ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 10(B), pp. 29043-29048 October, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

MODIFIED GRAPHITE PASTE ELECTRODE WITH POLY HYDROQUINONE /REDUCED GRAPHENE OXIDE FOR SIMULTANEOUS DETERMINATION OF BIO-COMPOUNDS

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DOI: http://dx.doi.org/10.24327/ijrsr.2018.0910.2783

ARTICLE INFO

Article History:

Received 13th July, 2018
Received in revised form 11th August, 2018
Accepted 8th September, 2018
Published online 28th October, 2018

Key Words:

Polyhydroquinone / reduced graphene oxide, Dopamine, Acetaminophen, Xanthine, simultaneous electrochemical determination

ABSTRACT

In this study, for the first time, poly hydroquinone / reduced graphene oxide (pHQ-rGO) composite was synthesized and used for modification of the graphite paste electrode (GPE)for simultaneous electrochemical determination of dopamine (DA), acetaminophen (AC), and xanthine (XN). A detailed investigation by X-ray diffraction spectroscopy (XRD), Fourier transform infrared spectroscopy(FT-IR), field emission electron microscopy (FESEM), transmission electron - microscopy (TEM), electrochemical impedance spectroscopy and electrochemistry methods such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) was performed in order to elucidate the preparation process and properties of the pHQ-rGOmodified graphite paste electrode (GPE/pHQ-rGO). The proposed modified electrode displays intense and indelible electrooxidation response for simultaneous determination of DA, AC and XN to three well-separated peaks in the potential range from 0.4 to 1.1V using CV and DPV methods in a phosphate buffer solution with pH 2.0. Some kinetic and thermodynamic parameters for the electrochemical oxidation of DA, AC, and XN were also determined. Under the optimum conditions, detection limits (S/N=3) of 60, 80and 90nM were obtained for DA, AC, and XN, respectively. Moreover, GPE/pHQ-rGOwas successfully used to determineDA, AC, and XN in real samples.

INTRODUCTION

Graphene is a two dimensional, one atom thick graphiteic material, which is the building block of graphite. Owing to its unique properties such as excellent optical, electrical and mechanical properties, graphene has considerable applications in many research areas such as solar cell, fuel cell, battery as well as optical and electrochemical sensors[1-4]. Graphene largely has been used as electrocatalyst. Electrocatalytic activity of Graphene mostly rises from its large surface area (theoretically about 2600 m^2/g) and good conductivity[5]. Some researcher used theses nano sheets as supports for decorating of nano particles on their surfaces, or mixed them with conductive polymers or redox polymers to prepare modifying composites for modification of electrodes[6-8]. A good idea for promoting the electrocatalytic activity of graphene, is it's functionalization. For example, Wang et. al prepared calixarene functionalized reduced graphene oxide modified glassy graphite electrode for simultaneous determination of Fe(III), Cd(II) and Pb(II) Ions[9].β-cyclodextrin functionalized reduced graphene oxide (rGO) modified glassy graphite electrode was used for simultaneous determination of Nitrophenol isomers [10]. Anthraquinone functionalized reduced graphene oxide was prepared as electrode material for aqueous and non-aqueous rechargeable batteries[11]. Immobilized tyreosinas 1-formylpyrene functionalized reduced graphene oxide was used for sensitive determination of phenol[12].

In mild basic pHs, molecules with electroactive functional groups such as dopamine and hydroquinone can be used for simultaneous reduction of GO and polymerization (polydopamine and polyhydroquinone) to prepare polymer/ graphene oxide composites[13, 14].

Dopamine (DA), as a hormone and neurotransmitter , has a main role in mammalian central nervous system [15]. It's low levels causes to Parkinson[16], also its low levels in urinary excretion indicates chronic renal parenchymal disease[17]. Acetaminophen (AC) is a popular anti-fever and pain drug[18], high levels of AC causes liver damages [19]. Xanthine (XN) is a product on the pathway of purine degradation. High levels of XN in human body fluids indicates xanthinuria pathologic state[20]. Thesimultaneous, rapid, sensitive and selective determination of theses co-existing compounds in biological fluids is important for inspection of their metabolism and diagnosing disease.

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Accordingly, in this work for the first time the HQwas used as reducing agent for reducing of GO as well as monomer for preparation of poly hydroquinone to prepare pHQ/rGO composite. Then, the pHQ /rGOcompositewas used as modifier for modification of graphite paste electrode for simultaneous electrochemical determination of DA, AC and XN in human urine and serum samples.

Experimental

Reagents

Graphite fine powder, sulfuric acid (95–98%), hydrochloricacid (36–38%), potassium permanganate (99.5%), sodium hydroxide (96%), Hydrogen peroxide (40%) were all analytically pure and purchased from Sigma-Aldrich Company. Dopamine (DA), Acetaminophen (AC), and Xanthine (XN), Sodium nitrate (99%) and Hydroquinone were obtained from Merck Company. Fresh urine and serum samples were obtained from the Omid Clinical Laboratory (Zahedan, Iran) without any pretreatments.

Instrumentation

Electrochemical measurements were carried out with a SAMA 500 ElectroAnalyzer (SAMA Research Center, Iran) controlled by a personal computer. All electrochemical experiments were carried out in a conventional three-electrode cell at room temperature. A platinum electrode and a silver/silver chloride electrode (Ag/AgCl) were used as the counter and reference electrodes, respectively. Electrochemical impedance spectroscopy (ESI) was performed with an Autolab PGSTAT 128N (EcoChemie, Netherlands) potentiostat/galvanostat controlled by NOVA 1.11 software. Electrochemical impedance measurements were performed in 5 mM[Fe (CN) $_6]^{3-/4-}$ prepared in 0.1 M KCl. EIS was performed overa frequency range of 0.1 Hz to 10 kHz with 0.02 V amplitude(rms).TEM images were taken using a Philips CM120 transmission electron microscopy with 2.5 angstrom (Å) resolution. An FESEM image was taken using a Philips XL30 Scanning electron microscope X-ray diffraction pattern was taken by Philips 1730 Diffractometer, the IR spectra were recorded as KBr pellets on an FTIR Perkin Elemerspectrophotometer and electronic spectra on a JASCO V-570 spectrophotometer. A Metrohm pH meter, model 744 was also used for pH measurements.

Preparation of modifiers

Synthesize ofpHQ-rGO composite

Graphite oxide was prepared by modified Hummer's method[21]. First, Graphite powder (5 g) was dispersedin a beaker containing 150 ml concentrated nitric acid and sulfuric acid (1:2 in volume) for 30 min. After that, this beaker was immersed in an ice bath and potassium permanganate (15 g) was added slowly. The resulting Brown paste was thoroughly washed with HCl (1M) and deionized water until pH 7.0and filtered to remove metal ions. The GO was suspended in water, and exfoliated through ultra-sonication for 1 h. Then the simultaneous reduction of GO and polymerization of HQ performed by refluxing the GO solution (5 % w/w) with HQ (1 M, pH = 9) for12 h. Then the final products were centrifuged, washed, and finally vacuum-dried. The resulting black powder was denoted as pHQ- rGO

Preparation of modified graphite paste electrode

5 mg of pHQ- rGO and 195 mg graphite were mixed together, then 5 mg paraffin oil was added. This mixture was ground by mortar and pestle for 10 min. There after, the resulting paste was packed into a polyethylene tube and a copper wire was inserted into the tube as an electrical contact. The new surface was obtained by pushing the graphite paste out of poly ethylene tube and polishing by weighing paper.

RESULT AND DISCUSSION

Morphological, Fourier transform infrared (FTIR) and XRD characterization of GO and pHQ-rGO

Figure 1S shows XRD pattern of as synthesized GO Nanosheets. This pattern shows the characteristic peak for GO at 10.3˚[22]. Figure 1 shows the FESEM and TEM image of pHQ-rGO composite. These figures clearly revealed the wrinkled sheet-like structure of HQ-rGO. Figure 2S shows FT-IR spectra of GO and pHQ-rGO, pHQis a polymer with hydroquinone and benzoquinone (BQ) electro active functional groups[13]. Comparatively both GO and pHQ-rGO show absorption bands at higher wave numbers attributed to OH and CH stretching vibrations. The absorption band at higher wave numbers for pHQ-rGO are stronger and broader due to presence of OH vibrations of HQ functional groups[23, 24]. pHQ-rGO shows absorption peaks at 1620 and 1725 cm^{-1} attributed to C=C aromatic rings and C=O groups respectively [24, 25].pHQ-rGO shows new strong peak at 1099 cm^{-1} attributed to C-O stretching vibrations which confirmed the nucleophilic substitution reaction between PhO-groups of pHQ and oxygen containing groups of GO. Phenolate anions act as nucleophilic reagent to functionalize GO[26].

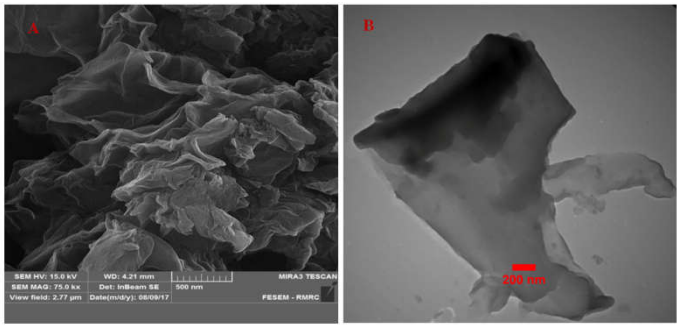


Fig 1 FESEM (A) and TEM images (B) of pHQ-rGO.

Electrochemical characterization of GPE/GO and GPE/ pHQ – rGO

Figure 3S shows CVs of GPE/GO and GPE/pHQ – rGO in 0.1 M PBS (pH=2). Based on this Figure, GPE/GO exhibits weak and broad redox peaks at 0.6 and -0.45 V attributed to the electro active functional groups at its surface. In the case of GPE/pHQ – rGO, these redox peaks were observed at the lower potential 0.34 and 0.25 V with strong current intensity and sharply. These peaks were attributed to the HQ/BQ functional groups in pHQ structure.

EIS Mesurments

Figure 2A shows the CVs ofbare GPE (BGPE) and different modified electrodes with GO and pHQ-rGO in 5 mM Fe (CN) $_6^{3-/4-}$in 0.1 M KCl. The results reveal a remarkable reduction in the peak potentials and increasingin the peak current for

different electrodes as follows: GPE/pHQ-rGO >GPE / GO>BGPE. Supporting evidence for these modified electrodes was found by EIS, which is a powerful technique to study of electrode-electrolyte interfacial features. As shown in Figure. 2B the Nyquist plot of BGPE comprises two parts: (1) thesemicircle at higher frequencies indicates charge transfer limitations and its diameter is equal to the charge transfer resistance (R_{ct}); (2) in the second part of theNyquist plot a straight line appears in low frequencies indicating mass transfer limitations[27].

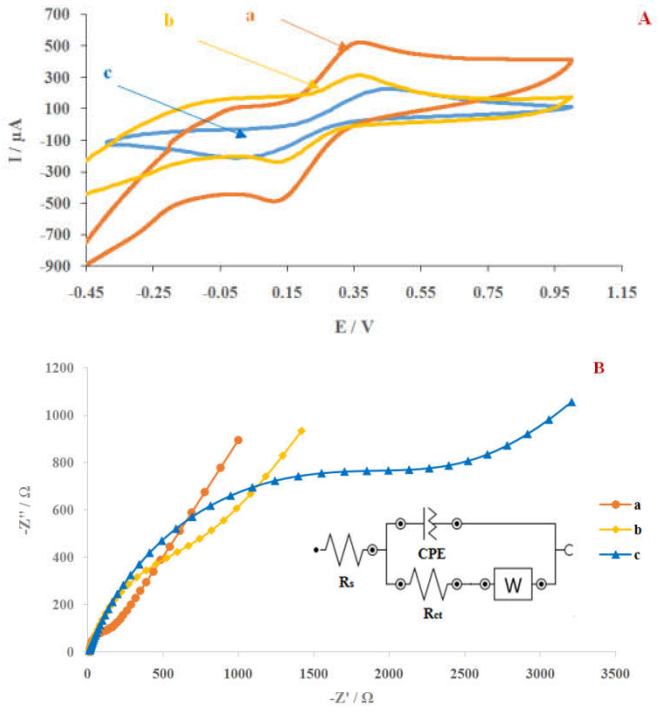


Fig 2 (A) CVs of 0.1M KCl containing 5 mM Fe $(CN)_6^{3-/4-}$ at at (a) GPE / pHQ-rGO, (b) GPE / GO, (c) BGPE, . Scan rate: 100 mVs^{-1} Fig (2.B). Nyquist plots of various electrodes (a-c) in 0.1M KCl containing 5 mM Fe $(CN)_6^{3-/4-}$ Fig 2.B (inset) equivalent circuit. R_s: solution resistance, R_{ct}: charge transfer resistance, W: Warburg element. GPE: Constant Phase Element

The NOVA software was used for fitting and simulation of EIS data and also the Randles equivalent circuit which was illustrated in the inset of Figure.2Bwas selected as anequivalent circuit for fitting and simulation of EIS data. The $R_{ct}$for GPE/pHQ-rGO, GPE/GO and BGPE was obtained 100, 800, 2500 Ωrespectively. The remarkable decreasing in$R_{ct}$of GPE/GO compare to BGPE is attributed to the large surface area and the presence of electroactive functional groups in the structure of GO[28]. Comparatively the observed decrease in $R_{ct}$of GPE / pHQ-rGO compared to other electrodes attributed to the presence of electro active HQ/BQ functional groups in the structure of pHQ-rGO, higher electrical conductivity and large surface area of rGO Nanosheets[5, 28].

Electrochemial charaterization of modifiedGPE for simultaneous determination of DA, AC, and XN

As shown in Figure 3, the electrocatalytic behavior of the electrodes in the stepwise modification of GPE was investigated.GPE / pHQ – rGOexhibited three well-defined peaks for three analytes and showed maximum peak separation among various electrodes.

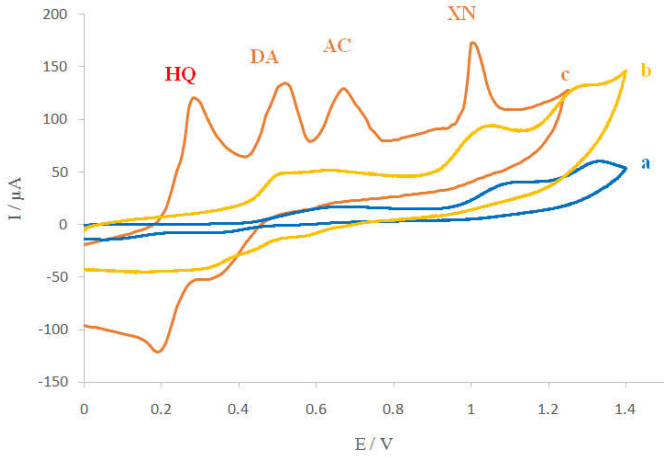


Fig 3 (A) CVs at (a) BGPE, (b) GPE / GO and (c) GPE / pHQ-rGO in 0.1M PBS (pH = 2) containing (DA (450µM), AC (300µM) and XN (200µM) (scan rate=100mV^{-1})

Figure 3 displays the CVs of a ternary mixture of DA, AC and XN in 0.1 M phosphate buffer solution (PBS, pH = 3.0) at BGPE, GO modified GPE and GPE / pHQ – rGO.BGPE showed broad and weak oxidation peaks for DA, AC and XN at 0.65, 1.1 and 1.3 V, respectively. Also, the GO modified GPE shows broad and weak oxidation peaks for DA, ACand XN at 0.5, 1.05 and 1.27 V, respectively. However, the pHQ – rGOmodified GPE showed strong well-separated oxidation peaks for DA, AC and XN at 0.5, 0.67 and 1.01V, respectively. Based on these results, GPE / pHQ – rGO exhibits higher electrocatalytic activity toward electro oxidation of these analytes, this effect attributed to the presence of electro active HQ/BQ functional groups in the structure of pHQ-RGO. Electroactive modifiers catalyze the electrochemical reactions of analytes either by inclusion in the electron transfer chain or by stabilization the intermediate products[29], higher electrical conductivity and large surface area of RGO Nanosheets[5].Therefore, the GPE / pHQ – rGO modified GPE has the highest sensitivity and selectivity for simultaneous electrochemical determination of DA, AC and XN.

The effect of scan rates

The effect of scan rate on the electrochemical oxidation of DA, AC and XNat the GPE / pHQ – rGOwas investigated by CV (Fig. 4SA-C). Based on this figures, the anodic peak currents of DA, AC and XN increased with higher scan rates. Also, the oxidation peak potentials for DA, AC, and XNshifted to more positive values with increasing scan rate, concerning the kinetic limitations of the electrochemical reaction. The plot of peak currentst (I_p) *vs.* the square root of scan rate ($v^{1/2}$) was linear in the range of 50 to 500 mV s^{-1}, suggesting that at sufficient overpotential, the process is diffusion- rather than surface-controlled (Fig. 4SD).

Chronoamperometric studies

As shown in figure 5S-7S,the diffusion coefficients of the analytes were determined by chronoamperometry and using the Cottrell equation which explained the variation in the current with time for diffusion controlled process [30] .

$$I = nFACD^{1/2}\pi^{-1/2}t^{-1/2} \quad (2)$$

where *n*, *F*, *A*, *C*, *D* and *t* are number of transferring electron, Faraday constant (96485$Cmol^{-1}$), electrode surface area (here

0.27 cm^2), analyte diffusion coefficient ($cm^2s^{-1/2}$), and time (s), respectively. Therefore the linear relationship between current and $t^{-1/2}$ indicates diffusion-controlled current. Plots of I vs. $t^{-1/2}$ were drawn for different concentrations of analytes .Then, the resulting slopes of trend lines were plotted against analyte concentrations. From the resulting slopes, according to the Cottrell equation. The values of D for DA, AC, and XN were found6.0×10^{-6}, 7.0×10^{-6} and 3.4×10^{-5} $cm^2.s^{-1}$ respectively.

pH effect

As shown in Figure4, pH effect on the electrochemical response of modified GPE in 0.1 M PBS containing DA (700 µM), AC (300µM), and XN (60µM) was investigated. Peak potential for three analytes shifted to negative potentials with increasing solution pH indicating the contribution of protons in their electrochemical reaction. For species in whose oxidation reaction, protons are transferred, the following formulas explain the relationship between peak potential and pH[31].

$$E'_{p(Red)} = E_{p(Red,pH=0)} - 2.303\frac{mRT}{nF}pH \quad (3)$$

$$\frac{dE_p}{dpH} = -2.303\frac{mRT}{nF} \quad (4)$$

$$\frac{dE_p}{dpH} = -0.059\frac{m}{n} \qquad \text{at } 25c^0 \quad (5)$$

Where $E_{p\ (Red,\ pH=0)}$ is the anodic peak potential for analyte at pH=0; m and n are the number of protons and electrons, respectively; and R ($8.314 jmol^{-1}k^{-1}$), T (K) and F ($96485 c.mol^{-1}$) have their normal meanings. Inset a of Fig.4 shows linear relationship between peak potentials and pH values for all species.

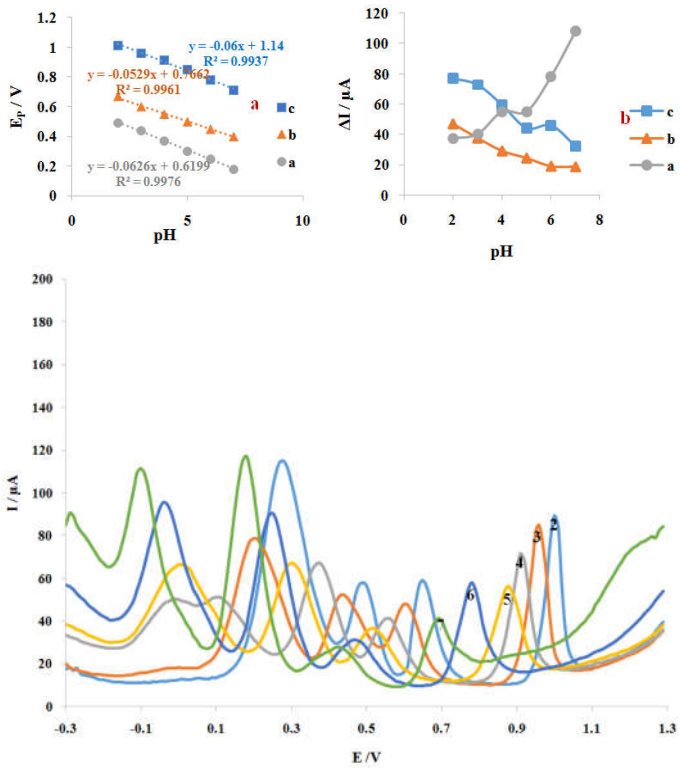


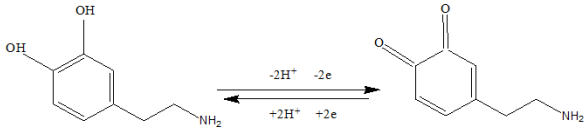
Fig 4 (A) DPVs of a solution containing DA(450µM), AC(450µM), and XN (140µM) at GPE / pHQ-rGO in 0.1M PBS at various pH values (a-g : 2,3,4,5,6,7) (inset. a). Plots of peak potential versus pH for three analytes (inset. b). plots of peak current versus pH for three analytes

According to the slopes of trend lines, it was suggested that the oxidation reaction of, DA, AC and XN involves two protons and two electrons (Eqs. 6-8). And the probable electrochemical reaction of DA, AC, and XA at surface of GPE / pHQ – rGO should be a two- electrons and two- protons process (Eqs. 9-11) [32, 33]. Based on Figure4, DPV related to pH=2 exhibited maximum peak separation, most strong (inset b of Fig.4) and sharpest oxidation peaks for AC and XN. Also, it exhibited almost strong but sharp oxidation peak for DA. Therefore, pH=2 was selected as optimum.

$$E_{(pa\ ,\ DA)}(V) = 0.6199 - 0.0626\ pH \qquad (r^2 = 0.9976) \quad (6)$$

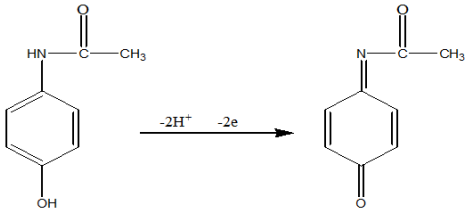
$$E_{(pa\ ,\ AC)}(V) = 0.7662 - 0.0529\ pH \qquad (r^2 = 0.9961) \quad (7)$$

$$E_{(pa\ ,\ XN)}(V) = 1.1400 - 0.0600\ pH \qquad (r^2 = 0.9937) \quad (8)$$



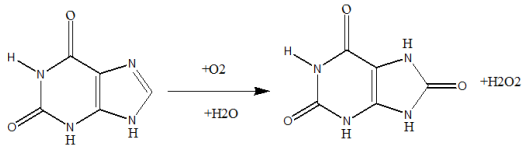
(9)

Dopamine



(10)

Acetaminophen



(11)

Xanthine

Simultaneous determination of DA, AC, and XN

Under optimum conditions, simultaneous determination of DA, AC, and XNwas carried out at the potential range of 0.4 to 1.1 V using DPV. The electro catalytic peak currents of DA, AC, and XN at the surface of GPE/pHQ-rGO were linearly dependent on the DA, AC, and XN concentrations. Figures (5A-B) show the DPVs and calibration curves of DA, AC, and XN at GPE / pHQ-rGO. The detection limit was calculated based on the relationship LOD = $3S_{blank}$ / m where S_{blank} is the relative standard deviation of blank signals (n=10) and m is the slope of calibration plot. The responses were linear with DA concentration in the range from 2.5 to 815 µM. While the dynamic ranges were linear with the AC concentration in the range from 2.5to 645 µM, the dynamic range for XN was linear from 2.5 to250 µM. The detection limits were determined to be 60, 80and 90nM for DA, AC, and XN, respectively.

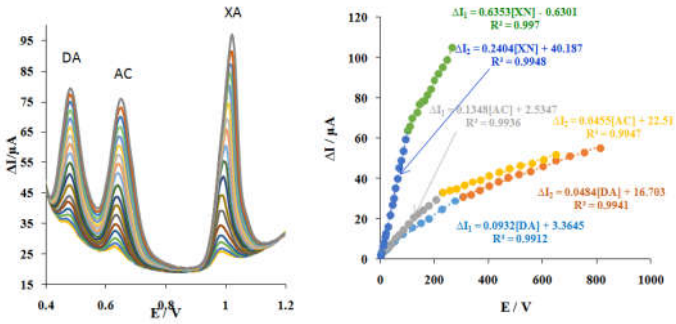


Fig 5 (A) DPVs of GPE / pHQ-rGO in 0.1 M PBS (pH = 2) containing mixed concentration of DA, AC and XN. [DA]: 3 – 814 µM, [AC]: 5 – 651 µM, [XN]: 2.5 –266 µM. (B). Plots of anodic peak current vs. concentration of DA, ACand XN.

The electrooxidation processes of DA, AC, and XN in the mixtures were also investigated when the concentration of one species changed and the other was kept constant; the results are shown in Fig. 8-10S. In a certain range of concentration, one analyte has no interference if it causes relative error lower or equal to ± 5 (%). Examination of Figure8-9S shows that the peak current of DA increased (5 to 815µM) with increasing DA concentration, whereas the concentrations of AC (50µM) and XN (20µM) remained constant. Similarly, as shown in Figures9S and 10S the oxidation peak currents of AC or XN increased linearly with increasing concentration in the presence of a constant concentration of the other two compounds. It was found that DA, AC, and XN had no interference in the simultaneous determination of each other in the linear ranges of DA, AC, and XN. Furthermore, the interfering effect of other probable coexisting species such as Uric acid, Caffeine and Ibuprofen was investigated and it was found that the maximum interfering effect was seen for Uric acid on the determination of DA (5% tolerable molar ratio > 15), for AC on the determination of AC (5% tolerable molar ratio >4) and on the determination of XN (5% tolerable molar ratio >10). Moreover, no significant interfering effects were seen for other common species. Based on this results, the simultaneous and quantitative determination of AP, Ph and NP was reliable.

Real sample analysis

To evaluate the practical applicability of the proposed modified electrode, the GPE / pHQ-rGO was examined for the simultaneous determination of DA, AC and XN in human urine and serum samples. The samples were diluted with PBS and DPVs were used for the simultaneous determination of DA, AC, and XN using the standard addition method. As depicted in Table 1, acceptable recovery values were obtained, pointing to the applicability of this modified electrode for trace amounts of these compounds in the real sample analysis.

Table 1 simultaneous determination of DA, AC and XN in human urine and serum samples (n = 3)

sample	Analyte	Analyte Concentration in the unspiked sample (µM)	Known spiked added Concentration (µM)	Analyte concentration in the spiked sample (µM)	Recovery (%)	RSD (%)
Urine	DA	_	100	97	97	1.3
	AC	_	200	196	98	1.6
	XN	_	150	154.5	103	1.4
Serum	DA	_	500	505	101	1.7
	AC	_	300	285	95	2.0
	XN	_	200	194	97	1.9

CONCLUSIONS

In this work, a simple modified electrode was prepared using GPE / pHQ-rGO and applied to the simultaneous determination of a ternary mixture of DA, AC, and XN. The pHQ-rGO improves the electrochemical catalytic activities towards the oxidation of DA, AC, and XN.The results showed low detection limits and good selectivity. In addition, this proposed method can be applied to the determination oftrace amounts of DA, AC, and XN in real samples with satisfactory results.

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How to cite this article:

Arezo Nouri and Meissam Noroozifar. 2018, Modified Graphite Paste Electrode With Poly Hydroquinone /Reduced Graphene Oxide For Simultaneous Determination of Bio-Compounds. *Int J Recent Sci Res.* 9(10), pp. 29043-29048.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0910.2783

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