Simultaneous voltammetric detection of dopamine, ascorbic acid and uric acid using a poly(2-(N-morpholine)ethane sulfonic acid)/RGO modified electrode

Keying Zhang, Na Zhang, Li Zhang, Hongyan Wang, Hongwei Shi and Qiao Liu

A poly(2-(N-morpholine) ethane sulfonic acid)/reduced graphene oxide (RGO) modified glassy carbon electrode (GCE) was prepared using an electropolymerization method, and was characterized by scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS). The electrochemical behaviors and simultaneous detection of ascorbic acid (AA), dopamine (DA) and uric acid (UA) at this electrode were studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Tests showed that this electrode exhibited excellent electrocatalytic activity towards the oxidation of AA, DA and UA. The oxidation peak currents of AA, DA and UA were proportional with their concentrations in the ranges 1.0 µM–30 µM (30 µM–100 µM), 0.05 µM–100 µM and 0.1 µM–100 µM, with detection limits of 0.43 µM, 0.0062 µM and 0.056 µM, respectively. In addition, this electrode exhibited an excellent selectivity, reproducibility and stability, and has been successfully used to determine real samples with satisfactory results.

1 Introduction

Ascorbic acid (AA), dopamine (DA) and uric acid (UA) are considered as important molecules for physiological processes in human metabolism. DA is one of the crucial catecholamine neurotransmitter molecules widely distributed in the mammalian central nervous system. It plays an important role in the function of the central nervous, renal, hormonal and cardiovascular systems. The dysfunction of the dopaminergic system in the central nervous system can result in some diseases or neurological disorders such as schizophrenia, Parkinson’s disease and HIV infection. AA, presenting in both the animal and plant kingdoms, is an essential vitamin for humans, and has been used for the prevention and treatment of the common cold, mental illness, infertility, cancer and AIDS. UA is the primary end product of purine metabolism. The extreme abnormalities of UA levels in the body are symptoms of several diseases including gout, hyperuricemia, and Lesch–Nyan disease. Therefore, real time monitoring of AA, DA and UA in biological samples and pharmaceutical preparations is of great significance, shows an important issue in diseases diagnosis. AA, DA and UA are of electrochemical active, therefore, electrochemical techniques have been considered as potential approaches to detect AA, DA and UA. However, it is a nontrivial task to simultaneously determine AA, DA and UA directly at ordinary (carbon and metal) electrodes, because these substances coexist in the extracellular fluid of the central nervous system and serum. Thus, the development of novel modified electrodes to distinguish AA, DA and UA in mixtures is a very active research area. Up to now, various modified electrodes, including nano-material, self-assemble monolayer, layer self-assemble and polymer films have been successfully constructed for individually and simultaneously detecting these substances. Among these modified electrodes, polymer film modified electrode has attracted researcher’s attention due to its advantages and wide applications in the chemical fields. Lin et al. used DNA/poly (p-aminobenzensulfonic acid) composite bi-layer modified electrode for determination of DA and AA under coexistence of AA; Zhang et al. used polyaniline nano-networks/p-aminobenzensulfonic acid functionalized electrode for the simultaneous determination of AA and UA; Hu et al. used poly(acid chrome blue K) modified electrode for simultaneous determination of DA, AA and UA; Chen et al. used poly (4 – amino – 1 ‘– azobenzene-3,4’-disulfonic acid) modified electrode for selective detection of DA; Ohsaka et al. used poly (N,N-dimethylaniline)-modified electrode for simultaneous electro-analysis of DA and AA; Huang et al. fabricated a poly(p-toluene sulfonic acid) modified electrode for simultaneous detection of DA and AA. Recently, nanomaterial-based electrochemical sensors have been pay wide attention to due to its excellent performances, various nanomaterials modified electrodes...
have been employed for AA, DA and UA analysis. RGO-based hybrids bring new opportunities for improving sensor performances due to these hybrids affording significant physicochemical properties.\(^{36}\) Among these RGO-based hybrids, combining RGO with conducting polymers show important potential application in sensor field.\(^{37}\) 2-(N-morpholine)ethane sulfonic acid (MES) with a morpholine ring, is often used as a buffering agent in biology and biochemistry. Previous study indicated that MES can be electropolymerized onto the electrode surface for silver electrodeposition providing good interface.\(^{38}\)

Herein, a poly(2-(N-morpholine)ethane sulfonic acid) (PMES)/RGO modified electrode was constructed and used for simultaneously detecting AA, DA and UA (Scheme 1). To the best of our knowledge, it was for the first time report its application for determination of AA, DA and UA. Based on experiment results, a sensitive method for simultaneous determination of AA, DA and UA was established for routine analysis.

2 Results and discussion

2.1 SEM characterization of PMES/RGO film

Fig. 1 showed the morphologies of RGO (a), PMES/RGO (b) characterized by SEM. Compared with RGO, a thin film layer was covered on its surface (b), because MES molecules were electropolymerized onto the RGO/GCE surface, indicated that PMES/RGO film could form on GCE surface.

2.2 Electrochemical impedance characterization of PMES/RGO film

The EIS can be used to characterize the electrode surface modification process based on the electron-transfer resistance change \((R_{et})\) which is the semicircle diameter on EIS curve. We utilized the \(R_{et}\) to observe the change of electronic transfer resistance. Fig. 2 exhibited EIS curves of different electrodes. It can be observed that a lower \(R_{et}\) for RGO/GCE, compared with bare GCE, indicated RGO has better conductivity. The modification of PMES on the RGO/GCE surface resulted in a larger \(R_{et}\), the reason may be that they have a charge repulsion role because of PMES film and redox probe are negatively charge. The above results indicated that PMES/RGO/GCE was successfully prepared.

2.3 Separation of the electrochemical responses of AA, DA and UA

Fig. 3 showed CVs of the mixture solution of AA, DA and UA at different electrodes in 0.1 M PBS (pH 7.0). A broad oxidation peak was observed (a), suggesting the peak potentials for AA, DA and UA are indistinguishable at the bare GCE. However, for PMES/RGO/GCE (c), three well-separated oxidation peaks corresponding to the electrooxidation of AA, DA and UA can be observed, which was enough to simultaneously detect them in mixture solution.

2.4 Single oxidation of AA, DA and UA

Fig. 4 showed CVs of AA (A), DA (B) and UA (C) at bare GCE and PMES/RGO/GCE in 0.1 M PBS (pH 7.0). Curves (a) and (b) correspond to bare GCE and PMES/RGO/GCE in the presence of AA, DA and UA, respectively. Compared with bare GCE, PMES/RGO/GCE can considerably enhance the oxidation peak currents of AA, DA and UA, and with more negative oxidation
peak potentials, indicating that PMES/RGO/GCE had excellent
electrocatalytic activities towards the oxidations of AA, DA and
UA.

2.5 Effect of pH

The effect of the solution pH on the response of AA, DA and UA
were investigated in the range of 4.0–9.0. Fig. 5 showed the
relationship of the oxidation peak currents of AA, DA and UA
with pH, respectively. The oxidation peak current of AA
decreased slightly with increasing pH until it reached 6.0, then
it increased until pH reached 8.0. Further increasing pH, the
oxidation peak current slightly decreased. For DA, the oxidation
peak current increased with increasing pH until pH reached 7.0,
and then it decreased when pH exceeded 7.0. For UA, the
oxidation peak current decreased with increasing pH. In addi-
tion, all the oxidation peak potentials for AA, DA and UA shifted
towards negative direction with increasing pH, showing that
protons have taken part in their electrode processes. PBS (pH
7.0), much closer to physical conditions, was chosen for the
following experiments.

2.6 Effect of scan rate

The dependence of oxidation peak current of AA, DA and UA on
scan rate was investigated as shown in Fig. 6. For DA and UA,
The oxidation peak current increased linearly with the increase
of scan rate, and the peak current ($I_p$) was proportional to scan
rate ($v$) from 20 to 200 mV s$^{-1}$, respectively. The linear regres-
sion equation was $I_p = 0.02 + 0.08v$ ($r = 0.9967$) and $I_p = 2.50 +
0.09v$ ($r = 0.9983$), respectively, suggesting an adsorption
controlled process. For AA, the oxidation peak current was
proportional to the square root of scan rate over the range of 20–
200 mV s$^{-1}$, the linear regression equation was $I_p = 1.67 +
0.74v^{1/2}$ ($r = 0.9912$), suggesting a diffusion controlled process.

2.7 Simultaneous determination of AA, DA and UA

Fig. 7A showed the peak current of AA increased with its
concentration increasing, when the concentration of DA and UA
were kept constant. In addition, the change of AA concentration
did not have significant influence on the peak currents and
peak potentials of the other two compounds. Similarly, as
shown in Fig. 7B and C, the oxidation peak current of DA or UA
increased with the increase of the concentration of DA or UA by
keeping the concentration of other two compounds constant.
with a detection limit of 0.43 µM (S/N = 3); \( i_{AA} \) (10 µA) = 1.3 + 0.01c\(_{AA} \) (c\(_{AA} \): 30 µM–100 µM) \( (r = 0.998) \). Compared with some reported methods\(^{13,17,39,40} \), this method had excellent analytical performances for detecting the AA, DA and UA (Table 1).

### 2.8 The reproducibility and stability

The reproducibility and stability of the modified electrode were investigated by CV response of 10 µM DA in 0.1 M PBS (pH 7.0). The fabrication of six modified electrodes, made independently, showed a well reproducibility with a relative standard deviation (RSD) of 3.4%. The stability of the modified electrode was studied by scanning for 30 continuous cycles at the potential between −0.2 and +0.6 V (vs. SCE) with a scan rate of 100 mV s\(^{-1}\), the peak heights of CV showed a negligible change. Therefore, the modified electrode exhibited an excellent reproducibility and stability.

### 2.9 Interference study and analytical application

The influence of various foreign species on the determination of 0.4 mM AA, 50 µM DA and 0.3 mM UA were investigated. The results indicated that the mutual interference from AA, DA and UA can be neglected. Other influences from common coexisting substances were also investigated. The experiment results indicated that no significant interference for the detection of AA, DA and UA for these compounds: L-lysine (20), L-cystine (20), L-tyrosine (20), glucose (20), where the data in the brackets were calculated by the regression equation of UA, and diluted to 20 µM, which was then used for testing recovery by the standard addition method. The experiment results were listed in Table 2.

The recovery values were reasonable, showing that the proposed method could be efficiently used for the real sample analysis. To further verify the reliability of this method and its potential in clinical diagnosis, the fresh serum samples from a healthy

### Table 1 Comparison of linear range and detection limit with some reported methods

<table>
<thead>
<tr>
<th>Modified electrode</th>
<th>Linear range (µM)</th>
<th>Detection limit (µM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc oxide/redox mediator/GCE</td>
<td>15–240 AA, 6–960 DA, 50–800 UA</td>
<td>1.4 AA, 0.7 DA, 4.5 UA</td>
<td>15</td>
</tr>
<tr>
<td>poly(amido-amine), multi-walled carbon nanotubes and Au nanoparticles functionalized reduced graphene oxide modified electrode</td>
<td>20–1800 AA, 10–320 DA, 1–114 UA</td>
<td>6.7 AA, 0.3 DA, 0.33 UA</td>
<td>37</td>
</tr>
<tr>
<td>Methylene blue/phosphorylated zirconia–silica composite electrode</td>
<td>10–1600 AA, 6–100 DA, 22–350 UA</td>
<td>8.3 ± 0.1 AA, 1.7 ± 0.1 DA, 3.7 ± 0.2 UA</td>
<td>39</td>
</tr>
<tr>
<td>Reduced graphene oxide/GCE</td>
<td>40–1000 AA, 0.1–100 DA, 0.8–800 UA</td>
<td>4.2 AA, 0.008 DA, 0.6 UA</td>
<td>40</td>
</tr>
<tr>
<td>PMES/RGO/GCE</td>
<td>30–100, 1–30 AA, 0.05–100 DA, 0.1–100 UA</td>
<td>0.43 AA, 0.0062 DA, 0.056 UA</td>
<td>This work</td>
</tr>
</tbody>
</table>
female individual were treated by centrifuging and filtering before the experiments. The concentration of UA in serum was calculated with the level of approximate 171 μM by this method, which was close to the hospital’s assay result (186 μM). Which indicated that the proposed method had the good reliability and potential in clinical diagnosis.

3 Experimental

3.1 Reagents and apparatus

AA, DA, UA and 2-(N-morpholine) ethane sulfonic acid were purchased from Sigma (USA), L-lysine, L-cystine, L-tyrosine, glucose and other reagents were purchased from Nanjing Chemical Reagent (Nanjing, China). 0.1 M phosphate buffer solutions (PBS) with different pH values were prepared by mixing the stock standard solution of Na₂HPO₄ and NaH₂PO₄ and pH was adjusted with H₂PO₄ or NaOH solution. All chemicals were of analytical reagent grade and used without further purification. All solutions were prepared with doubly distilled water.

CV was performed on a CHI660A electrochemical workstation (Shanghai Chenhua Instruments, China). The three-electrode system was used in the experiment with bare GCE or modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a platinum wire as counter electrode. All electrochemical measurements were carried out in a 10 mL electrochemical cell, where oxygen was removed with high-purity nitrogen for 20 min and a blanket of nitrogen was maintained over the solution during the measurements. All potentials given in this paper were referred to SCE. All experiments were performed in compliance with the ethical principles of human experimentation, and approved by the ethics committee at suzhou university. Informed consents of interest

There are no conflicts of interest to declare.

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Notes and references


Table 2 Detection of AA, dopamine hydrochloride injection and human urine in mixture

<table>
<thead>
<tr>
<th>Sample</th>
<th>Original (μM)</th>
<th>Added (μM)</th>
<th>Found (μM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>10.0</td>
<td>10.0</td>
<td>19.78</td>
<td>98.90</td>
</tr>
<tr>
<td>Dopamine hydrochloride injection</td>
<td>5.0</td>
<td>10.0</td>
<td>14.92</td>
<td>99.47</td>
</tr>
<tr>
<td>Human urine</td>
<td>20.0</td>
<td>20.0</td>
<td>41.34</td>
<td>103.35</td>
</tr>
</tbody>
</table>

The PMES/RGO/GCE was fabricated and used to detect AA, DA and UA and their mixture by DPV, exhibited a highly electrocatalytic activity for the oxidation of AA, DA and UA, and a large peak separations between AA, DA and UA. The modified electrode can individually or simultaneously detect AA, DA and UA with good reproducibility, stability, sensitivity and selectivity. In addition, this electrode can be applied to detect real samples with satisfactory results. The excellent performances of this method indicated a strong potential for application in the routine analysis of AA, DA and UA in clinical tests.