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ORIGINAL PAPER

# Simultaneous voltammetric determination of ascorbic acid and uric acid using a Nafion/multi-wall carbon nanotubes composite film-modified electrode

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**Abstract** A Nafion/multi-wall carbon nanotubes (MWNT) composite film-modified electrode was fabricated. The modified electrode showed excellent electrocatalytic activity toward ascorbic acid (AA) and uric acid (UA) in 0.1-mol $L^{-1}$ NaCl medium (pH 6.5). Compared to the bare electrode that only displayed a broad and overlapped oxidation peak, the Nafion/MWNT film-modified electrode not only remarkably enhanced the anodic peak currents of AA and UA but also avoided the overlapping of the anodic peaks of AA and UA with a 320-mV separation of both peaks. Under the optimized conditions, the peak currents of AA and UA were proportional to their concentration at the ranges of $8.0\times10^{-5}$ to $6.0\times10^{-3}$ mol $L^{-1}$ and $6.0\times10^{-7}$ to $8.0\times10^{-5}$ mol $L^{-1}$, respectively. The proposed method was used for the detection of AA and UA in real samples with satisfactory results.

**Keywords** Nafion · Multi-wall carbon nanotubes · Uric acid · Ascorbic acid · Simultaneous determination · Voltammetry

## Introduction

Ascorbic acid (AA) is widely present in many biological liquids, medicines, fruits, and beverages. It is one of the most important soluble vitamins and plays a significant role in the biological functioning, such as the supplement of inadequate dietary intake, wound healing [1, 2], prevention as well as treatment of common cold, mental illness, and infertility [3]. Uric acid (UA) is one of the principal end products of purine metabolism in human body. Abnormal levels of UA are symptoms of several diseases, like gout, hyperuricemia, and Lesch–Nyhan syndrome [4]. Hence, monitoring the concentration of UA in biological fluids has their clinical significance. Due to its crucial role in biological functioning and clinical significance, several traditional methods have been used for its determination, such as colorimetric, enzymatic, and electrochemical techniques. However, colorimetric techniques cannot afford the accurate concentration of UA [5], and enzymatic method has its inherent defects, for example, high cost and failure to achieve a rather high detection limit [6]. Instead, voltammetric techniques have been characterized by higher selectivity, lower cost, less time consumption, and real-time determination in vivo [7–9]. Electrochemical methods can be used to determine UA due to its electrochemical active. Generally, AA and UA always coexist in biological fluids such as blood and urine, and AA has a close oxidation peak potential of UA, which results in poor selectivity determination of AA or UA in real samples on conventional electrodes. Therefore, it is essential to exploit more sensitive, selective, and simple methods for the simultaneous determination of AA and UA.

Various modified electrodes have been developed to separate the overlapping anodic peaks of AA and UA, for example, nanogold modified electrode [10], carbon paste electrodes [11–13], $LaFeO_3$-based electrode [14], polymer-modified electrode [15–17], covalent monolayer film-modified electrode [18], Nafion film-modified electrode [19], chitosan incorporating cetylpyridine bromide-modified glassy carbon electrode (GCE) [20], etc. Although

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the voltammetric techniques focusing on the high-selective, low-cost, and little time-consuming determination of AA with various modified electrodes has already been reported, it is still significant to exploit more economical, efficient, and convenient voltammetry for simultaneous determination of AA and UA.

In recent years, carbon nanotubes (CNTs) have received more and more attention to the electrode preparation. Since being discovered by Lijima in 1991 [21], CNTs have obtained increasing applications in chemical, physical, and material fields owing to their unique structure and extraordinary properties. CNTs have excellent electronic properties, such as huge surface area and efficient catalytic activity, which suggest that they can promote charge transfer reaction when they are employed as electrode materials [22, 23].

Nafion, a perfluorinated sulfonated cation exchanger with characteristic properties, such as excellent antifouling capacity, chemical inertness, and high permeability to cations, has been extensively applied as an electrode modifier. CNTs can be homogeneously dispersed in Nafion solution because of the hydrophobic side chains and polar head groups of Nafion. Nafion/CNTs composite thin film-modified electrodes have their attractive effects in electroanalytical applications; for instance, they have been used as amperometric sensors for the trace detection of heavy metals [24–26] and dopamine [27], simultaneous determination of 2-nitrophenol and 4-nitrophenol [28], and the determination of clenbuterol [29]. Nafion has been used as the modifier coated on carbon paste electrode for the detection of UA in the presence of a high concentration of AA [19], but no investigation has been reported on the simultaneous determination of AA and UA by Nafion/MWNT composite film-modified electrodes.

In this paper, a simple method for the simultaneous determination of AA and UA on the Nafion/MWNT composite film-modified electrode was reported in 0.1-mol $L^{-1}$ NaCl medium (pH 6.5). The Nafion/MWNT composite film-modified electrode displayed high electrocatalytic activities toward the oxidation of AA and UA. The peak separation between AA and UA was 320 mV, which indicated the employment for the simultaneous voltammetric determination of AA and UA. The modified electrode was applied for the determination of AA and UA in real samples without any pretreatment.

## Experimental

### Reagents and chemicals

AA and UA were purchased from Alfa Aesar China (Tianjin) Co., Ltd, and used as received. The multi-wall carbon nanotubes (diameter, 10–20 nm; length, 1–2 μm; purity >95%) were obtained from Shenzhen Nanotech Port Co. Ltd, China. Nafion (wt.%, 5%) was purchased from Sigma. All the other chemicals used were analytical grade without further purification and prepared with double-distilled water. The pH of the solutions was adjusted with 0.1-mol $L^{-1}$ HCl and NaOH.

### Apparatus

RST3000 electrochemical system (Suzhou Risetech Instrument Co. Ltd., Suzhou, China) was employed for all the voltammetric measurement. A conventional three-electrode system was used, including a bare GCE ($d$=4 mm) or Nafion/MWNT film-modified GCE as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a platinum wire electrode as auxiliary electrode. All the pH values were measured with a PHS-3C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with standard buffer solution every day. The scanning electron microscopy (SEM) was performed with a Hitachi X-650 microscope.

### Preparation of Nafion/MWNT composite film-modified GCE

The bare GCE was pretreated carefully with 0.05-μm alumina slurry on a polishing cloth, rinsed thoroughly with1:1 $HNO_3$–$H_2O$ ($v/v$) and then washed with pure ethanol and redistilled water, respectively. Ten milligrams of the untreated MWNT was added to plentiful concentrated nitric acid (wt.%, 68%) and then sonicated for about 4 h. The mixture was filtrated and washed with doubly distilled water until the filtrate was litmusless. The treated MWNT were dried under an infrared lamp. Nafion/MWNT suspension was accomplished as follows: 5.0 mg of treated MWNT was sonicated in 10.0 mL (wt.%, 0.1%) Nafion methanol solution for about 30 min and then homogeneous suspension would be achieved. MWNT suspension was obtained with the same procedure, but 0.1% Nafion solution was replaced with *N*,*N*-dimethylformamide (DMF). The pretreated GCE was coated evenly with 10.0 μL of Nafion/MWNT suspension, and then methanol was evaporated at room temperature. For contrast, the Nafion/GCE was prepared with the same procedure without MWNT. MWNT/GCE was achieved after evaporating DMF under the ultraviolet lamp in this work. Before use, the modified electrodes were washed repeatedly with double-distilled water to remove the loosely combined modifiers.

According to the literature*s* [30], the microscopic area of the Nafion/MWNT modified GCE was calculated to be 0.8668 $cm^2$, which was 6.9 times greater than the bare GCE (0.1256 $cm^2$).

### Analytical procedures

Except as otherwise stated, 0.1-mol $L^{-1}$ NaCl medium (pH 6.5) was used as supporting electrolyte for the determinations of AA and UA. Voltammograms were obtained by scanning the potential from −200 to 800 mV (vs. SCE). The quantitative determinations of AA and UA were achieved by measuring the oxidation peak currents after background subtraction using differential pulse voltammetry (DPV). In order to fit into the linear range of the method, the urine sample employed for operation was accurately diluted by a factor of 1/100 (*v*/*v*) with the supporting electrolyte, vitamin C injection (standard concentration of AA 250-g $L^{-1}$, 2 mL per injection), by a factor of 1/10,000 (*v*/*v*). The dilution process can actually help reduce the matrix effect of real samples.

## Results and discussion

### Characterization of Nafion/MWNT modified electrode

Figure 1 showed the morphology of the Nafion/MWNT composite film on the GC electrode by using the SEM method. It can be seen that the Nafion/MWNT composite film was uniformly coated on the electrode surface and shaped as a spaghetti-like porous reticular formation. The special surface morphology presented a much larger real surface area than the apparent geometric area.

### Electrocatalytic oxidation of UA and AA

Figure 2A demonstrated the cyclic voltammetry (CV) curves of a mixture of UA and AA (containing 8.0×

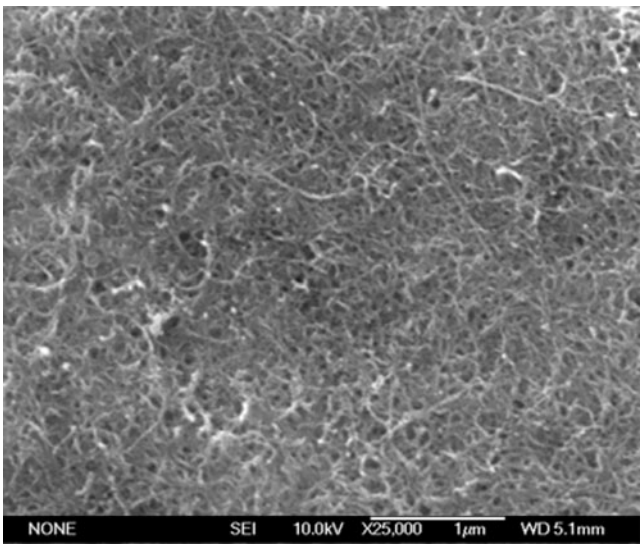



**Fig. 1** SEM image of Nafion/MWNT composite film on glassy carbon electrode

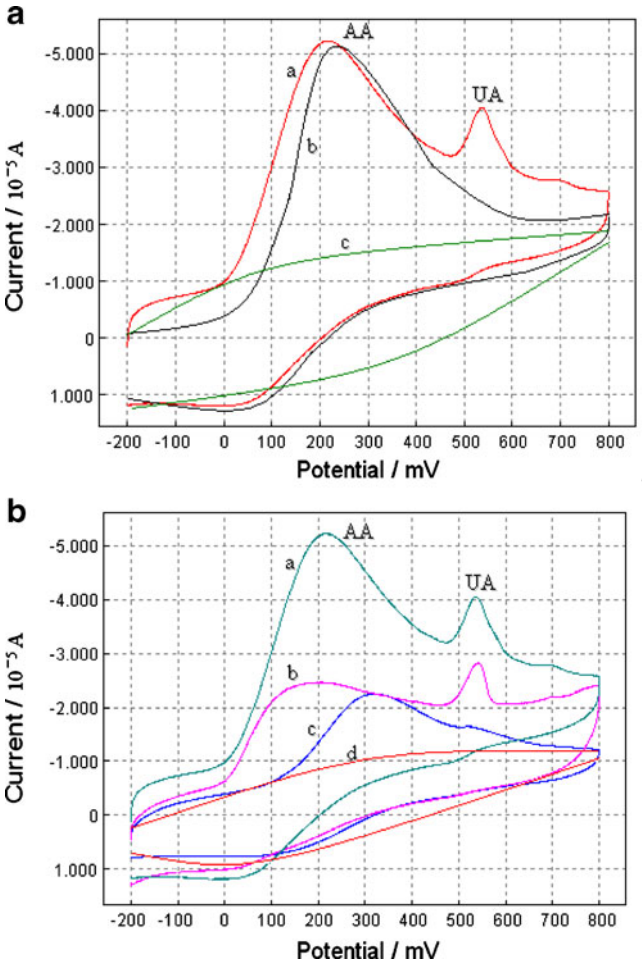
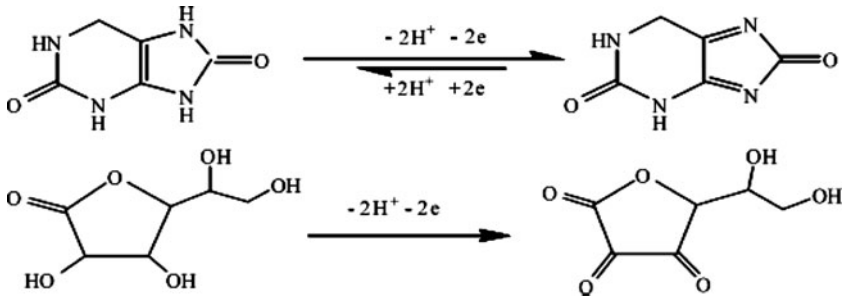



**Fig. 2** **A** CV curves of the mixture containing $1.0\times10^{-3}$ mol $L^{-1}$AA and $8.0\times10^{-5}$ mol $L^{-1}$ UA (*a*), $1.0\times10^{-3}$ mol $L^{-1}$ AA (*b*), and without AA and UA (*c*) in 0.1 mol $L^{-1}$ NaCl solution (pH 6.5) at the Nafion/MWNT/GCE. **B** CV curves of the mixture containing $1.0\times10^{-3}$ mol $L^{-1}$ AA and $8.0\times10^{-5}$ mol $L^{-1}$ UA in 0.1 mol $L^{-1}$ NaCl solution (pH 6.5) at the Nafion/MWNT/GCE (*a*), MWNT/GCE (*b*), bare GCE (*c*), and Nafion/GCE (*d*). Scan rate, 100 mV $s^{-1}$; rest time, 3 s

$10^{-5}$ mol $L^{-1}$ UA and $1.0\times10^{-3}$ mol $L^{-1}$AA) (a), $1.0\times10^{-3}$ mol $L^{-1}$ AA (b), and without AA and UA (c) in 0.1-mol $L^{-1}$ NaCl (pH 6.5) at Nafion/MWNT/GCE, respectively. Figure 2A (a) showed two anodic peaks at around the potential of 534 and 214 mV, which attributed to the oxidation of UA and AA with a 320-mV separation of both peaks. Figure 2B revealed a CV response of a mixture of UA and AA (containing $8.0\times10^{-5}$ mol $L^{-1}$ UA and $1.0\times10^{-3}$ mol $L^{-1}$ AA) in 0.1-mol $L^{-1}$ NaCl at pH 6.5 at Nafion/MWNT/GCE (a), MWNT/GCE (b), bare GCE (c), and Nafion/GCE (d). Under the same conditions, no anodic peak of AA or UA was observed at the Nafion/GCE. At the bare GCE, UA and AA exhibited an overlapped and broad anodic peak extended over a potential region of 98–580 mV with the mixed potential at 310 mV. However, CV for the Nafion/MWNT/GCE and MWNT/GCE showed two anodic

**Scheme 1** The oxidation mechanisms of AA and UA




peaks with a separation of about 320 mV toward UA and AA, which was broad enough for their simultaneous electrochemical determination. Nevertheless, at the Nafion/MWNT nanocomposite-modified electrode, the peak currents were significantly higher than those at the MWNT/GCE or the bare GCE. The oxidation mechanisms of AA and UA have been reported by Hu et al [10], as shown in Scheme 1.

## Effect of scanning rate

The effect of scan rate (in the range of 20–200 mV $s^{-1}$) on the peak currents and peak potentials at the Nafion/MWNT/GCE in 0.01 mol $L^{-1}$ NaCl (pH 6.5) containing AA and UA was investigated by cyclic voltammetry. As shown in Fig. 3, the peak potentials of AA ($E_{AA}$) and UA ($E_{UA}$) at the Nafion/MWNT/GCE shifted positively with the increasing of the scan rate. The anodic peak currents of AA ($i_{AA}$) and UA ($i_{UA}$) were found to be directly proportional to the square root of the scan rate ($v^{1/2}$; see Fig. 4), indicating a diffusion-controlled oxidation processes occurring at the Nafion/MWNT modified GCE, which was in good agreement with the previous report on the transport characteristics of AA on a chemically modified electrode [10].

## Effect of solution pH and stability of the electrode

The effect of pH on electrochemical reactions of AA and UA at the Nafion/MWNT/GCE were also investigated. With pH changing from 3.0 to 10.0, the $E_{AA}$ and $E_{UA}$ shifted toward more negative potentials. When pH was 6.5, the maximum peak currents $i_{AA}$ and $i_{UA}$ were achieved, suggesting that pH 6.5 should be selected as the optimum value for determination of AA.

## Simultaneous DPV determination of UA and AA

DPV was used for the simultaneous determination of AA and UA at the Nafion/MWNT/GCE by reason of its higher current sensitivity and better resolution than cyclic voltammetry. The determination of AA and UA in their mixtures was performed at the Nafion/MWNT/GCE when the concentration of one species changed, whereas the other species maintained a constant. Figure 5 displayed the DPV curves of different concentration AA at the Nafion/MWNT/GCE containing $8.0\times10^{-5}$ mol $L^{-1}$ UA. The results demonstrated that $i_{AA}$ was linear to the concentration of AA in the range of 8.0×

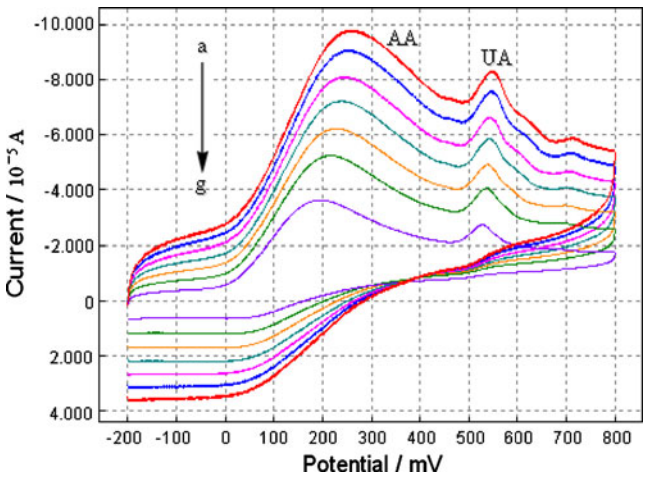



**Fig. 3** CV curves of the mixture containing $8.0\times10^{-5}$ mol $L^{-1}$ UA and $1.0\times10^{-3}$ mol $L^{-1}$ AA at the Nafion/MWNT/GCE at a different scan rate ($a\rightarrow g$): 140, 120, 100, 80, 60, 40, and 20 mV $s^{-1}$ in 0.1 mol $L^{-1}$ NaCl (pH 6.5)

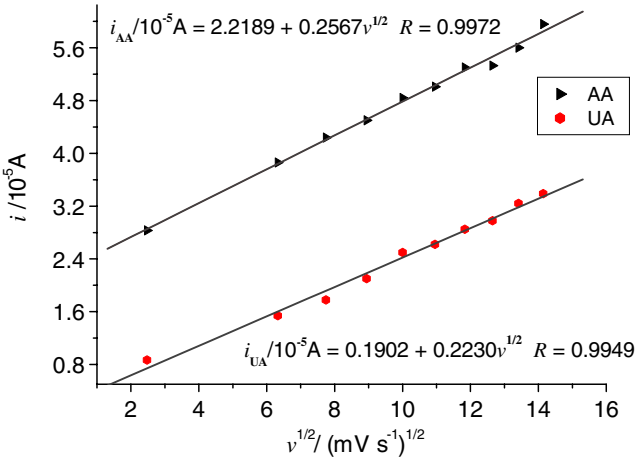



**Fig. 4** The plot of anodic peak currents of AA and UA vs. square root of the scan rate ($v^{1/2}$)

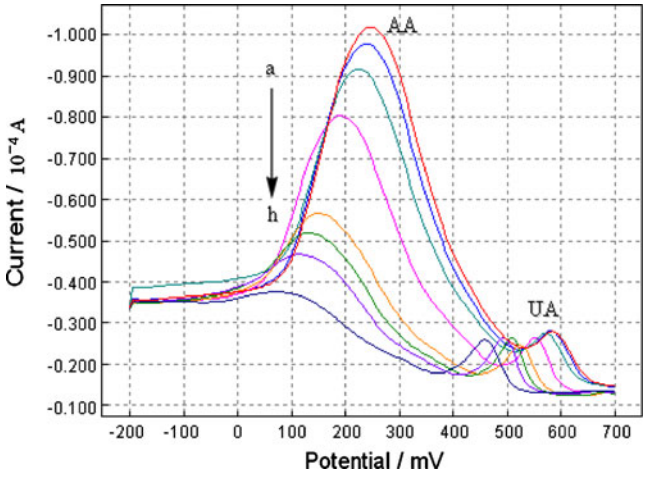



**Fig. 5** DPV curves of 0.1 mol $L^{-1}$ NaCl (pH 6.5) containing $8.0\times10^{-5}$ mol $L^{-1}$ UA with different concentrations of AA (*a*→*h*): 6.0, 5.0, 4.0, 2.0, 1.0, 0.6, 0.4, and $0.2\times10^{-3}$ mol $L^{-1}$

$10^{-5}$ to $6.0\times10^{-3}$ mol $L^{-1}$ (see Fig. 6), and the linear equation is as follows:

$$i_{AA}/10^{-5}\mathrm{A} = 0.0869C_{AA}\left(10^{-4}\mathrm{mol\,L^{-1}}\right) + 0.9442(R = 0.9988)$$

The detection limit was $4.0\times10^{-5}$ mol $L^{-1}$ ($S/N$=3). A similar experiment was carried out with UA. In the presence of $2.0\times10^{-3}$ mol $L^{-1}$ AA, the $i_{UA}$ was linear with the concentration of UA in the range of $6.0\times10^{-7}$ to $8.0\times10^{-5}$ mol $L^{-1}$ (see Fig. 6 insert). The following is the linear equation:

$$i_{UA}/10^{-6}\mathrm{A} = 4.4477\ C_{UA}\left(10^{-5}\mathrm{mol\,L^{-1}}\right) + 2.2506(R = 0.997)$$

The detection limit was $2.0\times10^{-7}$ mol $L^{-1}$ (S/N=3).

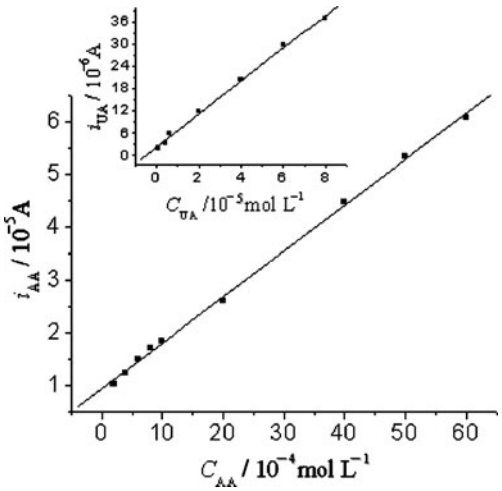



**Fig. 6** The plot of anodic peak currents ($i_{AA}$) of AA vs. the concentrations of AA ($C_{AA}$). *Insert*, $i_{UA}$ vs. $C_{UA}$

Reproducibility is the vital characteristic for the modified electrode, which should be investigated for analytical determination. The same Nafion/MWNT/GCE was used for five times successive measurement, and the relative standard deviation (RSD) of the peak current was 2.5% for $1.0\times10^{-3}$ mol $L^{-1}$ AA and 2.0% for $8.0\times10^{-5}$ mol $L^{-1}$ UA, revealing good reproducibility of the Nafion/MWNT/GCE.

## Interferences

For investigating the selectivity of the modified glassy carbon electrode for simultaneous determination of AA and UA, several potential coexistent interference compounds were selected. The tolerance limit ($C_{species}/C_{AA}$) was defined as the maximum concentration of the interfering substance that caused an error less than 10% for the determination of $1.0\times10^{-3}$ mol $L^{-1}$ AA in the presence of $8.0\times10^{-5}$ mol $L^{-1}$ UA. The following times did not show interference: KCl, $MgCl_2$, glucose, and $CaCl_2$, 100 times; glycine, 20 times; and $ZnCl_2$, 5 times.

## Real samples analysis

In order to fit into the linear range of the method, the urine sample and the vitamin C injection employed for detection were accurately diluted with the supporting electrolyte. The detected results are shown in Table 1. The total value of AA in vitamin C injection was 242 g $L^{-1}$, which was obtained by multiplying the detected value and the diluted factor. The total value of AA was in agreement with the declared content (i.e., 250 g $L^{-1}$). The total UA concentrations detected in the urine sample was $4.37\times10^{-3}$ mol $L^{-1}$, which was consistent with the containing level of a healthy human. In order to test the correctness of the results, the standard addition method was used to determine the AA (or UA) sample spiked with suitable AA (or UA). The experimental results were also displayed in Table 1. The results demonstrated that the proposed methods could be efficiently used for the determination of AA and UA.

**Table 1** Results of the determination of AA and UA in the real samples ($n$=5)

| Sample | Species | Species added (μmol$L^{-1}$) | Proposed method (μmol$L^{-1}$) | Recovery (%) | RSD (%) |
|---|---|---|---|---|---|
| Vitamin C injection | AA | – | 137.4 | – | – |
| | AA | 50.0 | 185.1 | 95.4 | 2.4 |
| | UA | 80.0 | 81.0 | 101.3 | 2.2 |
| Urine | UA | – | 43.7 | – | – |
| | UA | 50.0 | 93.4 | 99.4 | 2.0 |
| | AA | 1,000 | 976.5 | 97.7 | 1.8 |

## Conclusions

The Nafion/MWNT/GCE displayed good electrocatalytic activity for the oxidation of AA and UA. In addition, the modified electrode exhibited higher selectivity in the electroanalysis of AA and UA in their mixture solution with a 320-mV separation of their oxidation peaks, showing that Nafion/MWNT/GCE has the ability for the simultaneous determination of AA and UA. The proposed method was used for the determination of AA and UA in real samples with satisfactory results.

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