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Fabrication of microband glucose biosensors using a screen-printing water-based carbon ink and their application in serum analysis

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ABSTRACT

Microband glucose biosensors were fabricated by screen-printing a water-based carbon ink formulation containing cobalt phthalocyanine redox mediator and glucose oxidase (GOD) enzyme, then insulating and sectioning through the thick (20 μm) film to expose a 3 mm-long working electrode edge. The performance of these biosensors for glucose analysis was investigated at 25 °C. Voltammetry in glucose-containing buffer solutions established that an operating potential of +0.4 V vs. Ag/AgCl was suitable for analysis under both stirring and quiescent conditions. The influence of pH on biosensor performance was established and an operational pH of 8.0 was selected. Steady-state responses were obtained under quiescent conditions, suggesting a mixed mechanism predominated by radial diffusion, indicative of microelectrode behaviour. Calibration studies obtained with these biosensors showed steady-state currents that were linearly dependent on glucose concentration from the limit of detection (0.27 mM) up to 2.0 mM, with a precision for replicate biosensors of 6.2–10.7%. When applied to the determination of glucose in human serum, the concentration compared favourably to that determined by a spectroscopic method. These results have demonstrated a simple means of fabricating biosensors for glucose measurement and determination in situations where low-current real-time monitoring under quiescent conditions would be desirable.

1. Introduction

Electrochemical enzyme biosensors are commonly fabricated by the screen-printing of carbon inks as transducers in association with inorganic redox mediators, followed by the addition of a redox enzyme as the biological component. Since the screen-printing approach is adaptable to an industrial mass-production scale (Newman and Setford, 2006), it has been particularly suitable for producing low-cost disposable devices and has been employed to manufacture the very successful amperometric test-strips used by diabetics to monitor their blood glucose levels (Mathews et al., 1987). In the majority of glucose biosensors (Hart et al., 2004), screen-printed carbon electrodes (SPCEs) are used in combination with the enzyme glucose oxidase (GOD) (Wilson and Turner, 1992). An inorganic redox mediator such as ferrocene, ferrocyanide, Prussian blue or cobalt phthalocyanine (CoPC) is normally incorporated in order to act as an electrocatalyst, either for direct oxidation of the enzyme, or for the enzymatically produced hydrogen peroxide (H_2O_2). In this way, the operating potential for the biosensor can be reduced to below that at which other components within the sample matrix will be oxidised and produce interfering signals.

The reactions involved in the determination of glucose at carbon electrodes containing GOD, and CoPC as redox mediator (Gilmartin et al., 1995) are shown here:

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconolactone} + H_2O_2 \quad (1)$$

$$½H_2O_2 + Co^{2+} \rightarrow ½O_2 + Co^{+} + H^{+} \quad (2)$$

$$Co^{+} \rightarrow Co^{2+} + e^{-} \quad (3)$$

This sequence represents the enzymatic production of H_2O_2 via the GOD enzyme (Eq. (1)); this H_2O_2 is then oxidised electrocatalytically via its interaction with the central Co^{2+} ion of the CoPC mediator (Eq. (2)), followed by electrochemical reoxidation of Co^{+} at low overpotential (Eq. (3)).

Traditional carbon ink formulations contain organic solvents and require heat-curing at high temperature after printing. Since these features will result in protein denaturation, the enzyme component of the biosensor must be added in a separate fabrication step, often by drop-coating or printing over the carbon electrode. Several reports exist on the fabrication of biosensors from conducting biocomposite inks which have incorporated and retained the

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activity of an enzyme component and which have been screen-printed; incorporated enzymes used to date have included urease (Ogonczyk et al., 2005), HRP (Keay and McNeil, 1998; Ledru et al., 2006; Ghamouss et al., 2006), lactate oxidase (Ghamouss et al., 2006) and GOD (Koopal et al., 1994; Crouch et al., 2005a,b). Previous work in our laboratory, which was conducted through a collaborative project between the University of the West of England and Gwent Electronic Materials Ltd. (GEM), gave rise to a water-based carbon ink formulation. This formulation, with CoPC incorporated as the electrocatalyst, together with the enzyme GOD, was successfully screen-printed in a single fabrication step to produce planar 3 mm × 3 mm glucose biosensors (Crouch et al., 2005a). These biosensors maintained activity over an 18-month storage period, and were used amperometrically in stirred buffer solution to determine glucose concentrations in spiked bovine serum. In a follow-up study (Crouch et al., 2005b), using a slightly modified formulation, further devices were used successfully to determine glucose concentrations in human plasma samples by chronoamperometry in quiescent buffer solutions.

Amperometric responses obtained at planar electrodes, maintained at a constant operating potential, for reasons of diffusion-limitation, do not maintain steady-state current responses in quiescent solution; this precludes their use for continuous monitoring applications. Microelectrodes including microbands, on the other hand, which have at least one dimension of less than 50 μm size, provide the advantages of smaller double layer capacitance, low ohmic drop and highly efficient mass transport through radial diffusion (Pletcher, 1991; Amatore, 1995). The last results in steady-state current responses, allowing the possibility of making determinations in quiescent sample solutions and of continuous monitoring applications. There only appear to be a few reported examples of continuous monitoring using electrochemical glucose biosensors. These include amperometric biosensors based on GOD-coated silicon nanowires (Chen et al., 2006) and polymer-immobilised GOD on Pt/Ir wire (Lowry and Fillenz, 2001); also fermentation monitoring using the amperometric SIRE® glucose biosensor (Lidgren et al., 2006).

In order to obtain sensors exhibiting microelectrode behaviour using screen-printing technology with carbon inks, it is necessary to devise a means of obtaining a working electrode of micron-size in at least one dimension. Thick-film carbon electrodes are usually between 15 and 30 μm thick, but the minimum width of carbon ink that can be laid down accurately by traditional screen-printing methodology is at least 100 μm, and more usually millimetre dimensions are adopted for reproducible reasons. Hence the resulting surface areas are relatively large, approaching square millimetre size. Several approaches have been documented in which thick-film carbon layers have been processed, post-screen-printing, in order to create biosensors having microelectrode dimensions and behaviour. These have included the formation of microband electrodes by screen-printing successive conducting, then insulating layers onto alumina, followed by perpendicular cutting to expose microband electrode edges (Craston et al., 1991). Ultramicroband electrodes made from SPCEs have been described recently by other workers (Chang and Zen, 2006a); the low ohmic drop for these electrodes was exploited for the voltammetric determination of trace nitrite ions in low ionic strength solutions (Chang and Zen, 2006b). Recently in this laboratory we have examined the electrochemical behaviour of electrodes formed by drilling a small hole through screen-printed working and counter/reference electrode layers printed onto polyester. The resulting sensors, fabricated using a solvent-based CoPC-containing carbon ink from GEM, exhibited microelectrode-type behaviour and were capable of giving steady-state current responses in the presence of H_2O_2 (Rawson et al., 2007).

The aim of the work described in the present paper was to utilise the water-based CoPC/GOD-containing carbon ink formulation described above (Crouch et al., 2005a) to investigate the possibility of fabricating glucose microband biosensors possessing microelectrode-type behaviour. The following sections describe voltammetric and amperometric studies undertaken to investigate and optimise the performance of the resulting microbiosensors. These devices were then evaluated for use with human serum.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical reagent grade and were purchased from Sigma–Aldrich unless stated otherwise. The supporting electrolyte used throughout was 0.05 M phosphate buffer, prepared from 0.5 M stock solutions of Na_2HPO_4, NaH_2PO_4, Na_3PO_4 and H_3PO_4, which were mixed to obtain the desired pH, then diluted with deionised water (dH_2O; Purite R200 system, Purite Ltd., Oxon, UK). NaCl was added to the buffer solution as indicated. Beta-D-glucose was dissolved in water and allowed to mutorotate at room temperature overnight before being used to prepare standard solutions. Human type AB male serum was purchased from Biosera Ltd., UK and stored at −30 °C until use.

2.2. Apparatus

Experiments were performed using a water-jacketed glass electrochemical cell, or, for dipping experiments, a 12-well tissue culture plate (Greiner bio-one). Screen-printed electrodes were connected via gold edge connectors and insulated copper wire leads to a computer-controlled μAutolab Type II potentiostat (Windsor Scientific Ltd., Slough, UK): all experiments were conducted using a three-electrode system comprising working electrode, plus a screen-printed Ag/AgCl reference electrode and a Pt wire counter electrode. Water temperature was maintained using a circulating water-bath (Haake D3, Saddle Brook, USA). During experiments requiring stirred solutions, the cell contents were stirred at a constant rate using a PTFE-coated disc-shaped magnet and stirrer. Solution pHs were monitored using a pH meter (Fisherbrand Hydrus 400) and glass combination pH electrode (Russell, UK).

2.3. Biosensor fabrication

Reference electrodes were screen-printed first, using Ag/AgCl (60:40) ink (GEM product code: C61003P7), which was dried at 60 °C. Biosensors were then screen-printed in one step from water-based ink containing carbon, binder, CoPC and GOD (complete glucose biosensor ink formulation, code C2041124D3, available from GEM) (Crouch et al., 2005b) onto 0.5 mm-thick pre-heat-shrunk PVC card to form a 2 mm-wide track and square working area. This ink was dried at room temperature. For use as macroelectrodes, a dielectric layer of PVC tape was then applied, leaving a 3 mm × 3 mm working area exposed. This macroelectrode design has been described previously (Sprules et al., 1996).

The biosensors were configured as microband electrodes by first covering the working area and most of the track with a layer of insulating tape. The covered area was then cut transversely using a razor blade to expose a 3 mm-long working electrode edge (Fig. 2). The thickness of this edge was found to be 20 μm. Therefore the geometric area of these devices was 0.06 mm^2. Reference electrodes consisted of screen-printed 2 mm-wide Ag/AgCl tracks on PVC (60:40 ink; GEM product code: C61003P7).

2.4. Voltammetry

A macroelectrode, or microband, a screen-printed Ag/AgCl reference electrode, and a Pt wire counter electrode, were inserted at open circuit potential into a 10 ml volume of electrolyte solution (0.05 M phosphate buffer, pH 8, containing 150 mM NaCl, in the presence or absence of glucose) at a temperature of 25 °C and under constant-speed stirred or quiescent conditions as stated. After an equilibration time of 400 s, an appropriate potential step was applied and the resulting current response was monitored until it reached a steady state. The applied potential was then increased in 50 or 100 mV steps and the steady-state current was recorded each time. Corresponding blank voltammograms obtained in buffer were subtracted. The final voltammogram was obtained by plotting current response versus applied potential.

2.5. Calibration studies using amperometry

Amperograms for glucose were obtained using a 10 ml solution of 0.05 M phosphate buffer containing 150 mM NaCl at the pH specified, into which a biosensor, Ag/AgCl reference and Pt counter electrode were immersed. The solution was stirred continuously or unstirred, as stated. An appropriate operating potential was applied to the working electrode, and the system was left for the current response to reach a steady state (400–1500 s). Using a micropipette, a series of small-volume additions of a standard glucose solution was then made. Under stirred conditions, steady-state currents were awaited between additions; under quiescent conditions, the solution was stirred briefly to mix the solution, then the stirrer was switched off and steady-state currents were recorded. Calibration plots were constructed by plotting each resulting current response against the final cell glucose concentration.

2.6. Evaluation of the biosensors

2.6.1. Method of standard additions by dipping microbands directly into quiescent serum samples

The procedure for glucose analysis by dipping microband biosensors into quiescent glucose-containing solutions was as follows: 5 ml aliquots of 0.05 M phosphate buffer/150 mM NaCl were spiked with glucose over the concentration range 0–7.0 mM. A 3 ml volume of each aliquot was placed into a separate well of a tissue culture plate and held in a water-bath at 25 °C. A microband biosensor, plus reference and counter electrodes, were placed into the first, blank, well. An operating potential of +0.4 V was applied and 1500 s were allowed for the current response to reach steady state. The biosensor, reference and counter electrodes, were then lifted and transferred into the next well containing a 0.5 mM glucose spike; upon achieving a steady-state current response (after 400 s), subsequent transfers into successively stronger glucose solutions were made every 400 s. Amperograms were recorded for $n = 4$ replicate microband biosensors and the mean steady-state current responses were calculated for each added glucose concentration. A calibration plot was constructed and the limit of detection (l.o.d.) for the method was determined as that giving a current higher than the mean + 3 S.D. of the blank current.

Glucose determination in serum was performed using the same method: serum was diluted 1 in 4 in 0.05 M phosphate buffer containing 150 mM NaCl. 5 ml aliquots were spiked with increasing glucose concentrations over the range 0–7.0 mM and amperograms were recorded as above. The resulting mean current values were corrected for any contribution from other electroactive interfering species present in the serum. This was achieved by subtracting steady-state current responses recorded in unspiked serum aliquots using microband electrodes printed from water-based carbon/CoPC ink containing heat-inactivated enzyme. Lactate oxidase was used here as this could readily be deactivated, whereas GOD retained some activity under a variety of heating conditions.

Calibration plots were constructed from the current responses and regression analysis was used to determine the *x*-axis intercept. The original glucose concentration of the serum was then calculated, allowing for the dilution factor.

2.6.2. Method of standard additions by multiple spiking using a glucose standard

A microband biosensor was lowered into a 7.5 ml aliquot of quiescent pH 8 buffer solution in a water-jacketed cell held at 25 °C (three-electrode system). An operating potential of +0.4 V was applied, and the current response was allowed to reach a steady state (1000 s). A 2.5 ml aliquot of serum was then added, to give a final serum dilution factor of 1 in 4, and any change in current response was recorded, being the response due to any interferent species, plus endogenous glucose within the diluted serum. Standard additions of glucose solution were then made: the stirrer was activated for 15 s after each addition to mix the solution, then turned off to allow the response to reach a steady state under quiescent conditions. The steady-state current responses were recorded for each addition. Replicate amperograms were obtained using four separate biosensors on a serum sample which had been spiked with 5 mM glucose. The resulting mean current responses were calculated.

In order to compensate for background current due to interfering species, from that due to endogenous glucose, the above procedure was repeated in diluted serum using control electrodes printed from water-based carbon/CoPC ink formulation containing heat-inactivated (lactate oxidase) enzyme. Since there was no enzymatic production of hydrogen peroxide in response to glucose under these conditions, any difference in current response between the buffer only, and the final steady-state response with added serum, could be attributed to the oxidation of interferents. The mean background response for $n = 5$ electrodes was subtracted from the mean currents obtained for the biosensors.

A standard-addition plot was obtained by plotting mean steady-state current against added glucose concentration; this plot was used to determine the concentration of glucose in the original serum sample ($n = 4$).

2.7. Spectrophotometric glucose assay

The glucose concentration in the test serum was determined independently using a spectrophotometric method based on that published by Trinder (1969) and described earlier elsewhere (Crouch et al., 2005b). One millilitre volumes of the assay reagent, prepared as described (Crouch et al., 2005b) from phenol, 4-aminoantipyrine, glucose oxidase and peroxidase in phosphate buffer, were added to cuvettes containing 200 μl volumes of serum samples diluted fivefold in dH_2O. Cuvettes were incubated for 1 h at 37 °C to allow the reaction to reach end-point, then absorbances at 505 nm were read against a reagent blank. Absorbances due to serum components were corrected for by adding 1 ml of dH_2O instead of the reagent. Glucose concentrations in serum samples were determined using a calibration plot constructed for standard solutions of glucose.

3. Results and discussion

3.1. Effect of applied potential on biosensor response to glucose

It was the aim of this study to use our new microband glucose biosensors for serum analysis in a three-electrode system

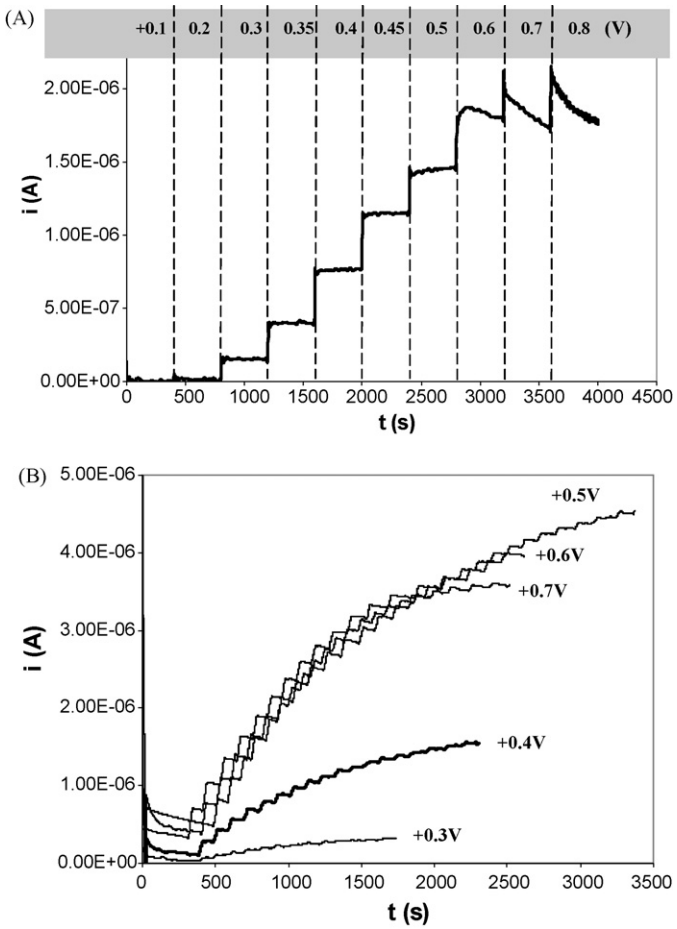

Fig. 1. (A) Hydrodynamic voltammogram (after blank subtraction) for 3 mm × 3 mm macroelectrode biosensors in 0.05 M phosphate buffer/150 mM NaCl, pH 8.0 containing 2 mM glucose. (B) Amperograms obtained for sequential (every 100 s) 70 μl additions of a 50 mM glucose solution using macroelectrodes under stirred conditions at various applied potentials.

comprising working electrode, screen-printed Ag/AgCl reference electrode and Pt counter electrode. In order to establish the applied potential required under these experimental conditions and to enable comparison with previous work (Crouch et al., 2005a,b), biosensors in the macroelectrode design were used. The amperometric responses at increasing potentials for a 2 mM glucose solution are shown in Fig. 1A. An anodic current response began at an applied potential of +0.3 V and increasing steady-state responses were observed up to +0.5 V. At higher potentials there was evidence of current decay. An applied potential between +0.5 and +0.6 V appeared to give the maximum available current response for the glucose biosensors.

This profile was similar to that shown previously for biosensors based on a macroelectrode and demonstrated that use in this case of a screen-printed Ag/AgCl reference electrode, in buffer containing NaCl, gave an equivalent voltammogram to that obtained with a saturated calomel reference electrode (Crouch et al., 2005a). It is well known that the half-cell potential of a Ag/AgCl reference electrode is influenced by chloride ion concentration. Therefore the influence of NaCl concentration on the current response was investigated with our screen-printed Ag/AgCl reference electrode using a potential of +0.4 V: no significant change in response was observed in glucose solutions containing 50–200 mM Cl^-. Thus, 150 mM NaCl was used in all further studies. Studies performed in this physiological concentration of chloride would therefore be suitable for optimisation of the biosensor performance in order to undertake measurements in serum.

Amperograms were obtained in stirred solutions for sequential additions of glucose using various applied potentials (Fig. 1B). The maximum responses were obtained for potentials of +0.5, +0.6 and +0.7 V, indicating that the position of the plateau for glucose determination begins at +0.5 V. A small response was obtained using +0.3 V. At +0.4 V, the response was about 50% of maximal sensitivity. From these results, it was deduced that an applied potential of +0.4 V would be suitable for future studies, being a compromise between sensitivity and selectivity; the latter is more important when analysing biological fluids due to interfering electroactive constituents within the sample matrix.

Having validated the proposed three-electrode system for glucose measurements, the next procedure was to reduce the dimensions of the working biosensor to microband geometry.

3.2. Microband biosensor fabrication

Microband electrodes were formed by covering the 3 mm × 3 mm screen-printed working area with PVC insulation tape, then cutting transversely across the centre of the working area as illustrated in Fig. 2. This procedure resulted in a 3 mm-long exposed edge of the working electrode.

3.3. Voltammetry of glucose at microband biosensors

In order to obtain the optimum operating potential for glucose determination using microband biosensors, voltammograms were obtained with a glucose solution, both under stirred (2 mM) and quiescent (2 mM and 5 mM) conditions (data not shown). Under stirring a current response for glucose was observed from +0.2 V, rising to a peak at around +0.45 V. From previous studies (Gilmartin et al., 1995) it seems that this peak corresponds to electrocatalysis of H_2O_2 via oxidation of Co^+ to Co^{2+}. Under stirring, a second wave at around +0.7 V, was also visible and probably corresponds to the subsequent oxidation of Co^{2+} to Co^{3+}. In quiescent solution, lower currents were obtained for glucose, indicating that the microband biosensor responses were not fully independent of stirring. The current response began around +0.3 V and increased to a plateau around +0.5 V. At more positive potentials the current response showed a decrease which may be related to the conversion of Co^{2+}

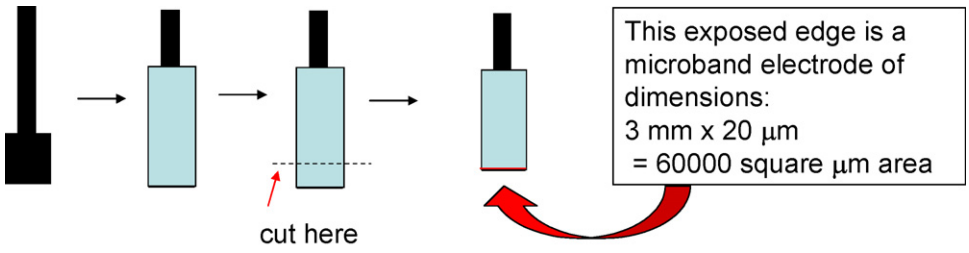

Fig. 2. Scheme illustrating microband biosensor production.

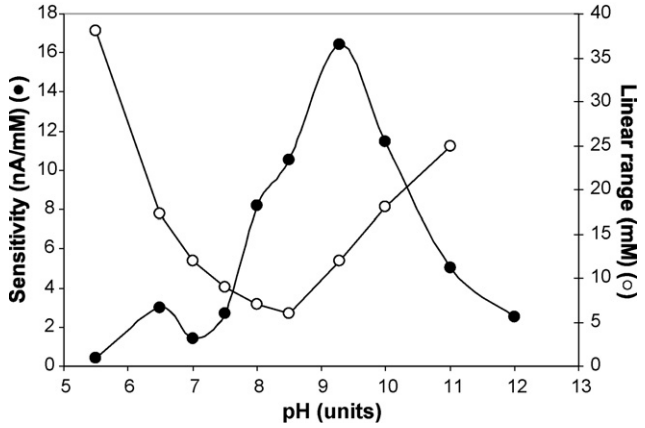

Fig. 3. Effect of pH on microband biosensor sensitivity (solid circles) and linear range (open circles) in quiescent buffer solution.

to Co^{3+}, as a consequence of which the pool of Co^{2+} available for electrocatalytic oxidation of H_2O_2 becomes diminished, leading to a reduced response. From these voltammograms, an applied potential of +0.4 V was indicated as the appropriate choice for subsequent experiments using microband electrodes for glucose measurement both under stirring and in quiescent conditions.

3.4. Effect of pH

The effect of pH on the performance of microband glucose biosensors in quiescent solution (stirred only to mix) was examined by carrying out glucose calibration studies over a range of pH values. Fig. 3 shows that the sensitivity was at a maximum (16.4 nA/mM)

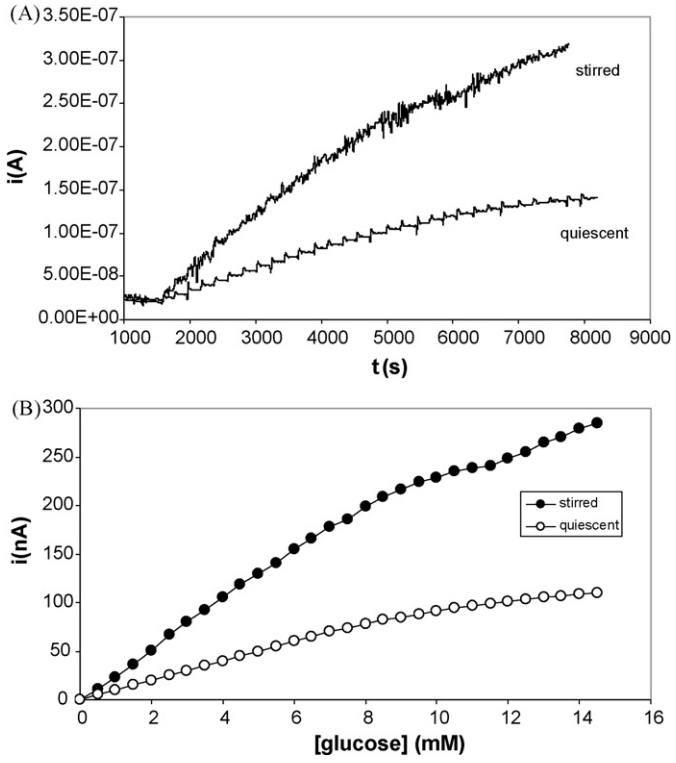

Fig. 4. (A) Amperograms obtained at WB-CoPC-GOD microband electrodes in stirred and unstirred (stirred to mix only) solution following successive additions (every 200 s) of 10 μl of 500 mM glucose into 10 ml 0.05 M phosphate buffer/150 mM NaCl, pH 8.0; E_{app} = +0.4 V vs. Ag/AgCl. (B) Corresponding calibration plots for stirred (closed circles) and unstirred (open circles) solutions.

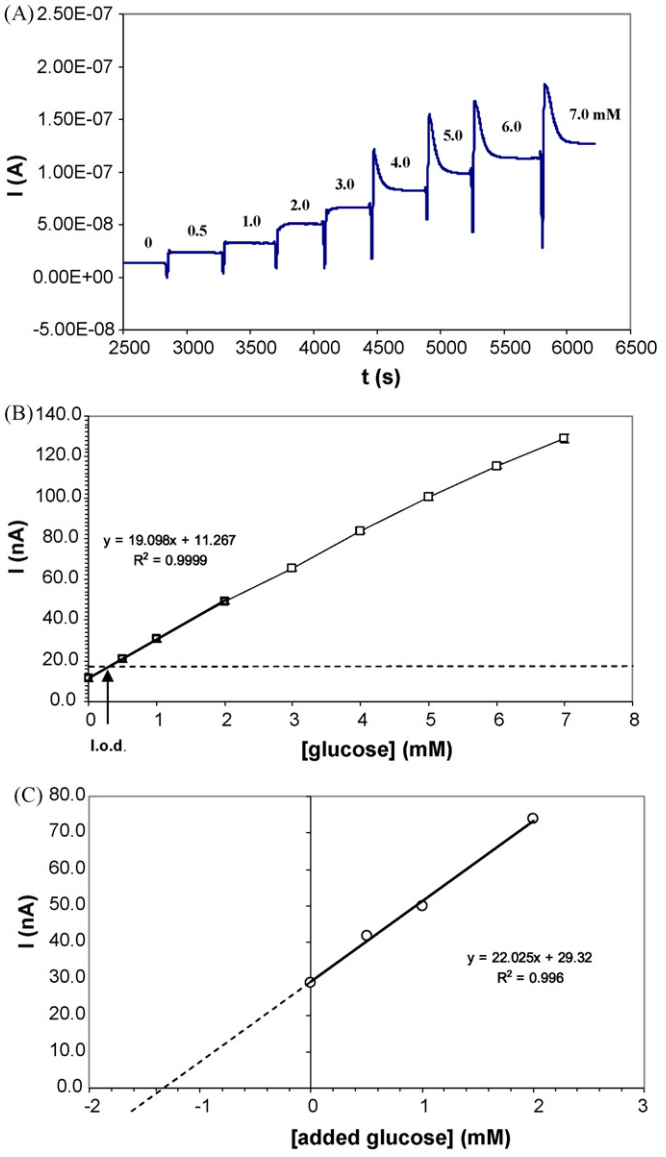

Fig. 5. (A) Amperogram obtained following sequential dipping of a single microband glucose biosensor into aliquots of buffer (pH 8.0) spiked with increasing millimolar concentrations of glucose (as indicated). (B) Corresponding calibration plot obtained for repeat (n = 4) microband biosensors. Error bars show ±1 standard error of the mean (S.E.M). (C) Regression plot constructed from replicate amperograms (n = 4) following dipping of microband biosensors into buffered serum aliquots spiked with increasing concentrations of glucose.

at around pH 9.0, with less sensitivity being obtained as pH was adjusted away from this value in either direction. The maximum linear range (up to 35 mM) was observed at the lowest pH (pH 5.5) at which the biosensors could function; at higher pH values, a trend for reduced linear range was observed, down to a minimal value (6 mM) at pH 8.5; at pH values greater than 8.5, the linear range again increased, to a value of around 25 mM at pH 11. These observations suggested that a pH of 8.0 would be suitable for further studies, conferring a suitable combination of sensitivity and linear range for glucose measurement in serum, whilst being relatively close to physiological pH.

3.5. Amperometry of glucose at microband biosensors

Representative amperograms obtained with microband biosensors in stirred and quiescent buffer solutions at an applied potential

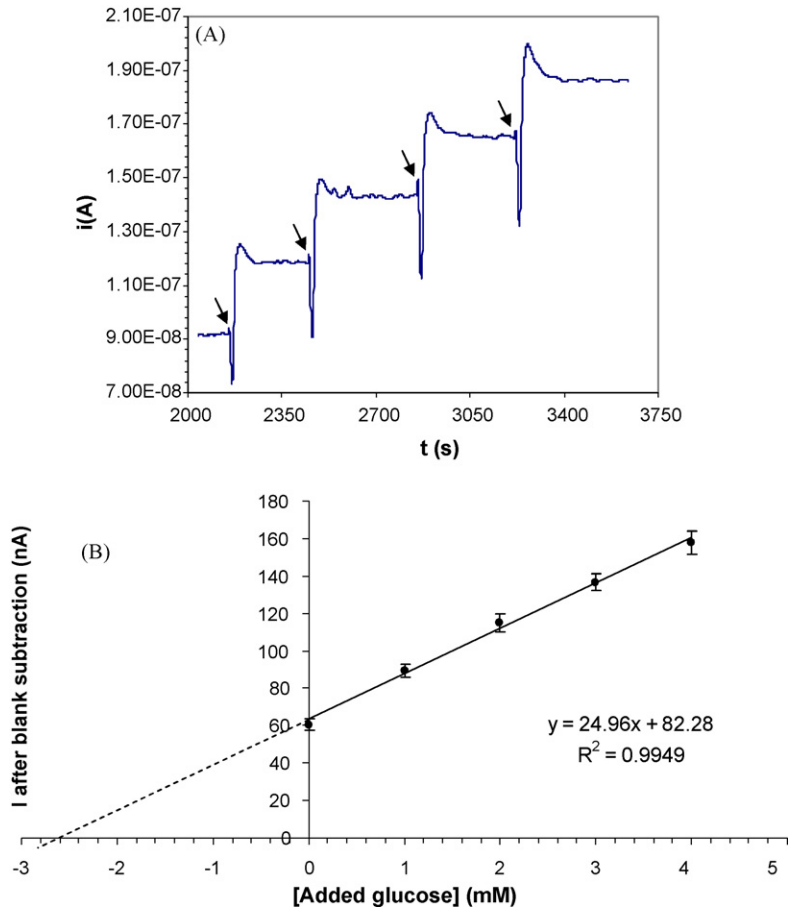

Fig. 6. Typical standard addition amperogram for a WB-CoPC-GOD microband biosensor in 25% spiked human serum in phosphate buffer, pH 8.0. Arrows indicate additions of 1 mM glucose, followed by stirring for 15 s. (B) Standard addition plot derived from mean values obtained using four separate biosensors. Error bars show ±1 S.D.

of +0.4 V are shown in Fig. 4A. The corresponding calibration plots are shown in Fig. 4B. These results demonstrated that microband biosensors were capable of being operated under either set of conditions. More sensitive current responses were obtained under stirring (26 nA/mM) than under quiescent conditions (10 nA/mM), but linear ranges were identical (up to 7 mM). With stirring, rapid current rises followed each glucose addition, with evidence of stirrer noise. However, the responses obtained under quiescent conditions (after briefly stirring to mix the solution) rapidly assumed steady-state status with little associated noise. Thus, microband biosensors were not fully independent of stirring, yet they showed microelectrode-like behaviour in assuming steady-state current responses. This combination indicated that a mixed mechanism of radial and planar diffusion was occurring. It also indicated that the microband biosensors would be suitable for use in unstirred applications.

An indication of the operational stability of the microband biosensors was obtained by allowing the amperometric currents to reach steady state, then testing subsequent changes in amperometric response to glucose in quiescent solution, either to glucose additions, or when transferred from one solution to another. In either case, full activity was maintained over an 8-h period.

3.6. Serum glucose determination using microband biosensors

3.6.1. Method of standard additions by dipping microbands directly into quiescent serum samples

A typical amperogram obtained using the dipping technique into buffer solutions containing increasing glucose concentrations is shown in Fig. 5A. Steady-state current responses were obtained at each concentration. The resulting calibration plot (Fig. 5B) was linear up to 2.0 mM glucose, with a l.o.d. of 0.27 mM. The working range was up to >7 mM. Precision (coefficients of variation) for replicate biosensors ranged from 2.2 to 11.1%. These results demonstrate that the microband biosensors are able, by virtue of their microelectrode-like behaviour to give concentration-dependent, steady-state current responses in quiescent solutions.

The dipping technique was then applied to aliquots of diluted serum at concentrations of glucose comparable to those used for Fig. 5A and B. In serum, as in buffer, stable steady-state current responses were obtained (amperogram not shown). The presence of serum had no measurable effect on the slope of the calibration plot, indicating the absence of a matrix effect on enzyme activity, electrocatalytic peroxide oxidation, or the position of the reference potential. The regression plot derived from the resulting current responses is shown in Fig. 5C. The precision for replicate biosensors ranged from 6.2 to 10.7%. Interpolating from this plot, and correcting for interference currents using blank biosensors, a value of 5.4 mM was obtained for endogenous serum glucose concentration. This compared favourably with the spectrophotometric assay determination of 5.7 mM.

3.6.2. Method of standard additions by multiple spiking using a glucose standard

Fig. 6A shows a typical amperogram obtained when performing standard additions (each of 1 mM glucose) using a microband biosensor in phosphate buffer containing 25% serum. After making each addition (arrows) and briefly stirring to mix the solution,

excellent steady-state current responses were obtained. The slopes of the standard addition plots derived from four replicate serum aliquots, each tested using a separate biosensor, showed good agreement, with a coefficient of variation of 7.0%. The mean plot is shown in Fig. 6B. The human serum was spiked with 5 mM glucose to bring the final concentration into the range expected in diabetic subjects. The glucose concentration was determined to be 10.4 mM, which indicates a recovery of 100% for the added glucose. This procedure was repeated four times and the coefficient of variation was calculated to be 3.8%.

4. Conclusions

Microband biosensors, prepared from a screen-printed water-based carbon ink, containing CoPC and enzyme (Crouch et al., 2005a,b), have been fabricated and investigated as amperometric devices for glucose measurement. The devices operate by producing H_2O_2 (from glucose), which is then electrocatalytically oxidised via the CoPC mediator. The applied potential (+0.4 V) selected for operation of these devices is sufficiently positive to enable the electrocatalytic oxidation of the H_2O_2, but sufficiently low to avoid any loss of sensitivity observed at higher potentials, particularly when in quiescent solutions. The studies presented here have demonstrated that they can be operated successfully under quiescent conditions. Indeed, stable steady-state responses to glucose are observed in both buffer solution and diluted serum. This observation indicates that the microbands are of sufficiently small size in at least one dimension to display predominantly radial diffusion. Such mass transport behaviour gives enhanced current density which leads to better signal-to-noise ratios.

Using these biosensors it has been possible to perform glucose determinations in serum under quiescent conditions and to quantify glucose over the concentration range of relevance to clinical normal and diabetic monitoring. The ability simply to dip the biosensors into a test solution suggests the potential for a simple versatile portable device. Alternatively, the ability to obtain steady-state responses at low current suggests the opportunity for continuous process-monitoring applications in situations where glucose consumption and perturbation of the system must be minimised.

The fabrication method employed here, using a single screen-printing step followed by a simple cutting modification, is relatively low-cost and may be adaptable to a more mass-production scenario. SPCE-based biosensors have been developed for analytes other than glucose, their specificity being conferred by addition of the appropriate enzyme (e.g. an oxidase) or biological molecule (Hart et al., 2007). In fact, some recent work in our laboratory indicates that lactate oxidase is stable in the water-based ink formulation. Investigations into alternative analytes for this microband biosensor approach are therefore under consideration.

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