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ORIGINAL PAPER

# Voltammetric determination of thiocytosine based on its electrocatalytic oxidation on the surface of carbon-paste electrode modified with cobalt Schiff base complexes

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**Abstract** The electrochemical oxidation of thiocytosine on the surface of carbon-paste electrode modified with Schiff base (salophen derivatives) complexes of cobalt is studied. The effect of the substituents in the structure of salophen on the catalytic property of the modified electrode is investigated by using cyclic and differential pulse voltammetry. Cobalt (II)-5-nitrosalophen, because of its electrophilic functional groups, leads to a considerable enhancement in the catalytic activity, sensitivity (peak current), and a marked increase in the anodic potential of the modified electrode. The differential pulse voltammetry is applied as a very sensitive method for the detection of thiocytosine. The linear dynamic range was between $1\times10^{-3}$ to $4\times10^{-6}$ M with a slope of 0.0168 μA/μM, and the detection limit was $1\times10^{-6}$ M. The modified electrode is successfully applied for the voltammetric detection of thiocytosine in human synthetic serum sample and also pharmaceutical preparations. A linear range from $1\times10^{-3}$ to $1\times10^{-5}$ M with a slope of 0.0175 μA/μM is resulted for the standard addition of thiocytosine spiked to the buffered human serum, which is differing from the curve in buffer solution about 4%. The electrode has a very good reproducibility (relative standard deviation for the slope of the calibration curve is less than 3.5% based on six determinations in a month), high stability in its voltammetric response and low detection limit for thiocytosine, and high electrochemical sensitivity with respect to other biological thiols such as cysteine.

**Keywords** Thiocytosine · Carbon-paste electrode · Modified electrode · Electrocatalysis · Cobalt salophen

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## Introduction

The development and application of chemically modified electrodes has continued to be of major concern in analytical chemistry. In recent years, chemically modified carbon paste electrodes (CMEs) have received increasing attention due to their potential applications in various analyses [1]. Modification of the paste matrix with various transition metal complexes [2–4], organic electron mediators [5–7], carbon nanotubes [8–10], and polymers [11–13] has been reported in recent years. The operation mechanism of such chemically modified electrodes depends on the properties of the modifier materials used to promote selectivity and sensitivity towards the target analytes [14]. This kind of electrode is inexpensive and possesses many advantages such as low background current, wide range of potential windows (in both cathodic and anodic region), easy fabrication, and rapid surface renewal. One of the most important properties of CMEs has been their ability to catalyze the electrode process via significant decreasing of overpotential with respect to the unmodified electrode. With respect to relatively selective interaction of the electron mediator with the target analyte, these electrodes are capable of considerably enhancing the selectivity in the electroanalytical methods. Transition metal complexes such as phthalocyanines [15–22], porphyrins [23–25], Schiff bases [26, 27], hexacyanometallates [28], and aquacobalamin [29] are well known as electron mediators in the

electrocatalytic oxidation of some biologically important compounds.

Given the wide spread involvement of thiols and the corresponding disulfides in many biological functions, much effort has been made to develop sensitive and selective methods for their detection. Investigations of the redox behavior of biologically occurring thiols by means of electrochemical techniques have the potential for providing valuable insights into biological redox reaction of such biomolecules.

Adenine, guanine, and their ribosides were more effective than cytosine, uracil, thymine, and their derivatives in preventing the inhibition due to 8-azaadenine and 8-azaguanine. Likewise, the inhibitory effects of 2-thiocytosine, 2-thiouracil, and 6-azauracil were overcame by the pyrimidines and their derivatives [30]. The derivatives of purines and pyrimidines are frequently used as antineoplastics. Analytical control of treatment with antineoplastics is fundamental from both bioanalytical and mechanistic views. Thiocytosine is one of the pyrimidine derivatives involving thiol group, which was investigated in this research. Some thiocytosine derivatives have shown enzymatic reactivity and antitumor activity [31]. The electroanalytical methods are very important owing to their great sensitivity and selectivity. There are some reports for the determination of thiocytosine by using electroanalytical methods. The adsorptive stripping voltammetric method has been applied for trace determination of thiocytosine using differential pulse voltammetry (DPV) at the hanging-drop mercury electrode [32]; in addition, voltammetric detection of thiocytosine using its enhancement effect on copper anodic stripping wave has been reported [33]. In another report existing, thiocytosine was determined using iodometric method [34].

Thiocytosine, as a derivative of canonic base cytosine, is known as an anti-leukemia drug detected in the RNA molecules of the *Escherichia coli* and also has shown potential anti-leukemic activity [35].

The aim of the present work is the development of a new sensitive method for the determination of thiocytosine (as a biologically active compound) based on a specially modified carbon paste electrode (CPE) in human serum and pharmaceutical samples. In this work, cobalt (II)-5-nitrosalophen (CoNSal) is used as a very efficient catalyst (modifier) in the matrix of the CPE and is applied for investigating the electrochemical oxidation of thiocytosine by using cyclic and differential pulse voltammetric methods.

## Experimental

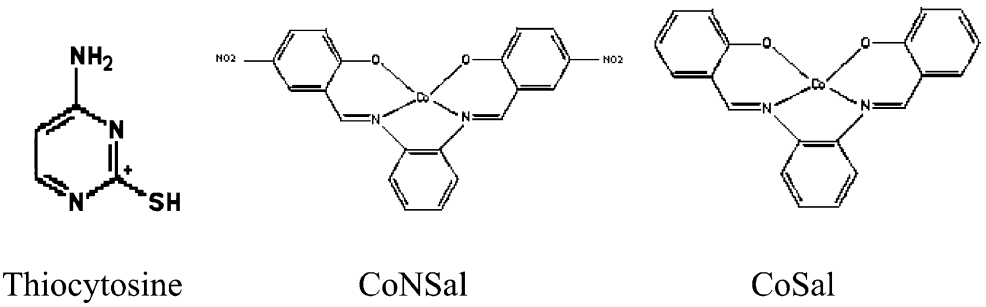
### Materials

Cobalt(II) acetate, 5-nitrosalicylaldehyde, and 1,2-phenylenediamine for synthesis of the Schiff base complex were purchased from Merck. The complexes *N*,*N*-bis (salicylidene)-1,2-phenylenediamino cobalt(II) acetate (cobalt(II) salophen, CoSal), *N*,*N*-bis (5-nitro-salicylidene)-1,2-phenylenediamino cobalt(II) acetate (cobalt(II)-5-nitrosalophen, CoNSal) were synthesized and purified as reported previously [36, 37]. Identification of the structure of synthesized complex was performed by infrared, $^{1}H$ and $^{13}C$ NMR, UV-Vis, and elemental analysis. Graphite powder and spectroscopic mineral oil (Nujol) were purchased from Merck. All chemicals were of analytical reagent grade from Merck. Human synthetic serum samples was diluted with appropriate buffer (100-folds) and used for recovery tests of the spiked thiocytosine. Voltammetric experiments were carried out in the buffered solutions of thiocytosine, deoxygenated by purging the pure nitrogen (99.999% from Roham Gas Company). During the experiments, nitrogen gas was passed over the surface of test solutions to avoid entrance of oxygen into the solution. The structures of thiocytosine and Schiff base complexes of cobalt are shown in the following scheme (Scheme 1).

### Apparatus

Voltammetric experiments were performed with a Metrohm Computrace Voltammetric Analyser model 757VA. A conventional three-electrode system was used with a carbon-paste-working electrode (unmodified or modified), a saturated Ag/AgCl reference electrode, and a Pt wire as the counter electrode. A digital pH/mV/Ion meter (Cyber-

**Scheme 1** Structures of thiocytosine and applied Schiff base complexes of cobalt



Scan model 2500) was applied for controlling pH buffer solutions, which were used as the supporting electrolyte in voltammetric experiments.

### Preparation of modified electrodes

The unmodified CPE was prepared by mixing graphite powder with appropriate amount of mineral oil (Nujol) and thorough hand mixing in a mortar and pestle (~75:25, w/w), and a portion of the composite mixture was packed into the end of a Teflon tube (about 2.5 mm i.d.). Electrical contact was made by forcing a copper pin down into the Teflon and into the back of the composite. The modified electrode was prepared by mixing unmodified composite with CoSal or CoNSal (2%, w/w), and then the composite was being dissolved in dichloromethane. The mixture was stirred by a magnetic stirrer until the solvent evaporated completely. The modified composite was then air dried for 24 h and used in the same way as unmodified electrode.

## Results and discussion

### Cyclic voltammetric studies of thiocytosine, selection of the appropriate modifier

To determine the best catalytic activity and sensitivity for the electrochemical oxidation of thiocytosine, preliminary studies were performed using cyclic voltammetry (CV) and DPV. Voltammetric responses were obtained on the surface of three different electrodes including unmodified, CoSal-modified, and CoNSal-modified CPEs. Figure 1a shows the cyclic voltammograms for 1 mM thiocytosine in buffered solution with pH 4.0 (0.1 M acetate) at the surface of unmodified (dotted line), CoSal-modified (dash point), and CoNSal-modified electrode (solid line) in the potential range of 0.2 to 0.9 V vs Ag/AgCl. The results show that the direct oxidation of thiocytosine at the surface of unmodified electrode is very sluggish, and an evident anodic peak cannot be obtained in the range of swept potential. On the surface of the CoSal-modified electrode, the kinetics of the electrode process is enhanced, and an anodic wave with peak potential of 676 mV is obtained for thiocytosine. On the other hand, a well-defined and relatively sharp irreversible oxidation peak at 586 mV (compared to unmodified and CoSal-modified electrode) is obtained using CoNSal-modified electrode. A similar effect for CoNSal-modified electrode, in comparison to the other studied electrodes, is resulted in DPV measurements (Fig. 1b). These results confirm that CoNSal, as an electron mediator in the matrix of the paste, shows more catalytic activity toward the thiocytosine oxidation, leading to a very sharp anodic wave with a considerably greater peak current in less positive potentials. For thiocytosine, no cathodic peak is observed on the reverse scan in various potential sweep rates (Fig. 2a). Such a behavior confirms the EC mechanism, which coupled irreversible chemical reaction hindered to the electron transfer step. The anodic peak current varied linearly with the square root of scan rate, suggesting that the thiocytosine oxidation follows a diffusion-controlled mechanism according to the linear relation $I_{p,a} = 3.674 + 1.756\nu^{1/2(\mathrm{mVs})^{1/2}}$ with a correlation coefficient of 0.9993 (Fig. 2b).

For CoNSal, the best voltammetric results are obtained with modified electrode containing 2% w/w modifier in the matrix of the paste. The results of our previous studies [15, 27] on various Schiff base complexes of cobalt showed that values of less than 2% w/w modifier lead to a considerable decrease in the peak current along with a broadening of peaks and a positive shift in peak potential. On the other

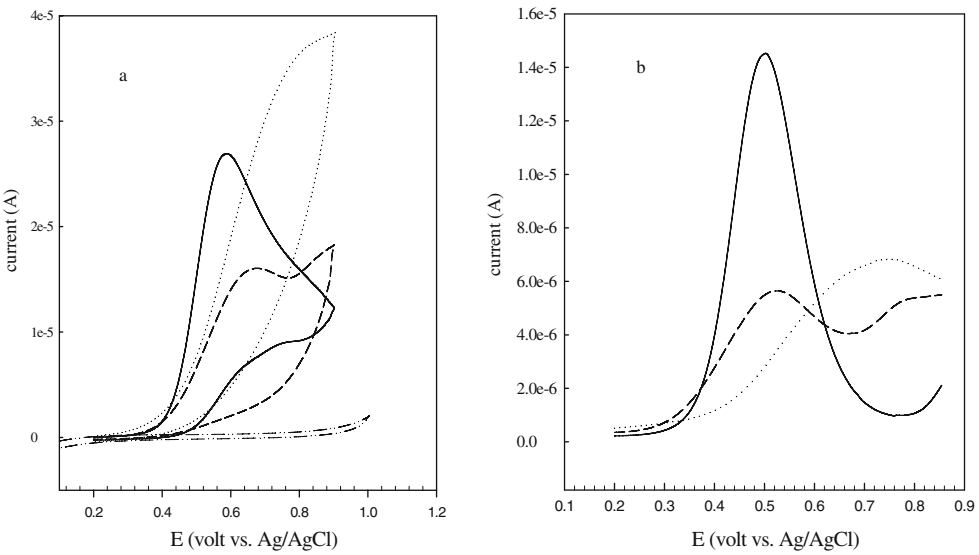



**Fig. 1** **a** Cyclic and **b** differential pulse voltammograms of 1 mM thiocytosine on the surface of three electrodes; unmodified CPE (*dotted line*), CoSal-modified CPE (*dashed line*), and CoNSal-modified CPE (*solid line*). *Curve with dashed-dotted line* in **a** represents the CV for modified electrode in background electrolyte. Supporting electrolyte was 0.1 M acetate buffer solution with pH 4.0, and sweep rate was 100 mV $s^{-1}$

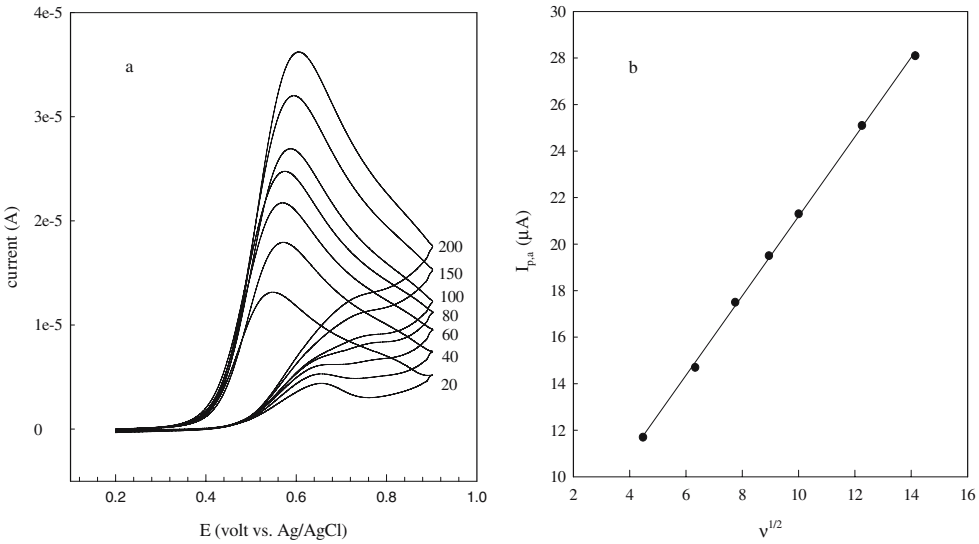



**Fig. 2** **a** Cyclic voltammograms of 1 mM thiocytosine at CoNSal-modified electrode in various potential sweep rates. **b** Variation of anodic peak current with the square root of the sweep rate ($\upsilon$). Electrolyte was 0.1 M acetate buffer solution with pH 4.0

hand, values of more than 2% w/w of modifier result in an increase in background current in conjunction with a graduate decrease in peak current [4].

## Polarization studies

The Tafel plot and its corresponding slope were used for elucidation of the mechanism of the electrode process. The results of polarization studies (logI/E plot) for the electrocatalytic oxidation of thiocytosine on the surface of the modified electrode were obtained in various sweep rates. The results of the slope of Tofel plots show values of $\alpha n_a$ between 0.52 to o.63 (in various pHs and potential sweep rates) on the surface of the modified electrode. By considering a value of 0.5 for $\alpha$ in these nearly symmetric waves, the one-electron transfer process is confirmed for the rate-determining step of processes. Results of the previous studies on the catalytic effect of phthalocyanine complexes of cobalt in the electrochemical oxidation of thiols have been shown that Co is capable to catalyze the thiol oxidation in both +2 and +3 oxidation states. The results showed that in acidic media, it is more probable that the oxidation process is caused by catalytic effect of Co(III)/Co(II) redox system as a mediator couple.

## The pH effect

The voltammetric response of a CoNSal-modified electrode was studied over a pH range between 3.0 and 7.0 in a solution containing 1 mM thiocytosine. As can be seen in Fig. 3, a negative shift in anodic peak has occurred by increasing of the pH of the buffer solution. The plot of $E_{p,a}$ vs pH (in the above mentioned pH range) has a slope of −51.2 mV with a correlation coefficient ($R^2$) of 0.9987. With respect to loss of an electron in the electro-oxidation of thiocytosine, contribution of a proton is accepted in the process. The proposed mechanism for the electrocatalytic oxidation of thiocytosine is similar to other biological thiols such as cysteine [4], penicillamine [16], and propylthiouracil [27] on the surface the electrodes modified with various complexes of cobalt. This mechanism proceeds by the deprotonation of thiol form (RSH) and formation of thiolate anion before the electron transfer step. The following

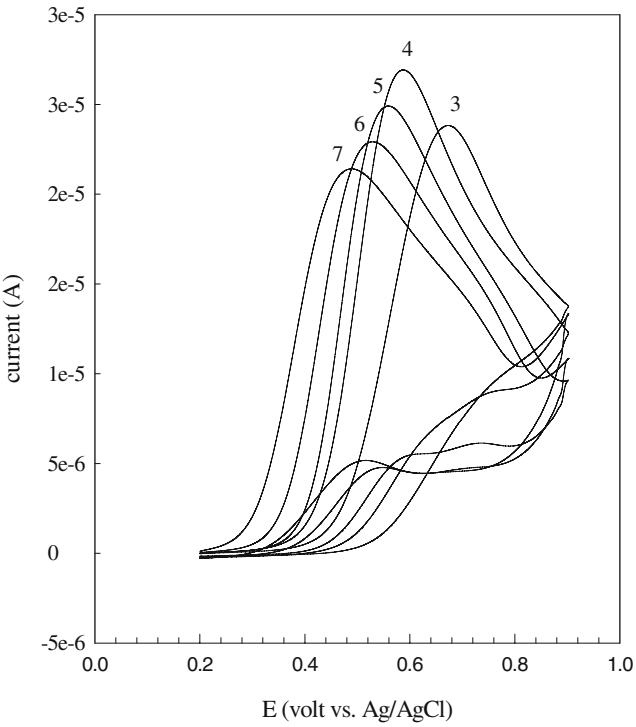



**Fig. 3** Cyclic voltammograms of 1 mM thiocytosine at CoNSal-modified electrode in various pHs of the buffered solution; 0.1 M acetate was used for pHs 4 and 5, others pHs are adjusted with 0.1 M phosphate as buffer solution. Potential sweep rate was 100 mV $s^{-1}$

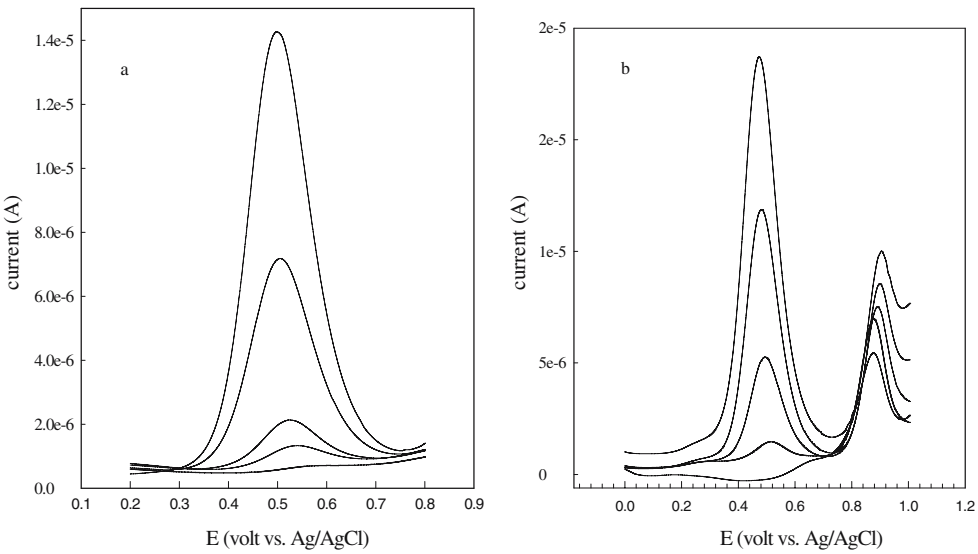



**Fig. 4** **a** DPVs for various concentration of thiocytosine in pH 4.0, *top to bottom*: $1\times10^{-4}$, $5\times10^{-5}$, $1\times10^{-5}$, $5\times10^{-6}$, and $1\times10^{-6}$ M. **b** DPVs for various concentration of thiocytosine added in a background of the human synthetic serum adjusted to pH 4.0 by 0.1 M acetate buffer solution, *top to bottom*: $1\times10^{-4}$, $6\times10^{-5}$, $2\times10^{-5}$, $8\times10^{-6}$, and $1\times10^{-6}$ M. Supporting electrolyte was 0.1 M acetate with pH 4.0, and pulse amplitude was 50 mV

equations can be presented for the interpretation of the mechanism:

$$RSH + H_2O \rightarrow RS^- + H_3O^+ \quad (1)$$

$$Co(II)NSal \rightarrow Co(III)NSal + e^- \quad (2)$$

$$Co(III)NSal + RS^- \rightarrow Co(II)NSal + RS^{\cdot} \quad (3)$$

$$2RS^{\cdot} \rightarrow RSSR \quad (4)$$

Differential pulse voltammetry; analytical applications

The DPV method was used as a very sensitive method with micromolar detection limit for determination of trace amounts of thiocytosine. DPV waves for thiocytosine in the range of $1\times10^{-6}$ to $1\times10^{-4}$ M and in 0.1 M acetate buffer (pH=4.0) as background electrolyte are shown in Fig. 4a. The calibration curve for the determination of thiocytosine exhibits a linear range of $4\times10^{-6}$ to $1\times10^{-3}$ M with a slope of 0.0168 and a correlation coefficient of 0.9995. The detection limit for the determination of thiocytosine (based on $3\sigma$ in 95% CI) using this method was $1\times10^{-6}$ M. An amperometric method using a preheated glassy carbon electrode modified with abrasive immobilization of multiwalled carbon nanotubes is reported for the determination of thiocytosine [38]. The presented method in this work showed remarkable advantages such as low background current, relatively large anodic peak currents, and sharp voltammetric signals, which can be conducted to improve the sensitivity, detection limit, and also the selectivity in analytical determinations. On the basis of five replicates, the RSD for the slope of the calibration curves (Ip vs concentration) for thiocytosine was 3.5%. The prepared modified electrode showed to be very stable and the RSD (%) for the slope variation based on six measurements in a period of 1 month was less than 3.5%.

In the electrochemical detection of thiocytosine in biological fluids (e.g., human serum samples), the presence of minor amounts of reducing agents, such as cysteine and tryptophan, is significant for the accuracy of the determinations. The CoNSal-modified electrode was used as the working electrode for the determination of minor amounts of thiocytosine spiked to human synthetic serum samples by DPV technique. The concentration of each component in this sample was chosen to be near to its normal, leveling the real sample [39]. The components were dissolved in acetate buffer with pH 4.0. Figure 4b represents the DPVs for solutions containing various concentrations of thiocytosine (in the range of $1\times10^{-6}$ to $1\times10^{-4}$ M) spiked to the synthetic serum sample. Results of our previous work on the electrocatalytic oxidation of cysteine on the surface of CPE modified with cobalt-4-methylsalophen showed that its anodic wave appeared in the same region of potential for thiocytosine [4]. However, the sensitivity of the electrode response for cysteine was remarkably lower than thiocytosine. Results of cyclic voltammetric measurements has been shown an anodic wave for 1 mM cysteine with an anodic peak current of about 5 μA. On the other hand, in the present work, for 1 mM thiocytosine the anodic peak current was more than 20 μA. Therefore, the presence of cysteine with a concentration of $7.1\times10^{-5}$ M (its normal level in the serum sample) cannot affect the response of the modified electrode. The calibration curve for thiocytosine

in the human synthetic serum as background electrolyte showed a linear range between $1\times10^{-5}$ to $1\times10^{-3}$ M. The slope of the calibration curve was 0.0175 μA/μM, and correlation coefficient ($R^2$) was 0.09952. The obtained slope differs with the slope of the calibration curve in background buffer about 4%.

Results in Fig. 4b show another anodic wave in more positive potentials, which is completely resolved from the wave of thiocytosine (between 0.8 to 1.0 V). Our investigations on various amino acids on the surface of CPE-modified salophen complex of cobalt showed that this wave is related to the anodic oxidation of tryptophan and tyrosine [40]. These results indicate that most components of the synthetic serum do not show any interference with the electrochemical response of thiocytosine on the surface of the modified electrode. The performance characteristics of the modified electrode in conjunction with the simplicity of its preparation and the renewability of its surface by simple polishing demonstrates its analytical utility as a voltammetric sensor for determination of thiocytosine in pharmaceutical and clinical samples.

## Conclusions

The results of the present work show the catalytic activity of the cobalt salophen complexes in the matrix of CPE toward the electrochemical oxidation of thiocytosine. These results prove that the presence of nitro functional group in the structure of Schiff base complex enhance the catalytic rule of the electron mediator, leading to lowering the anodic overpotential and increasing the sharpness and current of the anodic peak. The mechanism of the electrochemical oxidation of thiocytosine is investigated by the cyclic voltammetric studies in different pHs and potential sweep rates. The modified CPE is successfully applied as a very sensitive voltammetric sensor for the detection of micromolar amounts of thiocytosine. High sensitivity and low detection limit together with the very easy preparation and easy regeneration of the electrode surface, long time stability, and reproducibility make the discussed system useful in the construction of simple devices for the determination of thiocytosine. The modified electrode is successfully used for the recovery tests for thiocytosine added to the human synthetic serum samples and showed very good accuracies and precisions.

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