Fe₃O₄ nanoparticles decorated reduced graphene oxide & carbon nanotubes-based composite for sensitive detection of imatinib in plasma and urine

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Abstract
In this study, a new reduced graphene oxide (rGO) has been synthesized via a facile and environmentally friendly process using Callicarpa maingayi leaf extract. A novel magnetic catalyst based on Fe₃O₄ nanoparticles-reduced graphene oxide & carbon nanotubes ((Fe₃O₄-(rGO&CNT)) was prepared and characterized by hydrothermal method. The Fe₃O₄ nanoparticles with an average size of 25 to 40 nm were placed on carbon nanotubes and reduced graphene oxide sheets, while carbon nanotubes inserted between the reduced graphene oxide sheets effectively prevented their aggregation. The (Fe₃O₄-(rGO&CNT) composite has a large surface area and good electrocatalytic properties, suiting for the detection and determination of imatinib (IM) anticancer drug by voltammetry method. Under optimized conditions, good linearity was achieved in the concentration range of 0.1 to 40 μmol L⁻¹ and the limit of detection and sensitivity were 57 nmol L⁻¹ and 3.365 μA L μmol⁻¹, respectively. Furthermore, the fabricated sensor demonstrated acceptable reproducible behaviour and accuracy and a high level of stability during all electrochemical tests. In addition, the proposed method was applied for the detection of IM in biological samples and the recoveries were 94.0 to 98.5 %, with relative standard deviations of 2.1 to 4.4 %.

Keywords
Magnetite/carbon nanocomposite, electrochemical sensor; imatinib drug; real samples

Introduction
Cancer is one of the most important diseases facing humanity today. Cancer is lethal because its cells are uncontrolled and proliferate indefinitely and spread throughout the body [1,2]. Cytostatic agents are the most commonly used class of anticancer drugs. Their purpose is to inhibit the growth of cancer cells. However, the widespread use of cytostatic agents has caused some other effects, such as on environmental toxicology [3]. Among them, imatinib (IM), with the chemical structure shown in Figure 1, is a specific inhibitor. Imatinib (4-[(4-methylpiperazin-1-yl) methyl]-N-[4-methyl-
3-[(4-pyridin-3-yl)pyrimidin-2-yl]amino]phenyl]benzamide) is used to treat chronic myeloid leukemia (CML) and diseases such as gastrointestinal stromal tumours [4]. Imatinib, known as Gleevec, is a chemotherapy drug used to treat some cancers. This drug was approved by the Food and Drug Administration (FDA) to treat different cancers [5]. By inhibiting the activity of synthetic tyrosine kinase, specifically designed to inhibit Bcr-Abl fusion protein, it reduces cell growth or apoptosis in some cancer cells [6]. IM is a drug for anticancer treatment, used selectively to annihilate cancer cells instead of damaging them by rapidly dividing cells [7].

![Figure 1. Chemical structure of imatinib](image)

A number of analytical techniques have been applied to IM analysis, such as fluorescence, liquid chromatography, capillary electrophoresis and UV-Vis spectroscopy methods [8-12]. Owing to the sites with an electroactive nature on the IM surface, the electrochemical procedure has been offered for this drug detection. Therefore, selecting an appropriate method with sensitivity and efficiency in detection and reducing operating costs to evaluate lower IM values is a challenging endeavour. Several electrochemical investigations based on electrochemical sensors have been performed to date [13-16].

Carbon electrodes have a stable electrochemical window and do not react easily with other substances [17,18]. However, the electrochemical signal of ordinary carbon electrodes is weak, so it is difficult to meet the demand for highly sensitive detection [19]. Modification of the surface of ordinary carbon electrodes is a common method to improve the electrochemical activity of sensors. Recent studies have shown that modification of carbon nanomaterials on the surface of electrodes can increase electrical conductivity. Among carbon nanomaterials, carbon nanotubes (CNTs) are wholly employed as conductors due to their attractive and useful characteristics. The high chemical stability, excellent mechanical properties, high surface area, and prominent electrical conductivity make them appropriate for employment in electrochemical procedures [20]. It is known that CNTs promote electron transfer reactions when used as electrode-modifying material [21]. They display a highly hydrophobic surface by π-conjugative structure. This distinctive feature of CNTs could increase their ability to interact with some compounds via hydrophobic and/or π-π bonding.

Reduced graphene oxide (rGO) is a nanostructured material with high surface area, excellent thermal and mechanical stability, and remarkable electrical conductivity [22]. The rGO- has a more specific surface area and can easily be hybridized with Fe₃O₄ nanoparticles to form heterostructures [23]. The Fe₃O₄&rGO nanocomposites, with the advantages of magnetism and conductivity, could be easily adhered to the electrode surface to achieve the direct redox reactions and electrocatalytic behavior of analytes adsorbed on the modified surface [24]. Moreover, the functional groups (COOH and OH) of rGO allow it to conjugate with various molecules and act as an excellent performance carrier, while nanoparticles can be highly dispersed on its surface. The charge transfer at the interface of these hybrid materials can provide a synergistic effect to bring properties different from those of each component [25].
To the best of our knowledge, there are no prior reports in the literature on the electrocatalytic behavior of Fe$_3$O$_4$-(rGO&CNT) nanocomposites. In this work, the _Callicarpa maingayi_ leaf extract is chosen as a reducing agent to reduce graphene oxide to the reduced graphene oxide (rGO) by a one-step method. By combining CNT tubes, rGO sheets and Fe$_3$O$_4$ nanoparticles, an excellent nanocomposite was created with high surface area and good conductivity to improve the surface of electrodes. The prepared modified electrode demonstrated remarkable electrocatalytic activity for the detection of IM in biological samples with reasonable analytical results.

**Experimental**

**Materials**

Multi-walled carbon nanotubes (MWCNTs, outer diameter: 20-40 nm, length: 5-15 μm, >97 %) were purchased from Shenzhen Nano-Technologies Port Co., Ltd. (China). Graphene oxide was purchased from Nano Materials Pioneers (Iran). The _Callicarpa maingayi_ leaves were obtained from Kuala Lumpur, Malaysia. Imatinib (C$_{23}$H$_{21}$N$_{7}$O, 98 %), potassium hexacyanoferrate (II) ([K$_4$Fe(CN)$_6$]$_n$ 98.5 %), phosphoric acid (H$_3$PO$_4$, >99 %), sodium dihydrogen phosphate (Na$_2$HPO$_4$, >98 %), disodium hydrogen phosphate (Na$_2$HPO$_4$, >99 %), sodium phosphate (Na$_3$PO$_4$, >99 %) and graphite powder (99.99 %) were purchased from Sigma-Aldrich (Germany) and used as received. Human plasma samples were kindly provided by the clinical laboratory tests (Amol, Iran). The samples were stored at 4 °C.

**Apparatus**

Surface morphology and chemical composition of the nanocomposites were examined by a scanning electron microscope of MIRATESCAN-XMU (Czech Republic) combined with EDX (energy-dispersive X-ray spectroscopy) machine. Electrochemical measurements were performed with a potentiostat/galvanostat (Sama 500-c Electrochemical Analysis system, Sama, Iran). A conventional three-electrode configuration consisting of Ag|AgCl|KCl3M as the reference electrode, a platinum wire as the auxiliary electrode and Fe$_3$O$_4$-(rGO&CNT) modified CPE as the working electrode was employed. UV-Vis spectra of samples were recorded by UV-Vis spectrophotometer (UV-1900, Shimadzu Co., Japan). X-ray diffraction measurement was recorded on a Bruker D8-Advance X-ray diffractometer (Germany).

**Synthesis of Fe$_3$O$_4$-(rGO&CNT) composite**

The reduced graphene oxide was prepared according to previous works [26,27]. The amount of 10 g of fresh _Callicarpa maingayi_ leaves were crushed, transferred into a round bottom flask containing 100 mL ionized water, and then placed in an ultrasound bath (60 kHz frequency) at 50 °C for 60 min. After completion of sonication, the mixture was allowed to cool down to room temperature and filtered to obtain a yellowish-green colour extract. 10 mg GO was added to the 50 mL extract and sonicated for 30 min at 25 °C in order to disperse the GO uniformly throughout the extract. The mixture was refuxed for 6 h at 50 °C until the suspension solution colour changed to brown-black. The product (rGO) was filtrated, washed with water and dried in the oven at 100 °C overnight. To 10 mL of rGO (1 mg mL$^{-1}$), 10 mg ofCNT was added and the mixture was sonicated for 45 min at 25 °C. The product (rGO&CNT) was filtered, washed with water and dried in the oven at 100 °C overnight. For functionalization ofrGO&CNT composite by Fe$_3$O$_4$ nanoparticles, 5 mg of rGO&CNT was sonicated in 20 mL water at 25 °C for 1 h. Then, 10 mL of iron(II) sulphate heptahydrate (FeSO$_4$·7H$_2$O) solution (0.1 mol L$^{-1}$) was added to rGO&CNT suspension solution under
vigorously stirring. The pH of the solution was adjusted to 10 with NaOH, transferred to Teflon-lined stainless steel autoclave, and heated at 180 °C for 8 h. Water was used to wash the sample and then the product (Fe₃O₄-(rGO&CNT)) was dried at 60 °C overnight.

**Total phenolic content (TPC)**

The total phenolic content absorbed on rGO, rGO&CNT and Fe₃O₄-(rGO&CNT) samples was determined according to the Folin-Ciocalteu spectrophotometric method [28]. The 1 mL of samples (1 mg mL⁻¹) solution was mixed with 2.5 mL of Folin-Ciocalteu reagent 10 vol.% and neutralized with 2 mL of sodium carbonate 7.5 % (W/V). The reaction mixture was incubated at 45 °C for 40 min and the absorbance of solution was measured at 765 nm using a UV-Vis spectrophotometer. The total phenolic content is calculated based on gallic acid. To prepare a calibration curve from gallic acid, the results were expressed in terms of ppm of solutions. The test was done in triplicate.

**Fabrication of Fe₃O₄-(rGO&CNT) nanocomposite modified electrode**

The carbon paste electrode (CPE) was prepared as described in our previous reports [29,30]. The graphite powder plus paraffin was hand-mixed until a uniformly wetted paste was obtained. Then, the carbon paste was packed into a glass tube (with an internal radius 3 mm). Electrical contact was made by a copper wire. The new electrode surface was obtained by polishing it on a weighing paper. 1 mg of Fe₃O₄-(rGO&CNT) nanocomposite was added to 1 mL of water and sonicated for 30 min. 5 µL of this solution was drop-casted onto the CPE and allowed to dry in an oven at 50 °C for 30 min.

**Analysis of plasma and urine**

500 µL of human serum plasma or urine was transferred to the electrochemical cell containing 10 mL phosphate buffer solution (PB, 0.1 mol L⁻¹; pH 7.0) and the oxidation current was determined by cyclic voltammetry. Also, the samples were determined by UV-Vis spectroscopy at the wavelength of 243 nm with diluting 500 µL of plasma or urine in 5 mL PB (0.1 mol L⁻¹; pH 7.0).

**Results and discussion**

**Characterization**

Figure 2 (A) shows the electrochemical behaviour of *Callicarpa maingayi* leaf extract (100 mg L⁻¹) in 0.1 mol L⁻¹ PB, pH 7.0 at CPE. A pair of well-defined redox peaks was obtained in the cyclic voltamgram, where anodic peak potential at ~260 mV and cathodic peak potential at ~220 mV could be ascribed to many phytocompounds redox couples in 0.1 mol L⁻¹ PB that oxidize in the potential field. In order to realize the surface functional group information on samples, TPC on rGO, rGO&CNT and Fe₃O₄-(rGO&CNT) was calculated based on gallic acid (GA) with calibration linear function \( A = 0.022 \times C_{GA} - 0.008 \) and \( R^2 = 0.9915 \). TPC of rGO, rGO&CNT and Fe₃O₄-(rGO&CNT) was calculated as 0.93, 0.82 and 0.73 ppm, respectively compared with GO (0 ppm). *Callicarpa maingayi* leaf extract is a rich source of phytochemicals such as polyphenols, flavonoids and catechins, and shows remarkable performance as a reducing agent. The phytochemicals present in the *Callicarpa maingayi* leaf extract are of great medicinal value and also environmentally benign.

The formed rGO, rGO&CNT and Fe₃O₄-(rGO&CNT) are characterized by UV-Vis spectroscopy in the range of 200 to 600 nm (Figure 2B). The CNT shows an absorption broad peak at ~270 nm. The absorption peak of the rGO suspension is around 279 nm, which, on interaction with CNT, shifted towards 311 nm for rGO&CNT. The absorption peaks of Fe₃O₄-(rGO&CNT) at 271 nm and 320 nm belong to CNT and rGO, respectively, while the peaks of 361 and 376 nm are in good agreement with
Fe$_3$O$_4$ nanoparticles. Thus, the UV-Vis spectrum confirms the presence of the Fe$_3$O$_4$-(rGO&CNT) hybrid.

The electrochemical behaviour of CPE electrodes modified with rGO, CNT, rGO&CNT and Fe$_3$O$_4$-(rGO&CNT) through drop casting were tested using [Fe(CN)$_6$]$^{3-/4-}$ redox couple, and presented in Figure 2C. The redox current increased in the presence of Fe$_3$O$_4$-(rGO&CNT) and is larger than those recorded for bare CPE and rGO, CNT and rGO&CNT modified CPE. The good electronic properties of rGO and CNT support rapid electron transfer and are most likely responsible for the current enhancement. The real electrochemical active surface area of rGO/CPE, CNT/CPE, rGO&CNT/CPE and Fe$_3$O$_4$-(rGO&CNT)/CPE was determined by plotting the oxidation peak current as a function of the square root of the scan rate for [Fe(CN)$_6$]$^{3-}$ (2 mM). From the slopes of these graphs, the values of active surface area were obtained using equation (1) [27]:

$$A = \text{slope} / (268.6n^{3/2}D^{1/2}c)$$

where $A$ is the electrochemical active surface area (cm$^2$), $n$ is the number of electrons transferred ($n = 1$), $D$ is diffusion coefficient of [Fe(CN)$_6$]$^{3-}$ ($3.09\times10^{-6}$ cm$^2$ s$^{-1}$) and $c$ is concentration of [Fe(CN)$_6$]$^{3-}$. For rGO/CPE, CNT/CPE and rGO&CNT/CPE, active surface area values were 0.19, 0.22 and 0.25 cm$^2$, respectively, while for Fe$_3$O$_4$-(rGO&CNT)/CPE an increased surface area (0.27 cm$^2$) was obtained. The significantly high electroactive area of Fe$_3$O$_4$-(rGO&CNT)/CPE recommends Fe$_3$O$_4$-(rGO&CNT) as an efficient substrate to fabricate sensors and biosensors.
The powder X-ray diffraction (XRD) could provide effective evidence of synthesized Fe$_3$O$_4$-(rGO&cnt) hybrid. Figure 3 shows the XRD patterns of rGO, CNT, rGO&CNT and Fe$_3$O$_4$-(rGO&CNT) samples. As seen, rGO shows a diffraction peak at ~26° corresponding to the (002) reflection. CNT exhibited an intense diffraction pattern at 25.6° and a weak pattern at 44.4° originating from the (002) and (100), respectively, which, on interaction with rGO, shifted towards 2θ values of 26.14 and 43.3° for rGO&CNT. The diffraction peaks at 30.5, 36.1, 43.4, 53.8, 57.5 and 63.1 of Fe$_3$O$_4$-(rGO&CNT) could be indexed as that of face-centered cubic (fcc) spinel phase of Fe$_3$O$_4$ nanoparticles (JCPDS:19-0629) [31].

![Figure 3. XRD patterns of (a) rGO, (b) CNT, (c) rGO&CNT and (d) Fe$_3$O$_4$-(rGO&CNT)](image)

The morphology of rGO, CNT, rGO&CNT and Fe$_3$O$_4$-(rGO&CNT) is characterized with FE-SEM. As seen in Figure 4, the rGO shows the multilayered sheets onto each other, a rougher surface and wave-shaped corrugated structures. The CNTs are tubular and have open macropore structures. In contrast, the surface of rGO&CNT appears coarse, indicating most of the CNTs were relatively well dispersed in the rGO matrix. The image of Fe$_3$O$_4$-(rGO&CNT) shows that the rGO&CNT composite is decorated by sphere shape Fe$_3$O$_4$ nanoparticles with a diameter in the range of 25 to 40 nm.

Figure 5 illustrates the elemental analysis (EDX) of synthesized Fe$_3$O$_4$-(rGO&CNT) nanocomposite. The specific peak of Fe element in Figure 5 (a) indicates the presence of Fe$_3$O$_4$ nanoparticles on the rGO&CNT nanostructure. Also, the homogenous distribution of Fe can be seen in the elemental mapping of Fe$_3$O$_4$-(rGO&CNT) presented in Figure 5 (b).
Figure 4. FE-SEM images of: (a) rGO; (b) CNT; (c) rGO&CNT; (d) Fe$_3$O$_4$-(rGO&CNT); (e) Fe$_3$O$_4$-(rGO&CNT) with larger magnification

Figure 5. (a) EDX spectra of Fe$_3$O$_4$-(rGO&CNT) and (b) elemental mapping of Fe$_3$O$_4$-(rGO&CNT)
Voltammetric behavior of imatinib

The cyclic voltammetry (CV) method was used to investigate the electrochemical behaviour of IM on CPE, rGO/CPE, CNT/CPE, rGO&CNT/CPE and Fe$_3$O$_4$-(rGO&CNT)/CPE in 0.1 mol L$^{-1}$ PB, pH 7.0. The voltammograms obtained for 50 μmol L$^{-1}$ IM at 100 mV/s are shown in Figure 6(A). According to the results, the oxidation peak current of IM at the surface of the unmodified CPE is ~40 μA, while for rGO/CPE, CNT/CPE, rGO&CNT/CPE and Fe$_3$O$_4$-(rGO&CNT)/CPE electrodes, the oxidation peak current values are ~70, ~90, ~110 and ~170 μA, respectively. Moreover, the oxidation potential of IM on the surfaces of CPE and rGO/CPE is ~0.9 V, while the oxidation potential of IM on the surfaces of CNT/CPE, rGO&CNT/CPE and Fe$_3$O$_4$-(rGO&CNT)/CPE is ~0.86. Due to the high surface area of the electrode and the effects of rGO, CNT and Fe$_3$O$_4$ nanoparticle materials, the oxidation current of Fe$_3$O$_4$-(rGO&CNT)/CPE increases. In addition, these points improve the conductivity of Fe$_3$O$_4$-(rGO&CNT) composite, which results in a higher oxidation current on the surface of this electrode than on other electrodes.

The 0.1 mol L$^{-1}$ PB with pH 5.0-9.0 was used to detect IM response on the surface of Fe$_3$O$_4$-(rGO&CNT)/CPE. As shown in Figure 6 (B), the maximum oxidation current of IM at Fe$_3$O$_4$-(rGO&CNT)/CPE was obtained at pH 7.0, then used as the optimal pH for other tests. The dependence of the oxidation peak potential of IM on pH is also shown in Figure 6 (C). The slope of the line obtained for this relation was 54 mV per pH unit, which is close to the slope of the Nernst equation. Accordingly, the number of electrons and protons participating in the oxidation of IM is the same according to Scheme 1.

Figure 6. (A) Cyclic voltammograms of (a) CPE, (b) rGO/CPE, (c) CNT/CPE, (d) rGO&CNT/CPE and (e) Fe$_3$O$_4$-(rGO&CNT)/CPE in presence of 50 μmol L$^{-1}$ IM in 0.1 mol L$^{-1}$ PB, pH 7, at scan rate of 100 mV s$^{-1}$. (A) Plot of peak current for 50 μmol L$^{-1}$ IM in 0.1 mol L$^{-1}$ PB at the surface of Fe$_3$O$_4$-(rGO&CNT)/CPE at various pH: (a) 5.0, (b) 6.0, (c) 7.0, (d) 8.0 and (e) 9.0 at scan rate of 100 mV s$^{-1}$. (B) Plot of oxidation peak potential vs. pH of 50 μmol L$^{-1}$ IM in 0.1 mol L$^{-1}$ PB at the surface of Fe$_3$O$_4$-(rGO&CNT)/CPE.
Figure 7 (A) shows the CV curves of Fe₃O₄-(rGO&CNT)/CPE toward IM at different scan rates from 25 to 300 mV s⁻¹. It can be seen that the electrochemical oxidation current (I) of IM has a linear relationship with the $v^{1/2}$ (inset) while the oxidation potential is shifted positively with the increase of the scan rate, indicating that the electrochemical reaction of the IM obeyed the diffusion-controlled process. Also, the slope obtained from the graph of log I as a function of log $v$ is 0.537 (Figure 7B), which is very near to the generally expected slope value of 0.5, showing a purely diffusion-controlled system.

Figure 7. (A) Cyclic voltammograms of 30 μmol L⁻¹ IM in 0.1 mol L⁻¹ PB (pH 7.0) and 0.1 mol L⁻¹ KCl as supporting electrolyte at the surface of Fe₃O₄-(rGO&CNT)/CPE and various scan rates: (a) 25, (b) 50, (c) 75, (d) 100, (e) 125, (f) 150, (g) 200, (h) 250 and (i) 300 mV s⁻¹; inset: plot of peak currents vs. square root of scan rates. (B) Plot log I as a function of log $v$. (C) Chronoamperograms for Fe₃O₄-(rGO&CNT)/CPE in the presence of (a) 50, (b) 100 and (c) 200 μmol L⁻¹ IM; (inset) Cottrell’s plot for the data from chronoamperograms.
Chromatoamperometry and other electrochemical methods were usually employed to investigate electrode reactions at chemically modified electrodes. Chronoamperometric measurement of IM was done by setting the Fe₃O₄-(rGO&CNT)/CPE working electrode potential at 0.9 V vs. Ag/AgCl/KCl(3M) for various concentrations of IM (Figure 7(C)). For IM with a diffusion coefficient of D, the current for the electrochemical reaction at a mass transport limited rate is described by the Cottrell equation (2) [32]:

\[ I = nFAD^{1/2}c_0\pi^{1/2}t^{-1/2} \]

where \( n, F, A, c_0, D \) and \( t \) are the number of electrons transferred (2), Faraday constant (96485 C), electrode area (0.27 cm²), bulk concentration (50, 100 and 200 µmol L⁻¹), diffusion coefficient (cm² s⁻¹), and time (s), respectively.

Experimental plots of \( I \) vs. \( t^{-1/2} \) were employed, with the best fits for different concentrations of IM (inset). From the resulting slope and Cottrell equation, the mean value of \( D \) for IM was calculated as 5.7×10⁻⁵ cm² s⁻¹.

**Analytical performance of Fe₃O₄-(rGO&CNT)/CPE sensor**

The quantitative analysis of the IM was based on the dependence of the peak current value on the concentration of IM. Cyclic voltammograms depicting the systematic increase in the peak current values with an increase of the concentration in the linear working range 0.1 to 40 µM at Fe₃O₄-(rGO&CNT)/CPE are presented in Figure 8A. The linear calibration plot according to \( I / \mu A = 27.22 + 3.36c_\text{IM} \) with a correlation coefficient of 0.991 and error bars are presented in the inset. The detection limit (LOD) of 57 nmol L⁻¹ was calculated using the formula \( 3\sigma/b \), where \( \sigma \) is the standard deviation of the blank and \( b \) is the slope of the calibration curve. The calculated value for the limit of quantification (LOQ) is 187.5 nM, while the sensitivity was estimated to be 3.365 µA µM⁻¹.

Table 1 compares the analytical performance of Fe₃O₄-(rGO&CNT)/CPE for IM determination with these obtained by voltammetry techniques at some other electrodes [13,16,33-36]. Based on the results of this study, the Fe₃O₄-(rGO&CNT) modified CPE electrode appears to be comparable to other electrodes in terms of achieving low detection limit, wide range of concentrations (LDR) and high sensitivity. The LOD is, however, higher as compared to other sensors presented in Table 1, but the proposed modified electrode was prepared by a simple, easy, cost-effective and one-step procedure of making Fe₃O₄-(rGO&CNT)/CPE electrode that is more readily available than materials used in preparation of other electrodes. Moreover, there is no proof that a single electrode can work for a long period of time with almost the same performance, which gives the Fe₃O₄-(rGO&CNT)/CPE the privilege of being used for routine work.

The reproducibility of \( E_p \) and \( I_p \) values was tested by repeating five experiments with 20 µmol L⁻¹ of IM. The RSDs were calculated to be 3.43 % for \( I_p \) and 1.61 % for \( E_p \), respectively, using CV, which indicates excellent reproducibility. The RSD of the blank was 1.61 %, indicating that the electrode is stable.

In order to examine the selectivity of IM, it must be measured concurrently with compounds present in the body or compounds being administered simultaneously with IM. Many minerals in a body contain cations (Na⁺, Ca²⁺, Mg²⁺, etc.) and anions. For this reason, we investigated possible
interferents for IM detection, such as glucose, cysteine, lysine, Ca\(^{2+}\), K\(^{+}\), Na\(^{+}\), Mg\(^{2+}\), doxorubicin, acetaminophen, and ascorbic acid.

Some results of IM detection at the Fe\(_3\)O\(_4\)-(rGO&CNT)/CPE in 0.1M PB (pH 7.0) containing some interferents at equal concentrations as IM, are presented in Figure 8B. As seen in Figure 8B, glucose, cysteine, lysine, doxorubicin, acetaminophen, and ascorbic acid did not cause any interference.

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Moreover, some results (not shown here) indicated that the 500-fold higher concentrations of glucose and cations (Ca\(^{2+}\), K\(^{+}\), Na\(^{+}\) and Mg\(^{2+}\)) and 100-fold higher concentrations of lysine, ascorbic acid, acetaminophen and doxorubicin did not affect the determination of IM. However, 5-fold higher concentrations of cysteine showed interference in the determination of IM at Fe\(_3\)O\(_4\)-(rGO&CNT)/CPE.

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<th>Table 1 Analytical parameters for voltammetric determination of IM at different modified electrodes</th>
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<td>Electrode</td>
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<tr>
<td>MWCNT/SPCE</td>
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<tr>
<td>TbFeO(_3)/g-C(_3)N(_4)/GCE</td>
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<tr>
<td>MWCNT/NO-ZnO/GCE</td>
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<td>Cu-BTC/RGO/GCE</td>
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<td>RGO/AgNPs/GCE</td>
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<td>Cu-BTC/MWCNT/GCE</td>
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<td>Fe(_3)O(_4)-(rGO&amp;CNT)/CPE</td>
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\(^a\) Square wave voltammetry  
\(^b\) Differential pulse voltammetry

The accuracy of the proposed method was assessed by performing recovery experiments. Human plasma and urine samples were measured using the standard addition method at pH 7.0 on Fe\(_3\)O\(_4\)-(rGO&CNT)/CPE. Table 2 presents the concentrations of IM added to plasma and urine samples, along with their standard deviations and recovery percentages. According to the results, the mean recovery percentage in real samples is approximately 94-98.5, suggesting that the modified electrode can accurately measure IM. The results obtained from the proposed method were compared with the UV-Vis spectroscopy results (Table 2). It was observed that the results of electrochemical analysis show consistency with those obtained by the UV-Vis method (Figure 9) with acceptable recoveries.

![Figure 9](image-url)  

**Figure 9.** (A) The UV-Vis spectrum of IM (202.5 μM) in PB solution (pH 7.0); (B) calibration curve of IM determined by UV-Vis spectroscopy

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<th>Table 2. Determination of IM in urine and blood plasma samples at Fe(_3)O(_4)-(rGO&amp;CNT)/CPE in 0.1 mol L(^{-1}) PB solution (pH 7.0)</th>
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<td>Sample</td>
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Conclusion

In this study, new rGO sheets have been synthesized via a facile and environmentally friendly process using *Callicarpa maingayi* leaf extract. By combining CNT tubes, rGO sheets and Fe$_3$O$_4$ nanoparticles, an excellent nanocomposite on the CPE electrode was created with a high surface area and good conductivity, which led to satisfactory results in the IM determination. Due to the increased electron transfer rate at the surface of Fe$_3$O$_4$-(rGO&CNT)/CPE electrode at pH 7.0, the oxidation current of IM was higher than at CPE, rGO/CPE, CNT/CPE and rGO&CNT/CPE. The excellent results are attributed to the large electrode surface area provided by CNT, rGO and Fe$_3$O$_4$ nanoparticles, as well as their excellent catalytic properties. The proposed Fe$_3$O$_4$-(rGO&CNT)/CPE showed fast response, stable measurements, and excellent anti-interference properties. Due to the advantages of magnetism and conductivity, the prepared nanocomposite material could easily adhere to the electrode surface, offering good electrical contact. In addition, Fe$_3$O$_4$-(rGO&CNT) nanocomposite-modified CPE can be applied to detect of IM in real samples.

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