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Modified Graphite Paste Electrode with Strontium Phen-Dione Complex for Simultaneous Determination of a Ternary Mixture of Dopamine, Acetaminophen and Xanthine. Part II

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In this study, modified graphite paste electrode with $[Sr(OH_2)_4(phen\text{-}dione)_2](Cl)_2$ complex (Sr-Phen) was used for simultaneous electrochemical determination of dopamine (DA), acetaminophen (AC), and xanthine (XN). The proposed modified electrode was shown a sensitive and selective 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 3.0. Some kinetic and thermodynamic parameters for the electrochemical oxidation of DA, AC, and XN were also determined. Under the optimum conditions the calibration curves were linear up to 220, 150 and 60 µM with a theoretical detection limits (S/N=3) of 800, 90 and 60 nM for DA, AC, and XN, respectively. Moreover, GPE/Sr-Phen were successfully used to determine DA, AC, and XN in human urine and blood serum samples.

Keywords: Strontium Phen-dione, Simultaneous determination, Dopamine, Acetaminophen, Xanthine.

1. INTRODUCTION

Redox active electro catalysts at the surface of the modified electrodes catalyze the electrochemical reactions of analytes either by inclusion in the electron transfer chain or by stabilization the intermediate products [1]. Substances with electroactive quinone functional groups such as quinone containing polymers and ligands have long been used as electrocatalysts at the electrodes surfaces. For example, poly (aminoquinone) – multiwalled carbon nanotubes (MWCNTs) hybrid film modified glassy carbon electrode was used for electrooxidation of L-cysteine [2]. Goss et al. prepared some transition metal complexes of Phendione (Ru, Fe, Os, Co, and Cu) [3]. They concluded that metal complexes of phendione have electrocatalytic activity toward oxidation of Nicotinamide adenine dinucleotide (NADH). Ortho quinone functional groups containing electro catalysts such as phendione and poly (aminoquinone) exhibite quinone based redox activities. Their redox peak potentials and electrocatalytic activities can be controlled through complex formation as well as pH changes [3]. Phendione mostly chelates metal ions through Nitrogen atoms and the chemical structure of complex was shown in Figure 1.

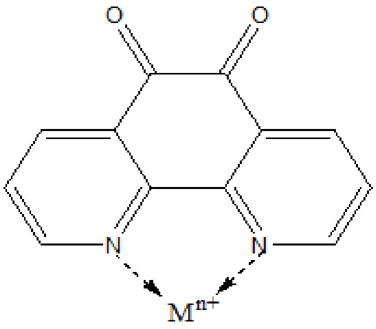


Figure 1. Chemical structure of phendione ligand coordinated to metal ion (M^{n+})

As a result complex formation increases the electron affinity of phendione ligand compare to free ligand [4]. In our previous work [5], we study the electrochemical property of $[Sr(OH_2)_4(phen\text{-}dione)_2](Cl)_2$ complex (Sr-Phen) and used it as modifier in a graphite paste electrode. The modified electrode was used for simultaneous determination of 4-aminophenol, Uric Acid and Tryptophan.

Dopamine is a well-known hormone and neurotransmitter that is naturally produced in the mammalian central nervous system [6]. Its low levels cause Parkinson and schizophrenia[7]. Its low levels in biological fluids (urine and serum) indicates parenchymal disease [8]. Acetaminophen (AC) is an anti-fever and pain drug. In high doses, it causes hepatotoxicity[9]. Xanthine (XN) is one of purines degradation products. Disorder in purine metabolism causes increased XN concentration in biological fluids resulting in xanthinuria [10]. Therefore, simultaneous sensitive and selective determination of these analytes is important.

In current work, we used modified graphite paste electrode with Sr-Phen for investigation of the effect of this complex on the electrocatalytic activity toward electrooxidation of DA, AC and XN. Based or results, the mentioned modified electrode is sensitive and selective for simultaneous determination of these bio-compounds in real samples.

2. EXPERIMENTAL

2.1. Reagents

Dopamine (DA), Acetaminophen (AC), and Xanthine (XN) were purchased from Sigma-Aldrich Company. Phosphoric acid, 1,10-phenanthroline 5,6-dione(Phen), Ethanol (99%), $SrCl_2 .6H_2O$ were purchased from Merck Company. Fresh urine and serum samples were obtained from the Omid Clinical Laboratory (Zahedan, Iran) without any pretreatments.

2.2. Instrumentation

Electrochemical measurements were carried out with a SAMA 500 Electro Analyzer (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.

2.3. Preparation of modified graphite paste electrode

The synthesize of [Sr $(OH_2)_4$(phen-dione) $_2$]$(Cl)_2$, (Sr/Phen) and modified graphite electrode is based on our provirus work [5].

3. RESULT AND DISCUSSION

3.1. Electrochemial charaterization of modified GPE for simultaneous determination

The cyclic voltammograms (CVs) of a mixture of DA, AC and XN of As using different electrode were shown in Figure 2A. Based on this Figure, the bare GPE (BGPE) showed a weak and broad overlapped oxidation peaks for DA and AC at 0.52 and 0.64 V and an oxidation peak at 1.06 V for XA. The electrocatalytic behavior of the electrodes in the stepwise modification of GPE was investigated. The modified GPE with Phen shows a broad overlapped oxidation peak for DA and AC at 0.71 and a broad oxidation peak at 1.07 V for XA. This modifed electrode can not separate the oxidation peaks of DA and AC. The modified GPE with Sr(Phen) exhibited three well-defined peaks for three analytes at 0.56, 0.71 and 1.05 V for DA, AC and XA. This modified electrode showed maximum peak separation among various electrodes. The first peak in the CVs of modified electrode with Phen at 0.35 V and Sr-Phen at 0.33 V was assigned to redox peak of free phen and Phen in Sr-Phen [3]. Based on these results, GPE/Sr-Phen exhibits higher electrocatalytic activity toward electro oxidation of these analytes, because the Coordinated ortho quinone groups in GPE/Sr-Phen structure have higher electron affinity compared to free ortho quinone groups in the GPE/Phen structure [5]. Therefore, the [Sr $(OH_2)_4$(phen-dione) $_2$]$(Cl)_2$ modified GPE has the highest sensitivity and selectivity for simultaneous electrochemical determination of DA, AC and XN. Also, Figure 2B displays the DPVs for the indivtual solution of DA, AC and XN and a mixture solution of DA, AC, and XN in the potential range of 0.4 - 1.1V. Based on this Figure, the peak potentials and peak currents of DA, AC and XN in both conditions are constant.

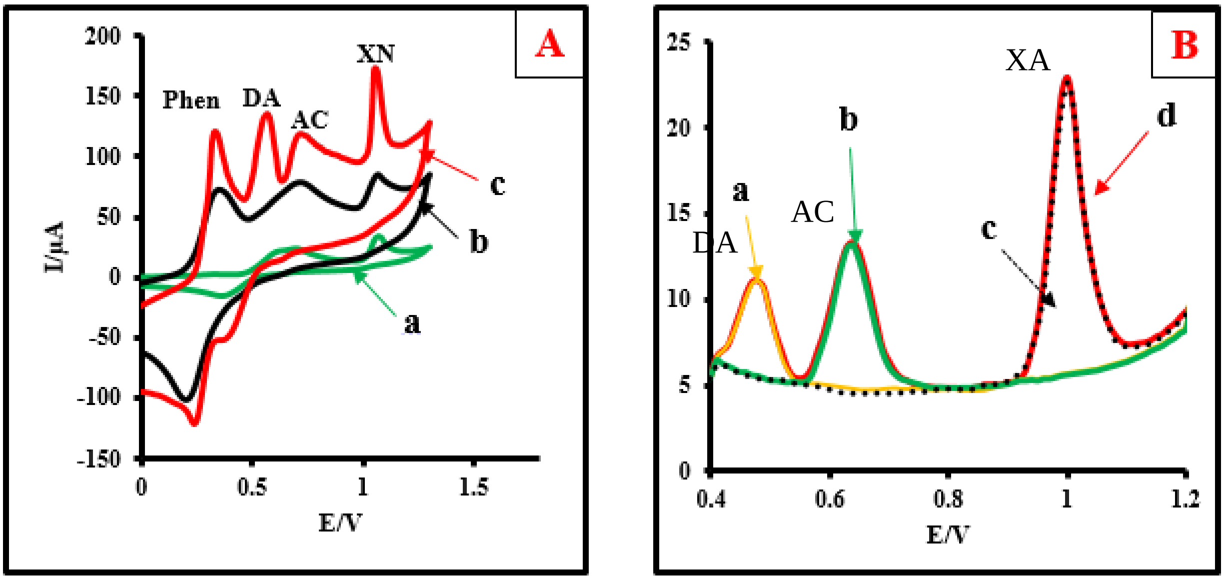


Figure 2. (A) CVs at (a) BGPE (b) GPE / Phen (c) GPE/ Sr-Phen in 0.1M PBS (pH = 3) containing DA (200µM) , AC (150µM) and XN (60µM) and (B) DPVs of GCE / PDA-MWCNT in 0.1 M PBS(pH = 3) containing (a) DA(50µM), (b) AC (50µM), (c) XN (30µM) (d) DA (50 µM) , AC (50 µM) and XN (30µM).

3.2. The effect of scan rates

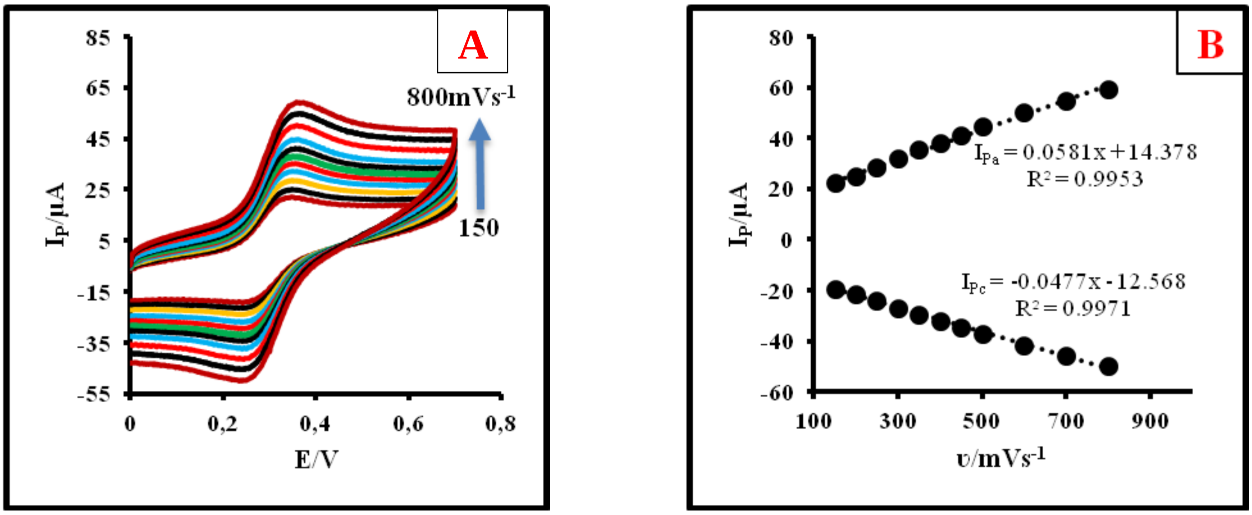


Figure 3. CVs of (A) modified GPE with Sr-Phen in 0.1 M PBS (pH=3) at various scan rates (from 150 to 800 mV.s^{-1}) (B) plot of anodic and cathodic peak currents vs. scan rate.

Figure 3 shows the CVs of GPE/Sr-Phen in 0.1 M PBS (pH=3) at various scan rates. The anodic and cathodic peak current ratio (Ipa/I_{pc}) is close to 1 and this result indicates the presence of diffusiosless and reversible surface process [11, 12]. The presence of these peaks is attributed to the ortho quinone groups of the Strontium-Phen dione complex [3, 5] and can be used for surface concentration of electroactive species at GPE/Sr-Phen by sharp equation as follow [13];

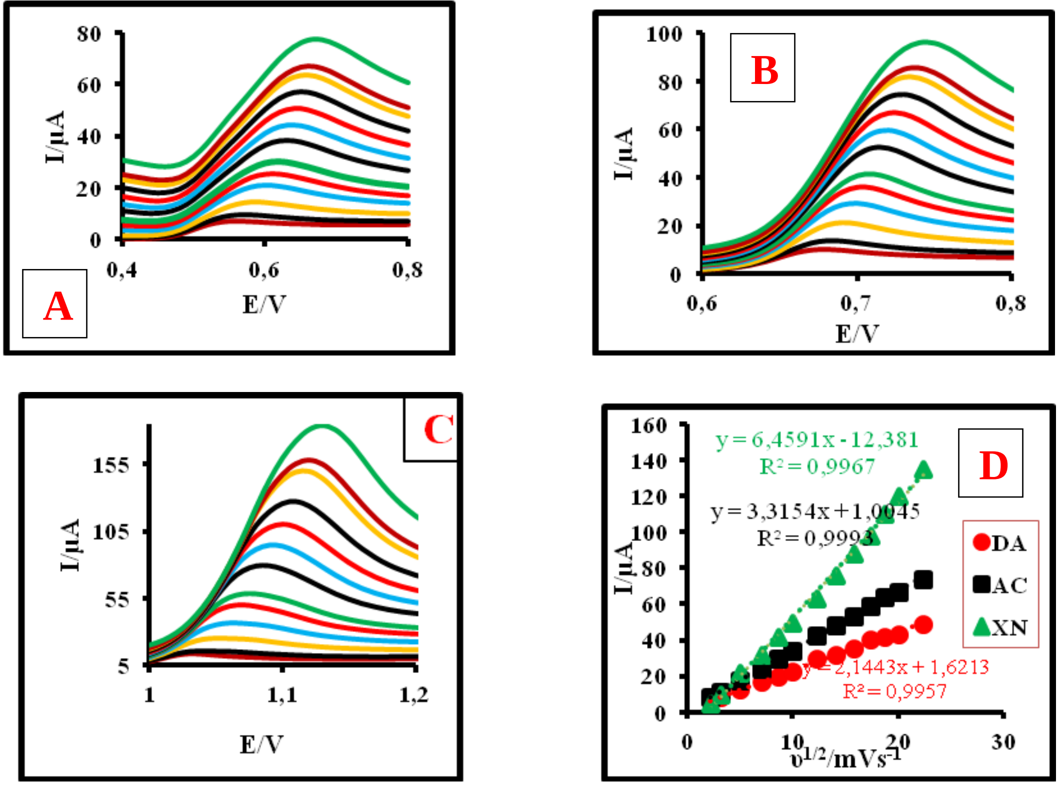
$$I_p = \frac{n^2F^2A\Gamma\upsilon}{4RT} \qquad (1)$$

where I_p (µA) is the peak current; n=2 is the number of transferring electrons [3]; A is the geometric surface area (here 0.0314 cm^2); υ (mV/s) is the scan rate; Γ (mol cm^{-2}) is the surface concentration of electro active species; and R (8.314 $jmol^{-1}k^{-1}$), F (96485 C/mol) and T (here 298 K) have their normal meanings as shown in Figure 3 From the slope of trend line of plotting I_p vs. υ, Γ was estimated as (6.12×10^{-9} $mol.cm^{-2}$).

The LSV for the oxidation of DA, AC and XN at the GPE/Sr-Phen in different scan rates wer shown in Figures 4A-C. As shown in thses Figs., the anodic peak currents of DA, AC and XN increased with increasing the scan rates and the anodic oxidation peak potentials for DA, AC, and XN shifted to more positive values with increasing scan rate, concerning the kinetic limitations of the electrochemical reaction. Fig. 4D was shown the plot of peak height (I_p) *vs.* the square root of scan rate ($v^{1/2}$) in the range of 25 to 600 mV s^{-1}. The linearity of these lines indicates that the electro transfer process at sufficient over potential is diffusion rather than surface-controlled. With these information and Tofel equation, the charge transfer coefficients (α) of analytes can be calculated using following equation [14]:

$$E_{pa} = E^{0'} + \frac{2.303RT}{(1-\alpha)nF} - \frac{2.303RT}{(1-\alpha)nF}\log\upsilon^{1/2} \qquad (2)$$

In this equation the E_{pa} (V), υ (V/s) α and n are the anodic peak potential, scan rate, charge transfer coefficient and the number of electrons transferred in the rate determining step (assuming n= 1), respectively.



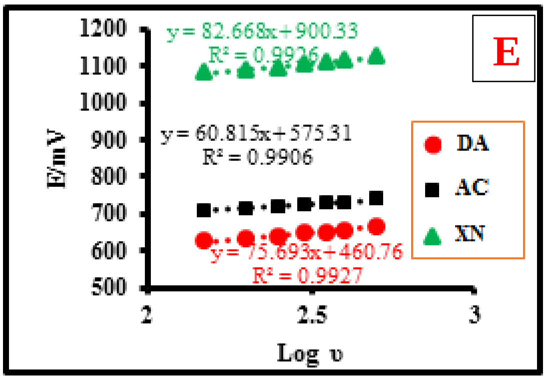


Figure 4. LSVs of modified GPE in 0.1 PBS (pH =3) containing DA (0.9 µM) (A), AC (1.25 µM) (B) and XN (4.5 µM) (C) at various scan rates (5 to 500 mVs^{-1}); Fig (D) plots of anodic peak potentials vs. logarithm of scan rate. Fig (E) plots of anodic peak current of modified vs. square root of scan rate.

The others parameters such as R (8.314 jmol^{-1}k^{-1}), T (here 298 K) and F (96485 C/mol) have their normal meanings. In Figure 4E, the anodic peak potential was plotted vs. log *v*. From the slope of trend lines, the α values for DA, AC and XN were obtained 0.576, 0.514 and 0.642, respectively.

3.3. Chronoamperometric studies

For determination of diffusion coefficient (D) of DA, AC and XA, the chronoamperometric measurements of the analytes at the GPE/Sr-Phen were carried out by setting the working electrode potentials at the 0.6, 0.75 and 1.1 V for the DA, AC and XA, respectively for the various concentrations of AA, AC and Trp in optimum pH 3.0 of PBS. The results were shown in Figs. 5A, 6A and 7A for the DA, AC and XA, respectively. The current observed for the electrochemical reaction under mass transport-limited conditions can be described by the Cottrell equation as follow;

$$I = nFACD^{1/2}\pi^{-1/2}t^{-1/2} \qquad (3)$$

In this equation the n, A (cm^2), C (mol.cm^{-3}), D (cm^2 s^{-1}), and t (s) are the number of electron, surface area of working electrode, concentration of analytes in the solutions, diffusion coefficient and time, respectively. The F (Faraday constant, 96,485 C mol^{-1}) and π (mathematical constant, 3.14) have their normal meanings. The diffusion coefficients of the analytes are calculated from information of these Figures and using the Cottrell equation which explained the variation in the current with time for diffusion controlled process. Experimental plots of I $_{vs.}$ t$^{-1/2}$ were drawn, and the best fits for different concentrations of DA, AC and XA were determined (see of Fig. 5B–7B). The slopes of the resulting straight lines were then plotted against DA, AC and XA concentration (insets Fig. 5C–7C). From the resulting slopes and based on the Cottrell equation the values of D for DA, AC, and XN were found 1.0 × 10^{-6} , 5.0 × 10^{-6} and 9.0 × 10^{-6} cm^2.s^{-1}, respectively.

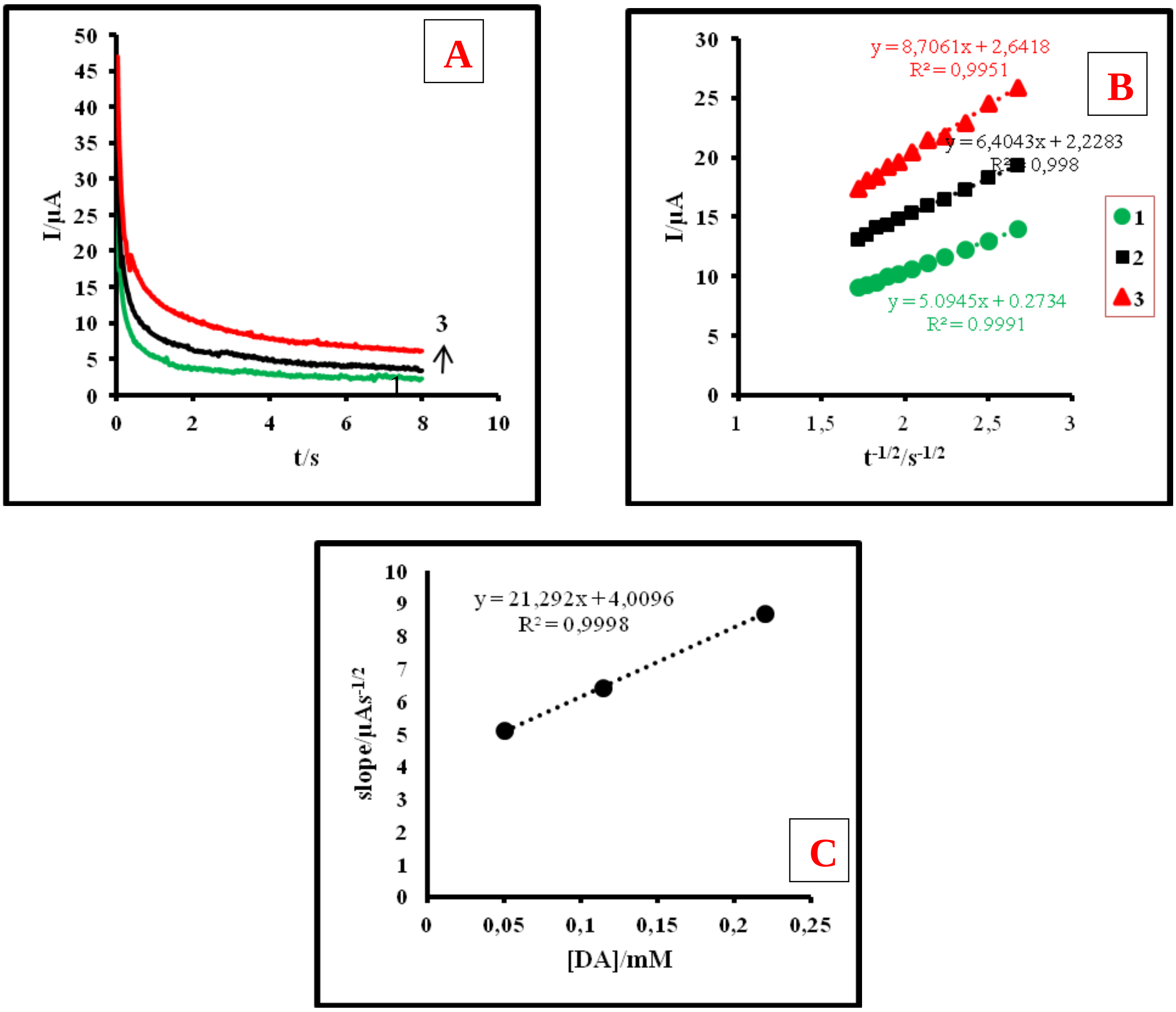
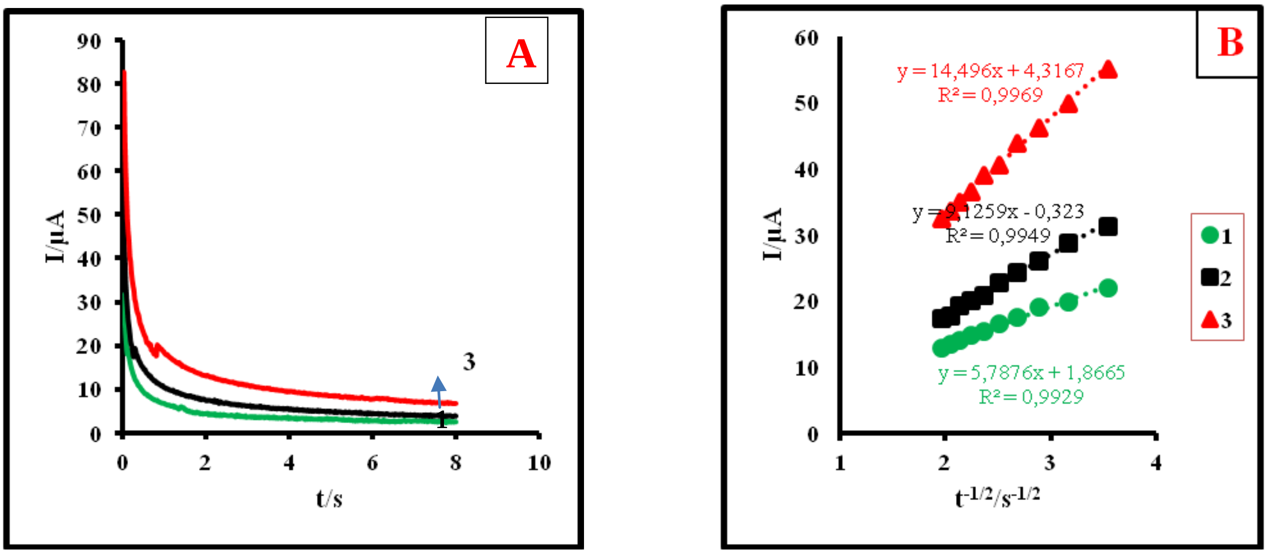


Figure 5. (A) Chronoamperograms obtained at the modified GPE containing different concentrations of DA: (1) 50, (2) 115, (3) 220 µM in buffer solution (pH= 3.0). (B) Cottrell's plots of I vs. $t^{-1/2}$ obtained from chronoamperograms and (C) plots of the slope of the straight lines against analyte concentration.



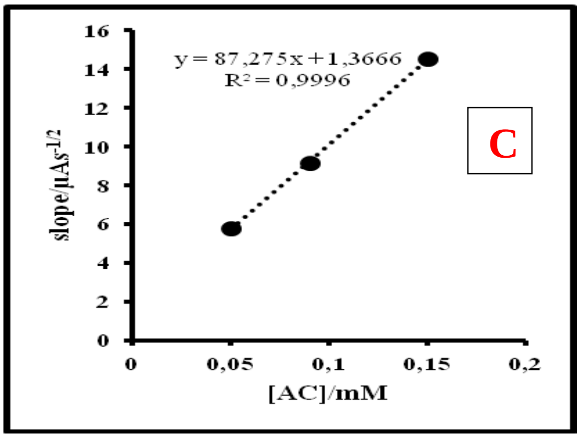


Figure 6. (A) Chronoamperograms obtained at the modified GPE containing different concentrations of AC: (1) 50, (2) 90, (3) 150 µM in buffer solution (pH =3.0). (B) Cottrell's plots of I vs. $t^{-1/2}$ obtained from chronoamperograms and (C) plots of the slope of the straight lines against analyte concentration.

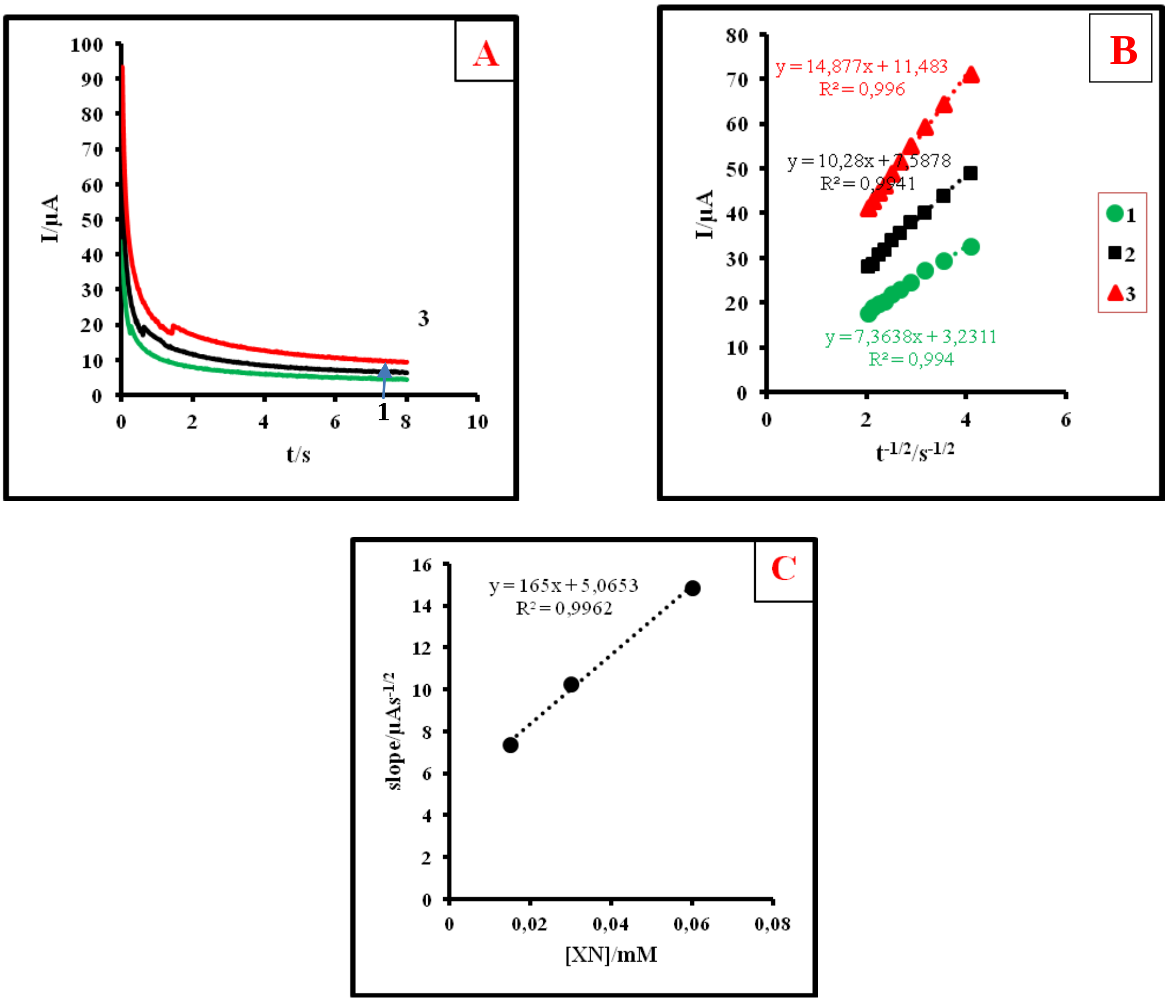


Figure 7. (A) Chronoamperograms obtained at the modified GPE containing different concentrations of XN: (1) 15, (2) 30, (3) 60 µM in buffer solution (pH =3.0). (B) Cottrell's plots of I vs. $t^{-1/2}$ obtained from chronoamperograms and (C) plots of the slope of the straight lines against analyte concentration.

3.4. pH effect

The pH effect on the electrochemical response of GPE/Sr-Phen in 0.1 M PBS containing with different pH for DA (220 µM), AC (150 µM), and XN (50 µM) were shown in Fig. 8. Based on this Fig., the peak potential for DA, AC and XN and also the peak potential of modifier, Sr-Phen, were shifted to negative potentials with increasing solution pH. These results are indicated that the contribution of protons in their electrochemical reactions. Figure 8B shows the linear relationship between peak potentials and pH values for DA, AC and XN and the lines equation (Eqs. 4-6) are as follow;

$$E_{(pa\,,\,DA)}\,(\,V\,) = 0.555 - 0.0590\ pH \qquad (\,r^2 = 0.9964) \qquad (4)$$

$$E_{(pa\,,\,AC)}\,(\,V\,) = 0.7575 - 0.0581\ pH \qquad (\,r^2 = 0.9998) \qquad (5)$$

$$E_{(pa\,,\,XN)}\,(\,V\,) = 1.0589 - 0.0596\ pH \qquad (\,r^2 = 0.9973) \qquad (6)$$

According to the slopes of trend lines with slopes 0.059, 0.0581 and 0.0596 for DA, AC and XN, that is close to theoretical slope 0.059, it was suggested that the oxidation reaction of, DA, AC and XN involves two protons and two electrons in these pH range [15]. Based on these results, the redox reactions in equations of 7-9 can be suggested for DA, AC and XN.

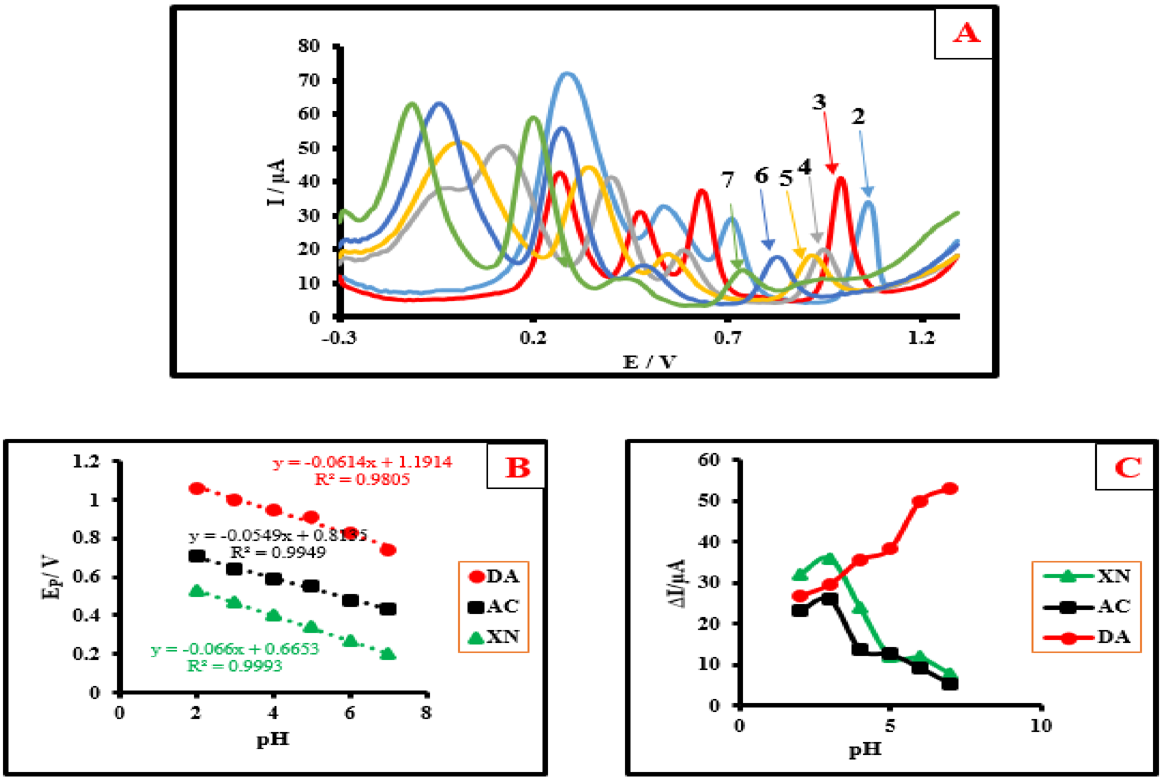
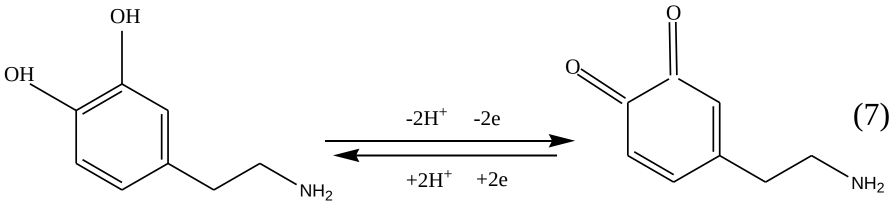
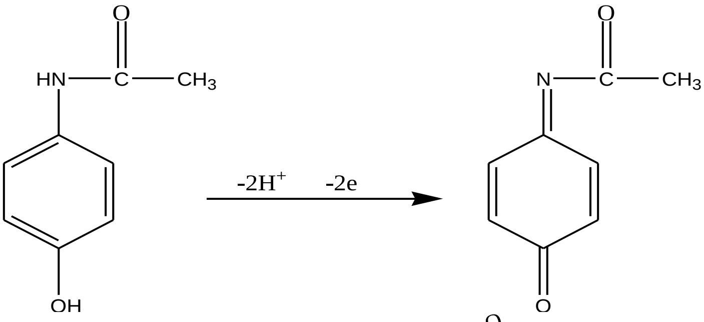


Figure 8. (A) DPVs of a solution containing DA(220µM), AC(150µM) and XN (50µM) at GPE/Sr-Phen in 0.1M PBS at various pH values (2-7) (B) plots of peak potential versus pH for three analytes; (C) plots of peak current versus pH for three analytes.



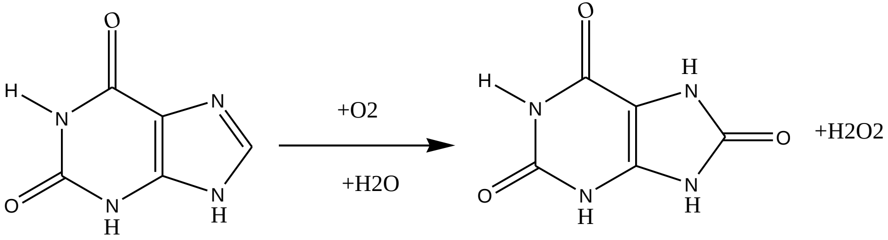
(7)

DA



(8)

AC



(9)

XN

Figure 8C was shown the plot of oxidation peak current of analytes *vs.* pH. Based on the this Figure, pH=3 exhibited maximum peak separation, high and sharpest oxidation peaks for them. Therefore, pH=3 was selected as optimum.

3.5. Simultaneous determination of DA, AC, and XN

Under optimum conditions, simultaneous determination of DA, AC, and XN was carried out at the potential range of 0.4 to 1.1 V using DPV method. The electrocatalytic peak currents of DA, AC, and XN at the surface of GPE/Sr-Phen were linearly dependent on the DA, AC, and XN concentrations. Figs.9A and B show the DPVs and calibration curves of DA, AC, and XN at GPE/Sr-Phen. 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 5 to 220 µM. While the dynamic ranges were linear with the AC concentration in the range from 0.8 to 150 µM, the dynamic range for XN was linear from 0.2 to 60 µM. The detection limits were determined to be 800, 90 and 60 nM for DA, AC, and XN, respectively.

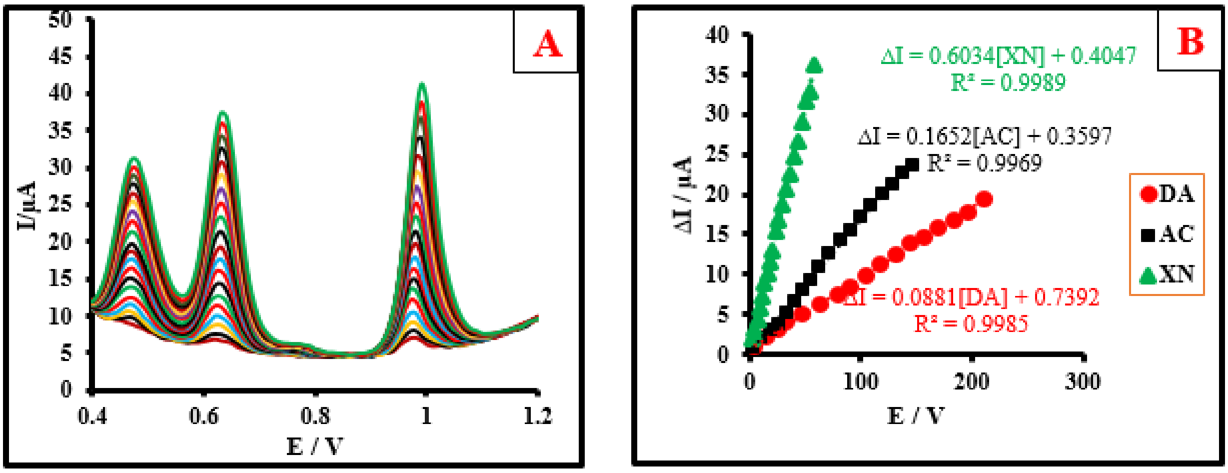


Figure 9. (A) DPVs of GPE / Sr-Phen in 0.1 M PBS (pH = 3) containing mixed concentration of DA, AC and XN; [DA]:5 – 220 μM, [AC]: 0.8 –150 μM, [XN]:0.2 – 60 μM; (B) plots of anodic peak current vs. concentration of DA, AC and XN.

A comparison study for proposed modified electrode with literature was shown in Table 1. Based on this table, the figures of merit of the proposed modified electrode such as modifiers, pH, linear range and detection limit were compared for the individual and simultaneous determination of the analytes, DA, AC and XN with some reported works. Based on the Table 1, the lower concentrations in the linear range of proposed modified electrode are better than the literature for AC [10, 19, and 21] and XN [10, 11] and satisfy in comparison of others works. The results for DA is satisfy in comparison of others works in Table 1. In addition, the detection limits of proposed method for AC and XN are lower than literature and also are satisfy for DA. Finally, the proposed modified electrode is cheap, simple and very stable and can be storage in DDW for the long time more than six month. Based on the above information, the proposed modified electrode is a good candidate for the individual and simultaneous determination of DA, AC and XN with satisfactory results in comparison of literature.

Table 1. Comparison of the proposed sensor with other electroanalytical methods for the simultaneous determination of DA, AC and/or XN

Electrode	Modifier	Method	pH	Analyte	Linear range(μM)	Detection Limit(μM)	Ref
GCE	Flavonoid nanostructured	CV/DPV	7	AC XN	0.9-80 1.0-70	0.78 0.65	10
GCE	B-Cyclodextrin	CV/DPV	5	DA XN	5-150 5-105	1.5 1.25	16

GCE	Poly(Methylene Blue)and electrochemically reduced Graphene oxide composite film	SWV/CV	3	XN	0.1-400	0.05	17
GCE	MWCNT/Poly (glycine)	CV/DPV	7	AC	0.5-200	0.5	18
GCE	MWCNTs	DPV	8	DA	3-200	0.8	19
				AC	3-300	0.6	
CPE	Pre-anodized	LSV	7	DA	2-500	0.42	20
				AC	2-400	0.21	
GCE	Poly (L-lysine)Graphene oxide	CV/DPV	5	DA	0.5-35	0.021	21
GCE	Nanoparticle film	CV/SWV/ LSV	5.5	XN	0.02-20	-	22
GPE	Sr-Phen	CV/DPV	3	DA	5-220	0.80	This work
				AC	0.8-150	0.09	
				XN	0.2-60	0.06	

3.6. Interference studies

The electro oxidation processes of DA, AC, and XN in the mixtures were also investigated intermolecular effect method and the results are shown in Figures 10 -12 for DA, Ac and XN, respectively. In a certain range of concentration, one analyte has no interference if it causes relative error lower or equal to ± 5 (%) for the peaks currents of the others analytes. Examination of Figure 10 shows that the peak current of DA increased (5 to 220µM) with increasing DA concentration, whereas the concentrations of AC (20 µM) and XN (10µM) remained constant.

Similarly, as shown in Figures 11 and 12 the oxidation peak currents of AC or XN increased linearly with increasing concentration in the presence of a constant concentration of the others 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.

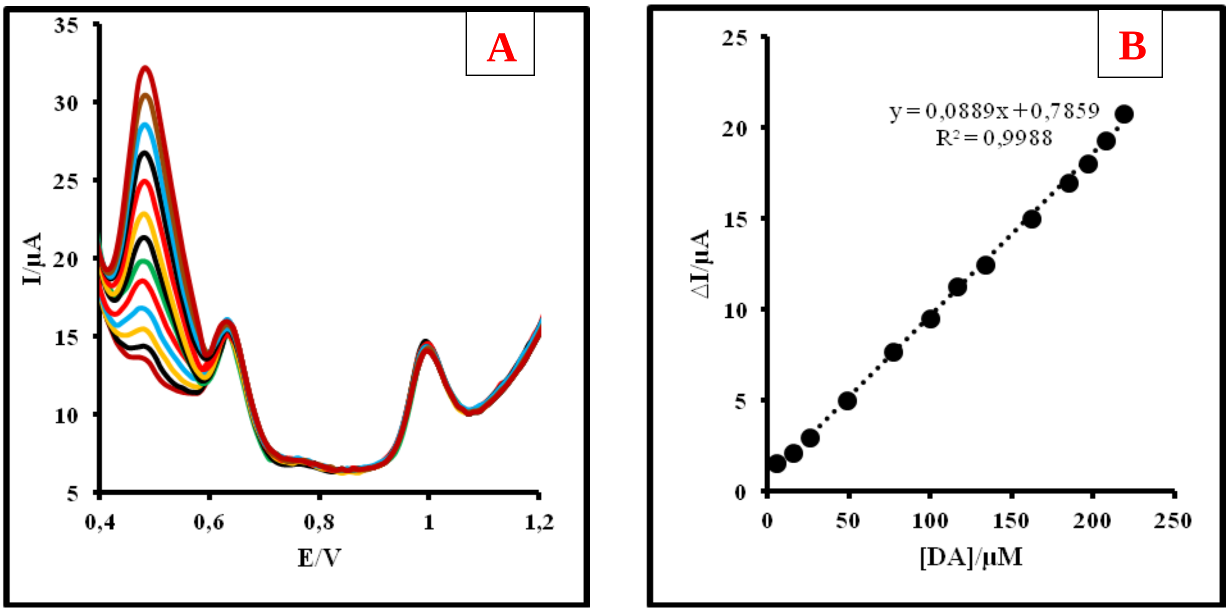


Figure 10. (A) DPVs at the modified GPE in 0.1 M (pH =3.0) containing AC (20µM), and XN (10µM) and different concentrations of DA in the range of 5-220 µM (from inner to outer). (B) plots of anodic peak current vs concentration of DA.

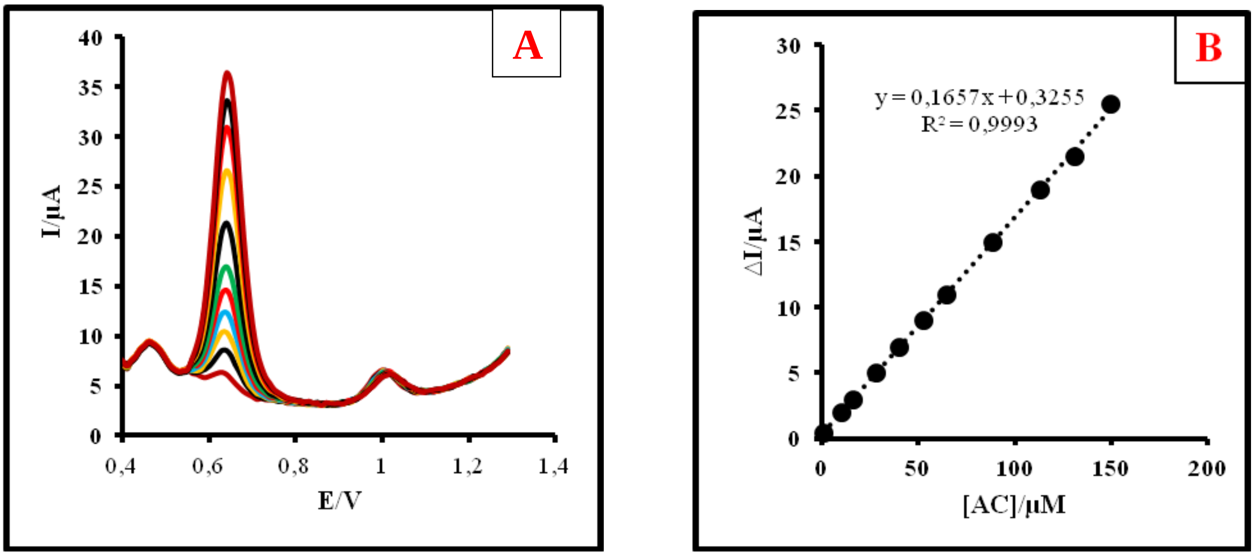


Figure 11. (A) DPVs at the modified GPE in 0.1 M (pH=3.0) containing DA (20µM) and XN (5µM) and different concentrations of AC in the range of 1-150 (from inner to outer). (B) plots of anodic peak current vs concentration of AC.

3.7. Real sample analysis

To evaluate the practical applicability of the proposed modified electrode, the GPE/Sr-Phen 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 2, acceptable recovery values were obtained, pointing to the applicability of this modified electrode for trace amounts of these compounds in the real sample analysis.

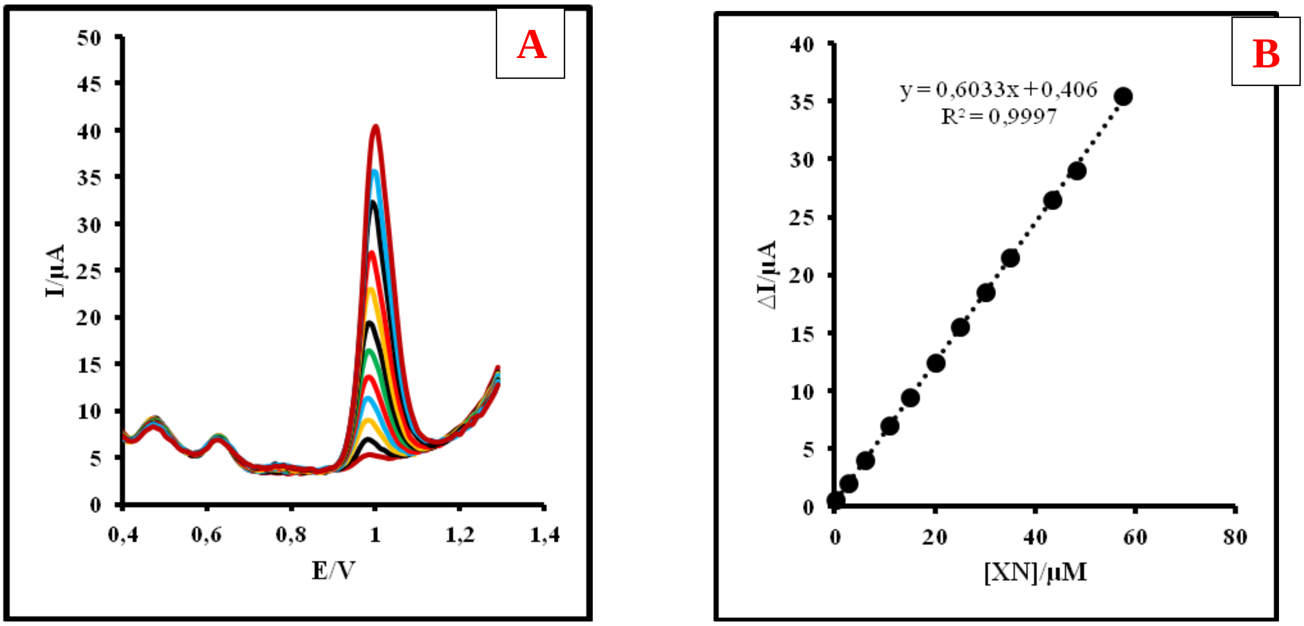


Figure 12. (A) DPVs at the modified GPE in 0.1 M (pH =3.0) containing DA (15µM), AC (10µM),) and different concentrations of XN in the range of 0.2-60 (from inner to outer). (B) plots of anodic peak current vs concentration of XN.

Table 2. simultaneous determination of DA, AC and XN in human urine and serum samples (n = 5)

Sample	Analyte	Detected (µM)	Spiked (µM)	Found (µM)	Recovery (%)	RSD (%)
Urine	DA	_	130	126.1	97	1.3
	AC	_	85	83.3	98	1.5
	XN	_	28	28.84	103	1.2
Serum	DA	_	150	151.5	101	1.6
	AC	_	70	66.5	95	2.0
	XN	_	36	34.92	97	1.3

4. CONCLUSIONS

In this work, GPE/Sr-Phen was applied for the simultaneous determination of a ternary mixture of DA, AC, and XN. The Sr-Phen improves the electrochemical catalytic activities towards the oxidation of DA, AC, and XN in comparison of BGPE and GPE/Phen. The calibration curve were liner up to 220, 150 and 60 µM with a detection limits 800, 90 and 60 nM for DA, AC and XN, respectively. Also**,** based on the Table 2, the GPE/Sr-Phen seems to provide a favorable alternative for the individual and simultaneous determination of AP, UA and Trp with better or satisfactory results than those described in the literature. In addition, this proposed method can be applied to the determination of trace amounts of DA, AC, and XN in real samples with satisfactory results.

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