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Short communication

# Easy modification of glassy carbon electrode for simultaneous determination of ascorbic acid, dopamine and uric acid

Soundappan Thiagarajan, Tsung-Hsuan Tsai, Shen-Ming Chen*

*Electroanalysis and Bioelectrochemistry Lab, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei 106, Taiwan, ROC*

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ABSTRACT

A glassy carbon electrode (GCE) has been modified by electrochemical oxidation in mild acidic media (0.1 mol $l^{-1}$ $H_2SO_4$) and could be applied for individual and simultaneous determination of ascorbic acid (AA), dopamine (DA) and uric acid (UA). Oxidized GCE shows a single redox couple ($E^{0\prime}$ = −2.5 mV) which is based on the formation functional groups during the electrochemical pretreatment process. Proposed GCE successfully decreases the over potentials for the oxidation process of these species (AA, DA and UA) comparing with bare GCE. The oxidized GCE has its own simplicity, stability, high sensitivity and possesses the potential for simultaneous determination of AA, DA and UA.



## 1. Introduction

Dopamine (DA), ascorbic acid (AA) and uric acid (UA) usually coexist together and considered as important molecules for physiological processes in human metabolism. DA, AA and UA deficiencies result in several diseases and disorders (Martin, 1998; Wightman et al., 1988; Heinz et al., 1995; Dutt and Mottola, 1974). Determination of these species exhibits overlapping oxidation peaks at solid electrodes. Thus, simultaneous determinations of these species are keen on a special interest in biosensors. Various film modified electrodes have been reported for simultaneous determination of these three compounds. For example, tetrabromo-p-benzoquinone modified carbon paste (Zare et al., 2005), carbon ionic liquid (Safavi et al., 2006) and carbon ceramic electrode (Salimi et al., 2006), ribonucleic acid (Kang and Lin, 2006), ruthenium oxide (Shakkthivel and Chen, 2007), poly(4-(2-pyridylazo)-resorcinol) (Chen et al., 2007), oracet blue (Zare et al., 2006), poly(eriochrome black T) (Yao et al., 2007), PtAu hybrid (Thiagarajan and Chen, 2007), single-wall carbon nanotubes with cetylpyridinium bromide (Zhang et al., 2007), polymer (Lin et al., 2008), $LaFeO_3$ nanoparticles (Wang et al., 2008) and zinc oxide composite modified glassy carbon electrode (GCE) (Tang et al., 2008) were reported.

In these, most of the films, fabricated on GCE, show the importance of GCE in the field of biosensors. GCE is made up of special type of carbon which fabricated by pyrolsis of polymer resin (Jenkins and Kawamura, 1976) exhibits good electrical conductivity with well-defined surfaces. The chemical and electrochemical pretreatment shows significant changes in physical and electrochemical properties of GCE. For example, surface properties of electrochemically pretreated GCE (Nagaoka and Yoshino, 1986) and electrochemical activation of GC (Barbero and Kotz, 1993; Alliata et al., 1999) were reported. In particular, electrochemical activation of GCE results in the generation of surface functional groups which could be used as capacitor electrodes (Sullivan et al., 2000a,b). Also, the anodized GCE for biologically important compounds (Maeda et al., 1999, 2000), interface between nafion and oxidized GC surfaces for oxygen reduction (Maruyama and Abe, 2002), electrochemically treated GC with Pt catalyst for methanol oxidation, platinum electrodeposited oxidized GC for formic acid oxidation were reported (Jovanović et al., 2004, 2005). Further the determination of vitamin B2 (Shiu and Shi, 2000), UA (Shi and Shiu, 2001) and AA and UA (John, 2005) have been reported using electrochemically activated GCE. These literature reports validate the surprising electrocatalytic properties of oxidized GCE. Thus, based on this context, first time we have employed the oxidized GCE for simultaneous determination of DA, AA and UA. Interestingly, the oxidized GCE successfully separates the electrooxidation of these species into well-defined three voltammetric peaks using CV.

## 2. Experimental

### 2.1. Apparatus

All electrochemical experiments were performed using CHI 1205a potentiostat (CH Instruments, USA). The BAS GCE ($\varphi$ = 0.3 cm

* Corresponding author. Tel.: +886 2 27017147; fax: +886 2 27025238.
*E-mail address:* smchen78@ms15.hinet.net (S.-M. Chen).

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in diameter, exposed geometric surface area 0.07 $cm^2$, Bioanalytical Systems, Inc., USA) was used. A conventional three-electrode system was used which consists of a Ag/AgCl (3 mol $l^{-1}$ KCl) as a reference, bare or oxidized GCE as working and platinum wire as counter electrode. Electrochemical impedance studies (EIS) were performed using ZAHNER impedance analyzer (Germany). The AFM images were recorded with multimode scanning probe microscope (Being Nano-Instruments CSPM-4000, China).

### 2.2. Chemicals and solutions

DA, AA and UA were purchased from Sigma–Aldrich (USA). All other chemicals (Merck) used were of analytical grade (99%). Double distilled deionized water was used to prepare all the solutions. A phosphate buffer solution (PBS) of pH 7.0 was prepared using $Na_2HPO_4$ (0.05 mol $l^{-1}$) and $NaH_2PO_4$ (0.05 mol $l^{-1}$). Pure nitrogen was passed through all the experimental solutions.

### 2.3. Fabrication of oxidized GCE

Prior to electrooxidation process, GCE was well polished with the help of BAS polishing kit with aqueous slurries of alumina powder (0.05 μm), rinsed and ultrasonicated in double distilled deionized water. Further the GCE was oxidized by performing 10 cycles in 0.1 mol $l^{-1}$ $H_2SO_4$ between 0 and 2 V at 0.1 V/s (inset of Fig. 1A). The oxidized GCE was washed with deionized water and transferred to pH 7.0 PBS for the further studies.

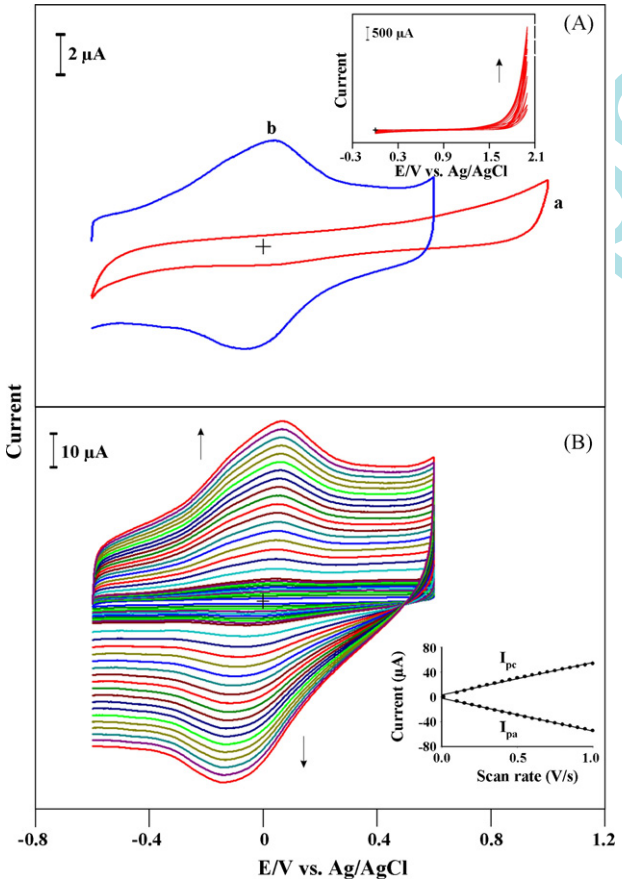


**Fig. 1.** (A) CV response of (a) bare and (b) oxidized GCE in pH 7.0 PBS. Inset of part (A) shows electrooxidation process of GCE in 0.1 mol $l^{-1}$ $H_2SO_4$ in the range of 0 and 2 V for 10 cycles at 0.1 V/s. (B) CV response of oxidized GCE surface in pH 7.0 PBS at different scan rates: 0.01–1.0 V/s. Inset shows the plot of cathodic and anodic peak currents of oxidized GCE vs. scan rate.

## 3. Results and discussion

### 3.1. Characterization of oxidized GCE

The inset of Fig. 1A shows the oxidation process of GCE. Here the increasing anodic peak current at 2.0 V indicates the oxidation process of GCE surface. To confirm the oxidized surface, it was transferred to pH 7.0 PBS for CV studies. In pH 7.0 PBS, the oxidized GCE displays a couple of well-defined and reversible redox peak (Fig. 1A, curve b) with the ($E^{0\prime}$) of about −2.5 mV (at 0.1 V, vs. Ag/AgCl). At the same time, well polished bare GCE (Fig. 1A, curve a) fails to exhibit such kind of redox peak in pH 7.0 PBS. This examination validates the oxidized surface of GCE. The electrochemical oxidation process of GCE results in the formation of functional groups like carbonyl, quinoid, carboxyl and hydroxyl radical species on the electrode surface (Nagaoka and Yoshino, 1986). In particular, the formation of these functional groups exhibits a redox peak and gives the special electrochemical properties. Further based on the previous literature reports (Sullivan et al., 2000a,b; Yamazaki et al., 2007) it was found that the important oxygen functional group found on the electrode surface supposed to be carboxyl and the remaining is carbonyl, etc. At the same time, we did not mean that other groups (carbonyl, quinoid and hydroxyl) did not involve in electrocatalytic process, but we are assuming that carboxyl group will be the major functional group for the proposed electrode. Further, (Sullivan et al., 2000a,b; Vettorazzi et al., 2008) clearly report about the surface porous nature of GC. They have claimed that anodic pretreatment process does not create any porous nature but opens the closed pores which already present on electrode surface (Braun et al., 1999). Thus, this type of oxidation will result in the formation of porous nature on electrode surface (Nagaoka and Yoshino, 1986). Also, the proposed GCE oxidation process is not new one (Shiu and Shi, 2000; Shi and Shiu, 2001). But this work follows the mild oxidation procedure to minimize surface damage and to possess the functional groups on GCE, respectively. Further the oxidized GCE was examined for different scan rate studies in pH 7.0 PBS. Fig. 1B exhibits the different scan rate studies of oxidized GCE in the range of 0.01–1 V/s. Here for the increasing scan rate, the redox peak increases and both the oxidation and reduction peaks shift to more negative and positive potentials. Also, the anodic and cathodic peak currents were linearly dependent on the scan rate (inset of Fig. 1B), which indicates that oxidized GCE surface reaction was a surface-controlled process. The Nyquist plot of electrochemical impedance spectra of bare GCE (Fig. 1A, curve a, Supplementary file) is smaller (curve a, $R_{et}$ = 3.03) compared with oxidized GCE (curve b, $R_{et}$ = 4.99) it indicates that the surface of oxidized GCE has been modified with the functional groups which make the electron transfer kinetics process as a slower one. Furthermore the oxidized GCE has been examined in various pH solutions (Fig. 1B, Supplementary file). From the plot of formal potentials ($E^{0\prime}$) vs. pH of oxidized GCE (inset of Fig. 1B, Supplementary file), the slope value was found as 48 mV/pH, which is close to the expected value of 59 mV/pH for one electron and one proton reaction process, respectively.

### 3.2. AFM analysis

AFM tapping mode was employed for the surface morphological analysis of GCE. The unmodified (Fig. 2A), oxidized (Fig. 2B) and well polished bare GCE (after oxidation) (Fig. 2C) were scanned for the comparison studies. Fig. 2A shows the surface nature of unmodified bare GCE. At the same time, the oxidized GCE (Fig. 2B) shows the rough surface with the formation of small grains on it. Next, the oxidized GCE was polished well and scanned again (Fig. 2C). Here the well polished GCE (Fig. 2C) exhibits its own smooth surface. These results validate the oxidation process on the GCE surface, respectively. Further the oxidized GCE possesses higher surface roughness

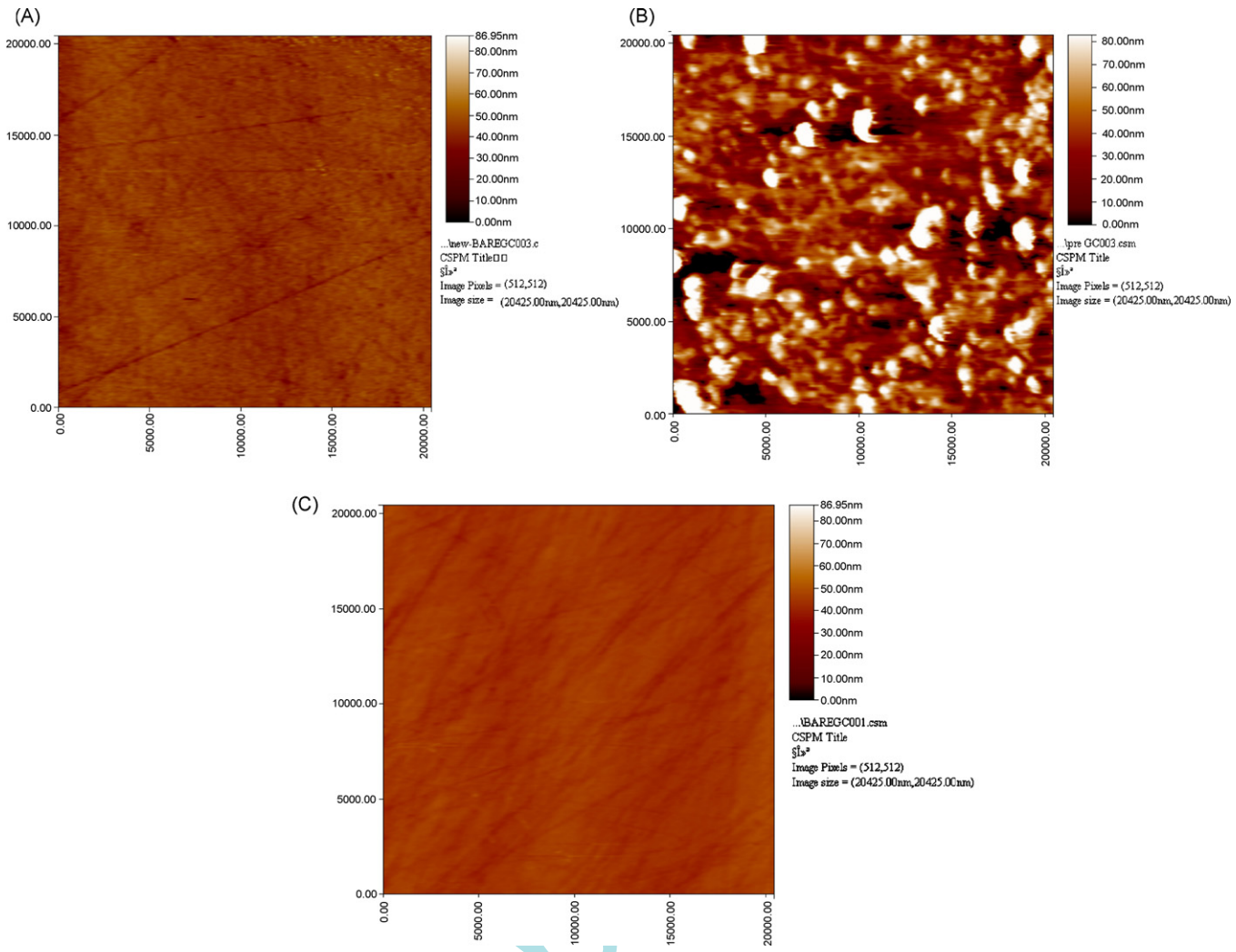


**Fig. 2.** AFM 2D images of (A) unmodified, (B) oxidized and (C) polished bare GCE.

comparing with unmodified and polished bare GCE. The average roughness values (Table 1, Supplementary file) were obtained from 2D image topographies of unmodified, oxidized and bare GCE surfaces. The surface roughness values are averages calculated from several images acquired in different regions of the respective samples. Here the unmodified GCE surface exhibits a slight roughness (2.02 nm) which can be seen in Fig. 2A. Further there is a great increase in the surface roughness (15.4 nm) for the oxidized GCE. At the same time, the well polished GCE possesses very low surface roughness (1.4 nm). Furthermore, the very low surface roughness of well polished bare GCE comparing with unmodified and oxidized GCEs proves that the own characteristic surface nature of the GCE could be retained. From the grain size analysis reports, the average heights of the unmodified, oxidized and well polished GCE were found as 7.5, 52.65 and 2.12 nm, respectively. The great decrease in the average height of well polished GCE resembles that we can access the habitual GCE surface by polishing. Furthermore, the average diameter of disordered grains which formed on the oxidized GCE surface was found as 340.69 nm. Finally, these AFM results clearly illustrate the oxidized surface, reproducible and reusable nature of oxidized GCE for continuous electrochemical applications.

### 3.3. Simultaneous determination of AA, DA and UA

The individual electrocatalytic oxidation of AA, DA and UA were investigated using CV (Fig. 2, Supplementary file). Here the

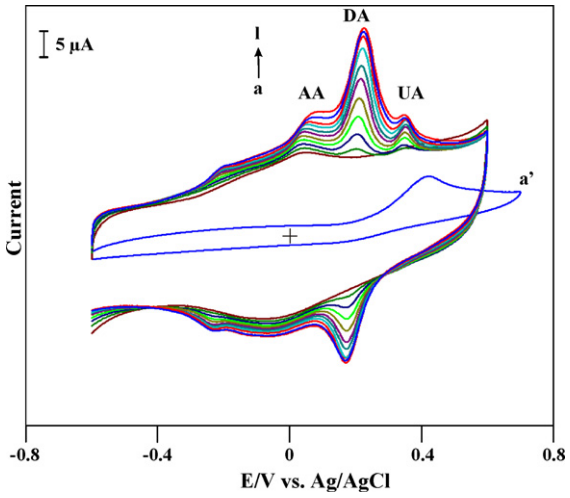


**Fig. 3.** CVs of simultaneous determination of AA, DA and UA using oxidized GCE. AA, DA and UA concentrations were in the linear range of AA (a-l: 0, 1, 1.97, 2.92, 3.85, 4.76, 5.66, 6.54, 7.4, 8.23, 9.06 and $9.88 \times 10^{-4}$ mol $l^{-1}$), DA (a-l; 0, 1, 1.97, 2.92, 3.85, 4.76, 5.66, 6.54, 7.4, 8.23, 9.06 and $9.88 \times 10^{-6}$ mol $l^{-1}$) and UA (a-l; 0, 1, 1.97, 2.92, 3.85, 4.76, 5.66, 6.54, 7.4, 8.23, 9.06 and $9.88 \times 10^{-5}$ mol $l^{-1}$) (a′ = bare GCE; AA, DA and UA; $9.88 \times 10^{-4}$, $9.88 \times 10^{-6}$ and $9.88 \times 10^{-5}$ mol $l^{-1}$).

oxidized GCE reduces the anodic over potentials and exhibits well-defined anodic peaks for AA, DA and UA oxidation (0.039, 0.20 and 0.34 V) comparing with bare GCE. The next attempt was employed for the simultaneous determination of AA, DA and UA. Here the oxidized GCE exhibits well-defined three separate anodic peaks for the simultaneous determination of AA, DA and UA using CV (Fig. 3). The presence of functional groups on oxidized GCE resolves the mixed voltammetric response of these species (AA, DA and UA) into three well-defined voltammetric peaks at the potentials of 0.064, 0.227 and 0.354 V, respectively. Further the peak separations between DA and AA (0.163 V), UA and DA (0.127 V) is sufficient enough to exhibit them as a well-defined three separate peaks. Here, DA exhibits both oxidation and reduction peaks in this process. It shows the reduction peak around 0.173 V. At the same time, the bare GCE fails to resolve all these three peaks and gives a single oxidation peak at around 0.422 V. Further the simultaneous determination of these species (AA, DA and UA) concentrations was in the linear range of $1.97–9.88 \times 10^{-4}$, $1.97–9.88 \times 10^{-6}$ and $1.97–9.88 \times 10^{-5}$ mol l$^{-1}$, respectively. Here all oxidation peak currents increase linearly with respect to their increasing concentrations. Also, from the calibration plots (figure not shown) the linear regression equations for AA, DA and UA were expressed as $I_{pa}$ (μA) = 0.0088$C$ ($10^{-4}$ mol l$^{-1}$) – 0.0377, $R^2$ = 0.995, $I_{pa}$ (μA) = 3.1575$C$ ($10^{-6}$ mol l$^{-1}$) – 0.7715, $R^2$ = 0.978 and $I_{pa}$ (μA) = 0.0833$C$ ($10^{-5}$ mol l$^{-1}$) – 0.4508, $R^2$ = 0.966, respectively. These results are comparable with previous literature reports (Zhang et al., 2008) (Table 2, Supplementary file). The relative standard deviation (% RSD) for all these species determination on oxidized GCE was less than 1.5% which shows the efficiency and linear nature of oxidized GCE. Also these results prove that the nature of the peak separation between DA and UA was possible in the higher concentrations of AA ($10^{-4}$ mol l$^{-1}$). It is also interesting to note that the individual or simultaneous determination of these three analytes was possible without any interference and suitable to detect these species in less or equal concentrations, respectively. Based on the previous literature report (Shi and Shiu, 2001) a scheme (Scheme 1, Supplementary file) has been proposed for the simultaneous determination of AA, DA and UA. Finally, from these CV results, it can be concluded that the oxidized GCE is a suitable mediator for the simultaneous determination of AA, DA and UA.

### 3.4. Stability and reproducibility

Stability of oxidized GCE was investigated by storing at room temperature in presence and absence of pH 7.0 PBS. It was stable for one month and after four weeks a gradual decrease (10%) has been found from the current initial values. Oxidized GCE can be prepared within 6.6 min and by manual polishing with alumina slurry we can retain the original nature of the GCE very easily. The background current variations of the oxidized GCE surface at five GCE were less than 2.0 % which validates the reproducible nature of the oxidized effect on the GCE surface. These results suggest that the oxidized GCE possesses the long term stability and reproducibility.

## 4. Conclusion

For the first time we are reporting a simple method for simultaneous determination of AA, DA and UA using oxidized GCE. The mild oxidized GCE shows efficient electrocatalytic activity for individual and simultaneous determination of AA, DA and UA. The proposed electrode was very easy to fabricate and eco-friendly and 100% reusable for multiple time analysis. Also, it overcomes the interest of using other film modified GCEs and found as novel and cheaper one for simultaneous determination of AA, DA and UA.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.12.010.

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