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Voltammetric Determination of 6-Mercaptopurine in Drugs and Blood Using a Glassy Carbon Electrode Modified with Chromium(III)hexacyanoferrate(II)

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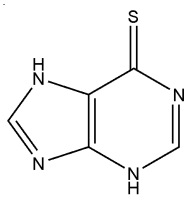
A Cr(III) hexacyanoferrate(II) (CrHCF)-modified glassy carbon electrode was used to determine 6-mercaptopurine in some drugs and blood by cyclic voltammetry. The influence of several parameters on the voltammetric electrode response was analyzed. The linear dynamic range was 2.30×10^{-7} mol L^{-1} to 7.27×10^{-6} mol L^{-1} of mercaptopurine, with r = 0.9976. The limit of detection was 1.3×10^{-7} mol L^{-1}. The voltammetric proposed method for determination of 6-mercaptopurine presented good accuracy and the experimental results demonstrated that the CrHCF-modified glassy carbon electrode has a large potential for the analysis of 6-mercaptopurine in real samples. Furthermore, it has the advantages of a fast response, a low detection limit, low cost, and simple development and application.

Key Words: 6-Mercaptopurine, Modified electrode, Voltammetry, Chromium(III)hexacyanoferrate(II).

INTRODUCTION

6-Mercaptopurine (6MP), a sulfur analogue of adenine (**I**) is one of the oldest anticancer drugs and immunosuppressive agents[1]. Since 1950s, 6-mercaptopurine has been applied to cure childhood and adult leukemias[2]. It has been established that 6-mercaptopurine and its metabolites exert their primary cytotoxicity through incorporation of deoxythioguanosine into DNA and inhibit the function of RNaseH in DNA-RNA heteroduplex molecules[3]. Different analytical methods have been used for the determination of 6-mercaptopurine. It was found that the iodine-azide reaction of 6-mercaptopurine could yield a cathodic stripping signal at a sliver electrode and the voltammetric response was used to detect 6-mercaptopurine over the range[4] of 0.2-6.0 mol L^{-1}. Removed protein from human blood plasma, 6-mercaptopurine and its metabolites could be detected in patient plasma by a reversed-phase high-performance liquid chromatography[5]. A DNA modified carbon paste electrode was applied for the determination of 6-mercaptopurine with a detection limit[6] of 2.0×10^{-6} mol L^{-1}.

Various other methods including high-performance liquid chromatography (HPLC)[7-11] and capillary electrophoresis[12,13] have been used for the detection of 6-mercaptopurine in plasma. Recently, in order to obtain an effective separation and sensitive detection, some coupled methods have been used to monitor the 6-mercaptopurine concentration such as capillary electrophoresis with laser-induced fluorescence (LIF)[14], liquid chromatography and electrochemical detection (LC-ECD)[15], liquid chromatography and electrochemical detection (LC- ECD) with microdialysis[16]. However, most of these methods need sample pretreatments or have relative low sensitivities. Therefore, it is necessary to develop a new direct, sensitive and facile assay method for 6-mercaptopurine.



Chemical structure of 6-mercaptopurine (**I**)

The working electrodes may be modified to improve the analytical signal, the detection range, the sensitivity and the selectivity of this technique.

The combined use of glassy carbon electrodes modified with hexacyanoferrate complexes may be instrumental in the determination of 6-mercaptopurine. Thus, the present work sought to use a glassy carbon electrode modified with Cr(III)-hexacyanoferrate(II) (CrHCF)[17] to determine 6-mercaptopurine in drugs and blood. The influence of parameters on the voltammetric electrode response, as well as pH, supporting electrolyte, scan rate, precursor reagent and the interference

of several compounds present in real samples containing 6-mercaptopurine have been evaluated.

EXPERIMENTAL

All solutions were prepared using deionized water and analytical grade reagents. Stock solutions sensitive to light were stored in dark glass flasks.

The standard solution of 6-mercaptopurine 1.00×10^{-3} mol L^{-1} was carefully prepared by dissolution of 6-mercaptopurine (Aldrich) in 100 mL deionized water. The solutions used in the interference study were prepared by dissolution of appropriate amounts of the species.

To prepare the CrHCF-modified glassy carbon electrode, 11 6.0×10^{-3} mol L^{-1} potassium chloride, pH 3.0 with 3.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$ and 1.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$ were used.

Potassium chloride solution prepared by dissolving the appropriate amount of salt in deionized water was used as supporting electrolyte and the pH was adjusted with HCl 0.10 mol L^{-1} and NaOH 0.10 mol L^{-1} solutions.

A Corning pH-meter, Model 140 with a glass electrode (conjugated with an Ag/AgCl reference electrode, Model 6.0232.100), was used to determine pH of the solutions. Voltamograms were obtained by using an EG and G instrument, Model 394 processor, with three electrodes consisting of a glassy carbon electrode as a working electrode, a Hg/Hg_2Cl_2 (3.0 M KCl) reference electrode and a platinum wire as a counter electrode that linked to a computer (Pentium III, 2.0 GHz).

Modification of working electrode: The glassy carbon electrode, with diameter area of 12.6 mm^2, was polished before modification with alumina paste, washed and cleaned by sonication in deionized water for 10 min.

Electrodeposition to generate CrHCF was carried out applying potential cycles between -0.2 and +1.0 V for 0.5 h in a KCl 0.10 mol L^{-1} (pH 3.0) solution containing 3.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$ and 1.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$. The electrodeposition scan rate was 40 mV s^{-1} under magnetic stirring at 400 rpm and the glassy carbon rotated disk at 30 rpm. After this step, the electrode was conditioned for 1 h in KCl solution 2.0×10^{-3} mol L^{-1} and pH 3.0[17].

Preparation of real samples: For drug analysis, the samples were powdered and the average mass corresponding to one tablet was weighted and dissolved in deionized water. The insoluble drug excipient was removed by filtration with a 45 μm Millipore membrane. The filtered material was colleted in a volumetric flask and diluted with deionized water up to 250 mL.

The voltammetric determinations of 6-mercaptopurine were carried out by cyclic voltammetry. The current value related to the mercaptopurine with modified glassy carbon electrode was that obtained by the difference of the current observed at 0.99 V in the presence and in the absence of 6-mercaptopurine.

Whole blood samples were obtained and stored frozen until the assay. Into each of 10 centrifugation tubes containing a certain concentration of 6-mercaptopurine, 1.0 mL of human serum/or whole blood sample was transferred and then mixed well with 9 mL methanol to precipitate blood proteins. The precipitated proteins were separated by centrifuging of the mixture for 20 min at 4000 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to produce protein-free serum/or whole blood.

RESULTS AND DISCUSSION

Modification of the glassy carbon electrode: The voltammograms obtained on modified and non-modified glassy carbon electrode is shown in Fig. 1. According to the potential values obtained, the resulting complex of $CrCl_3 \cdot 6H_2O$ and $K_3[Fe(CN)_6]$ deposition onto the glassy electrode is Cr(III) hexacyanoferrate(II) {$KCr[Fe(CN)_6]$} which is deposited in the proportion of 2:1 of $Cr(III)/[Fe(CN)_6]^{3-}$ that presents blue colour, which is in agreement with data present in the literature[17,18].

Cyclic voltammogram of KCl solution 0.10 mol L^{-1} at pH 3.0 as electrolyte on $CrCl_3$ modified glassy carbon electrode (Fig. 1a) shows that there is no peak, indicating the absence of electroactive species. Cyclic voltammogram of the precursor potassium hexacyanoferrate(III) reagent on the glassy carbon electrode (Fig. 1b) shows the appearance of two peaks relative to this reagent at 0.22 and 0.34 V that correspond to the redox process of $[Fe(II)(CN)_6]/[Fe(III)(CN)_6]$[17]. Peaks 1 and 2 at 0.22 and 0.88 V, respectively observed after the electrode surface modification (Fig. 1c), corresponds to the $K_2CrII[FeII(CN)_6]$ complex oxidation peaks, while their correspondent reduction peaks (3) and (4) were observed at 0.82 and 0.19 V, respectively. Thus, we have a system composed by stages with mixed valence represented by [CrIII-CN-FeII]/[CrII-CN-FeII] and [CrIII-CN-FeII]/[CrIII-CN-FeIII] and with two degeneracy states[19]. The small peaks (5 and 6) may be attributed to the inclusion and exclusion of potassium ions during the redox process[19].

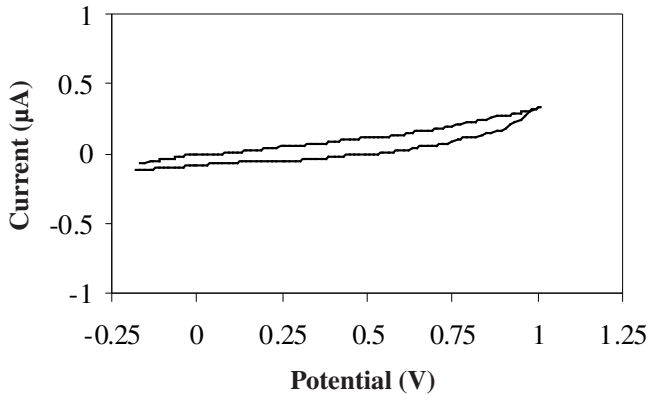
The probable electrode reaction is represented in eqn. 1. The electrochemical oxidation/reduction process is followed by the cation flux (K^+) provided by the supporting electrolyte solution, which helps to keep the electroneutrality of the system and works as a counter ion.

$$K_2Cr(II)[FE(II)(CN)_6]_{(s)} \rightleftharpoons KCr(III)[FE(CN)_6]_{(s)} + K^+_{(aq)} + e^-$$

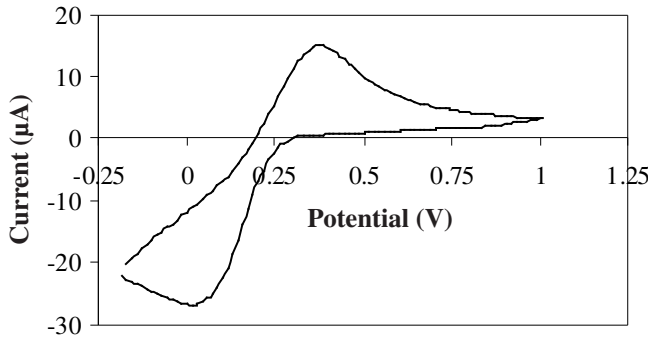
$$KCr(III)[Fe(II)(CN)_6]_{(s)} \rightleftharpoons Cr(III)[FE(III)(CN)_6]_{(s)} + K^+_{(aq)} + e^- \quad (1)$$

The applied conditioning reduction potential of -0.2 V resulted in the Cr(II) and Fe(II) complex and in the adsorbing of the CrHCF species at the electrode surface. As the scan progressed from the most negative to the most positive potentials, the metals oxidations occur and the oxidized species release the counter ion K^+(aq) as well as electrons. On the other hand, from the most positive to the most negative potential, the species were reduced.

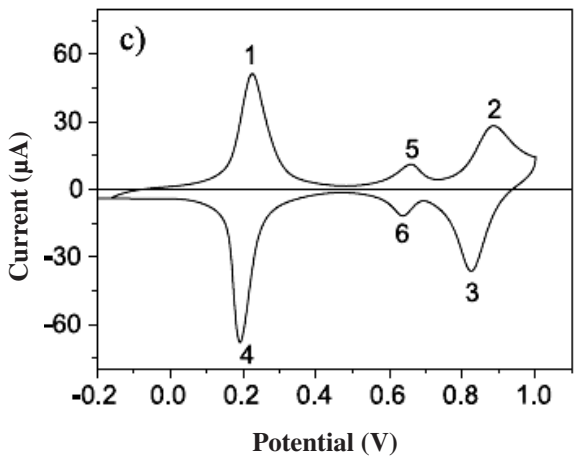
Fig. 2 shows the cyclic voltammogram of 6-mercaptopurine on the surface of modified glassy carbon electrode in the presence of 0.1 mol L^{-1} potassium chloride. It seems that the reversible couple of [Cr(III)-CN-Fe(II)]/[Cr(III)-CN-Fe(III)] acts as a mediator for the reduction of 6-mercaptopurine. This process accompany with the increasing on the oxidation current of [Cr(III)-CN-Fe(III)]/[Cr(III)-CN-Fe(II)].



(a)



(b)



(c)

Fig. 1. Cyclic voltammograms for: (a) $CrCl_3$ (3.0×10^{-2} mol L^{-1}); (b) potassium hexacyanoferrate(III) ((1.0×10^{-2} mol L^{-1}); (c) Cr(III) hexacyanoferrate(II) on glassy carbon electrode in an electrolyte solution containing 0.10 mol L^{-1} KCl. Scan rate 50 mV s^{-1}

Effect of operational parameters: In order to find the optimum conditions with highest sensitivity for determination of 6-mercaptopurine, the influence of various parameters including $CrCl_3 \cdot 6H_2O$ concentration, $K_3[Fe(CN)_6]$ concentration, KCl concentration in the presence of precursors, KCl concentration in the conditioning step, pH of the conditioning step, accumulation time, KCl concentration in the determination step, pH solution in the determination step and scan rate on the peak current were studied.

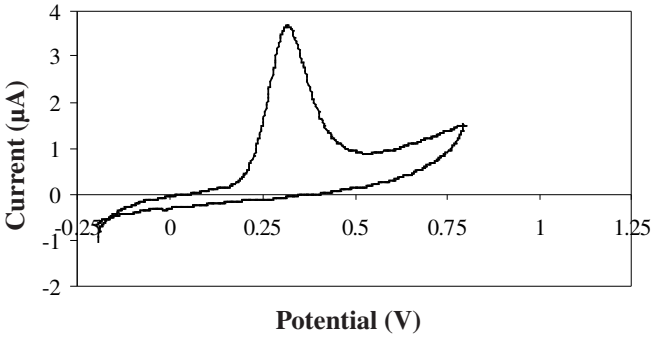


Fig. 2. Cyclic voltammetry of 10 μmol L^{-1} 6-mercaptopurine on the surface of modified electrode. All of the conditions are as Fig. 1

The precursor reagent concentration and electrodeposition time were investigated and it was observed a larger efficiency in the determination of 6-mercaptopurine with 3.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$, 1.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$ and 6.0×10^{-3} mol L^{-1} potassium chloride with deposition time of 0.5 h. The concentration of KCl in the conditioning step do not effect on the oxidation peak current of 6-mercaptopurine, therefore the concentration of 6.0×10^{-3} mol L^{-1} was chosen. The use of higher electrodeposition times results in excessively complex deposition on the electrode surface, leading to not reproducible current values. On the other hand, low precursor concentrations result in an electrode that cannot be applicable to the determination of 6-mercaptopurine due to generation of very low current values.

Effect of pH of the precursor reagent: Fig. 3 illustrates the dependence of the peak currents on pH of the precursor reagent. The peak current is at the maximum value at pH 3. This is probably due to stability of the CrHCF deposition layer. At pH values grater than 3, the peak current decreased about 90 %. Therefore, pH value of 3 was selected for precursor reagent.

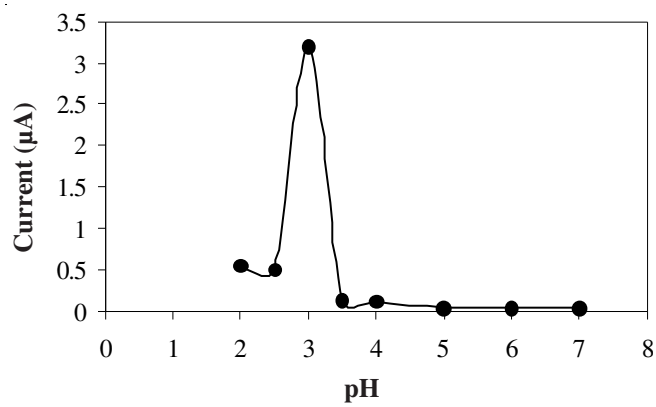


Fig. 3. Effect of pH of the precursor reagent on the peak current of 20 μmol L^{-1} 6-mercaptopurine in the presence of 3.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$, 1.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$, electrolyte solution containing 6.0×10^{-3} mol L^{-1} potassium chloride, deposition time of 0.5 h, pH 10.0 of the drug solution and scan rate of 40.0 mV s^{-1}

Effect of pH in the determination step: As it is shown the structure of mercaptopurine (**I**) is dependent to pH of the solution. Therefore, we have studied the influence of pH in the determination step on the oxidation peak current of mercaptopurine. It was found that with increasing pH up to 9.3, the

peak current is very small and independent to pH, however, at higher pH values the peak current increased (Fig. 4). This is due to the fact that at higher pH values (pH > 9) mercaptopurine is in the anionic form and can be solved in solution. But at pH values more that 11, the peak current decreased due to instability of CrHCF deposition layer. So pH value of 11.0 was selected for further optimization steps.

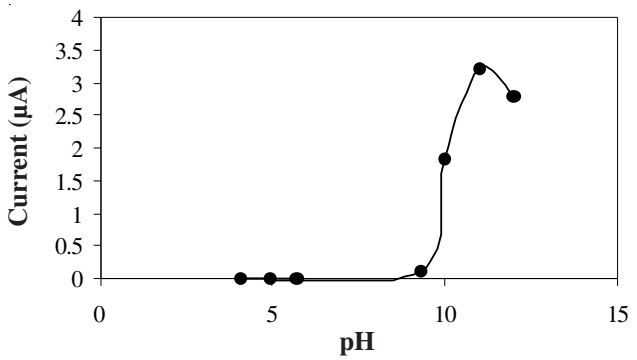


Fig. 4. Effect of pH of the 6-mercaptopurine solution on the peak current of 20 μmol L^{-1} 6-mercaptopurine after modification of glassy carbone electrode in the presence of 3.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$ and 1.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$ at pH 3, electrolyte solution containing 6.0×10^{-3} mol L^{-1} potassium chloride, deposition time of 0.5 h and scan rate of 40.0 mV s^{-1}

Effect of KCl concentration in the determination step: Fig. 5 illustrates the dependence of the peak currents on KCl concentration in the determination step. The peak currents increased up to 1.0×10^{-2} mol L^{-1} potassium chloride and then decreased. However, by increasing KCl concentration greater than 1.0×10^{-2} mol L^{-1}, the peak current decreased. This is probably due to irreversibility of the CrHCF deposition layer at high KCl concentrations.

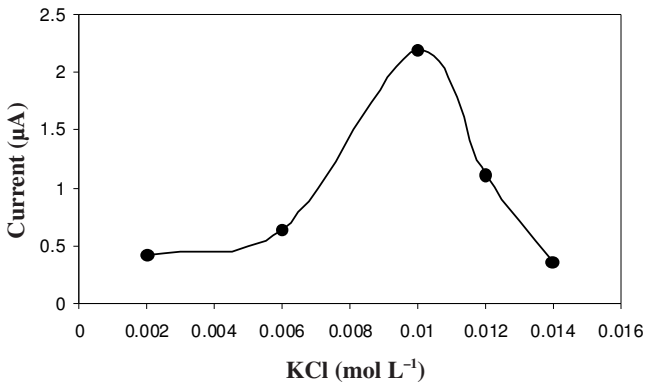


Fig. 5. Effect of KCl as the supporting electrolyte on the peak current of 20 μmol L^{-1} 6-mercaptopurine after modification of glassy carbone electrode in the presence of 3.0×10^{-2} mol L^{-1} $CrCl_3 \cdot 6H_2O$, 1.0×10^{-2} mol L^{-1} $K_3[Fe(CN)_6]$, electrolyte solution containing 6.0×10^{-3} mol L^{-1} potassium chloride, deposition time of 0.5 h, pH 11.0 of the drug solution and scan rate of 40.0 mV s^{-1}

Effect of scan rate: The influence of potential scan rate on the oxidation peak current of 6-mercaptopurine was also studied. As it is shown in Fig. 6, by increasing scan rate until 40 mV s^{-1} leads to increasing the oxidation peak current. Therefore, for taking high sensitivity, scan rate of 40 mV s^{-1} was selected for further studies.

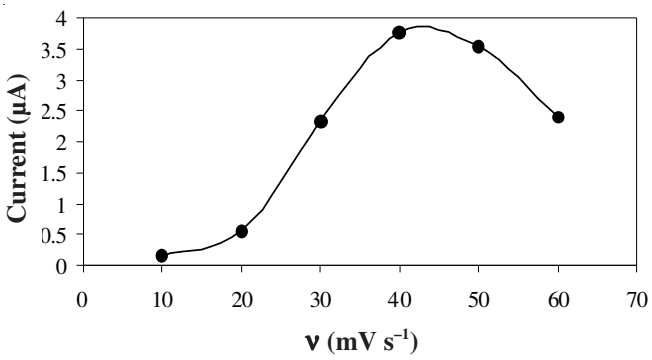


Fig. 6. Influence of scan rate on the oxidation peak current of 20.0 mol L^{-1} 6-mercaptopurine. All of the conditions are as Fig. 4

Figures of merit: Under the optimum conditions, a calibration graph for determination of 6-mercaptopurine was prepared according to the above recommended procedure using cyclic voltammetry on the modified glassy carbon electrode. The calibration plot was linear over the range of 1.6-40 μmol L^{-1} with regression equation of ($r^2 = 0.9952$) where I and C are current (μA) and concentration of 6-mercaptopurine (μmol L^{-1}), respectively.

The limit of detection ($3S_B/m$; S_B standard deviation of blank; m slope of the calibration curve)[20], was 0.13 μmol L^{-1}. The relative standard deviations (n = 3) for 6.0 and 10 μmol L^{-1} 6-mercaptopurine were calculated as 8.5 and 10.1 %.

Interferences: An attractive feature of an analytical procedure is its relative freedom from interferences. The selectivity of the proposed procedure for the assay of 6-mercaptopurine was identified by studying the effect of some compounds that may accompany with 6-mercaptopurine in pharmaceutical formulations and biological fluids. Samples containing 10 μmol L^{-1} of 6-mercaptopurine in the absence and presence of interferent species were analyzed by means of the proposed procedure. Tolerance limit was defined as the concentrations which give an error of ≤ 10 % in the determination of drug. The effects of all examined compounds at several molar ratios over 6-mercaptopurine on the measured analytical concentration are given in Table-1. The results show no significant interference from studied compounds. This is due to the fact that in alkali media (pH 11), many cationic ions precipitate and separate from the aqueous media.

TABLE-1
MAXIMUM TOLERABLE RATION CONCENTRATIONS OF INTERFERENCE SPECIES FOR DETERMINATION OF 10 μmol L^{-1} 6-MERCAPTOPURINE

Species	Tolerance ratio concentration (mol/mol)
NO_3^-, I^-, K^+, Mg^{2+}, Ca^{2+}, Na^+, CH_3COO^-, Cu^{2+}, Co^{2+}, Ag^+, Br^-, Cd^{2+}, Fe^{2+}, Zn^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, PO_4^{3-}, SO_4^{2-}, Mn^{2+}, CH_3OH, C_2H_5OH, glucose, sucrose	1000
Vitamin C, folic acid	100

Determination of 6-mercaptopurine in some real samples: The utility of the developed method was tested by determining 6-mercaptopurine in blood and pharmaceutical tablet (Table-2). The good agreement between these results

TABLE-2
DETERMINATION OF 6-MERCAPTOPURINE IN SOME REAL SAMPLES

Sample	C_{added} (μmol L^{-1})	C_{found} (μmol L^{-1})	Recovery (%)
Whole blood	–	< DL	–
	10.0	9.5	95.0
Tablet*	–	53.5 mg	107.0

*Real amount of 6-mercaptopurine was 50 mg/each tablet manufactured in Italy.

and known values indicates the successful applicability of proposed method for determination of 6-mercaptopurine in real samples. Standard addition method was used for omitting matrix effect in real samples.

Conclusion

The determination of 6-mercaptopurine in drugs and blood was possible due to the modification in the glassy carbon electrode with Cr(III) hexacyanoferrate(II). The concentration range was linear between 1.6×10^{-6} and 4.0×10^{-5} mol L^{-1} of 6-mercaptopurine, with $r^2 = 0.9952$. The limit of detection obtained was 1.6×10^{-7} mol L^{-1} mercaptopurine. In conclusion, the above system, offers a practical potential for selective determination of 6-mercaptopurine, high sensitivity and simplicity, good repeatability, suitable speed (less than 2 min for each test after preparation of modified electrode), good accuracy and lower detection limit that have not been presented together in the previously reported literatures.

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