Selective and Sensitive Determination of Uric acid in the Presence of Ascorbic Acid Using Gold Nanoparticles Modified Glassy Carbon Electrode

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Abstract—The present work reports a simple, facile and reproducible method for the fabrication of glassy carbon electrode (GCE) with AuNPs by drop casting method and its electrocatalytic application. AuNPs were directly deposited on GCE surface by cycling the potential between -0.2 V to 1.6 V for 15 potential cycles. The appearance for the formation of gold oxide and the respective gold oxide reduction peak confirmed the successful electrodeposition of AuNPs on GCE. Further, the size and morphology of AuNPs deposited electrode was characterized by SEM. The particles are spherical in nature with a diameter of 30 nm. The electrocatalytic behavior of AuNPs/GCE electrode was examined by studying the oxidation of ascorbic acid and uric acid as the probes. Since, AuNPs modified electrode greatly enhanced the oxidation potential of AA and UA, it is used for the simultaneous determination of them. The modified electrode also used for the selective determination of UA in the presence of high concentration of AA. Since the present modified electrode showed better electrocatalytic behavior at physiological pH, it can be used for the determination of AA and UA in the clinical analysis.

Keywords—Gold nanoparticles; Electrodeposition; Electrocatalysis; Scanning electron microscope; Ascorbic acid; Uric acid;

1. INTRODUCTION

I. Gold nanoparticles (AuNPs) are the most stable metallic nanostructures and have been intensively studied in materials science.1 The size and shape dependent electronic, optical and catalytic properties make AuNPs a fascinating material for various applications such as molecular recognization, drug delivery, biomedical imaging and photothermal therapy.2-5 Gold nanoparticles can be prepared using different chemical methods, such as direct electrodeposition, deposition–precipitation, sol–gel technique, impregnation, co-precipitation, metal organic-chemical vapor deposition, incipient wetness and dip-coating. In catalysis, the AuNPs-tailored electrodes find applications in the electrocatalytic reduction of oxygen6 and oxidation of CO,7 methanol,8 and the determination of various biologically important

biomolecules.9-12 For the applications of AuNPs in electrocatalysis, the AuNPs has to be immobilized into a conducting solid surface.13 Therefore, the AuNPs synthesized as colloidal solution must be immobilized by various methods such as layer-by-layer assembly, self-assembly of AuNPs on amine or thiol terminal of self-assembled monolayer of conducting solid surface, dry coating, spin coating and spray coating techniques.14-16 Instead of preparing AuNPs and immobilizing them onto solid surface, direct deposition of AuNPs from the Au3+ aqueous solution will be effective method to prepare AuNPs modified electrode in short time. Electrodeposition and electroless deposition are the suitable methods for the fabrication of AuNPs modified electrode directly from the precursor solution.17,18 Since it is difficult to control the deposition in electroless deposition which is also not reproducible, electrodeposition becomes a convenient method to prepare AuNPs modified electrode directly from the precursor solution.19,20 Various electrodeposition methods are available in the literature for the deposition of AuNPs directly in the solid surface including applying a potential step to a glassy carbon electrode,21,22 pulse techniques23 and applying a constant potential.24

II. Ascorbic acid (AA) is an unsaturated lactone. It is a soluble vitamin and an essential nutrient for humans. It is an antioxidant which can protect the body against oxidative stress.25 For adults, AA doses of 100-2000 mg/day are required to treat vitamin C deficiency.26 Higher intakes of AA have been associated with lower blood pressure, respiratory symptoms and cancer. Uric acid (UA) is an end product of purine metabolism and is related to the purine bases of nucleic acid.27 In normal serum, UA levels are less than 420 μmol/L in men and 330-360 μmol/L in women.27 High concentration of UA in blood can lead to a type of arthritis known as gout.28 Hyperuricemia may occur from excessive production of urate (overproduction) or decreased elimination (under excretion). Although several reports are available in the literature, the sensitivity and selectivity in the simultaneous determination of AA and UA still faces problems. Since AA usually coexist in higher concentration than UA in body fluids, the selective determination of UA in the presence of high concentration of AA is highly significant in the clinical point of view.29, 30 In this paper, AuNPs were directly deposited on GCE surface in acidic aqueous solution of Au3+ ions. The modified electrode was characterized by

SEM. It showed the uniformly distributed spherical AuNPs. The modified electrode was used for the simultaneous determination of AA and UA. It is also attempted to selectively determine UA in the presence of high concentration of AA.

2. EXPERIMENTAL

2.1 Materials

Hydrogen tetrachloroaurate (HAuCl4.3H2O) was purchased from Sigma. Ascorbic acid (AA) and uric acid (UA) were purchased from Sigma-Aldrich and were used as received. 0.2M phosphate buffer (PB) solution was prepared using Na2HPO4 and NaH2PO4. All other chemicals were of analytical grade and were used as received. Double distilled water was used for preparing all solutions. Indium tin oxide (ITO) plates were purchased from Asahi Beer Optical Ltd., Japan.

2.2 Instrumentation The electrochemical measurements and ac electrical impedance spectra were carried out with CHI electrochemical workstation (Model 643B, Austin, TX). Electrochemical measurements were performed in a conventional two-compartment three-electrode cell with GCE as a working electrode, platinum wire as a counter electrode and NaCl-saturated Ag/AgCl as a reference electrode. All the electrochemical experiments were carried out under a nitrogen atmosphere at room temperature. Before using GCE, it was polished using alumina slurry and sonicated in distilled water for 15min and checked with 1 mM [Fe(CN)6]3-/4- redox couple. Figure 2.1 shows the CVs obtained for GCE in a mixture of 1 mM [Fe(CN)6]3-/4- and 0.1 M KCl solution. Bare GCE shows a peak to peak separation of ~60 mV indicating the cleanliness of the GCE.

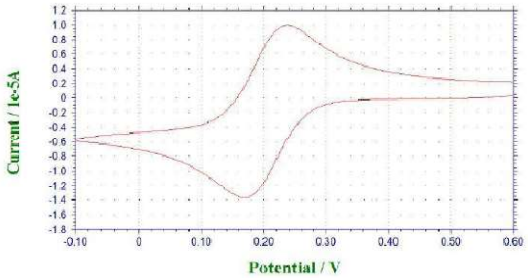

Figure 2.1. CV obtained for bare GCE in 1 mM [Fe(CN)6]3-/4- and 0.1 M KCl solution.

2.3. Electrodeposition of gold nanoparticles on GCE The well-cleaned GCE electrode was immersed into a solution of 1 mM HAuCl4.3H2O in 0.1M H2SO4 solution which acts as an electrolyte. Gold nanoparticles were electrochemically deposited at GCE surface by cycling the potential between -0.2 V to 1.6 V for 15 potential cycles at a scan rate of 50 mV s-1. After 15 cycles, the electrodes were cleaned with distilled water and used for further analysis. For morphological studies, AuNPs were deposited in ITO plates under similar condition for 15 cycles.

3. RESULTS AND DISCUSSION

3.1. Electrochemical deposition of gold nanoparticles

Gold nanoparticles were electrochemically deposited by the electrochemical reduction of Au3+ ions (HAuCl4.3H2O) by sweeping the potential from -0.2 V to 1.6 V. Figure 3.1 shows the continuous cyclic voltammograms obtained for GCE in 1mM HAuCl4 in 0.1 M H2SO4 at a scan rate of 50 mV s-1.

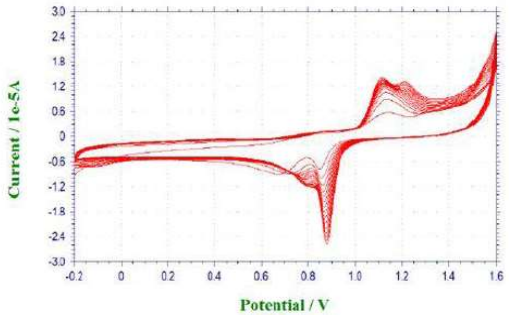

Figure 3.1. Continuous CVs obtained for GCE in 1mM HAuCl4 in 0.1 M H2SO4 at a scan rate of 50 mV s-1.

Since the starting potential is much low, the deposition of AuNPs begins at -0.2 V itself. In the first cycle, a shoulder peak at 0.8 V and two sharp peaks at 1.1 V and 1.3 V were observed in the forward cycle indicating the formation of gold oxide from the deposition AuNPs in 0.1M H2SO4 solution. The various gold oxide formation might be due to the various crystalline planes formed during the deposition of AuNPs. In the reverse cycle, two reduction peaks at 0.9 V and 0.7 V were observed indicating the reduction of gold oxide to nanogold. In subsequent cycles, the gold oxide formation and gold oxide reduction peak currents were increased indicating further deposition of AuNPs from HAuCl4 solution. After 15 cycles, the peak intensities were almost constant indicating the complete coverage of GCE surface with gold nanoparticles. The formation of gold oxide and its subsequent reduction indicated the successful deposition of AuNPs at GCE surface.

3.2. Morphological characterization using SEM

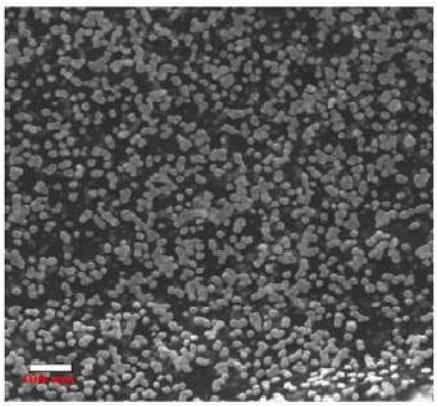

Figure 3.2. SEM images obtained AuNPs deposited ITO substrates

The surface morphology of AuNPs electrodeposited ITO substrates was analyzed by SEM. Figure3.2 shows the SEM images obtained for AuNPs deposited ITO substrates from HAuCl4 solution under identical condition used for the deposition of AuNPs at GCE. SEM image shows the uniform spherical AuNPs on ITO and the AuNPs were almost uniformly covered throughout the ITO substrate. The particles were well dispersed indicating that no aggregation of AuNPs takes place during electrochemical deposition. The size of the AuNPs was found to be 30 nm. From SEM images, it is clear that the particles are spherical in nature with 30 nm size and uniformly distributed without any significant aggregation. Since the deposition of AuNPs was successfully confirmed on GCE electrode, the AuNPs modified GCE electrode was used for electrochemical determination of AA and UA. The modified electrode is termed as GCE/AuNPs.

3.3 Electrochemical Oxidation of AA and UA at GCE/AuNPs and bare GCE

Further, the electrocatalytic activity of GC/AuNPs electrode was examined by taking AA and UA as probes. Figure 3.3 shows the CVs obtained for 0.5 mM AA at bare GCE and GCE/AuNPs electrodes in 0.2 M PB solution (pH 7.2). The oxidation of AA was observed at 0.36 V at bare GCE and for further potential cycles, decrease in peak current and shifting of oxidation potential to more positive potential were observed due to surface fouling effect caused by the oxidation products of AA. On the other hand, GCE/AuNPs electrode, AA oxidation was observed at 0.24 V with enhanced peak current compared to bare GCE and the oxidation peak was stable on repetitive measurements.

The enhanced oxidation current and the shifting of oxidation potential to less positive side compared to bare GCE might be due to the high surface area of AuNPs and also high conducting nature of AuNPs. The high conducting nature of AuNPs also prevents the surface fouling effect caused by the oxidation products of AA.

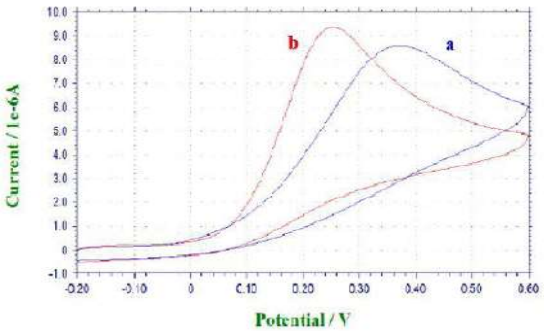

Figure 3.3 CVs obtained for 0.5 mM of AA at (a) bare GC and (b) GC/AuNPs in 0.2 M PB solution (pH 7.2)

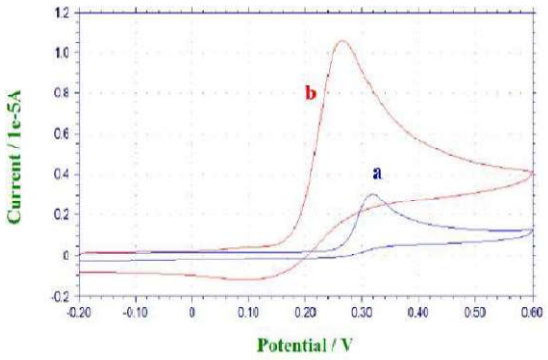

Figure 3.4 CVs obtained for 0.5 mM of UA at (a) bare GC and (b) GC/AuNPs in 0.2 M PB solution (pH 7.2).

Similarly, the oxidation of UA at bare GCE and GCE/AuNPs were also studied at PB solution. Bare GCE shows the oxidation of UA at 0.32 V (Figure 3.4). On the other hand, GCE/AuNPs electrode greatly enhanced the oxidation peak currents of UA and decreased the oxidation overpotential indicating the high catalytic activity of AuNPs towards UA oxidation.

Since GCE/AuNPs electrode successfully catalyses the electrochemical oxidation of AA and UA, it can be used for the simultaneous determination of AA and UA in a mixture at physiological pH. It is mentioned in the literature elsewhere that the purine metabolic final product UA level in urine and in human blood serum is an indication for several diseases like gout. AA usually coexists with UA and hence simultaneous determination of AA and UA is of highly important. In addition, the concentration of AA is usually higher than that of UA and hence selective determination of UA in the presence of AA is much significant in the clinical point of view.

3.4. Electrochemical Determination of UA at GC/AuNPs Electrode

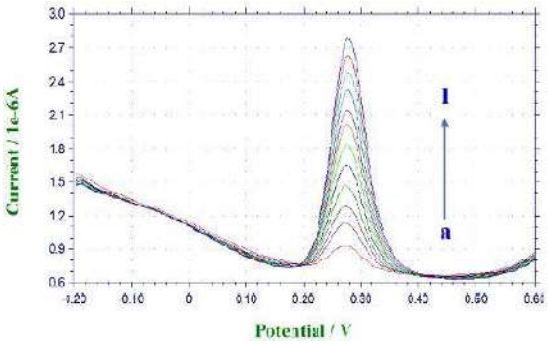

Figure 3.5. DPVs obtained for the increment of 5 µM UA at GC/AuNPs electrode (a-l) in 0.2 M PB solution.

Figure3.5 shows the differential pulse voltammograms (DPVs) obtained for the simultaneous increment of 5 µM UA at GC/AuNPs electrode in 0.2 M PB solution. The oxidation of UA was first observed at 0.29 V. On each addition of 5 µM UA, the peak current of UA was steadily increased without changing the oxidation peak potential. The peak currents were linearly increased with

UA concentration from 5 to 60 µM with a correlation coefficient of 0.9995 (Figure 4.6) indicating that GCE/AuNPs was highly suitable for the individual electrochemical determination of UA.

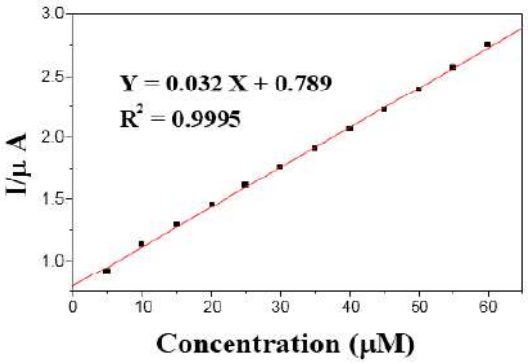

Figure 3.6. Plot of concentration versus UA oxidation peak current.

3.5. Simultaneous determination of AA and UA at GC/AuNPs Electrode

Further, GCE/AuNPs electrode was used for the simultaneous determination of AA and UA in 0.2 M PB solution. Figure 3.7 shows the simultaneous determination of 30 µM DA and 10 µM UA in PB solution. For the first addition of AA and UA, two well defined peaks at 0.05 V and 0.25 V were observed corresponding to the oxidation of AA and UA at GCE/AuNPs electrode. For the subsequent addition of 30 µM AA and 10 µM UA, the oxidation peak currents of both AA and UA were linearly increased. The oxidation of peak potential of UA was not changed during each addition whereas the oxidation peak potential of AA were slightly shifted to more positive potential. The plot of concentration of AA and UA versus oxidation peak current were linear for the range of 30-360 µM for AA and 10-120 µM for UA with a regression coefficient of 0.9991 and 0.9901, respectively (Figures 3.8 and 3.9). Since the AuNPs modified electrode successfully separated the oxidation peaks of AA and UA with a peak-to-peak separation of ~175 mV at physiological pH, this electrode can be used for the simultaneous determination of AA and UA in human body fluids like human urine and blood serum samples.

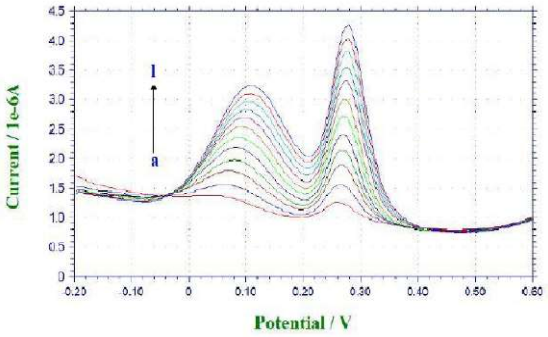

Figure 3.7 DPVs obtained for the increment of 30 µM AA and 10 µM UA at GC/AuNPs electrode (a-l) in 0.2 M PB solution.

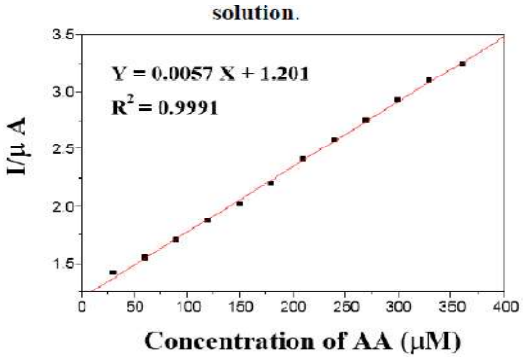

Figure 3.8. Plot of concentration versus AA oxidation peak current.

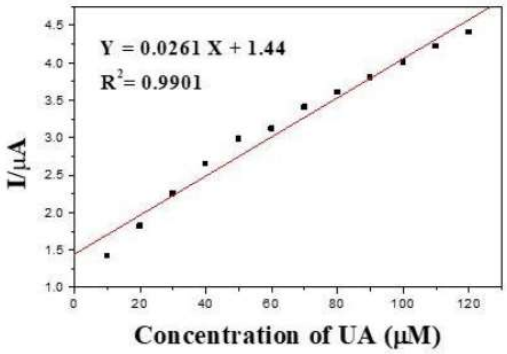

Figure 3.9. Plot of concentration versus UA oxidation peak current.

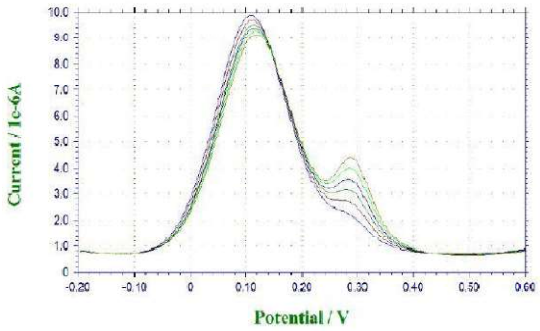

Figure 3.10 DPVs obtained for the increment of 20 µM UA in the presence of 1 mM AA at GC/AuNPs electrode in 0.2M PB solution.

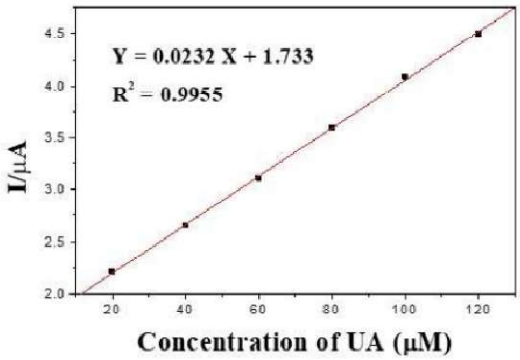

Figure 3.11. Plot of concentration versus UA oxidation peak current.

3.6. Selective determination of UA at GC/AuNPs Electrode

It is already stated that the concentration of AA usually higher than UA in human body fluids. Hence the determination of UA in the presence of high concentration AA is another goal of this present investigation.

Figure 3.10 shows the DPVs obtained for the addition of 20 μM UA in the presence of 1 mM of AA in 0.2 M PB solution. Even in the presence of 50 times higher concentration of AA, the UA peaks were clearly resolved indicating the successful selective determination of UA at GCE/AuNPs electrode. Further addition of UA increases the peak current of UA without affecting peak potential. For the concentration range of 20-120 μM UA in the presence of 1000 μM UA, the peak currents of UA were linear with a correlation coefficient 0.9955 (Figure 4.11). On the other hand, the oxidation peak potential were slightly shifted to more positive potential for AA and oxidation peak currents were slightly decreased due to the surface fouling effect. Although AA oxidation slightly suffered by fouling effect, it did not affect the oxidation of UA oxidation. From this, it is clear that the present modified electrode were highly suitable for the selective determination of UA in the presence of high concentration of AA.

4. CONCLUSIONS

In the present work, we have demonstrated the fabrication of GCE with AuNPs by electrodeposition method. SEM analysis showed that the particles are spherical in nature and uniformly distributed without any aggregation with a size of 30 nm. Further, AuNPs modified electrode were enhanced the oxidation peak currents of AA and UA and hence used for the determination of UA. The modified electrode separated the oxidation peak potential of AA and UA with a peak separation of 185 mV and used for the simultaneous determination of them. The present modified electrode was also successfully used for the selective determination of UA in the presence of high concentration of AA. The high conducting nature of AuNPs and high surface area of nanogold is the cause for the high electrocatalytic activity of AuNPs modified GCE.

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