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Comparisons of platinum, gold, palladium and glassy carbon as electrode materials in the design of biosensors for glutamate

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Abstract

Four electrode materials: Pt, Au, Pd and glassy carbon (GC), were studied to investigate their suitability as substrates in the development of two different classes of glutamate biosensor. Glutamate oxidase cross-linked onto poly(*o*-phenylenediamine) was chosen as the type 1 biosensor (PPD/GluOx), incorporating PPD as the permselective element to detect H_2O_2 directly on the electrode surface at relatively high applied potentials. GluOx and horseradish peroxidase/redox polymer modified electrodes (Os^{2+}PVP/HRP/GluOx) that relied on enzyme-catalysed H_2O_2 detection at lower applied potentials were used as type 2 biosensors.

The voltammetric and amperometric responses to the enzyme signal transduction molecule, H_2O_2, and the archetypal interference species in biological applications, ascorbic acid, were determined on the bare and PPD/GluOx-modified surfaces. The amperometric responses of these electrodes were stable over several days of continuous recording in phosphate buffered saline (pH 7.4). The sensitivity of the type 1 biosensors to H_2O_2 and glutamate showed parallel trends with low limits of detection and good linearity at low concentrations: Pt > Au ∼ Pd ≫ GC. Type 2 biosensors out-performed the type 1 design for all electrode substrates, except Pt. However, the presence of the permselective PPD membrane in the type 1 biosensors, not feasible in the type 2 design, suggests that Pt/PPD/GluOx might have the best all-round characteristics for glutamate detection in biological media containing interference species such as ascorbic acid. Other points affecting a final choice of substrate should include factors such as mass production issues.

Keywords: Poly(1,2-diaminobenzene); Poly(phenylenediamine); Glutamate oxidase; Horseradish peroxidase; Os^{2+}-polyvinylpyridine; Ascorbic acid; Amperometric detection; Cyclic voltammetry; Hydrogen peroxide

1. Introduction

The incorporation of enzymes into electrode designs (Clark and Lyons, 1962; Raba and Mottola, 1995) has led to the development of amperometric sensors for biomolecules that are either non-electroactive, such as glutamate, or whose surface electrochemistry is too complex to allow direct reliable detection, such as glucose (Hsiao et al., 1996; Jensen and Johnson, 1997). Although these biosensors offer the possibility of on-line monitoring, with a specificity linked to that of the redox reactions catalysed by the immobilised enzyme, problems of interference arise from direct surface electrochemistry of electroactive species, such as ascorbate and urate, present in biological tissues and samples (O'Neill et al., 1998). The most common class of enzyme used for biosensor fabrication are the oxidases (Raba and Mottola, 1995), many of which involve FAD-catalysed oxidation (reaction (1)) and the subsequent reduction of O_2 to form H_2O_2 (reaction (2)) (Kleppe, 1966), and the anodic detection of this enzyme-generated H_2O_2 has been the most widely used strategy for biosensor signal transduction (reaction (3)) (Updike and Hicks, 1967; Fraser, 1997; O'Neill and Lowry, 2000). The main problem with this approach is the high applied potential needed to oxidise H_2O_2 on most electrode materials, exacerbating electroactive interference, even at Pt where electrocatalytic reactions involving platinum oxides attenuate the overpotential significantly (Hall et al., 1998). This problem has been minimised by the use

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of permselective membranes to block access by the interference to the electrode surface, and a widespread strategy has been the electrosynthesis of ultrathin polymers directly on the electrode surface, especially poly(1,2-diaminobenzene) (Sasso et al., 1990; Malitesta et al., 1990; Wang and Wu, 1993; Cooper and Pritchard, 1994; Lowry et al., 1994, 1998; Ryan et al., 1997; Bartlett et al., 1998; Yao et al., 2001; Craig and O'Neill, 2003).

$$\text{substrate} + \text{EOx/FAD} \rightarrow \text{product} + \text{EOx/FADH}_2 \quad (1)$$

$$\text{EOx/FADH}_2 + \text{O}_2 \rightarrow \text{EOx/FAD} + \text{H}_2\text{O}_2 \quad (2)$$

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^- \quad (3)$$

Alternatively, an electrochemical signal from the reduced oxidase can be obtained by using a redox mediator to replace O_2 as the enzyme co-substrate. However, although these 'second generation' devices have the advantage of a lower operating potential and elimination of oxygen from the reaction scheme, they can suffer from a number of problems. These include leeching of mediator from the electrode surface or enzyme layer (Grundig and Krabisch, 1989), toxicity in biological tissues (Beh et al., 1991) and homogeneous interference, e.g. oxidised ferrocenes can be reduced by ascorbate (Wilson and Turner, 1992). Additionally, the complete insensitivity to oxygen tension sometimes claimed for this type of sensor has been questioned for certain mediators (Martens and Hall, 1994). A recent form of these mediator-based biosensors involves Os-containing redox hydrogels (Taylor et al., 1995; Sirkar et al., 2000; Mikeladze et al., 2002; Vilkanauskyte et al., 2002), in which the tethering of the redox couple overcomes many of the problems associated with freely-diffusing mediators. In this work, we investigate both strategies for the development of a glutamate biosensor suitable for incorporation into wells, that can be mass produced, for applications involving the release of glutamate from isolated cells. The importance of glutamate as a key target in bioanalytical applications is highlighted by the diverse studies on this amino acid, including cell perfusion media in vitro (White et al., 1995), and in vivo detection in discrete brain regions using both biosensor (Burmeister et al., 2002) and microdialysis techniques (Del Arco et al., 1999).

A main issue addressed is the choice of electrode material for enzyme immobilisation and electrochemical detection. Pt is a good candidate because of its efficient interaction with H_2O_2, but electrodeposited layers can be mechanically unstable (O'Connell et al., 1998). Pd, which has better electrodeposition characteristics (O'Connell et al., 1998), also displays high activity towards H_2O_2 electro-oxidation, but much of this literature involves particulate forms of the metal embedded in carbon (Xu et al., 2002; Miscoria et al., 2002; Chang et al., 2003) and conducting polymers (Yamato et al., 1997). Thus, as a step towards fabricating well-based biosensors, basic issues such as usable potential range, suitability for electrosynthesis of permselective polymers, and comparative sensitivity to H_2O_2 and interference, were studied here on solid Pd electrodes and compared to Pt, Au and glassy carbon (GC).

2. Materials and methods

2.1. Reagents and solutions

The enzyme L-glutamate oxidase (GluOx from *Streptomyces* sp. X-119-6, EC 1.4.3.11, 200 U ml^{-1} in 20 mM potassium phosphate buffer, pH 7.4) was obtained as a generous gift from Yamasa Corp., Chiba, Japan and stored at −20 °C (Kusakabe et al., 1983). All chemicals, including 1,2-diaminobenzene (*o*-phenylenediamine, oPD, Sigma), L-glutamic acid (Glu, Sigma), L-ascorbic acid (Aldrich) and HRP redox polymer, including surfactant, from BAS, were used as supplied. Stock solutions of 100 mM Glu and H_2O_2 were prepared in doubly distilled water, and stored at 4 °C; 0.01 M HCl was used for the 100 mM ascorbate solution to inhibit aerial oxidation. Phosphate buffered saline (PBS, pH 7.4) consisted of 0.15 M NaCl (Merck, Poole, UK), 0.04 M NaH_2PO_4 (Merck) and 0.04 M NaOH (Merck). Solutions were kept refrigerated when not in use. 20 mM phosphate buffer (PB, 50 mM potassium perchlorate, pH 7.4) was used in all experiments involving HRP-modified electrodes.

2.2. Instrumentation and software

Experiments involving oPD-modified electrodes were done in a 25-ml glass cell at room temperature using a standard three-electrode set-up with a saturated calomel electrode (SCE) as the reference and a stainless-steel needle as the auxiliary electrode. These experiments were microcomputer controlled with data acquisition achieved using a Biodata Microlink interface, a low-noise, low-damping potentiostat (Biostat II, Electrochemical and Medical Systems, Newbury, UK) and in-house software. Experiments involving HRP-modified electrodes were performed in a well of a 24-well polycarbonate plate at room temperature with a Ag/AgCl reference electrode (Harvard Apparatus Inc., Holliston, MA, type E215) and a Pt wire counter electrode connected to a potentiostat (Ministat, Newcastle upon Tyne, UK). The linear and non-linear regression analyses were performed using the graphical software package Prism (GraphPad Software, San Diego, CA, USA). Data are reported as mean ± S.D. in general, but S.E.M. values are used for slopes and other regression parameters.

2.3. Working electrodes and experiments

Pt and Au macrodisks (M), 1.6 mm diameter, 2.0×10^{-2} cm^2 area from BAS, and Pd and GC macrodisks (3.0 mm diameter, 7.0×10^{-2} cm^2 area from BAS), were prepared by polishing with alumina (0.05 μm, BAS) and rinsing with distilled water. These four electrode substrates

were used for the two distinct types of electrode investigated in this study: devices designed to detect H_2O_2 directly on the electrode surface, operating at high applied potentials and incorporating a permselective polymer (poly(*o*-phenylenediamine), PPD) electrosynthesised from oPD; and HRP/redox polymer modified electrodes that relied on enzyme-catalysed H_2O_2 detection at lower applied potentials. A SCE reference was used for the former, whereas a Ag/AgCl electrode (50 mV versus SCE) served as a reference for the latter type of biosensor. Amperometric electropolymerisation (700 mV versus SCE) onto the Pt, Au, Pd and GC electrodes was carried out potentiostatically using 300 mM oPD in PBS containing 5 mg/ml albumin (Alb), extracted from either chicken egg or bovine serum (Sigma). The presence of globular protein within the polymer (PPD) thus formed improves the compactness of the polymer matrix, providing increased permselectivity (Lowry and O'Neill, 1994; McAteer and O'Neill, 1996; Ryan et al., 1997; Craig and O'Neill, 2003). The enzyme GluOx was deposited by drop-evaporation (1 μl for Pt and Au, and 3.5 μl for Pd and GC—scaled geometrically) for PPD-coated disks, using the undiluted 200 U/ml solution supplied. The GluOx thus deposited was trapped on the M/PPD disks by exposure to glutaraldehyde vapour for 10 min. These devices are termed M/PPD/GluOx electrodes, and were compared with literature data for PPD/GluOx-modified 5 T Pt wire cylinder electrodes (4.0×10^{-3} cm^2), Pt(5 T)/GluOx/PPD (Ryan et al., 1997).

In separate experiments, HRP redox polymer (Os^{2+}-polyvinylpyridine) was deposited onto freshly cleaned working electrodes (Au, Pt, Pd and GC) as follows. Surfactant solution (1 μl) from the BAS kit was applied and allowed to dry at room temperature for 30 min. Then 1 μl of HRP redox polymer was dropped onto each electrode surface and allowed to dry at room temperature for 4 h. GluOx (1 μl) was then applied to the HRP-modified electrodes and allowed to dry overnight at 4 °C. These devices are termed M/Os^{2+}PVP/HRP/GluOx electrodes and were compared with the M/PPD/GluOx electrodes described above.

Voltammetric and amperometric experiments on PPD-modified electrodes were carried out in an electrochemical cell containing 20 ml of background electrolyte, PBS. Cyclic voltammograms were recorded between 0.0 and 0.8 V (versus SCE) at 20 mV/s in PBS before and after addition of analyte. Calibrations on these electrodes were carried out amperometrically at 700 mV, unless stated otherwise. These electrochemical measurements were recorded in quiescent solution. Aliquots of analyte (H_2O_2, Glu, ascorbate, etc.) were added using a 50 μl Hamilton syringe, and dispersed through the solution using a brief (ca. 5 s) period of pumped convection.

Amperometric measurements on HRP-modified electrodes were performed in a polycarbonate well containing 2 ml of PB, using an operating potential of 100 mV versus the Ag/AgCl. After achieving a stable baseline current in the buffer, aliquots of either H_2O_2 or glutamate were added and current changes monitored. These measurements were carried out in stirred solutions.

3. Results and discussion

3.1. CV studies on bare electrodes

3.1.1. Background electrolyte

Gold has a tendency to dissolve into solution at high anodic applied voltages, especially in basic solution (Gerlache et al., 1997). However, potentials as high as +800 mV (versus SCE) have been employed at pH 7.0, albeit in buffered acetate electrolyte (Wang and Du, 2002). To investigate the potential-dependent behaviour of Au and Pd under conditions appropriate to later biological applications (buffered chloride solution, pH 7.4), and to compare with more common Pt and GC substrates, CV experiments were carried out scanning between 0 and 0.8 V (versus SCE) in PBS for the four electrode materials (Fig. 1). The behaviour of Pt and Pd were similar in having pronounced cathodic arms associated with the reduction of oxides on the surface of both metals (Johnston et al., 1995; Hall et al., 1998), although the current density scale was a factor of five smaller for Pd. Au displayed a classically capacitance shape up to a late upswing, presumably associated with Au dissolution (Gerlache et al., 1997). The CV at GC consisted essentially of low capacitance currents. It appears, therefore, that +700 mV is the highest common applied potential that can be used safely for long-term amperometric experiments on all four electrodes in comparative studies.

Apparent double-layer capacitance values were calculated at a potential where the voltammetric currents were essentially horizontal for both the forward and backward sweeps (400 mV; see Fig. 1). The values (mF cm^{-2}, $n = 5$) were: 0.85 ± 0.05 (Pt); 0.28 ± 0.02 (Au); 0.19 ± 0.08 (Pd); and 0.040 ± 0.003 (GC).

3.1.2. Hydrogen peroxide

Since H_2O_2, the enzyme transduction molecule, is detected directly on the electrode surface in PPD/GluOx biosensors (reaction (3)), the potential dependence of H_2O_2 oxidation at the four electrode materials was investigated using CV (see Fig. 2). Following the pattern observed for CVs recorded in PBS only (Fig. 1), the voltammetric behaviour of H_2O_2 fell into two classes. At Pt and Pd, H_2O_2 oxidation occurred at relatively low applied potentials, consistent with electrocatalysis by surface oxides for both metals (Johnston et al., 1995; Hall et al., 1998); Au and GC displayed significantly higher overpotentials. In addition, there was a cathodic arm in the background-subtracted H_2O_2 CVs for Pt and Pd, absent for both Au and GC, which is due to the reduction of H_2O_2 (O'Connell et al., 1998). To quantify the position of a voltammetric wave on the potential axis when a peak is not present, the potential at which the current rises most steeply (potential of

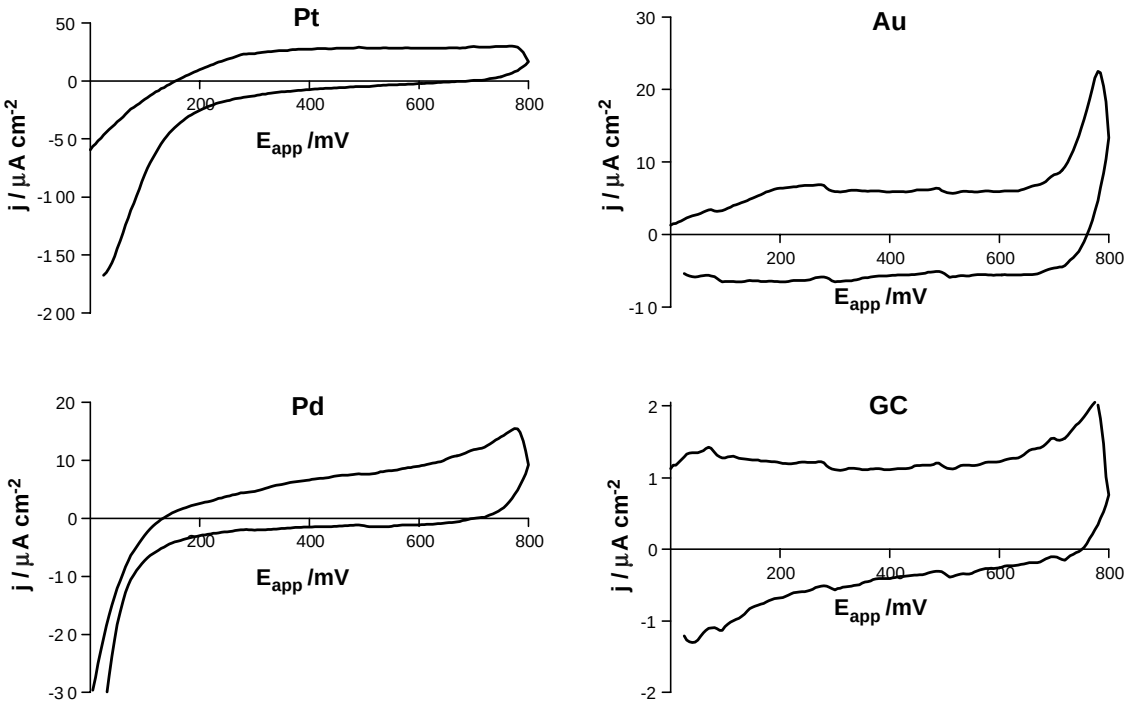

Fig. 1. CVs recorded at 20 mV/s in background electrolyte (PBS, pH 7.4) for Pt, Au, Pd and GC displayed on four current density scales. Scans, which were stable after a few cycles, were quite different in magnitude and shape for the four electrode materials.

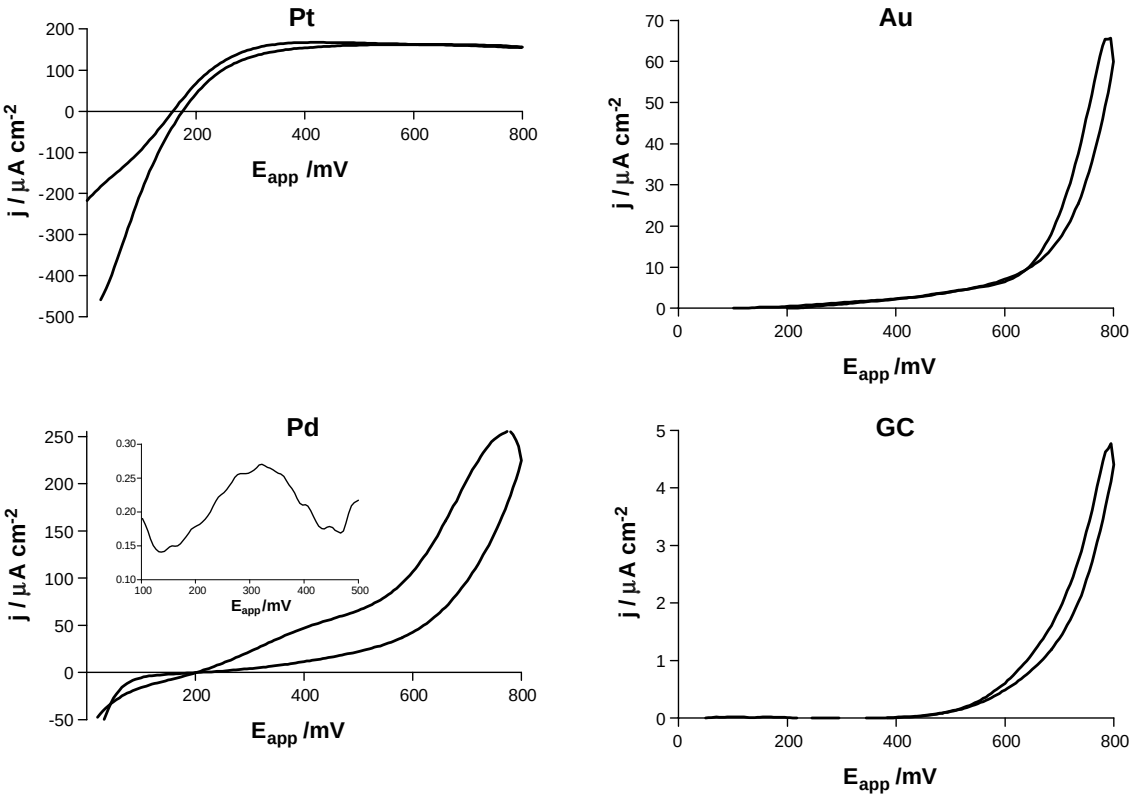

Fig. 2. CVs recorded at 20 mV/s in 1 mM H_2O_2 (PBS background current subtracted) for Pt, Au, Pd and GC displayed on four current density scales. Inset for Pd: differential of the first (minor) wave centred at ~300 mV highlighting its presence; *y*-axis label is $\partial I/\partial E_{app}$ ($\mu A\,cm^{-2}\,mV^{-1}$).

maximum slope, $E_{s,max}$) can be used (Oldham, 1985; Lyne and O'Neill, 1990). The following values of $E_{s,max}$ were obtained (all ±5 mV, $n = 10$): 155 mV (Pt); 770 mV (Au); 695 mV (Pd); and 770 mV (GC). The particular ability of Pt to facilitate the electro-oxidation of H_2O_2 is clear from its low $E_{s,max}$ value. The value for Pd was significantly higher, although less than that for Au and GC. The behaviour of H_2O_2 at Pd was complicated by the presence of two identifiable waves (Fig. 2, inset), the first (smaller) having an inflection point at ~300 mV. A clearer distinction between the H_2O_2 response at the four electrodes is provided by the slope of the wave ($\mu A\,cm^{-2}\,mV^{-1}\,mM^{-1}$) at $E_{s,max}$: 1.8 ± 0.2 (Pt); 0.62 ± 0.01 (Au); 1.2 ± 0.1 (Pd); and 0.044 ± 0.001 (GC). The ranking of H_2O_2 activity suggested by the trend in $E_{s,max}$ is re-enforced by this slope analysis: Pt > Pd > Au > GC. These results suggest that 700 mV is the optimum potential for efficient H_2O_2 detection in comparative amperometric experiments, *viz.* as high as possible for good H_2O_2 sensitivity and not too high for Au instability (see Fig. 1). However, voltammetric and amperometric behaviour might be different due to time-dependence of oxide states, etc. (Kuhn and Randle, 1985; Bolzan and Arvia, 1992; Burke and Buckley, 1996; Yang and Denuault, 1998). Therefore, this conclusion was tested in preliminary amperometric calibrations to compare H_2O_2 sensitivity at 600 and 700 mV for the four electrode types: the calibration slopes ($nA\,cm^{-2}\,mM^{-1}$) for Pt and Pd were not very different at 600 mV (144 ± 2, Pt; 31 ± 1, Pd) and 700 mV (134 ± 6, Pt; 39 ± 5, Pd), GC was increased ~60% from 1.0 ± 0.1 to 1.6 ± 0.1, whereas H_2O_2 sensitivity increased 7-fold at Au from 5.5 ± 0.2 to 37 ± 2 $nA\,cm^{-2}\,mM^{-1}$. This higher than expected sensitivity of Au maybe due to oxide states accumulated amperometrically at the higher potential (Burke, 1994). Thus, 700 mV was chosen for all further amperometric studies; the enhanced sensitivity of Au, observed at the bare electrode, was maintained in H_2O_2 and glutamate calibrations at polymer-modified Au biosensors (see below).

3.1.3. Ascorbic acid and 4-methylcatechol

Additional data on the relative electrochemical activities of the four materials was obtained by comparing CVs on potentially reversible (4-methylcatechol) and chemically irreversible (ascorbate) systems (Fig. 3). Ascorbate was also chosen as an important interference species in biosensor applications, and is chemically irreversible with an EC mechanism involving hydration (Counsell and Hornig, 1981). 4-Methylcatechol was quasi-reversible on Pt and Au with a well-defined re-reduction peak in each case, but was irreversible on Pd and GC; ascorbate oxidation was irreversible on all four surfaces. Because ascorbate has the lowest redox potential of the species studied here (−150 mV versus SCE under the present conditions (Ormonde and O'Neill, 1990; O'Neill, 1995), it displayed efficient oxidation on Pt at low applied potentials, with an $E_{s,max}$ value of 25 mV. Thus, even at the low potentials used in mediator-based devices, ascorbate interference could be problematic unless a

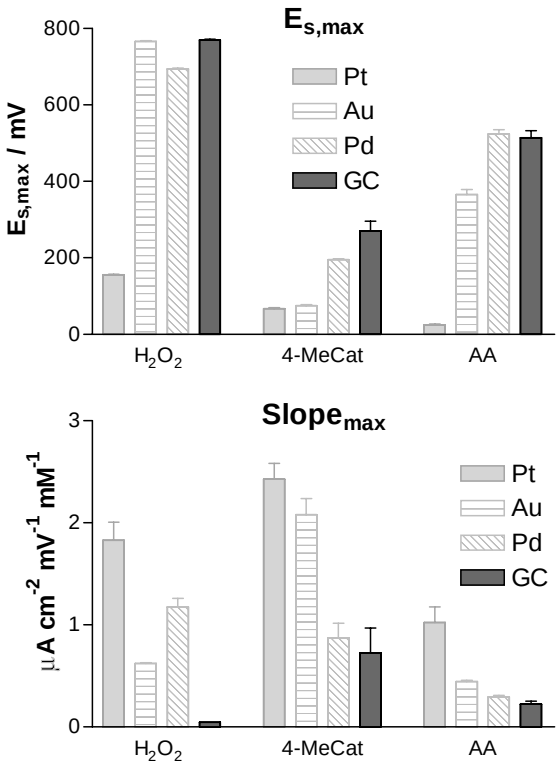

Fig. 3. The potential of maximum slope ($E_{s,max}$) and the maximum slope of the anodic sweep of the CV (see, for example, Fig. 2) recorded at 20 mV/s for 1 mM concentrations of three $2e^-$ oxidation species: H_2O_2, methylcatechol and ascorbic acid (background currents subtracted) at four bare electrode materials. The same scale is used for the four plots to enable direct 12-way first-approximation comparison of heterogeneous electron transfer kinetics between the three analytes and four electrode types.

permselective membrane is included in the biosensor design. To compare the activities of H_2O_2, 4-methylcatechol and ascorbate on the four electrodes, the steepness of the respective oxidation waves were compared, using the maximum slope ($\mu A\,cm^{-2}\,mV^{-1}\,mM^{-1}$) along the anodic sweep of the voltammogram (Fig. 3). This parameter is independent of the formal electrode potential of the redox couples and is a measure of relative electron-transfer kinetics (Oldham, 1985), if determined under the same conditions for couples with the same *z*-value, as was the case here. The overall picture presented by these data is that Pt showed the highest, and GC the lowest, activity towards all three analytes. The relative activities were mixed for Au and Pd.

3.2. Polymer (PPD) modification of the electrodes

CVs of oPD (1,2-diaminobenzene) showed that the oxidation of this monomer was facile on the four electrode materials with low values of $E_{s,max}$ in all cases: Pt, 195 mV; Au, 235 mV; Pd, 265 mV; and GC, 250 mV (±5 mV, first scan only due to passivisation). The electrodes were then re-polished and electropolymerisation carried out amperometrically at 700 mV over 20 min to achieve good permselective properties (Lowry and O'Neill, 1994; Ryan et al.,

Table 1
Parameters determined for biosensors of the type M/PPD/GluOx (M = Pt, Au, Pd, GC) by amperometric calibrations at 700 mV vs. SCE in PBS, pH 7.4 (mean ± S.E.M., $n = 4$)

	Pt	Au	Pd	GC
PBS background ($\mu A\,cm^{-2}$)	0.045 ± 0.009	0.070 ± 0.013	0.030 ± 0.004	0.025 ± 0.005
H_2O_2 slope ($nA\,cm^{-2}\,\mu M^{-1}$)	55.4 ± 3.3	42.9 ± 1.9	29.3 ± 3.8	0.90 ± 0.05
R^2 (linear)	0.996	0.997	0.995	0.997
Glu slope ($nA\,cm^{-2}\,\mu M^{-1}$)	26.8 ± 3.0	15.0 ± 5.0	16.4 ± 1.5	0.50 ± 0.08
R^2 (linear)	0.998	0.999	0.999	0.999

1997) because potential cycling has been shown to increase the permeability of electrosynthesised PPD to certain analytes (Centonze et al., 1994).

AA calibrations carried out at 700 mV at the M/PPD electrodes showed that the AA blocking ability of the polymer/albumin composite layer was similar for the three metals (AA sensitivity in $\mu A\,cm^{-2}\,mM^{-1}$): Pt, 0.5±0.1; Au, 0.5± 0.1; and Pd, 0.35±0.05. The particularly low AA sensitivity seen at the GC/PPD electrode (0.06±0.01 $\mu A\,cm^{-2}\,mM^{-1}$) was in part due to the poor AA oxidation on GC (see Fig. 3).

3.3. Enzyme (GluOx) modification of M/PPD electrodes

GluOx was immobilised onto M/PPD electrodes by drop evaporation and cross-linking with glutaraldehyde vapour. To determine the efficiency of the four types of M/PPD/GluOx electrodes to detect H_2O_2 amperometrically, H_2O_2 calibrations were carried out at 700 mV versus SCE (Table 1). The trends in amperometric H_2O_2 sensitivity are consistent with the CV data in Fig. 3, except that Au was more sensitive amperometrically than voltammetrically, which may be due to a build up of electrocatalytic oxides at the constant high applied potential used in the amperometric experiments (Burke, 1994).

The most important property of the M/PPD/GluOx sensors for applications in media containing low micromolar levels of glutamate is their sensitivity to glutamate at low concentrations. Table 1 gives the sensitivity slope up to 10 μM glutamate which shows that the trend is broadly in line with the H_2O_2 sensitivity. The low LOD for the metal-based biosensors (<0.5 μM) and good linearity suggest that this sensor design is appropriate for applications involving release of glutamate from isolated cells. Comparison of the linear sensitivity of Pt(M)/PPD/GluOx in the present studies (27 ± 3 $nA\,cm^{-2}\,\mu M^{-1}$, $n = 4$) with literature values for cylindrical wire Pt(5 T)/GluOx/PPD (28 ± 9 $nA\,cm^{-2}\,\mu M^{-1}$, $n = 9$) produced by dip evaporation of the enzyme solution (Ryan et al., 1997), shows that there was no difference in glutamate sensitivity in the linear range between these two methods of preparation.

The evolution of the biosensor parameters over four measurement days (three before a 13-day dry storage period, and one after) was determined. Only minor loss of sensitivity was observed over this period. For example, losses of 15 ± 6% in the linear H_2O_2 sensitivity and 23 ± 9% in the linear Glu response ($n = 4$ electrodes) were observed for the 14-day period of dry storage and re-wetting.

3.4. Electrodes modified with Os^{2+}PVP and HRP

The performance characteristics of M/Os^{2+}PVP/HRP/GluOx biosensors were studied on the four electrode types for comparison. In order to investigate the electrochemical enzyme activity of HRP after the modification, sensitivities to peroxide were obtained using all four electrodes. Au and Pd showed much higher sensitivity to peroxide compared with the sensitivities for Pt and GC (Table 2). This is due to the lower stability of the redox polymer on the surface of Pt and GC. The manufacture's manual states that the use of the polymer is optimised only for Au. However, after GluOx modification, all three metal electrodes showed similar sensitivity to peroxide.

For glutamate, stable initial steady-state baselines in buffer were achieved in less than 10 min and steady-state current re-achieved in 30 s after addition of glutamate samples; Au (<5 s), Pd (<10 s), Pt (<20 s) and GC (<20 s). In Table 2, Au/Os^{2+}PVP/HRP/GluOx electrodes show higher sensitivity (38 ± 8 $nA\,\mu M^{-1}\,cm^{-2}$) than Pt/Os^{2+}PVP/HRP/GluOx (24 ± 6 $nA\,\mu M^{-1}\,cm^{-2}$, $P < 0.02$). The results for the other electrodes are summarised in Table 2. For modified Pt electrodes, increasing the applied potential slightly improves its sensitivity (9% at 300 mV, 26% at 500 mV). However, the time taken to re-establish the steady-state response after addition of glutamate was significantly increased (more than 20 min), precluding these potentials in practical applications.

Table 2
Electrode sensitivity of HRP-based sensors to peroxide and glutamate after surface modifications determined at 100 mV vs. Ag/AgCl, pH 7.4 ($n = 5$)

Electrode	Sensitivity to peroxide ($nA\,cm^{-2}\,\mu M^{-1}$)		Sensitivity to glutamate ($nA\,cm^{-2}\,\mu M^{-1}$)
	HRP	HRP/GluOx	
Au	143 ± 21	48 ± 6	38 ± 8
Pt	51 ± 8	48 ± 12	24 ± 6
Pd	115 ± 14	52 ± 9	39 ± 9
GC	31 ± 11	24 ± 8	12 ± 7

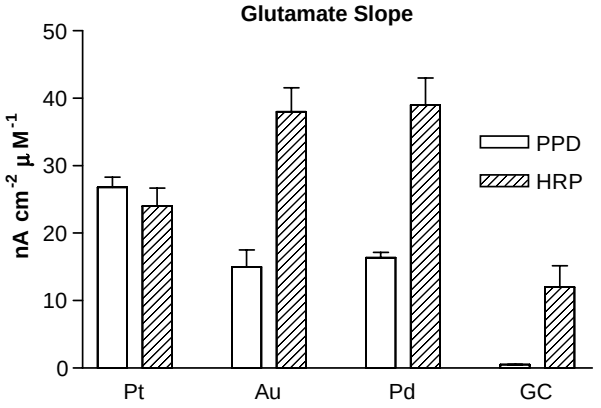

Fig. 4. Comparison of the glutamate calibration slopes in the linear response region for biosensors of type 1 (M/PPD/GluOx, $n = 4$) and type 2 (M/Os^{2+}PVP/HRP/GluOx, $n = 5$) based on the four electrode substrates: Pt, Au, Pd and GC.

4. Conclusions

Of the type 1 biosensors studied here, GC/PPD/GluOx appears too insensitive to Glu for applications in media of low glutamate concentration, with the highest LOD ($\sim$2 μM). The three metals, however, showed sufficient sensitivity and stability for biosensor applications. Pt/PPD/GluOx was the most sensitive, but Pt might be difficult to electrodeposit in custom-designed cell media wells. Type 1 Au- and Pd-based biosensors showed similar behaviours, and a final choice might depend on other considerations, such as compatibility with other chemistries in the detection cell.

When compared to type 2 (HRP) designs (see Fig. 4), only the Pt-based biosensors showed similar sensitivity to glutamate. However, although HRP-modified biosensors appear to outperform those based on the PPD design, the latter includes a permselective membrane making them suitable for applications in biological media containing high concentrations of ascorbate. Such a membrane would not be feasible for the HRP design because of the requirement for PVP-bound Os^{2+} to access the electrode.

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