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Amperometric Sensor for Glutathione Based on a Mechanically Immobilized Cobalt Hexacyanoferrate Modified Electrode

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A mechanically immobilized cobalt hexacyanoferrate modified electrode has been constructed. The modified electrode exhibits two reversible redox peaks in a 0.1 M NaCl medium, and can be used for the amperometric determination of glutathione. The modified electrode facilitates the catalytic oxidation of glutathione at a reduced overpotential compared with an unmodified graphite electrode. Glutathione was determined in the range of 4×10^{-5} to 5.2×10^{-4} M in 0.1 M NaCl and in 0.1 M KCl with correlation coefficients of 0.9991 and 0.9992, respectively. Relative standard deviations of 2.88% in NaCl and 3.27% in KCl were obtained for 4×10^{-4} M GSH ($n = 10$). The sensor exhibited excellent stability, reproducibility and immediate response, and can be used in flow-injection analysis.

The increasing use of sensors in the field of environmental, agricultural and medicinal analysis has encouraged analytical chemists to develop new sensors for fast, accurate, reproducible and selective determinations of important compounds. During the past two decades, considerable attention has been paid to the construction and application of chemically modified electrodes (CMEs), because they are used to improve the sensitivity and selectivity of electroanalysis. Immobilization of mediators on the electrode surface is a key process for preparing modified electrodes. There are a variety of techniques which allow the production of modified electrodes, ranging from simple chemisorption to a recently emerged sol-gel technique, and have led to the preparation of a variety of modified electrodes for various applications.[1–16] Among the techniques of incorporating electroactive species on conducting surfaces, the process of mechanical immobilization has gained considerable attention in recent years.[17–19] With this simple approach, we have constructed copper and nickel hexacyanoferrate modified electrodes for the determination of sulphur dioxide,[20] ascorbic acid,[21] cysteine[22] and thiosulphate.[23]

Despite the different mediators used for electrode modification, solid metal hexacyanoferrates have received considerable interest owing to their interesting chemical and electrochemical properties. Among the hexacyanoferrates, cobalt hexacyanoferrate (CoHCF) is an important member having similar characteristics as that of prussian blue (PB). It is a mixed-valence polymer with a zeolitic structure. Oxidation and reduction can proceed through a solid compound because the zeolitic structure allows a flux of cations to go through its channels and holes. A number of studies on CoHCF-modified electrodes based on spectroelectrochemistry have been reported.[24–27] However, reports on the two reversible peaks have been rather limited. Gomathi et. al. studied the cyclic voltammetric behavior of an electrochemically coated CoHCF electrode at different electrolytes.[28] Golabi and Mohammadi reported a CoHCF-modified electrode for the oxidation of hydrazine.[29] In the present work a CoHCF-modified electrode was constructed by mechanical immobilization using a paraffin-impregnated graphite electrode (PIGE), and was found have similar characteristics to those reported.[28,29] The modified electrode was then applied to the determination of glutathione (GSH). GSH is a tripeptide having a potential role in human metabolism. Although a number of reports on amperometric determination of GSH have appeared in the literature,[5,15,30,31] methods based on mechanically immobilized modified electrodes have not been reported so far. Despite different studies reported for GSH determination, the present method was found to be simple, reliable and promising.

Experimental

Chemicals and Apparatus. GSH was purchased from Sigma. All other chemicals were of analytical grade. All solutions were prepared with doubly distilled water. High-purity nitrogen was used for deaeration.

All electrochemical measurements were performed with a Potentiostat/Galvanostat Model 263A (EG & G PAR, USA) equipped with an IBM Personal Computer. All experiments were carried out with a conventional three-electrode system with a CoHCF-modified PIGE as the working electrode, a platinum wire as an auxiliary electrode and Ag/AgCl (saturated KCl) as a reference electrode.

Preparation of CoHCF Complex. The CoHCF complex was prepared by precipitation via the drop-wise addition of 0.1 M $Co(NO_3)_2$ to a well-stirred solution of 0.1 M $K_4Fe(CN)_6$ in a beaker. After the addition, the mixture was stirred well for 15 minutes and kept undisturbed for one hour; the precipitate was then centrifuged with repeated washing with 0.1 M KNO_3, followed by distilled water. An olive-brown CoHCF precipitate which formed was dried at room temperature under a vacuum, which was then crushed and milled to a fine crystalline powder.

Construction of a Modified Electrode. A modified electrode was prepared from PIGEs. The PIGEs were prepared by immersing graphite rods (0.3 cm circular diameter, 4.0 cm length) into molten paraffin wax under a vacuum until air bubbles ceased to evolve from the rods. After re-establishing atmospheric pressure, the rods were removed before the paraffin solidified. One end of the electrode was carefully polished on a smooth surface and with 0.5 μm alumina, washed with distilled water and dried in air.[17] The modified electrode was constructed by uniformly pressing and rubbing the polished surface of the PIGE onto a fine powder of CoHCF complex placed on a smooth glass plate. The resulting modified electrode with micro amounts of CoHCF on the surface was rinsed well in double distilled water and dried in air.

Results and Discussion

Figure 1 shows cyclic voltammograms of the CoHCF-modified electrode obtained in 0.1 M solutions of different cations, such as Na^+, K^+, NH_4^+, Li^+, Ba^{2+} and Ca^{2+}, at a potential scan rate of 10 mV/s. The figure shows that the number and position of the redox peaks vary with the nature of the cation of the supporting electrolyte. In contrast, the cyclic voltammograms of the modified electrode were not affected by the different types of anions of the supporting electrolytes (figure not shown). This behavior clearly indicates that the electrolyte cation should be responsible for maintaining the electroneutrality of the electrode surface, thus allowing redox reactions. In a NaCl medium, the cyclic voltammogram exhibits two sets of reversible redox peaks with formal potentials ($E_m = [(E_{p,a} + E_{p,c})/2]$) of about 0.34 V and 0.83 V. The first peak at a lower potential was assigned to the Co^{2+} / Co^{3+} transition by analogy with the case of a PB-modified electrode.[32,33] This assumption is also supported by reports of a cobalt oxidation peak observed in cobalt phthalocyanine and cobalt porphyrins.[34,35] The redox peak at a higher potential was assigned to the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ reaction by comparing the redox potentials of a low-spin iron transition in PB. A similar observation was reported for a CoHCF-modified electrode constructed by electrochemical coating.[28] In a KCl medium, the CoHCF-modified electrode exhibited two anodic peaks at potentials of 0.58 V and 0.69 V, and two cathodic peaks at 0.65 V and 0.32 V. In NH_4^+, a redox peak at a higher potential was observed. The cyclic voltammetric curves were broadened in other electrolytes. All of these phenomena were the result of cation transfer in the modified film. The peak currents in both of the reversible couples showed a linear dependence on the square root of the scan rate in the range between 5 to 200 mV/s. The square-root dependence suggests that the redox reaction is diffusion-controlled.

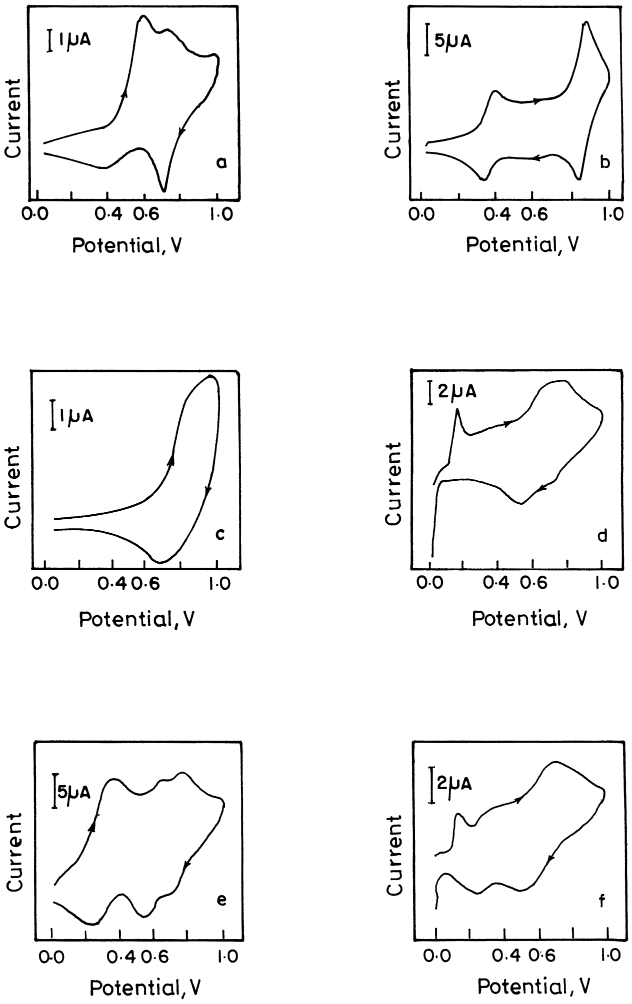

Fig. 1. Cyclic voltammograms of CoHCF modified electrode in 0.1 M solutions of (a) KCl, (b) NaCl, (c) NH_4Cl, (d) LiCl, (e) $BaCl_2$ and (f) $CaCl_2$ at 20 mV/s.

The electrochemical behaviour of the CoHCF-modified electrode depended on the concentration of the supporting electrolyte. When the concentration of NaCl changed from 1.0 to 0.01 M, both redox peaks showed a negative shift of the peak potential (0.39 V to 0.29 V for Co^{2+}/Co^{3+} and 0.87 V to 0.78 V for $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ system), and the shape of the cyclic voltammetric curves did not change greatly. Continuous scanning of the modified electrode for more than 100 cycles showed no considerable change in the peak current in both NaCl and KCl, thus suggesting its high stability during continuous use.

The performance of the CoHCF-modified electrode under different pH conditions was studied in the pH range of 2 to 12 (Fig. 2); it was found that the peak current and peak potentials did not vary much between pH 2 to 8, after which the peak current decreased and the peaks were broadened. The possible reason for the poor response at higher pH may be due to hydroxylation of the CoHCF in an alkaline medium. Hence, the

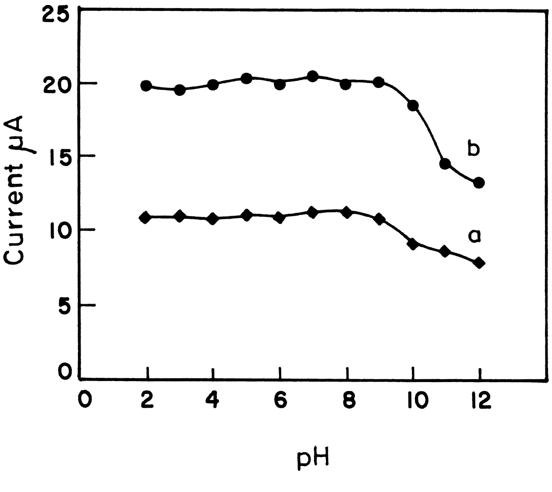

Fig. 2. Effect of pH on the anodic current of CoHCF modified electrode in 0.1 M NaCl; (a) Co^{2+}/Co^{3+} and (b) $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ redox peak; Scan rate: 20 mV/s.

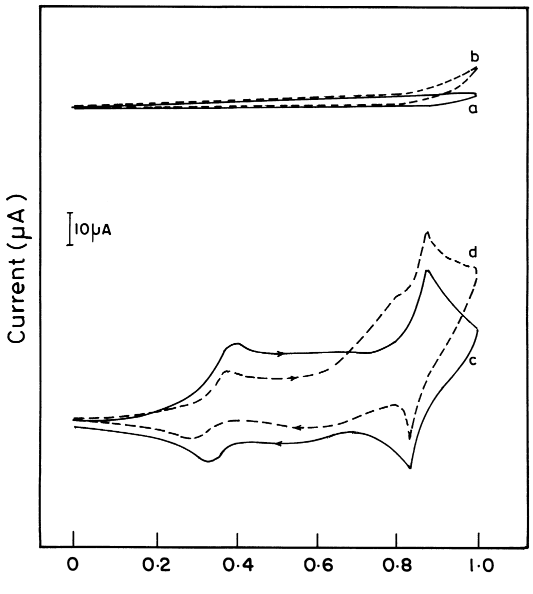

Fig. 3. Cyclic voltammograms in 0.1 M NaCl for (a) bare; (b) 4.8 × 10^{-4} M GSH at bare; (c) CoHCF modified and (d) 4.8 × 10^{-4} M GSH at CoHCF modified electrode; Scan rate: 20 mV/s; pH 6.5 (0.05 M Phosphate Buffer).

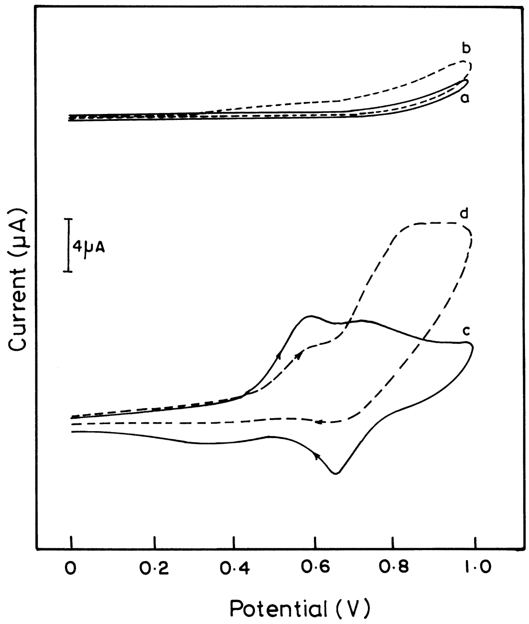

Fig. 4. Cyclic voltammograms in 0.1 M KCl for (a) bare; (b) GSH at bare; (c) CoHCF modified and (d) 4.8 × 10^{-4} M GSH at CoHCF modified electrode; Scan rate: 20 mV/s; pH 6.5 (0.05 M Phosphate Buffer).

CoHCF-modified electrode can be effectively used only under acidic and neutral conditions.

Electrocatalytic Oxidation of Glutathione. The feasibility of using a modified electrode to measure the GSH was investigated; the results are demonstrated in Fig. 3 (in 0.1 M NaCl) and Fig. 4 (in 0.1 M KCl). In both figures, curves (a) and (c) correspond to a bare and a CoHCF-modified electrode, and curves (b) and (d) correspond to GSH oxidation at a bare and at a modified electrode, respectively. It was observed that the bare electrode showed a poor response for the oxidation of GSH. However, an increase in the current response at the modified electrode was observed for GSH oxidation at 0.75 V in 0.1 M NaCl and 0.83 V in 0.1 M KCl. As can be seen from the figure, the catalytic oxidation peak was observed within the proximity of the Co^{2+}/Co^{3+} reaction (before the start of Fe(CN)$_6^{4-}$/Fe(CN)$_6^{3-}$ reaction) and, hence, the electrocatalytic oxidation reaction involves the Co^{2+}/Co^{3+} reaction, and not Fe(CN)$_6^{4-}$/Fe(CN)$_6^{3-}$. Studies on cobalt phthalocyanine and other cobalt complexes showed that the Co^{2+}/Co^{3+} reaction has been used for the electrocatalytic determination of various analytes.[31,34–36] However, the involvement of the Fe(CN)$_6^{4-}$/Fe(CN)$_6^{3-}$ reaction in electrocatalysis is uncertain in this case, though we have reported for copper and nickel hexacyanoferrates that the Fe(CN)$_6^{4-}$/Fe(CN)$_6^{3-}$ reaction is responsible for catalytic oxidation.[20–23] One possible reason may be because the Co^{2+}/Co^{3+} reaction is more favored than the Fe(CN)$_6^{4-}$/Fe(CN)$_6^{3-}$ reaction, and hence the GSH may become oxidised faster by Co^{3+} when it reaches the electrode surface. Furthermore, for each addition of GSH, an increase in the anodic current, followed by a decrease in the cathodic current, was observed, and the catalytic current was also found to be linear with the square root of the scan rate, indicating a diffusion-controlled electrocatalytic process.[31]

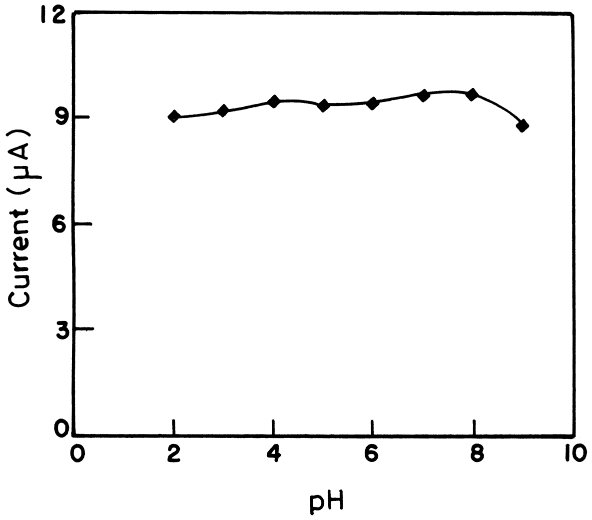

Fig. 5. Effect of pH on the anodic current for the catalytic oxidation of 4 × 10^{-4} M GSH; Electrolyte: 0.1 M NaCl; Scan rate: 20 mV/s.

The influence of the pH on the electrocatalytic oxidation of GSH was studied in a 0.1 M NaCl solution of varying pH (using HCl/NaOH); the results are shown in Fig. 5. It was found that catalytic oxidation was more favored under acidic and neutral conditions than in a basic medium.

Hydrodynamic voltammetry for GSH oxidation was performed to further investigate the analytical use of a modified electrode in flow systems; the results are shown in Fig. 6. The hydrodynamic voltammograms were registered with solution stirring, while measuring steady state currents at fixed values of the potential. A poor response to GSH oxidation was observed at a bare electrode (curve a). However, at a modified

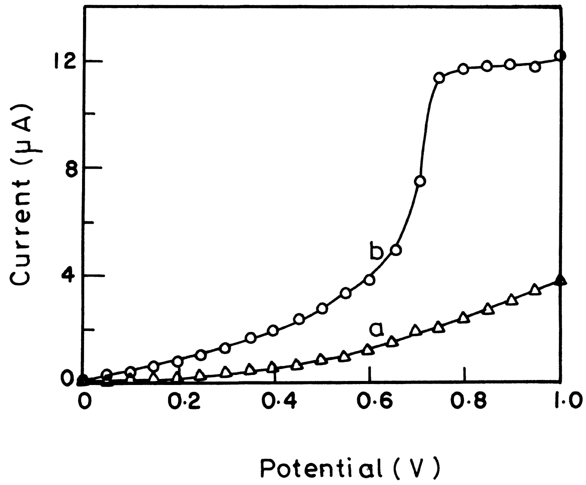

Fig. 6. Hydrodynamic voltammograms obtained in 0.1 M NaCl solution with (a) bare and (b) modified electrodes for 4.8×10^{-4} M GSH; pH 6.5; Stirring rate: 300 rpm.

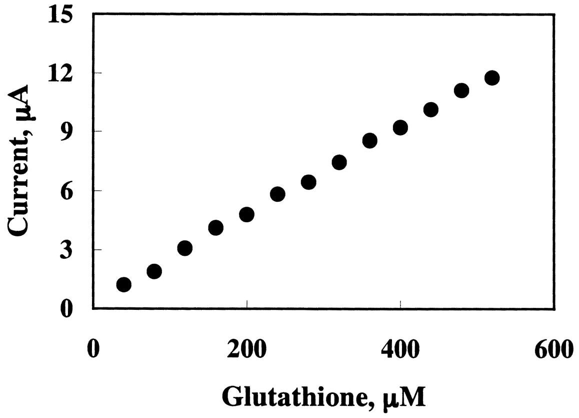

Fig. 7. Calibration plot for the determination of GSH.

electrode (curve b), an enhanced response to GSH oxidation was obtained at lower potentials, similar to that in an unstirred solution. Thus, a potential of 0.75 V can be applied for the determination of GSH using a modified electrode in flow-injection analysis. Also, because the response of the modified electrode is unaffected in a stirred condition, it is concluded that both the oxidised and reduced forms of the CoHCF film remain confined to the electrode surface, and do not appreciably dissolve or leach out into the electrolyte solution.

The modified electrode showed a linear response for GSH determination in the concentration range of 4×10^{-5} to 5.2×10^{-4} M in 0.1 M NaCl (Fig. 7) and in 0.1 M KCl with a correlation coefficient of 0.9991 and 0.9992, respectively. Relative standard deviations of 2.88% in 0.1 M NaCl and 3.27% in 0.1 M KCl were obtained for 4×10^{-4} M GSH ($n = 10$). The calculated detection limits were found to be 2.4×10^{-6} M and 2×10^{-6} M GSH in 0.1 M NaCl and 0.1 M KCl, respectively (S/N = 3). Though the determination of GSH in pico-molar concentrations has been reported based on carbon paste and electrochemically modified electrodes,[30,31,36] the advantages of the present sensor can be seen from its ease of preparation and long-time stable response for the GSH determination. No decrease in response about either peak potential or peak current was observed after the electrode was repeatedly used and stored either in air or in a 0.1 M solution of NaCl or KCl for more than 10 days.

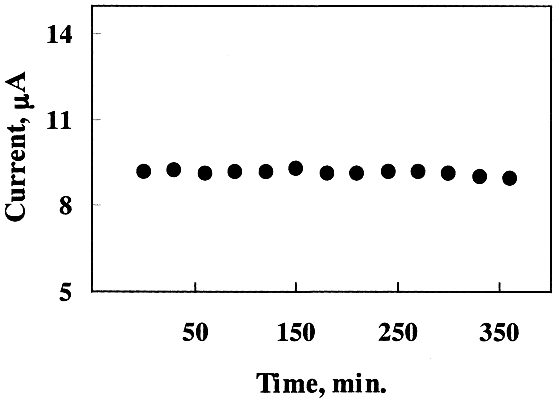

Fig. 8. CoHCF modified electrode response in 0.1 M NaCl solution for 4×10^{-4} M GSH versus time; pH 6.5; Potential: 0.75 V; Stirring rate: 300 rpm.

Figure 8 shows the result of experiments carried out to study the stability of the CoHCF-modified electrode towards the GSH response by measuring the response towards the catalytic oxidation of 4×10^{-4} M GSH for an extended length of six hours under hydrodynamic conditions. From the figure it was observed that the modified electrode exhibited good stability and retained its response for a long time. The stable response of the modified electrode reveals a stable confinement of the CoHCF on the electrode surface, which suggests its possible application to flow systems.

The proposed method has been applied to the determination of GSH in blood samples. However, the amount of GSH measured was found to be higher than that determined from spectroscopic studies. The possible reason may be due to the presence of other oxidisable compounds, like ascorbic acid. Hence, prior separation of the compounds is essential while applying this electrode for the analysis of real samples.

Conclusion

A CoHCF-modified electrode has been constructed by means of mechanical immobilization. The cyclic voltammetric characteristics of the modified electrode have been studied. The applicability of the modified electrode for the amperometric determination of GSH was evaluated. The electrochemical response of the modified electrode showed a higher current for GSH at a reduced overpotential when compared to that of an unmodified electrode. The possible application of the modified electrode in flow-injection analysis can be suggested from its stable response under hydrodynamic conditions. The results suggest that the present method is an easy, reliable and promising approach, and can be used for the determination of GSH in flow systems. For a real sample analysis, it will be necessary to couple the CME with HPLC. Efforts are being made to use the modified electrode for an HPLC system.

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