Voltammetric and Potentiometric Study of Cysteine at Cobalt(II) Phthalocyanine Modified Carbon-paste Electrode

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Cyclic voltammetry and potentiometry were used to investigate the electrochemical behavior of cysteine at a chemically modified electrode prepared by incorporating cobalt(II) phthalocyanine [Co(II)Pc] into carbon paste matrix. The modified electrode showed high electrocatalytic activity toward cysteine; the overpotential for the oxidation of cysteine was decreased by more than 100 mV, and the corresponding peak current increased significantly. The electrocatalytic process was highly dependent on the pH of the supporting electrolyte. The peak currents decreased when the pH was raised to 6 and totally disappeared at pH≥ 7, resulting from the autocatalytic oxidation of cysteine by Co(II)Pc at the electrode surface. Therefore, at pH values of 6 to 8, the modified electrode was used as a potentiometric sensor for quantitative measurement of cysteine in the presence of oxygen in air saturated solutions. In fact, the Co(II)Pc/Co(I)Pc couple acts as a suitable mediator for indirect oxidation of cysteine by dissolved oxygen at approximately neutral pH values. Under the optimized conditions, the potentiometric response of the modified electrode was linear against the concentration of cysteine in the range of 0.6 μM to 2 mM. The limit of detection was found to be 0.5 μM. The potentiometric response time was ≤15 s. The electrode showed long term stability; the standard deviation of the slope obtained after repeated calibration during a period of two months was 2.8% (n = 7). Application of the electrode in a recovery experiment for the determination of cysteine added to a synthetic serum sample is described.

INTRODUCTION

Sulfhydryl compounds are known to undergo electrochemical processes at solid electrodes, but their oxidation occurs at relatively high overpotentials [1,2]. Modification of electrodes with suitable electron transfer mediators enables the electrochemistry of sulfhydryl compounds to proceed at lower overpotentials with much higher sensitivities [2,3]. Among the sulfhydryl compounds, cysteine and its derivatives have attracted especial attention because of its involvement in many important biological

processes, and its chemical activity in the formation of complexes with various ionic species and biomolecules [4]. Cysteine is a highly significant bioactive compound, and is known to be an active site in the catalytic function of certain enzymes known as cysteine proteases and in many other peptides and proteins. It is also used in some proprietary antibiotic preparations used for the treatment of skin damages and as a radioprotective agent [3,5]. Therefore, investigation of the electrochemical behavior of cysteine and its determination in biological fluids and pharmaceutical preparations via a simple and reliable method is of great importance.

At most conventional electrodes, the oxidation of cysteine

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occurs at high positive potentials. However, several chemically modified electrodes (CMEs) have been designed and characterized for the electrocatalytic oxidation of cysteine [4,6-9], based on voltammetric and amperometric methods. The application of a less demanding potentiometric method appears to be advantageous from several stand points. First, the potentiometric method yields a continuous electrode response to the analyte, which is advantageous when monitoring over a long period of time over a wide concentration range is required [10]. Secend, the potentiometric method offers the advantages of a very simple instrumentation and the absence of deactivation processes that often accompany voltammetry at solid electrodes [11].

In previous publications, we have reported that some transition metal complexes, such as iron(II) and cobalt(II) phthalocyanines, show substantial electrocatalytic activity for the oxidation of ascorbic acid and several sulfur containing compounds [12-14]. Therefore, the modified electrodes were used as sensitive potentiometric sensors for fast and accurate determination of these species. We have also recently reported the use of a cobalt(II) salophen modified carbon paste electrode (CPE) for potentiometric, amperometric and voltammetric determination of cysteine [15]. To the best of our knowledge, there is no other report on the potentiometric determination of cysteine based on the electrocatalytic action of the modified electrodes. The present work describes the development of a potentiometric sensor for measurement of cysteine at a CPE modified with Co(II)Pc. This complex is more stable and less soluble compared to the Co(II) salophen used in our previous study [15], and therefore, the electrode modified with Co(II)Pc is expected to have long term stability and durability. Cyclic voltammetry was used to investigate the catalytic activity of the modified electrode toward cysteine.

EXPERIMENTAL

Reagents

Cobalt(II) phthalocyanine and spectroscopic grade mineral oil (Nujol) were obtained from Aldrich and used as received. Graphite powder (Cat. No. 104206 Merck, particle size <50 μm), amino acids, and all other chemicals were received from Merck and used without further purification. Doubly distilled, deionized water was used for preparing all of the solutions and throughout the experiments.

Cysteine solutions were freshly prepared in deoxygenated buffer solutions. The supporting electrolyte solutions were 0.05 M buffers of phosphate (pH 6-8) and acetate (pH $\leqq$5), and were prepared from 0.1 M solutions of reagent-grade chemicals using a pH meter. Voltammetric experiments were carried out in solutions deoxygenated by pure nitrogen. Stock solutions of interferences, 0.1 M, were prepared by dissolving the appropriate amount of each compound, usually their potassium salts, in water. Working solutions were prepared by successive dilution of the stock solutions.
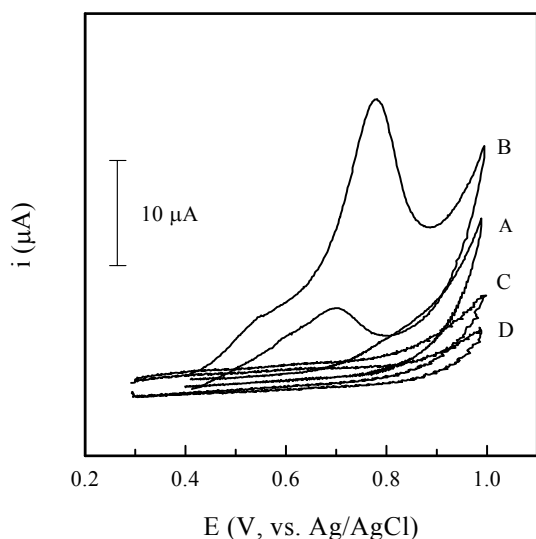
Apparatus

Cyclic voltammetric studies were carried out with a Bank Potentioscan model POS88 system, equipped with a DAS 1602 interface board. A conventional three-electrode cell was employed incorporating a carbon-paste working electrode (with or without Co(II)Pc), a saturated Ag/AgCl reference electrode, and a Pt-wire counter electrode.

Potentiometric measurements were performed with the following cell assembly: Co(II)Pc modified electrode | measuring solution (air sat'd) | KCl (sat'd) | AgCl | Ag. The cell consists of a Co(II)Pc modified carbon-paste as the indicator electrode, a saturated Ag/AgCl reference electrode and a magnetic stirrer. A Corning model 125 digital pH/mV meter was used for measuring pH and potentials. The meter was connected to a Philips model PM-8277 X-Y recorder for plotting potential-time data.

Modified Electrode Preparation

The general procedure to prepare the modified carbon-paste electrode was to mix graphite powder with Co(II)Pc (5% w/w) and mineral oil (Nujol, 30% w/w). After thorough hand mixing in a mortar and pestle to obtain a very fine paste, a portion of the composite mixture was packed into the end of a Pyrex glass tubing (ca. 3 mm i.d. and 15 cm long). Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. Unmodified electrodes were prepared in the same way without addition of Co(II)Pc. The working surface of the electrode was polished using a soft polishing tissue to obtain a shiny surface. Then the electrodes were rinsed with ethanol and distilled water, respectively.
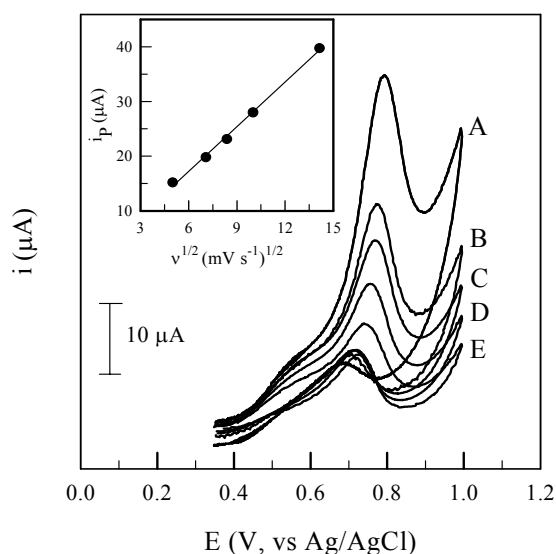
RESULTS AND DISCUSSION

Cobalt complexes, especially cobalt phthalocyanines, promote the oxygen reduction to give H_2O_2 or in some instances H_2O [16-18]. Cobalt complexes are also capable to oxidize sulfide to elemental sulfur and various sulfhydryl compounds to their disulfide derivatives [6-10,19-25]. Both of these diverse reactions have been separately studied at the surface of CMEs by various voltammetric methods. Lever and co-workers have shown that cobalt phthalocyaine modified electrodes, used to determine sulfide potentiometrically, must catalyze both the analyte oxidation and oxygen reduction in solutions containing both species [23]. Therefore, we were prompted to use Co(II)Pc as an electrocatalyst for potentiometric determination of cysteine in air saturated solutions.

Preliminary experiments for elucidation of the catalytic activity of the Co(II)Pc modified electrode toward cysteine were performed using cyclic voltammetry (CV). Cyclic voltammograms were recorded using unmodified and modified CPEs in deaerated solutions.

The processes associated with the electrochemical oxidation of cysteine are typically illustrated in Fig. 1 by CVs of 5.0 mM cysteine solution at pH 2.4. These voltammograms were recorded after several preliminary scans at the surface of unmodified (Fig. 1A) and modified (Fig. 1B) CPEs in the potential range of 0.4 to 1.0 V *vs*. Ag/AgCl reference electrode. As can be seen, electroactivity toward cysteine on unmodified electrode was insignificant (Fig. 1A), with a poorly defined peak potential, around +0.85 V *vs*. Ag/AgCl electrode. In addition, there was no measurable wave for the modified and unmodified electrodes in plain supporting electrolyte in the potential range studied (Figs. 1, C and D). However, the Co(II)Pc modified electrode exhibited a strong anodic peak at +0.75 V *vs*. Ag/AgCl electrode. A weak prepeak, observed as a shoulder during the forward scan, can be related to the product adsorption (RSSR) at the electrode surface. The current enhancement was remarkable, and additionally, a significant decrease in the oxidation potential of cysteine can be distinguished (more than 100 mV) when the electrode is modified with Co(II)Pc. This behavior, which was observed at different concentrations of cysteine and at several potential scan rates, clearly demonstrates that the Co(II)Pc

Fig. 1. Typical CVs for the oxidation of 5.0 mM cysteine at the (A) unmodified and (B) modified carbon-paste electrodes at pH 2.4 and scan rate (v) of 100 mV s^{-1}. Voltammograms (C) and (D) show the responses of the modified and unmodified electrodes, respectively, in supporting electrolyte in the absence of cysteine at the same experimental conditions.

Fig. 2. CVs obtained at different scan rates, v, at the Co(II)Pc modified electrode in 5 mM cysteine at pH 2.4; A, 200; B, 100; C, 70; D, 50; E, 20 mV s^{-1}. The inset figure shows the corresponding peak currents *vs*. v$^{1/2}$.

Table 1. Values of αn_a Calculated from the CV Data for 5 mM Cysteine at the Co(II)Pc Modified Electrode at Different pH Values and Scan Rates

Scan rate, ν (mV s^{-1})	pH			
	2.4	3.0	4.0	5.0
200	0.53	0.49	0.51	0.48
100	0.54	0.52	0.54	0.53
70	0.51	0.53	0.52	0.51
50	0.56	0.48	0.50	0.52
25	0.57	0.47	0.53	0.50
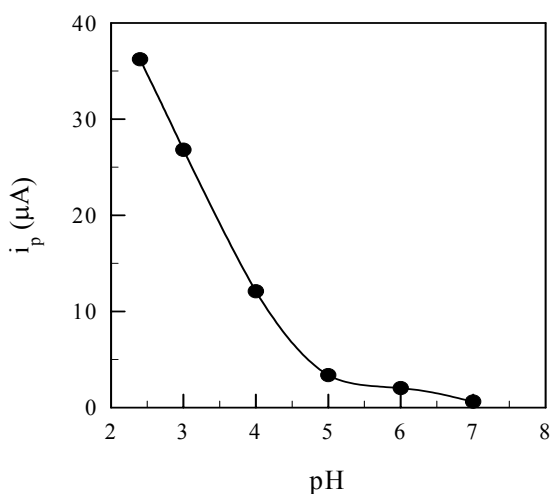
Fig. 3. Effect of pH on peak current for the electrocatalytic oxidation of 5 mM cysteine solution at the Co(II)Pc modified electrode. Scan rate, 100 mV s^{-1}.

mediator functions electrocatalytically toward cysteine. No cathodic peak was observed in the reverse scan, indicating that the oxidation of cysteine at Co(II)Pc modified electrode is an irreversible process. Occurrence of an anodic dip in current as the potential was cycled in the cathodic direction can be attributed to the involvement of a complexation step during catalysis by the Co(II)Pc [6,9].

In order to determine whether the current due to the oxidation process of cysteine was diffusion controlled, the effect of scan rate on the CVs for oxidation of 5.0 mM cysteine at the modified electrode was investigated. The linear dependence of peak current, i_p, vs. square root of the scan rate, $\nu^{1/2}$, suggests that the oxidation of cysteine at the CME is a diffusion controlled process. The results of the scan rates of 25 to 200 mV s^{-1} at pH 2.4 are shown in Fig. 2. Similar results were observed at other pH values up to a pH of 5.0.

Values of αn_a (where α is the transfer coefficient and n_a is the number of electrons involved in the rate determining step) were calculated for the irreversible oxidation of cysteine at several pH values according to the equation $\alpha n_a = 0.048/(E_p - E_p/2)$ [26], where $E_p/2$ is the potential corresponding to $i_p/2$. The calculated values of αn_a at the modified electrode using several potential scan rates are given in Table 1. In addition, the current function plot, $i_p/(C\nu^{1/2})$ vs. $\nu^{1/2}$, shows a negative slope at several pH values between 2.4 to 5.0, which is typical of a catalytic process for the oxidation of cysteine at the surface of the modified electrode [2,24,26]. The above results indeed suggest an EC′ (electron transfer process followed by a chemical reaction) mechanism, and a one electron transfer in the rate determining step for the electrocatalytic oxidation of cysteine (assuming that the transfer coefficient, α, is 0.5 for the irreversible system).

The effect of increasing pH on the cyclic voltammograms for the oxidation of cysteine at the surface of the modified electrode was a moderate shift of the anodic wave to lower potentials. The i_p values were also affected by pH and decreased significantly by raising the pH. The graph of peak current (i_p) vs. pH (Fig. 3) indicates that more acidic pH levels are preferred for the optimum sensitivity in the voltammetric or amperometric determination of cysteine at the Co(II)Pc modified electrode. The anodic waves completely disappeared at pH$\geq$ 7.0. Such a behavior can be related to the autocatalytic oxidation of cysteine to cystine by Co(II)Pc, which is known to occur at pH values greater than 4 according to Equation 1 [9,27].

$$2Co(II)Pc + 2RSH \rightarrow 2Co(I)Pc^- + RSSR + 2H^+ \qquad (1)$$
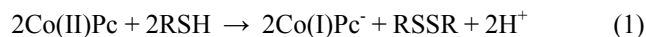
Therefore, for the potentiometric detection, which is based on the autocatalytic oxidation of cysteine, the sensitivity is expected to increase with increasing pH. In highly acidic media, the autocatalytic reaction does not occur and the oxidation of cysteine is catalyzed by the Co(III)Pc generated from the oxidation of Co(II)Pc at the electrode surface

(Equations 2 and 3).

$$Co(II)Pc \rightarrow Co(III)Pc^+ + e^- \qquad (2)$$

$$2Co(III)Pc^+ + 2RSH \rightarrow 2Co(II)Pc + RSSR + 2H^+ \qquad (3)$$
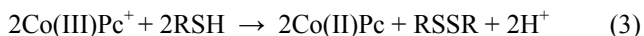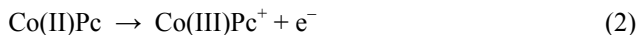
At pH values$\geq$ 7, the autocatalytic process overcomes the electrocatalytic oxidation of cysteine via Equations 2 and 3, and therefore, no voltammetric response is observed in the potential range studied at these conditions. In fact, the enhancement of the anodic currents observed in Fig. 1 at the CME in acidic media is a consequence of the electron transfer represented by the above equations. The observed anodic currents were reproducible and increased proportionally with the cysteine concentration. Therefore, the Co(II)Pc modified CPE can be used for the electrocatalytic determination of cysteine by voltammetric and amperometric methods. However, since the main purpose of the present work was potentiometry, we investigated the potentiometric response characteristics of the Co(II)Pc modified CPE at pH$\geq$ 6. The results are presented in the following section.

Potentiometric Response of the Modified Electrode

The Co(II)Pc modified electrode was successfully applied as a potentiometric sensor for determination of cysteine at pH 6.0-8.0. The modifier acts as a redox mediator for indirect oxidation of cysteine by oxygen. The direct oxidation of sulfhydryl compounds by oxygen in the absence of a catalyst is relatively slow [2,23]. The potentiometric method with an immobilized redox mediator represents a convenient way to measure the equilibrium potential of such slow and irreversible electrochemical systems [10]. The potentiometric response of the Co(II)Pc modified electrode can be related to the autocatalytic oxidation of cysteine to cystine according to Equation 1. The resulting Co(I)Pc can be re-oxidized back in a second step through a chemical reaction with oxygen, according to Equation 4.

$$2Co(I)Pc^- + O_2 + 2H^+ \rightarrow 2Co(II)Pc + H_2O_2 \qquad (4)$$

In fact the Co(II)Pc mediator provides a reversible redox couple, Co(II)Pc/Co(I)Pc, for measuring the equilibrium potential of the cysteine-oxygen system. As can be seen from
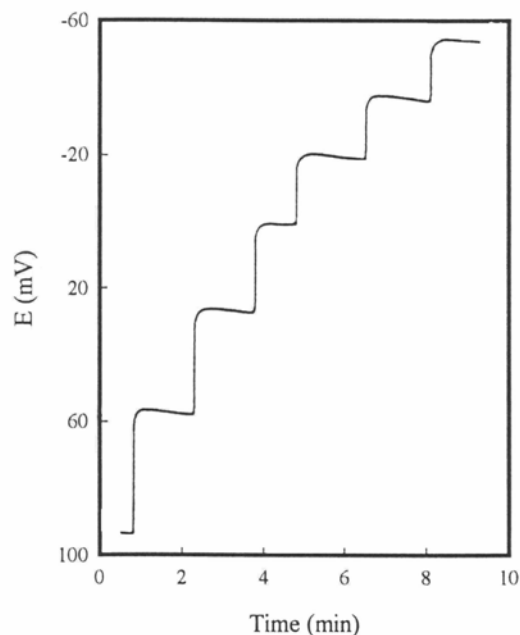
Fig. 4. The potential-time curve for the response of the Co(II)Pc modified electrode to successive additions of 100 μl 0.01 M cysteine to 25 ml of 10 μM cysteine in 0.05 M air saturated phosphate buffer, pH 7.0.
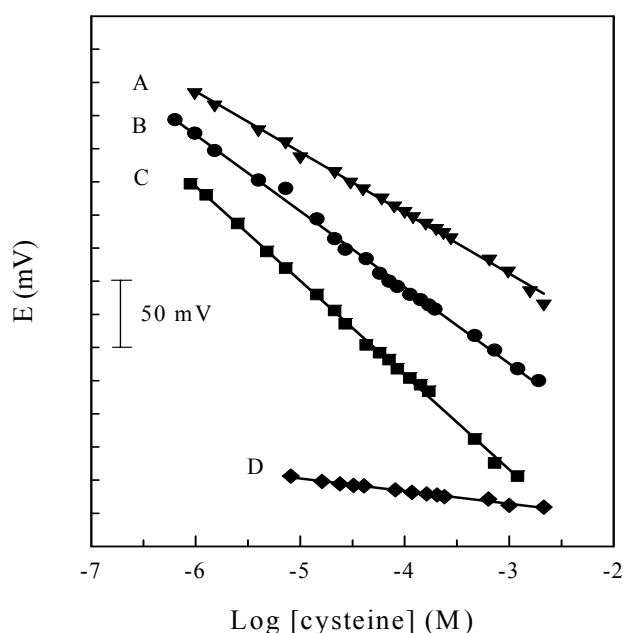
Equations 1 and 4, the potential of the electrode is determined by the Co(II)Pc/Co(I)Pc concentration ratio, which is determined by the cysteine concentration in air saturated solutions. A very weak response was observed in deaerated cysteine solutions, indicating that oxygen is a necessary reagent, as shown by Equation 4, for optimum sensitivity of the potentiometric response of the modified electrode.

The modified electrode was used as an indicator electrode in conjunction with a saturated Ag/AgCl reference electrode in a galvanic cell containing air saturated phosphate buffer for the determination of cysteine in stirred solutions. The response time of the modified electrode, tested by measuring the time required to achieve a steady state potential was within 15 s, depending on the efficiency of stirring during sample injection. A typical potential-time plot for the successive addition of 100 μl 0.01 M cysteine to a 25 ml solution of 10 μM cysteine at pH 7 is shown in Fig. 4, indicating that the electrode attains equilibrium in a very short time at different

concentrations of cysteine.

The calibration plots of potential of the modified electrode vs. logarithm of cysteine concentration at pH 6.0, 7.0 and 8.0 are shown in Fig. 5, demonstrating linearity in the range of 1.0 μM-2 mM, 0.6 μM-2 mM and 0.8 μM-1.2 mM at pH 6.0, 7.0 and 8.0, respectively. The reproducibility of the potential response of the electrode was evaluated by determining the standard deviation of slope of the calibration plots obtained in a period of two months (n = 7). The electrode retained its full activity during this period; the slopes of the calibration plots were reproducible to within 2.8% RSD at pH 7.0. Due to the high stability of the Co(II)Pc and its very low solubility in aqueous media, the modified electrode could be used for much longer periods. The response characteristics of the electrode together with the least squares analysis of the data are given in Table 2. The sensitivities, expressed as the slope of the calibration graph, were calculated to be between 46 to 71 mV/decade of cysteine concentration, depending on the solution pH. The improvement in sensitivity of the electrode at higher pH is associated with the formation of higher concentrations of cysteine anion, RS^-. Under nitrogen, the slope decreases to ~10 mV/decade of cysteine concentration (Fig. 5D), presumably due to a little change in the Co(II)Pc/Co(I)Pc concentration ratio, which is expected to occur by increasing the concentration of cysteine in solution.

The responses of the electrode to several common anions including nitrate, carbonate, sulfate, iodide, bromide, chloride and thiocyanate were investigated. These anions in the form of potassium salts were added to cysteine solutions to give a final concentration of 10 mM and the calibration plots were obtained. The electrode response was only slightly changed by the addition of these ions. These anions do cause a slight deviation in the slope and intercept of the calibration plot while maintaining linearity, but assuming calibration in their presence would not preclude the use of the electrode to analyze cysteine. Of course, compounds with reducing properties and redox potentials close to those of cysteine may interfere if they are present. Under such conditions, better

Fig. 5. Calibration plots of potential of Co(II)Pc modified electrode vs. logarithm of cysteine concentration in 0.05 M phosphate buffer at (A) pH 6.0, (B) pH 7.0 and (C) pH 8.0, all in air saturated solutions, and (D) at pH 7.0 in deaerated solutions.

Table 2. Potentiometric Response Characteristics of the Co(II)Pc Modified CPE toward Cysteine at Different pH Values

Parameter	pH		
	6.0	7.0	8.0
Slope (mV decade^{-1})	-45.8	-57.4	-71.1
Limits of linear range (M)	1.0×10^{-6}-2.0×10^{-3}	6.0×10^{-7}-2.0×10^{-3}	8.0×10^{-7}-1.2×10^{-3}
Limit of detection (M)	1×10^{-6}	5×10^{-7}	5×10^{-7}
Correlation coefficient (r)	0.9983	0.9985	0.9994

Table 3. Composition of Simulated Serum[a]

Compound	Concentration (mM)	Compound	Concentration (mM)
D,L-alanine	0.41	D,L-methionine	0.034
L-arginine	0.21	L-phenylalanine	0.16
L-aspartic acid	0.88	L-serine	0.16
L-cysteine	0.07	D,L-tryptophan	0.085
glycine	0.14	$NaHCO_3$	8.0
L-histidine	0.14	NaCl	88.8
L-lysine	0.20	Citric acid	0.17

[a] The simulated serum pH was 7.0.

Table 4. Potentiometric Determination of Cysteine Added to a Synthetic Serum Sample

Amount added (M)	Amount found[a] (M)	Recovery (%)
3.70×10^{-4}	$3.68 (\pm 0.11) \times 10^{-4}$	104.3
4.69×10^{-4}	$4.80 (\pm 0.14) \times 10^{-4}$	102.3
5.68×10^{-4}	$5.74 (\pm 0.16) \times 10^{-4}$	101.1
6.67×10^{-4}	$6.71 (\pm 0.18) \times 10^{-4}$	100.6

[a] Average of three determinations.

selectivity could be achieved using the modified electrode for amperometric or voltammetric determination of cysteine.

To investigate the applicability and selectivity of the proposed electrode as a potentiometric sensor for cysteine, experiments were conducted to measure the concentration of cysteine added to a synthetic serum sample, the composition of which is listed in Table 3. The concentration of each component was chosen to match its normal level in human serum [28]. The results of cysteine determination, summarized in Table 4, show an average recovery of 102%, indicating that the constituents of the synthetic serum sample do not significantly interfere with the determination of cysteine. Therefore, the method seems to be promising for the determination of cysteine in serum samples.

CONCLUSIONS

The Co(II)Pc modified CPE showed a high catalytic activity for the electrooxidation of cysteine. The electrocatalytic behavior of the electrode strongly depends on the pH of the supporting electrolyte. It was successfully applied as a sensitive sensor for potentiometric determination of cysteine at pH values between 6.0-8.0. The potentiometric response of the electrode results from the autocatalytic oxidation of cysteine by Co(II)Pc at the electrode surface, which is regenerated by oxidation with the oxygen present in air saturated solutions. The modified electrode shows high sensitivity, wide linear dynamic range (about 4 orders of magnitude) and low detection limit. The performance characteristics of the modified electrode in conjunction with simplicity of its preparation and re-newability of its surface by a simple polishing, demonstrates its analytical utility as a sensor for determination of cysteine at neutral pH.

ACKNOWLEDGMENT

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