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Sonochemically fabricated microelectrode arrays for biosensors offering widespread applicability

Part I.

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Abstract

A novel and patented procedure is described for the sonochemical fabrication of a new class of microelectrode array based sensor with electrode element populations of up to 2 x 10^5 cm^{-2}. For some years it has been accepted that microelectrode arrays offer an attractive route for lowering minimum limits of detection and imparting stir (convectional mass transport) independence to sensor responses; despite this no commercial biosensors, to date, have employed microelectrode arrays, largely due to the cost of conventional fabrication routes that have not proved commercially viable for disposable devices. Biosensors formed by our sonochemical approach offer unrivalled sensitivity and impart stir independence to sensor responses. This format lends itself for mass fabrication due to the simplicity and inexpensiveness of the approach; in the first instance impedimetric and amperometric sensors are reported for glucose as model systems. Sensors already developed for ethanol, oxalate and a number of pesticide determinations will be reported in subsequent publications.

Introduction

Biosensor technology has developed into an ever expanding and multidisciplinary field since the Clark enzyme electrode was first reported (Clark and Lyons 1962). Biosensors generically offer simplified reagentless analyses for a range of biomedical and industrial applications and for this reason this area has continued to develop into an ever expanding and multidisciplinary field during the last couple of decades. Since more than half of the sensors reported in the literature are based on electrochemical transducers (Meadows 1996) and at the time of writing approximately 85% of the world commercial market for biosensors is for blood glucose monitoring (Newman et al 2002), we will as the first model system of this sonochemically fabricated microelectrode array biosensor, report a glucose oxidase based impedimetric sensor. We have already within our laboratory demonstrated the fabrication of enzymatic and affinity based sensors that lend themselves to interrogation by either (i) amperometric or (ii) impedimetric approaches. Enzymatic sensors have been developed for analytes including ethanol, oxalate and a series of pesticides. Affinity sensors have been developed using antibody/antigen and DNA hybridisation based approaches. These will all be reported in a series of further publications.

Microelectrodes offer advantages over conventional larger working electrodes within biosensors since they experience hemispherical solute diffusional profiles, and it is this phenomenon that can impart stir-independence to sensor responses, whilst also offering lowered limits of detection.

Individual microelectrodes offer very small responses and one approach for overcoming this problem is to use many microelectrodes together in the form of an array to allow a cumulative and so larger response to be measured.

Microelectrode arrays may be fabricated by a number of approaches although techniques such as photolithography or laser ablation have to date proved cost prohibitive for the mass production of disposable sensor strips. We will within this paper describe a novel sonochemical fabrication approach

(Higson 1996) for the production of microelectrodes, that lends itself to the mass production of sensor arrays..

Experimental Section

Glucose oxidase from Asperigillus niger (80% protein, 132,000 units/g solid), was purchased from the Sigma Chemical Company (Poole, Dorset, UK), D-glucose, aniline, potassium hydrogen phthalate, disodium hydrogen orthophosphate 1-hydrate, sodium dihydrogen orthophosphate 12-hydrate, sodium chloride, and diaminobenzene dihydrochloride (all 'AnalaR' grade), were purchased from BDH (Poole, Dorset, UK). All chemicals were used without further purification.

Glucose oxidase was prepared using 250 units/ml, in distilled water for deposition at microelectrode arrays.

Anhydrous α-D-glucose solutions were prepared to a range of concentrations, from 1 to 40mM, in pH 7.4 phosphate buffer.

A small volume, 2.7 ml, PTFE cell was milled for the co-deposition of aniline/enzyme. The cell held the glass slide working and counter electrodes at a fixed distance from each other, whilst the reference electrode was clamped overhead.

A Sycopel PCI- 100 MK3 Potentiostat computer interface was used in conjunction with a 'Ministat Potentiostat', H.B. Thompson and Associates, (Newcastle-upon-Tyne) for all electrochemical studies: current/charge transients were recorded using a PC with dedicated software. AC impedance measurements were performed using a ACM Auto AC DSP frequency response analyser and potentiostat again linked to a PC.

A Philips XL30 FEG SEM was used for all scanning electron microscopy.

Electrode Preparation

Polymer modified insulated electrodes were prepared on gold-coated glass slides as reported earlier (Myler and Higson 1997).

Sonochemical Ablation of Polydiaminobenzene Ultra-Thin Films

The polymer modified electrodes were then immersed in a beaker containing distilled water, and then sonicated for 1s, 5s, 10s, 20s, 30s, 40s, 50s, 60s and 90s using a Camlab Transsonic T460, 25kHz sonic bath. This is set and calibrated by the manufacturer and we have verified this using a crystal microphone and recording the sound waves on a digital storage oscilloscope. The sonic bath employed 12 transducers geometrically arranged and bonded to the base of the stainless steel tank housing.

Polymerisation of microelectrode arrays

For the polymerisation of Glucose oxidase enzyme microelectrode arrays, a pH 4.5 phthalate buffer was first prepared using 0.2M potassium hydrogen phthalate and 0.2M NaCl. A 0.2M aniline solution was then prepared in the phthalate buffer. Glucose oxidase, (500 units ml^{-1}) was prepared in distilled water, to avoid denaturing the enzyme. 2.7 ml of the buffer and enzyme preparation (1.35 ml each) were mixed in a small volume PTFE cell, immediately prior to the electrochemical coating procedure being performed.

Aniline/GOD was polymerised by potentially sequentially cycling for 5 minutes between —200 and +800 mV vs. Ag/AgCl at 50 mVs^{-1}, film growths were terminated at —0.2V. Immediately following polymerisation, the working electrode was submerged in pH 7.4 phosphate buffer to prevent enzyme denaturisation.

Voltammetric Techniques

Hydrodynamic voltammetric techniques were used to examine the stir independent behaviour of the microelectrodes. Polydiaminobenzene coated electrodes, sonicated for 1, 10, 20, 30, 40, 50, 60, and 90 seconds, were placed in the glass stage within the cell along with a gold sputtered counter electrode and

Ag/AgCl reference electrode. The electrodes were submerged within a 5 mM ferricyanide/phosphate buffer solution previously purged for 20 minutes with N_2; the working electrode was then polarised at –300 mV vs. Ag/AgCl. A magnetic stirrer was used to agitate the solution. Current responses were recorded using a PC with dedicated software. We have determined the reproducibility of sensor responses to be <5% variability in a series of trials.

A C Impedance Analysis

A sealed glass cell assembly contained a gold sputtered auxiliary, a Ag/AgCI reference and an enzyme/polyaniline microelectrode array in pH 7.4 phosphate buffer solution previously purged with nitrogen for 20 minutes for the removal of oxygen prior to any electrochemical investigation. Serial additions of glucose were introduced to the cell giving 1 mM, 5 mM, 10 mM., 20 mM, 30 mM and 40 mM concentrations. AC impedance measurements were performed using an ACM Auto AC DSP frequency response analyser between the frequencies of 0.1 Hz and 10 kHz.

Results and Discussion

We have within our laboratory previously shown that 1,2-diaminobenzene dihydrochloride may be electropolymerised at conductive surfaces via a $2e^-$ process to form essentially defect free insulating polymer films of less than 100 nm thickness (Myler and Higson 1997).

The rationale underpinning this work is that sonochemical ablation of thin insulating polymer films at electrode surfaces may expose localised areas, each of which can act as localised microelectrodes and collectively as a microelectrode array.

Many other workers have studied the immobilisation of enzymes (e.g. the oxidases and dehydrogenases) within conducting polymers such as polyaniline or polypyrrole for use within sensors (Foulds and Lowe 1986, Shaolin et al 1992). We will, in this paper, describe a technique that allows the co-deposition of glucose oxidase within the conducting polymer, polyaniline, at conducting microelectrode cavities to form "mushroom" shaped microelectrode protrusions.

The interrogation of enzymatic microelectrode sensors of this type is described by both amperometric or AC impedance approaches to demonstrate their versatility. Scanning electron micrographs of electrode surfaces are included at each stage of the electrode fabrication process.

1,2-diaminobenzene was first electropolymerised on gold sputter coated ground glass slides as previously reported (Myler and Higson 1997) and this is shown schematically in Fig. 1(a). Cyclic voltammetry for the electrodeposition of insulating polydiaminobenzene film, Fig. 1(b), shows that diminishing peak currents are seen as the electrode becomes progressively insulated by the polymer film as it is deposited at the electrode surface (Myler and Higson 1997).

Scanning electron microscopy of a polydiaminobenzene coated gold substrate, Fig. 1(c), confirms that its surface is largely featureless and that the electrode is coated and so insulated by an essentially defect free

polymer film of polydiaminobenzene. Charge integration calculations for the current passed during the electropolymerisation would suggest that the polymer film is ~30-40 nm in thickness, in close agreement with previously calculated and experimentally determined thicknesses (Myler and Higson 1997).

Ultrasound (in the KHz range) passing through a solvent such as water causes thermal agitation and localised hotspots of up to several hundred to a few thousand K, which in turn gives rise to the formation of superheated vapour bubbles (Suslick 1990, Taleyarkan *et al* 2002). These bubbles are, however, cooled by the solvent at ambient temperature (in our studies 25^{o}C) and asymmetrically implode with the ejection of micro-jets of solvent at speeds of up to several hundred ms^{-1}. These micro-jets may cause the shattering of hard brittle solids and this is exploited, for example, within medicine for the shattering of kidney stones. Soft solid surfaces such as polymers may, however, be ablated by such jets (Suslick 1990).

Sonochemically fabricated microelectrodes were prepared via the ablation of polydiaminobenzene films using an ultrasonic frequency of 25 KHz as shown schematically within Fig. 1(d), for a range of differing time durations from 1 through to 90s.

Scanning electron micrographs for a 60s sonicated electrode assembly are shown in Fig. 1(e). These were chosen since these provided the clearest images of the cavitation at the polymer surface. There are several features of interest within these micrographs. Firstly the distribution of the pores is random since ultra-sonic cavitation is a chaotic process. It is also evident that almost all of the cavities are bimodal in size, possessing either ~3μm (+/–1μm) or sub-micron diameters. We believe that the smallest of the cavities observed are formed by the initial impact of the micro-jets of fluid (Suslick 1990). These cavities are known to act as nucleation sites for further bubble formation (Suslick 1990) and it is thought that the cavity grows as new bubbles implode within the confines of the original cavity. This process gives rise to a quantum enlargement in the diameter of a cavity. Since no larger pores are seen it is believed that the 3μm diameter pores no longer act as nucleation sites. In some instances there is evidence of a few pores joining to form dumbbell shaped cavities when two pores form in close proximity to each other, Fig. 1(e).

Fig 1(f) shows a close-up of the pores, showing how the polymer coating has been peeled back from the substrate.

Electrochemical characterisation

Electrochemical evaluation of microelectrode assemblies was performed for possible use within sensors or biosensors. Microelectrodes characteristically exhibit sigmoidal shaped cyclic voltammograms (Southampton 1985) for reversible solution bound redox couples such as $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$, in contrast to the characteristic diffusion limited reversible voltammograms expected at a planar electrode surface.

Fig. 2 shows a series of cyclic voltammograms recorded at a scan rate of 50 mVs^{-1} for a 1 mM solution of $Fe(CN)_6^{3-}$ at (i) planar gold electrodes, the (ii) sonochemically fabricated microelectrode arrays and (iii) polydiaminobenzene coated gold electrodes as a control. The voltammogram corresponding to the plain gold slides reveals an approximate 59 mV peak separation as would be expected for a $1e^-$ diffusional controlled reversible reduction/oxidation process. Cyclic voltammograms for the polymer insulated electrode and the microelectrode array are also shown in the enlarged bubble caption of Fig. 2 for clarity. Very little voltammetric response is observed at the polydiaminobenzene coated electrode, confirming that its has been insulated. The micro-electrode array electrodes yield sigmoidal shaped voltammetric profiles suggesting that all of the microelectrode arrays formed in this way were indeed exhibiting true microelectrode like behaviour. Individual microelectrodes would be expected to see forward (reductive) and reverse (oxidative) current transients that would fall almost on top of each other with little evidence of double-layer charging capacitance, however separate forward and reverse peaks displaying evidence of double layer charging are observed in this case due to the very large pore density (115,000 microelectrodes cm^{-2}) that will give rise to a significant cumulative double layer charging current.

Microelectrode Population Density and Reproducibility of Sensor Arrays.

It is important to understand in this context both how microelectrode population densities grow with increasing sonication time as well as the reproducibility of their formation. Population density may be

estimated via SEM imaging across a number of quantified cross-sectional areas. Fig. 3(a), shows that the calculated population density of the microelectrode arrays enlarges in a near linear manner as the sonication time increases from 1 through to 90s. Coefficient of variance of the charge densities for arrays was found to be <2% (at an electrode state E= -100mV vs Ag/AgCl for the reduction of 1 mM $Fe(CN)_6^{3-}$).

Convectional stir-independence of microelectrode arrays.

The convectional (stir-independent behaviour) of the microelectrode arrays was investigated since this is one of the most significant advantages that arrays could offer for use within sensors.

Amperometric responses of microelectrode arrays formed via a range of differing sonication times polarised at –600 mV vs. Ag/AgCl to 1 mM $Fe(CN)_6^{3-}$, were recorded in both unstirred (quiescent) and stirred solutions. If the microelectrodes were to become too closely packed together, eventually hemispherical diffusion profiles would begin to overlap and ultimately all microelectrode-like behaviour would be lost.

The calculation of the percentage change in amperometric response in a quiescent and a stirred solution for a given electrode allows us to assess when microelectrode behaviour begins to be lost, as microelectrode populations increase. The performance of electrodes prepared via different sonication times, Fig. 3(b) shows the percentage change in amperometric responses for electrodes when stirring is introduced (in excess-via the use of a mechanical stirrer) in comparison to the response for the same electrode in a quiescent (unstirred) solution.

Microelectrode arrays prepared via 20s or less sonication show no change in signal response to the reduction of $Fe(CN)_6^{3-}$, confirming that under these conditions the microelectrode arrays indeed show true stir independence in their response behaviour. Microelectrodes sonicated for 30s, give rise to a ~5% increase in response upon stirring the solution - and this variation increases progressively to a value in excess of 30% for electrode arrays prepared via 90s sonication. It is therefore clear that for electrodes

prepared via 30s or longer sonication, that the microelectrode arrays population density reaches a value where the hemispherical diffusional profiles of the individual microelectrodes begin to overlap and that this effect progressively leads to the loss of stir independent performance.

Electropolymerisation of enzyme containing polymer protrusions in microelectrode arrays.

Aniline solutions containing glucose oxidase were electropolymerised on microelectrode surfaces to form enzyme containing protrusions at each of the microelectrode cavities of the array as shown schematically, Fig. 4(a). Fig. 4(b), shows cyclic voltammograms recorded for the electropolymerisation of aniline from phosphate buffer containing glucose oxidase. The voltammogram is characteristic of an irreversible deposition with successive voltammograms progressively showing smaller peak currents (Foulds and Lowe 1986, Shaolin et al 1992). Scanning electron microscopy, Fig. 4(c), of these arrays following the electrodeposition of polyaniline shows that polymer protrusions are indeed formed via growth of the polymer from the sonochemically fabricated pores. Several interesting points should be noted from the electron micrograph of Fig. 4(c). Firstly it can be seen that all of the microelectrode cavitations appear to be filled with polyaniline, including the very smallest of the ~0.1μm diameter pores. This shows all ablative cavitations of the insulating polymer film lead to exposure of the underlying conductive surface, since aniline could not be electropolymerised without access to the underlying conducting surface.

Sensor Interrogation Approaches.

Enzymic microelectrode arrays of this type may interrogated by both (i) amperometric and (ii) impedimetric approaches. Microelectrode arrays prepared by 20s sonication were used throughout, since these offered the largest response whilst retaining stir-independent properties.

(i). Demonstration of Amperometric Interrogation

Enzyme electrodes were polarised at +650 mV vs. Ag/AgCl for the interrogation of glucose responses as a first demonstration of the enzymic microelectrode array described in this paper. Glucose oxidase catalyses

the production of H_2O_2 in the presence of glucose and oxygen, Eqn. 1. This approach allows for the interrogation of the sensor based on the amperometric monitoring of H_2O_2, Eqn. 2.

$$\textbf{Eqn. 1}.\ \text{glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconolactone} + H_2O_2.$$

$$\textbf{Eqn. 2}.\ H_2O_2 \xrightarrow{+650\ \text{mV vs. Ag/AgCl}} 2H^+ + O_2 + 2e^-$$

The calibration curve of Fig. 5(a), clearly demonstrates that an amperometric enzymatic sensor may be produced (with stir-independent responses) that could be exploited for a variety of applications in which various flow conditions may be encountered.

(i). Demonstration of Impedimetric Interrogation

Impedimetric measurements for enzyme electrode sensors were recorded in the plane of the working microelectrode array→analyte solution→electrode. Since the solutional impedance contribution will affect the final response of any sensor, defining and controlling the inter-electrode spacings of the working microelectrode array and counter electrode would clearly be crucial for all electrochemical investigations. A glass stage was therefore designed, to hold the working microelectrode array a fixed distance of 10 mm from the counter electrode within an analyte solution, with this assembly being designed so as to be held within a commercial Methrohm® glass cell as previously reported (Myler and Higson 1997).

Ac impedance spectra were first recorded for glucose oxidase/polyaniline sonochemically fabricated microelectrode arrays across a range of frequencies from 0.1 through to 10000 Hz upon ±5 mV ac excitation (0 V bias) (Teasdale and Wallace 1993), in order to characterise electrochemical redox behaviour and sensor performance for a range of differing concentrations of glucose within O_2 saturated conditions, Fig. 5(b). This bias potential allows monitoring of the impedance (conductivity) of the polymer without interference from, for example, the oxidation of H_2O_2 or biological interferents such as

ascorbate. It is clear that *ac* Bode impedance spectra show a clear trend towards lowered impedances upon increasing concentration of glucose, with lowered impedances also occurring at higher frequencies.

Control experiments were also performed by recording impedance spectra for enzyme free polyaniline microelectrode arrays (not shown for clarity) and showed minimal impedance changes to differing concentrations of glucose, confirming that the sensor responses observed within Fig. 5(b) were indeed enzymatic in nature.

It is firstly clear that increasing concentrations of glucose give rise to lowered impedances, via the enzymic activity of glucose oxidase.

A plot of total impedance vs. frequency is shown in Fig. 5(b) for a microelectrode exposed to 20 mM glucose with a corresponding Nyquist phase angle plot. For simplicity, only these plots are shown, since similar profiles are seen for all of the glucose concentration ranges studied. Fig. 5(c) shows a straight line profile of the real Z' vs. the imaginary Z" components with a slope close to unity, indicative of a diffusion controlled reversible process, where the phase angle (θ) profiles seen within Fig. 5(b) would appear to be dictated by the capacitance of the polymer film in conjunction with the double layer contribution within the circuit.

Until now, we have only considered how recorded impedance values, or components thereof, vary under differing conditions, as opposed to how percentage changes in impedimetric behaviour vary even though the total impedance measured may be dominated by the background impedance of the circuit. Percentage changes of microelectrode array glucose sensors across a range of glucose concentrations as a function of frequency under aerobic conditions are shown within Fig. 5(d). Percentage impedances are reported relative to the corresponding impedance at 0 mM glucose at a particular frequency, as shown in Fig. 5(d). These percentage changes may be plotted with respect to the analyte concentration in the form of a calibration plot, Fig. 5(e) clearly demonstrating this approach for interrogation.

The most prominent feature of this data is that impedance minima are observed at frequencies between 10 Hz and 100 Hz. Similar responses are seen under anaerobic conditions (not shown for clarity), although the percentage changes in response are not so pronounced. We have already shown that the impedance values are modulated by enzyme catalytic behaviour, although the nature of the impedance changes have not yet been considered.

At this stage the possible mechanisms by which the conductivity of the polyaniline may be modulated should be considered. Polyaniline has three differing forms (Eggins 1996).

The first mechanism by which glucose oxidase may give rise to redox modulated conductivities within the polyaniline involves the catalysed production of H_2O_2 (Eggins 1996). Previous workers (Cooper and Hall 1992) have shown that in aerobic conditions, H_2O_2 would be expected to play a major role in the modulation of the impedimetric behaviour of polyaniline. The responses observed under anaerobic conditions could clearly not be accounted for by such a mechanism, since without a supply of molecular oxygen as an electron acceptor from the enzyme, H_2O_2 could not be produced. Since we have established that the impedimetric responses are due to enzyme catalytic behaviour, it follows that electron donation from the enzyme must be occurring by some other mechanism. Since the sensor responses under aerobic conditions are always greater than those observed under anaerobic conditions, it is therefore probable that the generation of H_2O_2 in aerobic conditions might contribute to this behaviour - even if additional mechanisms are involved in the overall response.

A number of other possible mechanisms to explain anaerobic glucose oxidase responses have also been proposed by other workers. One of the most controversial of these is via a possible direct electron transfer occurring between the active site of enzyme and the polymer (Cooper and Hall 1992). The distance through which electrons would have to traverse would be in excess of 1.3 nm (www-biol.paisley.ac.uk)

and would on first reflection appear to be prohibitive. Other workers have shown, however, that electron tunnelling can occur across distances that had previously been thought impossible via complex pathways of unsaturated and delocalised bonds together even with some saturated bonds and free space (Moser et al 1992, Onuchic et al 1992).

It should not be forgotten that gluconolactone may be produced under anodic conditions so long as a surrogate electron acceptor for the enzyme is provided. Gluconolactone readily hydrolyses to form gluconic acid and this may easily protonate the polymer allowing another route for altering the conductivity of the polymer.

Skinner and Hall (1997) have previously shown that gluconolactone produced under anaerobic conditions may give rise to conductivity changes within the polymer via interaction of gluconolactone with the emeraldine base to form a zwitterion complex that itself may be readily oxidised through to the perigraniline form of the polymer.

While this behaviour would account for anaerobic impedimetric responses for our sensors to glucose, it would be difficult to fully explain why the response profiles we have observed exhibit minima at frequencies of approximately 10 Hz to 100 Hz. We believe that this behaviour could be linked to the hydrogen bonding of water to the imine centre of the polymer. These effects have been extensively studied and while these findings cannot be fully described here due to space limitations, they will form the basis of a subsequent publication.

Applications of Sonochemically Fabricated Sensors.

The focus of this report has been to show that sonochemical ablation of thin-polydiaminobenzene film coatings that insulate planar electrode surfaces can be exploited as a novel approach for the fabrication of microelectrode arrays. It was, at the outset, our intention to exploit these arrays as templates within a range of differing sensors and biosensors.

Enzymatic sensors of this type have so far been developed for ethanol, oxalate and a range of pesticides as well as DNA hybridisation and affinity antibody/antigen based sensors, all of which will be reported in forthcoming publications. These enhanced sensitivities and lower limits of detection have been made possible via the hemispherical diffusional profiles these microelectrode arrays experience.

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Legends to Figures.

Fig. 1. Deposition of polydiaminobenzene to form an insulating film.

(a) Schematic of polymer insulated electrode.

(b) Cyclic voltammogram for polymerisation of 5 mM *o*-phenylenediamine dihydrochloride at a potential scan rate of 50 mV s^{-1}.

(c) Electron micrograph of polydiaminobenzene insulated gold electrode surface.

(d) Schematic of microelectrode array

(e) Electron micrograph of sonochemically fabricated microelectrode array

(f) Close-up of sonochemically fabricated microelectrode array showing pore structure

Fig. 2. Cyclic voltammetry of ferri/ferrocyanide couple at:

(—) bare gold electrode, (---) polymer coated gold electrode and a 20 s sonochemically fabricated microelectrode array (- · - · -). Scan rate 50mV s^{-1}

Fig. 3.

(a) Population densities of sonochemically formed pores

(b) Stir-Dependence / Independence of Microelectrode arrays of these system with differing sonication times.

Fig. 4. Generation of enzyme containing - polyaniline protrusion microelectrode arrays.

(a) Schematic of sonochemically fabricated polyaniline / enzyme microelectrode array.

(b) Cyclic voltammetry (50 mV s^{-1}) for electropolymerisation of polyaniline and co-entrapment of glucose oxidase.

(c) Scanning electron micrograph of sonochemically fabricated polyaniline enzyme microelectrode array.

Fig. 5.

(a) Amperometric glucose calibration curve for polyaniline/glucose oxidase microelectrode array.

(b) Total impedance (solid line) and phase angle (dashed line) of microelectrode arrays immersed in glucose/phosphate buffer solutions.

Concentration of glucose 0 mM (■), 1 mM (♦), 5 mM (▲), 10 mM (*), 20 mM (□), 30 mM (△), 40 mM (X).

(c) Complex plane plot for a microelectrode array in 20 mM glucose.

(d) Percentage change in impedance for microelectrode arrays in various concentrations of glucose vs phosphate buffer.

Concentration of glucose 1 mM (■), 5 mM (♦), 10 mM (▲), 20 mM (□), 30 mM (△), 40 mM (X).

(e) Calibration plot of Impedance change vs glucose concentration for a 20s sonicated GOD microelectrode.

Figure 1

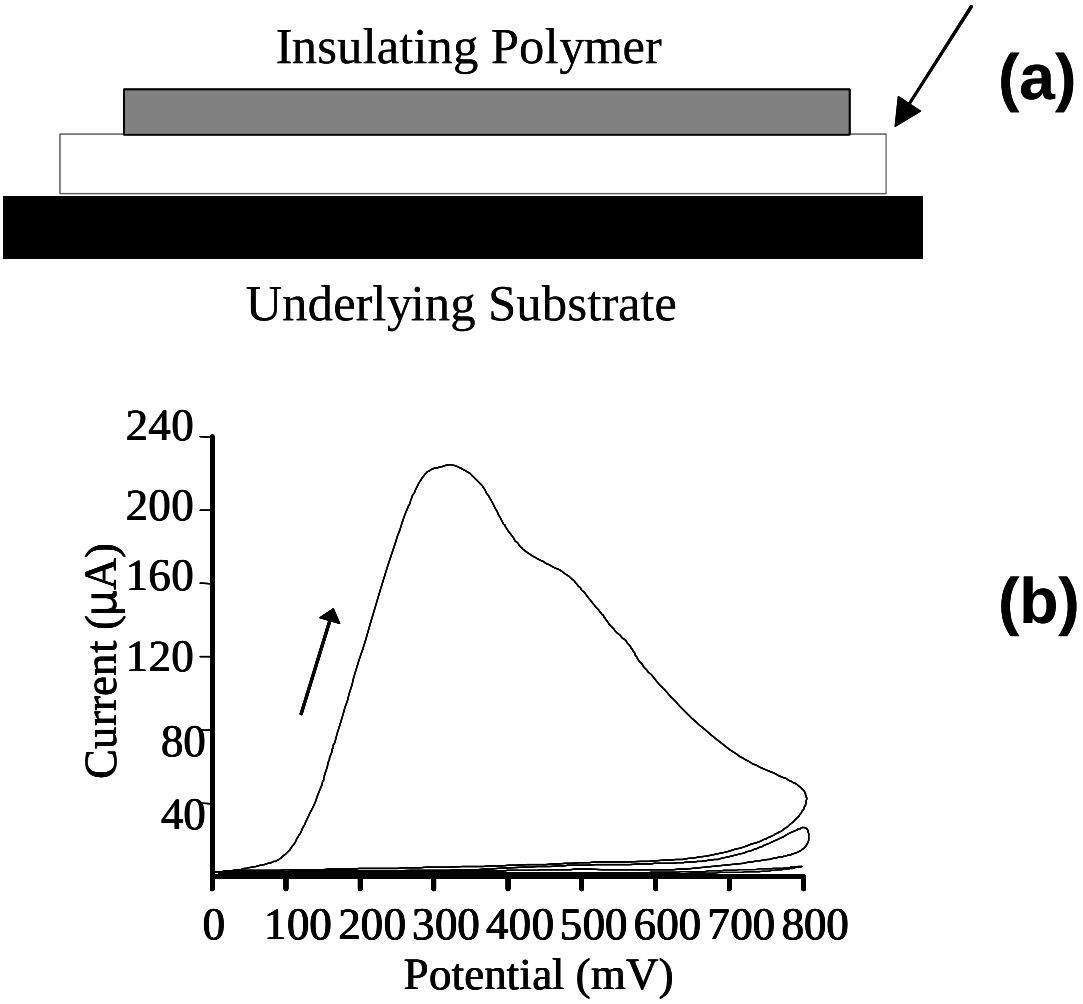

Insulating Polymer
(a)
Underlying Substrate
240
200
160
120
80
40
Current (µA)
0
100
200
300
400
500
600
700
800
Potential (mV)
(b)

Figure 1 cont..

(c)

Figure 1 cont..

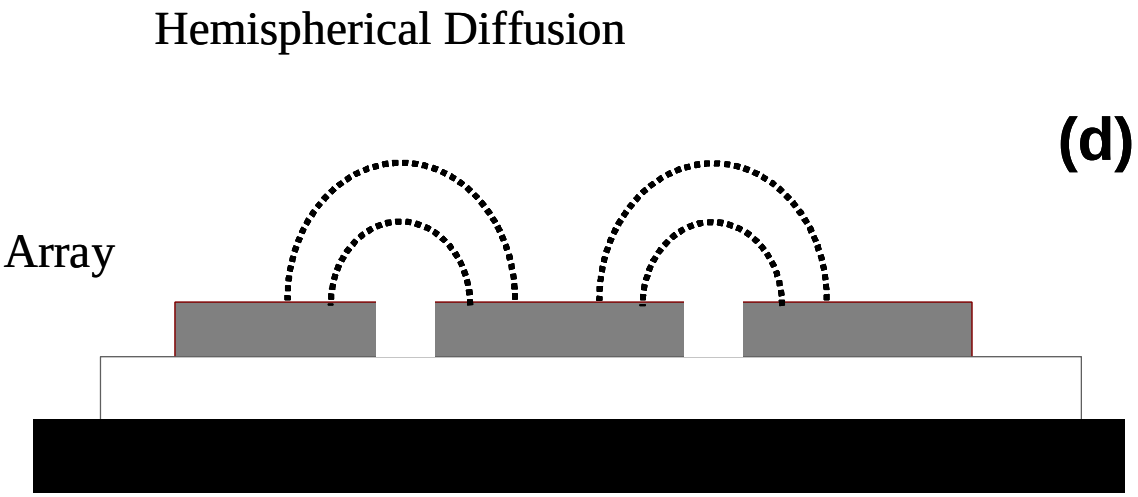
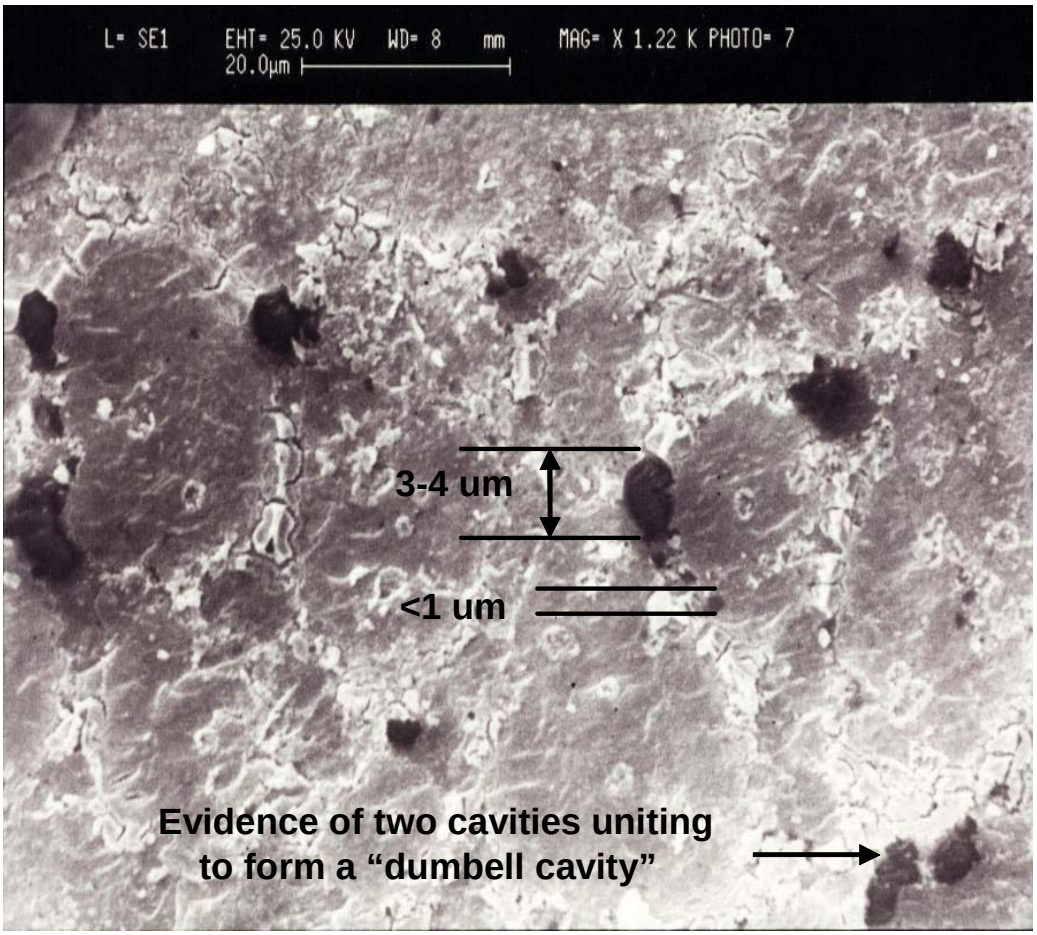
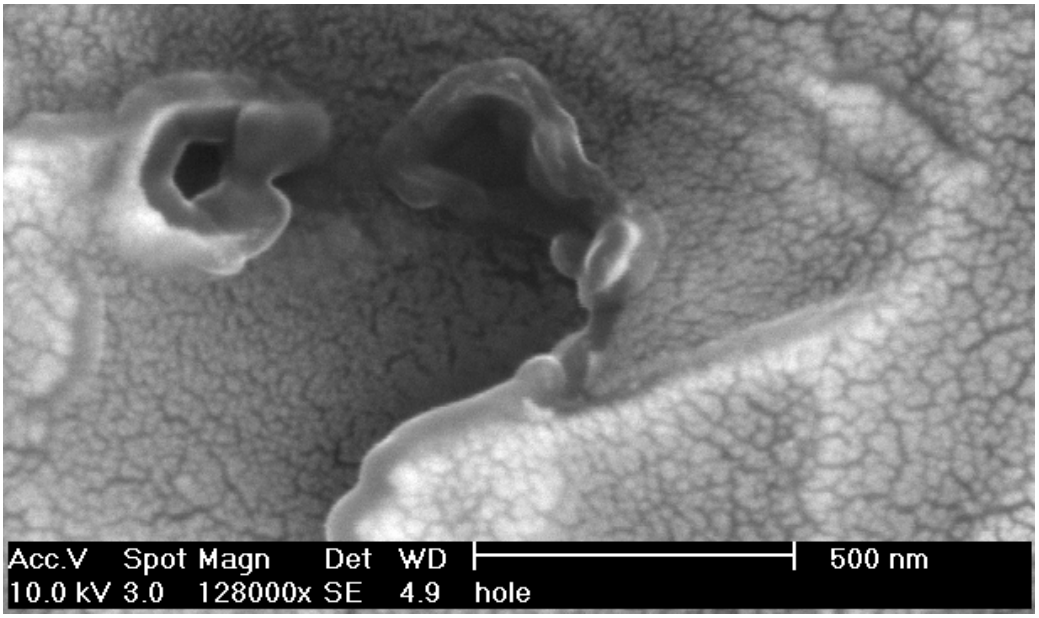


Figure 1 cont..

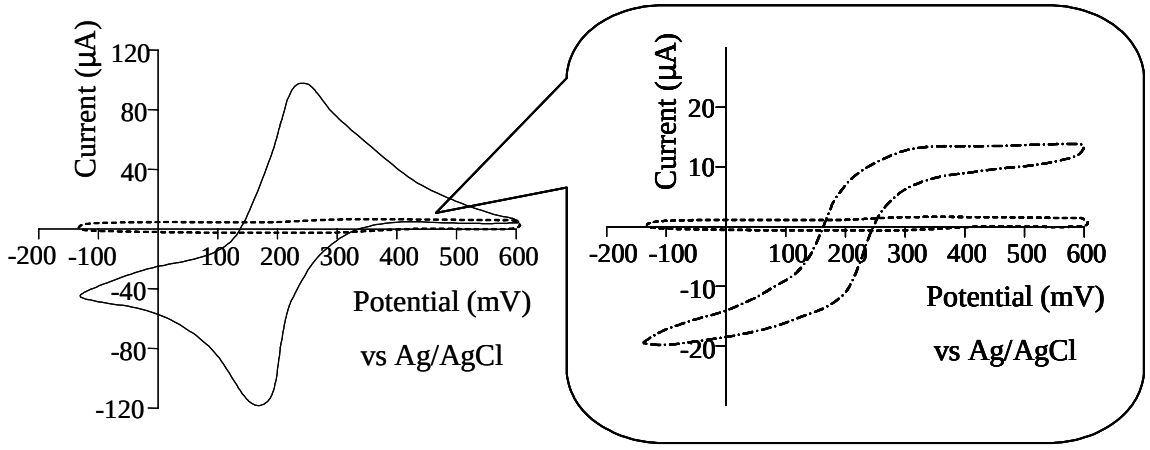


(e)



(f)

Figure 2



—— (i). bare gold

------- (ii). polymer-coated

–·–·–·– (iii). sonicated

Figure 3

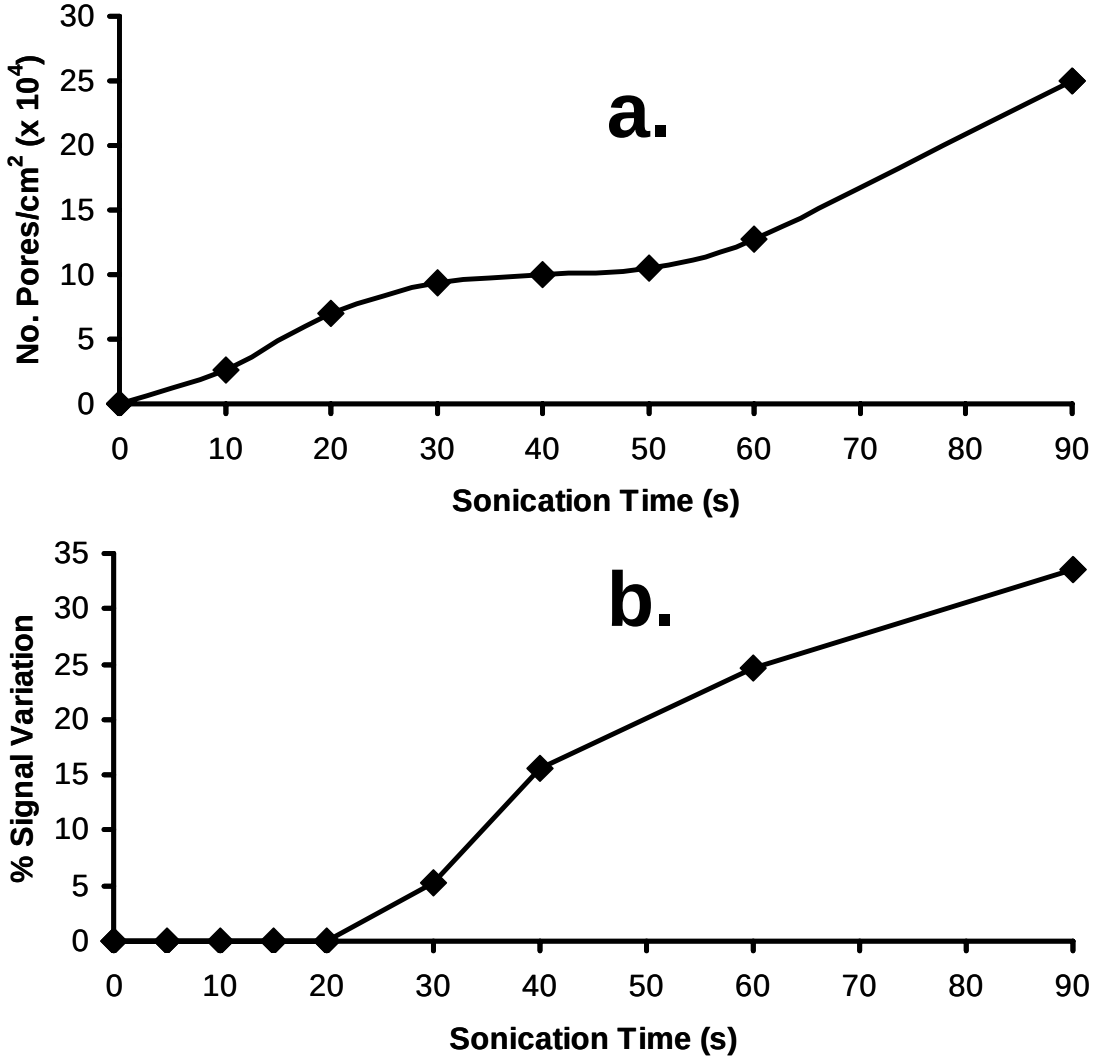

a.
No. Pores/cm^2 (x 10^4)
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
Sonication Time (s)
b.
% Signal Variation
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
Sonication Time (s)

Figure 4

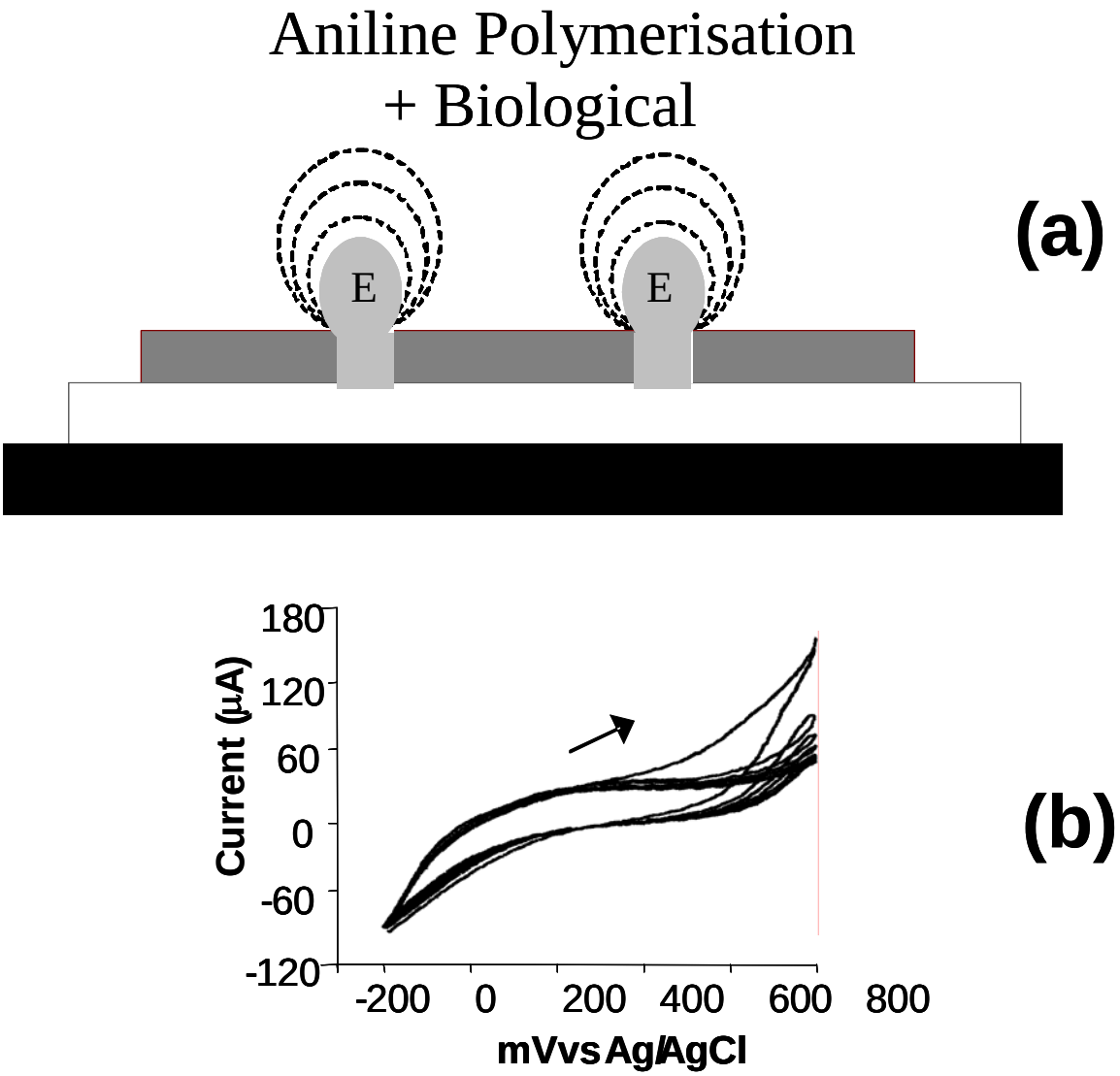
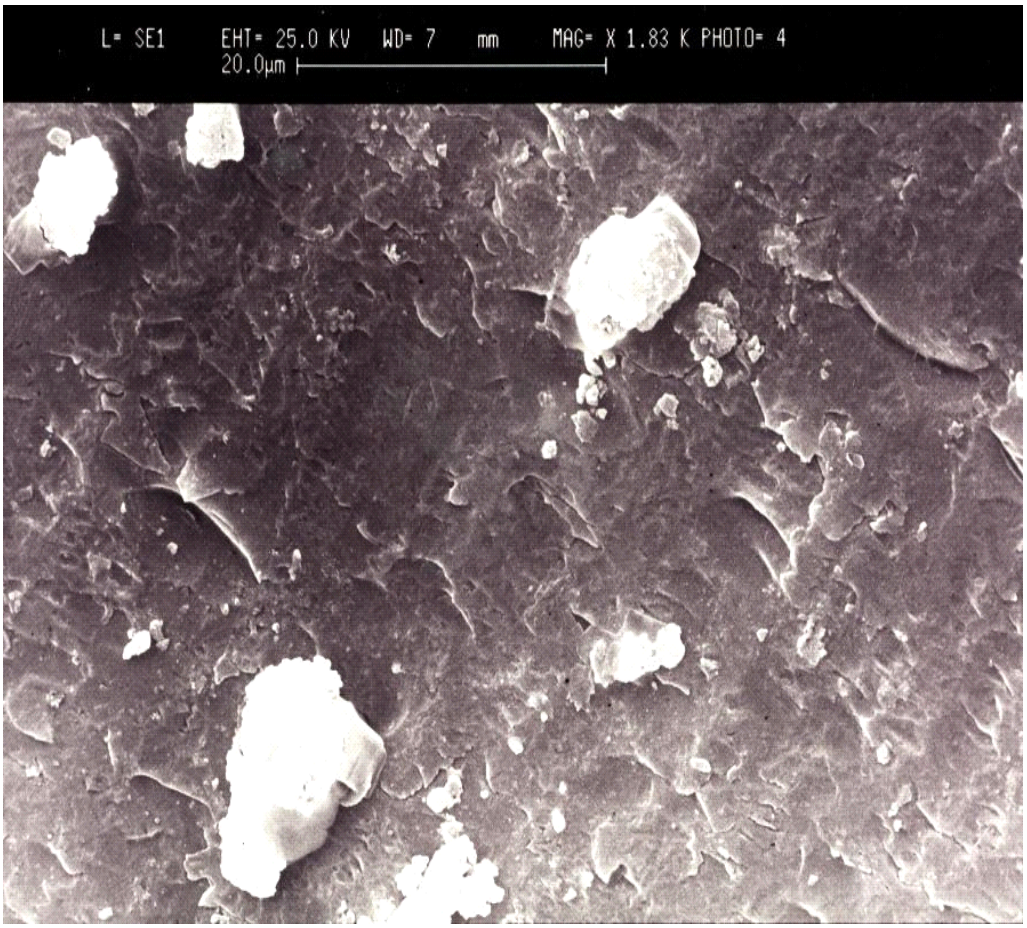
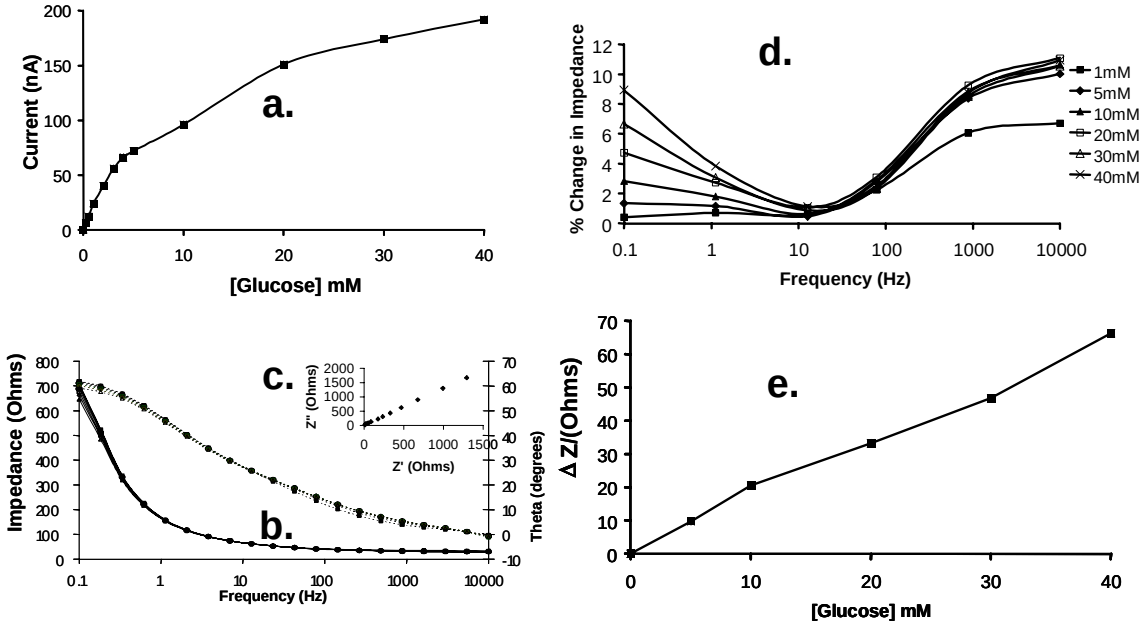

Aniline Polymerisation
+ Biological
E
E
(a)
180
120
60
0
-60
-120
Current (µA)
-200
0
200
400
600
800
mV vs Ag/AgCl
(b)

Figure 4 cont..



(c)

Figure 5


a.
Current (nA)
[Glucose] mM
d.
% Change in Impedance
Frequency (Hz)
1mM
5mM
10mM
20mM
30mM
40mM
c.
b.
Impedance (Ohms)
Theta (degrees)
Frequency (Hz)
Z'' (Ohms)
Z' (Ohms)
e.
Δ Z/(Ohms)
[Glucose] mM