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Study on the formation of the Prussian blue films on the polypyrrole surface as a potential mediator system for biosensing applications

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Abstract

The formation of a Prussian blue film under and above a polypyrrole film containing formate dehydrogenase and β -nicotinamide adenine dinucleotide has been investigated by electrochemical quartz crystal microbalance and cyclic voltammetry techniques. Two different deposition mechanisms of Prussian blue were identified and explained when the polypyrrole coated substrate electrode was used. Very stable current and frequency responses were obtained when the Prussian blue film was deposited under the polypyrrole film. This layered structure is potentially useful in solving the leakage problems of a biosensing system involves immobilised mediator. This advantage has been demonstrated using a formate biosensor fabricated with the enzyme, formate dehydrogenase, a co-factor, β -nicotinamide adenine dinucleotide and a Prussian blue mediator.

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Keywords: Biosensor; Prussian blue; Mediator; Polypyrrole; Quartz crystal microbalance

1. Introduction

The incorporation of a wide range of bioactive species into conducting electroactive polymers during electropolymerisation has been achieved by a number of workers [1–6]. The attraction of this form of immobilisation lies in a simple one step process. Moreover, the amount of bioactive species incorporated can be

quantitatively controlled during in situ polymerisation by varying the composition of the monomer solution as well as using appropriate electrochemical conditions [7]. However, the catalytic properties of bioactive species incorporated into conducting electroactive polymers such as polypyrroles are often insufficient for direct electrochemical biosensing of analytes [8,9]. Most enzyme-based amperometric biosensors therefore require co-immobilisation of an additional electron transfer mediator [9–11].

Electron mediation enables amplification of the analytical signal and also minimises the potential

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required for amperometric detection reducing the likelihood of interference in the electrochemical detection step. For example, in the case of an amperometric glucose sensor, electrochemical detection of H_2O_2 is usually carried out at electrode potentials greater than +0.60 V versus Ag/AgCl reference electrode. Many reductants in real samples can be oxidised at such a positive potential [9–11]. This is often the case for biological samples, which normally contain reductants such as ascorbate and bilirubin that can be easily oxidised at similar potentials. The use of an electron mediator in these cases enables signal generation at lower applied potentials with a concomitant increase in sensitivity. For example, when an electron transfer mediator, such as Prussian blue (PB), is employed, the detection of H_2O_2 can be accomplished at a potential as low as -0.2 V versus Ag/AgCl [9–11].

Prussian blue is an effective electrocatalyst [12–15] and the formation of PB films on the surface of noble metals or glassy carbon has been extensively investigated [12–15]. Nevertheless, there are only a few studies on the formation of PB on conducting electroactive polymer surfaces [9,10] and no detailed formation mechanism of PB has been described [16–22]. Since noble metals/glassy carbon and conducting electroactive polymers are very different in chemical and electrochemical nature, therefore, the deposition of PB onto these substrates may occur via different deposition mechanisms.

In this work, the electrochemical quartz crystal microbalance (EQCM) combined with various electrochemical techniques was used to investigate the mechanisms of the formation of PB film onto a polypyrrole coated formate biosensor electrode. The formate biosensor incorporated with an enzyme (formate dehydrogenase (FDH)), and a co-factor (β -nicotinamide adenine dinucleotide (NAD)) was used as a test case to study the activity of PB as an electron transfer mediator.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade, unless otherwise specified. All solutions were prepared with Milli-Q water (Millipore). Formate dehydroge-

nase (EC 1.2.1.2., F-8649) (FDH), β -nicotinamide adenine dinucleotide (N-7004) (β -NAD) and pyrrole were obtained from Sigma Chemical Co. All other chemicals were supplied by AJAX Chemical Pty Ltd.

2.2. Preparation of polypyrrole-based electrodes

Polypyrrole-based enzyme electrodes were prepared by galvanostatic polymerisation of pyrrole monomer from aqueous solution onto a gold substrate with a surface area of 7.0 mm^2 . A three-electrode cell, consisting of a working electrode, a coiled platinum auxiliary electrode and a Ag/AgCl (3 M NaCl) reference electrode was used. The working electrode was polished with $0.3\text{ }\mu\text{m}$ alumina, and then ultrasonicated for 5 min to remove any residue. Gold-coated quartz crystals (10 MHz, AT-cut) supplied by ICM (USA) were also used as working electrode (0.20 cm^2) for EQCM studies. Monomer solution containing 0.30 M pyrrole, 6 units/ml FDH and 5 mM β -NAD was used with a chosen applied current density of 0.20 mA/cm^2 to achieve electropolymerisation. Electrodeposition of Prussian blue (PB) onto the polypyrrole (PPy) layer was achieved potentiostatically or galvanostatically.

2.3. Electrochemical measurements

Cyclic voltammetric and amperometric experiments were carried out using a BAS CV-27 and a BAS LC-4C in conjunction with a MacLab[®]. A home-made QCM unit was used to measure the frequency responses. The output of the QCM unit is the frequency change and is directly proportional to the mass change of the working electrode. All experiments were performed in a mixture of 0.1 M KCl and 0.01 M phosphate buffer (pH 7.0), unless indicated otherwise.

3. Results and discussion

3.1. Formation of Prussian blue films

Deposition of PB on gold substrate electrode at a constant potential of +0.40 V was investigated using the EQCM (Fig. 1). A transient current response was observed within the first second of the potential application followed by a gradual increase in the cathodic current, then saturated. The current increase after the

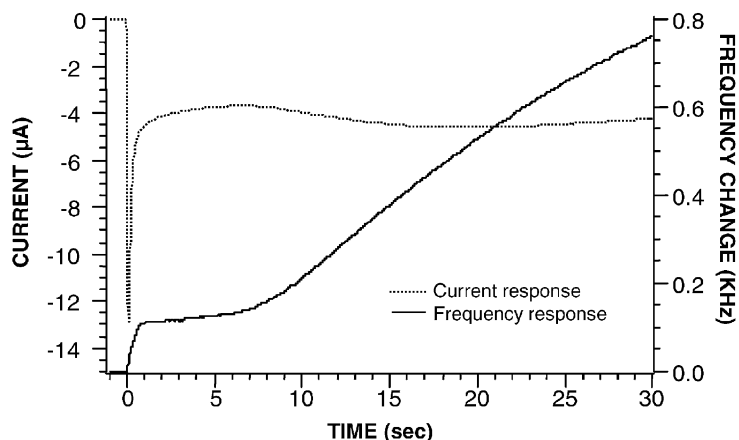


Fig. 1. Deposition of PB potentiostatically onto a gold substrate at a constant potential of +0.40 V vs. Ag/AgCl for 30 s from a solution containing 1.0 mM $K_3[Fe(CN)_6]$, 1.0 mM $FeCl_3$, 3.0 mM HCl and 1.0 M KCl.

transition period was due to the three-dimensional growth of a PB crystal on the electrode surface. This current response indicated that a typical nucleation/deposition process had occurred. The frequency response in Fig. 1 was sharply increased initially due to the absorption of negatively charged species onto the electrode surface. This was followed by a slow increase in the frequency response resulting from a slow nucleation process. Once the nucleation process was initiated, the frequency response increased almost linearly with time indicating the deposition of PB on the electrode surface. The current and

frequency responses match well and both indicate a slow nucleation process.

The deposition of a PB film onto the PPy-FDH/ β -NAD coated gold electrode was subsequently investigated (Fig. 2). In this case, the characteristics of the current response obtained were similar to the case where PB was deposited onto bare Au (see Fig. 1), except the magnitude of the transient current observed was much greater due to the large capacitance of the PPy layer. The frequency response observed increased linearly with time immediately after application of the potential. This was unlike the case of deposition of

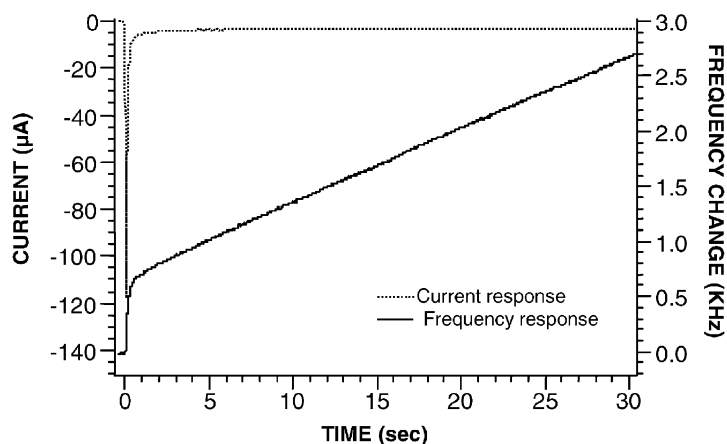
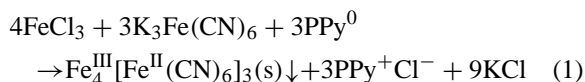


Fig. 2. Deposition of PB potentiostatically onto a PPy-FDH/ β -NAD coated Au electrode at a constant potential of +0.40 V vs. Ag/AgCl for 30 s from a solution containing 1.0 mM $K_3[Fe(CN)_6]$, 1.0 mM $FeCl_3$, 3.0 mM HCl and 1.0 M KCl.

PB directly onto a gold substrate electrode where involved a slow frequency increasing period resulted from a slow nucleation process (Fig. 1). It was also found that the value of the frequency change observed in the presence of PPy layer was doubled in comparison with the case of bare Au electrode indicating high deposition efficiency of PB on PPy surface. The enhancement of PB deposition on the PPy coated electrode surface may be attributed to that the polypyrrole plays an active role in facilitating the nucleation process and/or the increasing electrode surface area due to the polymer deposition.

Further investigations revealed that deposition of PB on top of the PPy layer was possible even without application of an external potential. The frequency changes were observed when the PPy coated electrode was simply immersed into a solution containing 1 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, 1 mM FeCl_3 , 3 mM HCl and 1 M KCl (Fig. 3). It was found that the frequency response increased almost linearly with time. The rate of deposition was about the same as the rate of deposition when a constant potential of +0.40 V was applied to the gold electrode (see Fig. 1). This result shows that PB can be chemically deposited onto polypyrrole surface. It is well known that polypyrrole is a redox polymer with an open-circuit potential between +0.3 and +0.45 V (versus a Ag/AgCl reference electrode) depending on the ratio of oxidised from (PPy^+) and neutral form (PPy^0) of polypyrrole. Given that $\text{FeCl}_3/\text{K}_3[\text{Fe}(\text{CN})_6]$ solution is a strong

oxidative solution with an open-circuit potential greater than +0.8 V versus a Ag/AgCl , a spontaneous reaction will occur when PPy is immersed in such a solution. As a result, PPy^0 is oxidised to PPy^+ and the insoluble PB, $\text{Fe}_4^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]_3$, is formed (see Eq. (1))



This result also explains the enhanced electrochemical deposition of PB on the PPy coated electrode where the enhancement was due to the combined contributions of two deposition processes (i.e. chemical and electrochemical).

3.2. Electrochemical behaviour

The electrochemical behaviour of the PB film was investigated using EQCM combined with cyclic voltammetry. Two pairs of redox peaks were observed when PB is subjected to a potential scan between −0.2 and +1.2 V [23]. It was predicted previously that these redox processes are accompanied by cation or anion movement [13,14,23].

The first pair of redox peaks around +0.2 V is due to the redox reaction of high-spin $\text{Fe}^{\text{III}}/\text{II}$.

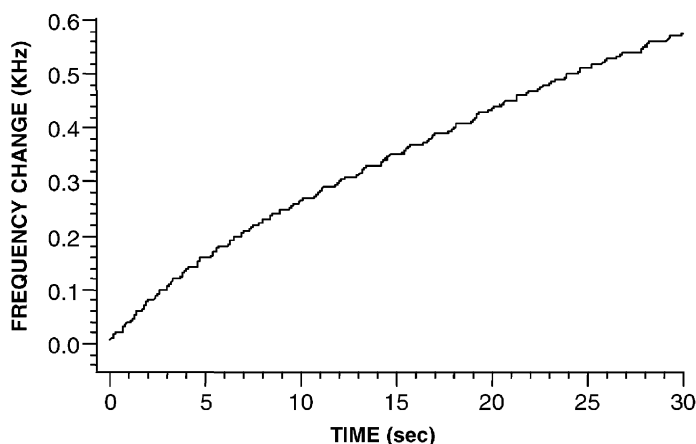
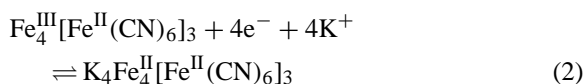
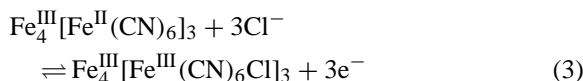


Fig. 3. Chemical (electroless) deposition of PB onto a PPy-FDH/ β -NAD coated Au electrode from a solution containing 1.0 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$, 1.0 mM FeCl_3 , 3.0 mM HCl and 1.0 M KCl .

This redox process involves cation movement in/out of the PB matrix [13,14,23,24]. The second pair of redox peaks around +0.9 V is attributed to the redox reactions of low spin $\text{Fe}(\text{CN})_6^{3-/2-}$ and is accompanied by anions moving in and out of PB matrix [23,24].



In this work, we will focus only on the first redox pair because the catalytic properties of this redox pair is normally the one of interest in analytical applications.

Fig. 4 shows the current and the frequency responses observed during oxidation/reduction of the PB film deposited on a gold electrode. A pair of reversible current peaks ($E_{\text{pa}} = +0.20$ and $E_{\text{pc}} = +0.15$ V) was observed. This is the same as previously reported [14,16,23]. The frequency response during the anodic potential scan shows a sharp decrease within the potential range of the anodic peak (+0.15 to +0.25 V) indicating a decrease in the mass of the electrode. This was due to K^+ moving out of the PB matrix during oxidation of the high-spin Fe^{II} (see Eq. (2)). During the cathodic potential scan, a sharply increasing frequency response was observed within the potential range of the cathodic peak (+0.25 to +0.04 V) indicating an increase in the mass of the electrode. This can be attributed to the K^+ moving into the PB matrix during the reduction of high-spin Fe^{III} (see Eq. (2)). This in situ EQCM result proves the previous

prediction of ions movement accompanying the redox state changes of PB films.

The electrochemical behaviour of the PB film deposited on the conducting polymer surface was also investigated. The layered structure electrode with PB exposed to solution was considered (Fig. 5). The current response observed during the first redox cycle showed the characteristics of both PPy and PB Fig. 5a). It is noticeable that the cathodic current peak of PB shifted to a more negative potential ($E_{\text{pc}} = 0.0$ V) in comparison with the case of PB on a Au electrode ($E_{\text{pc}} = +0.15$ V). This was due to the increase in the resistance caused by the reduction of polypyrrole [7]. The current response lost PB characteristics after 20 successive potential cycles Fig. 5b) indicating the loss of PB from the electrode.

The frequency (mass) change observed during the first redox cycle was predominant by the ion movement associated with PB oxidation/reduction. A sharp decrease in the frequency response corresponding to the range of anodic current peak of PB was due to the cation expulsion during the oxidation of high-spin Fe^{II} Eq. (2). The sharp increase in the frequency response within the cathodic current peak range was resulted from the cation incorporation accompanied with reduction of high-spin Fe^{III} Eq. (2). After 20 successive potential cycles, the mass change resulted from PB redox reaction was no longer obvious indicating the loss of PB from the electrode. The increase/decrease in frequency (mass) is consistent with cation/anion

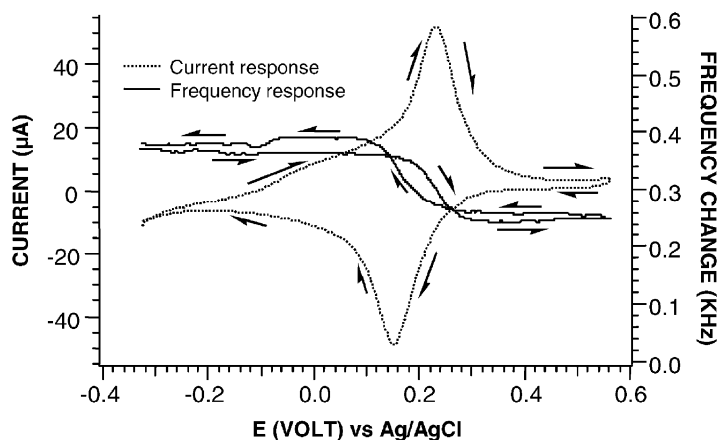


Fig. 4. The current and frequency responses of PB coated Au electrode obtained from a cyclic voltammetric experiment in a solution containing 0.1 M KCl and 0.01 M phosphate buffer. Scan rate: 50 mV/s.

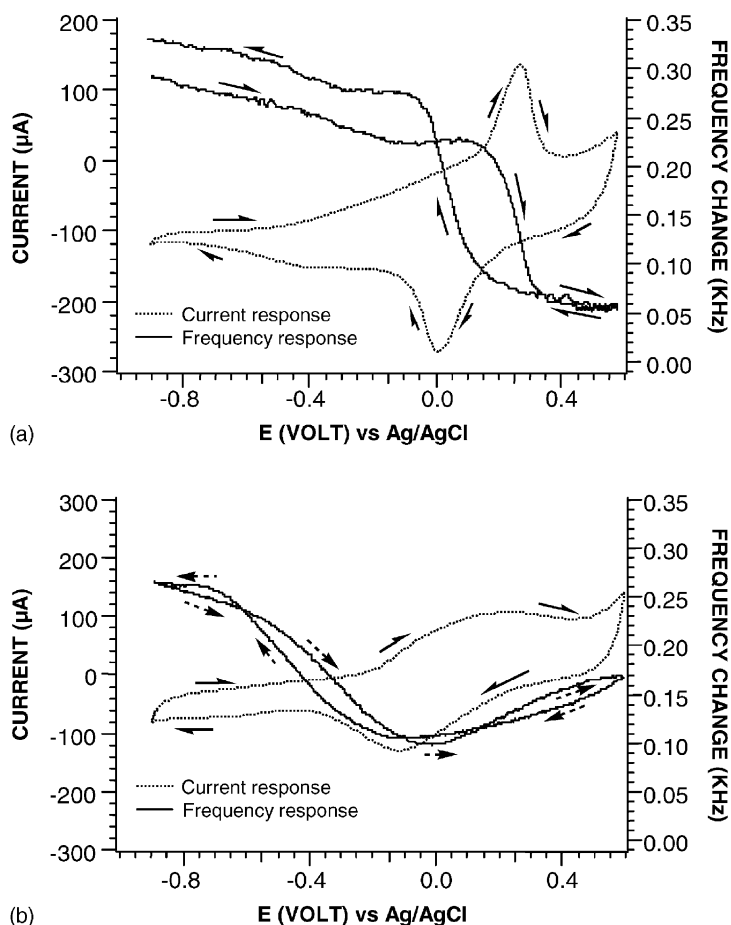
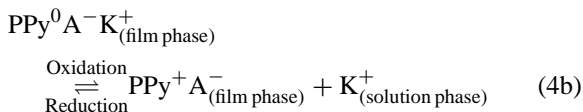
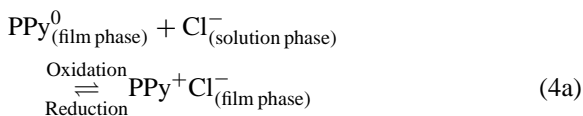


Fig. 5. The current and frequency responses of PB-PPy-FDH/NAD-Au bilayer electrode obtained from a cyclic voltammetric experiment in a solution containing 0.1 M KCl and 0.01 M phosphate buffer. Scan rate 50 mV/s. (a) The 1st potential cycle and (b) 20th potential cycle.

incorporation/expulsion upon reduction/oxidation of PPy Eqs. (4a) and (4b) [25].



where A^- is an anion in the solution.

The loss of PB from the polymer surface may be due to the fact that the water-insoluble PB, $\text{Fe}_4^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]_3$, was reduced to $\text{K}_4\text{Fe}_4^{\text{II}}[\text{Fe}^{\text{II}}(\text{CN})_6]_3$,

Prussian white, which is slightly soluble in 0.1 M KCl/0.01 M phosphate buffer (pH 7.0) [26,27].

The layered structure with PB deposited on gold first was then considered. The electrode was prepared by depositing a PB film first and then followed by a PPy-FDH/NAD film on the top. We expect this will protect water-soluble, $\text{K}_4\text{Fe}_4^{\text{II}}[\text{Fe}^{\text{II}}(\text{CN})_6]_3$, from leaching out of the membrane. Fig. 6 shows a little change in both current and frequency responses after 20 successive potential cycles indicating the loss of PB was minimised. It is important to note that with this configuration, both electrochemical characteristics of PB and polypyrrole were detected in the voltammograms by the current and frequency responses. The deposition of the PB film under the PPy-FDH/NAD



(b)

on a Au electrode and a Ppy-FDH/NAD layer was formed on the top of PB film. The detection principle of this PB mediator electrode can be described as:



As discussed previously, the high-spin iron (II) ions of Prussian blue can be oxidised at about +0.2 V. Steady-state current measurements for formate were therefore recorded at +0.4 V versus Ag/AgCl. The amperometric response was measured after 9 h of equilibration period to minimise the charging current

In order to evaluate the catalytic activity of PB as an electron mediator, a layered structure formate biosensor electrode was constructed. PB film was deposited

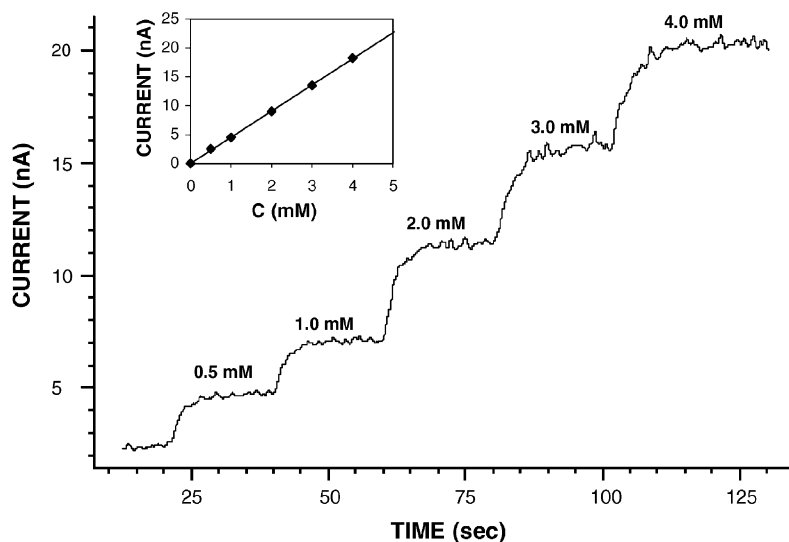


Fig. 7. Amperometric response of a PB-PPy-based formate biosensor in 0.1 M KCl and 0.01 M phosphate buffer in a stationary cell. Applied potential: +0.40 V vs. Ag/AgCl. The insertion is the calibration curve.

generated by polypyrrole. Fig. 7 shows a typical chronoamperogram of various concentrations of formate with the PPy-FDH/NAD-PB-Au electrode. The current increased linearly with increasing the concentration of formate indicating catalytic activities of PB.

Under the optimum conditions, the calibration curve obtained showed a linear concentration range up to 19 mM and the detection limit was 60 μ M with $S/N = 3$. A reproducibility of 2.3% R.S.D. was obtained from nine replicated injection of 1 mM formate. The stability of the electrode was also examined. The results revealed the sensitivity decreased by 70% during the operational stability test over 4 days period. However, the cyclic voltammetric responses obtained after 4 days of stability test showed less than 15% decrease in peak currents of PB suggesting the loss of sensitivity was due to the loss and/or denaturation of the enzyme and co-factor.

4. Conclusions

Two formation mechanisms of PB on polypyrrole surface have been identified and explained by EQCM technique. Detailed investigation of electrochemical behaviours of PB-PPy electrode, in particular, the

ionic species movements accompanying the redox processes have also been characterised using EQCM technique. The catalytic activities of Prussian blue as an electron mediator has been demonstrated by a layered structure amperometric biosensor for detection of formate.

References

- [1] M. Umana, J. Waller, *Anal. Chem.* 58 (1986) 2979.
- [2] H. Shinohara, T. Chiba, M. Aizawa, *Sens. Actuators* 13 (1988) 79.
- [3] S.B. Adeloju, S.J. Shaw, G.G. Wallace, *Electroanalysis* 6 (1994) 865.
- [4] S.B. Adeloju, J.N. Barisci, G.G. Wallace, *Anal. Chim. Acta* 332 (1996) 145.
- [5] G.G. Wallace, *Chem. Br.* November (1993) 967.
- [6] O.A. Sadik, G.G. Wallace, *Anal. Chim. Acta* 279 (1993) 209.
- [7] G.G. Wallace, M. Smyth, H. Zhao, *Trend Anal. Chem.* 18 (1999) 245.
- [8] A.A. Karyakin, E.E. Karyakina, L. Gorton, *Talanta* 43 (1996) 1597.
- [9] A.A. Karyakin, M.F. Chaplin, *J. Electroanal. Chem.* 370 (1994) 301.
- [10] A.A. Karyakin, O.V. Gitelmacher, E.E. Karyakin, *Anal. Chem.* 67 (1995) 2419.
- [11] Q. Chi, S. Dong, *Anal. Chim. Acta* 310 (1995) 429.
- [12] J.A. Cox, R.K. Jaworski, P.J. Kulesza, *Electroanalysis* 3 (1991) 869.

- [13] V.D. Neff, *Electrochem. Soc.* 125 (1978) 886.
- [14] D. Ellis, M. Eckhoff, V.D. Neff, *J. Phys. Chem.* 85 (1981) 1225.
- [15] V.D. Neff, *J. Electrochem. Soc.* 132 (1985) 1382.
- [16] W. Lu, G.G. Wallace, A.A. Karyakin, *Electroanalysis* 10 (1998) 472.
- [17] K. Ogura, M. Higasa, J. Yano, N. Endo, *J. Electroanal. Chem.* 379 (1994) 373.
- [18] K. Ogura, N. Endo, M. Nakayama, H. Ootsuka, *J. Electrochem. Soc.* 142 (1995) 4026.
- [19] L.D. Couves, S.J. Porter, *Synth. Met.* 28 (1989) C761.
- [20] M. Morita, *J. Appl. Polym. Sci.* 52 (1994) 711.
- [21] J.J. Garciajareno, J. Navarrolaboulais, F. Vicente, *Electrochim. Acta* 41 (1996) 2675.
- [22] R.J. Mortimer, *J. Electroanal. Chem.* 397 (1995) 79.
- [23] K. Itaya, H. Akahoshi, S. Toshima, *J. Electrochem. Soc., Electrochem. Sci. Tech.* 129 (1982) 1498.
- [24] K. Itaya, T. Ataka, S. Toshima, *J. Am. Chem. Soc.* 104 (1982) 4767.
- [25] H. Zhao, W.E. Price, G.G. Wallace, *J. Membr. Sci.* 148 (1998) 161.
- [26] A. Ludi, H.U. Gudel, *Struct. Bond. (Ber.)* 14 (1973) 1.
- [27] M.B. Robin, P. Day, *Adv. Inorg. Chem. Radiochem.* 10 (1967) 247.