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Simultaneous electrochemical determination of dopamine, ascorbic acid and uric acid in the presence of sodium dodecyl sulphate using multi-walled carbon nanotubes modified carbon paste electrode

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Abstract

Carbon paste electrode modified with multi-walled carbon nanotubes (CPE/MWCNT) was prepared as a biosensor for the simultaneous determination of dopamine (DA), ascorbic acid (AA) and uric acid (UA) in the presence of sodium dodecyl sulphate (SDS). The nature and surface morphology of the bare CPE and fabricated MWCNT were characterized by scanning electron microscopy (SEM). The CPE/MWCNT exhibited excellent electrocatalytic activity towards the oxidations of DA, AA and UA in the presence of SDS in 0.2 M phosphate buffer (PB) solution (pH 3.0). The separations of anodic peak potentials of AA/DA, DA/UA and AA/UA at the surface of modified electrode using differential pulse voltammetry (DPV) were obtained 108.0, 150.0 and 260.0 mV, respectively. The proposed method was successfully applied for the determination of DA, AA and UA in real samples with satisfactory results.

Keywords: Nanosensor, Sodium dodecyl sulphate, Dopamine, Ascorbic acid, Uric acid, Electrochemical analysis

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1. Introduction

Dopamine (3,4-dihydroxyphenyl ethylamine, DA), ascorbic acid (2-(1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3-one, AA) and uric acid (2,6,8-trihydroxypurine, UA) are biochemical compounds which play important roles in various biological processes. DA is an important neurotransmitter molecule of catecholamines which is widely distributed in the mammalian central nervous system for message transfer. Low levels of DA are related to neurological disorders such as Parkinson's disease, schizophrenia [1,2] and to HIV infection [3]. AA is a vital vitamin in human diet and is very popular for its antioxidant properties. It has been used for the prevention and treatment of common cold, mental illness, infertility, cancer and AIDS [4]. UA is a primary end product of urine metabolism and abnormal levels of UA exhibit symptoms of several diseases like gout, hyperuricaemia and Lesch-Nyhan syndrome [5,6]. It is one of the major parameters monitored in urine and in blood. UA concentration changes are associated with the altered metabolism of purines that are related to numerous illnesses and physiological disorders [7,8].

Surfactants, sometimes called surface-active agents, are among the most versatile chemicals available. These are amphiphilic molecules consisting of a hydrophilic head group and a hydrophobic (lipophilic) tail and are, thus, able to interact with both polar and non-polar compounds. Accordingly, surfactants are often classified as non-ionic and ionic (cationic, anionic or zwitterionic) [9].

Because of DA, UA and AA usually coexist in biological samples, simultaneous determination of them is very important. But, at unmodified electrodes the overlapping of their oxidation potentials and the pronounced electrode fouling often results in poor selectivity and reproducibility [10]. In addition, the oxidation products of DA can homogeneously catalyze the oxidation of AA, which will influence the

precise determination of AA and DA [11]. Therefore, designing a new method for the simultaneous determination of DA, UA and AA is a major goal of the research.

To achieve this goal, CPE/MWCNT was employed and low concentration of sodium dodecyl sulfate (SDS) as an anionic surfactant was added into the bulk solution, to change the electrode/solution interface as well as improve the electrochemical responses of DA, UA and AA [12-18]. It is found that well-defined and sensitive oxidation peaks is observed for DA, UA and AA at the CPE/MWCNT in the presence of SDS. In comparison with the absence of SDS, the oxidation peak current of DA, UA and AA significantly increases in the presence of SDS, suggesting that SDS facilitates the electron transfer of DA, UA and AA. Without a doubt, the sensitivity of determining DA, UA and AA must be greatly improved under the enhancement effect of SDS.

2. Experimental

2.1 Chemicals and reagents

DA, AA, UA and SDS were purchased from Merck. MWCNT with purity of 95% (40-60 nm in diameter) were obtained from the Chinese Academy of Sciences. Phosphate buffer (PB) solution (0.1 M) was prepared by dissolving 1.67 mL concentrated orthophosphoric acid in water and diluting to 250.0 mL in a volumetric flask. The buffer solutions were prepared by addition of sodium hydroxide to the phosphoric acid solution to reach appropriate pH values. All reagents were of analytical grade. All solutions were prepared with deionized water.

2.2. Instrumentation

All electrochemical measurements were carried out with a M273A Electrochemical Workstation (America, EG&G Corporation). A conventional three-electrode system was employed, consisting of a CPE/MWCNT as a working electrode, a saturated calomel reference electrode (SCE) (Metrohm, Switzerland) and a Pt wire counter electrode (Metrohm, Switzerland). Solution pH values were determined using a 691 pH meter combined with glass electrode (Metrohm, Switzerland)). Deionized water prepare by an ultrapure water system (smart 2 pure, TKA, Germany). MWCNT were dispersed with an ultrasonic bath (Sonorex Digital, 10P, Bandelin). The scanning electron micrographs (SEM) micrographs were obtained by scanning electron microscope (KYKY-EM3200).

2.3 Fabrication of CPE/MWCNT

For preparation of CPE/MWCNT, 6.00 mg MWCNT was dispersed into 5 mL of deionized water, and then sonicated for about 45 min to give a stable and homogeneous MWCNT suspension. This suspension was mixed with 0.50 gr graphite powder. After evaporation of the solvent in room temperature, a portion of it was mixed with Nujol in a mortar and pestle. A portion of the mixture was packed into the end of a polyethylene tube. Electrical contact was made by forcing a copper pin down into the tube and into the back of the composite, and the other end of the tube used as the electrode. For preparation of unmodified CPE the same procedure for preparation of CPE/MWCNT carried out except addition of MWCNT.

3. Results and discussion

3.1. Morphology characterizations of the CPE/MWCNT

The surface morphology of the unmodified CPE and CPE/MWCNT was analyzed by SEM (Fig. 1). The SEM images indicate coverage of CPE surface by MWCNTs (Fig. 1b). The formed CP/MWCNT nanocomposite possesses enlarged surface area, which can minimize the diffusion path and enhance the electrode-electrolyte interface for analytes to move in and out of the CPE/MWCNT, and thus favoring the electrochemical reaction of DA, AA and UA.

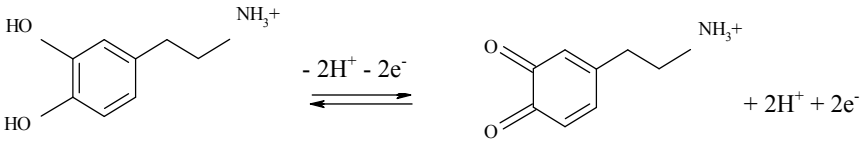
3.2. Electrochemical responses of DA, AA and UA in the presence of CPE/MWCNT and SDS

It is well known that the electrochemical detection of DA in the presence of high levels of AA on untreated carbon-based electrodes or on ordinary electrodes severely struggle due to the catalytic oxidation of AA by DA [19]. Fig. 2 shows electrochemical responses of DA (40.0 μM), AA (400.0 μM) and UA (20.0 μM) on the bare CPE, CPE/MWCNT and CPE/MWCNT in the presence of SDS. As can be seen, DA, AA and UA at the CPE show undefined and broad peaks, but in the presence of CPE/MWCNT and SDS the electrochemical responses of the three analyte occurred at different potential windows. This result means that the differential pulse voltammetric peaks of the three compounds can be well separated from their mixed solutions by CPE/MWCNT and SDS. Fig. 2 (curve a) depicts that DPV of the mixture only gives broad anodic peak by the bare CPE. On the other hand, the peak potentials for DA, AA and UA are indistinguishable at the bare CPE. Thus simultaneous determination of the three compounds is impossible. Fig. 2 (curve b) shows that two anodic peaks corresponding to the electrooxidation of the three compounds could be observed at the CPE/MWCNT. Although the CPE/MWCNT shows better electrocatalytic activity towards the oxidation of DA, AA and UA than

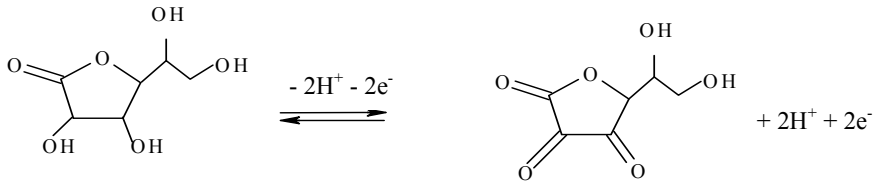
on the bare CPE, it is still difficult to simultaneous determination of DA, AA and UA from their mixture solutions. But, using of SDS in the mixture, the voltammetric peaks resolve into three well-defined peaks, at potentials around 400.0, 520.0 and 660.0 mV for AA, DA and UA, respectively (Fig. 2 (curve c)) at the CPE/MWCNT. The reason for this result is that SDS is an anionic surfactant but AA has positive charge, therefore AA is blocked by SDS. Thus more positive potential needs for oxidation of AA than the absence of SDS in solution. As a result, two peaks related to DA and AA separated using of SDS.

3.3. The influence of pH on the oxidation of DA, AA and UA

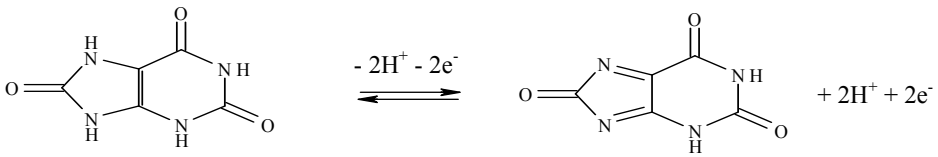
The effect of varying of pH (2.0-8.0) on the electrochemical responses of the CPE/MWCNT towards the simultaneous determination of DA, AA and UA in the presence of SDS has been shown in Fig. 3. It can be seen that the anodic peak current of AA and UA reaches to a maximum at pH 3.0, and then decreases gradually with increase of pH value. The influence of solution pH on the peak current of DA shows that it reaches the maximum at pH 5.0. In addition, the solution pH affected the anodic current peak potentials of DA, AA and UA. With increasing of pH, their oxidation peak potentials shift to less positive values, showing that protons take part in their electrode reactions. Thus, solution pH 3.0 was taken for the following simultaneous determination of the three compounds. Also, redox reaction mechanisms of DA, AA and UA have been presented in Scheme 1 [20], 2 [21] and 3 [22], respectively.



Scheme 1. Mechanism of DA oxidation at the CPE/MWCNT.



Scheme 2. Mechanism of AA oxidation at the CPE/MWCNT.



Scheme 3. Mechanism of UA oxidation at the CPE/MWCNT.

3.4. Effect of concentration of SDS

From Fig. 2, it is very clear that SDS can enhance the oxidation peak current of DA, AA and UA. However, the oxidation peak current of DA, AA and UA is found to be closely related to the concentration of SDS. Thus, the influence of SDS concentration on the oxidation peak current of DA, AA and UA was examined, and the results shown in Fig. 4. Based on Fig. 4, gradual increasing the concentration of SDS from 4.0 to 8.0 mM, the oxidation peak current of DA, AA and UA also increases gradually. However, the oxidation peak current of DA, AA and UA begins to decrease when the concentration of SDS is higher than 8.0 mM. Therefore, 8.0 mM of SDS is chosen in the research for simultaneous determination of DA, AA and UA.

3.5. Simultaneous determination of DA, AA and UA

The determination of DA, AA and UA in their mixtures was performed at the surface of CPE/MWCNT in solution contains 8.0 mM SDS when the concentration of one compound changed, whereas those of other two compounds are kept constant (Figs. 5, 6 and 7). As shown in Fig. 5, the peak current of DA is increased when its concentration increased. However, the changes of DA concentration showed an influence on the peak currents of AA and UA. Therefore, DA concentration influence on the peak currents of AA and UA were determined. Based on the data, the relative standard deviations (RSD) of the current signal for AA and UA were obtained between 1.4-3.2% and 0.8-2.5%, respectively. Similarly, as shown in Figs. 6 and 7, the oxidation peak currents of AA or UA increased linearly with increasing of the concentration of AA or UA when concentration of the other two compounds is kept constant. Fig. 6 shows that AA concentration can be effect on the peak currents of DA and UA. The interference on the current signals for DA and UA caused a RSD between 2.2-4.3% and 1.9-3.2%, respectively. Also, the influence of UA concentration on the peak currents of DA or AA was investigated in Fig. 7. According these data a RSD between 1.8-3.6% and 1.7-3.1%, was obtained for DA or AA, respectively. However, the voltammetric peaks for DA, AA and UA oxidation at the CPE/MWCNT are well separated from each other when they co-exist in buffer phosphate with pH 3.0. Therefore, it is possible to simultaneously determine DA, AA and UA in mixture samples using the CPE/MWCNT and SDS. According the studies, using DPV technique, the oxidation current peaks were linearly proportional to DA, AA and UA concentration. From analysis of these data, the lower limit of detection (S/N = 3) of DA, AA and UA were obtained 1.07, 18.85 and 2.86 μM, respectively.

3.6. Interferences

The ability of the proposed method for determination of DA, AA and UA in the presence of foreign substances was investigated by DPV technique. The experiments were carried out by analyzing a standard solution containing 30.0 μM DA, 300.0 μM AA, and 30.0 μM UA in the presence of SDS using increasing amount of interfering species. The tolerance limit was defined as the concentrations which give an error of <5.0%. No interference was observed for the following compounds (μM): K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, citric acid, cysteine, lysine and glucose. The results were listed in Table 1.

3.7. Samples analysis

3.7.1. Determination of DA in dopamine hydrochloride injection

In order to verify the reliability of the proposed method for analysis of DA in pharmaceutical product, the CPE/MWCNT was applied to determine DA in dopamine hydrochloride injection (10.0 mg mL^{-1}, 2.0mL per injection). The sample was diluted 10 times with deionized water, and then appropriate amounts of the diluted sample were transferred into the electrochemical cell for DA determination using DPV. The analytical results are summarized in Table 2. The recovery rates of DA were measured in this study by comparing the peak current of the DA treated with and without a specific interfering chemical and obtained from 98.6% to 101.2%. The results are acceptable and showing that the proposed method could be effectively used for the determination of DA in commercial samples.

3.7.2. Determination of UA and AA in human urine samples

The utilization of the proposed method in real samples analysis was also investigated by direct analysis of UA and AA in human urine samples. In order to fit

into the linear range of UA and AA, all the urine samples were diluted 200 times with 0.1 M PB solution (pH 3.0). The results were listed in Table 2. The recovery range of the spiked samples was between 99.6 and 100% that indicating the detection procedures are free from interferences of the urine sample matrix.

3.8. Stability and Repeatability of CPE/MWCNT in the Presence of SDS

The capability to create a modified electrode in the presence of SDS with a stable surface was tested in optimized experimental conditions, by successive DPV determination of DA for a three week period. The RSD for current signal were achieved 2.52%, whereas the peak potential for DA oxidation was unchanged. Additionally, the repeatability of the modified electrode was investigated by DPV data, from five separately prepared CPE/MWCNTs. From the data, the relative standard deviations (RSD) were obtained between 2.41 to 3.86%. These results indicate that reproducibility of the surface is satisfactory.

4. Conclusions

In conclusion, multi-walled carbon nanotubes were applied for the modification of the CPE. The modified electrode exhibited high electrocatalytic activities towards the oxidation of DA, UA and AA by significantly decreasing their oxidation overpotentials and enhancing the peak currents in the solution contains SDS. Large peak separation between DA, UA and AA could be obtained using DPV that indicating the CPE/MWCNT facilitated their simultaneous determination. The electrochemical sensor showed excellent selectivity and high sensitivity. Moreover, the proposed method has been applied to the determination of DA and UA in real samples with satisfactory results.

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References

[1] C. Xue, Q. Han, Y. Wang, J. Wu, Ti. Wen, R. Wang, J. Hong, X. Zhou, H. Jiang, *Biosens. Bioelectron.,* 2013, **49**, 199.

[2] O. Darbin*, Parkinsonism Relat. Disord.*, 2012, **18**, 426.

[3] P.R. Dalmasso, M.L. Pedano, G.A. Rivas, *Anal. Chim. Acta*, 2012, **710**, 58.

[4] O. Arrigoni, M.C. Tullio, *Biochim. Biophys. Acta*, 2002, **1569**, 1.

[5] N. Misraa,V. Kumar, L. Bordeb, L. Varshney, *Sens. Actuators. B*, 2013, **178**, 371.

[6] L. Tan, G.M. Yang, P. Wang, Z.Y. Xie, H.P. Bai, X.X. Lu, Y.H. Yang, *Anal. Lett.*, 2008, **41**, 2860.

[7] G. Hu, Y. Ma, Y. Guo, S. Shao, *Electrochim. Acta*, 2008, **53**, 6610.

[8] A. Khoobi, S.M. Ghoreishi, M. Behpour, *Analyst*, 2014, **139**, 4064.

[9] R. Vittal, H. Gomathi, K.J. Kim, *Adv. Colloid. Interface. Sci.*, 2006, **119**, 55.

[10] Z.Q. Gao, H. Huang, *Chem. Commun*., 1998, **19**, 2107.

[11] M.A. Dayton, A.G. Ewing, R.M. Wightman, *Anal. Chem.*, 1980, 52, 2392.

[12] Xiao-Lin Wen, Yun-Hua Jia, Zhong-Li Liu, *Talanta*, 1999, **50**, 1027.

[13] N.F. Atta, A. Galal, R.A. Ahmed, *J. Electrochem. Soc.*, 2011, **158**, F52.

[14] S.C. Avendano, G.A. Angeles, G.R. Pina, M.R. Romo, *ECS Trans.*, 2007, **3**, 23.

[15] J. Zheng, X. Zhou, *Bioelectrochemistry*, 2007, **70**, 408.

[16] B. Hoyer, N. Jensen, *Electroanalysis*, 2005, **17**, 2037.

[17] M. Demura,T. Yoshida, T. Hirokawa, Y. Kumaki, T. Aizawa, K. Nitta, I. Bitter, K. Tóth, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 1367.

[18] A. Cid, J.C. Mejuto, P.G. Orellana, O. López-Fernández, R. Rial-Otero, J. Simal-Gandara, *Food Res. Int.*, 2013, **50**, 143.

[19] Y. Wang, Y. Li, L. Tang, J. Lu, J. Li, *Electrochem. Commun.*, 2009, **11**, 889.

[20] M.H. Pournaghi-Azar, H. Dastangoo, R. Fadakar bajeh baj, *Biosens. Bioelectron.*, 2010, **25**, 1481.

[21] C. Li, Y. Ya, G. Zhan*, Colloids Surf. B*, 2010, **76**, 340.

[22] L. Zheng, S. Wu, X. Lin, L. Nie, L. Riu, *Electroanalysis*, 2001, **13**, 1351.

Figure captions

Fig. 1. SEM images of (a) CPE and (b) CPE/MWCNT.

Fig. 2. The DPV of 40.0 μM DA, 400.0 μM AA and 20.0 μM UA at a bare CPE (a), CPE/MWCNT (b), and CPE/MWCNT in the presence of 8.0 mM SDS (c), in pH 3.0 PB solution.

Fig. 3. Effect of pH on the peak current for the oxidation of DA, AA and UA. Concentrations: DA: 10.0 μM; AA: 600.0 μM; UA: 80.0 μM.

Fig. 4. Effect of the concentration of SDS on the oxidation peak current of DA (60.0 μM), AA (70.0 μM), UA (80.0 μM).

Fig. 5. (A) DPV of DA at CPE/MWCNT in the presence of 600.0 μM AA and 80.0 μM UA in pH 3.0. DA concentrations from a to d are corresponding: 20.0, 30.0, 40.0 and 50.0 μM, respectively. (B): I (μA) as a function of DA concentration (μM).

Fig. 6. (A) DPV of AA at CPE/MWCNT in the presence of 10.0 μM DA and 80.0 μM of UA in pH 3.0. AA concentrations from a to e are corresponding: 0.3, 0.4, 0.5, 0.7, and 0.9 mM, respectively. (B): I (μA) as a function of AA concentration (mM).

Fig. 7. (A) DPV of UA at CPE/MWCNT in the presence of 20.0 μM DA and 600.0 μM of AA in pH 3.0. UA concentrations from a to f are corresponding: 40.0, 60.0, 80.0, 100.0, 120.0 and 140.0 μM. (B): I (μA) as a function of UA concentration (μM).

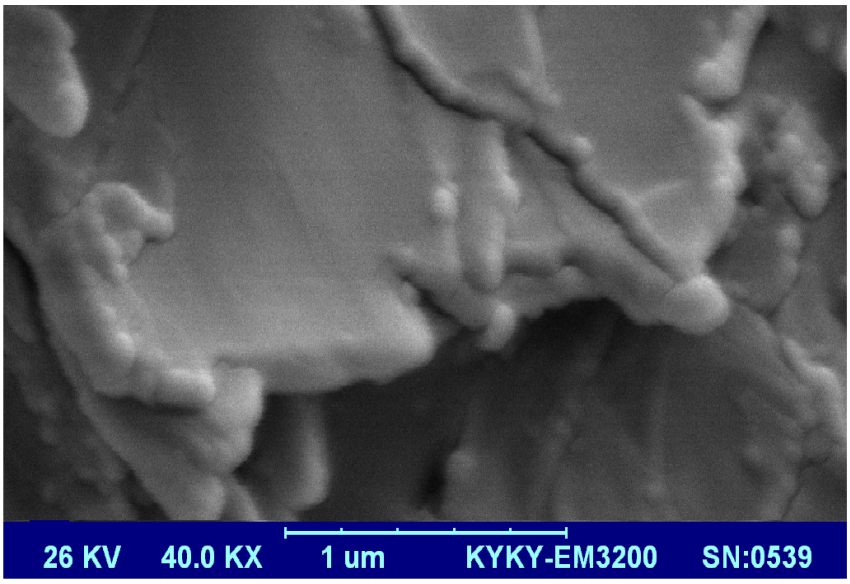
Table captions

Table 1. Interferences of some foreign substances for 30.0 μM DA, 300.0 μM AA and 30.0 μM UA.

Table 2. Determination DA, AA and UA in real samples.

Fig. 1

(a)



(b)

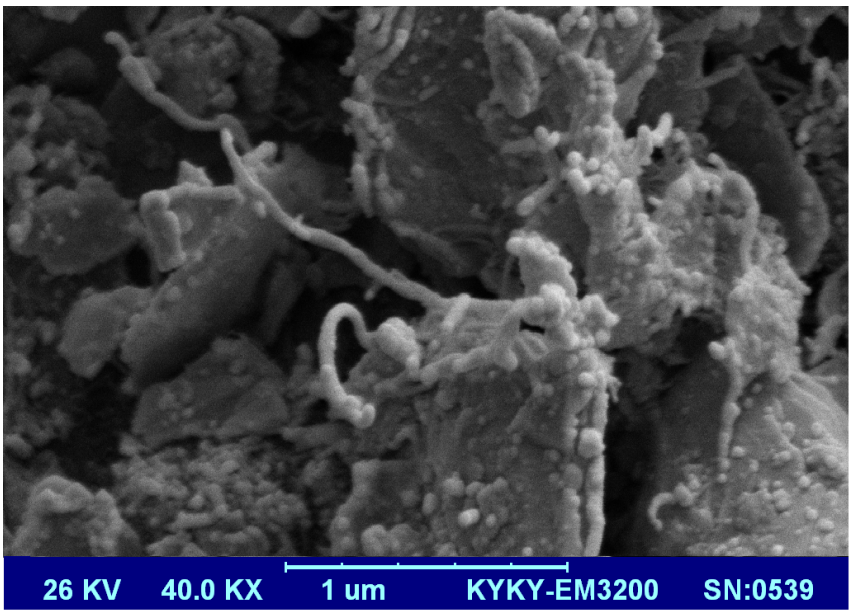


Fig. 2

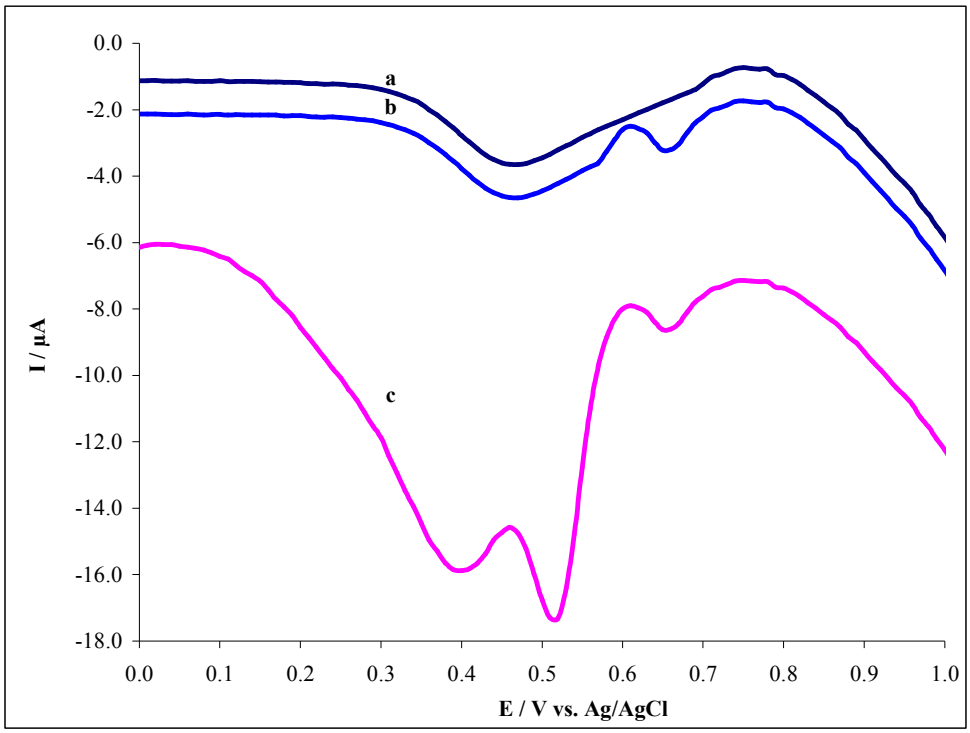


Fig. 3

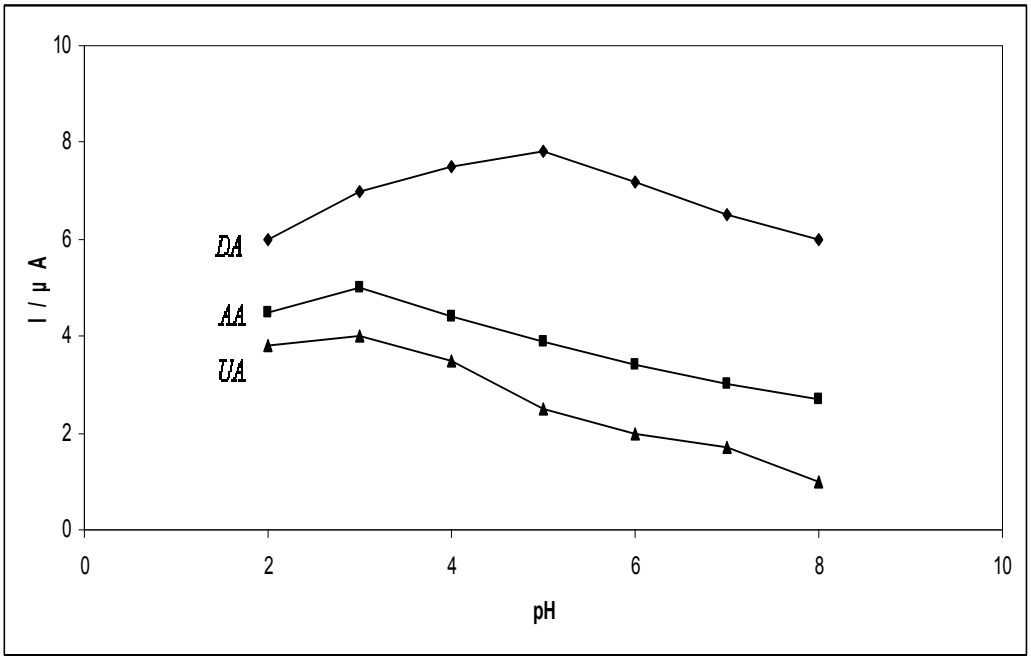


Fig. 4

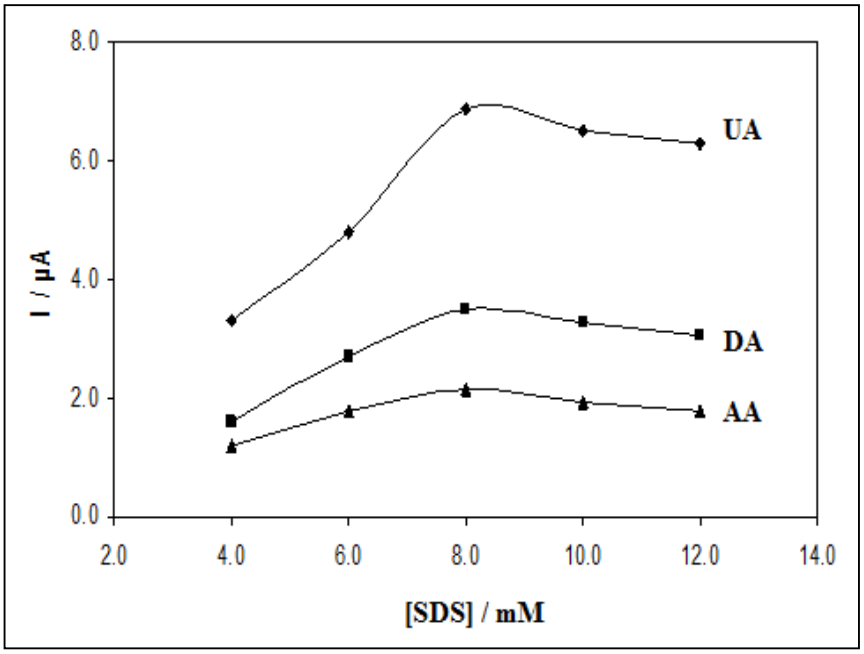


Fig. 5

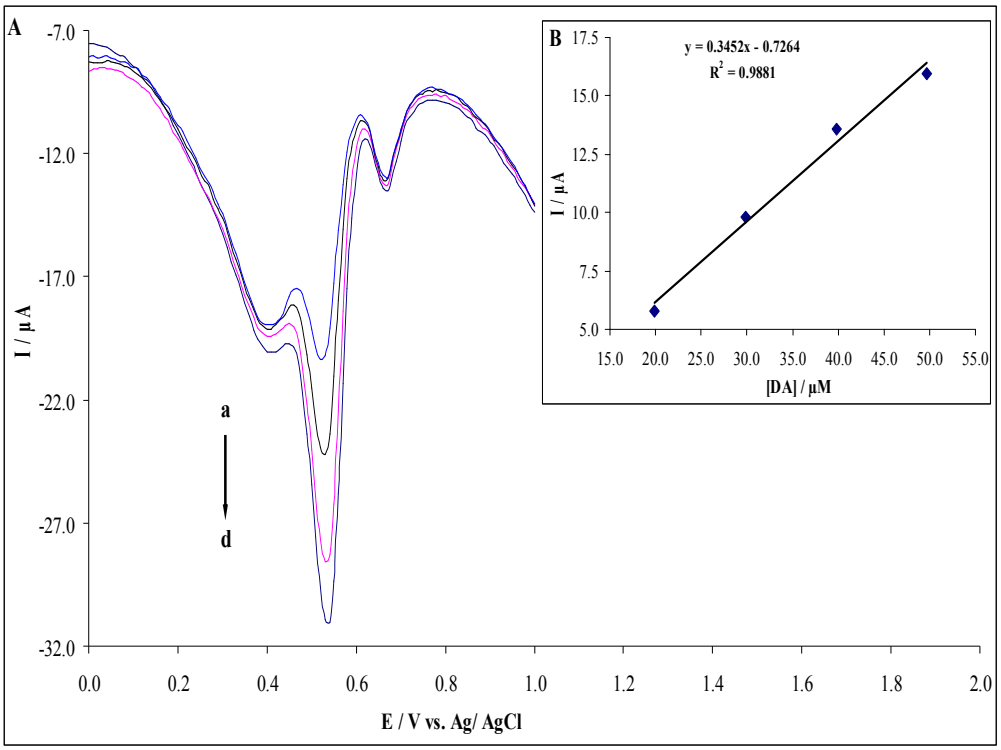


Fig. 6

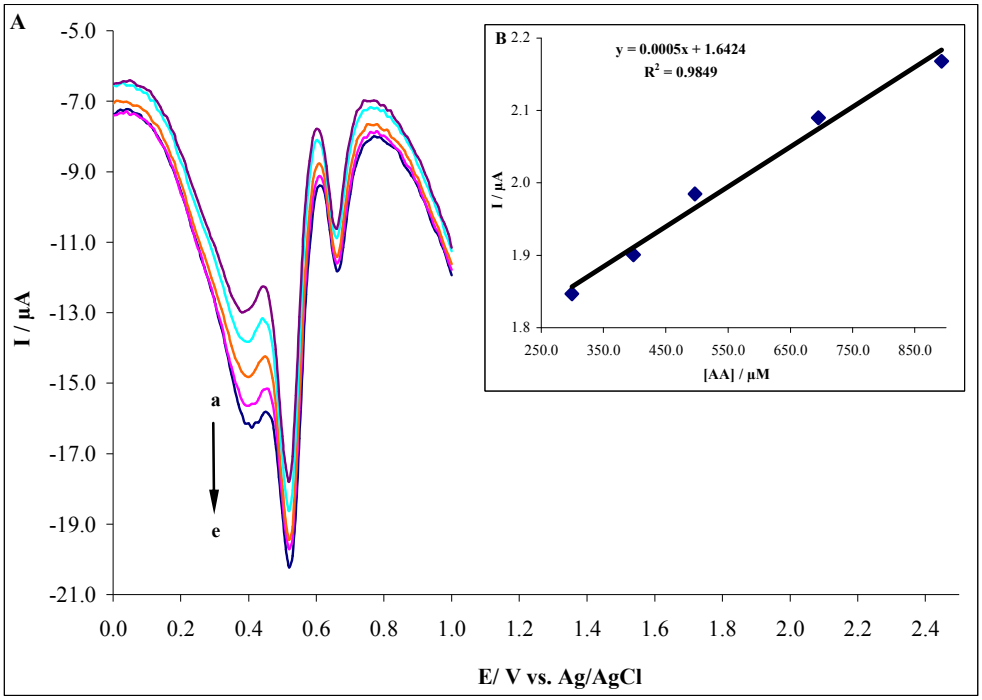


Fig. 7

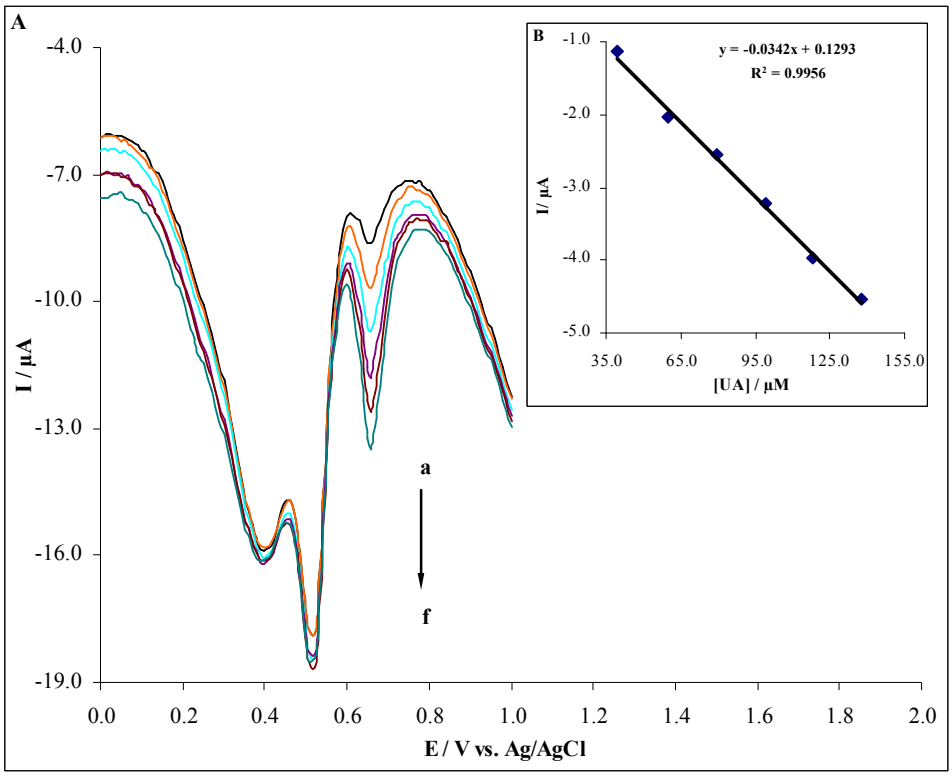


Table 1

Foreign substances	Tolerance (μM)
K^+, Na^+	300.0
Ca^{2+}, Mg^{2+}, Zn^{2+}	200.0
Citric acid	100.0
Cysteine, Lysine, Glucose	50.0

Table 2

Sample	Analyte	Content	Added (μM)	Found (μM)	Recovery (%)
Injections	***DA***	20.0	5.0	24.9	98.6
	DA	20.0	10.0	30.1	101.2
Urine 1	***UA***	15.2	5.0	20.2	99.9
	AA	2.1	5.0	7.1	100.0
Urine 2	***UA***	14.5	10.0	24.4	99.6
	AA	1.43	10.0	11.4	99.9