

Cobalt tetrasulphonated phthalocyanine immobilized on poly-L-lysine film onto glassy carbon electrode as amperometric sensor for cysteine

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Abstract

The present work describes the development of a simple and efficient method for the amperometric determination of cysteine (CySH) in wild medium at an applied potential of 0.150 V versus Ag/AgCl. In this sense, the electrocatalytic oxidation of cysteine (CySH) was carried out on a glassy carbon electrode modified with cobalt tetrasulphonated phthalocyanine (CoTSPc) and poly-L-lysine (PLL) film. The immobilization of CoTSPc in PLL film was performed by a simple evaporation of the solvent. The CoTSPc/PLL film, formed on the GC electrode showed an electrocatalytic activity to the CySH oxidation. The sensor presented its best performance in 0.1 mol l⁻¹ Pipes at pH 7.5. Under optimized operational conditions, the sensor provided a wide linear response range for CySH from 0.50 up to 216.0 μmol l⁻¹ with a sensitivity and detection limit of 157 nA cm⁻² l μmol⁻¹ and 150 nmol l⁻¹, respectively. The proposed sensor presented higher sensitivity when compared to the other modified electrodes described in the literature and showed a stable response for at least 200 successive determinations. The repeatability of the measurements with the same sensor and different sensors, evaluated in term of relative standard deviation, were 4.1 and 5.2%, respectively, for *n* = 10. The developed sensor was applied for the CySH determination in food supplement samples and the results were statistically the same to those obtained by a comparative method described in the literature at a confidence level of 95%.

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1. Introduction

Cysteine is one of the most important aminoacids. It plays an important role in biological systems and has been widely used in the medicine and food chemistry [1]. This thiol participates in a great number of biochemical processes, which depend directly on the particular reactivity of thiols [2]. Cysteine is also employed in several pharmaceutical applications; it is used in some antibiotics and treatment of skin damages [3]. Many methods for its determination have been reported including, spectrofluorimetry [4,5], high performance liquid chromatography [6,7] and electrochemical methods [8–11]. However, most of them suffer difficulties with sample preparation, necessity of molecules derivatization or the lack of sufficient sensitivity,

which limit their utility [12]. In general, electrochemical methods present the advantages of simplicity and high sensitivity. The major problem related to the electrochemical detection of thiols is the high overpotential required to the most conventional electrodes. In addition, the use of bare electrodes for detection has a number of limitations, such as low selectivity, reproductibility and the slow electron transfer reaction, thus the use of chemically modified electrodes has been proposed for the sensitive detection of thiols at lower potentials [13–15]. Thus, since the direct oxidation of thiols at solid electrodes is slow and requires high overpotentials [16], the study of electrocatalytical reactions of CySH is important in the electroanalysis of this thiol.

A wide variety of compounds has been used as electron transfer mediators for electrooxidation of CySH. Modified electrodes with lead ruthenate pyrochlore [17], pyrroloquinoline quinone [18], cobalt(II)-4-methylsalophen [19], fullerene-C₆₀ [20], oxovanadium(IV) complex of Salen [21], Pt microelec-

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trode modified with carbon nanotubes [22], ruthenium complex [23] and phthalocyanines [24–27] have been used for CySH detection. Among them, the phthalocyanines have acquired great interest due to their singular properties, including high thermal stability and catalytic efficiency for a great number of molecules [26]. However, in the specific case of cobalt phthalocyanine modified electrodes few of them were successfully employed in analytical chemistry, which can be related to its immobilization methods. In addition, the possibility of the complex leaching out from the electrode surface and the use of high potentials for CySH oxidation maybe the difficult for its application in biological and pharmacological analysis. Some methods to immobilize these complexes have been proposed such as physical adsorption, layer by layer and electropolymerization [26,28–30]. However, these preparation methods are generally unstable or lack sensitivity for real sample analysis. In this sense, the present work report the development of a simple and efficient method for immobilizing cobalt tetrasulphonated phthalocyanine with poly-L-lysine film onto glassy carbon electrode according to the recent works developed by our group [31,32]. The application of this electrode for CySH determination in samples of food supplement containing L-cysteine to show the reliability is also presented.

2. Experimental

2.1. Chemical and solutions

All chemicals were analytical grade. The food supplement sample was purchased from a local drugstore. Cysteine, poly-L-lysine hydrochloride (PLL), citric acid, Pipes [piperazine-*N-N'*-bis[2-ethanesulfonic acid]] and Hepes [*N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid)] were acquired from Sigma, St. Louis, USA. Disodium and monosodium phosphate (Na_2HPO_4 and NaH_2PO_4) and sodium chloride (NaCl) were acquired from Synth, São Paulo, Brazil. Cobalt(II) tetrasulphonated phthalocyanine (CoTSPc), was prepared and purified according to the Weber and Busch procedure [33]. The solutions were prepared by using water purified in a Milli-Q Millipore system and the actual pH of the buffer solutions were determined with a Corning pH/Ion Analyser model 350.

2.2. Preparation of CoTSPc/PLL glassy carbon electrode

A glassy carbon (GC) electrode acquired from Metrohm – Switzerland, with geometrical area of 0.196 cm^2 was used for the sensor preparation. Prior to the modification, the electrode surface was hand-polished on fine emery paper and chamois leather containing Al_2O_3 slurry [34]. After cleaning the electrode, $25\ \mu\text{l}$ of a mixed CoTSPc and PLL solution (1:1 v/v) in the concentrations: 0.3, 0.5, 0.8, 1.0 and 1.2 mmol l^{-1} for CoTSPc and 0.25, 0.4, 0.5, 0.6 and 0.75 mmol l^{-1} for PLL was put onto its surface and let to dry at $80\ ^\circ\text{C}$ during 8 min. CoTSPc and PLL solutions were prepared using deionized water.

2.3. Voltammetric measurements

The voltammetric measurements were carried out with a potentiostat PGSTAT-30 Model from Autolab Echo Chemie (Utrecht, The Netherlands) connected to a PC (Software GPES 4.9). An electrochemical cell with three electrodes was used with a Ag/AgCl electrode as reference, a Pt wire as auxiliary and the modified GC with 5 mm diameter as working electrode. The measurements were carried out, using 5.00 ml of buffer solution. Oxygen was removed by bubbling nitrogen through the solution.

2.4. Procedure for the sample preparation for amperometric and spectrophotometric determinations

For amperometric analysis the food supplement samples (Solaray Dietary Supplement L-cysteine (500 mg)) were prepared by dissolving the sample (one capsule of 500 mg) in 1000.00 ml of water and then the solution was filtered through a quantitative paper filter. After this step, an aliquot of 1.00 ml of the filtered solution was diluted to 10.00 ml. An aliquot of $50\ \mu\text{l}$ was added to the cell containing 5.00 ml of the electrolyte to be measured. For the spectrophotometric measurements the samples were diluted to 1000.00 ml with deionized water and then the solution was filtered through a quantitative paper filter. The other steps were done according to the procedure described in the literature [35], where an aliquot of the sample was transferred to a test tube plus $200\ \mu\text{l}$ of *p*-benzoquinone adjusting the volume with acetate buffer.

3. Results and discussion

3.1. Voltammetric behavior of the cobalt tetrasulphonated phthalocyanine immobilized in PLL film and electrocatalytic oxidation of CySH

Fig. 1a shows the cyclic voltammograms obtained with a CoTSPc/PLL modified electrode (full line) and bare electrode (dashed line) in phosphate buffer solution at pH 7.0. The voltammogram represented by full line shows a wave at $-0.3\ \text{V}$, probably associated to the reduction of immobilized $[\text{Co(II)TSPc}]^{2-}$ into $[\text{Co(I)TSPc}]^{3-}$ species [36], since that the reductions are well known to occur at the central metal in Co(II) phthalocyanine complexes [37,38]. On the other hand, the peak (about $-0.1\ \text{V}$) observed on the anodic scan, in this pH, was broad and presented poor definition in similar way to those observed by Limson and Nyokong [36]. Fig. 1b shows the cyclic voltammograms for CoTSPc/PLL modified (full line) and bare (dashed line) electrode in the presence of CySH. This figure clearly shows the role of the complex for the sensor response. As can be seen, a clear anodic peak is observed at the CoTSPc/PLL modified electrode when compared to the bare electrode.

It is well known that the interaction between cobalt(II) tetrasulphonated phthalocyanine $[\text{Co(II)TSPc}]^{2-}$ species and cysteine results in the reduced $[\text{Co(I)TSPc}]^{3-}$ species and the cysteine cation-radical ($\text{RS}^{\bullet+}$) [39]. The electrocatalytic oxidation of cysteine to cystine by $[\text{Co(II)TSPc}]^{2-}$ species is known to

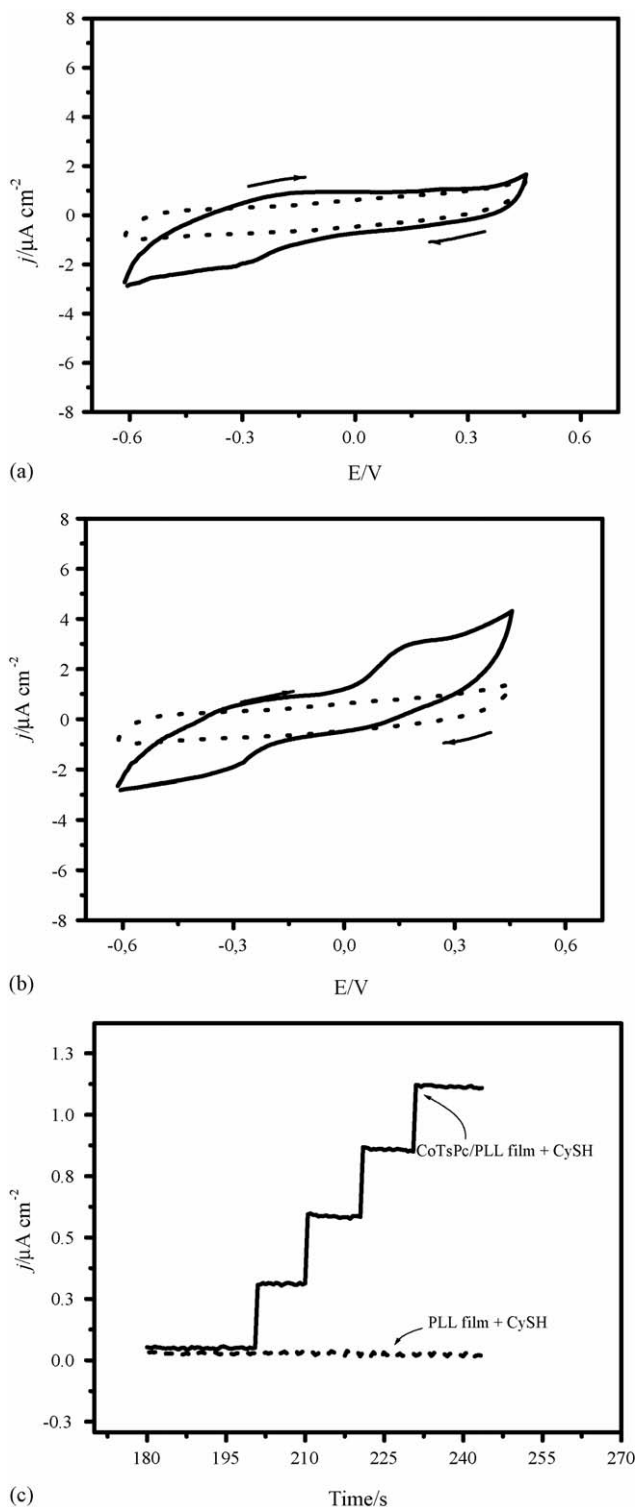
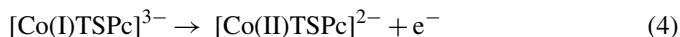
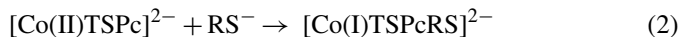


Fig. 1. Cyclic voltammograms obtained for: (a) CoTSPc/PLL modified electrode (full line) and bare electrode (dashed line), both in absence of CySH. (b) CoTSPc/PLL modified electrode (full line) and a bare electrode (dashed line), both in presence of $5 \mu\text{mol l}^{-1}$ CySH. Measurements carried out in 0.1 mol l^{-1} phosphate buffer solution at pH 7.0. Scan rate: 0.01 V s^{-1} . (c) Amperograms for PLL (dashed line) and CoTSPc/PLL (full line) modified electrode, referring to additions of $5 \mu\text{mol l}^{-1}$ CySH. $E_{\text{appl}} = 0.150 \text{ V}$ vs. Ag/AgCl.

occur according to Eqs. (1)–(4) [39]:



where RSH and $\text{RS}^{\bullet+}$ represent cysteine and its cation-radical form, respectively.

The enhancement of the anodic current observed in Fig. 1b (full line) on addition of cysteine is a consequence of the cyclic effects introduced by Eq. (4). The cysteine cation-radical species formed combining to give cystine as the product of the oxidation of cysteine remaining in solution and does not block the CoTSPc/PLL film since the electrode response is maintained.

After that, in order to verify the response of the GC electrode coated with PLL only, amperometric measurements were carried out using 0.150 V and no response was observed (Fig. 1c, dashed line). On the other hand, the amperogram with CoTSPc/PLL modified electrode in presence of $5 \mu\text{mol l}^{-1}$ CySH showed a high current density response (Fig. 1c, full line). These studies indicate that the GC electrode coated with PLL only does not catalyse the CySH oxidation. As can be seen, the current density for CySH oxidation is observed in the used potential range only when the complex is used. This behavior can also be associated to the catalytic effect of the CoTSPc film. In this sense this result suggests that the CoTSPc is working as catalyst for CySH oxidation on the sensor surface.

3.2. Influence of the CoTSPc and PLL concentrations in the sensor response

The concentration of complex and PLL is a control factor of great importance [40]. Firstly, the dependence of the CoTSPc concentration on the electrode response for CySH was investigated by using amperometry and solutions containing different concentrations of the complex ($0.3, 0.5, 0.8, 1.0$ or 1.2 mmol l^{-1}), maintaining the PLL concentration (0.5 mmol l^{-1}). The best amperometric response of the sensor was obtained using 0.8 mmol l^{-1} of CoTSPc solution ($\Delta j = 1.24 (\pm 0.04) \mu\text{A cm}^{-2}$). The membrane containing CoTSPc concentrations lower than 0.8 mmol l^{-1} presented a lower response, probably due to the lower amount of catalyst in the electrode surface. On the other hand, for concentrations higher than 0.8 mmol l^{-1} of the catalyst the sensor presented lower response and repeatability, presumably due to a poor adherence of the film on the electrode surface. Thus, the membrane prepared with CoTSPc concentration of 0.8 mmol l^{-1} was chosen to give the best sensor performance and repeatability.

In the next step, the influence of the PLL concentration on the sensor response was also investigated in the concentrations: $0.25, 0.4, 0.5, 0.6$ and $0.75 \mu\text{mol l}^{-1}$ maintaining the CoTSPc concentration in 0.8 mmol l^{-1} . The best amperometric response of the sensor was obtained with a PLL concentration of 0.6 mmol l^{-1} ($\Delta j = 1.26 (\pm 0.09) \mu\text{A cm}^{-2}$). When lower PLL concentrations were used a decrease in the responses were

obtained. This behavior suggests that the CoTSPc compound is leaching out from the electrode surface due to the PLL concentration become insufficient to the complex immobilization. However, using PLL concentration higher than 0.6 mmol l^{-1} resulted in a decrease of the responses, probably due to the barrier generated by the polymer film to the electroactive molecule. The sensors prepared in this way allowed to obtain modified electrodes with good sensitivity, repeatability and stability. In order to use the modified electrode to CySH determination, the optimization of the analytical procedure was performed by a systematic study of the experimental parameters that affect the sensor response, namely, the pH of the medium, the nature and concentration of the buffer solution.

3.3. Influences of the solution, pH, buffer nature and concentration

The influence of the solution pH in the voltammetric response of the sensor for CySH was studied using 0.1 mol l^{-1} MacIlvaine buffer at pH 6.0, 6.5, 7.0, 7.5 and 8.0 (Fig. 2). As can be verified, the current and potential of the peak depend on the solution pH. The peak current density increased with pH in the range from 6.5 up to 8.0 (Fig. 2a). However, the peak current density showed a significant increase until pH 7.5. Thus, was not necessary the use of higher values than 7.5 for further studies. A linear correlation obtained for E_p versus pH curve with a slope of 0.06 V/pH (Fig. 2b) from pH 6.0 up to 8.0, is close to that expected for a monoelectronic/monoprotonic electrode reaction 0.059 V/pH at 25°C . Thus, the number of proton involved in this process should be equal to electrons number, or $0.059 (n_p/n_e) \text{ V/pH}$, where $n_p = n_e$ [41].

The influence of the electrolyte on the sensor response was tested in MacIlvaine, phosphate, Pipes and Hepes solutions with concentrations of 0.1 mol l^{-1} and indicated that Pipes buffer solution gives the best amperometric response. In this sense, the Pipes buffer solution was chosen. Finally, in the study of the concentration of buffer solution (0.025 , 0.05 , 0.1 , 0.2 and 0.25 mol l^{-1}) the results indicated that concentrations from 0.1 up to 0.2 mol l^{-1} presented almost constant current density. In this way, the concentration of 0.1 mol l^{-1} was chosen for the further experiments.

3.4. Voltammetric studies

More detailed studies of peak current were performed by cyclic voltammetry in 0.1 mol l^{-1} Pipes buffer solution (pH 7.5) to obtain new insights to the CySH oxidation on the sensor surface. In a first step, the plot of peak current density (j_p) versus square root of the potential scan rate ($v^{1/2}$) in the rate from 0.01 up to 0.100 V s^{-1} (data not shown), resulted in a straight line expressed by $j_p (\mu\text{A cm}^{-2}) = 5.56 + 21.73 v^{1/2} (\text{V}^{1/2} \text{ s}^{-1/2})$, suggesting that the process is similar to those controlled by diffusion in the studied scan rate range as well as is under catalytic control in low scan rates (positive intercept). Using the angular coefficient of this expression could be possible to determine the number of electrons (n) involved in the CySH oxidation at the modified electrode. As can be seen in Fig. 1b, the cyclic voltam-

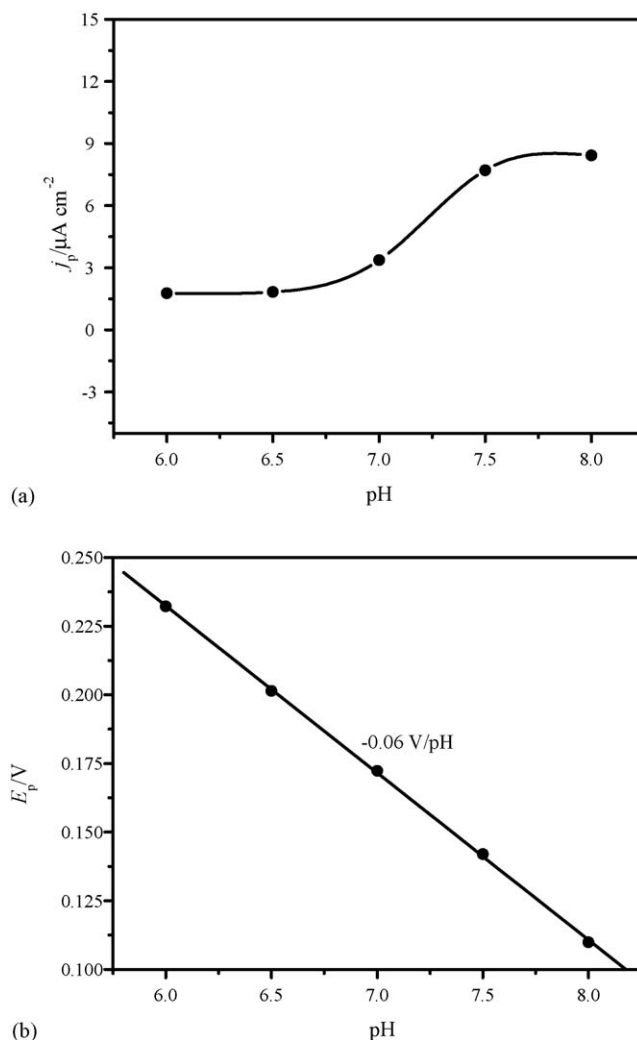


Fig. 2. (a) Influence of the solution pH on the sensor response and (b) influence of the solution pH on the peak potentials obtained by cyclic voltammetry in $10 \mu\text{mol l}^{-1}$ CySH. Measurements carried out in 0.1 mol l^{-1} phosphate buffer solution at pH 7.0.

metric experiments of CySH on CoTSPc/PLL film modified electrode presents one anodic and cathodic peak in the investigated potential range. However, one is provenient of the CySH oxidation process and the other is provenient of the CoTSPc reduction process. Thus, assuming an irreversible oxidation of CySH on the modified electrode the following equation was used [42]:

$$j_p = (2.99 \times 10^5) n(\alpha n_a)^{1/2} C_0^* D_0^{1/2} v^{1/2} \quad (5)$$

where j_p is the peak current density, n the number of total electrons involved in the reaction, α the electron transfer coefficient, n_a the number of electrons involved in the rate-determining step, " D_0 " the diffusion coefficient of the electroactive species ($\text{cm}^2 \text{ s}^{-1}$), C_0^* (mol cm^{-3}) the concentration of the electroactive species and v is the potential scan rate. The value of the concentration and diffusion coefficient used for CySH in aqueous solution were $10 \mu\text{mol l}^{-1}$ ($1 \times 10^{-8} \text{ mol cm}^{-3}$) and $4.8 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ [24,43], respectively.

Considering the necessary knowing of the αn_a value presented in Eq. (5) two approaches were employed, one based on the voltammogram shape factor and the other based on the shift of the peak potential as a scan rate function [43]. The first is based on the differences of the peak potential (E_p) and the half peak potential ($E_{p/2}$) as can be represented according to Eq. (5) $\alpha n_a = 47.7/(E_p - E_{p/2})$ [42]. In this way, the obtained value of αn_a was 0.95. Therefore, using this found αn_a value in Eq. (5) as well as the slope of the plot of j_p versus $\nu^{1/2}$ ($21.73 \mu\text{A s}^{1/2} \text{V}^{-1/2} \text{cm}^{-2}$) a value of $n = 1.08$ was obtained, suggesting an electron transfer mechanism of 1.0 electron for the electrocatalytic oxidation of CySH. This result is in agreement to the works reported in the literature based on the catalytic oxidation of CySH [16,44].

The second approach was based on the dependence of the position of the peak potential with the potential scan rate (E_{pa} versus $\log \nu$) and resulted in a regression equation represented by $E_p(\text{V}) = 0.22 + 0.033 \log[\nu(\text{V s}^{-1})]$ for scan rates between 0.025 and 0.150 V s^{-1} . In this sense, based on the simplified expression for an irreversible reaction the change in E_{pa} for each 10-fold increase in ν is $1.15RT/\alpha n_a F$ [42], the plot of E_{pa} versus $\log \nu$ indicates a linear variation with slope ($\Delta E_{pa}/\Delta \log \nu$) found to be 0.033 V/decade. Therefore, αn_a was calculated to be 0.96 and, a value of $n = 1.0$ was also determined. As can be seen, the values of αn_a obtained from both approaches are in agreement. If the electrons number determined in the CySH oxidation in this work was 1.0 as well as considering the behavior of the peak potential with the solution pH, there is only one possibility for pH between 6.0 and 8.0. The number of proton involved in this process should be also 1.0 or $0.059 (n_p/n_e) \text{V/pH}$ where the protons and electrons number involved in the electrode process are represented by n_p and n_e , respectively.

3.5. Stability of CoTSPc/PLL on the electrode surface

The stability of the CoTSPc/PLL film modified electrode was checked with successive CySH additions equivalents to $10 \mu\text{mol l}^{-1}$ in the electrochemical cell and recording the current density (Δj) associated to the analyte oxidation by amperometric measurements in 0.1 mol l^{-1} Pipes buffer solution (pH 7.5). After 200 measurements no significant change of the Δj was observed for the modified electrode. Indeed, when the modified electrode was stored at room temperature no significant change in the response was observed for months.

The modified electrode presented a good repeatability for CySH determination. The relative standard deviation (R.S.D.) for 10 determinations of $10 \mu\text{mol l}^{-1}$ CySH was 4.1%. Additionally, a series of 10 sensors prepared in the same manner and tested at Pipes buffer (pH 7.5) containing $10 \mu\text{mol l}^{-1}$ CySH gave responses with a relative standard deviation of 5.2%. These experiments indicate that the CoTSPc/PLL film modified electrodes have good stability and repeatability, probably due to the ability of PLL in fixing CoTSPc on the electrode surface in a stable and reproducible way. This behavior can be attributed to the strong interaction of PLL with the phthalocyanine by the ion-pairing between the amino group ($-\text{NH}_3^+$) of

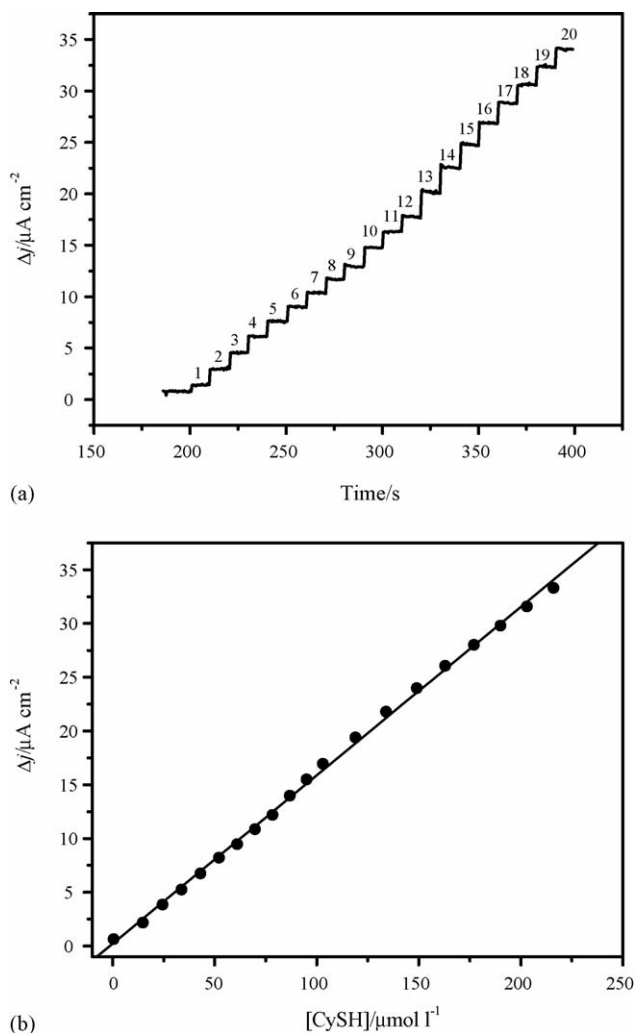


Fig. 3. Chronoamperometrics measurements for the electrooxidation of CySH obtained in Pipes buffer solution at pH 7.5 for concentration range between 0.5 up to $216 \mu\text{mol l}^{-1}$ and inset the calibration plot: (1) $0.5 \mu\text{mol l}^{-1}$, (2) $14.8 \mu\text{mol l}^{-1}$, (3) $24.4 \mu\text{mol l}^{-1}$, (4) $33.8 \mu\text{mol l}^{-1}$, (5) $43.1 \mu\text{mol l}^{-1}$, (6) $52.1 \mu\text{mol l}^{-1}$, (7) $61.0 \mu\text{mol l}^{-1}$, (8) $69.8 \mu\text{mol l}^{-1}$, (9) $78.3 \mu\text{mol l}^{-1}$, (10) $86.8 \mu\text{mol l}^{-1}$, (11) $95.0 \mu\text{mol l}^{-1}$, (12) $103.0 \mu\text{mol l}^{-1}$, (13) $119.0 \mu\text{mol l}^{-1}$, (14) $134.0 \mu\text{mol l}^{-1}$, (15) $149.0 \mu\text{mol l}^{-1}$, (16) $163.0 \mu\text{mol l}^{-1}$, (17) $177.0 \mu\text{mol l}^{-1}$, (18) $190.0 \mu\text{mol l}^{-1}$, (19) $203.0 \mu\text{mol l}^{-1}$ and (20) $216.0 \mu\text{mol l}^{-1}$. $E_{\text{appl}} = 0.150 \text{ V vs. Ag/AgCl}$.

the PLL and the anionic group ($-\text{SO}_3^-$) of the tetrasulphonated phthalocyanine, which should be very strong as recently verified [31,32] justifying the high stability of the proposed system.

3.6. Analytical characterization and application of the proposed sensor

In order to obtain an analytical curve for the developed sensor, chronoamperometric studies for CySH determination were carried out at different concentrations in 0.1 mol l^{-1} Pipes buffer at pH 7.5, using a potential of 0.150 V (Fig. 3a). The proposed sensor showed a linear response range from 0.5 up to $216 \mu\text{mol l}^{-1}$ (plot inset in Fig. 3b), which can be expressed according to the

Table 1
Experimental conditions and analytical parameters for CySH determination

Electrode	Method	E_p (V)	Electrolyte	LOD ($\mu\text{mol l}^{-1}$)	Dynamic range ($\mu\text{mol l}^{-1}$)	Sensitivity ($\text{nA } \mu\text{mol l}^{-1}$)
Nafion/indium hexacyanoferrate film modified electrode [1]	HPLC with electrochemical detection	0.600 vs. SCE	Phosphate buffer (pH 3.0)	0.0000016	1–1000	3.09
Pt microelectrode modified with carbon nanotubes [11]	Chronoamperometry	0.575 0.7 vs. SCE	0.2 M H_2SO_4	2	2–2800	–
Cobalt(II) salophen into carbon paste [13]	DPV chronoamperometry	– 0.600 vs. Ag/AgCl	Phosphate buffer (pH 6–8)	1 0.5	2–20 3–770 –	– ~22
Lead ruthenate pyrochlore-modified [17]	SWV flow injection analysis	0.550 1.0 vs. Ag/AgCl	Phosphate (pH 7.4)	1.91 0.0017	Up to 560 0.0001–100	32.5 78.8
Coenzyme pyrroloquinoline quinone into polypyrrole film on glassy carbon [18]	Chronoamperometry	0.500 vs. Ag/AgCl	Borate buffer (pH 8.2)	0.0006	–	4.71
Cobalt(II)-4-methylsalophen into carbon paste [19]	CV, DPV	0.500 vs. Ag/AgCl	Acetate buffer (pH 5.0)	0.2	0.5–100	0.036
Microcrystals fullerene- C_{60} adhered on glassy carbon electrode [20]	CV	0.580 vs. Ag/AgCl	Phosphate buffer (pH 7.0)	–	200–1200	15.5
Oxovanadium(IV) complex of Salen [21]	Chronoamperometry	0.650 vs. SCE	KCl (pH 5)	170	240–2300	4.7
Carbon ceramic with rutenium complex [23]	CV	~0.800 vs. Ag/AgCl	Phosphate (pH 2)	1	5–685	5
Octabutylthiophthalocyaninato-cobalt(II) self-assembled monolayer on gold [24]	CV	~0.400 vs. Ag/AgCl	Buffer (pH 4.0)	0.31	0.1–10	–
Self-assembled monolayer of cobalt an iron phthalocyanine complexes on gold [25]	CV	0.540 and 0.330 vs. Ag/AgCl	Buffer solution (pH 4)	0.52	–	–
Cobalt tetra-aminophthalocyanine adsorbed and electropolymerized on glassy carbon [26]	CV DPV DNP chronoamperometry	–0.130 –0.180 –0.180 vs. SCE	0.5 M NaOH	120 1100 450	–	–
Carbon electrodes bulk modified with cobalt phthalocyanine [27]	CV and chronoamperometry	0.400 vs. Ag/AgCl	Acetate buffer (pH 5.0)	0.2	1–12	8.89
Nickel tetrasulphonated phthalocyanine immobilized on silica gel with carbon paste [44]	CV	0.500 vs. SCE	Acetate buffer (pH 2.0)	–	1000–7000	233
Aquocobalamin adsorbed on glassy carbon [45]	Flow injection amperometric	0.800 vs. Ag/AgCl	Phosphate buffer (pH 7.0)	1.7	–	9.28
Cobalt tetrasulphonated phthalocyanine adsorbed on glassy carbon [46]	CV	0.820 vs. Ag/AgCl	0.05 M H_2SO_4	–	–	–
Molybdenum(V) phthalocyanines complexes in graphite powder [47]	CV	0.260 0.280 vs. Ag/AgCl	0.05 M H_2SO_4	0.1	20000–80000 2000–20000	–
Lead phthalocyanine incorporated into poly(vinylchloride) membrane on pyrolytic graphite [48]	Potentiometry	–	Phosphate buffer (pH 8.5)	~1	–	–
Boron-doped diamond electrode [49]	Voltammetry flow injection	0.570 vs. SCE	KHCO_3 (pH 9.0)	0.9 0.021	1–200 0.1–100	12–20 12
Hexacyanoferrate ion adsorbed on propylpyridiniumsilsesquioxane polymer film-coated $\text{SiO}_2/\text{Al}_2\text{O}_3$ [50]	CV	0.152 vs. SCE	KCl + Tris buffer (pH 7.0)	–	–	–
Substituted cobalt phthalocyanines polymerized on glassy carbon [51]	CV	0.800–0.900 vs. Ag/AgCl	Tris buffer and dimethylformamide (pH 3.5)	–	–	–
Palladium/iridium oxide electrodeposited on glassy carbon [52]	HPLC with electrochemical detection	0.850 vs. Ag/AgCl	Phosphate buffer (pH 3.0) + EDTA	0.5	4–200	–
Cobalt(II) tetrasulphonated phthalocyanine immobilized in poly-L-lysine film (this work)	Chronoamperometry	0.150 vs. Ag/AgCl	Phosphate (pH 7.5)	0.036	4.98–200	31

CV: cyclic voltammetry; HPLC: high performance liquid chromatography; DPV: differential pulse voltammetry; DNP: differential normal pulse.

Table 2
Cysteine determination in three food supplement samples in triplicate

Samples	Proposed method (w/mg)	Comparative method (w/mg) [33]
A	498 (±8)	499 (±13)
B	505 (±10)	500 (±25)
C	504 (±7)	507 (±18)

w: mass obtained per capsule.

following equation:

$$\Delta j_p (\mu\text{A cm}^{-2}) = 0.21(\pm 0.17) + 0.157(\pm 0.001) \times [\text{CySH}] (\mu\text{mol l}^{-1}) \quad (6)$$

with a correlation coefficient of 0.999 (for $n = 20$). A detection limit of $0.15 \mu\text{mol l}^{-1}$ was determined using $3\sigma/\text{slope}$ ratio and quantification limit was $0.50 \mu\text{mol l}^{-1}$ using $10\sigma/\text{slope}$, where σ is the standard deviation of the mean value for 10 amperograms of the blank, determined according to the IUPAC recommendations [53]. Table 1 shows the reported works involving modified electrodes with several mediators for CySH determination. According to this short reviewing fews works utilize neutral medium for CySH determination, although for the analysis of biological or pharmacological samples be necessary pH close to the physiological ones (pH 7.4). Besides, the oxidation potentials of the CySH are relatively high when compared to the present work. The detection limit and sensitivity of the present work are also better (Table 1) [11,13,19,21,23,27,45]. Such good sensitivity can be attributed to the efficiency of the electron transfer between CoTSPc and CySH as well as due to the simple and efficient immobilization method. It is important to relate that the proposed sensor also presented higher sensitivity than the works involving flow injection (Table 1). Although few works presented in this table did comment about the stability of the modified electrode, the proposed sensor presented better stability than those described in the literature.

3.7. Application of the proposed sensor in real samples

In addition, the proposed sensor was applied for CySH determination in three commercial food supplement samples (A, B and C). The concentrations of CySH were determined using the standard addition method and the results were compared to a spectrophotometric method, using the paired t -test and it showed that the results are statistically equal at a confidence level of 95% (Table 2). However the relative standard deviation was lower than those ones of the comparative method, suggesting a better precision. In this sense, it is clearly demonstrated that the glassy carbon electrode modified with CoTSPc/PLL is a feasible, sensitive, robust, stable, precise and rapid to CySH determination.

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