Journal of Chemical and Pharmaceutical Research, 2014, 6(12):823-831



Research Article

ISSN : 0975-7384 CODEN(USA) : JCPRC5

Simultaneous electrochemical determination of ascorbic acid, dopamine and uric acid using hollow gold nanospheres modified electrode

S. Basavanna^a, B. K. Chethan^b and Y. Arthoba Naik^{b*}

^aB. T. L. Institute of Technology & Management, Bommasandra Industrial Area, Bangalore, India ^bDepartment of Chemistry, Kuvempu University, Shankaraghatta, India

ABSTRACT

Hollow gold nanospheres (HGNs) were generated onto pencil graphite electrode (PGE) by electrodeposition and subsequent displacement reaction between the cobalt nanoparticles and Au^{3+} ions. HGNs/PGE displayed excellent electrochemical catalytic activities towards dopamine (DA), uric acid (UA) and ascorbic acid (AA). Differential pulse voltammetry was used for the simultaneous determination of DA, UA and AA. The peak separation between UA and DA, DA and AA was 132mV and 157mV, respectively. The lowest detection limits (S/N= 3) were 0.2, 0.7 and 2.5 μ M for DA, UA and AA respectively. With good selectivity and sensitivity, the present method has been applied to the determination of DA in injectable medicine and UA in human urine and serum samples.

Key words: Uric acid; Dopamine; Ascorbic acid; Hollow gold nanospheres; Pencil graphite electrode.

INTRODUCTION

Development of electrochemical techniques for the detection of ascorbic acid (AA), dopamine (DA) and uric acid (UA) in human urine samples and blood serum have received considerable attention in the literature [1-3]. AA is a very common electroactive biological molecule, which is very well known to prevent scurvy, and is vital to maintain good immune response and assist wound healing [4]. UA is the primary end product of purine metabolism [1]. Abnormal level of UA is a pathological indicator of several disease conditions, such as gout, immunodeficiency, and Lesch-Nyan syndrome [5]. DA, the most important among the class of catecholamine and it plays vital role in the functioning of the central nervous, renal, hormonal and cardiovascular systems [6-7]. Therefore, it is essential to develop a simple, rapid and reliable method for routine quantitative analysis of these molecules in the body fluids.

In the determination of UA or DA, a major obstacle usually encountered is the interference of AA, which is usually present in high concentrations in the body fluids and can be oxidized at a potential close to that of UA and DA. Various approaches have been attempted to minimize the interference of AA, such as electrodes modified with polymers [8], enzymes [9-10], and dye doped sol–gel [11] etc. Nanostructured materials have become prominent in recent years due to their practical applications in diverse fields, such as electronic nanodevices, molecular catalysts, and biosensors [12-14]. Gold nanoparticles have fascinating optical, electronic, and catalytic properties suitable for several biomedical applications [15-17]. More importantly, gold nanoparticles are complete biocompatible and stable of in-vivo.

Hollow metallic particles have been found to be attractive as drug carriers, electrocatalytic materials, etc., owing to the distinctive advantages of low density, high specific surface area, and reduced material costs. Application of hollow nanospheres in biosensor fabrication has just made a beginning. Shufeng Liu et al. [18] have reported the fabrication of DNA biosensor using hollow gold nanospheres (HGNs). Usually, these prepared HGNs are attached to the surface of gold electrode through thiol linkages.

In the present study, we have fabricated HGNs and attached them on to pencil graphite electrode (PGE) surface using a displacement reaction between the cobalt nanoparticles and HAuCl₄. The current research focuses on the determination of DA, UA and AA on the HGNs modified graphite electrode. We report here that, this approach provides biosensor for the three vital excellent biochemical molecules mentioned above.

EXPERIMENTAL SECTION

2.1. Apparatus and reagents

All the chemicals were of analytical-reagent grade and were used as received without any further purification. Dopamine hydrochloride, Potassium ferrycynide were purchased from Sigma-Aldrich. Uric acid, Ascorbic acid, Cobaltous chloride and Chloroauric acid were purchased from S.D. Fine Chem. Ltd. All the solutions were freshly prepared with double distilled water. The 0.1M phosphate buffer solutions were prepared from potassium phosphate and the different pH of solutions were adjusted by using H_3PO_4 or NaOH. A working PGE was constructed with a 2B graphite pencil rod (0.5 mm diameter) from Camlin Ltd. was placed into a plastic tube filled with epoxy resin.

All the voltammetric experiments were performed using CHI660D electrochemical work station (CH Instruments, USA) with a conventional three electrode system. Platinum wire and Ag/AgCl/saturated KCl electrodes were used as auxiliary and reference electrodes respectively. Bare or gold modified graphite electrode was used as working electrode. The surface morphology of the modified electrode was characterized by SEM (FEI-Quanta 200).

2.2. Preparation of HGNs modified pencil graphite electrode

The modified electrode was prepared by electrodeposition of cobalt particles on graphite electrode from 10 mM $CoCl_2.6H_2O$ solution at the potential -1.0 V for 4 minutes. After electrodeposition the electrode was dipped in 1mm $HAuCl_4$ solution for 10 min then washed with water. The modified electrode was activated in 0.2 M H_2SO_4 by cyclic voltammetry (10 cycles) in the potential range -0.3 to +0.7 V. Then electrode was stored in phosphate buffer of pH 7.0. The nitrogen gas was bubbled before electrodeposition and continued during the reaction in order to avoid the oxidation of cobalt particles by dissolved oxygen in the solution.

RESULTS AND DISCUSSION

3.1. Morphological characterization of the PGE and HGNs/PGE

The surface morphology of PGE and HGNs/PGE was characterized by scanning electron microscope (SEM). As shown in Fig. 1A, smooth surface was observed on the unmodified PGE surface. The Fig 1B is the SEM image shows the formation of HGNs on the surface of PGE. The figure also reveals that under the processing conditions the HGN particles distort to nearly spherical geometry.

3.2. Electrochemical behavior of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ couple

Figure 1C shows the cyclic voltammograms of 5 mM $K_3[Fe(CN_6)]$ in 0.1M KCl solution at PGE, HGNs/PGE and pure gold electrode. The cyclic voltammograms of $Fe(CN_6]^{3-}/[Fe(CN)_6]^{4-}$ couple show a peak to peak separation of 77.5 mV at PGE, 57 mV at HGns/PGE and 129.4 mV at gold electrode. The peak current intensity at HGNs/PGE was 1.9 times higher cathodically and 2.0 times higher anodically than that of gold and PGE electrodes. The increase in the peak current and reduction of peak to peak separation potential is observed at HGNs/PGE attributed to the large surface area and good electrocatalytic activity of the HGNs. The capacity of electron transfer of different electrodes was also investigated using electrochemical impedance spectroscopy [Fig. 1D]. Nyquist plots were almost completely lines suggesting that for the PGE and a pure gold electrode the electron transfer is via a characteristic mass diffusion process. In the case of HGNs/PGE, the Z'' -Z' curve is significantly non-linear with an initially rapid raise of Z''.



Fig. 1: SEM images of PGE (A) and HGNs/PGE (B); Cyclic voltammograms (C) and Nyquist plots (D) at gold electrode (a), PGE (b) and HGNs/PGE (c), in 5 mM K₃[Fe(CN)₆] + 0.1M KCl solution. (C) scan rate 50mVs⁻¹; (D) frequency range: 10 kHz-0.01Hz.

3.1. Electrochemical oxidation of DA, AA and UA

Figure 2A shows the cyclic voltammograms of AA at PGE and HGNs modified PGE in phosphate buffer solution. The oxidation peak of AA at the HGNs modified PGE occurred at a lower potential of -0.016 V. There is thus a 238 mV negative shift and is also associated with an enhanced anodic peak current to 1.5 times. The very large negative shift in the oxidation potential of AA could be explained on the basis of electrostatic interaction between AA and HGNs of modified electrode.

Electrochemical oxidation of DA at bare PGE and HGNs modified PGE are shown in Fig. 2B. At PGE the electrochemical response is evidently very poor. However the oxidation peak was observed at 0.17 V for PGE and at a slightly reduced potential (30mV) for HGNs/PGE. The sharp oxidation peak and increase in the peak height were observed at the potential 0.14V for DA, attributed to the large surface area of HGNs/PGE. This result confirms the fact that the oxidation reaction of DA at HGNs/PGE is more selective.

Figure 2C shows the oxidation reaction of UA at PGE and HGNs/PGE. The oxidation peaks of UA were observed at 0.274 V and 0.285 V respectively at PGE and HGNs/PGE. The oxidation peak current of UA was increased 4.7 times at HGNs/PGE than that of PGE. The enhancement in the oxidation peak current confirms the good electrocatalytic activity of HGNs.



Fig. 2. Cyclic voltammograms of 400µM AA, 100µM DA and 100µM UA at bare (a), and HGNs/PGE (b) in 0.1M PBS (pH 7.0). Scan rate: 50mVs⁻¹

3.2 Simultaneous determination of AA, DA and UA



 $\begin{array}{l} \mbox{Fig. 3. Cyclic voltammograms of 500 μmol L^{-1} AA, 100μmol L^{-1} DA and 100 μmol L^{-1} UA at (a) gold electrode (b) PGE and (c) $HGNs/PGE in 0.1M PBS (pH 7.0) and scan rate 50 mVs^{-1} \\ \end{array}$

Figure 3 shows the oxidation of AA, DA and UA mixture at the surface of PGE and HGNs/PGE. The PGE electrode shows only two oxidation peaks, which may be attributed to (AA+DA) center around 0.16 V and UA center around 0.27 V respectively. The oxidation peaks of AA (-0.015V), DA (0.142V) and UA (0.274V) are completely separated by the modified electrode. These shift in the peak potentials and enhanced peak currents on HGNs/PGE indicate an excellent electrocatalytic activity of the modified electrode and higher surface area available for oxidation of AA, DA and UA.

Figure 4 shows the differential pulse voltammogramms (DPV) for the oxidation of AA, DA and UA by varying the concentration of one species while the concentrations of the other two species are kept constant. Fig. 4A shows the peak current of AA increased with increasing its concentration, but those of DA and UA had no significant change. Simillar results were observed for the effects of DA and UA concentrations, when AA and UA or AA and DA concentrations remained constant as indicated in Figs. 4B and 4C, respectively. The increase in DA or UA concentrations led to the increase of their peak currents but had no significant influence on the peak currents of other two compounds. Thus, it is possible to determine AA, DA and UA simultaneously in their mixture solution at HGNs/PGE without significant cross interferences. The peak current of AA increases linearly with increase in AA concentrations from 50 to 600 μ M (I_p (μ M) = -0.0018c (μ M) - 1.214) (R^2 = 0.991) and a detection limit of 2.5 μ M (S/N = 3). Similarly, the currents corresponding to the oxidation of DA increased linearly with increase in DA concentration in the range of 20~120 μ M. The linear regression equation may be expressed as: $I_p(\mu M) = -0.03c$ $(\mu M) - 0.998$ (R² = 0.995) and the detection limit is 0.2 μM . The oxidation current peaks of UA increases with increase in the concentration of UA in the range of 20-120 μ M and the linear regression equation which fits the current behaviour is given by I_p (μ M) = -0.053c (μ M) -0.273 (R² = 0.996), the detection limit of 0.7 μ M was obtained. Together the results confirm that it is possible to determine AA, DA and UA simultaneously in the real samples using HGNs/PGE.



Fig. 4. Differential pulse voltammograms at HGNs/PGE in 0.1M pH 7.0 PBS: (A) containing 40 μM UA and 50 μM DA and different concentrations of AA: 50, 150, 300, 450, 600 μM (B) containing 300 μM AA, 40 μM UA and different concentrations of DA: 20, 40, 60, 80, 100, 120 μM and (C) containing 300 μM AA, 40 μM DA and different concentrations of UA: 20, 40, 60, 80, 100, 120 μM

3.3. Effect of PH

The effect of pH on the DPV signals for AA, DA and UA at the HGNs/PGE was examined, and the results are shown in Fig. 5A. It can be seen that oxidation peak potentials of AA, DA and UA are uniformly shifted towards more negative potentials with increase in pH from 3.5-8.0. These results confirm that the peak potential of AA, DA

and UA depend on the pH of the solution. Further, that the redox couples of AA, DA and UA include proton transfer in the redox process. The slopes from the plot of Ep versus pH are -46, -68, and -70 mV/pH unit of AA, DA and UA respectively, which are very close to the anticipated Nernstian value for a two-electron and two-proton process.

It can be seen from Fig. 5B that the peak current of AA decreases with increasing pH from 4.0; and that of UA increases with increasing pH value until it reaches 5.0, and then it decreases when the pH increases further. For DA, the maximum peak current is obtained at pH 5.5. It is well known that AA (pKa = 4.10), UA (pKa = 5.7) exist as anionic forms, whereas DA (pKa = 8.87) exists as cationic form in acidic solutions [19-20]. In view of simultaneous determination of AA, DA and UA, it is obvious that acidic solution is favorable for higher sensitivity and selectivity. However, in order to maintain the physiological environment, pH 7.0 was chosen in our present study.



Fig. 5. Effect of pH on the peak potential (A), and peak current (B) for the oxidation of AA, DA and UA. Concentrations: AA: 400µM; DA: 40µM; UA: 60µM

3.4. Interferences, Stability and reproducibility

For investigating the anti-interference ability of the HGNs/PGE, several co-existing substances were selected. No significant interference for the detection of AA(300 μ M), DA (30 μ M), and UA (40 μ M) was observed from the following compounds (μ M): NaCl (4000), KCl (5000), CaCl₂ (4000), ZnCl₂(4000) and glucose (900). The stability of the electrochemical sensor was also tested. After measurements, the modified electrode was stored in phosphate buffer at 4°C. The peak current intensity only decreased 7.4 %, 5.8% and 4.5% for AA, DA and UA after 10 days, respectively. In addition, this electrochemical response has good stability, as the peaks remain unchanged after successive 100 cyclic voltammetric scans. As the electrode fabrication is very easy and low cost, the present

modified electrode seems to be of great utility for making a voltammetric sensor for the detection of neurotransmitters.

3.5. Real sample analysis

Determination of DA in dopamine hydrochloride injections

Five milliliters of dopamine hydrochloride injection solution (40mg/mL) was diluted to 500 mL with double distilled water. Then 0.2 mL of this diluted solution was injected into 10 mL volumetric flask and made up to the volume with the 0.1M phosphate buffer solution (pH 7.0). This solution was transferred into the electrochemical cell for the determination of DA using the DPV technique. The results are presented in Table 1. The results are satisfactory, showing that the proposed methods could be efficiently used for the determination of DA in injections.

Determination of AA in vitamin C tablet

For tablet analysis, a tablet of vitamin C (labeled 150 mg) was finely ground and dissolved in 100 mL of distilled water. After filtration, 1.0 mL of the solution was diluted with 49mL of the buffer solution (pH 7.0) and the resulting solution was used for analysis. Then 10mL of the sample solution was transferred into the electrochemical cell for DPV scan. The results are listed in the Table 1. The average determination result of AA in vitamin C tablet is 157.9 mg per tablet, which is in agreement with the labeled value. To ascertain the validity of the proposed procedure, the sample was spiked (supplemented by the further addition) with standard AA solution and then the total amount of AA was measured. The enhanced signal intensity was equivalent to 100.53% of the added AA solution.

Table 1.Determination result of DA in injections, AA in Vitamin C tablets, UA in human Urine and blood serum samples

Samples	Analyte	Detected (µM)	Spiked (µM)	Found (µM)	Recovery %
Vitamin C	AA	179.33	100	280.82	101.49
Dopamine injection	DA	50.22	15	65.75	103.5
Urine 1(patient)	UA	55.76	40	92.23	91.2
Urine 2 (healthy)	UA	30.64	30	54.25	94.4
Blood serum 1	UA	12.47	10	22.07	96.0
Blood serum 2	UA	12.04	10	20.48	84.4

Determination of UA in human urine and blood serum

In order to assess its potential application, the HGNs/PGE was used to detect the contents of UA in human urine and blood serum samples. The urine samples were collected from the lab personnel, and the blood serum samples were obtained from the affiliated Health Centre of Kuvempu University. Urine samples were analyzed directly after dilution by 50 times with the buffer solution (pH 7.0) without any treatment. To ascertain the correctness of the results, the samples was as before spiked with fixed amounts of UA and then total values were detected (Table 1). The recovery rates of the spiked samples were determined and found in the range 91.2 - 94.4 %.

Human serum samples were selected for analysis by the proposed method using the standard addition method. In order to avoid the interferences of the real sample matrix and fit into the linear range of UA. Only 0.2 mL of the serum was added to the electrochemical cell containing 5 mL of buffer (pH 7.0) solution. The determined results are listed in Table 1. The recovery of the spiked samples ranged between 84.4% and 96%, indicating the detection procedures are free from interferences from other components of the serum sample.

CONCLUSION

In the present work HGNs have been generated onto pencil graphite electrode by displacement reaction between cobalt nanoparticles and Au (III) ions. This modified electrode exhibited high electrocatalytic activities towards the oxidation of DA, UA and AA by significantly decreasing their oxidation over potentials and enhancing the peak currents. Large peak separation between DA, UA and AA could be obtained using CV or DPV, indicating that the HGNs/PGE facilitated their simultaneous determination. Moreover, the proposed method has been applied to the determination of AA, DA and UA in real samples with satisfactory results. The high surface area and nanostructures of HGNs make it a promising candidate for electro-catalytic and biomedical applications.

Acknowledgement

The authors gratefully acknowledge to Kuvempu University, Department of Science and Technology, New Delhi for providing the facilities to carry out this work. Authors also thank Prof. K. J. Rao Emiritus Professor, Indian Institute of Science for support, discussion and correction of the manuscript.

REFERENCES

- [1] Jianshe Huang; Yang Liu; Haoqing Hou; Tianyan You, Biosens. Bioelectron., 2008, 24, 632–637.
- [2] Lei Zhang; Chunhua Zhang; Jiying Lian, Biosens. Bioelectron., 2008, 24(4), 690-695.
- [3] Haiqing Liu; Yang Tian. Electroanalysis, 2008, 20(11), 1227-1233.

[4] GF Combs. The Vitamins: Fundamentals Aspects in Nutrition and Health, 2nd ed. Academic Press, San Diego, CA. **1992**.

- [5] VSE Dutt; HA Mottola, Anal. Chem., **1974**, 46, 1777-1781.
- [6] RM Wightman; LJ May; AC Michael, Anal. Chem., 1998, 60, 769A-779A
- [7] A Liu; I Honma; HS Zhou, Biosens. Bioelectron., 2005, 21, 809-816.
- [8] PR Roy; T Okajima; T Ohsaka. J. Electroanal. Chem., 2004, 561, 75-82.
- [9] T Hoshi; H Saiki; Anzai, J. Talanta., 2003, 61, 363–368.
- [10] FF Zhang; XL Wang; SY Ai; ZD Sun; Q Wan; ZQ Zhu; YZ Xian; LT Jin; K Yamamoto, Anal. Chim. Acta, 2004, 519, 155-160.
- [11] SB Khoo; F Chen, Anal. Chem., 2002, 74, 5734-5741.
- [12] CR Raj; T Okajima; T Ohsaka. J. Electroanal. Chem., 2003, 543, 127-133.
- [13] RW Siegel. Nanostruct. Mater., 1993, 3(1-6), 1-18.
- [14] MA Hayat. Colloidal Gold: Principles, Methods and Applications, Vol. 1, Academic Press, New York, 1989.
- [15] F Kim; JH Song; P Yang, J. Am. Chem. Soc., 2002, 124, 14316-14317.
- [16] JB Pendry, Science, 1999, 285, 1687-1688.
- [17] Y Tian; T Tatsuma. Chem. Commun., 2004, 1810-1811.
- [18] Shufeng Liu; Jing Liu; Xiaoping Han; Yanning Cui; Wei Wang; Biosens. Bioelectron., 2010, 25(7), 1640–1645.
- [19] A Balamurugan; SM Chen, Anal. Chim. Acta., 2007, 596, 92-98.
- [20] H Zhao; Y Zhang; Z Yuan, Anal. Chim. Acta., 2001, 441(1), 117-122.