

## Chromium(III) hexacyanoferrate(II)-based chemical sensor for the cathodic determination of hydrogen peroxide

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**A cathodic scheme to measure hydrogen peroxide by utilizing a membrane free chromium(III) hexacyanoferrate(II) based chemical sensor is described. The cluster is prepared simply by generating a chromium(III) hexacyanoferrate(II) cluster electrochemically on a rotating disk glassy carbon electrode. Subsequently, the hydrogen peroxide can be measured at 0 V versus Ag/AgCl. This approach significantly reduces interferences, especially those by easy oxidizable compounds such as catechol and ascorbic acid. Also, no significant oxygen interference is observed at this working potential. Several electrolytes were carefully investigated. The calibration curve was linear up to 1.3 mM ( $r = 0.9992$ ) with a typical response of the sensor of about 5.6 s with injection of 0.05 mM hydrogen peroxide. The detection limit of this chemical sensor is  $3.0 \times 10^{-8}$  M ( $S/N = 3$ ) with a low-pass filter (time constant 0.1 s). The sensitivity of the sensor is  $11.52 \times 10^{-6}$  A  $1 \text{ mmol}^{-1} \text{ mm}^{-2}$  ( $r = 0.9992$ ). The RSD of this sensor is 1.1%.**

**Keywords:** Chromium(III) hexacyanoferrate(II)-modified electrode; hydrogen peroxide; chemical sensors

Measurement of hydrogen peroxide is of particular importance in both biomedical and environmental studies, with many applications in the plastics and food processing industries, etc. Hydrogen peroxide also is a by-product of many biological oxidative reactions and is therefore also a valuable parameter for monitoring biological reactions. Numerous schemes based on various principles have been developed for hydrogen peroxide determination, such as fluorimetric,<sup>1–3</sup> fiber-optic,<sup>4–6</sup> chemiluminescence<sup>7–10</sup> and electrochemical<sup>11–18</sup> approaches, for both liquid and air samples.

Owing to the high overvoltage nature of hydrogen peroxide and possible interferences with detection, a direct oxidative detection scheme for hydrogen peroxide in the presence of easily oxidizable interference does not seem feasible. A catalyst-modified electrode may provide an adequate solution to reducing the high overvoltage problem. Recently, Wang and co-workers<sup>19–21</sup> utilized a series of precious metals, such as Pd and Rh, to reduce the applied potential for the determination of hydrogen peroxide through the coupling of a flavin-containing oxidase.

A mixed-valence cluster is a polynuclear compound with two or more metal centers linked by a bridging ligand.<sup>22</sup> Usually, a mixed-valence compound is prepared simply by mixing two solutions of an anionic metal ion and a cationic metal ion with a ligand to form an immediate precipitate. The electrons are delocalized over the whole molecule and the proof of this electronic interaction is observed from the separation of the redox potentials of the two metal centers.

This characteristic of mixed-valence compounds, such as cobalt(III) hexacyanoferrate(II),<sup>23</sup> shows catalytic properties for

the reduction of hydrogen peroxide. Recently, Chi and Dong<sup>24</sup> and Karyakin *et al.*<sup>25</sup> developed a 'first generation' of glucose biosensors with Prussian Blue. However, such schemes are subject to interference from either ascorbic acid or oxygen. The basic nature of the redox signal is propagated directionally through chromium(III) hexacyanoferrate(II) at the control electrode. The basic electrochemical preparation of chromium(III) hexacyanoferrate(II) has been investigated previously.<sup>26,27</sup>

In this paper, we report on the catalytic properties of chromium(III) hexacyanoferrate(II) obtained by replacing Fe<sup>III</sup> with Cr<sup>III</sup> ions during the formation of a mixed-valence compound. The modification procedure results in an energy change of the catalytic surface from the Fe–CN–Fe cluster and lower oxygen interference is experienced. Hence a sensing scheme with a lower overvoltage of hydrogen peroxide may be designed. The characteristic properties of chromium(III) hexacyanoferrate(II) and the feasibility of utilizing this new catalytic material in a chromium(III) hexacyanoferrate(II)-based sensor at cathodic potentials are demonstrated. In contrast to previous sensors, the significant improvements achieved with this new heterogeneous metal hexacyanoferrate-based chemical sensor offer low overvoltage, low background current, rapid response time, fewer interferences and membrane-free advantages.

### Experimental

#### Chemicals and reagents

All the reagent solutions were prepared with doubly distilled, de-ionized water obtained using a Milli-Q Reagent Water System (Millipore, Bedford, MA, USA). All the measurements were conducted in 0.1 M KCl (Riedel-de Haën, Seelze, Germany) (pH 3) or succinic acid (Riedel-de Haën) buffer solution (pH 6). A 20 ml volume of plating solution containing both 10 mM chromium(III) nitrate [ $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ] (Riedel-de Haën) and 5 mM potassium hexacyanoferrate(III) [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] (Riedel-de Haën) was used for the preparation of the chromium(III) hexacyanoferrate(II)-modified electrode. Hydrogen peroxide solution (0.1 M), prepared by dissolving 86  $\mu\text{l}$  of 35% hydrogen peroxide (Riedel-de Haën) in 10 ml of doubly distilled water, was stored at 4 °C when not in use. Ascorbic acid (0.2 M) (Riedel-de Haën), uric acid (Sigma, St. Louis, MO, USA), acetaminophen (Sigma), dopamine (Sigma), 1,4-dihydroxyquinone (Junsei, Tokyo, Japan), spermine (Sigma) and spermidine (Sigma) stock standard solutions were prepared with doubly distilled water just before use.

#### Preparation of modified working electrode

The chromium(III) hexacyanoferrate(II)-modified glassy carbon electrode was prepared in a 0.1 M KCl solution (pH 3) containing 10 mM  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and 5 mM  $\text{K}_3\text{Fe}(\text{CN})_6$ . The electrode can be prepared either at a constant potential,  $-0.1$  V, or by cycling the potential between  $-0.2$  and  $+0.95$  V versus Ag/AgCl for 1 h at 400 rev  $\text{min}^{-1}$ . Subsequently, the modified

electrode was conditioned in 0.1 M KCl electrolyte (pH 3) for an additional 1 h.

### Apparatus

For the steady-state amperometric measurement of hydrogen peroxide, a bipotentiostat (Model PAR 366A, EG&G Princeton Applied Research, Princeton, NJ, USA) was used to control the applied voltage in a three-electrode system for all amperometric experiments. The detection temperature of the electrochemical cell was maintained at 25 °C with a circulator (Model B402, Firstek Scientific, Taipei, Taiwan). The measurement cell containing a glassy carbon ring electrode (RDE 0032, EG&G Princeton Applied Research) with a motor-controlled rotor (Model 636, EG&G Princeton Applied Research), a laboratory-made 3 M Ag/AgCl reference electrode and a platinum wire counter electrode (local supplier) were used for rotating disk experiments. A cyclic voltammetric analyzer (BAS 100W, Bioanalytical Systems, West Lafayette, IN, USA) was used to check the glassy carbon electrode after polishing and to conduct all the cyclic voltammetric experiments. The data from steady-state amperometric experiments were recorded with a strip-chart recorder (Linear Chart Recorder MF 1201, Alltech, Deerfield, IL, USA). pH measurements were carried out with a Suntex pH meter (local supplier). Elemental analysis of chromium(III) hexacyanoferrate(II) was conducted with a Heraeus (South Plainfield, NJ, USA) CHN-O Rapid Element Analyzer. An infrared spectrometer (FTD-40, Bio-Rad Labs., Richmond, CA, USA) was used to characterize the characteristic absorption of the chromium(III) hexacyanoferrate(II).

### Procedure

The glassy carbon working electrode surface was polished with  $1 \times 10^{-6}$  m diamond solution (Bioanalytical Systems) and then sonicated for 5 min in de-ionized water. The working electrode surface was polished with  $1 \times 10^{-5}$  m alumina powder and then sonicated for 5 min twice. Subsequently, the electrode was checked with the cyclic voltammetric analyzer.

A commercial glassy carbon electrode of 3 mm diameter (MF2012, Bioanalytical Systems) or a 0.221 in diameter commercial rotating glassy carbon electrode (RDE0032, EG&G Princeton Applied Research) was used to study the electrochemical characteristics on a glassy carbon electrode on a BAS100W cyclic voltammetric analyzer. Solutions of 10 mM  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and 5 mM  $\text{K}_3\text{Fe}(\text{CN})_6$  were prepared in 0.1 M KCl solution (pH 3). The potential range of the working electrode was scanned between  $-200$  and  $+950$  mV at a scan rate of  $50 \text{ mV s}^{-1}$ .

### Results and discussion

In the proposed scheme, a membrane-free chromium(III) hexacyanoferrate(II)-based chemical sensor was prepared on both a laboratory-made rotating graphite disk electrode and a conventional glassy carbon electrode (from Bioanalytical Systems). This  $(\text{CN})_5\text{-Cr-NC-Fe-(CN)}_5$  cluster possesses semiconductor-like properties. The responses at various carbon electrodes, such as glassy carbon, graphite and carbon ink electrodes, are similar. However, the response on the platinum electrode is significantly lower than that on carbon, which may be attributed to the difference in the electronic structures of the electrode materials. Chromium(III) hexacyanoferrate(II) was prepared by two different methods. In the first, the cluster was prepared by mixing chromium(III) nitrate and potassium hexacyanoferrate(III). In contrast, the second method utilized a plating solution containing chromium(III) chloride and potassium hexacyanoferrate(III). The first results indicated that the former solution provides a better response to the injection of  $5$

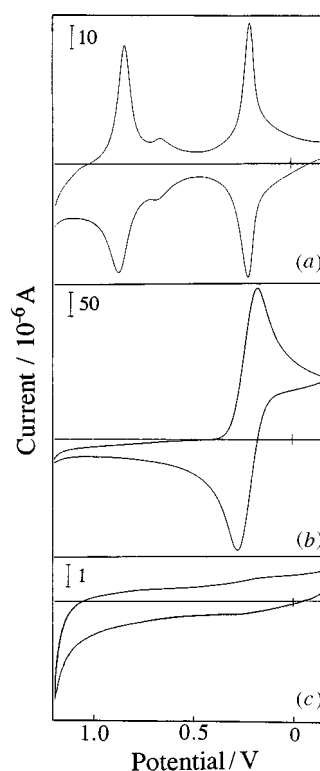
$\times 10^{-5}$  M hydrogen peroxide. In subsequent investigations, the deposition solution always involved a mixture of chromium(III) nitrate and potassium hexacyanoferrate(III) to generate the compound.

### Electrochemical characteristics of chromium(III) hexacyanoferrate(II)

In our recent studies of chromium(III) hexacyanoferrate(II) and cobalt(II) hexacyanoferrate(II), we found that these clusters possess the unique property of acting as a catalyst for the cathodic reduction of hydrogen peroxide at a controlled working potential. This property of chromium(III) hexacyanoferrate(II) is of particular interest in chemical sensor development. In this compound, the hybrid orbitals provide suitable new orbitals, based on crystal field theory, for the catalytic reduction of hydrogen peroxide. In other words, the properties of this newly generated compound may provide a suitable energy surface for the catalytic path to occur.

Chromium(III) hexacyanoferrate(II)  $\{\text{KCr}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6] \cdot 8\text{H}_2\text{O}\}$  modified rotating glassy carbon disk chemical sensors can be prepared by scanning the potential, between  $-0.2$  and  $+0.95$  V (scan rate  $50 \text{ mV s}^{-1}$ ) or by applying constant voltage of  $-0.1$  V in 20 ml of 0.1 M KCl electrolyte (pH 3) containing both 10 mM  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  and 5 mM  $\text{K}_3\text{Fe}(\text{CN})_6$ . However, potential cycling provides a less noisy surface. Hence, subsequently, all the electrode preparations were carried out by the cyclic voltammetric method.

A typical cyclic voltammogram characteristic of the chromium(III) hexacyanoferrate(II) modified glassy carbon electrode in 0.1 M KCl electrolyte is shown in Fig. 1(a). The voltammogram indicates that the two oxidative peaks of  $\text{Cr}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]$  are at  $+225$  and  $+880$  mV. The two reductive



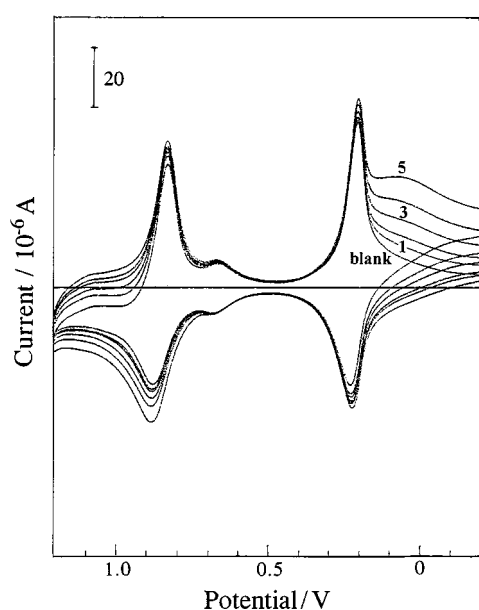
**Fig. 1** Typical cyclic voltammograms for chromium(III) hexacyanoferrate(II) (a), potassium hexacyanoferrate(III) (b) and chromium nitrate (c) on glassy carbon electrode in an electrolyte solution containing 0.1 M KCl (pH 3). Scan rate,  $50 \text{ mV s}^{-1}$ ; operating temperature, 25 °C.

peaks are at +185 and +840 mV, respectively, which are significantly different from the responses from the voltammograms of either potassium hexacyanoferrate(III) or chromium(III) nitrate in Fig. 1(b) and (c), respectively. These two peaks are attributed to different mixed-valence stages such as (2,2) to (2,3) or (3,2) to (3,3). The structure of chromium(III) hexacyanoferrate(II) is face-centered cubic, based on a previous report.<sup>28</sup> In contrast, we found that the response of iron hexacyanochromate-modified glassy carbon electrode is less sensitive to the injection of hydrogen peroxide if the *N*-terminus of the ligand faces the iron atom. We also confirmed that the composition of this cluster is  $\text{KCr}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6 \cdot 8\text{H}_2\text{O}]$  with a Heraeus CHN-O Rapid Analyzer and from the typical infrared absorptions at  $2083\text{ cm}^{-1}$  and  $530/539\text{ cm}^{-1}$  for CN and FeCN/FeC, respectively. These results indicate that the cluster we obtained is a  $\text{Cr}[\text{Fe}(\text{CN})_6]$  species, which is in agreement with previous studies.<sup>29</sup>

### Catalysis of hydrogen peroxide

The feasibility of using the chromium(III) hexacyanoferrate(II) based chemical sensor to measure hydrogen peroxide was investigated. The increasing responses, between +0.3 and -0.20 V upon five successive additions of 0.25 mM hydrogen peroxide, in the cyclic voltammogram in 0.1 M KCl electrolyte, are shown in Fig. 2. These successive cyclic voltammetric responses indicate the feasibility of utilizing this chromium(III) hexacyanoferrate(II) compound to develop an effective electrochemical scheme for hydrogen peroxide determination at potentials between +0.3 and -0.2 V.

The cluster of chromium(III) hexacyanoferrate(II) is at its reduced stage (2,2) when the potential of the modified electrode is held at -0.1 V. The hydrogen peroxide oxidizes chromium(III) hexacyanoferrate(II) on the electrode from a (2,2) trapped state cluster into a (2,3) mixed-valence state. Subsequently, an electron is forced to add to the oxidized cluster through the mixed-valence state at the applied reductive potential. Hence, the characteristics and the optimum conditions for the chromium(III) hexacyanoferrate(II)-based electrode for hydrogen peroxide measurement were investigated.



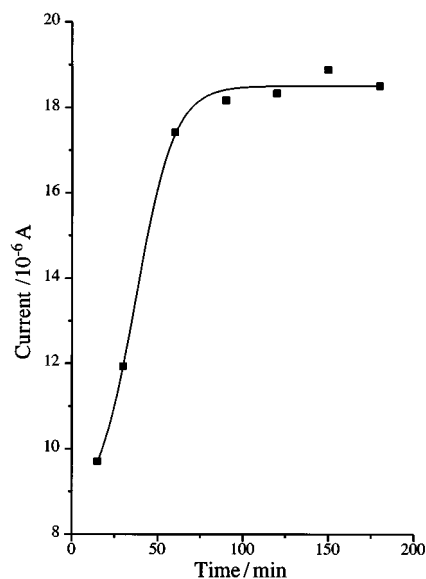
**Fig. 2** Six typical successive cyclic voltammograms from the mixed-valence cluster of the chromium(III) hexacyanoferrate(II)-modified glassy carbon electrode (blank) and five successive additions of 0.25 mM hydrogen peroxide (1–5). Other conditions as in Fig. 1.

### Optimization study of hydrogen peroxide chemical sensor

The sensitivity of the chromium(III) hexacyanoferrate(II)-modified chemical sensor to the determination of hydrogen peroxide is dependent on the cluster amount. The deposition time for generating sufficient  $\text{KCr}^{\text{III}}[\text{Fe}^{\text{II}}(\text{CN})_6]$  was studied in 0.1 M KCl solution (pH 3). Fig. 3 shows the response current from the modified electrode to the addition of  $5 \times 10^{-5}\text{ M}$  hydrogen peroxide at various deposition times at 25 °C. The optimum deposition time of the modifying process is about 1 h and the sensitivity soon reaches the steady state thereafter. Also, the effect of the rotation speed was investigated. The Koutecky–Levich plot [current *versus* rotation speed in  $(\text{rev min}^{-1})^{-1/2}$ ] indicates a linear relationship up to  $400\text{ rev min}^{-1}$  (data not shown). This result shows that the response current approaches the steady state after  $3600\text{ rev min}^{-1}$  and indicates that the electrode reaction was maintained in the mass transport-controlled region at  $900\text{ rev min}^{-1}$ . Subsequently, all the experiments were carried out at  $900\text{ rev min}^{-1}$ .

Other parameters that affect the amperometric response of the chemical sensor are electrolyte, pH and applied potential. In the systematic studies, we found that the sizes of the cation and anion have profound effects. In the latter instance, chloride ion provides a greater significant current response than other potassium salts, such as potassium nitrate, acetate and phosphate. In the former instance, potassium was found to be the most suitable cation, followed by lithium, sodium, rubidium, cesium and ammonium ions. The anion effect may result from the size of the anions. In contrast, the potassium ion has the most compatible size for the counter ion, chloride. The electrolyte studies indicated that KCl electrolyte ( $16.38 \times 10^{-6}\text{ A}$ ) provides the best response to the injection of  $5 \times 10^{-5}\text{ M}$  hydrogen peroxide (pH 3). Fig. 4 shows the dependence of the solution pH on the injection of  $5 \times 10^{-5}\text{ M}$  hydrogen peroxide. The results indicate that the optimum pH range is  $> 6$ ; the noise level gradually increases at  $\text{pH} > 7$ , which may be attributed to the conversion of chromium(III) hexacyanoferrate(II) into a chromium gel.

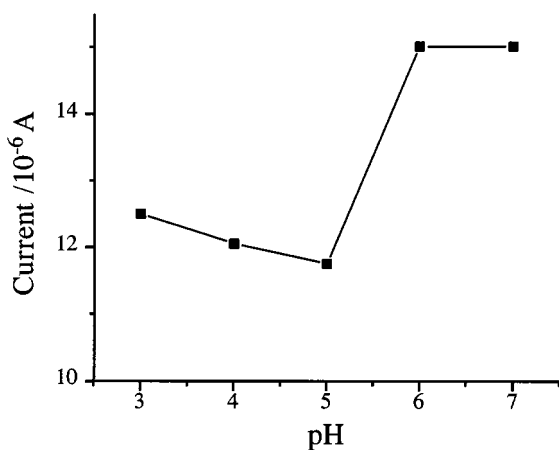
Table 1 summarizes the various electrolytes' effects. The results are in agreement with previous investigations.<sup>30</sup> Other electrolytes investigated included succinic acid buffer (pH 6)



**Fig. 3** Electrodeposition time study. The optimum deposition time for the chromium(III) hexacyanoferrate(II) modified glassy carbon electrode was evaluated with the steady-state response to the injection of  $5 \times 10^{-5}\text{ M H}_2\text{O}_2$  at various deposition times. The potential of the rotating disk electrode was scanned between -0.2 and +0.95 V (*versus* Ag/AgCl) at  $400\text{ rev min}^{-1}$ . Other conditions as in Fig. 1.

( $16.13 \times 10^{-6}$  A), phosphate buffer (pH 6) ( $13.63 \times 10^{-6}$  A), imidazole buffer (pH 6) ( $9.82 \times 10^{-6}$  A) and tris(hydroxymethyl)aminomethane (pH 6) ( $11.43 \times 10^{-6}$  A). In order to maintain pH stability throughout the experiments, a succinic acid buffer was used as electrolyte/buffer in subsequent studies. The ionic strength of the buffer solution was also investigated. The results indicated no significant response current change between 0.05 and 0.1 M. The response decreased gradually at electrolyte concentrations  $>0.1$  M (data not shown). In subsequent studies, 0.05 M succinic acid buffer (pH 6) was used. The solution pH was maintained at 6 in the succinic acid buffer ( $pK_{a1} = 4.2$  and  $pK_{a2} = 5.6$ ) for further investigations.

The detection potential of this sensor is of particular importance since both the oxygen and hydrogen peroxide can act as oxidizing agents in this scheme. Hence proper selection of the potential to distinguish these two species is extremely critical, and a series of investigations were conducted. Fig. 5 shows the results for the chromium(III) hexacyanoferrate(II)-based chemical sensor with respect to both oxygen (B) and hydrogen peroxide (C). Fig. 5 indicates that there is no interference from oxygen at potentials  $>0.0$  V and the reduction response of hydrogen peroxide decreases as the



**Fig. 4** Effect of pH on the chromium(III) hexacyanoferrate(II)-modified electrode sensor for determining  $5 \times 10^{-5}$  M  $H_2O_2$ . Electrolyte, 0.05 M succinic acid buffer; applied potential, 0.0 V (versus Ag/AgCl); rotation speed, 900 rev  $min^{-1}$ ; operating temperature, 25 °C.

**Table 1** Electrolytes studied

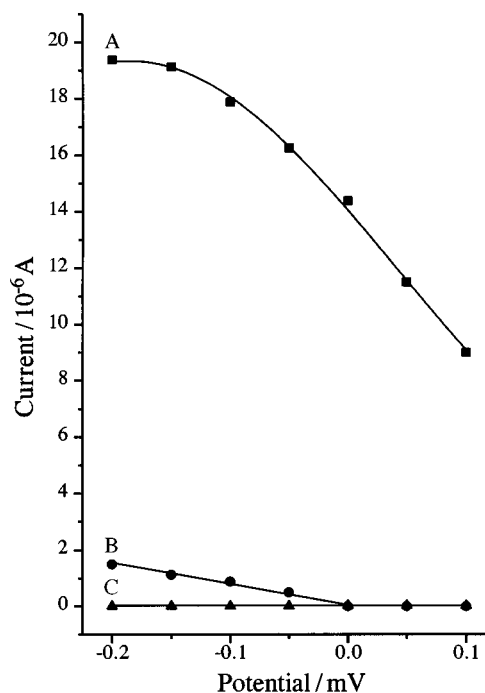
pH 3	Response ( $10^{-6}$ A)	pH 6	Response ( $10^{-6}$ A)
Acetate buffer	12.63	Succinic acid buffer	16.13
Glycine buffer	10.58	Succinic acid + acetate	11.78
Phosphate buffer	11.25	Succinic acid + ammonium chloride	15.00
Citric acid	13.54	Succinic acid + potassium chloride	16.40
Boric acid	12.38	Ammonium chloride	26.13
Succinic acid buffer	13.88	Ammonium chloride + phosphate	14.05
Ammonium chloride	21.67	Ammonium chloride + acetate	19.63
Ammonium chloride + citrate	18.35	Phosphate	13.63
Ammonium chloride + acetate	21.04	Imidazole	9.82
		Tris(hydroxymethyl)-aminomethane	11.43

Response current was obtained on injection of  $5 \times 10^{-5}$  M  $H_2O_2$  under various buffer conditions; applied potential, 0.0 V (versus Ag/AgCl); other conditions as in Fig. 4.

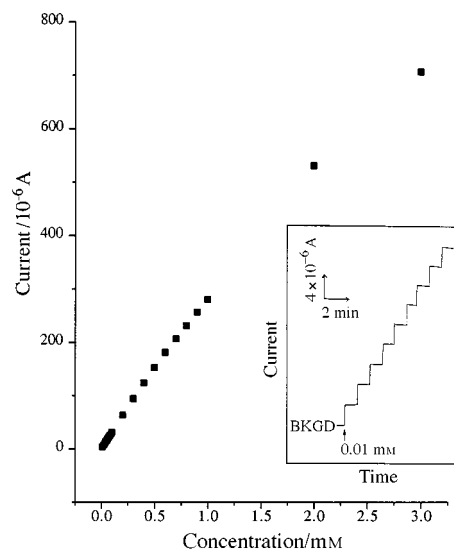
applied potential becomes more positive (oxidative). Hence a detection potential of 0.0 V was adopted in subsequent studies.

### Analytical preference

The deposited chromium(III) hexacyanoferrate(II) possesses a unique catalytic property which is of paramount importance for developing a rapid electrochemical scheme. The calibration plot was conducted by sequential injection of  $1 \times 10^{-5}$ –3 mM hydrogen peroxide and the linear range was evaluated from the calibration curve. Fig. 6 indicates that the linear range of the calibration plot is up to 1.3 mM. A typical response time of 5.6



**Fig. 5** Effect of potential on the chromium(III) hexacyanoferrate(II)-modified electrode sensor for determining  $5 \times 10^{-5}$  M  $H_2O_2$  (A) and ambient  $O_2$  (B) and the bare electrode (C) sensor for  $5 \times 10^{-5}$  M  $H_2O_2$ , using 0.05 M succinic acid buffer (pH 6). Other conditions as in Fig. 4.



**Fig. 6** Typical calibration curve for the determination of  $H_2O_2$  using the chromium(III) hexacyanoferrate(II)-modified glassy carbon electrode cathodically. Other conditions as in Fig. 5.

s was observed. This result indicates that a rapid measurement scheme for hydrogen peroxide and oxidase-based biosensors is possible for future development. A least-squares treatment of the standard addition data yielded a slope of the initial portion of the calibration plot of  $285.2 \times 10^{-6} \text{ A l mmol}^{-1}$  ( $y = 285.24753x + 3.2588$ , with a correlation coefficient  $r = 0.9992$ ) or a current sensitivity per unit area of  $11.52 \times 10^{-6} \text{ A l mmol}^{-1}$ . The sensitivity is comparable to that with Prussian Blue<sup>25</sup> (10.00) or our previous result for cobalt(II) hexacyanoferrate(II)-based sensors<sup>23</sup> (11.80). However, this study does not just concern an additional mixed-valence compound-based sensor. This report indicates that the operational voltage can be adjusted through the proper selection of the metal ion pairs to modify the surface energy of the catalyst in the measurement schemes. Hence this surface modification reduces the measurement voltage effectively down to 0.0 V, which results in few interferences being observed. In contrast to Prussian Blue, the significance of the chromium(III) hexacyanoferrate(II) scheme is the effectively decreased applied voltage and minimization of easily oxidizable compounds, such as ascorbic acid.

Injection of  $5 \times 10^{-8} \text{ M}$  hydrogen peroxide was used to evaluate the detection limit in the presence of a low-pass filter (time constant = 0.1 s). The signal-to-noise characteristics ( $S/N = 3$ ) indicate that a detection limit of  $3.0 \times 10^{-8} \text{ M}$  can be achieved. In contrast, on injection of  $1 \times 10^{-6} \text{ M}$  hydrogen peroxide in the absence of a low-pass filter, the detection limit is  $4.6 \times 10^{-7} \text{ M}$ .

This chemical sensor shows few interferences in the presence of  $5 \times 10^{-5} \text{ M}$  hydrogen peroxide. The interferents investigated included easily oxidizable compounds, such as L-ascorbic acid, cysteine, uric acid, tyrosine, acetaminophen, dopamine, 1,4-dihydroxyquinone, spermine and spermidine. The experiments were conducted by two different procedures, first, the addition of  $2 \times 10^{-4} \text{ M}$  interferent to a solution containing  $5 \times 10^{-5} \text{ M}$  hydrogen peroxide was investigated, second, the investigations were carried out in the absence of hydrogen peroxide. The results from both experiments indicated that no significant interferences were observed. This may be attributed to low operational voltage of 0.0 V (*versus* Ag/AgCl).

The results of a series of 20 successive injections of  $5 \times 10^{-5} \text{ M}$  hydrogen peroxide solution were utilized to evaluate the precision of the response currents (conditions as in Fig. 6). The mean peak current was  $16.10 \times 10^{-6} \text{ A}$ , with a range of  $15.80\text{--}16.44 \times 10^{-6} \text{ A}$  and an RSD 1.1%. The half-life,  $t_{50\%}$ , of the chromium(III) hexacyanoferrate(II)-based chemical sensor preserved in the dry state after use and at room temperature, was found to be 14 d. However, if the chemical sensor was kept under vacuum the half-life was 16 d. The difference may be attributed to the gradual loss of chromium(III) hexacyanoferrate(II) during day-to-day operation.

## Conclusion

We have presented a simple scheme to create a membrane-free mixed-valence based chemical sensor. Chromium(III) hexacyanoferrate(II) possesses catalytic properties with respect to hydrogen peroxide which can be utilized to design an interface for a chemical sensor. The chromium(III) hexacyanoferrate(II) containing interface was oxidized on contact with hydrogen peroxide. Subsequently, the oxidized cluster received an electron from the electrode at a reductive potential. This interface offers hydrogen peroxide specificity and without any significant oxidative interference from oxygen or other easily oxidizable compounds. Additionally, this work has successfully demonstrated the advantages of a low overvoltage for hydrogen peroxide determination, which may benefit the further implementation of this technique in both biological and environmental applications.

It is important to investigate the nature of this catalytic reaction with hydrogen peroxide to design a better interface for hydrogen peroxide detection. We have utilized chromium(III) to replace iron(III) in Prussian Blue and successfully decreased the catalytic potential from +100 down to 0.0 mV. The small decrease in potential, however, significantly lowers the potential interference from easily oxidizable compounds, such as ascorbic acid. The characteristic properties of various mixed-valence clusters and their catalytic characteristics were carefully investigated before coupling an additional biological recognition element, flavin-containing enzymes, to this hydrogen peroxide-specific chemical sensor to develop chromium(III) hexacyanoferrate(II) biosensors.

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