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ORIGINAL PAPER

Electrochemical determination of thiamazole at a multi-wall carbon nanotube modified glassy carbon electrode

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Abstract A simple and convenient method is described for voltammetric determination of thiamazole, a commonly used anti-hyperthyroid drug, based on its electrochemical oxidation at a multi-wall carbon nanotube modified glassy carbon electrode. Under optimized conditions, the proposed method exhibited acceptable analytical performances in terms of linearity (over the concentration range from 1.0×10^{-7} to 5.0×10^{-4} mol L^{-1}, $r = 0.9983$), detection limit (3.0×10^{-8} mol L^{-1}) and reproducibility (RSD = 2.64%, $n = 10$, for 5.0×10^{-5} mol L^{-1} thiamazole). To further validate its possible application, the method was used for the quantification of thiamazole in pharmaceutical formulations and biological fluids.

Keywords Thiamazole · Voltammetric determination · Multi-wall carbon nanotube · Modified electrode

1 Introduction

Thiamazole (1-methyl-2-mercaptoimidazole, methimazole, Fig. 1) is an orally active drug widely used in the therapy of hyperthyroidism or in biomedical studies as a model substance for endocrine disruption [1]. The action of thiamazole is to slow down the iodide integration into thyrosine and thus to inhibit the production of thyroid hormones. It has been reported that thiamazole may cause side effects such as irritation of skin, decrease of white blood cells in the blood, impaired taste, olfaction, allergies and pharyngitis with fever, and in rare occasions, nephritis and liver cirrhosis [2]. Therefore, the determination of thiamazole is of great importance in clinical chemistry and pharmaceutical formulation analysis. Numerous analytical techniques have been described for the determination of thiamazole, which include high-performance liquid chromatography–mass spectrometry (HPLC–MS) [3, 4], gas chromatography–mass spectrometry (GC–MS) [5], HPLC with ultraviolet detection [6, 7], flow-injection with chemiluminescence detection [8], resonance light scattering spectroscopy [9], fluorescence probe method [10], potentiometric and voltammetric [11], titrimetric [12], etc. Electrochemical strategies for determining thiamazole have also been tried either using a silver-silver sulphide solid state electrode [13] or a carbon paste electrode [14], showing limited success.

Carbon nanotubes (CNTs) represent a very important class of nanomaterials, which are divided into multi-wall carbon nanotubes (MWCNTs) and single-wall carbon nanotubes (SWCNTs) based on the number of carbon atom layers of the wall of the nanotubes. Since the discovery of CNTs [15], they have attracted tremendous research interest due to their unique structural, mechanical, electronic and chemical properties [16]. Because they impart strong electrocatalytic activity and minimization of surface fouling onto electrochemical devices [17], CNTs have been exploited as the electrode material for promoting the electron transfer reaction of many electroactive or non-electroactive analytes in complex sample matrices [18]. In this study, the electrochemical behavior of thiamazole at the MWCNT modified glassy carbon electrode (GCE) was

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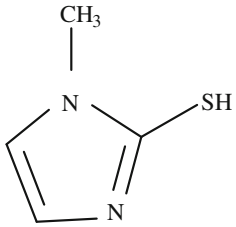
Fig. 1 Chemical structure of thiamazole



investigated. The results showed that MWCNT modified GCE enhanced the oxidation peak current of thiamazole as compared to bare GCE. Based on this, we developed a simple and sensitive voltammetric method for determining thiamazole in pharmaceutical formulations and biological fluids.

2 Experimental

2.1 Reagents and apparatus

MWCNTs were kindly provided by the Laboratory of Life Analytical Chemistry, Nanjing University, China. They were synthesized with a catalytic pyrolysis method and then purified with concentrated HNO_3 [19]. The chemical treatment causes segmentation and carboxylation of MWCNTs at their terminus. Dihexadecyl hydrogen phosphate (DHP), a hydrophobic surfactant, was purchased from Fluka (Buchs, Switzerland) and used as a solubilizing agent to prepare MWCNT–DHP suspension.

Thiamazole (standard), purchased from Material Medical and Biologic Product Verification Institution of China, was dissolved in water to prepare a stock solution of 1.0×10^{-3} mol L^{-1} and stored at 4 °C for use. The working standard solutions were freshly prepared by serial dilution of the stock solution with 0.1 mol L^{-1} phosphate buffer solution (PBS, pH 6.86). The commercially available drug tablets of thiamazole (Beijing Taiyang Pharmaceutical Industry Co., Beijing, China) were purchased from a local pharmacy. All other reagents were of analytical grade and used as received without further purification. Double-distilled water was used throughout. A 0.1 mol L^{-1} PBS (pH 6.86) served as the supporting electrolyte unless otherwise stated.

All the electrochemical measurements were performed with a CHI 660A Workstation (CH Instruments, Texas, USA). A conventional three-electrode system, consisting of a MWCNT modified GCE (3-mm-diameter) as the working electrode, a Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode, was employed. All the potentials were recorded versus Ag/AgCl.

Surface characterization was accomplished by using a JEM-1230 transmission electron microscope (JEOL Instrument, Inc., Japan) with a CCD high-resolution camera at the acceleration voltage of 100 kV, and a JEM-T300 scanning electron microscope (JEOL Instrument, Inc., Japan) at the acceleration voltages of 3–20 kV, respectively.

2.2 Fabrication of MWCNT modified GCE

Five milligrams each of MWCNT and DHP were dispersed into 5 mL of water by ultrasonication for about 20 min to give a stable and homogeneous MWCNT–DHP suspension of 1 mg mL^{-1}. Prior to modification, a GCE was mechanically polished with alumina slurry of different grades to a mirror finish, and rinsed in water under sonication for 3 min. A MWCNT modified GCE was prepared by first dropping 15 μL of 1 mg mL^{-1} MWCNT–DHP suspension onto the GCE surface and then allowing water to quickly evaporate under an infrared lamp in the air. The electrochemical active surface areas of bare GCE and MWCNT modified GCE were measured to be 0.041 and 0.068 cm^2, respectively, according to the procedures described in the previous report [20].

2.3 Analytical procedure

A desired volume of thiamazole standard or sample solution was pipetted to a 10 mL electrolytic cell containing 0.1 mol L^{-1} PBS (pH 6.86), followed by deaeration with pumping oxygen-free nitrogen for 10 min. An accumulation step was then conducted with stirring of the solution for 3 min at potential −0.40 V. After a quiescent interval of 30 s, linear sweep voltammograms from 0.20 to 0.60 V were recorded. Prior to each measurement, the MWCNT modified GCE was activated by successive cyclic voltammetric sweeps from 0.00 to 0.60 V at 100 m Vs^{-1} in the electrolyte solution until the voltammograms kept unchangeable, indicating that a fresh electrode surface had been reproduced.

3 Results and discussion

3.1 Surface characterization of the MWCNT modified GCE

The representative transmission electron microscopy (TEM) image of MWCNT–DHP suspension (Fig. 2a) demonstrates that MWCNTs are dispersed homogeneously into water with the aid of DHP and no essential agglomeration is in evidence. It also can be seen from the image that the MWCNTs are cylindrical and, mainly, folded in shape, with outer diameters around 20 nm and lengths ranging from 0.1 μm to several micrometers.

The typical scanning electron microscopy (SEM) image (Fig. 2b) shows that the GCE surface is coated with a

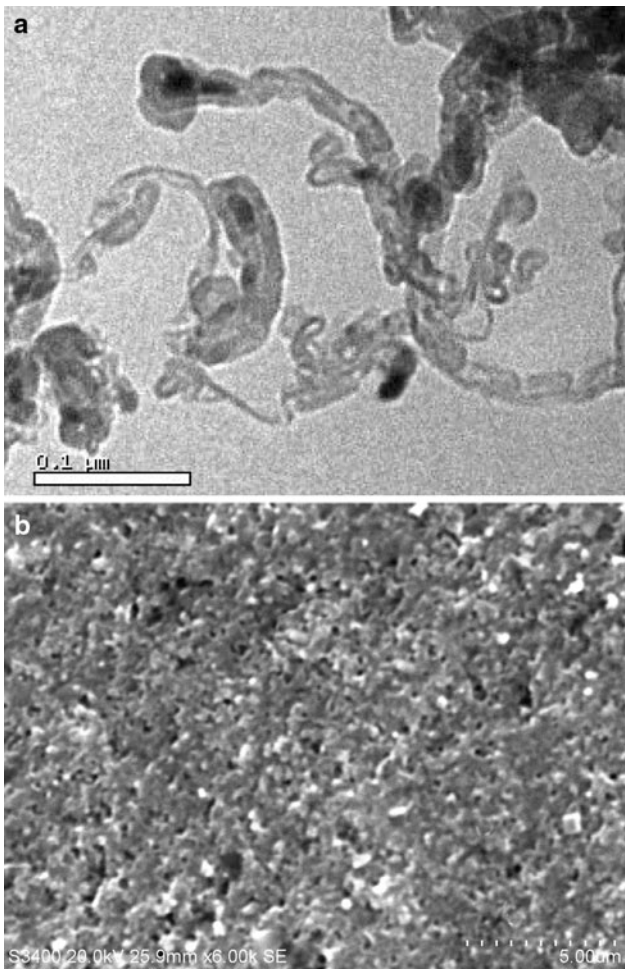


Fig. 2 TEM image of the MWCNTs (**a**) and SEM image of the MWCNT modified GCE (**b**)

uniform MWCNT thin film, which had a rough, mesoporous, matted structure featured by an increased size of the effective surface area.

3.2 Electrochemical behavior of thiamazole at the MWCNT modified GCE

The electrochemical behavior of thiamazole at the MWCNT modified GCE was examined by using cyclic voltammetry within a certain potential window. Figure 3 compares cyclic voltammograms of the MWCNT modified GCE in 0.1 mol L^{-1} PBS (pH 6.86) in the absence (a) or presence (b) of 5.0×10^{-5} mol L^{-1} thiamazole. No observable redox peaks appeared in the blank PBS. Upon addition of 5.0×10^{-5} mol L^{-1} thiamazole, a sensitive, well-defined oxidation peak was observed at the potential of 0.43 V during the first anodic sweep from 0.00 to 0.60 V, but no corresponding reduction peak was observed on the reverse scan, implying that the electrode reaction of thiamazole was totally irreversible. The oxidation peak

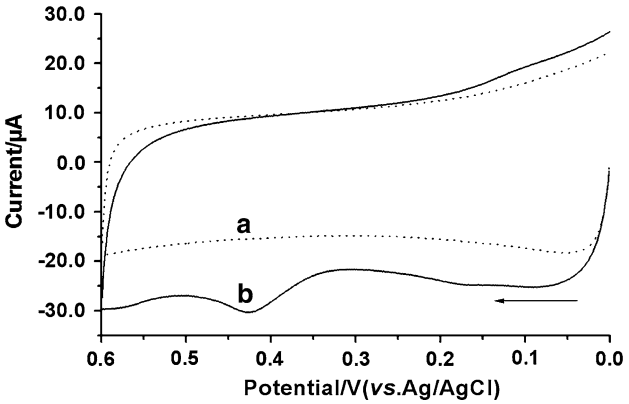


Fig. 3 Cyclic voltammograms of the MWCNT modified GCE in 0.1 mol L^{-1} PBS (pH 6.86) without (*a*) and with (*b*) 5.0×10^{-5} mol L^{-1} thiamazole. Scan rate: 100 mV s^{-1}

current of thiamazole dramatically decreased during the successive cyclic voltammetric sweeps. After four potential sweeps with a scan rate of 100 mV s^{-1}, the peak current remained nearly unchanged. This phenomenon might be ascribed to the adsorption of thiamazole itself and its oxidation product onto the surface of the MWCNT modified GCE and the resultant inactivation of the electrode surface.

To have more insight into the significance of MWCNT for the analysis of thiamazole, the oxidation behaviors of thiamazole at bare GCE and MWCNT modified GCE were compared by differential pulse voltammetry (DPV). At the bare GCE, 5.0×10^{-5} mol L^{-1} thiamazole exhibited a very ill-defined oxidation peak with very low current at about 0.45 V (Fig. 4b). However, under the same

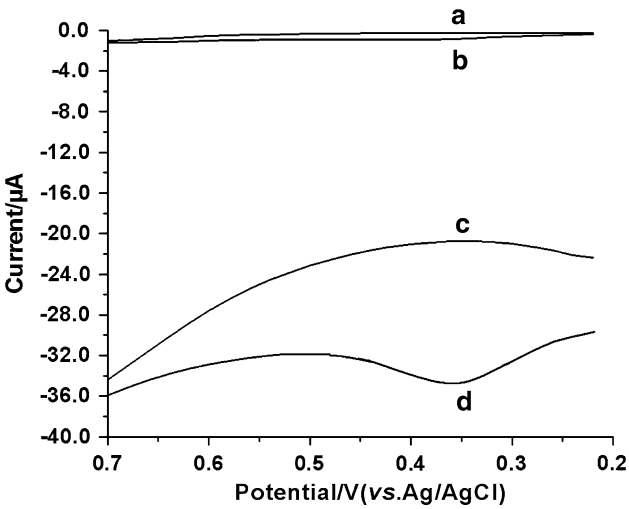


Fig. 4 Differential pulse voltammograms of the bare GCE (*a*, *b*) or the MWCNT modified GCE (*c*, *d*) when placed in 0.1 mol L^{-1} PBS (pH 6.86) in the presence (*b*, *d*) and absence (*a*, *c*) of 5.0×10^{-5} mol L^{-1} thiamazole. Accumulation time: 3 min, pulse amplitude: 50 mV, scan rate: 20 mV s^{-1}, pulse width: 50 ms

conditions, the oxidation peak current of thiamazole increased significantly at the MWCNT modified GCE (Fig. 4d). The current density of oxidation peak at the bare GCE and the MWCNT modified GCE was measured to be 3.2 and 16.1 μA/cm^2, respectively. In other words, compared to the bare GCE, the MWCNT modified GCE induced about fivefold increase of the peak current density as well as a negative shift of the peak potential from 0.45 to 0.36 V. Obviously, the electrocatalytic action of the MWCNT modified GCE stems from the unique properties inherent to MWCNTs [21], including their better wetting performance due to porous structure of bundle CNTs, and their surface electronic structure due to the helicity, low dimensionality and possible topological defects.

3.3 Optimization of experimental variables

3.3.1 Effect of supporting electrolytes

The electrochemical oxidation of 5.0×10^{-5} mol L^{-1} thiamazole in different supporting electrolyte solutions, including pH 5.00–8.00 PBS, pH 2.00–10.00 Britton-Robinson buffer, pH 1.00–5.00 sodium citrate–HCl buffer, pH 4.00–6.00 HAc–NaAc buffer (0.1 mol L^{-1} of each buffer), were examined by cyclic voltammetry. The best oxidation response was obtained in pH 6.86 PBS in that the electrochemical response was well defined with the highest peak current as compared to that in other buffer systems.

It was also demonstrated that in 0.1 mol L^{-1} PBS the oxidation peak current of thiamazole gradually increased as pH values increased from 5.00 to 6.86 and then leveled off within the pH range from 6.86 to 8.00. Hence, 0.1 mol L^{-1} PBS (pH 6.86) was chosen as the supporting electrolyte for the determination of thiamazole. Moreover, the dependence of the oxidation peak potential (E_{pa}) on the pH value was examined. The pH value strongly affected the E_{pa} of thiamazole. It showed that the E_{pa} shifted negatively with increasing pH from 5.00 to 8.00, and a good linear relationship was observed between pH value and E_{pa} with a slope of −55 mV/pH. This slope value indicates that identical numbers of protons and electrons are involved in the electrode process.

3.3.2 Effect of amount of MWCNT–DHP suspension

The thickness of the MWCNT–DHP film on the GCE surface is determined by the amount of MWCNT–DHP suspension dropped on the GCE surface. The peak current significantly increased with increasing the amount from 0 to 10 μL. As the amount of suspension further increased, the peak current changed very slightly, and when the amount of suspension exceeded 17.5 μL, the peak current conversely decreased. This is probably attributed to the compromising effects of DHP on the electrochemical performance of the composite film due to the hydrophobic and insulating actions of DHP. As a result, an appropriate amount for the fabrication of MWCNT modified GCEs was determined as 15 μL of 1 mg mL^{-1} MWCNT–DHP suspension.

3.3.3 Effect of accumulation potential or accumulation time

Accumulation prior to voltammetric measurements could influence the electrooxidation of thiamazole at the MWCNT modified GCE. When accumulation potential varied from 0.60 V to −0.60 V, the peak current increased and finally reached a maximum value at about potential −0.40 V, and then decreased when the accumulation potential became more negative. Thus, the optimum accumulation potential of −0.40 V was used for the determination of thiamazole.

As to the effect of the accumulation time on the oxidation peak current, the current increased with the accumulation time in the range of 3 min; when the accumulation time exceeded 3 min the current reached a plateau, suggesting that the accumulation process of thiamazole had achieved its saturation adsorption on MWCNT–DHP film. Therefore, 3-min accumulation was used to improve sensitivity of the method and shorten the time consumption.

3.4 Electrode reaction mechanisms of thiamazole

In order to understand the mechanisms responsible for the electrochemical reactions of thiamazole at the MWCNT modified GCE, cyclic voltammograms of 5.0×10^{-5} mol L^{-1} thiamazole at different scan rates (10, 25, 50, 100, 125, or 150 mV s^{-1}) were recorded. The oxidation peak current (i_p) was found to be proportional to the scan rate (v) with a regression equation of i_p (μA) = 1.48 + 0.012v (mV s^{-1}) (r = 0.9968), providing further evidence that the electrode reaction of thiamazole at the MWCNT modified GCE is adsorption-controlled.

The values of αn (α is the electron transfer coefficient and n is the number of electrons involved in the rate-determining step) were calculated for the irreversible oxidation of thiamazole according to the equation $\alpha n = 0.0477/(E_{pa} - E_{pa/2})$ [22], where E_{pa} is the oxidation peak potential, and $E_{pa/2}$ is the half peak potential. In the range of potential sweep rate from 10 to 200 mV s^{-1}, the values of αn at the modified electrode were found to be from 0.48 to 0.56. Generally, α is assumed as 0.5 for the totally irreversible electrode process. Consequently, our data indicated that the rate-determining step in the electrocatalytic oxidation of thiamazole might be a one-electron transfer process. Combining the fact that identical numbers

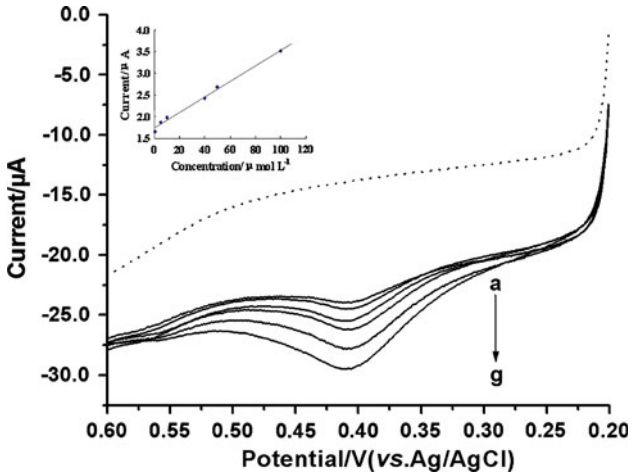


Fig. 5 Linear sweep voltammograms of thiamazole at the MWCNT modified GCE in 0.1 mol L^{-1} PBS (pH 6.86) containing 0 (*a*), 5.0×10^{-7} (*b*), 5.0×10^{-6} (*c*), 1.0×10^{-5} (*d*), 4.0×10^{-5} (*e*), 5.0×10^{-5} (*f*), 1.0×10^{-4} (*g*) mol L^{-1} thiamazole, respectively. Accumulation time: 3 min, scan rate: 100 mV s^{-1}. Also shown is the resulting calibration plot (*inset*)

of protons and electrons were involved in the electrode process, we conclude that the electrochemical reaction of thiamazole at the MWCNT modified GCE might be realized through the oxidation of sulfhydryl groups in the molecule [11, 13, 14, 23]:

$$RSH \rightarrow RS^{\bullet} + H^{+} + e^{-} \quad \text{(RSH is thiamazole)} \qquad (1)$$

$$2RS^{\bullet} \rightarrow RSSR \qquad (2)$$

3.5 Analytical performance

3.5.1 Calibration plot and stability

Under the optimized experimental conditions, the linear sweep voltammograms of thiamazole with different concentrations at the MWCNT modified GCE were recorded (Fig. 5). The peak current increased linearly with incremental concentration of thiamazole in the range from 1.0×10^{-7} to 5.0×10^{-4} mol L^{-1}, giving a regression equation of i_p (μA) = $1.74 + 1.79 \times 10^{4}C$ (mol L^{-1}) ($r = 0.9983$). The detection limit was found to be 3.0×10^{-8} mol L^{-1} (according to $S/N = 3$). The relative standard deviation (RSD) of 2.64% for 5.0×10^{-5} mol L^{-1} thiamazole ($n = 10$) indicated a good reproducibility.

For evaluating the long-term stability of the MWCNT modified GCE, it was stored in the air and used for monitoring 5.0×10^{-5} mol L^{-1} thiamazole daily over a period of 4 weeks. The deviation of current responses was only 5.82%.

3.5.2 Interference

A systematic study was carried out to evaluate the interferences of foreign species on the determination of thiamazole at the level of 1.0×10^{-6} mol L^{-1}. We found that 200-fold concentration of Na^{+}, K^{+}, Mg^{2+}, Pb^{2+}, Ca^{2+}, Al^{3+}, Fe^{3+}, Cl^{-}, Br^{-}, I^{-}, NO_3^{-}, or SO_4^{2-}, 50-fold concentration of uric acid, oxalic acid, citric acid, lactic acid, tartaric acid, glucose, lactose, saccharose, starch, or carboxymethylcellulose, and 20-fold concentration of vitamin B_1, vitamin B_2, vitamin B_{12}, or vitamin C in the solution had almost no influences on the determination of thiamazole (signal change below 5%). However, some thiocompounds such as cysteine, glutathione, 6-thiopurine could interfere with the determination of thiamazole.

3.5.3 Applications

Ten thiamazole tablets were weighed and powdered in an agate mortar. A certain amount of powder sample was transferred into a 100 mL flask to be dissolved in water, and the resulting solution was properly diluted. After being sonicated, the sample solutions were filtered, and subjected to detection at the MWCNT modified GCE by linear sweep voltammetry. The amount of thiamazole was calculated from the calibration equation (Table 1). Furthermore, the results obtained by our proposed method were compared with those determined by the pharmacopeia method [12]. The two method results were in agreement with each other, suggesting that the MWCNT modified GCE had great promise for practical application in pharmaceutical formulation analysis. In order to establish the suitability of the proposed method, the known amounts of the standard thiamazole were added into the analyte solution made by pharmaceutical products for determination of thiamazole at

Table 1 The data of determining thiamazole in pharmaceutical formulations (percentage of the declared content, %) and the recovery validated by standard addition

Sample	Detected content by this method (%)	Detected content by the pharmacopeia method [12] (%)	Recovery of this method (%)
1	98.2	99.6	98.6
2	97.9	98.5	100.4
3	99.5	99.8	99.8
4	102.0	101.4	103.1
5	103.6	100.6	101.3

Table 2 The data of determining thiamazole in human serum or urine samples

Sample	Added (μmol L^{-1})	Found (μmol L^{-1})[a]	Recovery (%)	RSD (%)
Serum				
1	0.3	0.293	97.7	2.64
2	1.0	1.02	102.0	1.32
3	5.0	5.04	100.8	1.67
Urine				
1	0.3	0.312	104.0	3.12
2	1.0	0.985	98.5	2.88
3	5.0	4.97	99.4	1.85

[a] Average of five assays

the MWCNT modified GCE. Recoveries were found to be in the range of 98.6 and 103.1% (Table 1), indicating that the proposed method had good accuracy and satisfactory repeatability.

The proposed method was also applied to the determination of thiamazole in human serum and urine samples which were obtained from volunteers. For sample pretreatment, the serum and urine samples were diluted 50 and 20 times, respectively, with 0.1 mol L^{-1} PBS (pH 6.86), and the urine samples were further centrifuged for 5 min at 4,000 rpm to remove the suspended particles. The determination of thiamazole in biological fluid samples was performed by a standard addition method. The determination recoveries were found to be in the range from 97.7 to 104.0% (Table 2).

4 Conclusion

In this study, a MWCNT modified GCE was fabricated for the voltammetric determination of thiamazole. The enhancement in the oxidation current of thiamazole and the negative shift of peak potential of thiamazole at the MWCNT modified GCE might be attributed to the unique physicochemical properties of MWCNTs. This newly developed method is sensitive, convenient, rapid and suitable for determining thiamazole in pharmaceutical formulations and biological fluids.

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