



Voltammetric determination of cysteine using carbon paste electrode modified with Co(II)-Y zeolite

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ABSTRACT

A novel zeolite modified electrode for use in voltammetric determination of L-cysteine (CySH) was described. The electrode comprises a Co(II)-exchanged zeolite Y as modifier in carbon paste matrix. First, the electrochemical behavior of Co(II) in modified carbon paste electrode was studied. The results demonstrated that diffusion can control the redox process of cobalt cations at the surface of the modified electrode. Then, the behavior of the electrode in the presence of CySH was studied by using cyclic voltammetry and a novel behavior was observed. In high concentration of CySH (above 10 mmol L⁻¹), one pair of semi-reversible electrochemical extra peak was observed which was assigned to the processes of oxidation–reduction of CySH at the unmodified and modified electrode. Acidic conditions with respect to the neutral one cause an increase in the electrode response. The modified electrode showed a suitable linear calibration graph in the concentration range of 1.0×10^{-9} – 1.0×10^{-3} mol L⁻¹ cysteine with a detection limit of 2.37×10^{-10} mol L⁻¹. The influence of potential interfering substances on the peak current was studied and the results showed that the method was highly selective for determination of CySH. Thus, the proposed electrode was used for the determination of CySH in real samples including human blood serum, urine, N-acetylcysteine tablet and powdered poultry feed and the satisfactory results were obtained. Typical features of the sensor can be summarized as: low cost, simple preparation, fast response, good stability and selectivity, wide linear range, low detection limit and high reproducibility.

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1. Introduction

Cysteine ([[(R)-2-amino-3-mercaptopropanoic acid]], one of about 20 amino acids commonly found in natural proteins, is a sulfur-containing amino acid. Cysteine (CySH) is critical for the proper metabolism of a number of essential biochemicals such as: heparin, biotin, lipid acid, coenzyme A and glutathione. It has been used as a prospective radiation protector and cancer indicator in a number of pathological conditions including Parkinson's and Alzheimer's diseases as well as autoimmune deficiency syndrome (AIDS) [1,2]. Moreover, it has also been widely used in the food and pharmaceutical industries as an antioxidant and biomarker, respectively [3]. Low level of CySH causes certain diseases including slow growth in children, depigmentation of hair, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and weakness [4]. Therefore, the detection of CySH in body fluids has become more important from biological and pharmacological stand points. Several methods for its detection have been

reported including flow injection [5,6], high performance liquid chromatography (HPLC) [7,8], colorimetry [9,10], chemiluminescence [11], spectrofluorimetry [12] and electrochemistry [13–18]. Compared with the other methods, electrochemical techniques offer several advantages such as simplicity, ease of preparation and method, high sensitivity and selectivity, and relatively low cost [19,20].

Chemically modified electrodes (CMEs) have recently attracted much attention due to their significant advantages [21–26]. So, various chemically modified electrodes have been developed for the oxidation and detection of CySH [27,28]. As a subcategory of CMEs, zeolite-modified electrodes (ZMEs) have been greatly studied [29–31]. ZMEs have some advantages over other CMEs due to their ion-exchange capacity and the molecular (size, shape and charge) selectivity properties of zeolites [32]. Particularly, substantial efforts have been dedicated to the development of sensors and biosensors using ZMEs [33–37].

In this work, we constructed a novel modified carbon paste electrode by incorporation of Co(II)-exchanged zeolite Y (Co²⁺Y/ZMCPE). The Co(II)-Y was obtained by ion exchanging of the parent Na-Y zeolite in a Co(II) solution as nitrate salt. Our preliminary experiments showed that the obtained Co²⁺Y/ZMCPE electrode exhibits good voltammetric response towards CySH.

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Hence, the fabricated electrode was used for the determination of CySH. Finally, the analytical performance of the electrode was evaluated for the determination of CySH in the patient blood serum, urine, N-acetylcysteine (ACC) tablet and powdered poultry feed (PPF) samples.

2. Experimental

2.1. Reagents

The Na-Y zeolite (Cat. No. 33444-8) and the spectroscopic grade mineral oil (Nujol) were obtained from Aldrich. All reagents were of analytical grade from Merck, Fluka, or Aldrich companies. Tablets of ACC and PPF as pharmaceutical samples were purchased respectively from Flumucil Company of Swiss and Razak Company of Iran. Also, patient blood serum and urine as clinical samples were taken from local sources. Triple-distilled water was used throughout the experiments. Buffer solutions were prepared from acetic acid, sodium acetate and NaOH in the pH range of 2.2–9.3. Stock solutions of CySH were freshly prepared and purged with pure nitrogen gas (99.99%) for 5 min before the voltammetric measurements and diluter solutions were prepared by serial dilution of the stock solution. To prevent the diffusion of oxygen into the solution, nitrogen gas was passed over the surface of test solutions during the experiments.

2.2. Apparatus and procedure

Electrochemical experiments were performed by using a potentiostat/galvanostat (PerkinElmer, EG&G 273 A). A three-electrode system was used containing the $\text{Co}^{2+}\text{Y/ZMCPE}$ as the working electrode, a platinum rod electrode as the counter electrode, and an Ag/AgCl as the reference electrode. The pH of solutions was adjusted using a digital pH meter (Jenway 370). An appropriate amount of the standard or the measuring solution of CySH was added into the cell containing 25 mL of the acetate–hydrochloric acid mixture (pH 2.2) as the supporting electrolyte. The solutions were prepared immediately before use, and it was deaerated with purified nitrogen to prevent the oxidation of CySH to cystine (oxidized form of CySH) by atmospheric oxygen (especially in basic solutions). Cyclic voltammetry experiments were performed by immersing the modified electrode together with the reference and the counter electrodes into the measuring solution and then scanning the potential in the range of +1.0 to -0.2 V vs. Ag/AgCl. All electrochemical measurements were conducted at room temperature. In case of observing low sensitivity and slow or noisy response, it was only necessary to renew the electrode surface by a slight rubbing of the electrode on a soft paper and rinse with ethanol and water. The electrodes were kept in open air when not in use. To determine the cobalt loading of zeolite Y, 1.000 g of the Co(II) exchanged zeolite Y was digested in a platinum crucible by adding a mixture of 1:1 concentrated nitric and perchloric acids. After drying the mixture on a soil bath, a mixture of 1:1 concentrated hydrochloric and hydrofluoric acids was added and it was again heated until it was dried. Finally, the remained content was dissolved in a 10% hydrochloric acid solution and then it was filtered and washed with water in a 100 mL volumetric flask. The obtained solution was used for determining cobalt by atomic absorption spectrometer (Perkin Elmer AAnalyst 300).

2.3. Electrode preparation

To prepare Co(II) -exchanged of zeolite Y, 1 g of the Na-Y zeolite was lightly grounded and it was added to 250 mL 0.01 mol L^{-1} $\text{Co(NO}_3)_2 \cdot 6\text{H}_2\text{O}$ solution and it was shaken magnetically for 48 h. The exchanged zeolite was carefully washed with dilute HCl

solution (pH 2.0) to remove occluded materials and surface-adherent salts, and then it was washed with water till free of Cl^- and dried in air. The loaded cobalt extent into the exchanged form was determined by atomic absorption spectroscopy and it was 5.9% (w/w). An appropriate amount of the Co(II) -exchanged zeolite (5–20 weight% with respect to carbon paste) was mixed with 100 mg graphite powder and then Nujol was added. After thorough hand mixing in a mortar to obtain a fine paste, a portion of the composite mixture was packed into the end of a Teflon tube. Electrical contact was made by forcing a copper wire positioned into the glass tubing, down the Teflon tube and into back of a paste. Unmodified electrodes were prepared in a similar way, using a carbon-paste with unmodified zeolite (Na-Y). The electrode surface was polished using a soft paper and then rinsed with ethanol and water, respectively.

2.4. Preparation of real samples

ACC tablets: 20 mg portion of a finely powdered sample was dissolved in a 10 mL of acetate–hydrochloric acid solution (pH 2.2) and then 0.25 mL of the prepared solution was transferred to the cell containing 25 mL of 0.2 mol L^{-1} acetate–hydrochloric acid solution (pH 2.2) and finally cyclic voltammograms were recorded.

PPF: 151.15 mg portion of a finely powdered sample was dissolved in a 25 mL of acetate–hydrochloric acid solution (pH 2.2) and then 0.25 mL of the prepared solution was transferred to the cell containing 25 mL of 0.2 mol L^{-1} acetate–hydrochloric acid solution (pH 2.2) and then cyclic voltammograms were recorded.

Human blood serum: To prepare the blood serum of a patient, 15 mL of the blood sample was separated and centrifuged after putting the sample in room temperature for 15 min. A 2.5 mL portion of the serum was diluted to 25 mL in a volumetric flask with 0.2 mol L^{-1} acetate–hydrochloric acid solution (pH 2.2) and it was transferred into the voltammetric cell for recording the cyclic voltammograms.

Urine: To prepare the urine, a 2.5 mL portion of urine was diluted to 25 mL in a volumetric flask with 0.2 mol L^{-1} acetate–hydrochloric acid solution (pH 2.2) and it was transferred into the voltammetric cell for recording the cyclic voltammograms.

3. Results and discussion

3.1. Voltammetric response of the $\text{Co}^{2+}\text{Y/ZMCPE}$ in the absence of CySH

3.1.1. Effect of modifier

In preliminary experiments, effect of modification of the electrode on the voltammetric response was studied. The voltammetric behavior of the CPE and $\text{Co}^{2+}\text{Y/ZMCPE}$ in a 0.2 mol L^{-1} acetate–hydrochloric acid solution (pH 2.2) with a scan rate of 25 mV s^{-1} was studied and the corresponding voltammograms, which were recorded after several preliminary scans, are collected in Fig. 1. As the results show, no voltammetric response was observed for unmodified CPE (Fig. 1a) which is desirable. While, a well defined two sequence reduction peaks with peak potentials of 0.067 and 0.27 V and also oxidation peak with a peak potential of 0.475 V were observed for the Co(II) -Y modified electrode, assigned to the processes of oxidation–reduction of Co(II) at the surface of the modified electrode (Fig. 1c).

3.1.2. Effect of electrolyte

Supporting electrolyte has a significant role on the behavior of ZMEs with respect to other CMEs, because ion exchange process between the cations of the electrolyte solution and cations of the zeolite has considerable effect on the voltammetric behavior of these zeolitic modified electrodes. Due to definite pore

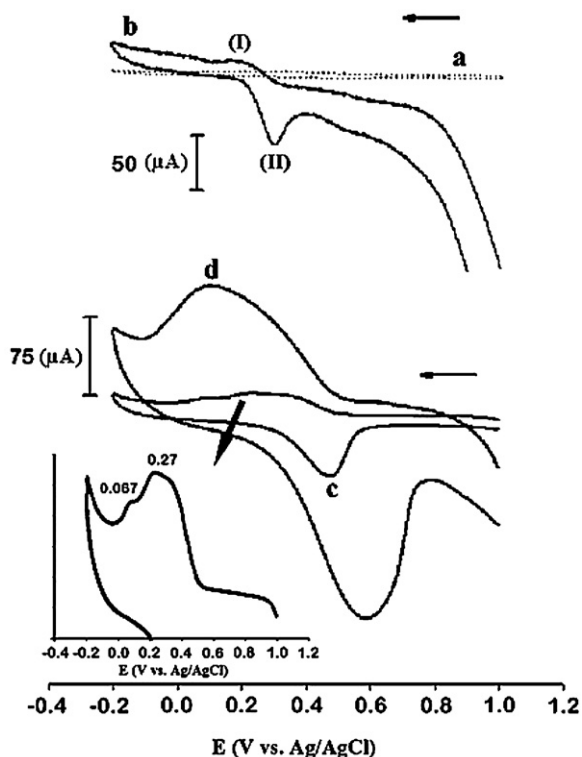


Fig. 1. Cyclic voltammograms of (a, b) CPE and (c, d) $\text{Co}^{2+}\text{Y/ZMCPE}$. (a, c) In the absence and (b, d) in the presence of 10 mmol L^{-1} CySH (in 0.2 mol L^{-1} acetate-hydrochloric acid solution (pH 2.2)), scan rate: 25 mV s^{-1} . Inset: magnification of voltammogram (c) in the range of -0.2 to 1.0 V .

sizes of zeolite pores, ion exchange process of zeolites is very selective to the nature of supporting electrolytes regarding to the size, charge and shape of their content cationic species. Experimental results showed that the CV peak height at the modified electrode depends on the type of the supporting electrolyte. Several electrolytes, including 0.2 mol L^{-1} of MgCl_2 , CaCl_2 , NaNO_3 , KNO_3 solutions, as separate, and also an acetate-hydrochloric acid solution (0.2 mol L^{-1} acetate, pH 2.2) were investigated (voltammograms not shown). The results of these studies are summarized in Table 1. Although, there is a significant difference between the hydrated radii of cations of supporting electrolytes (H_3O^+ : 9, Mg^{2+} : 8, Ca^{2+} : 6, Na^+ : 4 and K^+ : 3 Å) there is no considerable difference between the observed peak currents as we expected. We suggest that other factors such as charge of the cations, the counter anion and especially the ionic strength of the solutions also affect the ion exchange extent. According to the results, it is concluded that the electroactive Co(II) species existing in the zeolite were exchanged with the electrolyte cations, and then escape from the zeolite to be reduced at the interface of electrode-solution. It should be mentioned that no cobalt species was initially present at the surface of the electrode due to probably surface adsorbed process. As described in Section 2.3, after preparation of Co(II)-Y modified zeolite, it was thoroughly washed with dilute HCl

Table 1
Effect of supporting electrolyte on E_p and I_p of Co(II)/Co(0) at $\text{Co}^{2+}\text{Y/ZMCPE}$.

Supporting electrolyte	MgCl_2	CaCl_2	KNO_3	NaNO_3	Acetate-hydrochloric acid solution (pH = 2.2)
I_{pc}^a (μA)	8.15	9.02	9.69	10.58	12.94
E_{pc} (mV)	-29.97	6.23	10.27	15.02	41.02
I_{pa} (μA)	-51.22	-52.38	-54.47	-56.52	-61.73
E_{pa} (mV)	653.72	650.47	634.22	614.97	529.97

^a Current and potential peak related to reduction of Co(I) to Co(0) .

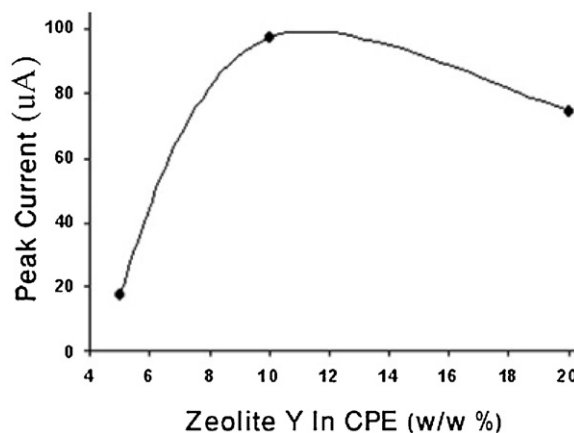


Fig. 2. The effect of amount of zeolite Y in the CPE on the electrode response, scan rate: 25 mV s^{-1} ; 0.2 mol L^{-1} acetate-hydrochloric acid solution (pH 2.2).

solution to remove the surface adsorbed species. Hence, the observed voltammetric current is only resulted from the ion exchanged Co(II) cations into the electrode surface. The transfer mechanism is in conformity with the extra zeolite electron transfer mechanism which has been described by Bessel and Rolison [38]. As the results show, the response of the electrode depends on the type of cation in the supporting electrolyte solution. The highest current was observed when the modified electrode was exposed to the acetate-hydrochloric acid solution containing 0.2 mol L^{-1} Na^+ (pH 2.2) which the current was more than of that observed for a neutral 0.2 mol L^{-1} NaNO_3 electrolyte with the same concentration of Na^+ . This can be related to cooperation of hydronium ion in displacing of Co(II) from zeolite pores to the surface of the electrode.

3.1.3. Effects of electrode ingredient

The composition of electrode has significant effects on the resistance, hydrophilicity and especially the voltammetric response of CMEs. In ZME studies this also affects on the ion exchange extent which in turn plays a significant role on the electrode response. To study these effects, cyclic voltammetry responses of the modified electrodes containing 5, 10 and 20% $\text{Co}^{2+}\text{Y/ZMCPE}$ (w/w, with respect to carbon paste) were examined in acetate-hydrochloric acid solution (pH 2.2) at the same experimental conditions. The results of these studies showed that the optimum proportion of the zeolite to carbon paste was 10:90 (corresponds to 0.59% of Co(II) in the paste) (Fig. 2). Lower amounts of the modifier decrease the extent of ion exchange while the higher amounts increase the resistance of the electrode; both of which decrease the sensitivity of the electrode response. This is in agreement with the literature [34,36].

3.1.4. Effects of scan rate

Effect of scan rate on the behavior of the modified $\text{Co}^{2+}\text{Y/ZMCPE}$ electrode was studied in acetate-hydrochloric acid solutions and the obtained cyclic voltammograms are shown in Fig. 3. Stability of the modified electrode was long, so that the current response remained almost unchanged after several determinations, during which the electrode was stored in air. The current produced by

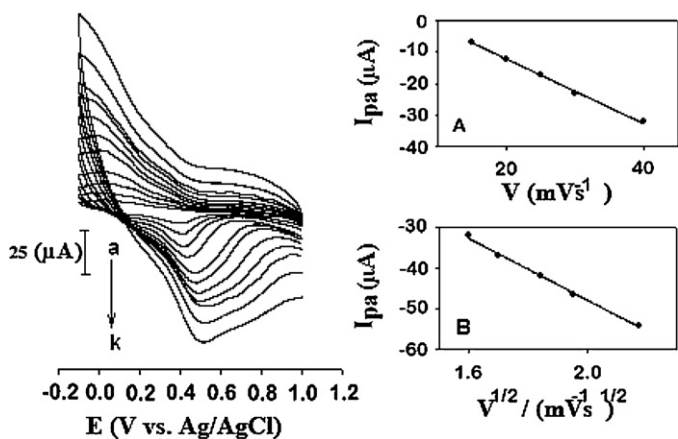


Fig. 3. Cyclic voltammograms of $\text{Co}^{2+}\text{Y/ZMCPE}$ in 0.2 mol L^{-1} acetate-hydrochloric acid solution (pH 2.2) at various scan rates (from inner to outer curves): (a) 15, (b) 20, (c) 25, (d) 30, (e) 40, (f) 50, (g) 70, (h) 90, (i) 110, (j) 150 and (k) 200 mV s^{-1} . Inset: (A) the dependency of anodic peak currents to the scan rate at lower values ($15\text{--}40 \text{ mV s}^{-1}$) and (B) the anodic peak currents to the square roots of scan rate at higher values ($50\text{--}150 \text{ mV s}^{-1}$).

Co(II) did not decreased any more when the ion exchange and electro-redox attained steady state. The high stability obtained at the $\text{Co}^{2+}\text{Y/ZMCPE}$ was primarily due to the strong affinity of zeolite Y for Co(II) .

At low scan rates, the plot of the anodic peak current was linearly dependent on the scan rate (ν) with a correlation coefficient of 0.9986 (inset A in Fig. 3), which indicates a surface-confined redox couple [39]. Moreover, at high scan rates, anodic peak current was linearly dependent on square root of the scan rate (ν) with a correlation coefficient of 0.9966 (inset B in Fig. 3), suggesting that the reaction is mass transfer controlled [40].

As shown by Laviron [41], linear potential sweep voltammetry appears as a convenient method for determination of the characteristics of the electrochemical reactions (the transfer coefficient and the rate constant) in diffusion-less electrochemical systems. For $\text{Co}^{2+}\text{Y/ZMCPE}$, the peak-to-peak separation potential ($\Delta E_p = E_{pa} - E_{pc}$) of the cyclic voltammograms recorded at a low scan rate (15 mV s^{-1}) was 0.19 V in the presence of acetate-hydrochloric acid solution with pH 2.2 as the supporting electrolyte. In addition, when the scan rate was increased, the wave shape was distorted severely which indicates that the electrode reaction becomes electrochemically irreversible at the higher scan rates. In the scan rates above 50 mV s^{-1} , the peak separations also begin to increase, indicating the limitation arising from the charge transfer kinetics [42].

For the case of surface confined electroactive species with a concentration small enough, Laviron derived general expressions for the linear potential sweep voltammetric response as:

$$E_{pa} = E^{o'} + \left[\frac{RT}{(1-\alpha)nF} \right] \ln \left[\frac{RTk_s}{(1-\alpha)nF} \right] + \left[\frac{RT}{(1-\alpha)nF} \right] \ln(\nu) \quad (1)$$

$$E_{pc} = E^{o'} + \left(\frac{RT}{\alpha nF} \right) \ln \left[\frac{RTk_s}{\alpha nF} \right] - \left(\frac{RT}{\alpha nF} \right) \ln(\nu) \quad (2)$$

$$\text{For } E_{pa} - E_{pc} = \Delta E_p > 200/n \text{ mV}$$

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log \left(\frac{RT}{nF\nu} \right) - \alpha \frac{(1-\alpha)nF \Delta E_p}{2.3RT} \quad (3)$$

From these expressions, it is possible to determine the electron transfer coefficient (α) by measuring the variation of the peak

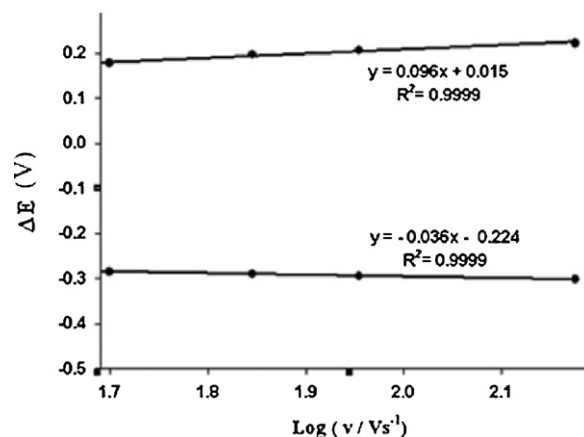


Fig. 4. Experimental variations of ΔE vs. the logarithm of the scan rate.

potentials as a function of scan rate (ν) as well as the apparent charge transfer rate constant (k_s) for electron transfer between the electrode and the surface deposited layer. We have found that for scan rates above 50 mV s^{-1} ; the values of $\Delta E = (E_p - E^{o'})$ were proportional to $\log(\nu)$ as shown by Laviron. According to Fig. 4, the plot yields two straight lines with slopes equal to $-2.3RT/\alpha nF$ and $2.3RT/(1-\alpha)nF$ for the cathodic and anodic peaks, respectively.

The evaluated values for the cathodic electron transfer coefficient, α_c , and the anodic electron transfer coefficient, α_a (using such a plot and Eq. (3)) were 0.82 and 0.66 (assuming $n_\alpha = 2$) for the $\text{Co}^{2+}\text{Y/ZMCPE}$ in the presence of acetate-hydrochloric acid solution with pH=2.2, respectively. This suggests the difference between the rate-limiting steps for the reduction and oxidation processes [43]. The mean value of k_s for anodic peak was obtained 45.15 s^{-1} .

The $\text{Co}^{2+}\text{Y/ZMCPE}$ electrode with the above characteristics was successfully applied for the determination of CySH, the results of which are presented in the following sections.

3.2. Electrochemical behavior of $\text{Co}^{2+}\text{Y/ZMCPE}$ in the presence of CySH

3.2.1. Effect of modification

After investigation of the voltammetric behavior of the modified electrode, its behavior was studied in the presence of CySH as the investigating analyte. The cyclic voltammograms of the CPE and $\text{Co}^{2+}\text{Y/ZMCPE}$ in the presence and absence of CySH in

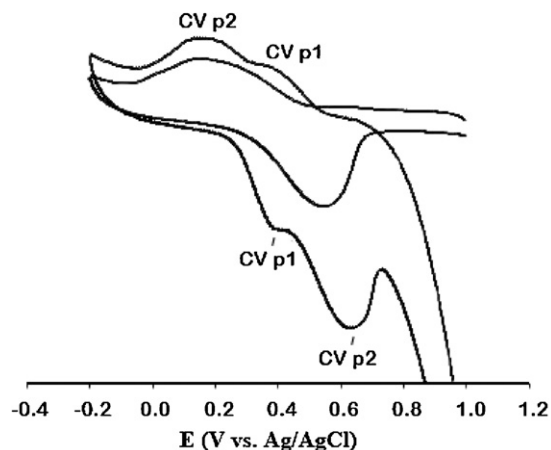
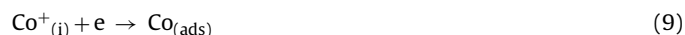
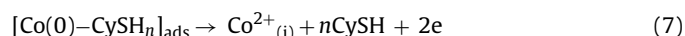
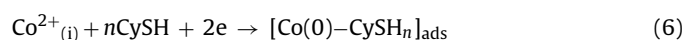
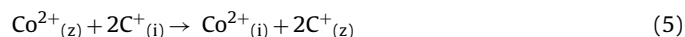


Fig. 5. Cyclic voltammograms of $\text{Co}^{2+}\text{Y/ZMCPE}$ in the presence of (a) $3 \mu\text{mol L}^{-1}$ and (b) 30 mmol L^{-1} CySH 0.2 mol L^{-1} acetate-hydrochloric acid solution (pH 2.2), scan rate: 25 mV s^{-1} .

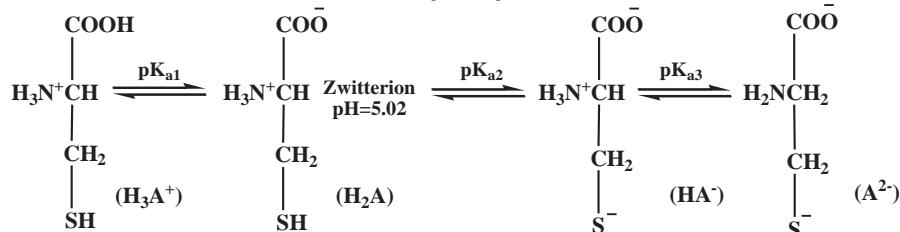
an acetate–hydrochloric acid solution (pH 2.2) were obtained (Fig. 1). Fig. 1b shows the results of the oxidation–reduction of the electroactive species in acetate–hydrochloric acid solution at the surface of the unmodified CPE. According to the previous reports, the electrochemical reaction of CySH is an irreversible process and an oxidation peak could only be observed at different electrodes [44,45]. It was also reported that cystine (CySSCy) could be reduced on a mercury electrode or a modified electrode because some metal ions such as mercury and cadmium are involved in the reduction process of CySSCy [46,47]. Due to the absence of such metal ions, the reduction of CySSCy could not be observed at Pt and Au electrodes. However, in this work (like the obtained results by Dong et al. [48]), one pair of redox peaks were obtained at the CPE, related to the electrochemical reaction of CySH at the CPE in the proposed solution. As shown, the separation between peak potentials, $\Delta E_p = (E_{pa} - E_{pc})$ with $E_{pa} = 0.30$ V and $E_{pc} = 0.18$ V vs. Ag/AgCl, is greater than the $59/n$ mV ($n=2$) as expected for a reversible system. This result shows that the redox couple of CySH obeys a quasi-reversible process in an acidic solution at the surface of the CPE. Eq. (4) shows the electrochemical reaction of CySH at CPE (in pH 2.2) [49]:



In fact, with applying positive potentials at the beginning of sweep (from +1.0 V), some CySH molecules were oxidized on the surface of the electrode to CySSCy, and with sweeping potential towards more negative values, CySSCy was reduced at the surface of the electrode at potential about 0.3 V (peak I). The $\text{Co}^{2+}/\text{Y}/\text{ZMCPE}$ in the acetate–hydrochloric acid solution (pH 2.2) exhibited a well-defined redox reaction as shown in Fig. 1c. Upon the addition of 10 mmol L^{-1} CySH, both cathodic and anodic peak currents increased (Fig. 1d). It can be seen, in the presence of CySH, cathodic and anodic peak potentials shifted towards more negative and positive values, respectively (increase of overvoltage). Experimental results illustrated that the electron transfer step occurs at the zeolite–electrode–solution interface after ion exchange between the electroactive species and the cations in the solution. According to the results, the following mechanisms which are known as extra-zeolite electron transfer [50] can be predicted [51,52] (Eq. (5)–(10)):



where C^+ stands for a singly charged cation and the descriptors z , s and i stand for zeolite, solution and zeolite–solution interface,



respectively. In low concentration of CySH, all $\text{Co}(\text{II})$ ions on the surface of the electrode do not take part in the process of complex formation with CySH. Therefore according to Eqs. (8) and (9), free $\text{Co}(\text{II})$ ions along with the formed complex are reduced.

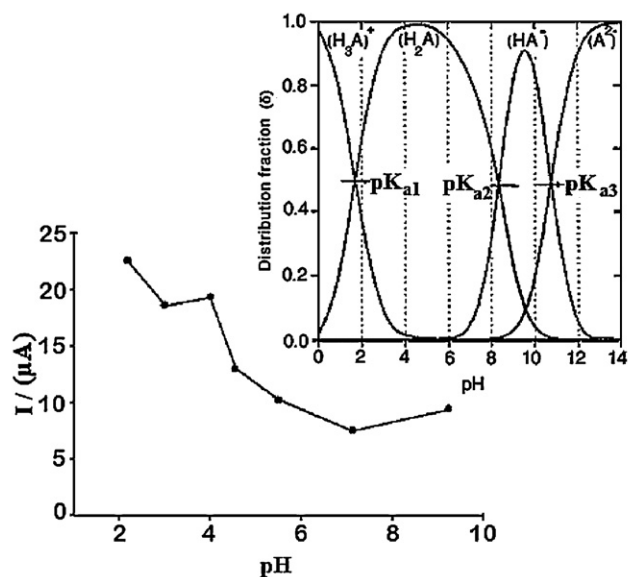


Fig. 6. Influence of pH on the anodic peak current of $\text{Co}^{2+}/\text{Y}/\text{ZMCPe}$ in solution containing 0.01 mol L^{-1} CySH at scan rate of 25 mV s^{-1} (inset: distribution fraction (δ) for various CySH species vs. its pH).

In fact, the presence of CySH makes more escape of Co^{2+} from the zeolite pores to the electrode surface which in turn increases the related cathodic and anodic peak currents. In high concentration of the CySH (above 10 mmol L^{-1}), a pair of extra peak appears (CV p1 and CV' p1) at $\text{Co}^{2+}/\text{Y}/\text{ZMCPe}$ (Fig. 5b). This pair relates to the direct redox process of electroactive species of CySH on the surface of the electrode, as represented in Eq. (4).

3.2.2. Effects of pH

To investigate the effect of pH on the results, the behavior of the $\text{Co}^{2+}/\text{Y}/\text{ZMCPe}$ in the acetate–hydrochloric acid solutions with different pH values covering the range from 2.2 to 9.3 containing 0.01 mol L^{-1} CySH was studied and the results are shown in Fig. 6. As shown, in pH ranges from 7.1 to 2.2 and also from 7.1 to 9.3, the anodic peak current increases.

The ion exchange process is the most important process in ZMEs performance (especially with an extra-zeolite electron transfer mechanism). It is apparent that as pH of the environment decreases (increase in concentration of hydronium ion in the solution), ion exchange process will be at higher rate and a large number of $\text{Co}(\text{II})$ cations will be on the surface of the electrode. Thus, increase in current at lower pH is absolutely reasonable.

Because of CySH functional groups of $-\text{COOH}$, $-\text{SH}$ and $-\text{NH}_2$ (with pK_a of 1.29, 8.37 and 10.70, respectively), the ionization of CySH depends on the pH of aqueous solutions, which can be described as follow [53,54]:

The previous studies have shown that $\text{Co}(\text{II})$ coordinates with nitrogen (N) and sulfur (S) atoms of CySH [55,56]. In the basic solution because of deprotonated of thiol groups (SH), CySH easily coordinates with $\text{Co}(\text{II})$, while in a highly acidic pH all the three binding sites of CySH remains protonated (pK_a value of a COOH

Table 2
Comparison of methods for the determination of CySH.

Detection method	Linear range (mol L ⁻¹)	LOD (μmol L ⁻¹)	Samples detection	Ref.
HPLC	50 × 10 ⁻⁶ –500 × 10 ⁻⁶	0.12	Urine	[7]
Colorimetry	10 × 10 ⁻⁶ –100 × 10 ⁻⁶	–	Urine	[9]
Spectrofluorimetry	0–1.82 × 10 ⁻⁵	40	Protein hydrolysate and cystine electrolyte	[12]
Voltammetry	1 × 10 ⁻⁶ –13 × 10 ⁻⁶	0.13	Urine	[13]
Voltammetry	5 × 10 ⁻⁶ –37 × 10 ⁻⁶	0.48	Urine and serum blood	[15]
Voltammetry	8 × 10 ⁻⁵ –2.25 × 10 ⁻³	61.7	Not given	[17]
Voltammetry	5 × 10 ⁻⁶ –685 × 10 ⁻⁶	1	Not given	[28]
Voltammetry	7.8 × 10 ⁻⁷ –2 × 10 ⁻⁴	0.26	Syrup	[44]
Voltammetry	0.2 × 10 ⁻³ –1.2 × 10 ⁻³	–	Vitamin pill, hydrolyzed casein, syrup and Soya bean milk	[45]
Voltammetry	1 × 10 ⁻⁹ –1 × 10 ⁻³	0.237 × 10 ⁻³	Human blood serum, urine, ACC tablet and PPF	This work

of CySH is 1.8). Thus, when CySH is reacted with Co(II) at low pH (pH = 1.0), there is no distinct complex formation [57]. According to the proposed mechanism (Eqs. (3) and (4)), the formation of Co(II)–CySH complex is responsible for the increase in the anodic current. Inset of Fig. 6 shows the distribution fraction (δ) of different chemical species of CySH [CySH₂⁺ (H₃A⁺), CySH (H₂A), CyS⁻ (HA⁻) and CyS²⁻ (A²⁻)] against pH [49,53]. In pH 2.2, only 35% of CySH is as cationic form and other is neutral. Based on the above discussion, the cationic form of CySH (H₃A⁺) will not make complex with Co(II). As a result, the formed complex is the result of the reaction of Co(II) with the natural form of CySH. In alkaline pH (above 7.1), the distribution fraction of anionic species of CySH (HA⁻) in solution increases (pH = 9.3, δ = 0.85). The HA⁻ has high tendency to make a complex with Co²⁺ ions [56]. This makes more Co(II) taken out of zeolite, and as a result, anodic peak current increases. Of course, in alkaline pH, not only Co(II)–CySH complex but also hydroxide cobalt [Co(OH)₂] is formed [58,59]. This decreases the anodic peak current in comparison with the acidic pH condition.

According to the previous reports, in the pH range of 2.2–9.3, CySH₂⁺ (H₃A⁺) and CyS⁻ (HA⁻) are only electroactive substances among the four chemical species of CySH [49]. Therefore, the mechanism of direct redox and reversible process of CySH at the CPE and Co²⁺/Y/ZMCPe in pH 2.2 are shown by the following Eqs. ((11)–(13)):



Therefore, the pH 2.2 was chosen as the working pH value in the next studies.

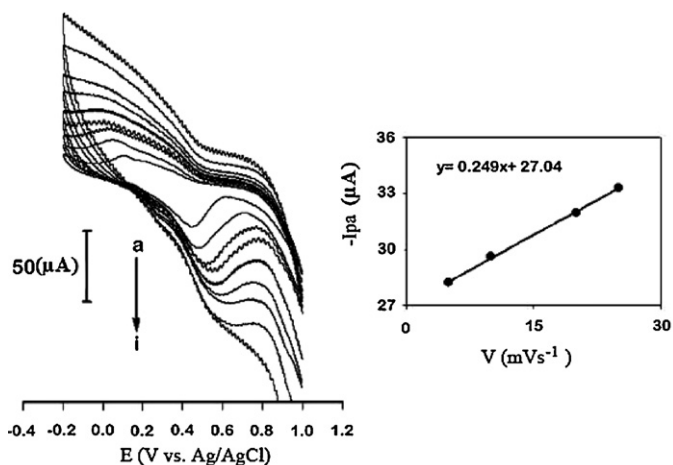


Fig. 7. Cyclic voltammograms of 0.01 mol L⁻¹ CySH at Co²⁺/Y/ZMCPe with different scan rates in 0.2 mol L⁻¹ acetate–hydrochloric acid solution (pH 2.2). (a–i) 5, 10, 15, 20, 25, 35, 45, 65 and 108 mV s⁻¹, respectively (inset: the anodic peak current vs. scan rate).

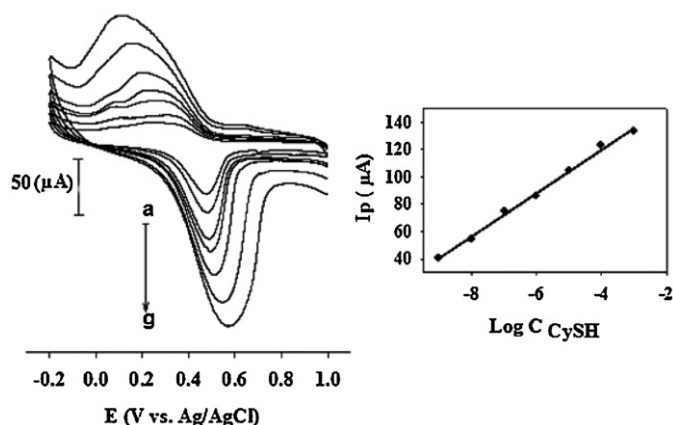


Fig. 8. Cyclic voltammograms of Co²⁺/Y/ZMCPe in the presence of CySH concentrations (a–g): 1 × 10⁻⁹, 1 × 10⁻⁸, 1 × 10⁻⁷, 1 × 10⁻⁶, 1 × 10⁻⁵, 1 × 10⁻⁴ and 1 × 10⁻³ M, respectively. Scan rate: 25 mV s⁻¹, in 0.2 mol L⁻¹ acetate–hydrochloric acid solution (pH 2.2), inset: the variations of anodic peak currents vs. CySH concentration.

3.2.3. Effects of scan rate in the presence of CySH

The effect of scan rate on the peak current of Co(II)–CySH complex was studied. Fig. 7 shows the cyclic voltammograms of 0.2 mol L⁻¹ acetate–hydrochloric acid solution (pH 2.2) containing 0.01 mol L⁻¹ CySH at ν = 5–108 mV s⁻¹. By increasing in the scan rate from 5 to 25 mV s⁻¹, the anodic peak current increases (inset of Fig. 7). The anodic peak current at the modified electrode was linear with the scan rate in the range of 5–30 mV s⁻¹ ($y = 0.249x + 27.4$, $R^2 = 0.9985$). This linear behavior may suggest that the peak current comes largely from the surface adsorbed species [39] which indicates the formation of the Co(II)–CySH complex on the surface of the modified electrode. Since with these experimental conditions, the highest analytical signal and also very good cyclic voltammograms profiles were obtained, a scan rate of 25 mV s⁻¹ was chosen for the next studies.

Table 3

Interference study for the determination of 3 × 10⁻⁶ mol L⁻¹ CySH under the optimized conditions.

Species	Tolerance limits ($W_{\text{species}}/W_{\text{CySH}}$)
Cd ²⁺ , Ni ²⁺ , Mg ²⁺ , K ⁺ , NH ₄ ⁺ , SO ₄ ²⁻ , NO ₃ ⁻ , CO ₃ ²⁻ , Cl ⁻	1400 ^a
Na ⁺ , Ca ²⁺ , Mn ²⁺ , ClO ₄ ⁻ , Urea	1200
L-Methionine, Glucose, CN ⁻	1100
L-Serine, BrO ₃ ⁻ , Cu ²⁺	1000
Citric acid	800
L-Alanine, L-Glycine	700
L-Histidin	600
Fe ³⁺	300
L-Ascorbic acid	200

^a Maximum concentration of the species tested.

Table 4
Determination of CySH in real samples.

Sample ^a	Human blood serum	Urine	ACC tablet	PPF
Measured value	244 (± 0.06) $\times 10^{-6}$	26 (± 0.08) $\times 10^{-6}$	5.06 (± 0.09) $\times 10^{-3}$	598 (± 0.07) $\times 10^{-3}$
Real value ^b	261.40 $\times 10^{-6}$	22.60 $\times 10^{-6}$	5.09 $\times 10^{-3}$	600 $\times 10^{-3}$ (mg CySH/1gPPF)

^a Result values at 95% confidence limits, obtained from five replicated determination per sample.

^b Real value of CySH in human blood serum and urine [60].

Table 5
Comparison of the obtained values with other references.

Sample	Modifier	Obtained value (mol L ⁻¹)	Ref.
Human blood serum	Co(II)-Y	244 (± 0.06) $\times 10^{-6}$	This work
	Nile blue A	178 (± 4.8) $\times 10^{-6}$	[62]
	Ferrocenedicarboxylic acid	460 (± 0.4) $\times 10^{-6}$	[63]
	Quanzarin	140 (± 0.08) $\times 10^{-6}$	[64]
Human urine	Co(II)-Y	26 (± 0.08) $\times 10^{-6}$	This work
	Copper, zinc superoxide dismutase	50 (± 0.41) $\times 10^{-6}$	[65]
	Copper hexacyanoferrate nanostructure	<Detection limit (0.13 $\mu\text{mol L}^{-1}$)	[13]
ACC tablet	Co(II)-Y	5.06 (± 0.09) $\times 10^{-3}$	This work
	1-[4-(Ferrocenyl ethynyl) phenyl]-1-ethanone	5.05 (± 0.3) $\times 10^{-3}$	[66]
	Ferrocenedicarboxylic acid	5.04 (± 0.25) $\times 10^{-3}$	[63]
	Quinizarine	5.05 (± 0.04) $\times 10^{-3}$	[64]

3.2.4. Calibration, reproducibility and lifetime of ZMCPE

Since the oxidation peak was proportional to the concentration of CySH, the peak current can be used for the determination of CySH. Using cyclic voltammetry under the optimum conditions selected as pH 2.2 (0.2 mol L⁻¹ acetate–hydrochloric acid solution), the modified electrode containing 10% modifier and a scan rate of 25 mV s⁻¹ (Fig. 8), the regression equation obtained: “ I_{pa} (μA) = 15.88 log $C_{[\text{CySH}]}$ – 183.1 with $R^2 = 0.9995$ ($n = 6$) for the CySH over the concentration range of 1.0×10^{-9} – 1.0×10^{-3} M (inset of Fig. 8). The relative standard deviations (RSD %) for five replicated analyses of $(3.0, 5.0$ and $7.0) \times 10^{-6}$ mol L⁻¹ CySH at 25 mV s⁻¹ were 0.4, 0.6 and 0.9%, respectively. The limit of detection (LOD), which was defined as $3S_b/m$ (where S_b is the standard deviation of the blank signal and m is the slope of the calibration curve ($n = 10$)), was 2.37×10^{-10} mol L⁻¹ which could be comparable to the most sensitive methods have reported for the detection of CySH (Table 2). The lifetime of the proposed electrode was also over nine months.

3.2.5. Interference studies

The influence of various substances as potential interference compounds on the determination of CySH under the optimum conditions with 3.0×10^{-6} mol L⁻¹ CySH was studied. The tolerance limit was defined as the maximum concentration of the potential interfering substance that caused an error less than 3% for the determination of CySH. The results are given in Table 3, which show the peak current of CySH was not affected by more the tested amino acid, cations, anions, and organic substances. L-Ascorbic acid and Fe(III) interfere in the determination of CySH, in concentrations more than 200 and 300 fold of CySH, respectively. Thus, the results demonstrated the good selectivity of this method for the voltammetric determination of CySH.

3.2.6. Real sample analysis

As a practical use, the proposed method was also used for the determination of CySH in real samples. The determination of CySH in these samples was performed by the standard addition method in order to prevent any matrix effects. The sample solutions were prepared as described before (Section 2.4). The total concentration of CySH in the human blood serum and urine samples was found to be $244.00 (\pm 0.06) \times 10^{-6}$ mol L⁻¹ and $26.00 (\pm 0.08) \times 10^{-6}$ mol L⁻¹, respectively. The results are presented in Table 4. The levels of CySH are close to the previous reported values [60,61]. The results of the proposed method are also compared with the data which

have reported in the previous works and the obtained results are summarized in Table 5. The comparison suggests that the proposed method is very reliable and sensitive in the determination of CySH in different real samples.

4. Conclusions

In the present work a novel electrode based on Co(II) doped zeolite modified carbon paste was constructed. By forming of complex between Co(II) and CySH on the electrode surface, the oxidation–reduction peaks of Co(II) was increased in the presence of CySH. The electrode showed high stability in repetitive experiments due to the high affinity of zeolite Y for Co(II). The proposed electrode showed excellent analytical performance characteristics especially a very low detection limit with the very easy preparation, surface regeneration and the reproducibility of the voltammetric response. The proposed system is very useful as a simple device for the determination of CySH in the clinical and pharmaceutical samples, especially without the necessity for sample pretreatment or any time-consuming extraction or evaporation steps prior to the analysis, with satisfactory recovery.

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