Novel functionalized multiwalled carbon nanotube-glassy carbon electrode for simultaneous determination of ascorbic acid and uric acid

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Abstract In this study the electrochemical behavior of ascorbic acid (AA) and uric acid (UA) at the surface of glassy carbon electrodes (GCEs) modified by multi-walled carbon nanotubes (MWCNTs) functionalized with Fe3+ complex was investigated. The voltammetric studies using the modified electrode showed two well-resolved anodic peaks for AA and UA with a potential difference of ∼0.4 V, revealing the possibility of the simultaneous electrochemical detection of these compounds. First, the electrochemical behavior of ferric/ferrous at Fe3+ complex/MWNTs/nafion (FeCMN) modified electrode was studied. The results showed an adsorption-controlled reaction at the modified electrode. Then, the behavior of ascorbic acid and uric acid at the modified electrode was investigated. The optimum analytical conditions were sought. Linear calibration plots were obtained over the range of 4.0 to 600 µmol l−1 and 0.3 to 490 µmol l−1 with detection limits (3σ) of 2.57 µmol l−1 and 0.137 µmol l−1 for AA and UA, respectively.

The electrode with the best conditions was applied for selective determination of AA and UA in biological matrices.

1. Introduction

Ascorbic acid (AA) is a soluble vitamin widely present in many biological systems and in multivitamin preparations; it is commonly used to supplement inadequate dietary intake and as an anti-oxidant (Yu and Chen, 1997). AA has been used for prevention and treatment of common cold, mental illness, infertility, cancer, and in some clinical manifestations of HIV infections (Arrigoni and Tullio, 2002). Uric acid (UA) is the primary product of purine metabolism in the human body...
(Kaur and Halliwell, 1990). It has been proved that abnormalities of UA levels are symptoms of gout, hyperuricemia (Harper, 1977), Lesch–Nyhan syndrome (Nyhan, 2005), multiple sclerosis (Toncev et al., 2002) and oxidative stress (Becker, 1993). Other diseases such as leukemia and pneumonia are also associated with enhanced urate levels (Miland et al., 1996). As UA and AA are usually coexistent in biological fluids of blood and urine, it is important to develop a technique to selectively detect UA and AA conveniently in routine assay (Kalimuthu et al., 2006). Among several methods of determination, electrochemical methods have received much interest because they are more selective, less expensive, less time consuming and can potentially be applied to a real-time determination in vivo (Matos et al., 2000). Because of irreversible oxidation of UA and AA in aqueous solution, electrochemical procedures have been greatly developed to determine UA and AA based on their electrochemical activities. However, direct electro-oxidation of UA and AA requires high overpotentials at bare electrodes (Gao et al., 1997; Kachoosangi et al., 2006), in addition, UA and AA are oxidized at a very close potential valve, which results in poor selectivity for simultaneous determination of UA and AA. To solve this problem, most of the attention has been focused on electrochemical procedures using modification of electrode surfaces for selective electrocatalytic determination of AA and UA (Shahrokhiian and Gholkhanl, 2006; Yao et al., 2007; Zen and Hsu, 1998; Gilmartin et al., 1992).

Nowadays, carbon nanotubes (CNTs) have attracted much interest directed toward exploiting unique thermal, mechanical, electronic, and chemical properties (Popov, 2004) since they were first discovered. Due to these unique properties, CNTs have received great attention for the preparation of electrochemical sensors (Gooding, 2005; Wang, 2005). CNTs can be functionalized with organic compounds without any damage to their electrical and chemical properties (Wang, 2005; Sun et al., 2006). Functionalization of carbon nanotubes is an effective way to enhance their physical properties and improve their solubility. However, the aromatic character of nanotubes is a restriction to any possible additional reactions. Some of the functionalization approaches were through the formation of covalent bonds (Bahr et al., 2001; Pompeo and Reasaco, 2002; Dyke and Tour, 2003), while others had utilized noncovalent interactions (O’Connell et al., 2001; D. Chattopadhyay et al., 2003). Both noncovalent and covalent modifications of the surface were developed to improve solubility.

In the present study, glassy carbon electrodes (GCEs) were modified by multi-walled carbon nanotubes (MWCNTs) functionalized with Fe3+/2-(5-bromo-2-pyridylazo)-5-diethyl amino phenol (5-Br-PADAP) complex.

Our results showed that Fe3+ complex/MWCNTs/nafion modified GCE (FeCMN/GCE) could be used for the determination of AA in the presence of UA. The peak separation between AA and UA was wide enough to provide an attractive ability for the simultaneous determination of AA and UA, with lower detection limit and excellent selectivity. The proposed modified electrode could be applied to the simultaneous determination of AA and UA concentrations in real samples with satisfactory results.

2. Experimental

2.1. Reagents

MWCNTs (95% purity) with an average outer diameter of 3–20 nm, length of 1–10 μm, number of walls 3–15 and surface area of 350 m2 g−1 were obtained from Plasma Chem. GmbH (Berlin, Germany). UA and AA were purchased from Merck and used as received. Nafion perfluorinated ion exchange resin (5.0 wt% solution in lower aliphatic alcohols/H2O mixture) and 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) (Fig. 1) were purchased from Sigma. 2-(5-bromo-2-pyridylazo)-5-diethyl aminophenol solution (1.0 mmol l−1) was prepared in ethanol. Other reagents used in this study were of analytical grade and were used as received. Doubly distilled, deionized water was used for all experiments. Phosphate buffer solutions (PBS) were prepared from H3PO4 and NaH2PO4 (0.1 mol l−1); we adjusted the pH range with 0.1 M H3PO4 and NaOH solutions and used the solutions as supporting electrolytes. All solutions were prepared with doubly distilled water. The electrolyte solutions were deoxygenated with nitrogen bubbling before each voltammetric experiment. All experiments were performed under nitrogen atmosphere at room temperature.

2.2. Apparatus

Voltammetric experiments were performed using a Computrace Voltammetric Analyzer (Model 757 Metrohm). All voltammograms were recorded with a three-electrode system consisting of an Ag/AgCl electrode as the reference electrode, a platinum wire as the auxiliary electrode, and the modified GC electrode as the working electrode. A Metrohm 710 pH meter was used for pH adjustments. All the electrochemical experiments were carried out under pure nitrogen atmosphere at room temperature (23 ± 1 °C).

2.3. Preparation of functionalized MWCNTs with Fe3+/5-Br-PADAP complex

Raw MWCNTs were heated at 350 °C for 30 min to remove amorphous carbon. Prior to use, MWCNTs were oxidized with concentrated HNO3 according to the literature, in order to create binding sites onto the surface of MWCNTs (Tan et al., 2005). The treatment was carried out by the dispersion of 50 ml of concentrated HNO3 to 5.0 g of MWCNTs, and then refluxing for 5 h at 80 °C. Afterward, the oxidized

![Figure 1](http://dx.doi.org/10.1016/j.arabjc.2014.12.039)
MWCNTs were washed with distilled water until removing any excess nitric acid (neutral pH of solution). The treated MWCNTs were dried at 80°C and stored for further use. Through these steps, the carboxylic acid groups were introduced onto the cross sections of the MWCNTs.

According to the study by (Taher and Puri, 1996; Fu-sheng et al., 1981), Fe³⁺–5-Br-PADAP complex is formed by stirring stoichiometric amounts of Fe³⁺ and 5-Br-PADAP under the optimum conditions.

The complex solution was added to 2 g of prepared nanotube, and the suspension was sonicated for 60 min, then the modified MWCNTs were filtered and washed with doubly distilled, deionized water and got dried at room temperature.

2.4. Preparation of Fe³⁺-complex/MWCNTs/nafion modified GCE (FeCMN/GCE)

A 1.0 wt% nafion solution was prepared by diluting the 5.0 wt% nafion solution with ethanol. 0.02 g of the functionalized nanotubes was added to 10 ml 1.0 wt% nafion solution and then ultrasonicated for 30 min in order to form a homogeneous Fe³⁺-complex/MWCNTs/nafion (FeCMN) solution. The GCE was polished to a mirror-like surface with 0.05 μm Al₂O₃ and then rinsed ultrasonically with doubly distilled, deionized water and absolute ethanol. The GCE was immersed in a phosphate buffer solution (pH 4), and cyclic voltammetry was performed from 0 V to 1.0 V until a stable cyclic voltammogram (CV) was obtained. FeCMN/GCE was prepared by dropping 15 μl of the FeCMN suspension on the surface of a GCE and allowing the ethanol to evaporate at room temperature. Finally, the modified electrode was thoroughly rinsed with distilled water and placed in the electrochemical cell as the working electrode.

3. Results and discussion

3.1. Voltammetric response of FeCMN/GCE

The cyclic voltammograms of both modified and unmodified electrodes were recorded in phosphate buffer solution (pH 4). Fig. 2 shows the resulted voltammograms. As can be seen, no voltammetric response was observed for the unmodified electrode (Fig. 2a). However, a well-defined redox couple with anodic and cathodic peak potentials at 0.11 V and 0.245 V was observed when the FeCMN/GCE was applied (Fig. 2b).

3.2. The effect of the amount of FeCMN suspension on the electrode response

The effect of the amount of FeCMN modifier suspension was investigated by differential pulse voltammetry (DPV). The oxidation peak current of UA and AA increased as the volume of
FeCMN suspension on the GCE surface increased from 0 to 15.0 l and then reached an approximate plateau from 15.0 to 20.0 l. However, the peak current decreased when the volume exceeded 20 l, because the mass transport and charge transfer rate may decrease when the FeCMN film is too thick. Therefore, the optimized amount of the modifier suspension was chosen as 15 l.

3.3. Voltammetric behavior of ferric/ferrous at the FeCMN/GCE

Fig. 3 shows the cyclic voltammograms of the modified electrode in phosphate buffer solution (pH 4) obtained by scanning the potential from −1.0 to +0.5 V at various scan rates. The peak currents have a linear relationship with scan rates, indicating an adsorption-controlled reaction.

An approximate amount of electroactive species on the modified electrode (surface coverage of the electrode) was estimated by applying the method used by Sharp et al. (1979). According to this method, the peak current is related to the surface concentration of electroactive species by the Eq. (1):

\[ I_p = n^2 F^2 A \Gamma / 4RT \]  

(1)

Where \( n \) represents the number of electrons involved in reaction (one for Fe redox system), \( A \) is the surface geometrical area (≈0.0314 cm²), \( \Gamma \) (mol cm⁻²) is the surface coverage, and other symbols have their usual meaning. From the slope of anodic peak currents versus scan rates (Fig. 4) the surface concentration of Fe was calculated about \( 1.69 \times 10^{-6} \) mol cm⁻².

3.4. Electrocatalytic oxidation of AA at the FeCMN/GCE

The cyclic voltammograms of the FeCMN/GCE in the absence and presence of 0.5 mmol l⁻¹ AA are shown in Fig. 5a and c, respectively. As it is shown, after immobilization of FeCMN on the surface of the GCE, a redox couple with anodic and cathodic peak potentials at 0.11 V and −0.245 V, was seen (Fig. 5a). This redox couple can be associated with the immobilized Fe complex on the carbon nanotubes. By adding of 0.5 mmol l⁻¹ AA, a significant enhancement in the anodic peak current was observed while the cathodic peak current decreased (Fig. 5c), a process representing an electrocatalytic oxidation process. The anodic peak potential for the oxidation of AA at the FeCMN/GCE was about 0.103 V, whereas, at the unmodified GC electrode, AA was not oxidized before 0.5 V (Fig. 6b). Thus, a considerable decrease in overpotential and a significant enhancement in the peak current were achieved by using FeCMN/GCE.
The mechanism of electrocatalytic oxidation of AA at the modified electrode can be written as below:

\[
\text{Fe}^{3+}(\text{ox}) + \text{AA} \rightarrow \text{Fe}^{2+}(\text{red}) + \text{DAA}
\]

These results showed that the presence of the multi-walled carbon nanotubes functionalized with Fe\(^{3+}\)-5-Br-PADAP complex caused the high electrocatalytic activity toward AA oxidation. The high activity of the FeCMN/GCE for the oxidation of AA in aqueous solutions could be associated with the low charge transfer resistance of the FeCMN system as well as better catalyst dispersion on MWCNTs.

3.5. Electrochemical properties of FeCMN/GCE toward AA and UA oxidation

In order to obtain more information about the catalytic activity of FeCMN/GCE, several CVs were performed for a mixture of UA and AA (containing 52.0 \(\mu\text{mol l}^{-1}\) UA and 30 \(\mu\text{mol l}^{-1}\) AA) in phosphate buffer solution (pH 4) using different electrodes (Fig. 6). At the bare GCE, UA and AA exhibited an overlapped and broad anodic peak extended over a potential region of 0.054–0.490 V (Fig. 6a). Cyclic voltammograms for the same mixture performed at GCE modified by oxidized MWCNT dispersed in nafion, presented two peaks at 0.267 and 0.498 V for AA and UA electrooxidation, respectively (Fig. 6b). Fig. 6c shows the cyclic voltammograms for the same mixture performed at FeCMN/GCE. Two clearly distinguished peaks at 0.5 and 0.11 V attributed to the oxidation of UA and AA were observed respectively. As shown in this figure, the presence of FeCMN on the glassy carbon surface not only makes the determination of UA and AA more sensitive, but also allows the discrimination of both oxidation processes due to the considerable decrease in the over-potential for the oxidation of AA. The probable electrocatalytic oxidation reaction of UA and AA at the FeCMN/GCE can be described as Scheme 1.

![Scheme 1](attachment:image.png)

3.6. Effect of scanning rate

Cyclic voltammograms of UA and AA at the FeCMN/GCE were recorded at different potential scan rates (Fig. 7). With the increase of scan rate, the peak current of UA and AA also increased. The relationship between the peak currents and the square root of scan rate for UA and AA was linear in the range of 10–200 (mV s\(^{-1}\)), with the correlation coefficient of 0.998 for UA, and 0.997 for AA. Therefore, it could be concluded that the process of the electrode reaction was controlled by the diffusion of UA and AA. (See Fig. 8).

3.7. The effect of solution pH

In most cases, the solution pH is an important factor which influences the electrochemical reaction. Cyclic voltammetry
was carried out to characterize the effect of solution pH on the electrochemical behavior of UA and AA at the FeCMN/GCE. It was found that the peak potential shifted negatively with the increase of solution pH. The anodic peak potentials (Epa) of UA and AA were proportional to the solution pH in the range of 1.0–8.0 (Fig. 9). The linear regression equations were $Epa(UA) = -0.055\, pH + 0.697$ for UA and $Epa(AA) = -0.0584\, pH + 0.326$ for AA, respectively, demonstrating that the total number of electrons and protons in the UA and AA oxidation mechanism were the same. As the oxidation of UA and AA is known to occur by a two-electron transfer, the number of protons involved is also predicted to be two (as shown in Scheme 1). In addition, the relationship between the peak currents of UA (or AA) and the pH was explored. Fig. 9 shows the dependence of anodic peak current of UA and AA on the solution pH in the range of 1–8. The larger anodic peak current for AA and UA was obtained at pH 4. So the pH 4 was chosen in the electrochemical determination of AA and UA.

3.8. Simultaneous DPV determinations of UA and AA

The determination of UA and AA at the FeCMN/GCE was performed using differential pulse voltammetry (DPV). DPV curves of different concentrations of UA (or AA) were recorded in the presence of AA (or UA) in phosphate buffer solution (pH 4). Fig. 10 shows the DPV curves of different concentrations of UA at the FeCMN/GCE coexisting with 100 $\mu$mol l$^{-1}$ AA. The results showed that Ipa was proportional to the concentration of UA in the range of 0.3–490 $\mu$mol l$^{-1}$, and the linear equation obtained was $Ipa(UA) = 0.151\, c_{UA}(\mu$mol l$^{-1}) + 5.82$ with a correlation coefficient of 0.999. A similar experiment was carried out with AA (Fig. 11) in the presence of 320 $\mu$mol l$^{-1}$ UA, and as the figure shows the Ipa was proportional to the concentration of AA in the range of 4.0–600 $\mu$mol l$^{-1}$. The linear equation obtained was $Ipa(AA) = 0.0698\, c_{AA}(\mu$mol l$^{-1}) + 9.9817$ with a correlation coefficient of 0.999.

The detection limit of AA or UA was obtained by using Eq. (2):

$$\text{Limit of Detection} = 3S_b/m$$  \hspace{1cm} (2)

where $S_b$ represents standard deviation of blank measurements, and $m$ is the slope of calibration curve. To obtain the $S_b$, seven blank solutions (phosphate buffer solutions (0.1 M)) were prepared, and the general procedure was applied to those solutions. After recording the differential pulse voltammograms, anodic background currents were measured at +0.12 V for UA and at 0.50 V for AA.

The detection limits of 0.137 $\mu$mol l$^{-1}$ and 2.57 $\mu$mol l$^{-1}$ were calculated for UA and AA based on Eq. (2).

The relative standard deviation of seven successive determinations was 1.36% for 400 $\mu$mol l$^{-1}$ AA and 1.04% for 100 $\mu$mol l$^{-1}$ UA. The experimental results indicated that the FeCMN/GCE possessed an excellent sensitivity and reproducibility for the simultaneous determination of UA and AA.

3.9. Real sample analysis

The proposed FeCMN/GCE was applied for the determination of UA and AA in human urine using the standard addition method. Table 1 presents the results obtained from the five parallel measurements. The recovery of sample solutions of different concentrations of UA (or AA) was between 98.5% and 104%, with a less relative standard deviation. The results showed that the proposed method could be efficiently used for UA and AA determination in biological matrices.

### Table 1 Determination of UA and AA in human urine (n = 5).

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<th>Sample</th>
<th>UA added ((\mu)mol l$^{-1}$)</th>
<th>AA added ((\mu)mol l$^{-1}$)</th>
<th>Ipa found ((\mu)A)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
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<td>30.0</td>
<td>100.7</td>
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* ND: not detected.

### References


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