reduced starting from 0.31 V vs. SCE. The total charge passed to deposit the polymer film was 0.7 mC.

reaction is initially driven by a large potential difference between the polymer and the mediator. As this potential difference becomes less, the driving force of the reduction decreases. The difference in plateau potential arises because the redox potential of *p*-aminophenol is pH dependent as shown in Fig. 7. Comparing Figs. 6 and 7, we can see that at high pH, when the redox potential of *p*-aminophenol is more cathodic, the plateau potential of the poly(aniline) film is also more cathodic. These data confirm that the device operates as intended, reduction of the *p*-aminophenol on the large collector electrode in the buffer solution leads to reduction of the poly(aniline) film, with the ultimate potential of the poly(aniline) being related to the pH-dependent redox potential of the *p*-aminophenol (note that the two potentials will not necessarily be identical because there may be liquid junction potentials associated with the salt bridge between the two solutions).

Since it was first introduced as an electrochemical mediator by Thompson et al. [22] in 1991, *p*-aminophenol has been extensively used in the field of electrochemical immunoassays. Today most antibodies are commercially available with alkaline phosphatase attached as a labelling agent. By using *p*-aminophenyl phosphate as a substrate for the enzyme, these protein conjugates can be used in electrochemical immunoassays without further modification. Several papers have been published reporting the degree of signal amplification that could be obtained by using *p*-aminophenol phosphate with alkaline phosphatase and recycling the electrode reaction product with another enzyme at alkaline pH in electrochemical immunoassays [15]. Christie et al. [15] showed that *p*-aminophenyl phosphate was very efficient for the detection of alkaline phosphatase in human serum by measuring the rate of increase of *p*-aminophenol concentration in patient samples.

The sensitivity of *p*-aminophenol detection can be significantly increased if a recycling reaction, such as that

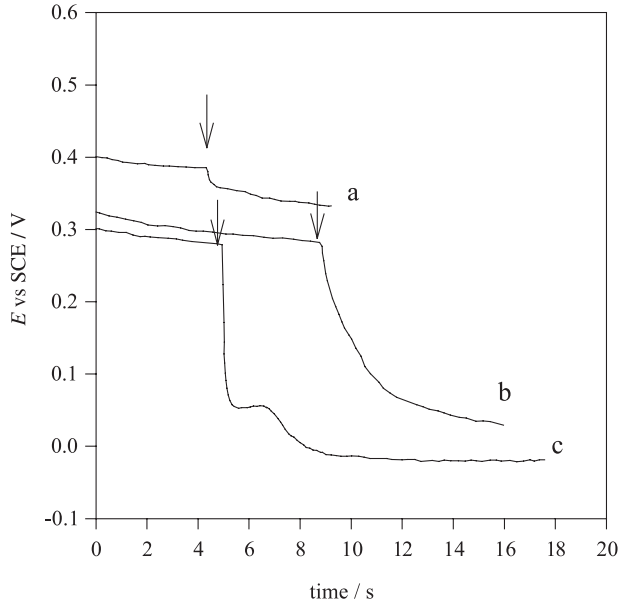

Fig. 6. Plot of the potential of the poly(aniline) film in 2 M sulfuric acid as a function of time following addition of *p*-aminophenol to the buffer solution in cell B. The buffer solution was (a) pH 2 HCl/KCl, (b) pH 7 citrate phosphate, and (c) pH 9.5 Tris–HCl. All buffers were 0.1 M. The arrows indicate the addition of *p*-aminophenol to give a concentration of 10 μM. The collecting electrode area was 1 cm^2 and the polymer deposition charge 0.7 mC.

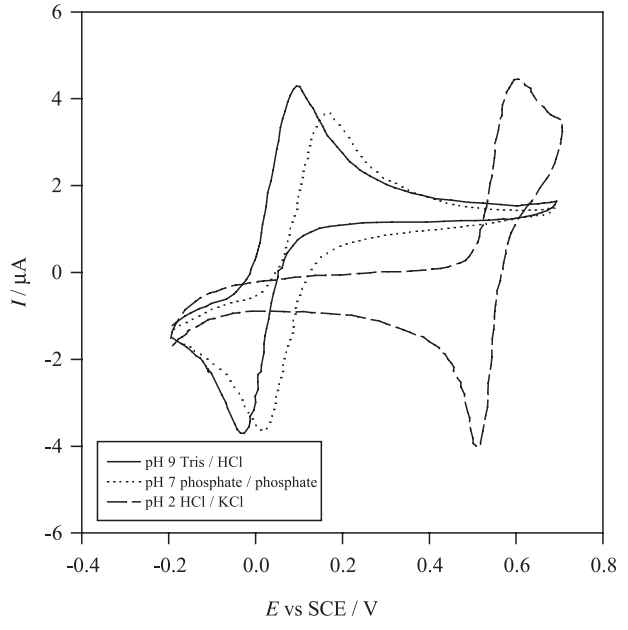

Fig. 7. Cyclic voltammetry of 10 μM *p*-aminophenol in pH 2 HCl/KCl, pH 7 phosphate/phosphate and pH 9 Tris/HCl buffer at 50 mV/s recorded on a 3-mm-diameter glassy carbon electrode.

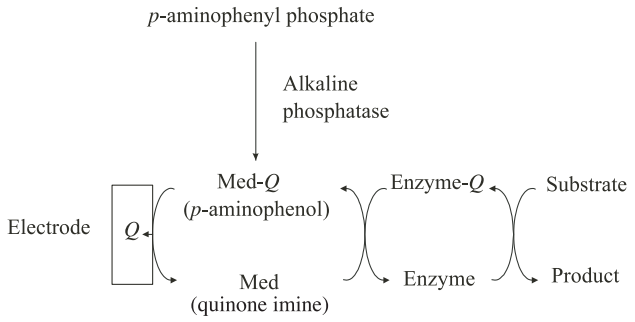

Fig. 8. Scheme for the alkaline phosphatase catalysed hydrolysis of *p*-aminophenyl phosphate to give *p*-aminophenol, which is oxidised at the electrode. The electrode reaction product, quinine imine, is then recycled by glucose oxidase (recycling enzyme) leading to current amplification.

shown in Fig. 8, is employed. This combined system will generate a higher current, which can be used to detect lower concentrations of mediator [16]. Using this type of approach, Thompson et al. [22] have reported zeptomolar detection. Since we can select the pH of the buffer solution in the collection compartment independent of that needed for operation of the poly(aniline) microelectrochemical transistor, we are able to use the enzymatic amplification scheme shown in Fig. 8 with our device.

Fig. 9 shows a set of response curves obtained from the addition of different concentrations of *p*-aminophenol for the buffer solution at pH 9.5 containing glucose and glucose oxidase. Upon addition of *p*-aminophenol, the drain current decreases as before because the poly(aniline) film is reduced and converted from the conducting to insulating state. However, in these experiments the response is 10–100 times more sensitive to *p*-aminophenol concentration than for the experiments shown in Fig. 6 because of the presence of glucose and glucose oxidase in the buffer solution which recycles the oxidised *p*-aminophenol back to the reduced form. The shape of the drain current transients is determined by the reaction on the collecting electrode which, in turn, is being polarised by the polymer. As the reaction proceeds the polymer potential decreases and therefore the driving force for the reduction of *p*-aminophenol decreases. This effect accounts for the curved shape of the drain current transient towards the end of the reaction. For our analytical measurements, we take the slope of the transient at the start of the reaction, where the transient is approximately linear.

Fig. 10 shows a calibration curve constructed by plotting the initial slopes of the drain current transients as a function of the *p*-aminophenol concentration. The results show a linear response over the range 100 to 350 nM *p*-aminophenol. All the slopes are calculated from drain current transients recorded for less than 30 s. It is worth noting at this point that the detection level of the device can be altered by altering the volume of the polymer film and the area of the collecting electrode.

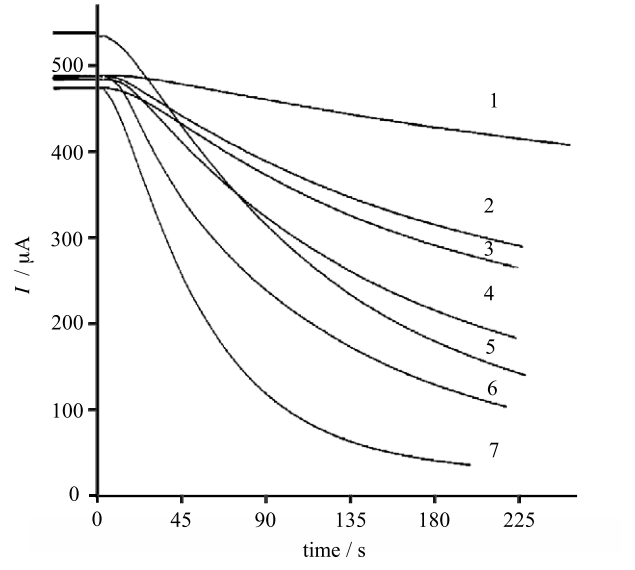

Fig. 9. Drain current transients obtained for different concentrations of *p*-aminophenol: 1, blank; 2, 100 nM; 3, 150 nM; 4, 200 nM; 5, 300 nM; 6, 450 nM; 7 900 nM. The polymerisation charge used to deposit the poly(aniline) on the microelectrochemical transistor was 0.78 mC and the drain voltage was 30 mV. The same microelectrochemical transistor was used for all seven measurements. Between measurements the device was reset by holding the potential of the polymer at 0 V vs. SCE (for 1 min), 0.35 V (for 1 min), and 0.2 V (for 1 min) in 2 M sulfuric acid. The *p*-aminophenol was injected into the buffer (0.1 M Tris–HCl) solution at time zero in each case. The collecting electrode is a 1-cm^2 basal plane graphite electrode with a freshly cleaved surface.

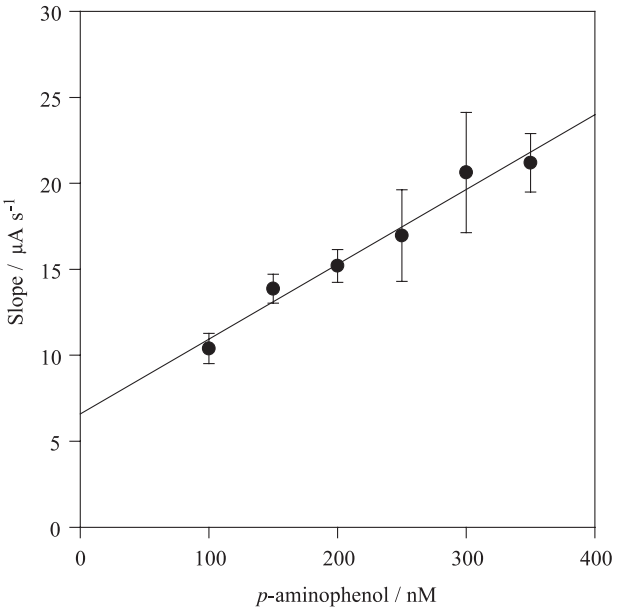

Fig. 10. Calibration curve for *p*-aminophenol using the initial rate of change of the drain current. Each point is the average of four separate measurements made on different days and data for each concentration of *p*-aminophenol were obtained on different days using freshly prepared solutions. The buffer solution contained 100 mM glucose, 38 μM glucose oxidase in 0.1 M Tris–HCl at pH 9.5. The polymer deposition charge was 0.7 mC and the collecting electrode was 1-cm^2 basal plane graphite.

4. Conclusions

We have shown that poly(aniline) microelectrochemical transistors operating in acidic solution can be used to detect low concentrations of a mediator at any pH by making use of a novel two compartment cell. The separation of the microelectrochemical transistor from the biological buffer solution allows us to use a very small microelectrochemical transistor combined with a large collecting electrode. Using this approach, combined with the use of enzyme amplification, we obtain a linear calibration of *p*-aminophenol in pH 9 buffer over a concentration range from 0 to 400 nM with a detection time of under 30 s in an un-optimised system.

Acknowledgements

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