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ORIGINAL PAPER

Simultaneous electrochemical determination of ascorbic acid, epinephrine, and uric acid using a polymer film-modified electrode based on Au nanoparticles/poly(3,3′,5,5′-tetrabromo-*m*-cresolsulfonphthalein)

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Abstract A new polymer film-modified electrode based on the Au nanoparticles/poly(3,3′,5,5′-tetrabromo-*m*-cresolsulfonphthalein)/glassy carbon electrode (Au-NPs/poly(BCG)/GCE) was prepared for the simultaneous determination of ascorbic acid (AA), epinephrine (EP), and uric acid (UA). The prepared electrode, Au-NPs/poly(BCG)/GCE, was characterized by scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS), and attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR). The poly(BCG) film showed an efficient electrocatalytic activity for the oxidation of AA, UA, and EP. In addition, the prepared electrode separates the oxidation peak potential of AA-EP by 140 mV, and EP-UA by 140 mV, while the bare GCE cannot resolve them.

Keywords Ascorbic acid · Epinephrine · Uric acid · 3,3′,5,5′-Tetrabromo-*m*-cresolsulfonphthalein · Au nanoparticles

Introduction

Epinephrine (EP) is an important neurotransmitter in mammalian and many diseases which are related to change of the EP concentration in mammals. Thus, the determination of EP is very important in diagnosis and controlling medicine. The two main problems for determination of EP in vivo are its very low concentration and a large number of interfering substances such as ascorbic acid (AA) and uric acid (UA) [1]. UA is produced in the purine metabolism, and therefore its determination in the body fluids is essential [2, 3]. AA is an effective reducing agent in the human diet. Electrochemical determination of AA, EP, and UA is possible because they are electrochemically active. But there are two major problems here. First, EP is coexisting with AA and UA in the biological fluids such as blood and urine. Second, the oxidation potentials of UA, EP, and AA are very close at the bare electrode surface [4]. Therefore, their voltammograms are overlapped, and therefore are difficult to analyze. So, it is important to develop an electrochemical technique for the selective determination of EP in the presence of AA and UA.

Recently, the chemically modified electrode surfaces have been developed for selective determination of biomolecules. Electrochemical techniques using modified electrodes have been interested more and more, because this approach does not require pretreatment of samples and may be performed in situ. Carbon and metal electrodes may become oxidized at the surface that can add various kinds of groups (such as carboxyl, quinoidal, and phenolic functionalities) on their surfaces. Several electrodes such as Ag nanoparticles/SiO_2/graphene oxide [5], graphene-modified electrode [6], ruthenium oxide/hexacyanoferrate film [7], and conductive polymer electrodes [8, 9] are applied for selective determination of EP in the presence of UA and/or AA. Modified electrodes with conductive polymer or redox polymer have been widely used owing to their excellent unique physical and chemical properties [10–12]. They are good approaches for selective determination of some biomolecules, because the surface characteristic

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Table 1 Comparison of some analytical characteristics of the different modified electrodes for the determination of AA, EP, and UA

Sensitivity (μA/μmol L^{-1})			Limit of detection (μmol L^{-1})			Linear dynamic range (μmol L^{-1})			Interferences	Reference
AA	EP	UA	AA	EP	UA	AA	EP	UA		
0.026	0.51	0.12	0.40	0.030	0.0090	1.0–100.0	0.2–3.0	0.02–70.0	Dopamine	1
0.010	0.13	0.021				100.0–2000.0	3.0–175.0	70.0–2000.0		
0.0090	0.13	0.031	7.0	0.20	0.60	20.0–1000.0	2.0–80.0	5.0–300.0	L–Lysine, glucose	17
–	1.5×10^{-3}	–	–	0.065	–	–	0.1–0.65	–	Not reported	18
–	8.1	8.6	–	0.0030	0.0030		0.01–10	0.01–60.0	Not reported	19
–	0.13	–	–	0.45	–	–	2.4–3.0	–	Not found	20
–	–	0.16	–	–	–	–	–	5.0–45.0	Not found	21
–	0.71	–	–	0.10	–	–	0.5–500.0	–	Dopamine	22
–	0.41	–	–	0.40	–	–	1.0–800.0	–	Not found	23
–	0.098	–	–	0.94	–	–	4.0–100.0	–	Dopamine	24
–	2.3	0.45	–	0.060	0.032	–	0.70–7.0	0.20–7.0	Dopamine	25
0.0080	0.026	0.034	0.20	0.010	0.0040	5.0–1320.0	4.0–903.0	7.0–1500.0	Dopamine	This work

on electrode can be modulated by introducing various chemicals with reactive group [13, 14]. The polymer-modified electrodes show broad potential windows and can catalyze some electrochemical reactions which have a high over-potential and a poor selectivity [15].

3′,3″,5′,5″-tetrabromo-*m*-cresol-sulfonephthalein (BCG) is used as a pH indicator which has one sulfonate, two hydroxyls, and four bromides. Application of poly(BCG) for determination of biomolecules is rarely seen in the literature. Recently, Ai [16] prepared poly(BCG) on the surface of a multi-walled carbon nanotubes/GC electrode for electrochemical determination of glutathione. For the first time, we report here the preparation of a new polymer film-modified electrode based on the Au nanoparticles/poly(3,3′,5,5′-tetrabromo-*m*-cresolsulfonphthalein)/glassy carbon electrode (Au-NPs/poly(BCG)/GCE) for the simultaneous electrochemical determination of EP, AA, and UA. The prepared electrode, Au-NPs/poly(BCG)/GCE, not only can catalyze the oxidation of AA, EP, and UA, but also can efficiently resolve their oxidation peaks. Our proposed method can be used for the simultaneous determination of these biomolecules in the urine and plasma samples. Table 1 shows a comparison of our proposed method with several reported electrochemical methods for determination of EP, AA, and UA [17–25].

Experimental

Apparatuses and reagents

All electrochemical experiments including cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed using a Metrohm instrument, Model 797 VA processor. A conventional three-electrode cell was used for all electrochemical experiments, which consisted of a working electrode (Au-NPs/poly(BCG)/GCE), a platinum wire as the counter electrode, and Ag/AgCl (3.0 M KCl) as a reference electrode. A GCE with a formal surface area of 0.0314 cm^2 was used as the basal working electrode. All potentials were measured against the Ag/AgCl electrode.

All chemicals and solvents were commercially available and used without further purification. Doubly distilled water was used throughout the experiments. UA, 3,3′,5,5′-tetrabromo-*m*-cresolsulfonphthalein, and EP were purchased from Sigma-Aldrich. AA was obtained from Merck. A stock solution of AA (0.010 M) was prepared daily by dissolving a suitable amount of the reagent in water. The UA solution (0.010 M) was prepared by dissolving the solid in a small

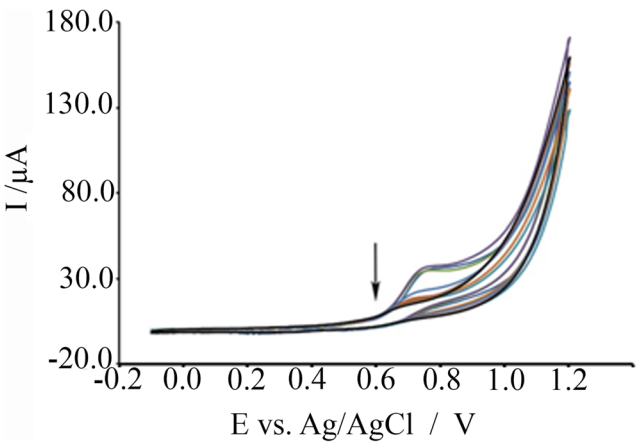


Fig. 1 Cyclic voltammograms of the electropolymerization reaction of BCG at the surface of GCE in a solution containing 0.0050 M BGC in 0.010 M NaOH at 100 mV s^{-1}

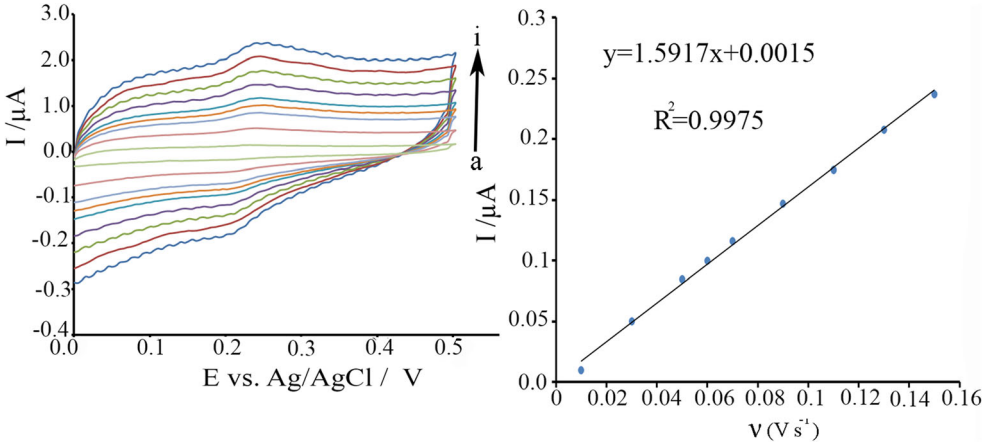


Fig. 2 Cyclic voltammograms of Au-NPs/poly(BCG)/GCE in PBS (pH 6.0) at various scan rates: **a** 10, **b** 30, **c** 50, **d** 60, **e** 70, **f** 90, **g** 110, **h** 130, and **i** 150 mV s^{-1}

volume of 0.1 M NaOH, and then the solution was diluted with water to the desired concentration. A Stock solution of EP (0.010 M) was prepared daily by dissolving a suitable amount of EP in a small volume of 0.10 M H_3PO_4 and the

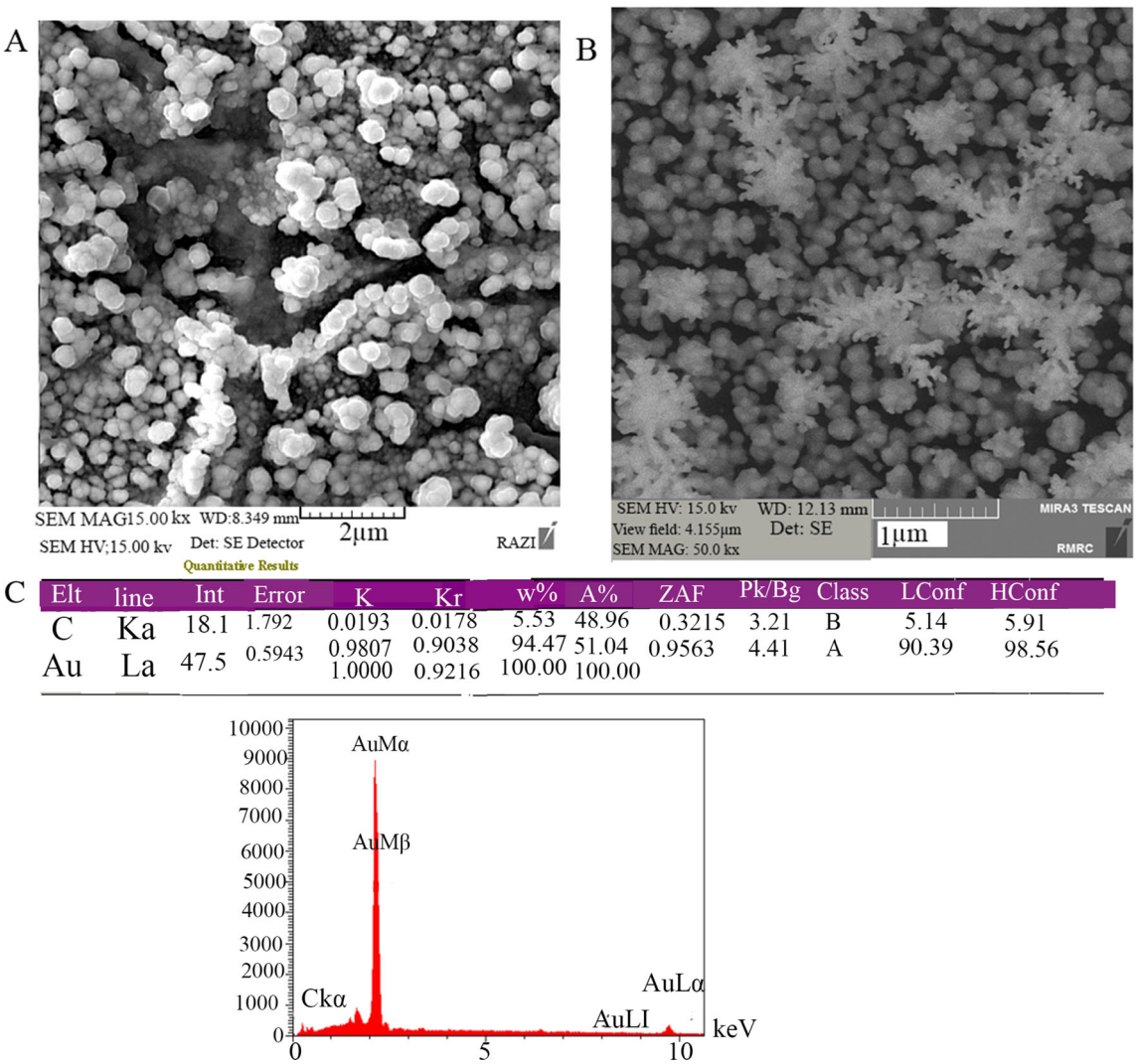


Fig. 3 SEM images of **a** Au-NPs/GCE, **b** Au-NPs/poly(BCG)/GCE, and **c** the corresponding EDX spectrum taken from the whole area of (**b**)

resulting solution was diluted with water. The phosphate buffer solutions (PBS) with different pH levels were prepared by mixing 0.10 M Na_2HPO_4 and 0.10 M NaH_2PO_4 solutions at different ratios. The pH was adjusted by adding 1.0 M H_3PO_4 and/or NaOH. The working standard solutions were prepared by appropriate dilution of the stock solutions with phosphate buffer, pH 6.0.

Preparation of Au-NPs/poly(BCG)/GCE

Prior to the modification, the bare GCE was carefully hand-polished with alumina slurry (6, 1, and 0.05 μm) on a polishing cloth. The electrode was then successively sonicated in ethanol and doubly distilled water for 5 min to remove the adsorbed particles. The electrode was subsequently placed in a 0.01-M NaOH solution containing 0.005 M 3,3′,5,5′-tetrabromo-*m*-cresolsulfonphthalein (BCG), and a cyclic potential sweep was applied in the range of −0.1 to 1.2 V for 25 cycles at 100 mV s^{-1}. Then, the electrode was rinsed and immersed in 0.1 M KNO_3 containing 0.4 g L^{-1} $H[AuCl_4]$ to electro-deposit the Au-NPs for 60 s at −0.2 V [24]. The resulting electrode, Au-NPs/poly(BCG)/GCE, was activated by several successive voltammetry cycles from −0.5 to 1.0 V at 100 mV s^{-1} in a buffer solution (pH 6.0) until a steady-state voltammogram was obtained.

Preparation of real samples

The applicability of Au-NPs/poly(BCG)/GCE was assessed by determination of AA in tablets, EP in hydrochloride injection, and UA in human urine samples. The epinephrine hydrochloride injection solution (specified content of EP is 1.00 mg mL^{-1}) was analyzed after suitable dilution with buffer solution (pH 6.0). An aliquot of 10 mL of this test solution was transferred to the electrochemical cell, and EP was determined according to the recommended procedure.

The urine samples were collected from healthy volunteers and diluted to 25 times with PBS (pH=6.0) without any further pretreatment. Then, an aliquot of 10 mL of this test solution was transferred to the voltammetric cell.

For the analysis of AA, ten tablets (labeled 500 mg AA/tablet, Osvah Pharmaceutical Company, Tehran, Iran) were completely ground and homogenized. Then, 200 mg of the powder was accurately weighed and dissolved by sonication for 4 min in 25 mL of water. Finally, a suitable volume of the resultant solution plus 5 mL of the phosphate buffer solution (pH 6.0) was diluted with water in a 10-mL volumetric flask, and the resulting solution was used for the analysis of AA.

Results and discussion

Fabrication of the poly(BCG)-modified GCE

It is proven that BCG is a symmetric monomer. According to Dyson [26], the electropolymerization of symmetric monomer is done easier. Furthermore, the resulted polymer exhibits better mechanical and electrocatalytic properties. The present electrochemical sensor was optimized in terms of the thickness of polymer film, the potential ranges, and the pH of polymerization. The effect of the film thickness was determined by the number of polymerization scans. The poly(BCG) film was prepared on an activated GCE in 0.005 M BCG and 0.01 M NaOH, with cycles ranging from 2 to 30. The I_{pa} value increases after increasing the cycle number up to 25 cycles. A further increase in the cycle number causes the current response to be slightly lowered. This behavior indicates that the activity of the polymer film is dependent on its thickness, most probably due to a barrier for electron transfer in the thick films. Figure 1 shows 25 continuous CVs of the BCG polymer formation onto a GCE surface by scanning potential over the range of −0.1 to +1.2 V at

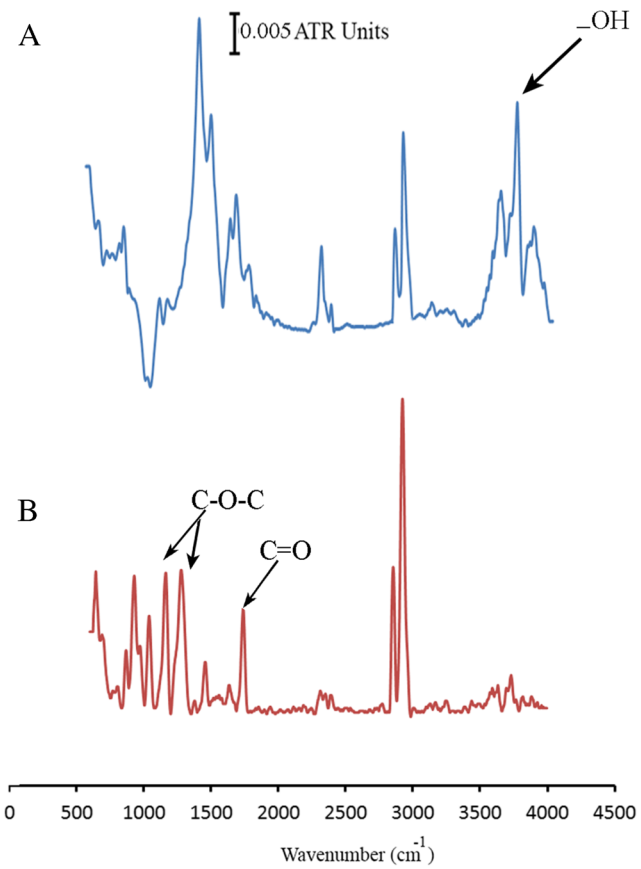


Fig. 4 ATR-FTIR spectra of **a** BCG and **b** poly(BCG) (electropolymerized in 0.01 M NaOH solution) at the surface of a glassy carbon electrode

100 mV s^{-1} for 25 cycles in a solution containing 0.005 M BCG in 0.01 M NaOH. The CVs show that an irreversible anodic peak current appearing at +0.74 V (due to the oxidation of an OH group) gradually tends to be stable after 25 scans. This suggests that the initial-formed poly(BCG) film has a leaching process with the scan cycle increasing up to 25 times, which may be implied by the self-adjustment of the polymer film thickness at the GC electrode. The potential scan range is also an important factor in the preparation of the poly(BCG) film. If the positive potential value is below +1.1 V or if the lower range is above −0.1 V, no polymerization occurs. Therefore, a potential window from −0.1 to +1.2 V was selected as the electropolymerization range in this paper. Figure 1 depicts the electropolymerization of BCG on the GCE surface over the potential range from −0.10 to +1.20 V.

The electropolymerization of BCG varies with the pH of the solution and the electrochemical properties of poly(BCG) film also depends on the film preparation conditions. The optimum pH for the electropolymerization of BCG on the surface of GCE was determined using various electrolytes with different pH values. The pH of the electrolytes was altered from an acidic pH of 4.0 to an alkaline pH of 13.0 using phosphate buffer and NaOH containing 0.005 M BCG. The best response of AA, EP, and UA was found on the surface of poly(BCG) polymerized at pH 12.0. In addition, among cresol derivatives (such as cresol red, bromocresol purple), the BCG has the maximum number of bromide. The substituted bromides as electron withdrawing groups help to facilitate the formation of carbonyl functional group in polymer body.

The typical voltammograms of the poly(BCG) modified electrode in the range of 0.0 to +0.5 V at various sweep rates in pH 6.0 were also investigated (Fig. 2). The cyclic voltammograms show a reversible redox couple at E_{Pa}=0.20 V and E_{Pc}=0.23 V. The plot of I_{Pa} vs. υ at low scan rates (10–150 mV s^{-1}) exhibits a linear dependence of I_{pa} on υ and the ratio of I_{Pa} to I_{Pc} nearly equal to unity. This behavior is consistent with a diffusionless, reversible electron-transfer process [27]. It is also suggested that the reaction of the poly(BCG) film-modified electrode is a two-electron-transfer process (n=2), because the oxidized form also holds the aromaticity [28, 29]. Furthermore, the anodic peak current (I_{Pa}) was linearly dependent on the scan rate (ν) with the regression equation of I (μA)=1.59 ν (V s^{-1})+0.00150 (R^2=0.9975).

Figure 3 shows the morphology of the electrode surface for (A) Au-NPs/GCE and (B) Au-NPs/poly(BCG)/GCE, which were characterized by scanning electron microscopy (SEM). When a smooth surface of the bare GC electrode was modified, the small particles appeared. In addition, it can be seen clearly that the Au-NPs are distributed on the polymer layer surface (Fig. 3b). The FE-SEM images also show the

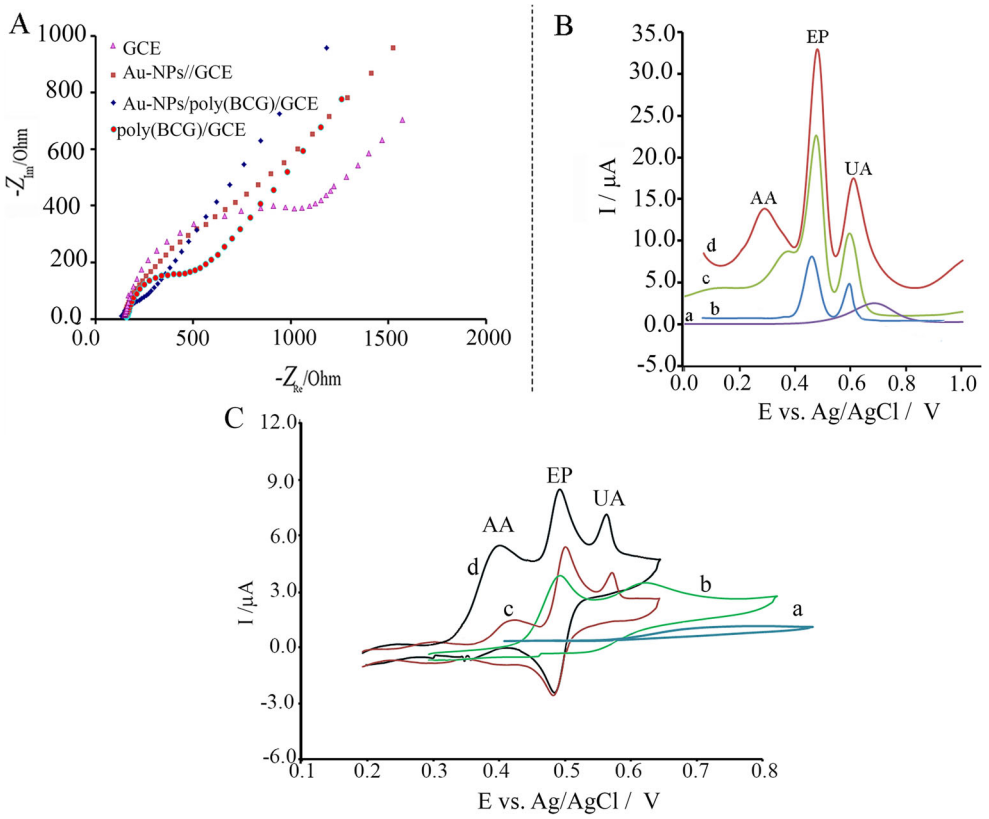
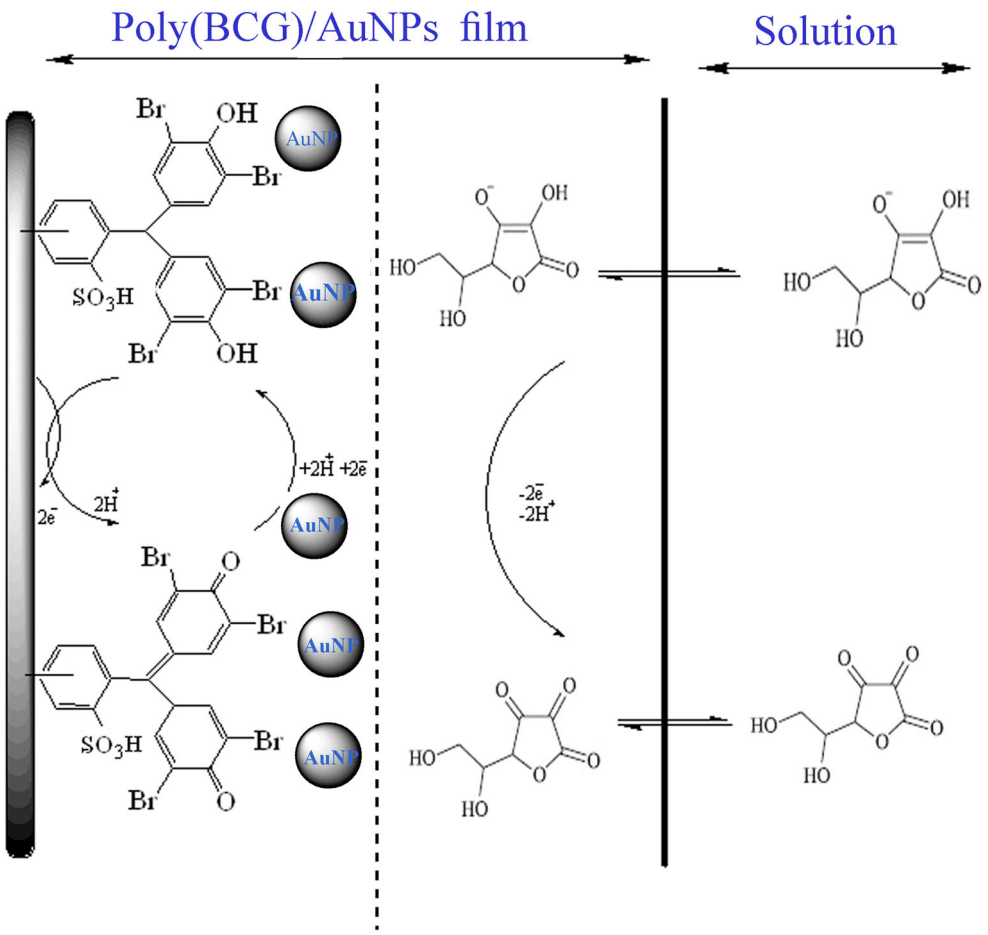


Fig. 5 **a** Nyquist plots in impedance measurements of different electrodes in 5.0 mmol L^{-1} $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ containing 0.10 M KNO_3. **b** Differential pulse voltammograms. **c** Cyclic voltammograms of 300.0 μmol L^{-1} AA, 40.0 μmol L^{-1} EP, and 40.0 μmol L^{-1} UA, at the surface of **a** bare GCE, **b** Au-NPs/GCE, **c** poly(BCG)/GCE, and **d** Au-NPs/poly(BCG)/GCE, at pH 6.0. DPV experimental conditions: voltage step of 5 mV and scan rate of 55 mV s^{-1}. CV experiments: *scan rate*, 55 mV s^{-1}

formation of dendronized polymer with nanosize pores on the surface of the electrode. The resulted substrate provides a large surface area for deposition of Au-NPs and induces the formation of more Au-NPs with smaller size [30]. This leads to a better electrocatalytic property of the modified electrode. The diameter range of the Au-NPs is 200–300 nm. Deposition time is a critical parameter to form nanoparticles on the different surfaces. It can be seen that the increasing in the deposition time results in the augmentation of the average particle size of the Au-NPs. Furthermore, the energy-dispersive X-ray spectroscopy (EDX) has also confirmed the formation of the Au-NPs on the surface of GCE (Fig. 3c).

To further confirm the formation of poly(BCG) on GCE, the attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) technique was employed. The ATR-FTIR spectra of BCG and poly(BCG) were directly recorded on the surface of GCE. The ATR-FTIR spectra of BCG (Fig. 4a) and poly(BCG) (Fig. 4b) were obtained using the spectral subtraction technique. By comparing Fig. 4b with a, it can be seen that a strong and broad absorption band at 3747 cm^{-1} in Fig. 4a is possibly from the stretching vibration of the hydrogen bonds and –OH groups. The disappearance of this band and appearance of a new band at 1747 cm^{-1} for the stretching vibration of the C=O group, confirm the formation of poly(BCG) at the surface of GCE. Two new bands at 1168 and 1284 cm^{-1} are assigned to the stretching vibration of C–O–C in poly(BCG). This new functional group in the ATR-FTIR spectra confirms the formation of poly(BCG) at the surface of GCE by the electropolymerization reaction.

The effect of the pH value of the supporting electrolyte on the electrochemical behavior of the Au-NPs/poly(BCG)/GCE was also investigated. The results show that the anodic peak potential is negatively shifted at higher pH values. Also, the anodic peak current is gradually decreased with increasing pH from 6.0 to the higher values. With increasing pH, the rate of the electron transfer of the poly(BCG) film is gradually decreased, which is a disadvantage to the electrocatalytic reaction of EP at the poly(BCG)/Au-NP-coated electrode. The alkaline –NH group of EP (pK_a=8.55) can accept a proton to form an EP^+ cation. The bromine atoms on poly(BCG) have an affinity to the EP^+ cations and can catalyze and promote the oxidation of EP at pH 6.0. Due to the presence of these four bromine atoms, the cresol derivative shows a significant electrocatalytic activity which is comparable with those reported



Scheme 1 Mechanism of the electropolymerization reaction of BCG at the surface of GCE.

in the literature [28, 29]. However, at pH >6.0, the decrease in the peak current of EP is due to the decrease of the anodic peak current of poly(BCG). Therefore, for the simultaneous determination of these compounds, a pH of 6.0 (PBS, 0.1 M) was selected for further study.

The effect of scan rate on the anodic peak current of EP at the Au-NPs/poly(BCG)/GCE surface was also investigated. The results show that by increasing the scan rate, the anodic peak current (I_{Pa}) is gradually increased. A linear relationship between $\nu^{1/2}$ and I_{Pa}, at scan rates from 10 to 170 mV s^{-1}, confirms a diffusion-controlled process on the modified electrode (R^2=0.9968).

Electrooxidation of EP, AA, and UA at Au-NPs/poly(BCG)/GCE

The electrochemical impedance spectroscopy was used to characterize the interface properties of the Au-NPs/poly(BCG)/GCE surface during different modification processes. The Nyquist plot (with a semicircle portion at higher frequencies corresponds to the electron transfer limited process and a linear portion at lower frequencies) indicates the diffusion process. Figure 5a shows the typical diagrams of 0.10 M KNO_3 solution containing 5.0 mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ at GCE, poly(BCG)/GCE, Au-NPs/GCE, and Au-NPs/poly(BCG)/GCE. The charge-transfer resistance ($R_{c.t}$) of the bare GCE is estimated to be 1100 Ω cm^{-1}. After polymerization with BCG (25 cycles), the $R_{c.t}$ value is decreased (500 Ω cm^{-1}), suggesting that the conducting polymer enhances the electron-transfer rate. The electrodeposition of Au-NPs in 0.4 g L^{-1} $H[AuCl_4]$ and 0.1 M KNO_3 causes further decrease of $R_{c.t}$ (250 Ω cm^{-1}) and greatly improves the conductivity of the electrode surface.

The differential pulse and cyclic voltammograms of the oxidation of different concentrations of AA, EP, and UA at the surface of (a) GCE, (b) poly(BCG)/GCE, (c) Au-NPs/GCE, and (d) Au-NPs/poly(BCG)/GCE are shown in Fig. 5b, c. The results show that all three compounds are oxidized with well-defined and distinguishable sharp peak potentials at the Au-NPs/poly(BCG)/GCE surface. Whereas, these peak potentials are indistinguishable and broad at the bare GCE, indicating a slow electron-transfer rate. Also, the oxidation peak potentials of AA, EP, and UA separate completely into three well-defined peaks using Au-NPs/poly(BCG)/GCE. At the bare GCE in pH 6.0, the oxidation peak potentials are 0.61, 0.64, and 0.73 V for AA, EP, and UA, respectively. The separation of the oxidation peak potentials for AA-EP and EP-UA were about 140 and 140 mV, respectively. In addition, the deoxygenation of the analyte solution did not affect the peak potentials and/or the peak currents of AA, EP, and UA. The DPV parameters including pulse amplitude, pulse time, and voltage step time were changed when the concentration of AA, EP, and UA on the cell were 100, 50, and 50 μmol L^{-1}. The results showed that a maximum peak current was obtained with a pulse amplitude of 40 mV, a pulse time of 0.03 s, and a voltage step time of 0.1 s. These values were selected for further studies. According to the reported works [16, 28], poly(BCG) is firstly deposited on the surface of GCE and oxidized to form a quinine moiety. Then, the oxidized polymer undergoes a catalytic reaction by analytes (AA, EP, and UA) back to phenol form, which can then be electrochemically reoxidized to produce an enhancement in the anodic peak currents (Scheme 1). Based on these results, the following catalytic diagram (EC′, catalytic mechanism) describes the voltammetric response of the

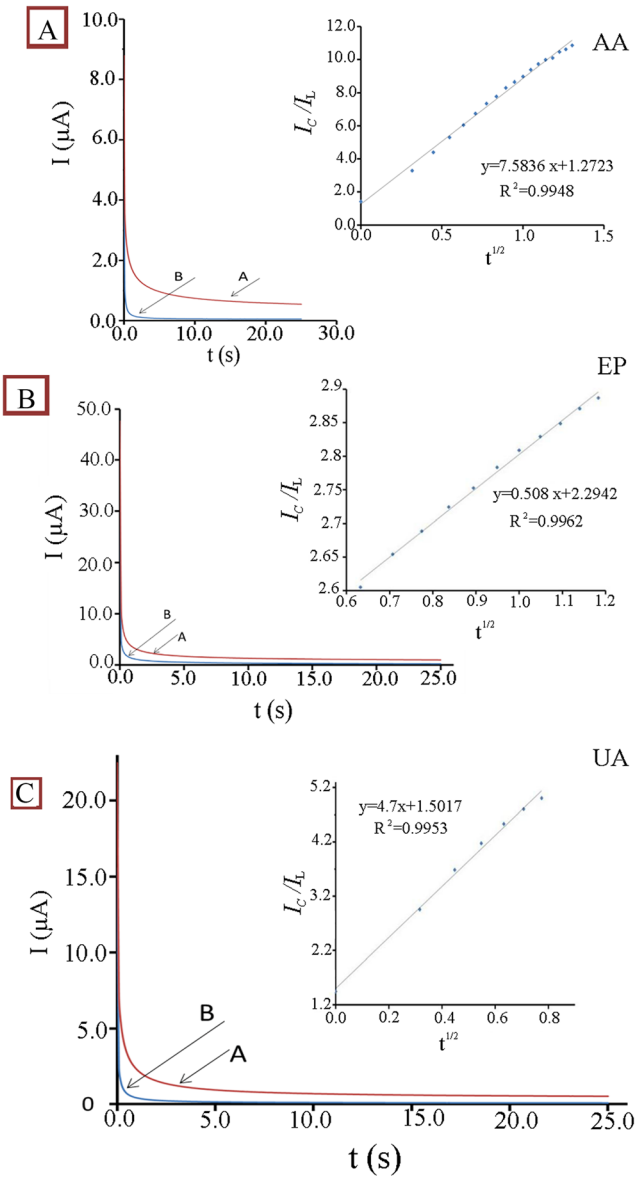


Fig. 6 Dependence of I_C/I_L on the $t^{1/2}$ driven from the chronoamperograms data at Au-NPs/poly(BCG)/GCE in the **a** absence and **b** presence of *a* 200 μmol L^{-1} AA, *b* 100 μmol L^{-1} EP, and *c* 300 μmol L^{-1} UA

electrochemical oxidation of AA, EP, and UA at Au-NPs/poly(BCG)/GCE. In addition, all three peak potentials have positive potential shifts. These shifts in the oxidation peak potentials accompanied by an increase in the peak currents for Au-NPs/poly(BCG)/GCE indicate that the modified electrode has a catalytic effect on the oxidation of AA, EP, and UA, but the catalytic role of Au-NPs/poly(BCG)/GCE for AA is stronger than EP and UA.

$$\mathrm{Poly(BCG)} \rightleftarrows \mathrm{poly(BCG)}^{+2} + 2e + 2H^{+} \quad (E)$$

$$\mathrm{Poly(BCG)}^{+2} + (\mathrm{analyte})_{\mathrm{Red}} \rightarrow (\mathrm{analyte})_{\mathrm{OX}} + \mathrm{poly(BCG)} \quad (C')$$

Chronoamperometric studies

The single potential-step chronoamperometry was applied for the calculation of the diffusion coefficient and the rate constant of AA, EP, and UA at Au-NPs/poly(BCG)/GCE by setting the working electrode potential at 0.30, 0.40, and 0.53 V, respectively (Fig. 6). The rate constant between these analytes and Au-NPs/poly(BCG)/GCE can be evaluated according to the Galus method [31]:

$$I_C/I_L = \gamma^{1/2}\left[\pi^{1/2}\mathrm{erf}\left(\gamma^{1/2}\right) + \exp(-\gamma)/\gamma^{1/2}\right] \quad (1)$$

Where, I_C is the catalytic current of Au-NPs/poly(BCG)/GCE in the presence of analyte, I_L is the limited current in the absence of analyte, and $\gamma = K_h C_b t$ is the argument of the error function (C_b is the bulk concentration of the analyte, M). If γ exceeds 2, the error function is almost equal to 1 and the above equation can be simplified to the following equation:

$$I_C/I_L = \pi.^{1/2}\gamma^{1/2} = \pi.^{1/2}(k_h C_b t)^{1/2} \quad (2)$$

Where, k_h and t are the catalytic rate constant ($M^{-1}\ s^{-1}$) and time elapsed (s), respectively. Equation 2 can be used to calculate the rate constant of the catalytic process, k_h. From the slope of I_C/I_L vs. $t^{1/2}$ plot, the value of k_h can be simply calculated for a given concentration of the substrate. The calculated values of k_h were equal to 9.2×10^4, 8.2×10^2, and $2.3\times10^4\ M^{-1}\ s^{-1}$ for AA, EP, and UA, respectively. The values of k_h also explain the sharp feature of the catalytic peak observed for the catalytic oxidation of AA, EP, and UA at the surface of

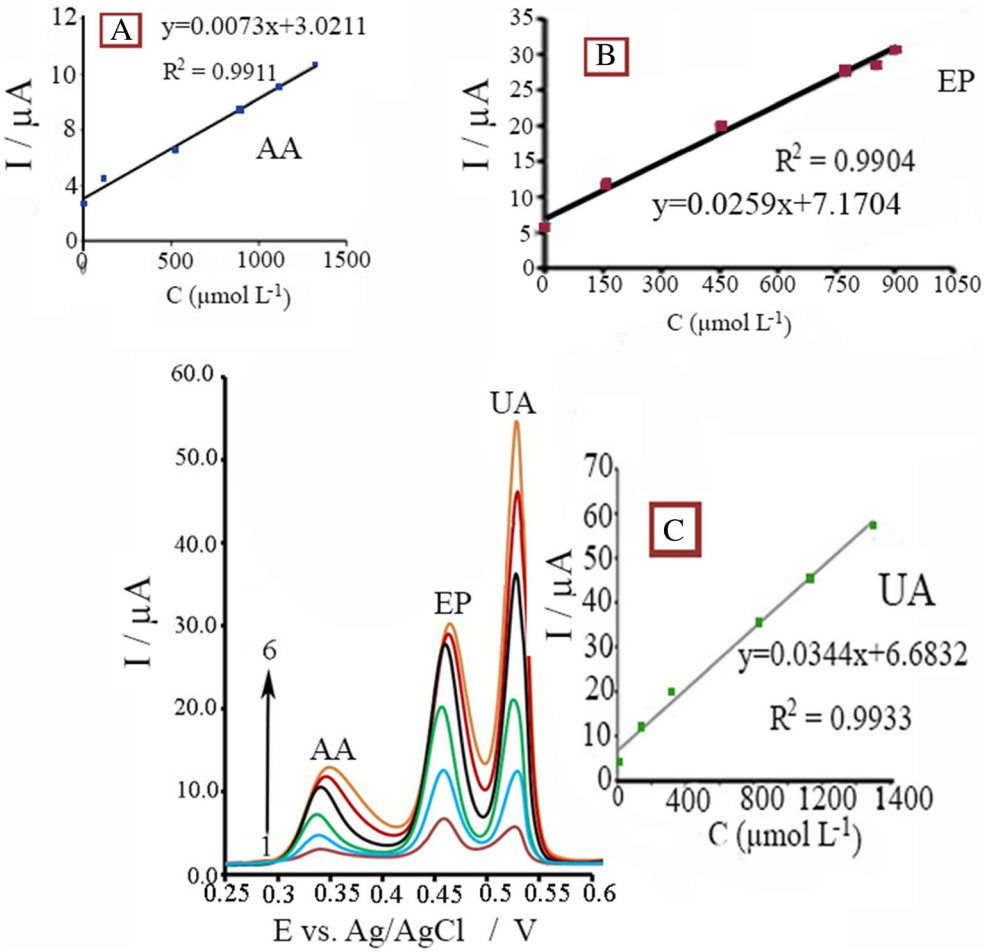


Fig. 7 Differential pulse voltammograms of Au-NPs/poly(BCG)/GCE in a 0.10-M phosphate buffer solution (pH 6.0) containing different concentrations of AA, EP, and UA. *Numbers 1–6* correspond to **a** 5.0–1320.0 µmol L^{-1} of AA, **b** 4.0–903.0 µmol L^{-1} EP, and **c** 7.0–1500.0 µmol L^{-1} UA

poly(BCG)/Au-NPs. The catalytic oxidation of AA, EP, and UA at poly(BCG)/Au-NPs was also studied by chronoamperometry in different concentrations of these three analytes. The experimental plots of I vs. $t^{-1/2}$ were employed with the best fits for different concentrations of AA, EP, and UA. The slopes of the resulting straight lines were then plotted *vs.* AA, EP, and/or UA concentrations, respectively. From these results, we calculated the diffusion coefficients of 2.9 (±0.10)×10^{-6} cm^2 s^{-1} for AA, 8.5 (±0.10)×10^{-6} cm^2 s^{-1} for EP, and 1.5 (±0.090)×10^{-6} cm^2 s^{-1} for UA.

The simultaneous determination of AA, EP, and UA

The results described in the previous section clearly indicate that AA, EP, and UA can be independently and simultaneously detected on the Au-NPs/poly(BCG)/GCE surface using DPV. We further studied the simultaneous and quantitative determination of all three compounds on Au-NPs/poly(BCG)/GCE. The next attempt was made to detect AA, EP, and UA simultaneously using poly(BCG)/Au-NPs. Figure 6a shows the DPVs obtained for the oxidation of different concentrations of AA, EP, and UA at Au-NPs/poly(BCG)/GCE. As can be seen, there are three well-distinguished anodic peaks at potentials of 330, 460, and 590 mV corresponding to the oxidation of AA, EP, and UA, respectively. The calibration curves for AA, EP, and UA are linear for the concentration ranges of 5.0–1320.0 μmol L^{-1} for AA, 4.0–903.0 μmol L^{-1} for EP, and 7.0–1500.0 μmol L^{-1} for UA. Also, the sensitivity of the modified electrode for determination of AA, EP, and UA in the presence (Fig. 7) and absence of another two analytes (Fig. 8) is almost the same. Therefore, it is possible to individually or simultaneously determine AA, EP, and UA in different mixtures at poly(BCG)/Au-NPs without cross interferences. The anodic current of AA peaked at 0.33 V and increased linearly as the concentration of AA increased while the peak current of both EP (at 0.46 V) and UA (at 0.59 V) remained essentially unchanged. We observed similar patterns for EP and UA without mutual interference. Detection limits were obtained as 0.2, 0.01, and 0.004 μmol L^{-1} for AA, EP, and UA, respectively, according to the definition of $Y_{LOD}=Y_B+3S_B$, where, Y_{LOD} is a signal for limit of detection, Y_B is average blank signal (n=10), and S_B is the standard deviation of the blank signal [32]. These values are much lower than the previously reported values for the simultaneous determination of AA, EP, and UA [17–25]. The analytical parameters for the simultaneous determination of AA, EP, and UA are given in Table 1.

The reproducibility expressed in terms of relative standard deviation (RSD) of the same electrode in 10 successive measurements was 0.37, 0.14, and 0.31 % for AA, EP, and UA, respectively. Furthermore, the RSD value for four electrodes prepared in the same conditions was 1.7, 1.9, and 2.1 % for AA, EP, and UA, respectively, indicating that the proposed method is highly reproducible. The anodic peak currents of AA, EP, and UA were reduced to 98.9, 98.7, and 96.35 % of the initial responses, respectively, after 80 consecutive CV measurements in the same conditions. This indicates a good performance stability of the proposed sensor. In addition, the storage stability of the modified electrode was also examined. When the sensor was stored in pH 6.0 PBS for 3 weeks at 4 °C, it retained more than 93 % of its initial current response.

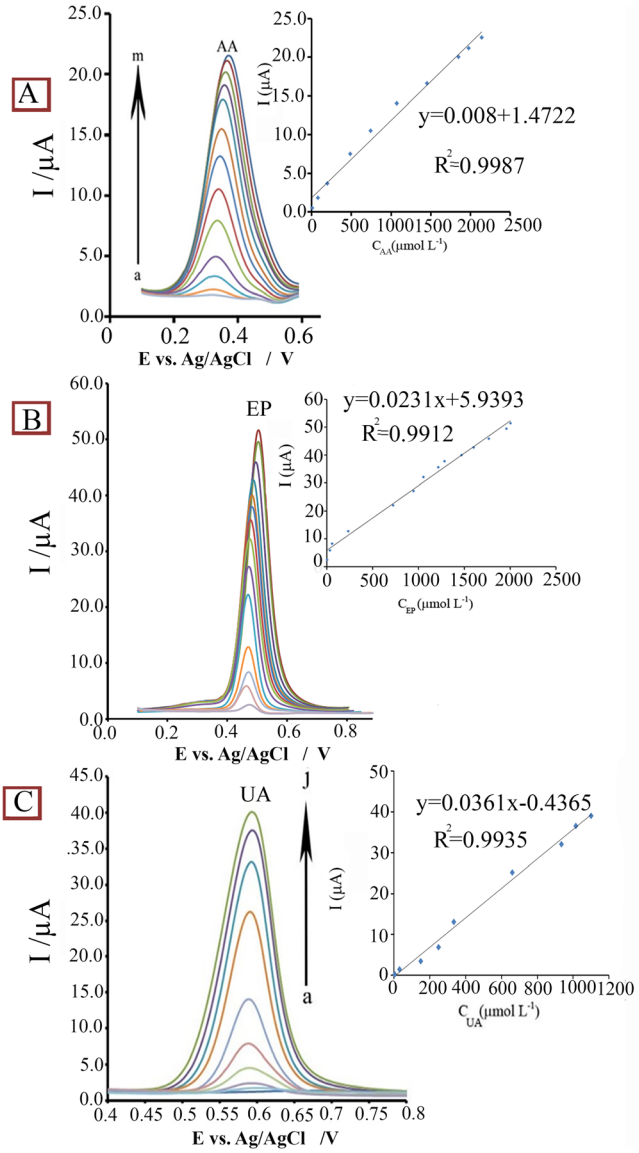


Fig. 8 DPVs of Au-NPs/poly(BCG)/GCE at pH 6.0 in (**a**): *a* 5.0, *b* 79.3, *c* 196.0, *d* 485.0, *e* 740.7, *f* 1071.4, *g* 1453.0, *h* 1853.0, *i* 1980.0, and *j* 2140 μmol L^{-1} AA; **b** *a* 2.0, *b* 30.0, *c* 56.0, *d* 230.0, *e* 720.0, *f* 943.0, *g* 1053.0, *h* 1214.0, *i* 1280.0, *j* 1468.0, *k* 1597.0, *l* 1764.0, *m* 1955.0, and *n* 2002 μmol L^{-1} EP; **c** *a* 6.0, *b* 32.0, *c* 150.0, *d* 250.0, *e* 334.0, *f* 934.0, *g*1015.0, and *h* 1100.0 μmol L^{-1} UA

Table 2 Calibration curves parameters for determination of AA, EP, and UA under the optimum conditions

Analyte	Regression equation	R^2	RSD (%)	Limit of detection (μmol L^{-1})	Linear dynamic range (μmol L^{-1})
AA	$Y=(7.0\pm0.90)\times10^{-4}X+3.0$ (±0.060)	0.9911	0.37	0.2	5.0–1320.0
EP	$Y=(2.6\pm0.40)\times10^{-2}X+7.1$ (±0.070)	0.9904	0.14	0.01	4.0–903.0
UA	$Y=(3.4\pm0.80)\times10^{-2}X+6.7$ (±0.20)	0.9933	0.31	0.004	7.0–1500.0

RSD relative standard deviation

This indicates that the proposed sensor has good storage stability for the simultaneous and quantitative determination of AA, EP, and UA.

Interference study

The influence of various foreign species on the determination of 100.0 μmol L^{-1} EP, 200.0 μmol L^{-1} AA, and 30.0 μmol L^{-1} UA was investigated. The potentially interfering substances were chosen from the group of substances commonly found with EP, AA, and UA in the pharmaceuticals and/or biological fluids. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5 % relative error in the determination of EP, AA, and UA. After the experiments, we found that Mg^{2+}, Ca^{2+}, SO_4^{2-}, Br^-, K^+, NO_3^-, ClO_4^-, glycine, alanine, glucose, sucrose, lactose, fructose, valine, aspartic acid, urea, and saturated starch solution did not interfere with the determination of these three compounds. However, the greater amounts of 100-fold oxalate ion, 80-fold cysteine, 50-fold citric acid, and 50-fold methionine cause

Table 3 Simultaneous determination of EP, AA, and UA in real samples and mixture synthesis samples

Real sample				Added (μmol L^{-1})			Proposed method (μmol L^{-1})	Recovery (%)	Official method (μmol L^{-1})
Vitamin C[a]	AA			–			96.2±1.8	–	99.3±4.0
Vitamin C[a]	AA			100.0			196.3±10.1	98.5	–
Urine 1	UA			–			8.2±0.3	–	7.8±0.1
	UA			100.0			108.8±0.6	100.9	–
	EP			–			<DL	–	<DL
	EP			50.0			48.5±0.7	93.0	–
	AA			–			<DL	–	<DL
	AA			70.0			66.5±3.0	95.0	–
Urine 2	UA			–			7.2±0.5	–	6.4±0.2
	UA			200.0			201.3±14.0	102.5	≤DL
	EP			–			<DL	–	
	EP			5.0			4.9±0.009	98.0	4.7±0.9
Epinephrine[c]	EP			–			36.5±3.0	–	36.8±0.5
Epinephrine[c]	EP			10.0			44.9±2.1	95.7	–
Epinephrine[d]	EP			–			9.3±.7	–	10.3±0.2
Epinephrine[d]	EP			40.0			51.3±3.3	102.0	–
Synthesis sample	Added			Found			Recovery (%)		
	AA	EP	UA	AA	EP	UA	AA	EP	UA
1	10.0	40.0	7.0	9.4±0.06	35.7±0.7	6.3±0.2	94.0	89.2	90.0
2	500.0	200.0	30.0	488.9±15	198.6±11	30.3±0.9	97.8	99.3	101.0
3	1000.0	1.0	150.0	1017.1±32.3	0.88±0.09	150.7±12	101.7	88.0	100.5

Number in the parenthesis shows the standard deviation for $n=5$

[a] Vitamin C: Swiss Natural Sources (500 mg)

[b] Less than limit of detection

[c] Epinephrine ampoule Darou Pakhsh–Iran (1 mg/mL)

[d] Epinephrine ampoule Streuli Pharma AG, Uznach (1 mg/mL)

interference in the simultaneous determination of EP, AA, and UA. However, equal amounts of dopamine (DA) interfered with the EP signal, because of its similar catecholamine structure. This compound shows interference in the simultaneous determination EP, AA, and UA. Despite its interference, it is not present at significant levels in the urine and serum samples.

Analysis of real samples

In order to evaluate the applicability of Au-NPs/poly(BCG)/GCE, the determination of AA, EP, and UA were done in synthetic mixtures and real samples using the standard addition method. The synthetic mixtures containing different concentrations of EP, AA, and UA were prepared with phosphate buffer solution (pH 6.0). These solutions were analyzed using DPV and the concentrations of EP, AA, and UA were determined. The results are presented in Table 2. Comparison of the proposed method with the official methods [33–35] also confirms the accuracy of our results.

In order to determine the linear range of the method, the urine sample and EP hydrochloride injection were accurately diluted with buffer solution. These solutions were analyzed using DPV at Au-NPs/poly(BCG)/GCE. In fact, the potentials were controlled between 0.0 and +0.8 V at 55 mV s^{-1}. The I_{pa} value was measured at the oxidation potentials of EP and UA. The results are shown in Table 2. This procedure was repeated five times and the relative standard deviation obtained was 2.4 % (Table 3). Different standard concentrations of EP were added to the diluted EP hydrochloride injection and the recovery was between 89.2 and 102.5 % for five measurements (Tables 2).

Conclusions

New electrochemical sensor based on a poly(BCG)/Au-NPs film was prepared. This electrode is electrochemically active for the oxidation of AA, EP, and UA and can resolve their overlapped oxidation peaks. The excellent electrocatalytic activity of the modified electrode is due to a combination of an organic polymer and Au-NPs. Also, the sensor has simple fabrication procedure, high stability, high sensitivity, and wide linear range, which can be applied for the simultaneous determination of AA, EP, and UA in different biological samples.

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