Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Synthesis of ZnO nanorods and its application to construct a nanostructured base electrochemical sensor for determination of levodopa in the presence of carbidopa

Elahe Molaakbari [a,b], Ali Mostafavi[a], Hadi Beitollahi[*c], Reza Alizadeh[d]

[a]*Department of Chemistry, Shahid Bahonar University of Kerman, P.O. Box 76175-133, Kerman, Iran*

[b]*Young Researchers Society, Shahid Bahonar University of Kerman, P.O. Box 76175-133, Kerman, Iran*

[c]*Environment Department, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran*

[d] *Department of Chemistry, Faculty of Science, Qum University, Qum, Iran*

Abstract

A novel carbon paste electrode modified with ZnO nanorods and 5-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid (3,4´AAZCPE) was fabricated. The electrochemical study of the modified electrode, as well as its efficiency for electrocatalytic oxidation of levodopa, is described. The electrode was employed to study the electrocatalytic oxidation of levodopa, using cyclic voltammetry (CV), chronoamperometry (CHA), and square-wave voltammetry (SWV) as diagnostic techniques. It has been found that the oxidation of levodopa at the surface of modified electrode occurs at a potential of about 370 mV less positive than that of an unmodified carbon paste electrode. SWV exhibits a linear dynamic range from 1.0×10^{-7} M to 7.0×10^{-5} M and a detection limit of 3.5×10^{-8} M for levodopa. In addition, this modified electrode was used for simultaneous determination of levodopa and carbidopa. Finally, the modified electrode was used for determination of levodopa and carbidopa in some real samples.

Keywords: Levodopa, Carbidopa, ZnO nanorods, Electrochemically Modified Electrode, Electrochemical Sensors

*corresponding author:

E-mail address: h.beitollahi@yahoo.com

Fax No: +98 3426226617, Tel No: +98 3426226613

1. Introduction

Patients suffering from Parkinson's disease have a significant depletion of dopamine in their brains. Dopamine can not be administered directly because this neurotransmitter cannot cross the blood–brain barrier into the central nervous system and cannot be employed to restore its normal level.[1] Levodopa (Scheme 1) is a precursor of the neurotransmitter dopamine, widely used in the clinical treatment of Parkinson's disease.[2] It could be converted to dopamine by DOPA decarboxylase and capable of crossing the protective blood-brain barrier.[3] After administration, levodopa is converted into dopamine through enzymatic reaction catalyzed by dopadecarboxylase. Some side effects of systemic dopamine can appear if levodopa is taken at high dosages because of the metabolism of levodopa being extracerebral. Administration of levodopa in combination with carbidopa (Scheme 1) an inhibitor of the decarboxylase enzyme, which does not cross the blood-brain barrier, helps to control dopamine levels in appropriate manner and reduces side effects.[4] In order to achieve better curative effect and lower toxicity, it is very important to control the content of levodopa and carbidopa in pharmaceutical tablets.

A number of methods like spectrophotometry,[5] high performance liquid chromatography (HPLC) [6] and chemiluminescence (CL) [7] have been reported in literature for the determination of levodopa or carbidopa in biological samples and pharmaceutical formulations.

Nevertheless, each technique has often suffered from diverse disadvantages with regard to cost and selectivity, the use of organic solvents, complex sample preparation procedures, and long analysis time. Electrochemical methods have also been used and attracted enormous interest due to its advantages of simplicity, rapid response, excellent reproducibility, good stability, low cost and low detection limit, etc..[8-16]

ZnO is one of the most attractive semiconductors, and has wide band gap (3.37 eV) with hexagonal wurtzite structure, high exciton binding energy (60 meV), and a wide range of applications such as sensors, optoelectronic devices, photonic detectors, polarized light emitting devices, catalysis, photovoltaicsetc.[17] ZnO can be synthesised in different single crystal forms such as thin films,[18] and it is enriched with number of one dimensional morphologies including nanobelts,[19] nanorods,[20] and nanowires.[21] These nanostructures possess high surface area to volume ratio and outstanding mechanical stability which strongly favours for the design of sensors based on the ZnO nanomaterial [22] and suitable candidate for the modification of electrodes. [23]

Carbon paste electrode (CPE) is a special kind of heterogeneous carbon electrode consisting of mixture prepared from carbon powder (as graphite, glassy carbon and others carbonaceous materials) and a suitable water-immiscible or non-conducting binder.[24–29]

The use of carbon paste as an electrode was initially reportedin 1958 by Adams.[30] In afterward researches awide variety of modifiers including enzymes, [31] polymers [32] and nanomaterials [33–36] have been used with these versatile electrodes. CPEs are widely applicable inboth electrochemical studies and electroanalysis thank to their advantages such as very low background current (compared to solid graphite or noble metal electrodes), facility to prepare, low cost, large potential window, simple surface renewal process and easiness of miniaturization. Besides the advantageous properties and characteristics listed before, the feasibility of incorporation different substances during the paste preparation (which resulting in the so-called modified carbon paste electrode), allow the fabrication of electrodes with desired composition, and hence, with pre-determined properties.[37]

The electrochemical methods using chemically modified electrodes (CMEs) have been widely used as sensitive and selective analytical methods for the detection of the trace amounts of biologically important compounds. One of the most important properties of CMEs has been their ability to catalyze the electrode process via significant decreasing of overpotential respect to unmodified electrode. With respect to relatively selective interaction of the electron mediator with the target analyte in a coordination fashion, these electrodes are capable to considerably enhance the selectivity in the electroanalytical methods.[38–41]

On the other hand, a practical mediator needs to have a low relative molar mass while being reversible, fast reacting, regenerated at low potential, pH independent, stable in both oxidized and reduced forms, unreactive with oxygen and nontoxic. [42-45]

Electrochemical techniques in the field of pharmaceutical analysis have developed due to their simplicity, reasonable accuracy and precision, low cost, and rapidity. There is no need for derivatization or time-consuming extraction steps in comparison with other techniques because of less sensitivity of electroanalytical methods to the matrix effects. [44-66]

In the present work, we describe the preparation of a new electrode composed of ZnO nanorods carbon paste electrode (ZCPE) modified with 3-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid (3,4′AAZCPE) and investigate its performance for the electrocatalytic determination of levodopa in aqueous solutions. We also evaluate the analytical performance of the modified electrode for quantification of levodopa in the presence of carbidopa.

2. Experimental

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 101, Eco Chemie, the Netherlands). The experimental conditions were controlled with Nova 1.6 software. A conventional three electrode cell was used at 25±1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the 3,4´AAZCPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 827 pH/Ion Meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. Levodopa, carbidopa and all other reagents were of analytical grade from Merck (Darmstadt, Germany). Graphite powder and paraffin oil (DC 350, density = 0.88 g cm^{-3}) as the binding agent (both from Merck, Darmstadt, Germany) were used for preparing the pastes.

2.2 Synthesis of ZnO nanorods

The growth solution was prepared by modifying the method reported by Vayssiers. Briefly, a typical synthesis involved the preparation of a 100 ml aqueous solution of 0.005 M $Zn(NO_3)_2.6H_2O$ and HMT in a closed Pyrex bottle equipped with an autoclavable screw cap. The above fibers were kept immersed in this solution for 4 hours at 90 ^oC. the precipitate was collected by centrifugation process at 15000 rpm. A typical SEM for synthesized ZnO nanorods is shown in Fig. 1.

2.3. Synthesis of 3-(4'-Amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid

For preparing of the title compound, 2.18 g (20 mmol) of 2-aminophenol, 4.54 g (20 mmol) of 4-bromocinnamic acid and 4.62 g of $Pd(PPh_3)_4$ into a 50 ml conical vial and add 20 ml of dimethylacetamide (DMA). And a magnetic spin vane to the conical vial and attach a water-cooled condenser. The mixture was heated at about 90 ◦C for at least 12 hours. The progress of reaction was monitored by TLC. After completion of reaction, remove the apparatus from the heater and allow it to cool for a few minutes. Collect the $Pd(PPh_3)_4$ by vacuum filtration using a Hirsch funnel. Chloroform was added to the mixture and filtered to recover the catalyst and the

crude product recrystallized from iso-propanol and chloroform (20:80) to afford pure 3-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid with 73 % yields.

FTIR(KBr,cm^{-1}): 3428, 3365, 3302, 3030, 2975, 2830, 2750, 2615, 1680, 1622, 1605, 1575, 1525, 1490, 1467, 1425, 1403, 1333, 1310, 1290, 1285, 1250, 1223, 1210, 1178, 989, 925, 880, 767, 692, 614, 496.

^{1}H-NMR(400MHz, DMSO-d_6): 4.62(br,2H), 6.50 (d, J = 6.4, 1H), 6.73(d, J = 8.4, 1H), 6.76 (d, J = 8.4, 1H), 6.90 (s,1H), 7.23 (d, J = 8.6, 2H), 7.50 (d, J = 8.6, 2H), 7.55 (d, J = 6.4, 1H), 9 (br, 1H), 12.35 (br, 1H).

2.4. Preparation of the electrode

The 3,4´AAZCPEs were prepared by hand mixing 0.01 g 3,4´AA with 0.94 g graphite powder and 0.050 g ZnO nanorods with a mortar and pestle. Paraffin oil was added to the above mixture and mixed for 20 min until an uniformly wet paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, 3,4´AA modified CPE electrode (3,4´AACPE) without ZnO nanorods, ZnO nanorods carbon paste electrode (ZCPE) without 3,4´AA, and unmodified CPE in the absence of both 3,4´AA and ZnO nanorods were also prepared in the same way.

3. Results and discussion

3.1. Electrochemical properties of 3,4´AAZCPE

To the best of our knowledge there is no prior report on the electrochemical properties and, in particular, the electrocatalytic activity of 3,4´AA in aqueous media. Therefore, we prepared 3,4´AAZCPE and studied its electrochemical properties in a PBS (pH 7.0) using CV (Fig.2 A). It should be noted that one of the advantages of 3,4´AA as an electrode modifier is its insolubility in aqueous media. Experimental results showed reproducible, well-defined, anodic and cathodic peaks with E_{pa}, E_{pc} and E°´ of 270, 130 and 200 mV vs. Ag/AgCl/KCl (3.0 M) respectively. The observed peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 140 mV, was greater than the value of 59/n mV expected for a reversible system,[66] suggesting that the redox couple of 3,4´AA in 3,4´AAZCPE has a quasi-reversible behavior in aqueous medium. The effect of the

potential scan rate (ν) on electrochemical properties of the 3,4´AAZCPE was also studied by CV. Plots of the both anodic and cathodic peak currents (I_p) were linearly dependent on ν in the range of 10 to 700 mV s^{-1} (Fig. 2 B), indicating that the redox process of 3,4´AA at the modified electrode are those anticipated for a surface-confined redox couple.[66]

In addition, the longterm stability of the 3,4´AAZCPE was tested over a 3-week period. When CVs were recorded after the modified electrode was stored in atmosphere at room temperature, the peak potential for levodopa oxidation was unchanged and the current signals showed less than 2.1% decrease relative to the initial response. The antifouling properties of the modified electrode toward levodopa oxidation and its oxidation products were investigated by recording the CVs of the modified electrode before and after use in the presence of levodopa. CVs were recorded in the presence of levodopa after having cycled the potential 20 times at a scan rate of 10 mV s^{-1}. The peak potentials were unchanged and the currents decreased by less than 2.4%. Therefore, at the surface of 3,4´AAZCPE, not only the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decreases.

3.2. Influence of pH

The electrochemistry of 3,4´AA molecule is generally pH dependent. Thus, the electrochemical behavior of 3,4´AAZCPE was studied at different pHs using CV (Fig. 3). It was observed that the anodic and cathodic peak potentials of 3,4´AAZCPE shift to less positive values with increasing pH. Inset of Fig. 3 shows potential-pH diagrams constructed by plotting the anodic, cathodic and half-wave potential values as the function of pH. As can be seen the slopes are 54.5, 60.71and 57.6 mV/pH for E_{pa}, E_{pc} and $E_{1/2}$ respectively, indicating that the system obeys the Nernst equation for an equal electron and proton transfer reaction.[66]

3.3. Electrocatalytic oxidation of levodopa at a 3,4´AAZCPE

The electrochemical behavior of levodopa and 3,4´AA are dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of levodopa. Thus the electrochemical behavior of levodopa was studied in 0.1 M PBS in different pH values ($2.0 < pH < 11.0$) at the surface of 3,4´AAZCPE by CV. It was found that the electrocatalytic oxidation of levodopa at the surface of 3,4´AAZCPE was more favored under neutral conditions than in acidic or basic medium. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak

current in the CVs of 3,4′AAZCPE. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of levodopa oxidation at the surface of 3,4′AAZCPE.

Fig. 4 depicts the CV responses for the electrochemical oxidation of 50.0 μM levodopa at unmodified CPE (curve b), ZCPE (curve d), 3,4′AACPE (curve e) and 3,4′AAZCPE (curve f). Also, curve a shows unmodified CPE in 0.1 M PBS (pH 7.0).

As it is seen, while the anodic peak potential for levodopa oxidation at the ZCPE, and unmodified CPE are 590 and 640 mV, respectively, the corresponding potential at 3,4′AAZCPE and 3,4′AACPE is ~ 270 mV. These results indicate that 3,4′AA can act as a good mediator and peak potential for levodopa oxidation at the 3,4′AAZCPE and 3,4′AACPE shift by ~ 320 and 370 mV toward negative values compared to ZCPE and unmodified CPE, respectively. However, 3,4′AAZCPE shows much higher anodic peak current for the oxidation of levodopa compared to 3,4′AACPE, indicating that the combination of ZnO nanorods and the mediator (3,4′AA) has significantly improved the performance of the electrode toward levodopa oxidation. In fact, 3,4′AAZCPE in the absence of levodopa exhibited a well-behaved redox reaction (Fig. 4, curve c) in 0.1 M PBS (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of 50.0 μM levodopa (curve f), which can be related to the strong electrocatalytic effect of the 3,4′AAZCPE towards this compound (Scheme 2).

The effect of scan rate on the electrocatalytic oxidation of levodopa at the 3,4′AAZCPE was investigated by linear sweep voltammetry (LSV) (Fig. 5). As can be observed in Fig. 5, the oxidation peak potential shifted to more positive potentials with increasing of scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) vs. the square root of scan rate ($\nu^{1/2}$) was found to be linear in the range of 2-35 mV s^{-1}, suggesting that, at sufficient overpotential, the process is diffusion controlled rather than surface controlled (Fig. 5 inset).[66]

Fig. 6 shows the LSV of 3,4′AAZCPE obtained in 0.1 M PBS (pH 7.0) containing 50.0 μM levodopa, with a sweep rate of 2 mV s^{-1}. The points show the rising part of the voltammogram (known as the Tafel region), which is affected by the electron transfer kinetics between levodopa and 3,4′AAZCPE. If deprotonation of levodopa is a sufficiently fast step, the number of electrons involved in the rate determining step can be estimated from the slope of the Tafel plot. The inset of Fig. 6 shows a Tafel plot that was drawn from points of the Tafel region of the LSV. The Tafel slope of 92.0 mV obtained in this case agrees well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of α=0.36.

3.4. Chronoamperometric measurements

Chronoamperometric measurements of levodopa at 3,4´AAZCPE were carried out by setting the working electrode potential at 0.35 V (at the first potential step) and at 0.1 V (at second potential step) vs. Ag/AgCl/KCl (3.0 M) for the various concentration of levodopa in PBS (pH 7.0) (Fig.7). For an electroactive material (levodopa in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation.[66] Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of levodopa (Fig. 7A). The slopes of the resulting straight lines were then plotted vs. levodopa concentration (Fig. 7B). From the resulting slope and Cottrell equation the mean value of the D was found to be 9.9×10^{-6} cm^2/s.

3.5. Calibration plot and detection limit

Square wave voltammetry (SWV) method was used to determine the concentration of levodopa (Initial potential= -0.02 V, End potential=0.43 V, Step potential=0.001 V, Amplitude=0.02 V, Frequency=10 Hz) (Fig. 8). The plot of peak current vs. levodopa concentration consisted of two linear segments with slopes of 0.467 and 0.105 μA μM^{-1} in the concentration ranges of 0.1 to 5.0 μM and 5.0 to 70.0 μM, respectively. The difference in the slopes for the calibration curves is due to the different activity of the electrode surface with low and high concentration of the analyte. In the lower levodopa concentration, due to a high number of active sites (in relation to the total number of the analyte molecules), the slope of the first calibration curve is high. While in the higher levodopa concentration, due to decreasing active sites (in relation to the total number of analyte molecules, mainly at the surface of the electrode), therefore the slope of the second calibration decreased too.

Also, the detection limit, C_m, of levodopa was obtained using the following equation:[66]

$$C_m = 3s_b/m \qquad (1)$$

In the above equation, m is the slope of the calibration plot (0.467 μA μM^{-1}) in the first linear range (0.1 to 50.0 μM), and s_b is the standard deviation of the blank response which is obtained from 20 replicate measurements of the blank solution. The detection limit (3σ) of levodopa was found to be 3.5×10^{-8} M. These values are compared with values reported by other research groups for electrocatalytic oxidation of levodopa at the surface of chemically modified electrodes by other mediators (Table 1).

3.6. Simultaneous determination of levodopa and carbidopa

One of the main objective of this study was to detect levodopa and carbidopa simultaneously using 3,4´AAZCPE. This was performed by simultaneously changing the concentrations of levodopa and carbidopa and recording the SWVs (Initial potential= -0.02 V, End potential=0.75 V, Step potential=0.001 V, Amplitude=0.02 V, Frequency=10 Hz). The voltammetric results showed well-defined anodic peaks at potentials of 210 and 490 mV, corresponding to the oxidation of levodopa and carbidopa respectively, indicating that simultaneous determination of these compounds is feasible at the 3,4´AAZCPE as shown in Fig. 9.

The sensitivity of the modified electrode towards the oxidation of levodopa was found to be 0.106 μA μM^{-1}. This is very close to the value obtained in the absence of carbidopa (0.105 μA μM^{-1}, see Section 3.5), indicating that the oxidation processes of these compounds at the 3,4´AAZCPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

3.7. Interferences study

The influence of various substances as compounds potentially interfering with the determination of levodopa was studied under optimum conditions with 50.0 μM levodopa at pH 7.0. The potentially interfering substances were chosen from the group of substances commonly found with levodopa in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5 % in the determination of levodopa. According to the results, glucose, sucrose, lactose, fructose or citric acid, nor a 600-fold excess of methanol, ethanol, Mg^{2+}, SO_4^{2-}, Al^{3+}, NH_4^+, Fe^{2+}, Fe^{3+}, CO_3^{2-}, Cl^- or F^-, alanine, methionine, phenylalanine, glycine, or folic acid (vitamin B_9), saturated starch solution, urea did not interfer with the determination of levodopa. But methyldopa, ascorbic acid, epinephrine and norepinephrine with equal molar showed interference in determination of levodopa. Although ascorbic acid showed interference, this interference could be minimized, if necessary, by using ascorbic oxidase enzyme, which exhibits a high selectivity to the oxidation of ascorbic acid.

3.8 Real sample analysis

3.8.1 Determination of levodopa and carbidopa in pharmaceutical products

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of levodopa and carbidopa in Parkin C fort tablet purchased from Alborz Darou (Each tablet contains 250 mg levodopa and 25 mg carbidopa).

Based on the repeated SVW responses (n = 3) of the diluted analytes and the samples that were spiked with specified concentration of levodopa and carbidopa, measurements were made for determination of levodopa and carbidopa concentrations. The results are listed in Table 2.
The reliability of the proposed modified electrode was also evaluated by comparing the obtained results with those declared in the label of the pharmaceutical preparations (Table 2). The results in Table 2 show the relative standard derivations (RSD%) and the recovery rates of the spiked samples are acceptable. Also, the data in Table 3 indicate that the results obtained by utilizing 3,4´AAZCPE are in good agreement with those declared in the label of the preparations. Thus, the modified electrode can be efficiently used for individual or simultaneous determination of levodopa and carbidopa in pharmaceutical preparations.

3.8.2 Determination of levodopa and carbidopa in human blood serum and water samples

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of levodopa and carbidopa in human blood serum and water samples. The results for determination of the two species in real samples are given in Table 4. Satisfactory recovery of the experimental results was found for levodopa and carbidopa. The reproducibility of the method was demonstrated by the mean relative standard deviation (RSD).

4. Conclusion

This work demonstrates the construction of a chemically modified carbon paste electrode by the incorporation of ZnO nanorods and 5-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid as modifying species. The electrochemical behavior of the levodopa was studied by CV. The results showed that the oxidation of levodopa is catalyzed at pH 7.0, with the peak potential of levodopa shifted by 370 mV to a less positive potential at the surface of the modified electrode. Potential differences of 280 mV between levodopa and carbidopa were detected, which was large enough to determine levodopa and carbidopa individually and simultaneously. Finally, the modified

electrode was used for determination of levodopa and carbidopa in pharmaceutical product, human blood serum and water.

References:

1 D.G. Standaert, A.B. Young, in: J.G. Hardman, L.E. Limbird, P. B. Molinoff, R.W. Ruddon and A.G. Gilman (Eds.), The McGraw-Hill companies, New York, 9th edn, 1996.

2 X.Q. Chen, Y.Y. Jin and G. Tang, New Pharmacology, Beijing, 15th edn, 2003.

3 N.L. Benowitz and B.G. Katzung, Basic and Clinical Pharmacology, The McGraw-Hill Companies, New York, NY, 9th edn, 2004.

4 M. Blanco, J. Coello, H. Iturriaga, S. Maspoch and N. Villegas, *Anal. Lett.,* 2000, **33**, 2701-2718.

5 W.H. Kim, M.M. Karim and S.H. Lee, *Anal. Chim. Acta,* 2008, **619**, 2-7.

6 F. Bugamelli, C. Marcheselli, E. Barba and M.A. Raggi, *J. Pharm. Biomed. Anal,.* 2011, **54**, 562-567.

7 S. Zhao, W. Bai, B. Wang and M. He, *Talanta,* 2007, **73**, 142-146.

8 A. Babaei, A.R. Taheri and I.K. Farahani, *Sens. Actuators B,* 2013, **183**, 265-272.

9 A. Babaei, M. Sohrabi and A.R. Taheri, *J. Electroanal. Chem.,* 2013, **698**, 45-51.

10 J.B. Raoof, R. Ojani, M. A. Aref and M. Baghayeri, *Sens. Actuators B*, 2012, **166–167**, 508-518.

11 M. Mazloum-Ardakani, M. A. Sheikh-Mohseni, M. Abdollahi-Alibeik and A. Benvidi, *Analyst,* 2012, **137**, 1950-1955.

12 X. Yan, D. Pan, H. Wang, X. Bo and L. Guo, *J. Electroanal. Chem.,* 2011, **663**, 36-42.

13 P. Prabhu, R. Suresh Babu and S. Sriman Narayanan, *Sens. Actuators B*, 2011, **156**, 606-614.

14 G. Hu, L. Chen, Y. Guo, X. Wang and S. Shao, *Electrochim. Acta,* 2010, **55**, 4711-4716.

15 S. Shahrokhian and E. Asadian, *J. Electroanal. Chem.,* 2009, **636**, 40-46.

16 M. Mazloum. Ardakania, Z. Taleata, A. Khoshroo, H. Beitollahi and H. Dehghani, *Biosens. Bioelectron.,* 2012, **35**, 75-81.

17 S.J. Pearton, D.P. Norton, K. Ip, Y.W. Heo and T. Steiner, *Superlattices Microstruct.,* 2003, **34**, 3-32.

18 V. Khranovskyy, R. Minikayev, S. Trushkin, G. Lashkarev, V. Lazorenko, U. Grossner, W. Paszhkowicz, A. Suchocki, B.G. Svensson and R. Yakimova, *J. Cryst. Growth.,* 2007, **308**, 93-98.

19 Z.W. Pan, Z.R. Dai and Z.L. Wang, *Science,* 2001, **291**, 1947–1949.

20 V. Khranovskyy, I. Tsiaoussis, G.R. Yazdi, L. Hultman and R. Yakimova, *J. Cryst. Growth.,* 2010, **312**, 327–332.

21 M. Huang, Y. Wu, H. Feick, N. Tran, E. Weber and P. Yang, *Adv. Mater.,* 2001, **13**, 113–116.

22 S.J. Pearton, F. Ren, Y.L. Wang, B.H. Chu, K.H. Chen, C.Y. Chang, W. Lim, J. Lin and D.P. Norton, *Prog. Mater. Sci.,* 2010, **55**, 1.

23 A. Taherkhani, T. Jamali, H. Hadadzadeh, H. Karimi-Maleh, H. Beitollahi, M. Taghavi and F. Karimi, *Ionics*, 2013, DOI 10.1007/s11581-013-0992-0.

24 S. Tajik, M.A. Taher and H. Beitollahi, *Sens. Actuators B,* 2013, **188**, 923– 930.

25 E. Molaakbari, A. Mostafavi and H. Beitollahi, *Mater. Sci. Eng., C,* 2014, **36**, 168-172.

26 Z. Taleat, M. MazloumArdakani, H. Naeimi, H. Beitollahi, M. Nejati and H. R. Zare, *Anal. Sci.,* 2008, **24**, 1039-1044.

27 H. Beitollahi, J.B. Raoof and R. Hosseinzadeh, *Electroanalysis*, 2011, **23**, 1934–1940.

28 M. Mazloum-Ardakani, H. Beitollahi, M. K. Amini, F. Mirkhalaf, M. Abdollahi-Alibeik, *Sens. Actuators B,* 2010, **151**, 243–249

29 W. Yantasee, Y. Lin, T. S. Zemanian and G. E. Fryxell, *Analyst*, 2003,**128**, 467-472.

30 R.N. Adams, *Anal. Chem.,* 1958, **30**, 1576.

31 J.V.B. Kozan, R.P. Silva, S.H.P. Serrano, A.W.O. Lima and L. Angnes, *Anal. Chim. Acta,* 2007, **591**, 200–207.

32 R. Fonseca do Nascimento, T. Matheus G. Selva, W. F. Ribeiro, M. F. Belian, L. Angnes and V. Bernardo do Nascimento, *Talanta*, 2013, **105**, 354-359.

33 A. Mokhtari, H. Karimi-Maleh, A. A. Ensafi and H. Beitollahi, *Sens. Actuators B,* 2012, **169**, 96–105.

34 L. Tang, Y. Zhou, G. Zeng, Z. Li, Y. Liu, Y. Zhang, G. Chen, G. Yang, X. Lei and M. Wu, *Analyst*, 2013,**138**, 3552-3560

35 J.B. Raoof, R. Ojani and H. Beitollahi, *Electroanalysis,* 2007, **19**, 1822–1830.

36 M. Behpour, S. M. Ghoreishi, E. Honarmand and M. Salavati-Niasari, *Analyst*, 2011,**136**, 1979-1986.

37 J. B. Raoof, R. Ojani, H. Beitollahi and R. Hossienzadeh, *Electroanalysis,* 2006, **18**, 1193–1201.

38 S. Mehretie, J. Losada, M. Tessema, S. Admassie, T. Solomon, J. Perez-Pariente and I. Díaz, *Analyst*, 2012, **137**, 5625-5631

39 S. Mohammadi, H. Beitollahi and A. Mohadesi, *Sensor. Lett.,* 2013, **11**, 388–394.

40 H. Beitollahi, M. A. Taher, M. Ahmadipou and R. Hosseinzadeh, *Measurement,* 2014, **47**, 770–776.

41 J. R. Windmiller, N. Zhou, M.C. Chuang, G. Valdés-Ramírez, P. Santhosh, P. R. Miller, R. Narayan and J. Wang, *Analyst*, 2011,**136**, 1846-1851

42 H. Beitollahi, J.B. Raoof and R. Hosseinzadeh, *Anal. Sci.,* 2011, **27**, 991-997.

43 N. F. Atta, A. Galal and E. H. El-Ads, *Analyst*, 2012, **137**, 2658-2668.

44 J.B. Raoof, R. Ojani, H. Beitollahi and R. Hosseinzadeh, *Anal. Sci.,* 2006, **22**, 1213-1220.

45 M.H. Habibi, B. Karimi, M. Zendehdel and M. Habibi, *Spectrochim. Acta A*, 2013, **116**, 374-380.

46 V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon and S. Agarwal, *Anal. Biochem.,* 2011, **408**,179-196.

47 V.K. Gupta, A.K. Jain, G. Maheshwari, Heinrich Lang and Z. Ishtaiwi, *Sens. Actuators B,* 2006, **117**, 99-106.

48 V. K. Gupta, A. K. Singh, S. Mehtab and B. Gupta, *Anal. Chim. Acta,* 2006, **566**, 5-10.

49 V.K. Gupta, A.K. Jain and P. Kumar, *Sens. Actuators B,* 2006, **120**, 259-265.

50 V.K. Gupta, A.K. Jain, P. Kumar, S. Agarwal and G. Maheshwari, *Sens. Actuators B,* 2006, **113**,182-186.

51 R. N. Goyal, V. K. Gupta and S. Chatterjee, *Biosens. Bioelectron.,* 2009, **24**, 3562-3568.

52 R. N. Goyal, V. K. Gupta, N. Bachheti and R. Avatar Sharma, *Electroanalysis,* 2008, **20**, 757-764.

53 R.N. Goyal, V.K. Gupta and N. Bachheti, *Anal. Chim. Acta,* 2007, **597**, 82-89.

54 V.K. Gupta, A.K. Singh, M. Al Khayat and B. Gupta, *Anal. Chim. Acta,* 2007, **590**, 81-90.

55 V.K. Gupta, A.K. Singh and B. Gupta, *Anal. Chim. Acta,* 2007, **583**, 340-348.

56 V. K. Gupta, S. Chandra and H. Lang, *Talanta,* 2005, **66**, 575-580.

57 V. K Gupta, R. Prasad and A. Kumar, *Talanta*, 2003, **60**, 149-160.

58 V. K Gupta, S. Chandra and R. Mangla, *Electrochim. Acta,* 2002, **47**, 1579 - 1586.

59 V. K. Gupta, R. Mangla, U. Khurana and P. Kumar, *Electroanalysis*, 1999, 11, 573-576.

60 V. K. Gupta, S. Jain and U. Khurana, *Electroanalysis,* 1997, 9, 478-480.

61 V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon, S. Agarwal, *Anal. Biochem.,* 2011, **408**, 179-196.

62 V.K. Gupta, M.R. Ganjali, P. Norouzi, H. Khani, A. Nayak and S. Agarwal, *Critical Rev. Anal. Chem.,* 2011, **41**, 282-313.

63 V.K. Gupta, A. Nayak, S. Agarwal and B. Singhal, *Combinatorial Chem. High Throughput Screen.*, 2011, **14**, 284–302.

64 V. K. Gupta, L.P. Singh, R. Singh, N. Upadhyay and S.P. Kaur, B. Sethi, *J. Mol. Liq.,* 2012, **174**, 11-16.

65 V.K. Gupta, B. Sethi, R.A. Sharma, S. Agarwal and A. Bharti, *J. Mol. Liq.,* 2013, **177**, 114-118.

66 A.J. Bard and L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, second ed., Wiley, New York, 2001.

67 M. Mazloum-Ardakani, H. Beitollahi, M.A. Sheikh-Mohseni and H. Naeimi, *J. Iran. Chem. Soc.* 2012, **9**, 27-34.

68 F.R. Figueirêdo Leite, C.M. Maroneze, A.B. Oliveira, W.T.P.d. Santos, F. S. Damos and R.C.S. Luz, *Bioelectrochemistry,* 2012, **86**, 22-29.

69 A. Abbaspour, E. Dehghani and A. Noori, *Electroanalysis* 2011, **23**, 2878-2887.

70 X.X. Yan, D.W. Pang, Z.X. Lu, J.Q. Lu and H. Tong, *J. Electroanal. Chem.* 2004, **569**, 47-52.

Legend for the figures:

Fig. 1 SEM image of synthesized ZnO nanorods.

Fig. 2. (A) CVs of 3,4′AAZCPE in 0.1 M PBS (pH 7.0), at various scan rates, numbers 1–9 correspond to 10, 50, 100, 200, 300, 400, 500, 600 and 700 mV s^{-1}. (B) Variation of anodic and cathodic peak currents vs. scan rate.

Fig. 3. CVs (at 20 mV s^{-1}) of 3,4′AAZCPE at various buffered pHs. The numbers 1–7 correspond to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 pHs, respectively. Inset: plot of E_{pa}, E_{pc} and $E_{1/2}$ vs. pH.

Fig. 4. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0), (b) unmodified CPE in 50.0 μM levodopa, (c) 3,4′AAZCPE in 0.1 M PBS, (d) ZCPE in 50.0 μM levodopa, (e) 3,4′AACPE in 50.0 μM levodopa, and (f) 3,4′AAZCPE in 50.0 μM levodopa. In all cases the scan rate was 10 mV s^{-1}.

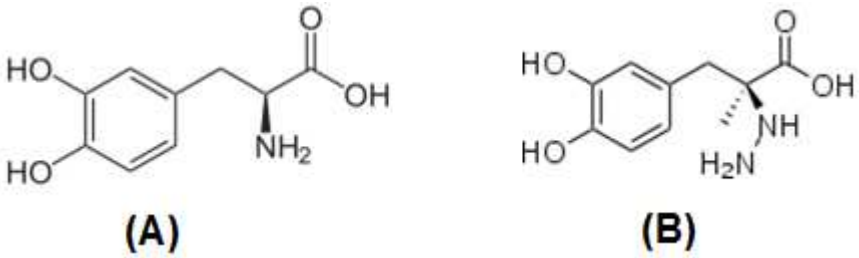
Fig. 5. LSVs of 3,4′AAZCPE in 0.1 M PBS (pH 7.0) containing 50.0 μM levodopa at various scan rates; Numbers 1-9 correspond to of 2, 4, 6, 8, 10, 14, 18, 25 and 35 mV s^{-1}, respectively. Inset Variation of anodic peak current vs. $\nu^{1/2}$.

Fig. 6. LSV (at 2 mV s^{-1}) of an 3,4′AAZCPE in 0.1 M PBS (pH 7.0) containing 50.0 μM levodopa. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV.

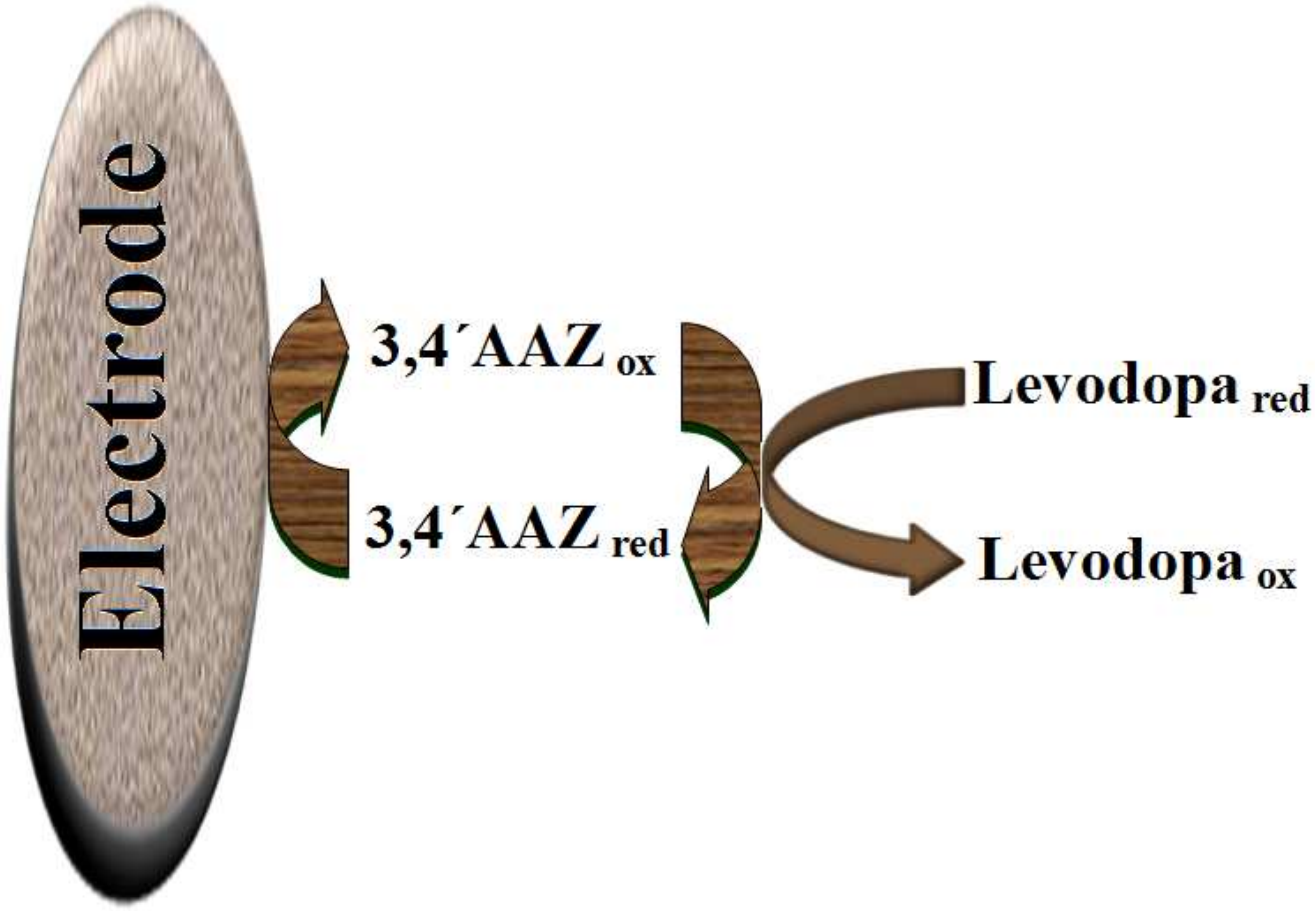
Fig. 7. Chronoamperograms obtained at 3,4′AAZCPE in 0.1 M PBS (pH 7.0) for different concentration of levodopa. The numbers 1–6 correspond to 0.0, 0.2, 0.6, 1.0, 1.4 and 1.6 mM of levodopa. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 2–6. (B) Plot of the slope of the straight lines against levodopa concentration

Fig. 8. SWVs of 3,4´AAZCPE in 0.1 M PBS (pH 7.0) containing different concentrations of levodopa. Numbers 1-13 correspond to 0.1, 0.5, 1.2, 2.5, 5.0, 7.5, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0 and 70.0 μM of levodopa. Insets show the plots of the electrocatalytic peak current as a function of levodopa concentration in the range of 0.2-5.0 μM (A) and 5.0-70.0 (B).

Fig.9. SWVs of 3,4´AAZCPE in 0.1 M PBS (pH 7.0) containing different concentrations of levodopa+carbidopa in μM, from inner to outer: 5.0+250.0, 10.0+500.0, 20.0+1000.0, 30.0+1500.0, 60.0+3000.0 and 70.0+3500.0 respectively. Insets (A) and (B) plots of I_p vs. levodopa and carbidopa concentrations respectively.



Scheme 1. Structures of levodopa (A) and carbidopa (B).



Scheme 2. Electrocatalytic oxidation of levodopa at the 3,4´AAZCPE.

Table 1 Comparison of the efficiency of some modified electrodes used in the electrocatalysis of levodopa

Electrode	Modifier	LOD (M)	LDR (M)	Ref.
Glassy carbon	Poly-pyrrole doped with tiron	1.0×10^{-7}	1.0×10^{-6}-1.0×10^{-4}	15
Carbon paste	2,2′-(1,2 butanediylbis(nitriloethylidyne))-bis-hydroquinone	2.0×10^{-7}	2.0×10^{-6}-4.0×10^{-4}	67
Basal plane pyrolytic graphite electrode	Chloro(pyridine)bis(dimethylglyoximato)cobalt(III)	8.6×10^{-7}	3.0×10^{-6} - 1.0×10^{-4}	68
Carbon paste	ß-cyclodextrin/poly(N-acetylaniline)(ß-CD/PNAANI)	2.0×10^{-7}	5.0×10^{-7}–1.17×10^{-4}	69
Glassy carbon	Single-wall carbon nanotube	3.0×10^{-7}	5.0×10^{-7}–2.0×10^{-5}	70
Carbon paste	3,4′AA	3.5×10^{-8}	1.0×10^{-7}-7.0×10^{-4}	This work

Table 2. Determination of levodopa and carbidopa in Parkin C fort tablet. All the concentrations are in μM (n=3).

Sample	Spiked		found		Recovery (%)		R.S.D. (%)	
	LD	CD	LD	CD	LD	CD	LD	CD
Parkin C fort tablet	0.0	100.0	4.8	100.3	96.0	100.3	2.69	2.41
	5.0	250.0	9.7	247.5	97.0	98.0	2.22	1.96
	10.0	500.0	15.2	504.7	101.3	100.9	2.07	1.89
	15.0	750.0	19.4	744.6	97.0	99.3	1.93	1.77

Table 3 Comparison of the total values of levodopa and carbidopa in Parkin C fort tablet using 3,4´AAZCPE with declared values in the lable of the sample (n=3).

Sample	Declared value	Found value	RSD%
Levodopa (mg per tablet)	250.0	244.6	2.2
Carbidopa (mg per tablet)	25	24.9	2.0

Table 4. Determination of levodopa and carbidopa in human blood serum and water samples. All the concentrations are in μM (n=3).

Sample	Spiked		found		Recovery (%)		R.S.D. (%)	
	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa
human blood serum								
1	0.0	0.0	ND	ND	-	-	-	-
2	5.0	400.0	4.9	410.2	98.0	102.5	3.1	2.4
3	10.0	600.0	10.2	606.5	102.0	101.1	2.1	1.8
4	15.0	800.0	15.1	812.8	100.7	101.6	2.5	2.8
water								
1	0.0	0.0	N.D	N.D	-	-	-	-
2	5.0	300	5.1	295.9	102.0	98.6	2.4	1.9
3	10.0	450	9.9	448.6	99.0	96.7	1.9	2.2
4	15.0	600	15.2	609.3	101.3	101.5	2.0	1.8

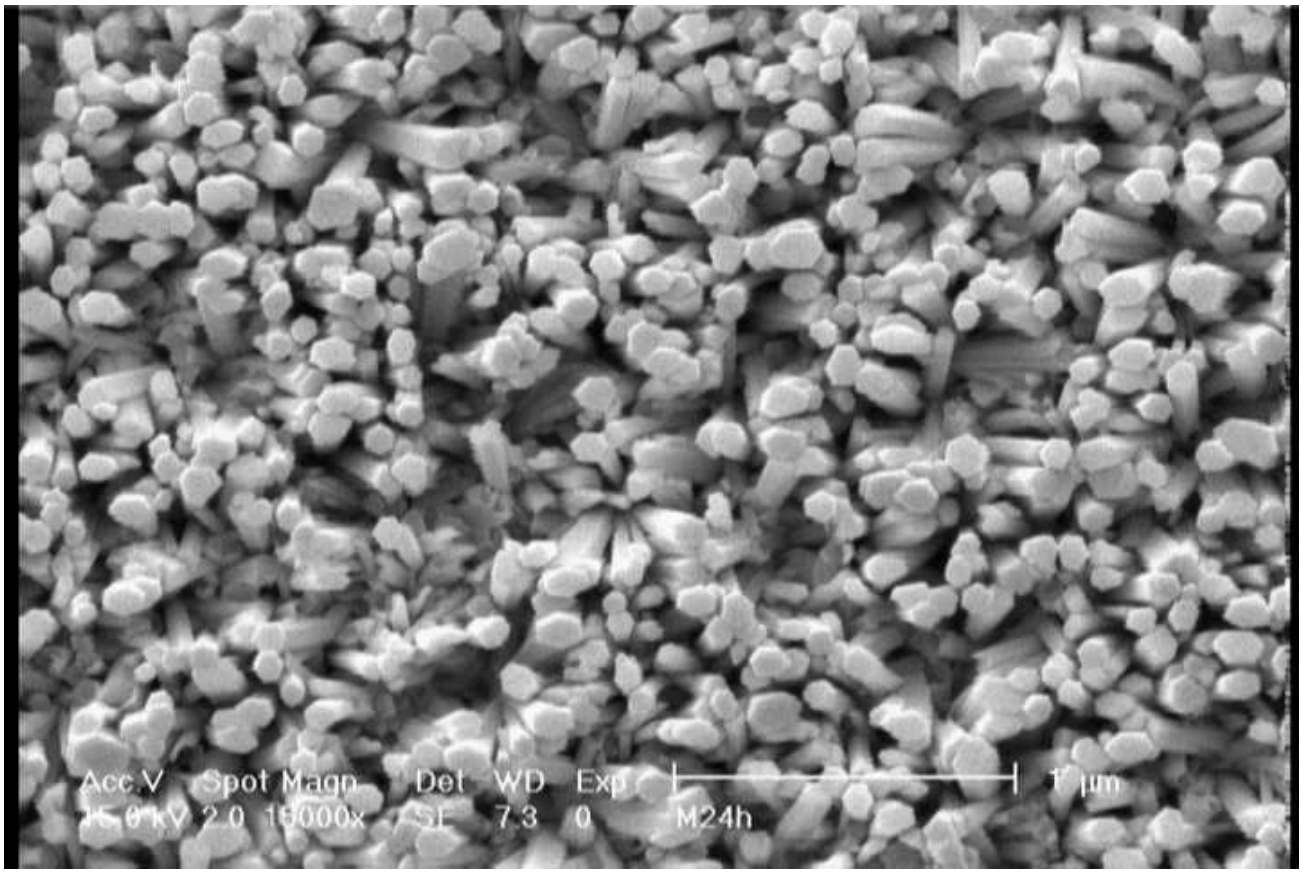


Fig. 1

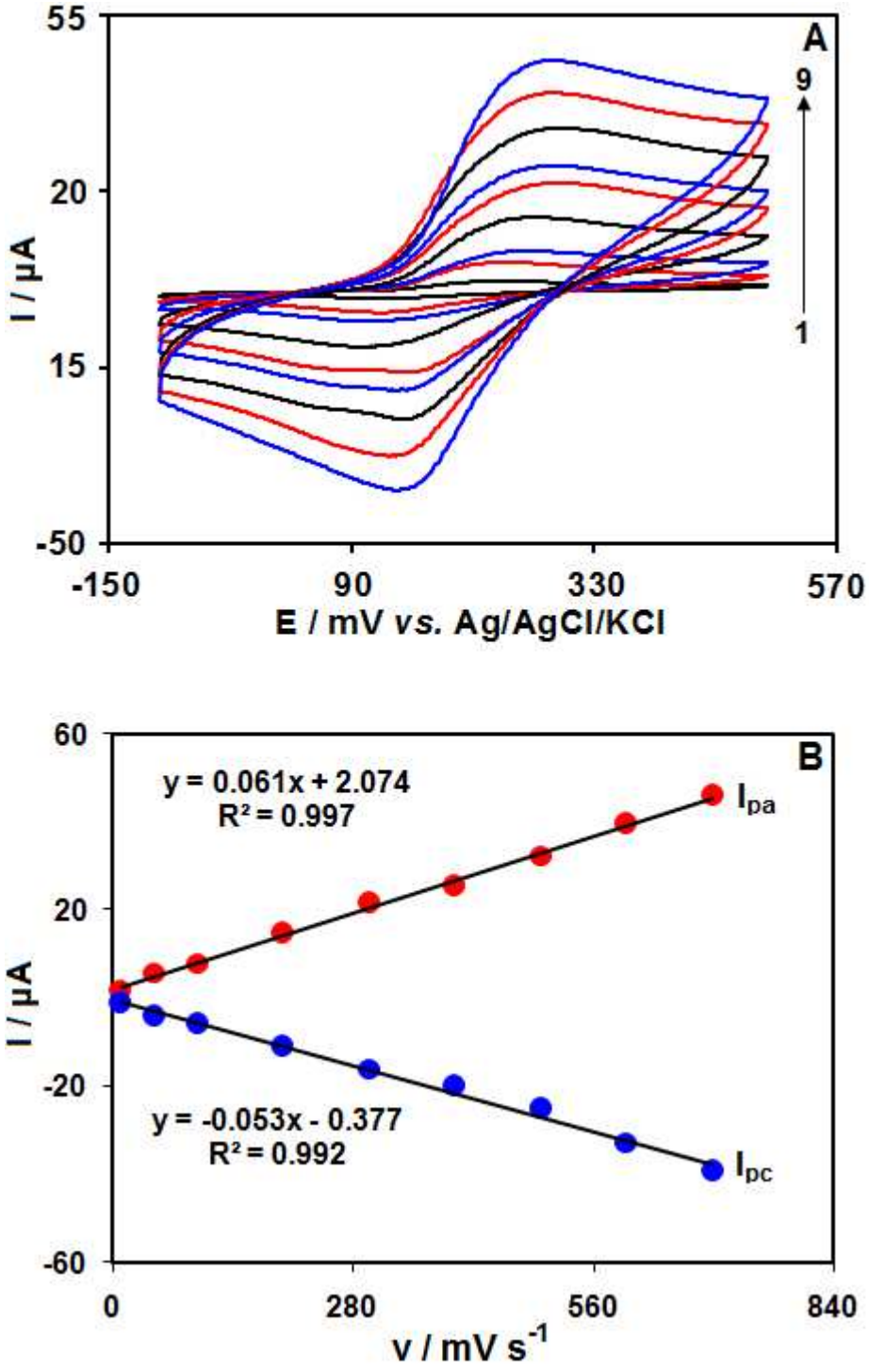


Fig. 2

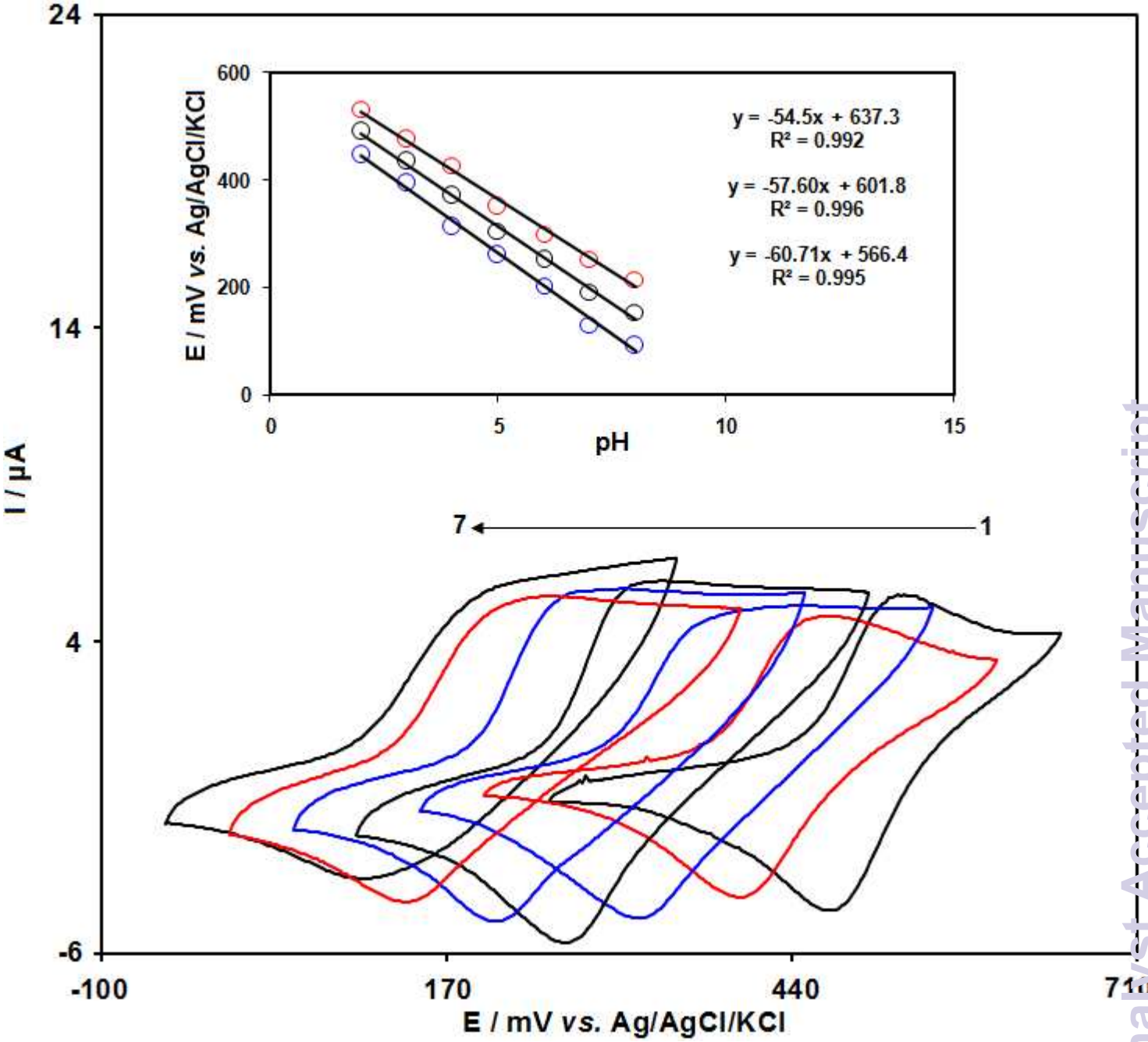


Fig. 3

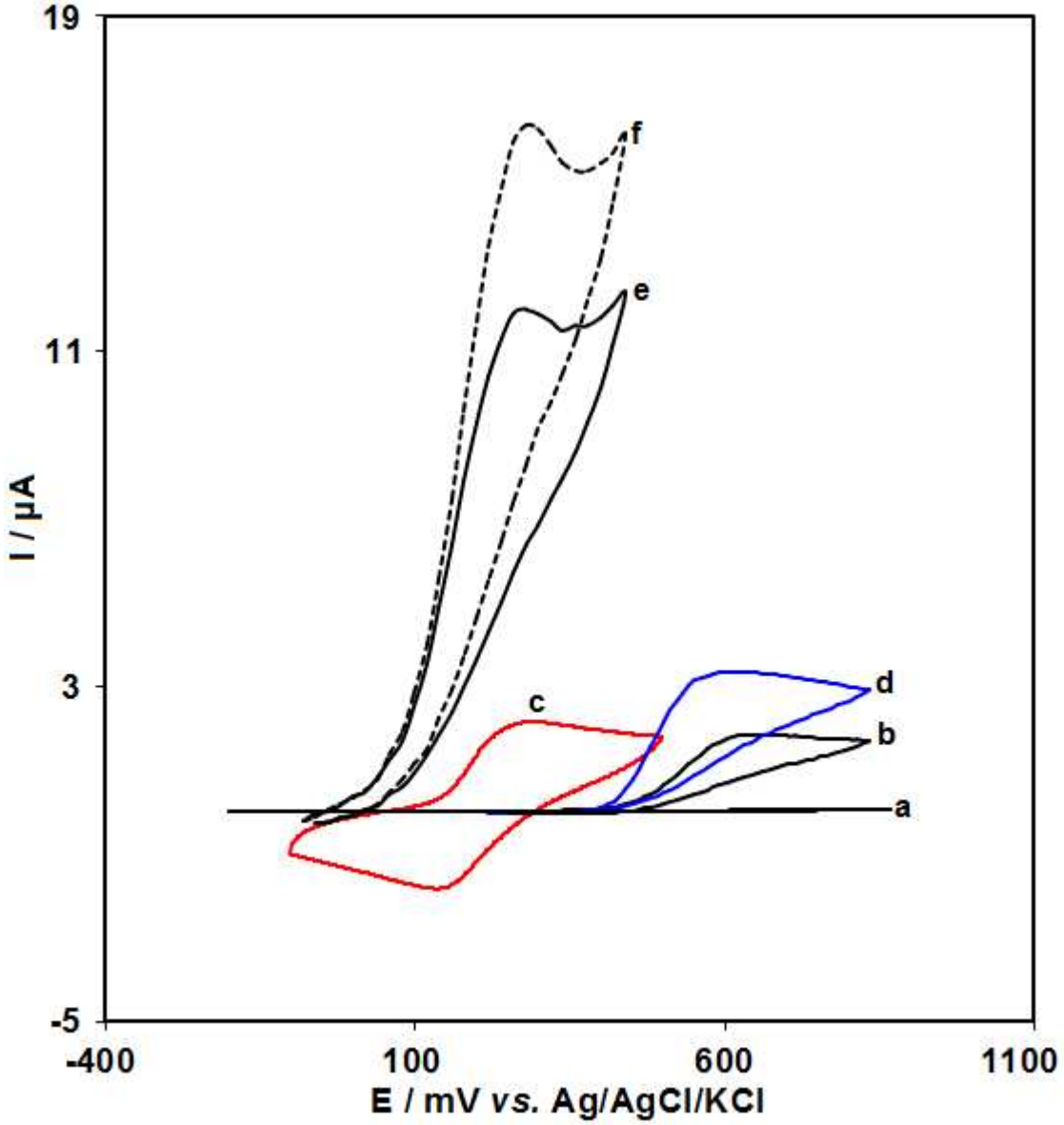


Fig. 4

.

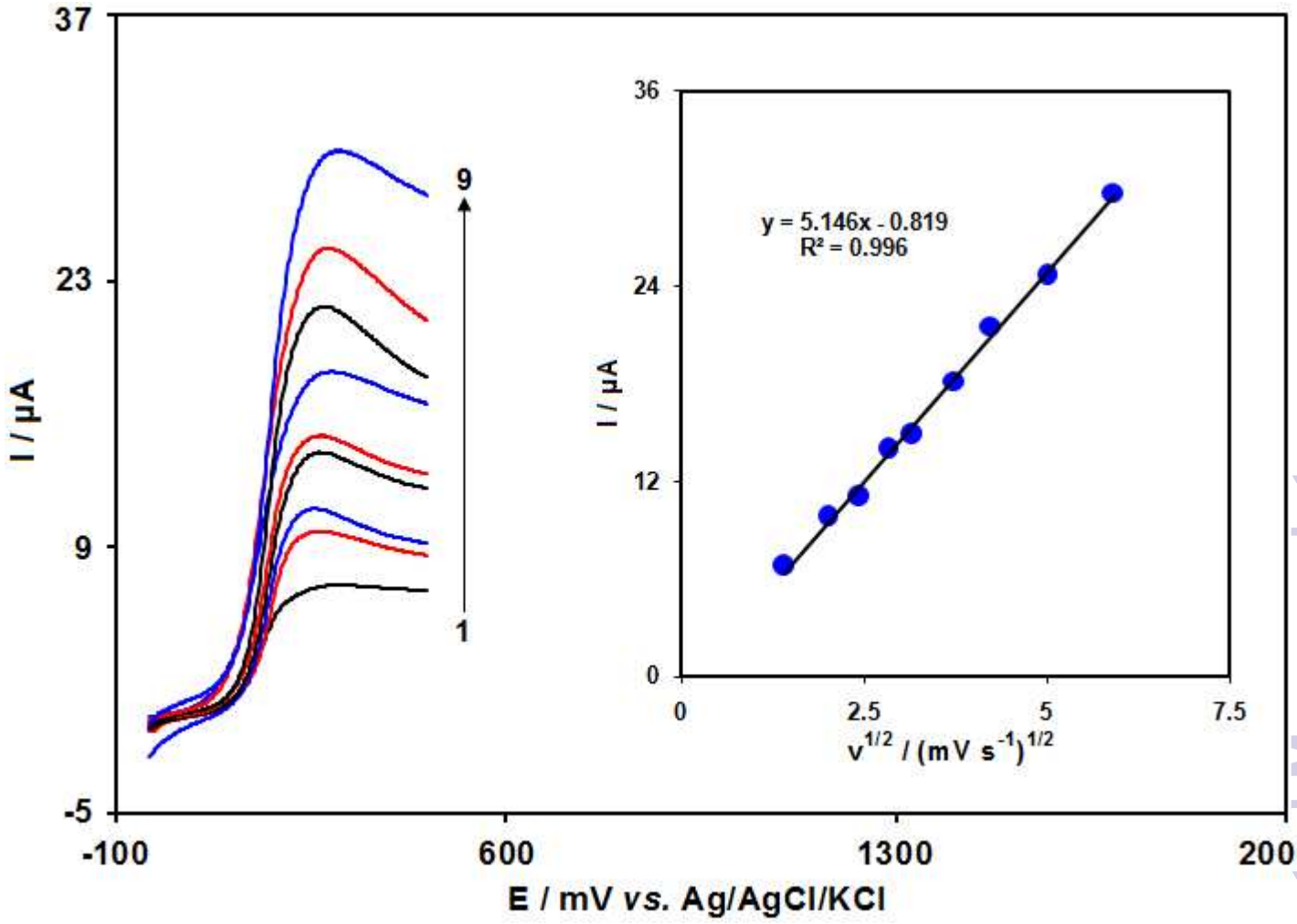


Fig. 5

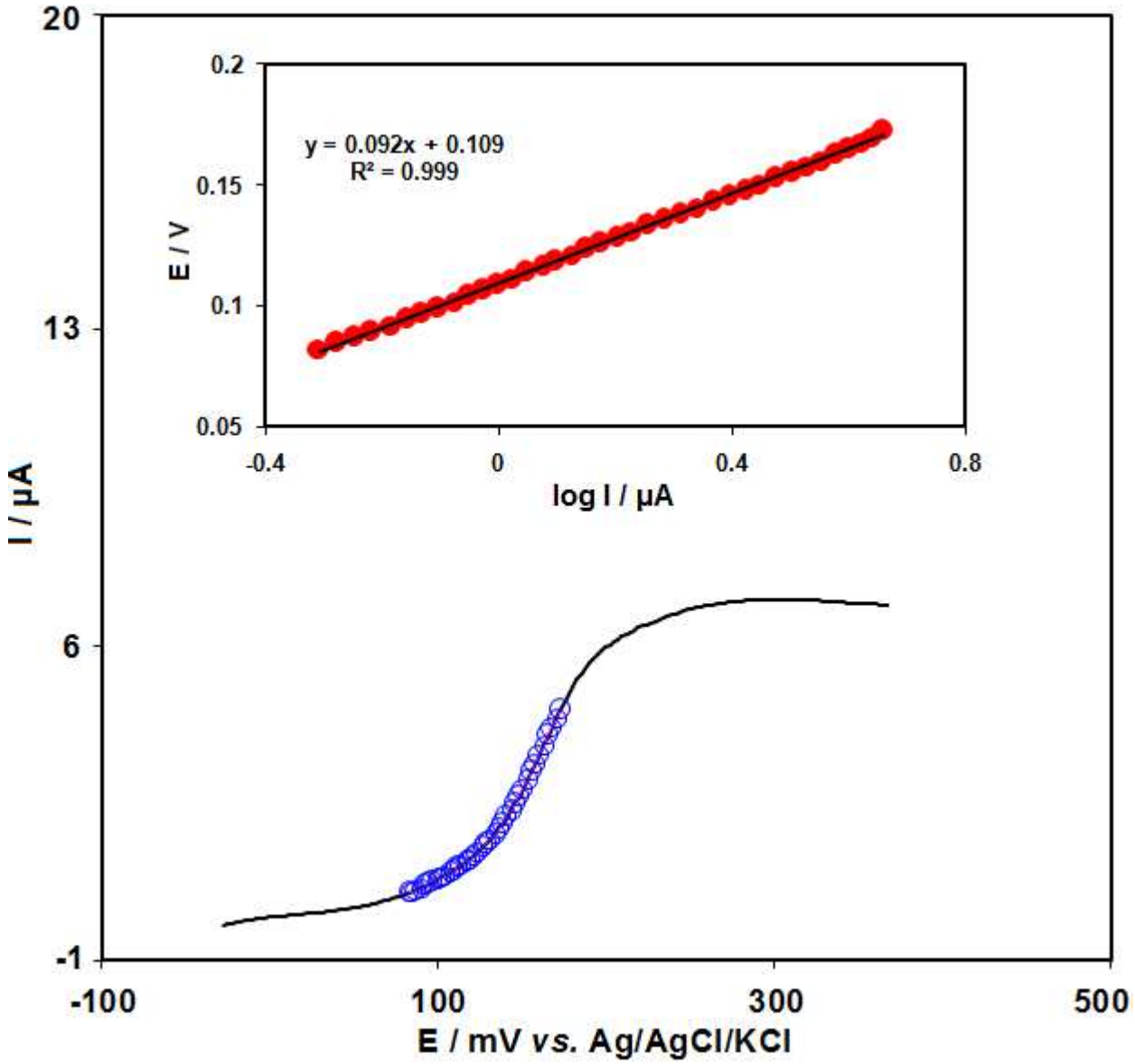


Fig. 6

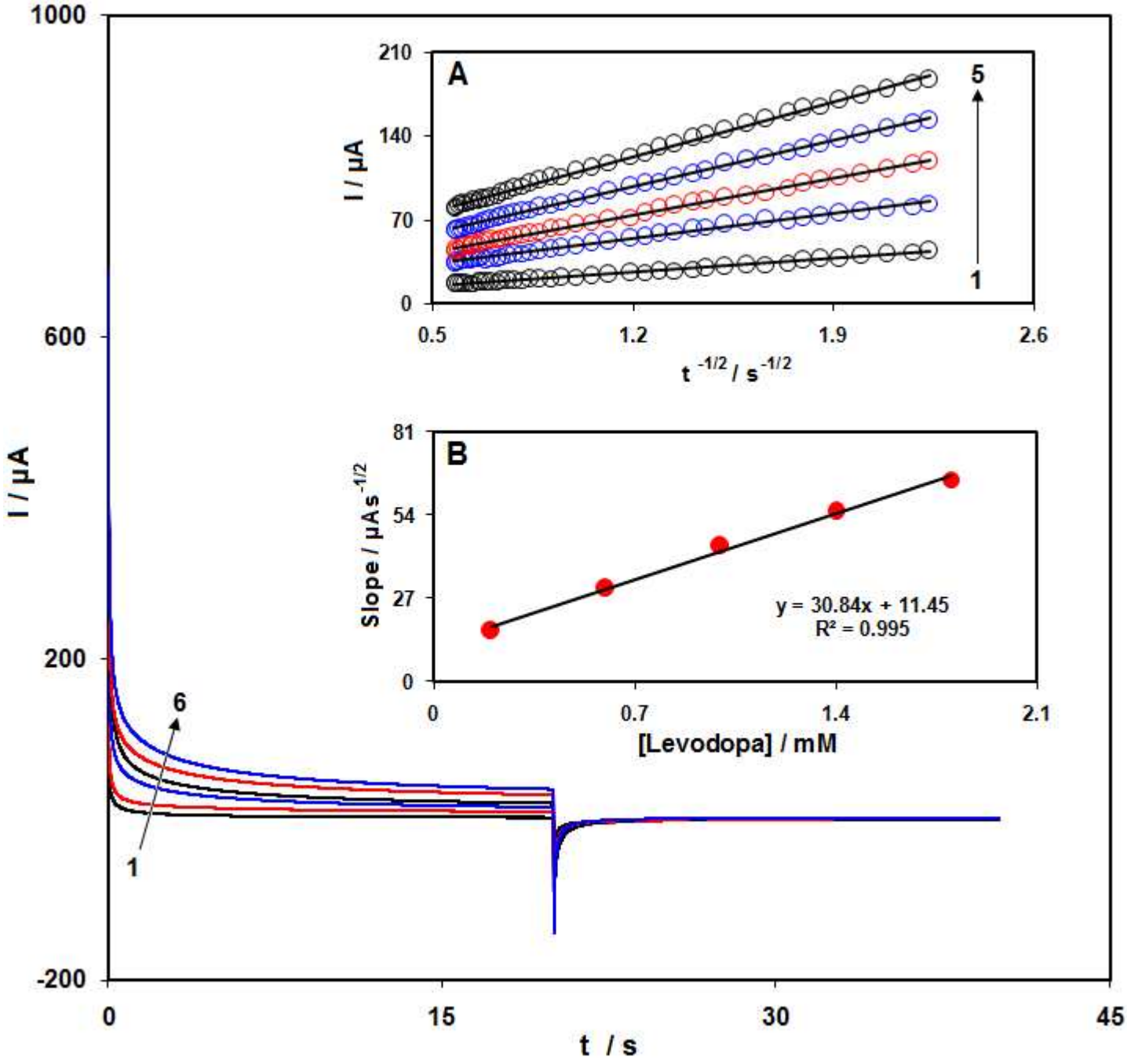


Fig. 7

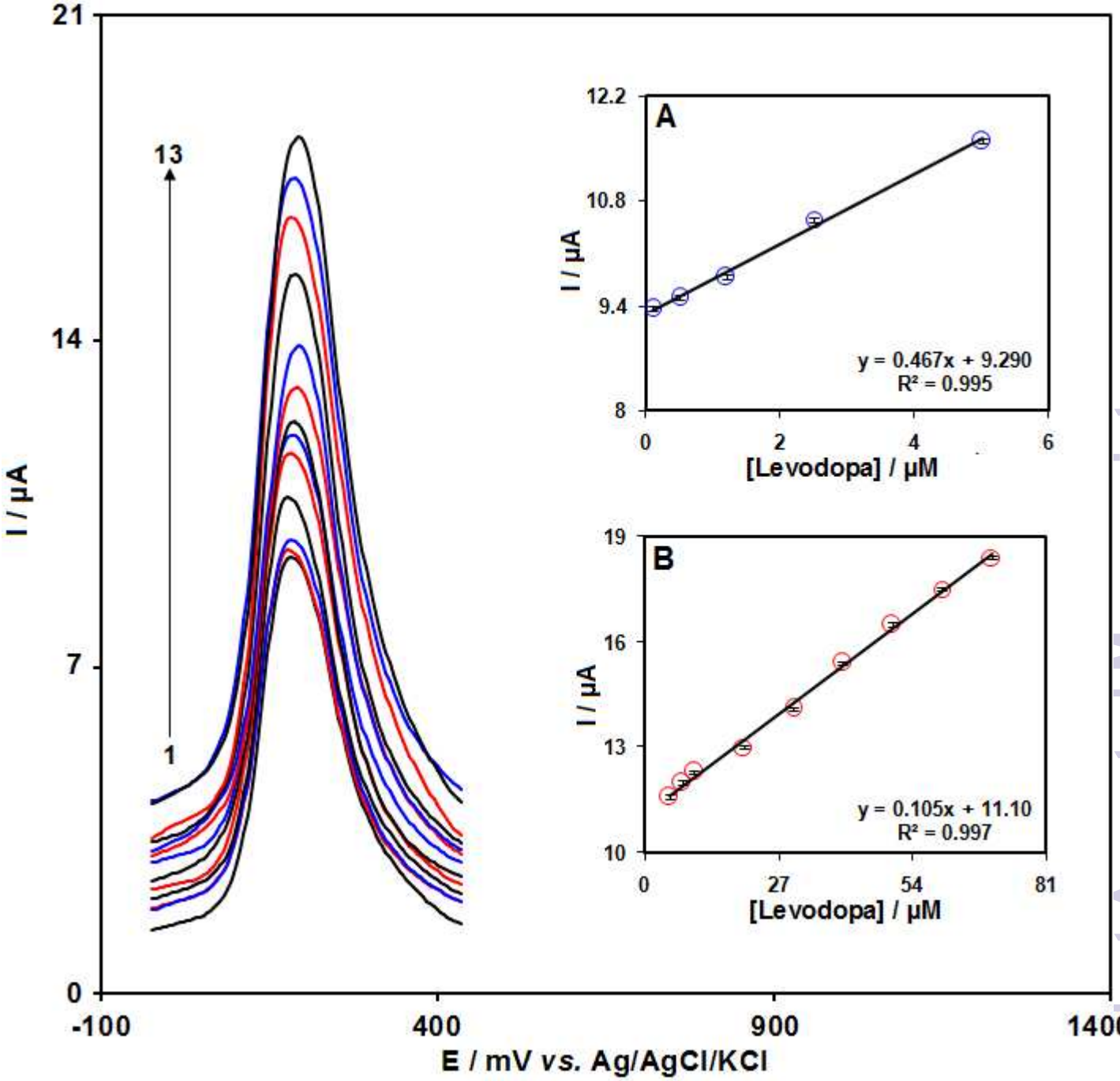


Fig. 8

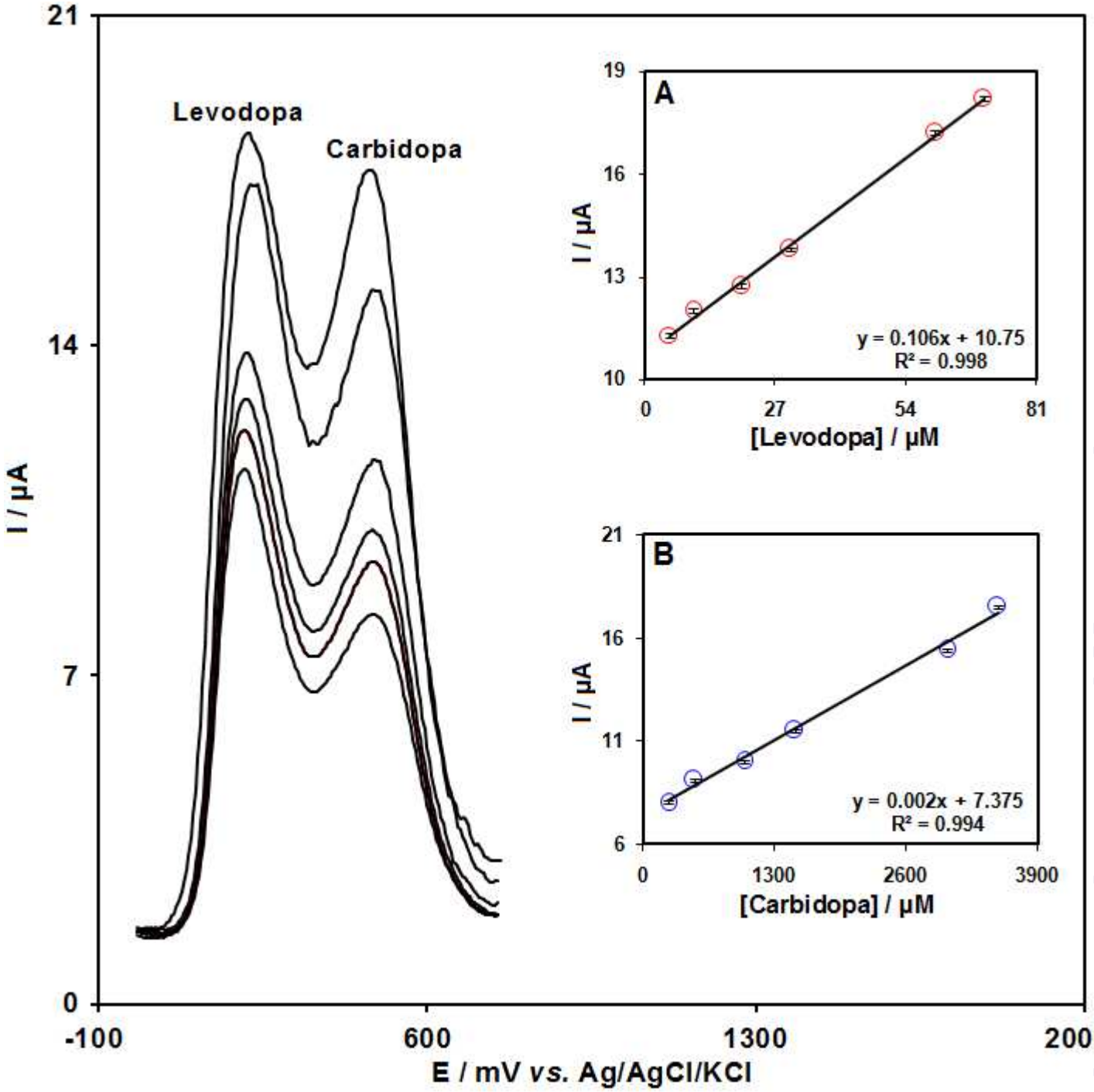


Fig. 9