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Simultaneous Determination of Thiocyanate and Oxalate in Urine using a Carbon Ionic Liquid Electrode Modified with TiO₂-Fe Nanoparticles

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A carbon ionic liquid electrode (CILE) modified with TiO₂-Fe nanoparticles was constructed by incorporating TiO₂-Fe nanoparticles into the paste matrix. Under the optimized experimental conditions, using differential pulse voltammetry (DPV), the oxidation of thiocyanate and oxalate were occurred at potentials of 0.740 V and 1.010 V, respectively, at the surface of the modified electrode. Observing a high peak current along with a suitable peak separation allowed introducing the first voltammetric sensor for simultaneous determination of these anions with high sensitivity without mutual interference. A wide linear range of 1.0×10^{-5} - 1.8×10^{-3} M for thiocyanate with a detection limit of 6.4×10^{-6} M and a wide linear range of 5.0×10^{-5} - 3.0×10^{-3} M for oxalate with a detection limit of 2.3×10^{-5} M were obtained. The modified CILE showed good reproducibility, repeatability and storage stability as well as good selectivity. The proposed sensor was successfully applied for analysis of thiocyanate and oxalate in urine samples.

Keywords: Thiocyanate, Oxalate, Simultaneous determination, TiO₂-Fe nanoparticles, Carbon ionic liquid electrode

INTRODUCTION

Thiocyanate and oxalate are common components of human urine and their analysis is very important in clinical diagnosis and treatment. Thiocyanate is the end product of detoxification of cyanide compounds which excreted in urine, saliva and blood serum [1]. Since tobacco smoke has been shown to be a major contributor to the production of thiocyanate, *in vivo*, the levels of urinary thiocyanate is regarded as a good means of distinguishing between smokers and non-smokers [2]. Moreover, chronically elevated levels of thiocyanate in body fluids are known to be toxic and its relation to local goiter, vertigo, or unconsciousness has been pointed out [3]. Oxalates can be ingested as dietary constituent or produced as the final product of amino and ascorbic acids metabolisms [4]. This anion is potentially toxic to humans because they form insoluble precipitates with divalent cations such as calcium and produce renal stones. The determination of oxalate in

urine is very important for the clinical diagnosis and treatment of various forms of hyperoxaluria and urinary tract stones [5]. Thus, development of a convenient, accurate, simple, and rapid method for determination of both thiocyanate and oxalate is significant in medicine and in the life sciences.

Many analytical methods such as UV-Vis spectrophotometry [6-10], spectrofluorimetry [11], gas chromatography [12-14], flow-injection analysis [15,16], enzymatic methods [17,18], electrogenerated chemiluminescence [19] and potentiometric methods [1,20] have been proposed to quantify both thiocyanate and oxalate. Each of these methods suffers from problems such as poor selectivity, pretreatment requirement, time-consuming sample detection, complicated procedure and expensive instrumentation. The chromatographic methods such as HPLC [21-24], capillary electrophoresis [25-27] and ion chromatography [28-30] are commonly used in analysis of real mixtures for both ions. To the best of our knowledge, few reports have been published on simultaneous determination of these anions. In this regard, Bord *et al.*

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[31] used capillary electrophoresis with indirect UV detection and in Jin *et al.* [32] used ion-exclusion chromatography with electrochemical detection.

Electroanalytical techniques are of particular advantage because of their practicality, simplicity, low-cost, good sensitivity, precision and rapidity for real-time monitoring of drugs and biological compounds. The electrocatalytic oxidation of oxalate has been investigated at metal electrodes such as platinum [33] as well as carbon electrodes such as highly boron doped diamond (BDD) electrodes [34,35]. The role of different electrode materials such as Ti/PbO₂, BDD, Pt, Au and Ti/IrO₂-Ta₂O₅ on the electrochemical oxidation of oxalic acid has also been investigated [36].

Electrochemical oxidation of thiocyanate at a bare glassy carbon (GCE) electrode and traditionally carbon paste electrodes (CPE) suffers from sluggish electron transfer kinetics and large oxidation overpotential. To overcome these limitations, different electrodes such as gold electrode modified with iron phthalocyanine [37], thin film modified glassy carbon electrode [38] and carbon paste electrode modified with cobalt phthalocyanine [39] have been proposed. Nanoparticle-modified electrode has been developed for electrooxidation of oxalate and thiocyanate when they provide high effective surface area and more electrocatalytic effect compared to macroelectrodes. In this regard, palladium nanoparticle-loaded carbon nanofiber modified electrode [40], palladium-based modified glassy carbon electrode [41] and carbon paste electrode with deposited palladium nanoparticles [42] were introduced for electrooxidation of oxalate, while Ag-nanoparticles modified GCE [43] was introduced for sensitive determination of thiocyanate. To the best of our knowledge, up to now the simultaneous voltammetric determination of oxalate and thiocyanate has not been reported may be due to their ill-defined, large oxidation peaks with low peak separation which causes mutual interferences. Moreover, considering this fact that the majority of separation-based methods use electrochemical detection system (*i.e.* amperometry), fabrication of electrodes with high sensitivity and long term stability is highly demanded. However, careful selection of both substrate electrode and modifier is necessary for fabricating a satisfactory electrode.

Carbon ionic liquid electrode (CILE) known as suitable substrate electrode for to be modified with different

nanoparticles [44-46]. The TiO₂-based nanoparticles have recently attracted growth attention in the fields of physics, chemistry and biology because of great stability, biocompatibility and conductivity [47]. They are extensively used in modification of sensors and biosensors but, as far as we aware there is no report on application of Fe-TiO₂ nanoparticles as electrode modifiers.

Our recent studies proved the excellent performance of CILE as when was modified with Fe₃O₄ nanoparticles for sensitive determination of nitrite [48]. In the present study the chemically-synthesized TiO₂-Fe nanoparticles simply incorporated into the substrate CILE. In this way, the modified electrode (TiO₂-Fe/CILE) combines the benefit of high surface area of TiO₂-Fe nanoparticles with high electric conductivity, good viscosity and chemical stability of IL. The constructed electrode has been used for investigating simultaneous determination of thiocyanate and oxalate. The effect of variables such as pH, supporting electrolyte and scan rate are evaluated. The possible interferences of other coexistence species on the both analytes peak currents are studied.

EXPERIMENTAL

Reagents

Potassium thiocyanate, potassium oxalate, sodium hydroxide, Fe(NO₃)₃.9H₂O, isopropanol, nitric acid (60%), 1-iodooctane, pyridine, ammonium hexafluorophosphate, graphit powder (mesh size <100 μm), boric acid, acetic acid (99-100%) were purchased from Merck. Sulfuric acid (98%, AR grade) and titanium tetraisopropoxide (TTIP, 97%) were obtained from Sigma-Aldrich. Universal buffer (0.04 M, pH 7.0) was used as supporting electrolyte.

Synthesis of TiO₂-Fe

The doped TiO₂ nanoparticles were synthesized by sol-gel method [49,50]. In a typical experiment, 62 ml TTIP is dissolved in 120 ml isopropanol. Two other solutions in separate burette are added to this solution dropwisely with vigorous stirring. One solution is the mixture of 0.4 ml concentrated nitric acid in 25 ml water and the other is 15.2 g of Fe(NO₃)₃.9H₂O in 120 ml isopropanol. The resultant mixture is stirred for 2 h and aged for 1 day at room temperature. The obtained precipitate are filtered and rinsed

with water and isopropanol repeatedly and dried at 110 °C in an oven for 12 h in order to vaporize most of isopropanol and water. Then calcinated and crystallized at 500 °C for 4 h.

Synthesis of Ionic Liquid

The N-octylpyridinium hexafluorophosphate (OPFP) was synthesized and purified based on a previously reported procedure [51]. Briefly, N-octylpyridinium iodide ([C₈Py] [I]) is prepared by adding equimolar amounts of pyridinium to 1-iodooctane in a round-bottomed flask while stirring for 2 days. To have a pure product ([C₈Py] [I]), the greasy mixture is washed several times with pure diethyl ether and is dried under vacuum at room temperature. The OPFP is prepared slowly by adding a solution of ammonium hexafluorophosphate to [C₈Py] [I]. After stirring for 2 h, the white precipitate is washed with water. The produced ionic liquid (OPFP) is dried under vacuum for 24 h.

Electrode Preparation

Carbon ionic liquid electrode was prepared by hand-mixing in a mortar. The graphite powder and IL ([OPy][PF₆]) were in a 50:50 weight ratio [52]. The resulting paste was packed firmly into the cavity (1.6-mm diameter) of a Teflon holder. The electrode was then heated in an oven at 80 °C for 2 min. After heating, the electrode was left to be cooled to room temperature. The electrical contact was established *via* a stainless steel handle. A new smooth surface was obtained by polishing the electrode against a smooth surface such as a regular paper. For preparation of the modified electrode, TiO₂-FeNPs were added to graphite powder and IL so that their weight percent, respectively, were 10.0, 45.0 and 45.0% with a total mass of 50.0 mg. The mixture was mixed well to provide a uniform paste. The resulting paste was packed firmly into the cavity (1.6-mm diameter) of a Teflon holder. This electrode was then heated and treated similarly as mentioned for the CILE.

Apparatus

Voltammetric measurements were performed with a PGSTAT302N Electroanalyser systems controlled by a personal computer. The electrochemical cell was assembled with a conventional three-electrode system. An

Ag/AgCl/KCl (3.0 M) electrode was used as a reference electrode (Metrohm) and a platinum wire as the counter electrode. The working electrode was the carbon ionic liquid electrode modified with TiO₂-Fe nanoparticles (TiO₂-FeNPs/CILE). The pH measurements were made with a Metrohm 780 pH meter using a combined glass electrode. The scanning electron microscopy (SEM) images were taken by a VEGA TESCAN microscope. The X-ray diffraction (XRD) measurements were performed on an XRD Bruker D8 Advance with copper as anode at wavelength of 1.54 Å. Cyclic voltammograms were recorded in the potential range of 0.4-1.3 V with a scan rate of 100 mV s⁻¹. For DPV method, the range of potential was 0.40-1.17 V with modulation amplitude of 0.05 V and an interval time of 0.5 s.

RESULTS AND DISCUSSION

Characterization of TiO₂-Fe Nanoparticles and TiO₂-FeNPs/CILE

The particle size of TiO₂-Fe nanoparticles were determined by SEM. Figure 1a shows the SEM photograph of the synthesized nanoparticles. The particles have a narrow size distribution; most of them have the diameter below 100 nm. However, some large aggregates can be easily observable. The X-ray diffraction technique was used to determine the crystalline structure of the TiO₂-Fe nanoparticles Fig. 1b. Five characteristic peaks for TiO₂-Fe ($2\theta = 25.25, 38.85, 48.15, 62.65$ and 68.80) corresponding to anatase phase and other five characteristic peaks ($2\theta = 27.40, 36.15, 41.40, 54.20$ and 75.10) corresponding to rutile phase are clearly observed in the pattern [53]. However, no crystalline phase containing Fe could be observed. It indicates that the iron ions are highly dispersed on TiO₂, and XRD is not sensitive enough to detect such minor changes to TiO₂ phases. On the other hand, the radius size of Ti⁴⁺ (0.68 Å) and Fe³⁺ (0.64 Å) was almost the same, therefore, it can be inferred that the Fe³⁺ ions might insert into the structure of titania and located at interstices or occupied some of the lattice sites of TiO₂, forming an iron-titanium solid solution [54]. The surface morphology of TiO₂-FeNPs/CILE was investigated by SEM. SEM image of bare CILE (Fig. 1c) indicates that uniform and smooth surface has been achieved due to the infiltration of IL into

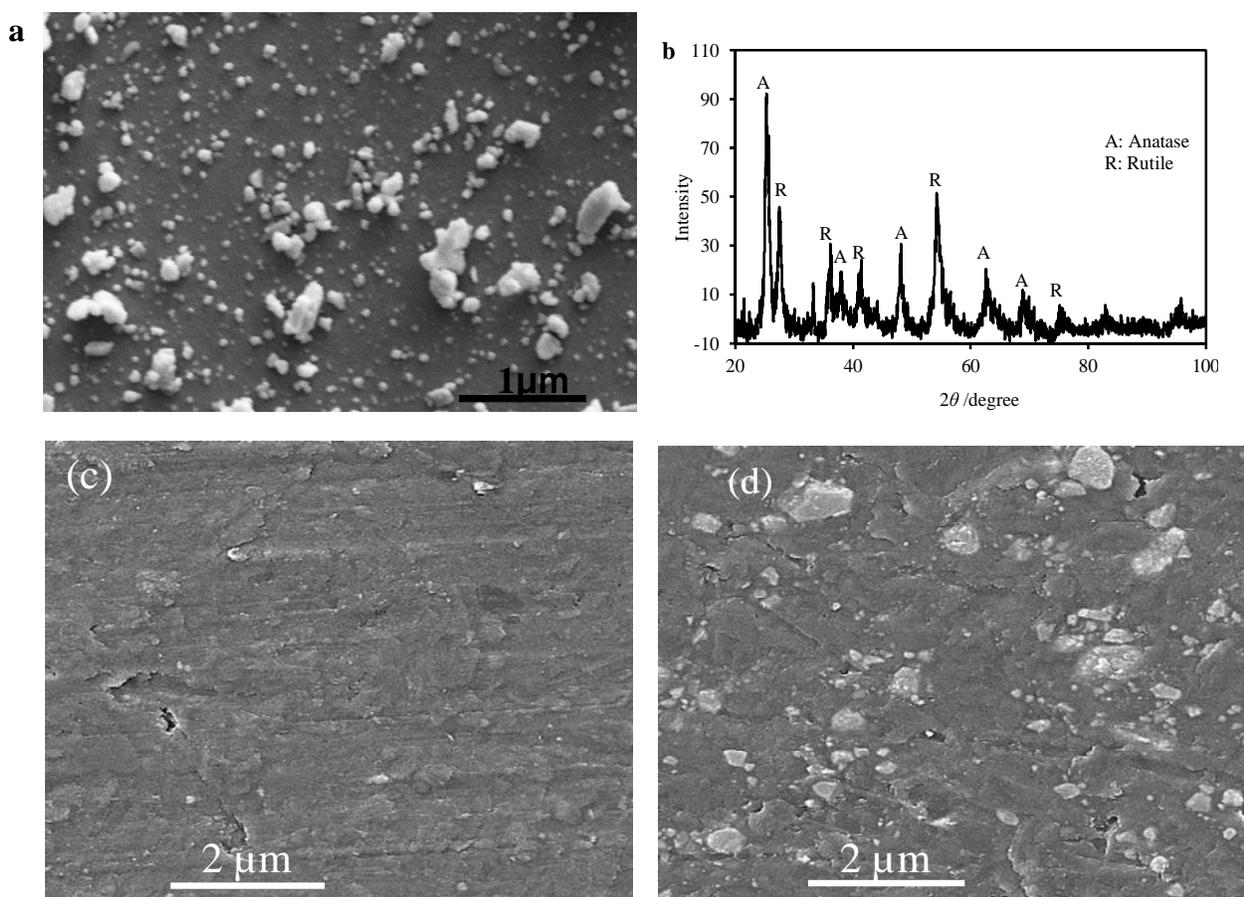


Fig. 1. The SEM image of $\text{TiO}_2\text{-Fe}$ nanoparticles (a), the XRD pattern of $\text{TiO}_2\text{-Fe}$ nanoparticles (b), the SEM image of CILE (c), and the SEM image of $\text{TiO}_2\text{-Fe}$ NPs/CILE (d).

the void spaces of the bulk graphite of the CILE. When $\text{TiO}_2\text{-Fe}$ NPs were incorporated into the paste, the uniformity of the surface was unchanged while the surface roughness is increased by appearing small $\text{TiO}_2\text{-Fe}$ clusters in nano/micro scales throughout the surface of the electrode (Fig. 1d).

CYCLIC VOLTAMMETRIC STUDIES

Electrochemical Behavior of Oxalate and Thiocyanate on $\text{TiO}_2\text{-FeNPs/CILE}$

The electrochemical behavior of thiocyanate and oxalate at bare CILE and $\text{TiO}_2\text{-FeNPs/CILE}$ was investigated by using cyclic voltammetry in universal buffer solution (0.04 M, pH 7.0) both individually as well as simultaneously. To

better understanding the performance of CILE, as substrate electrode, for modification in comparison with CPE, the cyclic voltammograms on both bare CPE and $\text{TiO}_2\text{-FeNPs/CPE}$ were recorded similarly. As can be seen from Fig. 2, both analytes show irreversible weak oxidation peaks at relatively high overpotentials of 1.23 V for oxalate and 1.04 V for thiocyanate. However, modification of this electrode has not improved the sluggish electron transfer and low oxidation peak current of the analytes. Although for oxalate a little decrease in overpotential is observed, the electrooxidation of thiocyanate seems to worse on the modified electrode.

When CILE was used for electrooxidation of the individual analytes, a negative shift for the oxidation peak (120 mV for oxalate and 176 mV for thiocyanate) was

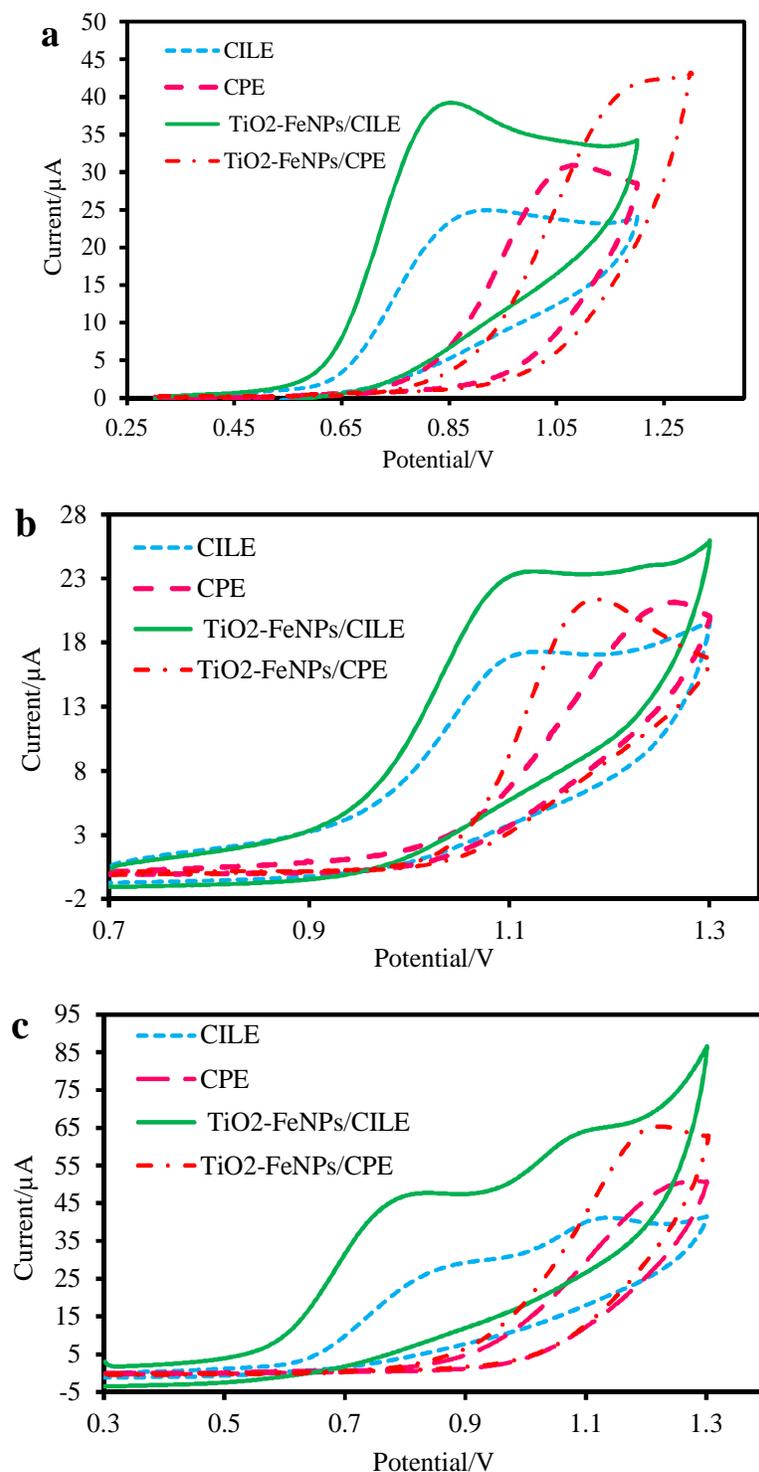


Fig. 2. Cyclic voltammograms recorded at different electrodes as indicated in universal buffer (0.04 M, pH 7.0) containing, (a), 1.0 mM oxalate; (b), 1.0 mM thiocyanate; and (c), mixture of 1.0 mM oxalate and 1.0 mM thiocyanate. Scan rate was 100 mV s^{-1} .

observed. This indicates that the CILE had an electrocatalytic effect compared to traditional CPE. As it has been reported, by mixing IL with graphite powder, it not only acts as a binder, but also fills up the void spaces of the graphite powder and provides an excellent charge-transfer bridge in the bulk of the CPE [55,56]. By incorporating TiO₂-FeNPs into the paste of CILE, although no significant change in oxidation overpotential was observed, there was an increase for the peak currents of each analyte which could be due to extending of the active surface area by nanostructures. By exploring the redox peak current with the scan rate for K₃[Fe(CN)₆], based on Randles-Sevcik equation, the average electroactive area for both CILE and 10% TiO₂-Fe in TiO₂-FeNPs/CILE were calculated and found to be 2.87 and 5.74 mm², respectively. This result indicates that TiO₂-FeNPs has greatly improved the effective surface area of the electrode. However, the attraction of anions toward Fe³⁺ presents in TiO₂-FeNPs which improves the sensitivity of the measurement cannot be ignored. The cyclic voltammograms at the different electrode were also obtained for a mixture containing both oxalate and thiocyanate in order to evaluate the possibility for simultaneous determination of these anions. As depicted in Fig. 2c, separate oxidation peaks was not observed at CPE and TiO₂-FeNPs/CPE, but one oxidation peak was observed at 1.19 V. However, both CILE and TiO₂-FeNPs/CILE provided well-separated oxidation peaks with peak separation of 300 mV; Moreover, the later seems to be more sensitive evidenced by higher peak currents for both analytes. It could be concluded that the combination advantages of electrocatalytic activity of CILE and high effective surface area imparted by TiO₂-FeNPs makes the fabricated electrode a promising for tool for simultaneous measurement of oxalate and thiocyanate.

Effect of pH

The effect of pH on the electrooxidation currents and potentials of 1.0 mM of both thiocyanate and oxalate, in the pH range of 2.0-8.0, was investigated at TiO₂-FeNPs/CILE by cyclic voltammetry. Based on the physiological and analytical perspective, all the remaining experiments were carried out in universal buffer solution (0.04 M, pH 7.0). The results showed that the oxidation peak potential has not varied significantly for thiocyanate (Fig. 3a), indicating that

this anion is in its anionic form throughout the examined pH range. Although, thiocyanate showed a well-defined oxidation peak at all pH values, the oxidation peak was not well-defined for oxalate at pH < 6.0. For oxalate, however, the peak potential is sensitive to pH variation in the range of 2.0-4.0; *i.e.* decreases from 1.198 V in pH 2.0-1.072 V in pH 4.0 V. The peak potential decreasing with pH at pH < 4.0, with a slope of 47.6 (mV pH⁻¹), suggested that equal number of protons and electrons are involved in the overall oxidation process [57]. The linear regression equation between the oxidation peak potential and pH (in the pH range of 2.0-4.0) was calculated as E_{pa} (mV) = -47.6 pH + 136.04 (with $R^2 = 0.9944$) reflects the known pK_{a1} (1.25) and pK_{a2} (4.20) values of oxalic acid [58]. At pH values higher than 4, the oxidation potentials were approximately constant indicating that the process was not pH-dependent as shown in Fig. 3a.



For thiocyanate, the peak current decreased with increasing pH in the range of 2.0-8.0. For oxalate it showed a maximum at pH of about 7.0 as shown in Fig. 3b. In the pH range of 2.0-8.0, thiocyanate is in its anionic form. Electrode surface is more alkaline at pH > 5.0 so that Fe³⁺ has tendency to be complexed by hydroxide. As a result, the electrode surface would be rich in OH⁻ ion and diffusion of SCN⁻ ion toward electrode surface becomes slower; as a result the peak current is reduced [59].

Effect of Scan Rate

Cyclic voltammograms of 1.0 mM of both thiocyanate and oxalate on TiO₂-FeNPs/CILE with different scan rate (10-200 mV s⁻¹) in 0.04 M universal buffer (pH 7.0) are shown in Fig. 4. It can be seen that with increasing the scan rate (v), the oxidation peak current increases gradually. The peak current increases linearly with the square root of scan rate with mathematical equations of $I_{pa} = 1.192 v^{1/2} + 0.1598$ ($R^2 = 0.9972$) for thiocyanate and $I_{pa} = 0.8046 v^{1/2} + 0.3010$ ($R^2 = 0.9941$) for oxalate. These results suggest that the

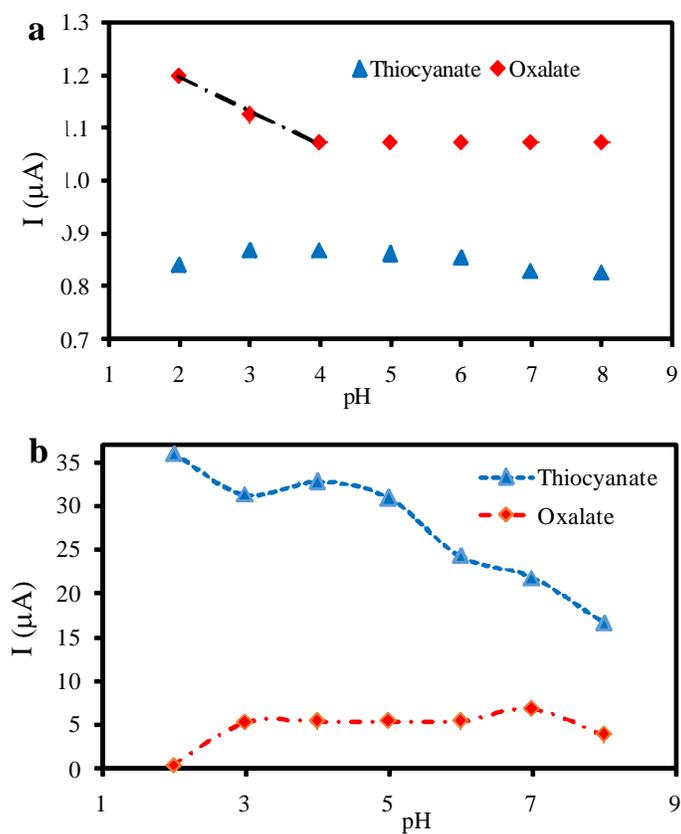


Fig. 3. (a) Effect of pH on the catalytic peak currents of thiocyanate (\blacktriangledown) and oxalate (\blacklozenge) at 100 mV s^{-1} in 0.04 M universal buffer at pH range of 2.0-8.0. (b), Effect of pH on the oxidation peak potentials of thiocyanate (\blacktriangledown) and oxalate (\blacklozenge) at 100 mV s^{-1} in 0.04 M universal buffer at pH range of 2.0-8.0

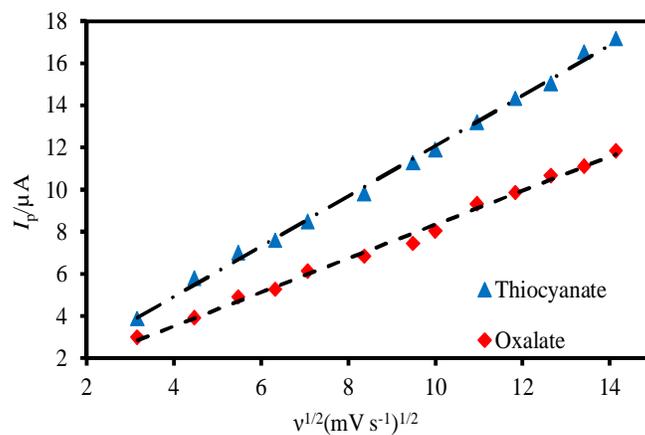


Fig. 4. Plot of peak current vs. square root of scan rate in the range of $10\text{-}200 \text{ mV s}^{-1}$ for 1.0 mM of oxalate (\blacklozenge) and 1.0 mM thiocyanate (\blacktriangledown).

kinetics of oxidation reactions for both analytes at the TiO₂-FeNPs/CILE, is controlled by diffusion process [60]. Additionally, with increasing scan rate, the oxidation peak potential (E_p) shifted to positive values indicating that the electrooxidation process is irreversible for both analytes. From the plot of E_{pa} vs. $\log v$, slopes of 107.2 and 105.6 mV decade⁻¹ were achieved for thiocyanate and oxalate, respectively. Assuming one electron transfer in the rate determining step ($n = 1$), anodic transfer coefficient (α) was calculated as 0.45 and 0.44 for thiocyanate and oxalate, respectively, according to the following equations which are valid for a totally irreversible diffusion controlled process [61]:

$$E_p = \frac{b}{2} \log v + K \quad (4)$$

$$b = \frac{2.3 RT}{(1-\alpha)n_a F} \quad (5)$$

In another experiment, the slope of E_p vs. $\log i$ plot [$d(E_{pa})/d(\log(i))$], *i.e.* the Tafel slope, was obtained to be 106 mV decade⁻¹ for thiocyanate and 103.8 mV decade⁻¹ for oxalate from the data of the rising part of the current-voltage curve at a scan rate of 20 mV s⁻¹. The anodic transfer coefficients (α) were calculated as 0.44 and 0.43 for both thiocyanate and oxalate, respectively, which are in agreement with the values obtained above.

Differential Pulse Voltammetric Analysis

Simultaneous electrochemical behaviors of thiocyanate and oxalate, at both modified and unmodified electrodes were investigated by using differential pulse voltammetry (DPV). As shown in Fig. 5a, the peak potentials for both species are almost the same; however, the currents are significantly higher on the modified electrode. On TiO₂-FeNPs/CILE, two oxidation peaks were observed, at 0.740 V for thiocyanate and at 1.01 V for oxalate; the peak potential separation (ΔE_p) was 270 mV. Because the peak potential separation is appropriate, for optimization of the experimental conditions, the DPV method was preferred.

It has been reported [52] that the CILE prepared with a graphite/OPFP ratio of 50% (w/w) offered the lowest background as well as good mechanical stability, so it was used for further studies. On the other hand, finding the

optimum amount of modifier in electrode composition is essential for practical applications. To do this, simultaneous electrocatalytic response of TiO₂-FeNPs/CILE to thiocyanate and oxalate was studied on the electrodes with different weight percent of TiO₂-FeNPs. It was found that the oxidation peak currents on an electrode containing 10 percent TiO₂-FeNPs was higher. However, it was observed that when weight percent of TiO₂-FeNPs was more than 10%, the background current greatly increased. The increase in background current could be attributed to the increase in surface area of the composite and to the increase in the double-layer capacitance and charge build-up at the double-layer interface as reported for electrodes modified with MWCNTs [62]. Thus, a weight percent of 10% TiO₂-Fe was selected for further investigations.

The other factor which is important in simultaneous voltammetric analysis is the nature of the electrolyte, since it can affect both current response and peak separation. The DPVs were recorded for individual buffer solutions including acetate, citrate, phosphate, Tris and universal buffers at pH 7.0. The maximum current with the highest ΔE_p value was observed for the universal buffer solution.

Calibration Curve

Differential pulse voltammetry was used for simultaneous determination of thiocyanate and oxalate at TiO₂-FeNPs/CILE in universal buffer solution (0.04 M, pH 7.0) under the optimized experimental conditions while the concentration of one analyte changed in the presence of a fixed concentration of the other. Figure 5b shows the DPVs of thiocyanate at different concentrations in the presence of 0.1 M oxalate. The peak current linearly increased with increasing concentration from 1.0×10^{-5} to 1.8×10^{-3} M. Figure 5c displays the DPVs of oxalate in the presence of 0.1 M thiocyanate indicating that its peak current is linearly proportional to concentration in the range of 5.0×10^{-5} - 3.0×10^{-3} M. The linear regression equation and correlation coefficients are as follow:

$$I_{pa} = 3.354 C_{Oxalate} + 0.103, (R^2 = 0.997)$$

$$I_{pa} = 3.323 C_{Thiocyanate} + 0.273, (R^2 = 0.998)$$

where I_{pa} is the anodic peak current in μ A and C is the

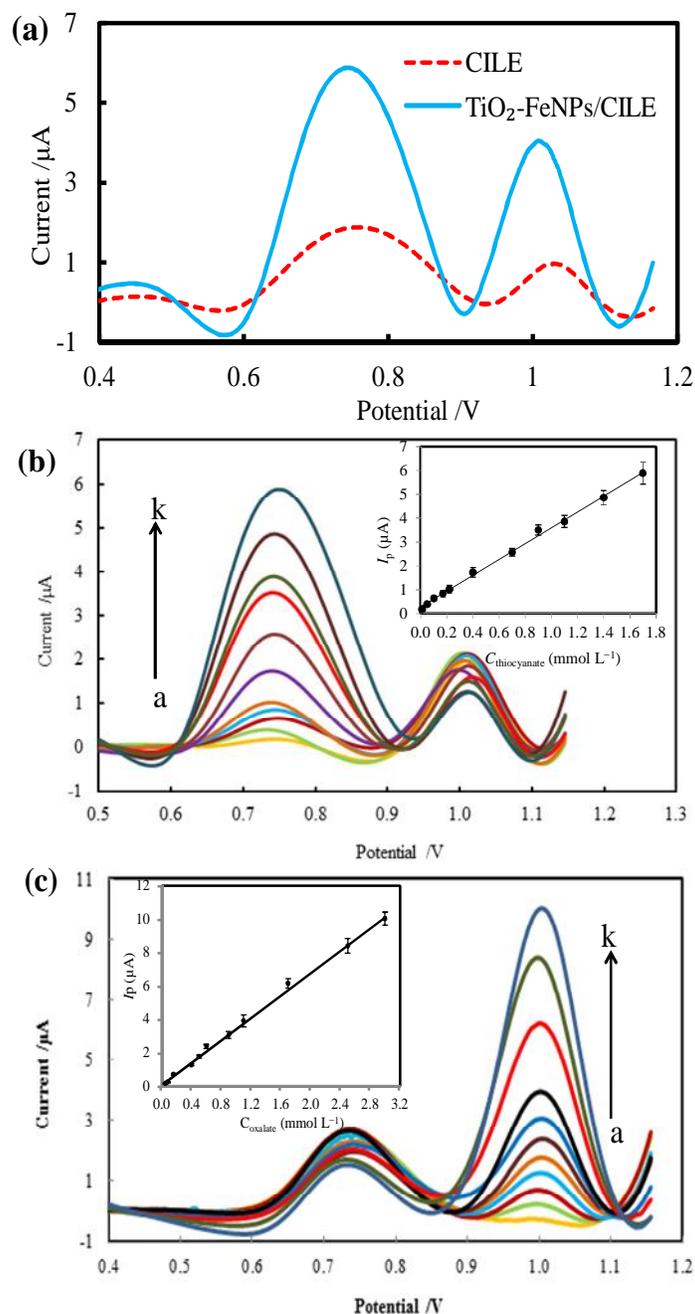


Fig. 5. (a), Simultaneous differential pulse voltammetry of mixture containing 1.0 mM thiocyanate and 1.0 mM oxalate in 0.04 M universal buffer (pH 7.0) at CILE (dotted line) and TiO₂-FeNPs/CILE (solid line). (b), Differential pulse voltammograms of thiocyanate in TiO₂-FeNPs/CILE in the presence of 5.0×10^{-4} M oxalate in universal buffer (pH 7.0). Thiocyanate concentrations (from a to k): 1.0×10^{-5} , 5.0×10^{-5} , 1.0×10^{-4} , 1.7×10^{-4} , 2.2×10^{-4} , 4.0×10^{-4} , 7.0×10^{-4} , 9.0×10^{-4} , 1.1×10^{-3} , 1.4×10^{-3} , 1.8×10^{-3} M. (c), Differential pulse voltammograms of oxalate in TiO₂-FeNPs/CILE in the presence of 2.0×10^{-4} M thiocyanate in universal buffer (pH 7.0). Oxalate concentrations (from a to k): 5.0×10^{-5} , 8.0×10^{-5} , 1.6×10^{-4} , 4.0×10^{-4} , 5.0×10^{-4} , 6.0×10^{-4} , 9.0×10^{-4} , 1.1×10^{-3} , 1.7×10^{-3} , 2.5×10^{-3} , 3.0×10^{-3} M. Insets show the corresponding calibration curves.

concentration of analytes in the unit of mM. The detection limits for thiocyanate and oxalate in their linear range regions were found to be 6.4×10^{-6} M and 2.3×10^{-5} M, respectively.

Stability, Reproducibility and Fouling Study

The constructed sensor was stored in air at ambient condition and its sensitivity was checked every week. The response of the sensor was 95.4% of the initial value after 40 days indicating to an excellent stability of the electrode. Five successive cyclic voltammetric measurements of 0.1 mM of thiocyanate and oxalate mixture solutions on modified electrode yielded reproducible currents with relative standard deviations (RSD) of 2.2% and 3.2%, respectively, for thiocyanate and oxalate. This demonstrated that the electrode was not poisoned by the oxidation products and can be used repeatedly for simultaneous detection of thiocyanate and oxalate. In addition, four modified electrodes based on the same fabrication procedure were prepared and used for simultaneous determination of 0.1 M thiocyanate and oxalate mixture solution. The RSD of the peak currents, for all electrodes, were calculated and found to be 4.4% and 6.4%, respectively, for thiocyanate and oxalate.

Interference Study

The influence of various foreign ions in simultaneous determination of thiocyanate and oxalate was studied under the optimum experimental conditions (Tables 1 and 2). The tolerance limit was defined as the maximum concentration of the foreign species that causes an error less than $\pm 5\%$ for determination of 1.0×10^{-4} mM of both thiocyanate and oxalate. It should be mentioned that most of the examined species were not electroactive in the potential window in which the differential pulse voltammograms have been recorded. However, the analytical signal decreases in the presence of some anions such as I^- , S^{2-} and $Cr_2O_7^{2-}$. It seems that the electrocatalytic effect of the ionic liquid as well as the strong attraction of Fe^{3+} ion toward thiocyanate and oxalate are responsible for relatively selective sensing of both analytes

Simultaneous Determination of Thiocyanate and Oxalate in Urine Sample

The constructed sensor was applied to determine thiocyanate and oxalate simultaneously in a synthetic urine sample with composition presented in Table 3. Standard addition method was adopted to estimate the accuracy. To 3.0 ml of the urine sample, 7.0 ml universal buffer (0.04 M,

Table 1. Results of Interference Study for the Determination of 0.10 mM Thiocyanate under the Optimum Experimental Conditions

Foreign Species	TConcentration of Additive/Thiocyanate
K^+ , Na^+ , Mg^{2+} , Cl^- , PO_4^{3-} , SO_4^{2-} , ClO_3^- , Urea	500
NO_3^- , Ethanol	300
Ascorbic acid, NH_4^+ , HCO_3^- , SO_3^{2-} , Glucose	100
Uric acid	70
CN^-	50
S^{2-}	10
CrO_4^{2-} , $Cr_2O_7^{2-}$	3
I^-	1

Table 2. Results of Interference Study for the Determination of 0.10 mM Oxalate under the Optimum Experimental Conditions

Foreign Species	Concentration of Additive/Oxalate
K ⁺ , Na ⁺ , Mg ²⁺ , Cl ⁻ , PO ₄ ³⁻ , SO ₄ ²⁻ , ClO ₃ ⁻ , Urea	500
SO ₃ ²⁻ , NO ₃ ⁻ , Ethanol	300
HCO ₃ ⁻ , Glucose	100
NH ₄ ⁺	50
CrO ₄ ²⁻	30
Uric acid	20
Ascorbic acid, CN ⁻	5
Cr ₂ O ₇ ²⁻ , S ²⁻	3
I ⁻	1

Table 3. Simultaneous Determination of Thiocyanate and Oxalate in Urine Sample by Standard Addition Method

Sample	Analyte	Analyte added (μM)	Analyte found by TiO ₂ ⁻ FeNPs/CILE (μM) ^a ± S.D.	Recovery (%)
Urine ^b	Thiocyanate	0.00	98.7 ± 5.5	-
		100.0	188.7 ± 1.1	90%
		200.0	279.8 ± 1.1	90%
		300.0	387.6 ± 6.6	96%
	Oxalate	0.0.0	111.2 ± 0.5	-
		100.0	192.7 ± 5.3	81%
		200.0	292.4 ± 3.7	90%
		300.0	381.2 ± 1.2	90%

^aMean value ± standard deviation (three replicate analysis). ^bUrine components: uric acid = 0.034; creatinine = 0.090; Na₃C₆H₅O₇·2H₂O = 0.297; urea = 2.42; NaCl = 0.634; KCl = 0.450; NH₄Cl = 0.161; MgSO₄·7H₂O = 0.100; NaHCO₃ = 0.034; Na₂C₂O₄ = 0.0086; NaSCN = 0.0052; Na₂SO₄ = 0.258; NaH₂PO₄·H₂O = 0.100; Na₂HPO₄ = 0.011 g in 200 ml of deionized water [63].

Table 4. Comparison of Thiocyanate and Oxalate Determination with Different Electrodes

Electrode	Anion	Interfering ion	pH	Detection limits (M)	Linear range (M)	[Ref.]
Selective polymeric membrane containing a Nickel(II)-Azamacrocyclic complex coated on a graphite electrode	Thiocyanate	Oxalate	5	4.8×10^{-8}	$1 \times 10^{-7} - 1 \times 10^{-1}$	[64]
Silver nanoparticles modified electrode	Thiocyanate	Oxalate	6	4.0×10^{-8}	$5.0 \times 10^{-7} - 4.0 \times 10^{-4}$	[43]
Selective sensor based on tripodal zinc complex	Thiocyanate	Oxalate	5.5	3.16×10^{-7}	$6.3 \times 10^{-7} - 1.0 \times 10^{-2}$	[1]
Membrane electrode based on 2,2-[1,4-butandiyli bis(nitrilo propylidine)]bis-1-naphtholato copper(II)	Oxalate	Thiocyanate	7	5.0×10^{-8}	$5.0 \times 10^{-8} - 1.0 \times 10^{-1}$	[59]
TiO ₂ -FeNPs/CILE	Thiocyanate and oxalate	-	7	6.38×10^{-6} & 2.31×10^{-5}	$1.00 \times 10^{-5} - 1.75 \times 10^{-3}$ & $5.00 \times 10^{-5} - 3.00 \times 10^{-3}$	This work

pH 7.0) as well as known amount of thiocyanate and oxalate in urine samples. The low recoveries are perhaps due to the effect of the urine matrix that could be improved by applying a pre-separation step such as that reported by Xu *et al.* [32].

CONCLUSIONS

According to the results of this work, it has been shown that thiocyanate and oxalate can be determined simultaneously by using voltammetric method on the basis of their oxidation processes at TiO₂-FeNPs/CILE electrode with wide linear ranges of 1.0×10^{-5} - 1.8×10^{-3} M and 5.0×10^{-5} to 3.0×10^{-3} M and good detection limits of 6.4×10^{-6} M and 2.3×10^{-5} M for thiocyanate and oxalate, respectively. The characteristics of the modified electrode such as high sensitivity, easy fabrication, good reproducibility and stability, indicated that the constructed electrode is promising for simultaneous determination of

thiocyanate and oxalate in urine samples. For the electrodes (Table 4) that have been applied for individual determination of thiocyanate and oxalate, it is frequently reported that both oxalate and thiocyanate act as interfering species for each other. So, the benefit of the modified electrode which is presented in this research is its ability for simultaneous determination of oxalate and thiocyanate, which is demonstrated for the first time.

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