Graphene Oxide-Templated Polyaniline Microsheets toward Simultaneous Electrochemical Determination of AA/DA/UA

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Abstract
Graphene oxide-templated polyaniline (GO-PAN) microsheets were prepared via oxidation polymerization of aniline with graphene oxide (GO) as template. By applying this approach, irregularly shaped GO-PAN microsheets were obtained which was revealed by scanning electron microscopy (SEM). Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements demonstrated that the resulted GO-PAN was electroactive and could be further used as electrocatalyst in simultaneous determination of ascorbic acid (AA), dopamine (DA) and together with uric acid (UA).

Keywords: Polyaniline, Graphene oxide, Dopamine, Ascorbic acid, Uric acid

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Graphene in Electroanalysis

1 Introduction
Ascorbic acid (AA), dopamine (DA) and uric acid (UA) are essential biomolecules coexisting in body fluids [1]. However, electrochemical determination of these species based on anodic oxidation suffers from oxidation peaks overlapping at solid electrodes [2,3]. Till now, various modified electrodes have been attempted to solve the problems encountered in simultaneous determination of AA, DA and UA, for example, PDDA modified graphite electrode [4], amorphous silica gel modified carbon paste electrode [5], carbon-nanofiber modified carbon paste electrode [6], poly(acrylic acid)-multiwalled carbon-nanotubes composite-coated glassy-carbon disk electrode [7] and natural antibiotic lasalocid A cast glassy carbon electrode [8], etc. However, development of new materials to accomplish simultaneous electrochemical determination has still been one of the attractive subjects which researchers ever strived.

Polyaniline (PAN) is one of the most significant conducting polymers due to its facile preparation, tunable conductivity and high environmental stability. Thus, considerable efforts have been devoted to improve its unique electronic [9–11], optical [12–14] and electrochemical properties [15,16], as well as the extensive applications in optical sensing [17,18] and bioelectrochemical sensing [15,16]. However, PAN was reported to be insensitive to DA. This situation could be improved by introducing suitable functional groups into the polymer backbone [19] or incorporating PAN with other materials which are sensitive to DA, such as gold nanoparticles [20], carbon nanotubes [21,22], etc. Another route [23] to enhance the selectivity is to overoxidize poly(N-acetylaniline) to exclude the negative ascorbic ions from the electrode while accumulate the positive DA ions due to the charged characters of DA and AA.

In recent years, a burst of reports has been published on graphene. Graphene is known as a new class of two dimensional carbon material, according to its unique nanostructure, excellent mechanical properties, unusual electronic and transport properties [24–26]. Researchers mainly focused on the preparation and functionalization of graphene layers to improve their solubility, dispersibility, mechanical properties and electrochemical activity [27–32]. For instance, Shang and coworkers [30] reported an electrochemical method on selective detection of dopamine by using multilayer graphene nanoflake films which were fabricated via chemical vapor deposition. The determination resulted in obvious potential separation which indicates the excellent selectivity of this system. Selective detection of dopamine by graphene-chitosan [32] was achieved by eliminating AA in the system, which was
attribute to the different charged characters of DA (pK_a = 8.87) and AA (pK_a = 4.10). Further more, gra

2.1 Materials

Graphite and hydrazine solution (50 wt%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Ani

c line (from Beijing Xingjin Chemical Plant) was distilled under reduced pressure and kept below 0°C before use. A

cetic acid (AA) was purchased from Fluka. Dopamine (DA) and uric acid (UA) were obtained from Sigma. Ammonium peroxydisulfate (APS), phosphoric acid (H_3PO_4), sulfuric acid (H_2SO_4), NaOH, KH_2PO_4 and Na_2HPO_4 (from Beijing Chemical Plant) were of analyti-cal reagent grade and used without further purification. Phosphate buffer solution (PBS, 0.05 M, pH 7.4) was pre-

PBS, 0.05 M, pH 7.4) was prepared with KH_2PO_4 and Na_2HPO_4. PBS of different pH value were prepared from stock solutions of 0.1 M H_3PO_4, KH_2PO_4, Na_2HPO_4 and NaOH. All the aqueous solutions were prepared with double-distilled water from a Millipore system (>18 MΩcm).

2.2 Instruments

Scanning electron microscopy (SEM) images were obtained from an XL30 ESEM FEG scanning electron mi-

croscope operating at 20 kV on the microstructures sput-
er-coated with gold. Transmission electron microscopy (TEM) image was imaged by Hitachi H600 transmission electron microscope operating at 100 kV. Fourier transform infrared spectra (FTIR) were collected on a Bruker Vertex 70 spectrometer (4 cm^{-1}) by coating sample/water dispersions on CaF_2 plates. Cyclic voltammetry measurements were performed in a conventional three-electrode cell with a platinum wire as auxiliary electrode and an Ag|AgCl (in 3 M KCl) as reference electrode on a CHI 920C Electrochemical Workstation (CHI, USA). The working electrodes were bare or modified glassy carbon electrodes (GCE, ) for a few minutes, the vial was kept in an oil bath (~95°C) for 1 h. After this, the system turned its color from brown into black, which indicated the reduction of GO to graphene.

2.3 Polymerization

Graphene oxide (GO) was synthesized from graphite by a modified Hummers’ method [37,38]. The as-purified graphene oxide suspensions were dispersed into water to create a 0.05 wt% dispersion by ultrasonication for 30 min. The resulted brown dispersion was then subjected to 30 min of centrifugation at 3000 rpm to remove any un-exfoliated graphite oxide.

Graphene-templated polyaniline (GO-PAN) was synthesized as following: typically, 5 mL GO dispersion was added into 10 mL of 0.05 M aniline, 0.05 M H_2SO_4 aqueous solution with vigorous stirring. Then, 1 mL 0.5 M APS aqueous solution which served as oxidant was added into the above mixture dropwise within one minute. After stirring for 24 hours, the green product was collected by centrifugation. After thoroughly rinsing with ethanol and deionized water, the resulted sample was vacuum-dried at room temperature. As contrast, a similar procedure was carried out during the polymerization of aniline only without GO and the resultant was abbreviated as PAN.

Unfunctionalized, chemically converted graphene sheets (GS) was synthesized from as-purified graphene oxide nanosheets [27]. Briefly, 10 mL of graphene oxide sheets (in water, 0.05 wt%) was mixed with 10 mL of water and 6 μL of hydrazine solution (50% in water) in a 50 mL glass vial. After being vigorously shaken or stirred for a few minutes, the vial was kept in an oil bath (~95°C) for 1 h. After this, the system turned its color from brown into black, which indicated the reduction of GO into graphene.

2.4 Preparation of the Modified Electrodes

A certain amount of GO-PAN was ultrasonicated in dis-

tilled water to form a homogenous dispersion. Then, 5 μL of the above solution was drop-cast onto the surface of a freshly polished glassy carbon electrode and dried in ambient atmosphere for 12 h. The PAN, GO and GS modi-fied electrodes were prepared by using the same proce-
dure and the corresponding modified electrodes were de-noted as GO-PAN/GCE, PAN/GCE, GO/GCE and GS/ GCE, respectively.

3 Results and Discussions

3.1 Morphology and Properties

Figure 1 presents the SEM images of GO-PAN (A) and PAN (B), the TEM image of GO (C). In Figure 1C, the

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TEM image reveals that the successful exfoliation of graphite down to individual GO nanosheets. It is easy to see that the GO layers are sheets that are several hundreds or thousands of nanometers thick with some pleats. They are transparent and very thin, which can be seen from the fringe of GO sheets. As shown in Figure 1A, the GO-PAN exhibited irregular shape in morphology and the sample sheets were also several hundreds or thousands of nanometers in size, which was consistent with the TEM result of GO. And PAN pillars were found homogeneously covered on graphene oxide template. While without adding GO, the obtained PAN (Figure 1B) was typical nanowire structured with 40–50 nm in diameter, which is in accordance with the well-accepted intrinsic fibril growth mechanism in PAN polymerization as previous reports [39,40]. Due to the distinct differences in morphology of GO-PAN and PAN, it is confirmed that GO played a key role in the synthesis of GO-PAN microsheets.

Molecular structures of GO, PAN and GO-PAN were characterized by FT-IR spectra. As shown in Figure 2, the spectrum of GO (curve a) illustrates the presence of C/C/O (νC-O at 1101 cm⁻¹), C/C/O/C (νC-O-C at 1230 cm⁻¹), C/OH (νC-OH at 1418 cm⁻¹), and C=O in carboxylic acid and carbonyl moieties (νC-O at 1728 cm⁻¹). The peak at 1588 cm⁻¹ may be attributed to skeletal vibrations of unoxidized graphitic domains [41]. PAN (curve b) exhibits the characteristic peaks of PAN at 1591 cm⁻¹ and 1504 cm⁻¹ which were corresponding to the stretching of quinoid (Q) and benzenoid (B) rings, respectively [42]. The bands situated at 1157 and 1309 cm⁻¹ are attributed to the vibration mode of C/H (in-plane) and stretching of the C-N bonds of the aromatic secondary amines, respectively. The FT-IR spectrum of GO-PAN (curve c) is almost the same as PAN (curve b). The above analysis indicates that the surface of the GO was completely wrapped in PAN, so there is no peak around 1700 cm⁻¹ which is due to C=O vibration. In this system, graphene oxide played a key role during the polymerization process. GO served as the hard template during the growth of GO-PAN microsheets.

3.2 Electrocatalytic Oxidation of AA, DA and UA

Figure 3 shows the voltammetric responses of the bare GC electrode and GO-PAN modified GC electrode toward AA, DA and UA. At the bare GC electrode (Figure 3A), AA demonstrates a broad oxidation peak at
264 mV. DA shows quasi-reversible electrochemical behavior with the anodic and cathodic peak potential at 190 mV and 114 mV, respectively. And UA shows an oxidation peak at 318 mV. It seems that the separation of the anodic peak potentials for these three species is not enough to obtain good selectivity at the bare GC electrode. In contrast, for the GO-PAN modified GC electrode (shown in Figure 3B), the anodic oxidation peaks of AA, DA and UA appear at about 25 mV, 249 mV and 373 mV, respectively. A distinct negative shift of AA oxidation peak and a positive shift of DA and UA oxidation peaks are found. The difference between AA and DA peak potentials is above 200 mV, which is enough to distinguishing AA from DA. The currents observed at GO-PAN/GCE (Figure 3B) are much enhanced compare with the bare GCE. This may be due to the increased surface area and/or the catalytic activity of the GO-PAN modified GC electrode. The above results demonstrate that the GO-PAN can not only accelerate the oxidation of AA, DA and UA, but also dramatically enlarge the peak separations among AA, DA and UA. The enlarged separations of the anodic peak potentials, coupled with the increased sensitivity, facilitate simultaneous determination of AA, DA and UA.

3.3 Electrochemical Behavior of AA, DA and UA in a Ternary Mixture

Differential pulse voltammetry (DPV) is a sensitive technique which has been usually undertaken for electrochemical detections [43], and it also have been often applied for simultaneous detection of AA, DA and UA. Figure 4 shows the DPV of the mixture solution of AA, DA and UA at bare GCE, GO/GCE, GS/GCE, PAN/GCE and GO-PAN/GCE. The oxidation peaks of AA and DA completely overlapped and show a broad overlapped peak at 180 mV at the bare GCE. In contrast, the DPV peaks are well-resolved at GO-PAN/GCE with the peak potentials at about −55 mV, 195 mV and 385 mV for AA, DA and UA, respectively. And the separations of the oxidation peak potentials for AA-DA, DA-UA and AA-UA are 250 mV, 190 mV and 440 mV, respectively. The large separations of the peak potentials allow selective determination of AA, DA and UA in the presence of the other two species, or simultaneous detection of them in their mixture.

Up to now, only DA has been detected on the graphene-chitosan modified GC electrode by Li’s group. However, AA has been completely eliminated by the use of graphene-modified electrode [32]. On the contrary, the oxidation of AA was reported on sulfonated polyaniline/graphene-modified GC electrode [36]. It is worthwhile noting that simultaneous determination of AA, DA and UA is achieved at the GO-PAN modified GC electrode. The reasons are concluded as the following aspects. First, molecular structures of DA and AA are distinct from each other. The π–π interaction between phenyl structure of DA and polyaniline make the easy arrival of DA molecules to the surface of modified electrode. Second, although the π–π interaction between penta-heterocycle of AA and polyaniline is weak, the presence of cationic emeraldine salt provides a remarkable electrostatic attraction to negatively charged AA and UA, but rejection to positively charged DA in PBS (pH 7.4) due to the charged characters of AA (pK_a = 4.10), DA (pK_a = 8.87) and UA (pK_a = 3.70). The electrostatic force leads to the realization AA oxidation on the GO-PAN modified GC electrode. Third, in the control experiments, only one distinct anodic peak is observed for GO-modified GC electrode at around 255 mV, and there are two anodic peaks observed for GS-modified GC electrode at around 150 mV and 275 mV, indicates that the oxidation peaks of AA and DA can not distinguish with each other on GO/GCE and GS/GCE. As shown in Figure 4, it is apparently that the oxidation peak currents at the GS/GCE are much higher than other modified electrodes. This may be attributed to the unusual electronic conductivity and high specific surface area of graphene, which reveals the preferable electrocatalytic ability towards AA, DA and UA. For GS/GCE, we used drop-casting method to prepare the modified electrode. However, the prepared graphene sheets tend to form irreversible agglomerates or even re-stack to form graphite through van der Waals interactions.

Fig. 3. CVs of 2.0 mM AA, 1.0 mM DA and 1.0 mM UA at bare GCE (A) and GO-PAN/GCE (B) in 0.05 M (pH 7.4) PBS. Scan rate: 50 mV s^{-1}.
during the drying process of the electrode, which caused the losing of the catalytic activity towards oxidation of AA, DA and UA. It seemed that it is possible for PAN/GCE to discriminate individual analyte. However, compared to GO-PAN/GCE, the AA oxidation peak is much lower, and the DA-UA peak-to-peak separation is smaller. GO-PAN has electrochemical activity in neutral solution, and its electrochemical activity has been greatly enhanced than the neat PAN fibers. The possible reason for this preferable electrochemical behavior may be attributed to the microsheets nanostructure, which provided large surface area to enhance the sensing signal and speeded up the electron transporting derived from enhanced basal planes of PAN backbones due to strong π–π stacking between PAN and GO (or graphene) [36]. In addition, we consider that the increased electrocatalytic activity may also be derived from the graphene component in the composite. During the aniline monomer oxidation polymerization process, GO might be reduced to graphene at the same time. Moreover, PAN can also served as the dispersant for graphene. In the composite, the active graphene surface not fully covered by PAN can also provide catalytic ability towards the oxidation of AA, DA and UA. Therefore, GO-PAN modified GC electrode can simultaneously detection AA, DA and UA in their mixture.

3.4 Effects of the Ratio of GO to Aniline and pH on the Electrochemical Response of AA, DA and UA

Figure 5 shows DPV curves of AA, DA and UA in their ternary mixture on the GO-PAN/GCE with different ratio of GO to aniline during the polymerization process. The ratio of GO to aniline (mass ratio) in GO-PAN-1, GO-PAN-2 and GO-PAN-3 are 1/10, 1/20 and 1/40, respectively. As shown in Figure 5, the anodic peak potentials for these three species are well-separated in the three ratios, and the maximal peak-to-peak separations are obtained on GO-PAN-2 modified GC electrode. GO-PAN-2 modified GC electrode can effectively resolve the merged voltammetric peak into three well-defined oxidation peaks at potentials around −55 mV, 195 mV and 385 mV for AA, DA and UA, respectively. And the separations of the oxidation peak potentials for AA-DA, DA-UA and AA-UA is 250 mV, 190 mV and 440 mV, respectively. These large anodic peak potential separations imply that GO to aniline ratio 1/20 is a very suitable stuff ratio for this system. And all the works in this paper were carried out under the condition of this.

In addition, the effect of solution pH value on the determination of AA, DA and UA in the mixture at GO-PAN/GCE was carefully investigated by DPV in a wide pH range from 3.0 to 9.0. When the solution pH was below 6.0, the anodic oxidation peaks of AA and DA were overlapped. When the solution pH was between 7.0 and 9.0, all the peak potentials for AA, DA and UA shifted negatively with higher pH value, which indicated that protons take part in their electrode reaction process [44].
The maximum separations of the peak potentials for AA-DA and DA-UA are 300 mV and 195 mV, obtained at pH 7.0 and pH 8.0, respectively. In order to obtain a high selectivity within the biological neutral matrices, pH 7.4 was finally selected as an optimum pH value for the determination of AA, DA and UA in their mixture in our experiments.

### 3.5 Simultaneous Determination of AA, DA and UA

Figure 6 shows DPV responses of GO-PAN-modified GC electrode towards AA, DA or UA when the concentration of one species changes with the other two species keeping constant. The peak current of AA increase linearly with the increasing of AA in concentration from 150 μM to 1050 μM (I (μA) = -10.884 - 0.008 C (μM)). Similarly, the oxidation peak currents of DA and UA are also proportional to their concentrations in the range of 1–14 mM for DA (I (μA) = -6.324 - 0.265 C (μM)) and 3–26 mM for UA (I (μA) = -5.129 - 0.209 C (μM)). The detection limits for AA, DA and UA are found to be 50 μM, 0.5 μM and 1 μM, respectively. To evaluate the reproducibility of GO-PAN/GCE, the peak currents of 10 successive measurements by DPV in ternary mixture solution of 1 mM AA, 0.5 mM DA and 0.5 mM UA were determined. And the relative standard deviation (RSD) obtained were 1.45%, 4.75% and 4.22% for AA, DA and UA, respectively, which indicate that the GO-PAN/GCE is not subject to surface fouling by the oxidation product. These results strongly suggest that AA, DA and UA can be selectively and sensitively determined at GO-PAN/GCE in their ternary mixture.

### 4 Conclusion

In summary, nanostructured polyaniline microsheets were generated chemically with GO sheets serving as templates via typical APS oxidation of aniline monomer. The resulted GO-PAN microsheet showed preferable electroactivity even in neutral media and displayed excellent electrocatalytic activity towards oxidation of AA, DA and UA. Furthermore, in AA, DA and UA ternary mixture, three well-defined peaks located at about −55 mV, 195 mV and 385 mV were obtained. Therefore, it demonstrated that this material competent for the determination of AA, DA and UA simultaneously. And this GO-PAN based material could also be expected to become a promising candidate for electroanalytical and clinical applications in the future.

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