Mechanically Immobilized Copper Hexacyanoferrate Modified Electrode for Electrocatalysis and Amperometric Determination of Glutathione

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A new copper hexacyanoferrate modified electrode was constructed by mechanical immobilization. The modified electrode was characterised by cyclic voltammetric experiments. Electrocatalytic oxidation of glutathione was effective at the modified electrode at a significantly reduced overpotential and at broader pH range. The modified electrode shows a stable and linear response in the concentration range of 9×10^{-5} to 9.9×10^{-4} M with a correlation coefficient of 0.9995. The modified electrode exhibits excellent stability, reproducibility and rapid response and can be used in flow injection analysis for the determination of glutathione.

Keywords : Modified electrode, Mechanical immobilization, Glutathione, Copper hexacyanoferrate.

Introduction

Recent innovations are expected to provide the necessary improvements in convenience, cost, turnout time and user friendly methodology and to have a major impact on science and technology in analysis of the compounds of biological and environmental interest. Among the different analytical approaches, one field that offers great potential is chemically modified electrodes (CMEs).[1-4] The development of CMEs by insoluble metal hexacyanoferrates have attracted wide spread attention due to their interesting chemical and electrochemical characteristics.[4-6] Among them, copper hexacyanoferrate (CuHCF) has received considerable interest in electroanalysis due to its remarkable electrocatalytic property.[7,8] It is an ion conductor with redox active sites. The oxidation and reduction of its redox sites can proceed without dissolution of the solid compounds. These interesting features motivated the construction of modified electrode by mechanical immobilization.[9] Recent developments in the qualitative and quantitative electroanalysis of solid compounds by means of mechanical immobilization have opened a new range of possibilities for the construction of electrochemical sensors.[10-13]

In the present study, a CuHCF modified electrode was constructed by mechanical immobilization. The analytical applicability of the modified electrode was evaluated for the determination of glutathione. Glutathione (γ-glutamylcysteinylglycine, GSH) is a physiologically important aqueous antioxidant, capable of scavenging oxygen-derived free radicals, which are thought to contribute to the development of many common diseases including cancer, heart attack and stroke.[14,15] Thus there is always an increasing demand for simple and reliable sensor for determination of GSH. A variety of electroanalytical methods have been developed for the determination of GSH.[2-4] GSH requires higher overpotential for electrooxidation at conventional electrodes. Consequently their electrochemical quantification following liquid chromatography is usually performed at the mercury[16] or mercury amalgam[17] electrodes at which the mercury sulphide formed can be oxidised at a comparatively low potential. However, methods based on mercury electrodes may be undesirable either because of their possible toxicity or because of rapid deteriroration of the electrode response. Halber and Baldwin have developed a carbon paste electrode containing cobalt phthalocyanine to decrease the overpotential for the determination of GSH.[18] Wring *et al.* demonstrated the determination of GSH following liquid chromotography at a carbon epoxy resin composite electrode modified with cobalt phthalocyanine.[19] A Ru(III) diphenyldithiocarbamate modified carbon paste electrode was constructed in our laboratory for the monitoring of GSH and cysteine.[20] Despite the different sensors, the present sensor has the advantages of ease of fabrication, reliable sensitivity and excellent stability.

Experimental Section

Materials and Instrumentation. Glutathione was purchased from Sigma. All other chemicals were of analytical grade and were used as received. All solutions were prepared from double distilled water. The pH of the medium was adjusted with 0.05 M phosphate buffer ($K_2HPO_4 + KH_2PO_4$).

The CuHCF complex was prepared by precipitation via drop-wise addition of 0.1 M $CuNO_3$ to a well-stirred solution of 0.1 M $K_4Fe(CN)_6$ in a beaker. After the addition, the mixture was stirred for 15 minutes and kept undisturbed for one hour and the precipitate was centrifuged with repeated washing with 0.1 M KNO_3 followed by distilled water. The reddish brown precipitate formed was dried at room temperature under vacuum, which was then crushed and milled to fine crystalline powder.

Electrochemical experiments were performed with EG & G PAR electrochemical system (Model 263A) equipped with GPIP (IEEE-488) interface port and IBM personal computer. The modified electrode was used as working electrode, while Ag/AgCl (saturated KCl) and a platinum wire were used as reference and counter electrode respectively. All

measurements were carried out under an atmosphere of high purity nitrogen.

Construction of Modified Electrode. The modified electrode was constructed from paraffin impregnated grahite electrodes (PIGEs). The preparation of PIGE has been carried out by as reported elsewhere.[10] Prior to surface modification, the PIGE (3.0 mm diameter) was mechanically polished with emery paper followed by alumina slurry on a microcloth polishing pad, rinsed well with water and sonicated for one minute in doubly distilled water. The immobilization of the CuHCF mediator onto the electrode surface was effected by uniformly rubbing the PIGE onto a fine powder of the complex placed on a smooth glass plate.[10,11] The modified electrode was then rinsed well with double distilled water.

Results and Discussion

Figure 1 shows the cyclic voltammograms (CVs) of the CuHCF modified electrode in 0.1 M KNO_3 solution at the potential scan rates from 2 to 50 mV/s. A reversible redox couple with a mid-point potential ($E_{pa} + E_{pc}/2$) of ~0.7 V corresponding to $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ reaction was observed. It is noteworthy that in the entire potential range studied, there was no redox signal for copper, which bears with the witness to the extreme stability of the solid compound. However when a CuHCF complex was prepared with the 2 : 1 ratio of $Cu(NO)_3$ and $K_4Fe(CN)_6$, a redox peak corresponds to the Cu^+/Cu^{2+} reaction with a mid point potential of ~0.2 V was observed in 0.1 M NH_4Cl solution.[11] It was assumed that the copper ion responsible for this behavior is not the N-coordinated one but is present in the holes of the cubes (interstitial metal ions). The possible application of redox reaction of the interstitial copper ions for the determination of ascorbic acid was evaluated.[11] However no such behavior was observed in the present system as the complex was prepared in the 1 : 1 ratio of $Cu(NO)_3$ and $K_4Fe(CN)_6$. It was observed that the peak current of the redox couple of the CuHCF modified electrode increase with the increase of scan rates and are linearly proportional to the square root of scan rates in the range from 2 to 500 mV/s in 0.1 M KNO_3, suggesting that the redox reaction was diffusion controlled. This implies that the diffusion of electrolyte ions into and out of the CuHCF film is essential for charge compensation during redox process.

The electrochemical behaviour of the modified electrode in electrolytes of different cations and anions was studied. It was observed that the modified electrode exhibit different voltammetric behaviour in electrolytes of different cations. However there was no appreciable change in the CVs when the anions of the electrolytes were varied indicating that the cations of the electrolytes involve in the redox reaction of the CuHCF film. Figure 2 depicts the CVs of the CuHCF modified electrode in 0.1 M solutions of different cations such as NH_4^+, Na^+, Li^+, Ca^{2+} and Ba^{2+}. It was observed that the modified electrode exhibit good and stable response in electrolytes of K^+ and NH_4^+. The effect of cations on the current and potential response of the CuHCF film showed that the ability of the electrolyte cation to fit within the interior of the unit cell was the deciding factor of the peak potential and current. The length of the edge of the unit cell and the diameter of the cage for bulk CuHCF are 10.0 and 3.2 Å respectively.[7] Both the K^+ and NH_4^+ ions have hydrated diameter of 2.4 Å, and hence can easily fit well within the

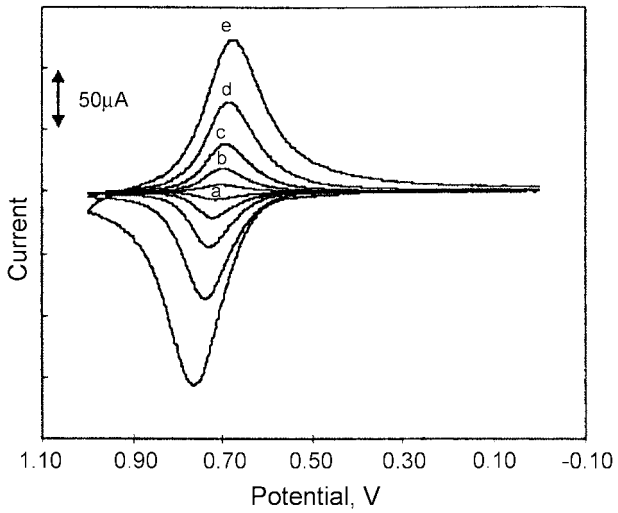

Figure 1. Cyclic voltammograms of CuHCF modified electrode in 0.1 M KNO_3 solution at different scan rates: (a) 2, (b) 5, (c) 10, (d) 20 and (e) 50 mV/s.

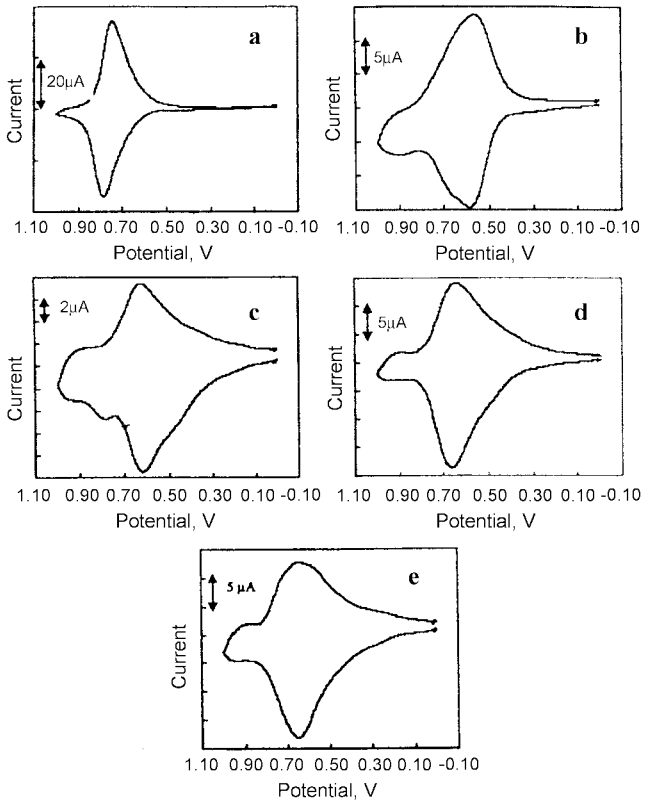

Figure 2. Cyclic voltammograms of CuHCF modified electrode in 0.1 M solution of (a) NH_4Cl, (b) NaCl, (c) LiCl, (d) $BaCl_2$ and (e) $CaCl_2$; Scan rate: 10 mV/s.

cage to allow for generation of faradic current upon electrochemical cycling. As the hydrated diameter of other cations are higher than the zeolitic cage diameter, their movement is restricted and hence the redox behaviour of CuHCF modified electrode was not well defined and also less stable in presence of other cations.

The effect of solution pH on the electrochemical behaviour of the CuHCF modified electrode was studied by cyclic voltammetry in 0.1 M KNO_3 solution at various pH values adjusted with 0.05 M phosphate buffer (figure not shown). The result indicated that the electrochemical response of the modified electrode remains almost same in the pH range between 2 to 8. A change in the CV response with reduced currents and broad peaks were observed after pHs higher than 8. The possible reason for poor response at higher pHs may be due to the hydroxylation of the CuHCF film in alkaline medium as given below:

$$Fe(CN)_6^{4-} + 2OH^- \rightarrow Fe(OH)_2 + 6CN^-$$
$$2Fe(OH)_2 + H_2O + 1/2O_2 \rightarrow 2Fe(OH)_3$$

The stability of the CuHCF modified electrode was examined by repetitive scans in 0.1 M KNO_3 solution at a scan rate of 100 mV/s. The peak current remained constant for nearly hundred cycles. In addition, the response of the modified electrode remain unchanged when stored in air or dipped in electrolyte solution for two weeks and more than 90% of the response was retained upto one month. The results indicate the remarkable stability of the CuHCF modified electrode.

To address the analytical applicability of the CuHCF modified electrode, CVs were recorded for the catalytic oxidation of GSH and the result was shown in Figure 3. The curves (a) and (c) correspond to CVs of bare and CuHCF modified electrode respectively. Curves (b) and (d) compare the typical CVs recorded for the oxidation of 8.1×10^{-4} M GSH at a bare graphite and at CuHCF modified electrode respectively. As can be seen from the figure, on scanning from 0.0 V to 1.0 V, at the bare electrode, relatively small anodic peak at higher potential was observed for the oxidation of GSH. Whereas at CuHCF modified electrode, a large enhancement in anodic current at 0.72 V with a corresponding decrease in the cathodic current was observed. The increase in current at the modified electrode in presence of GSH is due to the catalytic oxidation of GSH by the mediator. With the modified electrode a clear shift in the oxidation potential for GSH towards cathodic direction was observed compared to bare electrode. The results of reduced overpotential and increased current response are the clear evidence for the catalytic effect of the CuHCF film present on the electrode.

Oxidation of GSH on CuHCF modified electrode is a two step electrocatalytic process, initiated by the electrochemical oxidation of ferrocyanide to ferricyanide followed by the chemical oxidation of glutathione by ferricyanide and the regeneration of ferrocyanide. It was also observed that the catalytic current increases with increase of scan rates and the current display a good linearity with square root of scan rate in the range of 10 to 500 mV/s, which illustrates that the electrocatalysis was diffusion controlled.[8,11] It was suggested that GSH was diffuse to the electrode surface where it was oxidised to oxidised glutathione (GSSG) by the CuHCF film as given below:

$$Fe(CN)_6^{4-} \rightarrow Fe(CN)_6^{3-} + e^-$$
$$2Fe(CN)_6^{3-} + 2GSH \rightarrow GSSG + 2Fe(CN)_6^{4-} + 2H^+$$

The effect of pH on the electrocatalytic oxidation of GSH at the modified electrode was studied in the pH range between 2 to 9. Figure 4 shows the plot of pH versus the catalytic current observed for 1×10^{-4} M GSH oxidation with the modified electrode. It was found that the catalytic oxidation of GSH at CuHCF modified electrode occurs effectively in neutral and acidic media (pH 2-8). The reduced

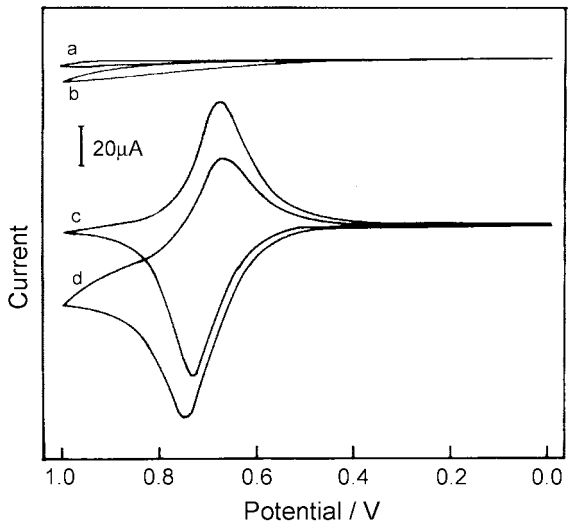

Figure 3. Cyclic voltammograms of (a) bare electrode, (b) 8.1×10^{-4} M GSH at bare electrode, (c) CuHCF modified electrode and (d) 8.1×10^{-4} M GSH at modified electrode; Supporting electrolyte : 0.1 M KNO_3; pH 6.5 (0.05 M phosphate buffer); Scan rate: 20 mV/s.

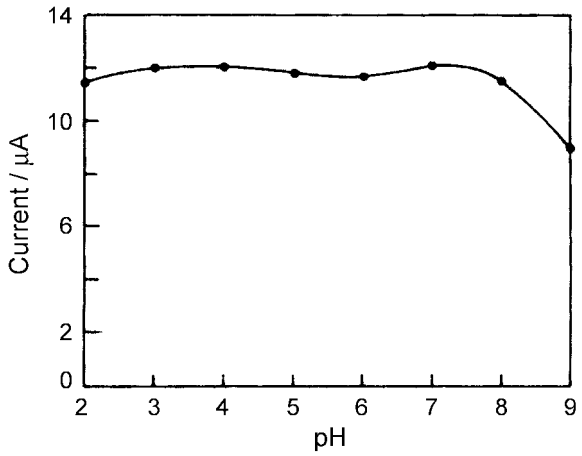

Figure 4. Effect of pH on the anodic peak current of 3.6×10^{-4} M GSH oxidation at the CuHCF modified electrode; Scan rate: 20 mV/s.

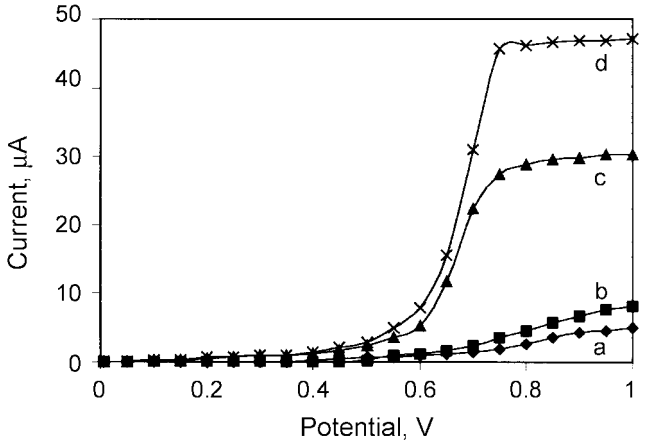

Figure 5. Hydrodynamic voltamograms in 0.1 M KNO_3 solution for (a) bare electrode, (b) 8.1×10^{-4} M GSH at bare electrode, (c) modified electrode and (d) 8.1×10^{-4} M GSH at modified electrode; pH 6.5 (0.05 M phosphate buffer); Stirring rate: 300 rpm.

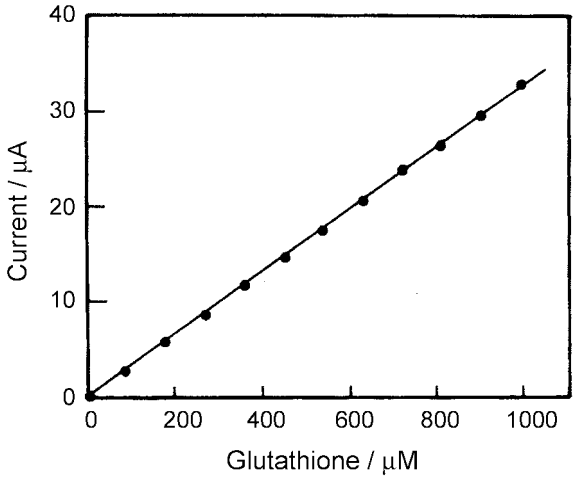

Figure 6. Calibration plot for the determination of GSH.

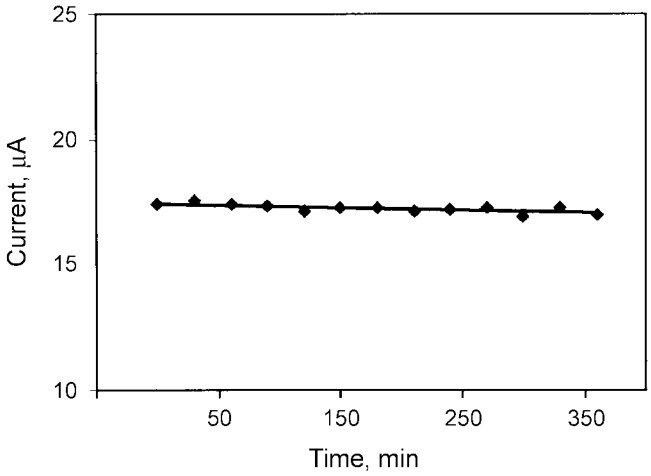

Figure 7. CuHCF modified electrode response in 0.1 M KNO_3 solution for 5.4×10^{-4} M GSH versus time; pH 6.5 (0.05 M phosphate buffer); Potential: 0.75 V.

catalytic current at higher pHs is due to the dissolution of the CuHCF film due to hydrolysis in alkaline medium as described earlier.

Hydrodynamic voltammograms (HDVs) were recorded in stirred solution of 0.1 M KNO_3 (pH 6.5, phosphate buffer) to study the effect of potential on the catalytic oxidation of GSH. In Figure 5, the curve (a) and (c) corresponds to HDVs of bare and modified electrode without GSH and the curves (b) and (d) corresponds to HDVs obtained in presence of 8.1×10^{-4} M GSH. It was noticed that with the modified electrode, the catalytic current increases when the potential was increased from 0.0. V to 1.0 V with a plateau at 0.75 V, so we select this as the working potential for GSH determination. As expected, a poor response for GSH oxidation was observed with the unmodified electrode, which offers detection of the GSH only at more positive potentials with lesser sensitivity. In contrast, the electrocatalytic action of the CuHCF permits convenient detection of GSH at lower potentials with high sensitivity.

Figure 6 shows the calibration plot for the determination of GSH with the modified electrode. A linear response for the GSH in the concentration range from 9×10^{-5} to 9.9×10^{-4} M with a correlation coefficient of 0.9995 was observed. To study the precision of the method, 10 successive measurements of 2.0×10^{-4} M GSH was carried out and a relative standard deviation of 1.86% was found. The detection limit was 3.2×10^{-5} M GSH (S/N=3).

The stability of the modified electrode towards GSH response was evaluated by measuring the response towards the catalytic oxidation of 5.4×10^{-4} M GSH for an extended length of six hours under hydrodynamic conditions (Figure 7). The stable response of the modified electrode shows the better confinement of the mediator at the electrode surface resulting in good long-term stability of the modified electrode, which suggests its possible application in flow systems.

An attempt was made to use the modified electrode for the determination of GSH in blood samples. It showed good sensitive response to GSH in the linear range mentioned. However ascorbic acid and other antioxidants were found to interfere with the determination of GSH. Hence for blood sample analysis, prior separation by HPLC is essential. Number of reports with good sensitivity for the determination of GSH were reported.[3,4,19] Halbert and Baldwin reported a detection limit of 3.7 pmol for GSH using cobalt phthalocyanine modified carbon paste electrode.[18] A detection limit of 10 ng for GSH determination was demonstrated by Hou and Wang using Nafion coated prussian blue modified electrode.[21] A Ru(III)Diphenyl dithiocarbamate modified carbon paste electrode constructed in our laboratory showed a detection limit of 15 ppm.[20] Though the sensitivity is little less compared to the previous reports, the simplicity and stability of the present sensor make it more attractive. Moreover there are possibilities to enhance the sensitivity of the sensor by altering the experimental factors such as the electrode area and the amount of mediator.

Conclusion

In this work we have demonstrated a novel amperometric sensor for glutathione determination based on mechanically immobilized CuHCF modified electrode. Its performance rests on a number of features: the good electrochemical properties of CuHCF, the low solubility of both oxidised and reduced forms of CuHCF resulting in the effective confinement of the mediator on the electrode surface. These features combine to give an electrode which permits rapid and reproducible analysis of GSH in solution. The modified electrode exhibit high stability, sensitivity, portability and simple to use. The hydrodynamic voltammetry result suggests that the modified electrode could be developed as a useful sensor for the on-line monitoring of glutathione in flow systems.

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References

1. Murray, R.W. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1984; Vol. 13, p 191.
2. Baldwin, R. P.; Thomsen, K. N. *Talanta* **1991**, *38*, 1.
3. Wring, S. A.; Hart, J. P. *Analyst* **1992**, *117*, 1215.
4. Cox, J. A.; Tess, M. E.; Cummings, T. E. *Rev. Anal. Chem*. **1996**, *15*, 173.
5. Krishnan, V.; Xidis, A. L.; Neff, V. D. *Anal. Chim. Acta* **1990**, *239*, 7.
6. Lin, C.; Bocarsly, A. B. *J. Electroanal. Chem*. **1991**, *300*, 325.
7. Siperko, L. M.; Kuwana, T. *Electrochim. Acta* **1987**, *32*, 765.
8. Zhou, J.; Wang, E. *Electroanalysis* **1994**, *6*, 29.
9. Scholz, F.; Meyer, B. In *Electroanalytical Chemistry: A Series of Advances*; Bard, A. J., Ed.; Marcel Dekker: New York, 1998; p 1.
10. Shankaran, D. R.; Narayanan, S. S. *Fresenius J. Anal. Chem*. **1999**, *364*, 686.
11. Narayanan, S. S.; Scholz, F. *Electroanalysis* **1999**, *11*, 465.
12. Kulesza, P. J.; Malik, M. A.; Berrettoni, M.; Giorgetti, M.; Zamponi, S.; Schmidt, R.; Marassi, R. *J. Phys. Chem. B* **1998**, *102*, 1870.
13. Shankaran, D. R.; Narayanan, S. S. *Sensors and Actuators B* **1999**, *55*, 191.
14. Meister, A. *J. Biol. Chem*. **1988**, *263*, 17205.
15. Adams, J. D.; Johannessen, J. N.; Bacon, J. P. *Clin. Chem*. **1987**, *33*, 1675.
16. Rabenstein, D. L.; Saetre, R. *Anal. Chem*. **1977**, *49*, 1036.
17. Allison, L. A.; Shoup, R. E. *Anal. Chem*. **1983**, *55*, 8.
18. Halbert, M. K.; Baldwin, R. P. *Anal. Chem*. **1985**, *345*, 43.
19. Wring, S. A.; Hart, J. P.; Birch, B. J. *Analyst* **1989**, *114*, 1563.
20. Nalini, B.; Narayanan, S. S. *Electroanalysis* **1998**, *10*, 779.
21. Hou, W.; Wang, E. *J. Electroanal. Chem*. **1991**, *316*, 155.