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Simultaneous Determination of Pyridine-2-aldoxime Methochloride and Dopamine at Poly(3,4-ethylenedioxythiophene) Modified Glassy Carbon Electrode

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Abstract- A simple and sensitive voltammetric method was developed for simultaneous determination of pyridine-2-aldoxime (PAM-2) and the neurotransmitter dopamine (DA) at poly(3,4-ethylenedioxythiophene) (PEDOT) modified glassy carbon electrode. The electrochemical behavior of DA and PAM-2 were investigated using cyclic voltammetry. PAM-2 was irreversibly oxidized at 650 mV while DA was reversibly oxidized and reduced at about 185 mV and 147 mV, respectively. The observed significant oxidation peak potential difference between PAM-2 and DA allowed the simultaneous determination of both species using square wave adsorptive stripping voltammetry. Under optimized conditions, the voltammetric responses gave linear ranges of 3.0×10^{-6}–1.5×10^{-4} M and 1.0×10^{-7}–1.0×10^{-4} M with detection limits of 1.9×10^{-7} M and 3.1×10^{-8} M for PAM-2 and DA, respectively. The determination of PAM-2 and DA in human blood serum samples was successfully carried out with a very good recovery result 95.17% and 101.6%, respectively.

Keywords- Pyridne-2-alidoximemethochiloride, Dopamine, Adsorption stripping voltammetry, Poly(3,4-ethylenedioxythiophene)

1. INTRODUCTION

Pyridine-2-aldoxime methyl chloride (PAM-2) belongs to a family of oximes formed from the reaction of picolinaldehyde with hydroxylamine while dopamine (DA) is a catechol structure with one amine group attached *via* an ethylene-chine. Acetylcholine

(ACh) is an ester of acetic acid and choline. Both ACh and DA are neurotransmitter in our body. Acetylcholine esterase (AChE) enzyme is used to hydrolyze ACh into acetic acid and choline in order to have proper functioning of the nervous system [1].

Deactivation of the AChE enzyme due to the exposure of nerve agents or organophosphate pesticides affects the level of acetylcholine molecule in the nervous system. A major repercussion of this abnormal level of ACh is that it can cause imbalance between ACh and dopamine (DA). Failure of ACh and DA balance results in improper functioning of the nervous system such as Parkinson's diseases [2]. One way to balance ACh and DA is to use a drug such as PAM-2 which reactivates AChE enzyme whose active sites are blocked by organophosphate pesticide or other toxic substances. But, PAM-2 can be rapidly excreted in the urine as is or as a metabolite produced by the liver [3]. Hence, a simple and sensitive method is needed to simultaneously detect PAM-2 and DA in clinical samples.

Several methods such as high performance liquid chromatography (HPLC) [4-5], capillary electrophoresis [6-7], and spectroscopic techniques [8-9] have been used to determine PAM-2 and DA. However, chromatographic technique needs rigorous sample preparation and costly instrument, capillary electrophoresis requires skilled personnel, and spectroscopic detection needs derivatization to determine the analyte of interest.

To address these problems, electroanalytical method has been used because of its simplicity and sensitivity. Hence, several modified electrodes such as the poly(p-toluene sulfonic acid) modified GCE [10], multi-walled carbon nanotube modified platinum [11] iron(III) doped zeolite modified GCE [12] and multi-walled carbon nanotube-modified gold electrode (MWCNT/Au) [13] have been employed for the determination of PAM-2. Similarly, many modified electrodes have been studied for the determination of DA such as β-Cyclodextrin modified GCE [14], iron oxide/reduced grapheme oxide modified GCE [15], Azure A-interlinked multi-walled carbon nanotube/gold nanoparticles composite modified electrode [16], Nafion/carbon nanotubes coated poly(3-methylthiophene) modified electrode [17], SnO_2/chitosan (CHIT) nanocomposite modified glassy carbon electrode [18], A composite comprising reduced graphene oxide (RGO) and titanium nitride (TiN) glassy carbon electrode (RGO–TiN/GCE) [19], N,N-bis(indole-3-carboxaldimine)-1,2-diaminocyclohexane deposited glassy carbon electrode(ICDACH/GCE) [20], poly-4-amino-6-hydroxy-2-mercaptopyrimidine film modified glassy carbon electrode (Poly-AHMP/GCE) [21] and carbon-supported Ni nanoparticles (Ni/C) [22].

Hence, this work reports the potential application of the most widely used polymer, poly (3,4-ethylenedioxythiophene), owing to its stability, simple electropolymerization, and high conductivity [23]. However, to the best of our knowledge, PEDOT modified electrode has not been used for the simultaneous determination of DA and PAM-2. Hence, we report the application of PEDOT modified electrode for the determination of DA and PAM-2 in blood serum samples under optimized conditions.

2. EXPERIMENTAL PART

2.1. Reagents and apparatus

PAM-2 (Sigma–Aldrich, UK), DA (Sigma, Germany), tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (Sigma–Aldrich, Germany), acetonitrile (Scharlau Chemie, Spain), dipotasium hydrogen phosphate (Techno Pharmchem, India), potassium dihydrogenphosphate (BDH, England), hydrochloric acid (Riedel-deHaen, Germany), sodium hydroxide (BDH, England), uric acid and ascorbic acid (Sigma–Aldrich, Germany) were used as received without any purification. 3,4-Ethylenedioxythiophene (EDOT) was purified by repeated distillation until a colorless liquid was obtained and then kept in the dark. PAM-2 and DA solutions were prepared in phosphate buffer solution (PBS) by mixing 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4. Basi Epsilon-Electrochemical Worksation instrument with a conventional three-electrode system was employed for the electroanalytical measurements. The constituents of the electrochemical cell were Ag/AgCl/KCl(satd.) as reference, platinum wire as counter, and PEDOT modified GCE as working electrode. The pH of the buffer solutions was adjusted with a Jenway model 3510 pH meter.

2.2. Preparation of the modified electrode

The modification of the glassy electrode was carried out as reported in our previous paper [24]. Briefly, 0.01 M EDOT monomer was electropolymerized by running successive cycles between -0.20 V and 1.30 V versus Ag/AgCl/KCl(satd.) at a scan rate of 50 mVs^{-1}. The electropolymerization was performed in a non-aqueous solution containing 0.1 M Bu_4NBF_4 dissolved in acetonitrile. Finally, stabilization of the modified electrode was done by running cyclic voltammetry in PBS (pH 7.0).

2.3. Blood serum sample preparation and recovery test

The human blood serum samples were collected from Ethio-Tebib hospital and transferred to a serum separator vacuum tube containing a gel material. Then the sample was kept at room temperature until it was clotted. After the samples were centrifuged at 5000 rpm for 5 min, the serum part of the sample was taken and diluted ten times with 0.1 M PBS (pH 7.0). A recovery test was done by spiking a solution containing 60, 120 and 180 µL of 5.0 mM PAM-2 and 20, 40 and 60 µL of 5.0 mM DA into 25 mL serum sample. This spiking procedure was repeated for 25 mL of 0.1 M PBS as a control.

2.4. Electrochemical measurements

Square wave anodic stripping measurements were carried out for both PAM-2 and DA by sweeping the potential from -0.20 V to 0.80 V using the square wave parameters of step

potential 5 mV, amplitude 25 mV and frequency 15 Hz. To regenerate the modified electrode, the potential was scanned from -0.20 V to 0.80 V in PBS until stable background current was obtained.

3. RESULTS AND DISCUSSION

The cyclic voltammetry of 0.5 mM PAM-2 and 0.05 mM DA in PBS pH 7.0 at the bare and PEDOT modified GCE were shown in Figure 1. Only a single oxidation peak potential was observed for 0.5 mM PAM-2 both at the bare and PEDOT modified glassy carbon electrode. The peak potentials for PAM-2 at the bare and modified electrodes appeared at 710 mV and 650 mV, respectively. The 60 mV shift of peak potential to the negative direction and 9 times increment of peak current of PAM-2 at the modified electrode indicate the electrocatalytic behavior of the PEDOT modified electrode. This behavior of the modified electrode is the result of an increase in surface-active area as reported in our previous papers [24,25].

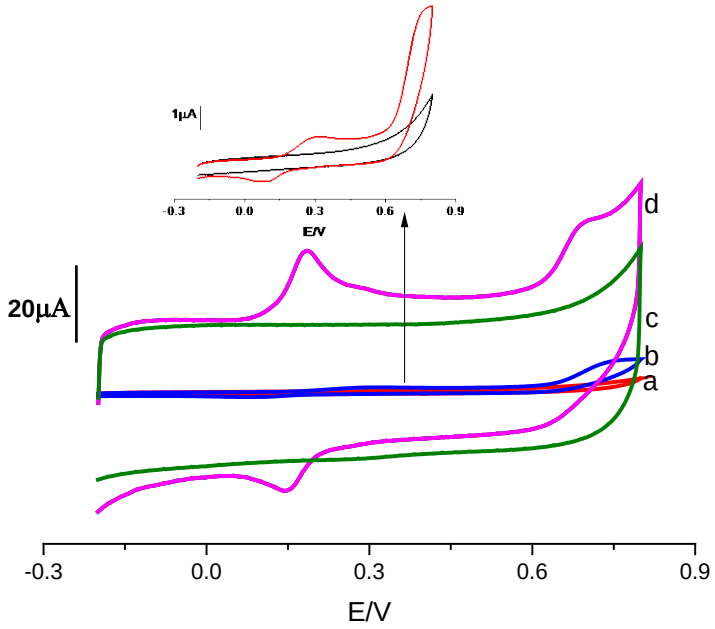


Fig. 1. CV responses at the bare GC (a) and PEDOT modified GCE (c) in PBS without analyte and CVs of the bare (b) and PEDOT-modified GCE (d) in a mixture of DA (0.05 mM) and PAM-2 (0.5 mM) at a scan rate of 50 mV/s

The oxidation and reduction peak potentials of DA at bare electrode appeared at 316 mV and 74 mV, respectively, while that of the modified electrode was observed at about 185 mV and 147 mV. The peak potential difference (ΔE_p) for the bare electrode nearly equals to 240 mV (see inset in Figure 1) and 38 mV for that of the modified electrode (see in Figure 1d). Hence, DA exhibits quasi-reversible reaction at the bare electrode and a reversible behavior

at the modified electrode. Moreover, ΔE_p shifts with scan rate for the unmodified electrode while no shift was observed in the case of the modified electrode. This further confirms that the electrochemical reaction of DA is quasi-reversible at the unmodified electrode and reversible at the modified electrode [26].

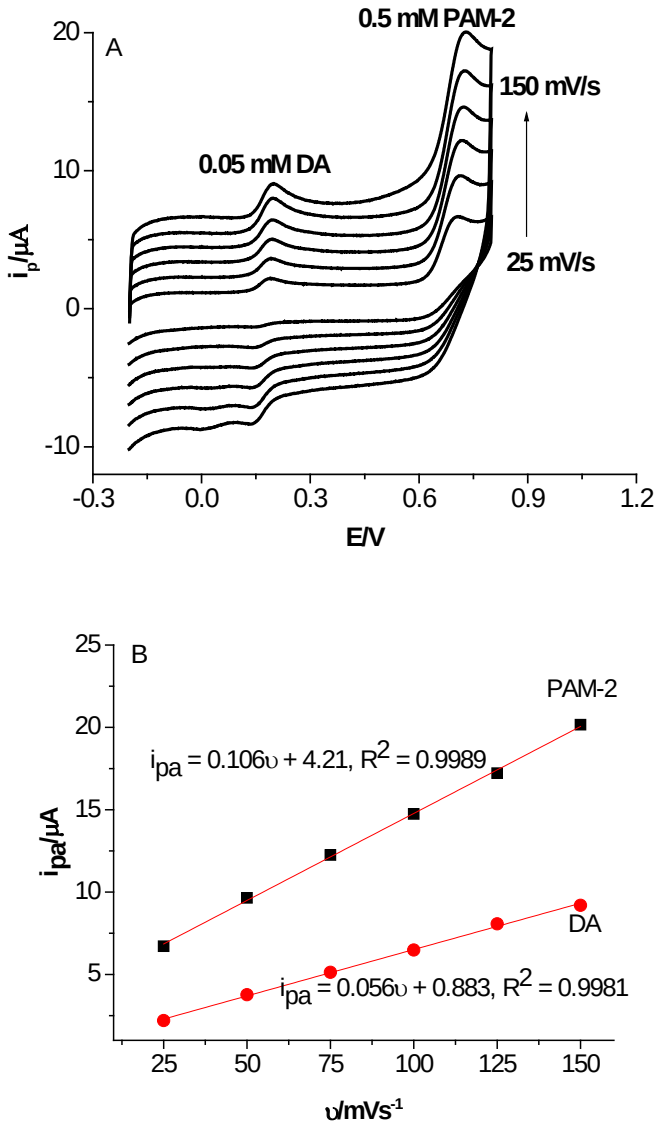


Fig. 2. A) CVs of the PEDOT modified GCE for DA (0.05 mM) and PAM-2(0.5 mM) in 0.1 M PBS (pH 7.0) at scan rates from 25 to 150 mV/s, and B) The plots of peak currents of *versus* scan rates for both DA and PAM-2

The oxidation peak potentials of PAM-2 and DA at the PEDOT modified electrode appeared at 650 mV and 185 as shown in Figure 1d, respectively. There is about 465 mV potential difference between their peak potentials. Hence, simultaneous determination of

PAM-2 and DA can be carried out in the same solution without any interference from one another.

3.1. Effect of Scan Rate

In order to investigate the electrochemical behaviors of the analytes at the modified electrode, cyclic voltammograms were recorded at different scan rates as shown in Figure 2A. The peak currents of 0.5 mM PAM-2 and 0.05 mM DA *versus* scan rates were linear with the expressions of i_{pa}=0.106υ+4.21 (R^2=0.9989) for PAM-2 and i_{pa}=0.056υ+0.883 (R^2=0.9981) for DA (Figure 2B). In both cases, good linearity was observed indicating a surface controlled electrochemical reactions of PAM-2 and DA at the modified electrode [26].

3.2. Effect of pH

The cyclic voltammetric responses for PAM-2 (0.5 µM) and DA (0.5 mM) at the PEDOT-modified GCE were studied in the pH range 5.5–8.0. Figure 3 shows that the anodic peak current increased with increasing pH until 7.0 and then decreased afterwards. The analytes are present in protonated forms below pH 7.0 [27], which could result in electrostatic repulsion with oxidized PEDOT modified electrode. When the solution is greater than pH 7.0, the competitive effect of hydroxide ion over the analytes causes less sensitivity to the modified electrode. Hence, pH 7.0 was taken as an optimum pH for further experiments.

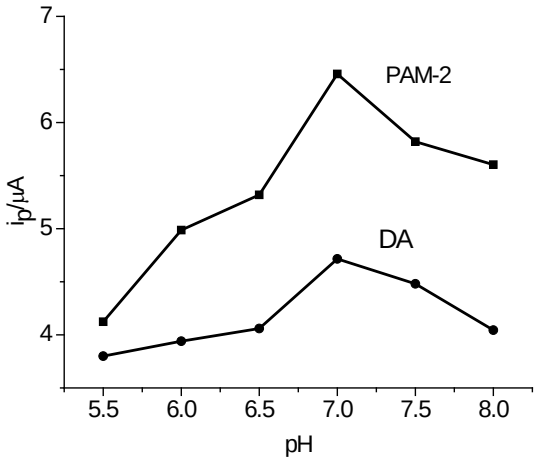
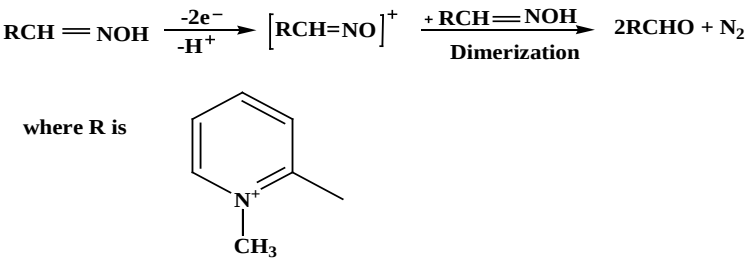


Fig. 3. The plot of peak current versus pH for DA and PAM-2 at scan rate of 50 mV s^{-1}

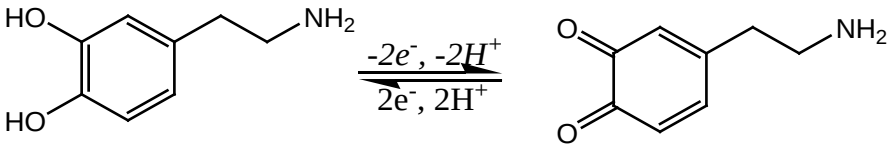
The change in the oxidation peak potential PAM-2 and DA with pH is shown in Figure 4 where the peak potentials for both PAM-2 and DA shifted negatively as the pH was increased. The plot of peak potential of PAM-2 *versus* pH (from 5.5 to 8.0) is depicted in Figure 4A. The peak potentials varies linearly with pH (E_p=0.895-0.032pH; R^2=0.9948). The slope 32.4 mV per unit pH indicates that the electrochemical reaction involves unequal

electrons and protons. The result is consistent with the literature report [12] and the reaction of PAM-2 at the modified electrode proceeds as shown in scheme 1:



Scheme 1. Mechanism of PAM-2 oxidation at the PEDOT modified GCE[5]

The variation of peak potential *versus* pH for DA gave a linear equation of E_{pa}= 0.558−0.0502 pH with a correlation coefficient 0.9993 (see Figure 4B). The slope of –50.2 mV/pH is close to the theoretical value of –59 mV/pH, suggesting that the electron transfer step is being preceded by a protonation step with equal number of protons and electrons. This is in agreement with the proposed mechanism reported in the literature [28] as shown in scheme 2:



Scheme 2. Mechanism of DA oxidation at the PEDOT modified GCE

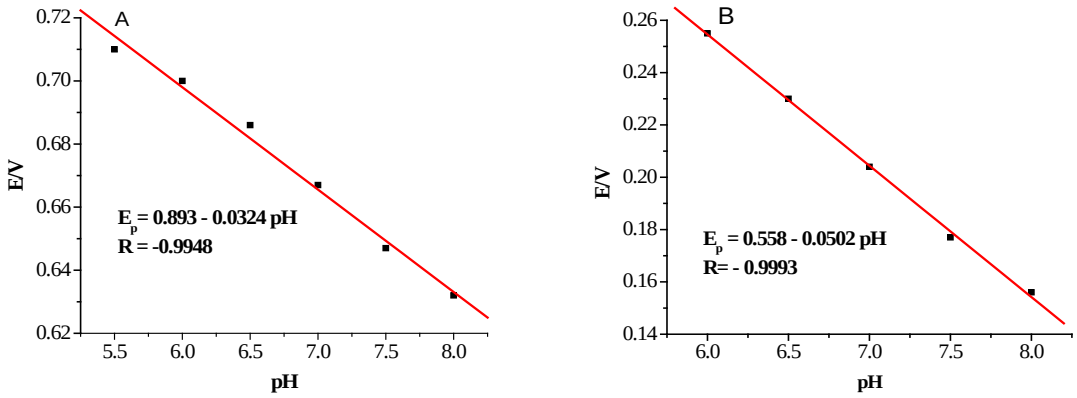


Fig. 4. Plot of peak potential versus pH for (A) PAM-2 and (B) DA

3.3. Effect of deposition time and accumulation potential

Figure 5 shows the voltammetric responses of 0.05 mM DA and 0.5 mM PAM-2 at the PEDOT-modified GCE for the deposition time range from 30 s to 210 s and accumulation potential range from -50 mV to -700 mV. The peak current increased with deposition time up to 150 s and then after no significant change in the peak current was observed (see the blue line of Figure 5). Hence, the deposition time of 150 s was used for subsequent experiments. Similarly, the oxidation peak current varied with accumulation potential and reached maximum at a potential of 0.55 mV which was selected as an optimum potential for subsequent experiments (see the red line of Figure 5).

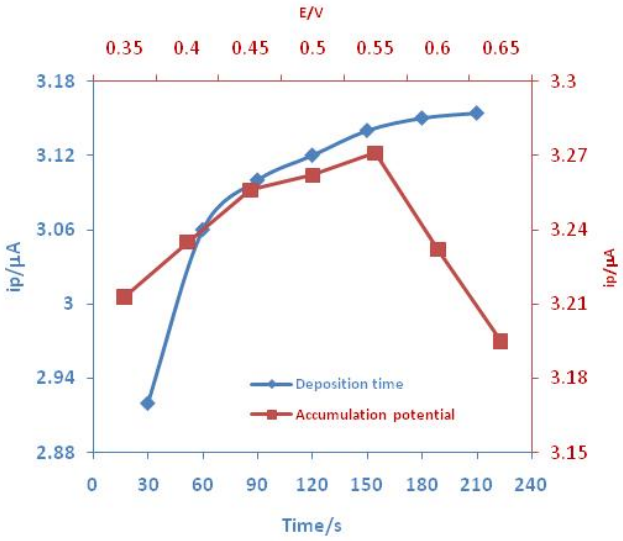


Fig. 5. The optimization of anodic peak currents as function of deposition time and accumulation potential

3.4. Analytical performances

Typical calibration plots for the anodic stripping peak currents of different concentrations of DA with fixed concentration of PAM-2 and different concentrations of PAM-2 with fixed concentration of DA are shown in Figure 6 (A and B), respectively. The anodic peak currents of DA were linearly related to its concentration (1.0–100 µM) in the presence of a fixed amount of 50 µM PAM-2 (inset Figure 6A). The linear regression equation obtained was i_{pa}=0.271+0.105c, with a correlation coefficient (R^2) of 0.9879.

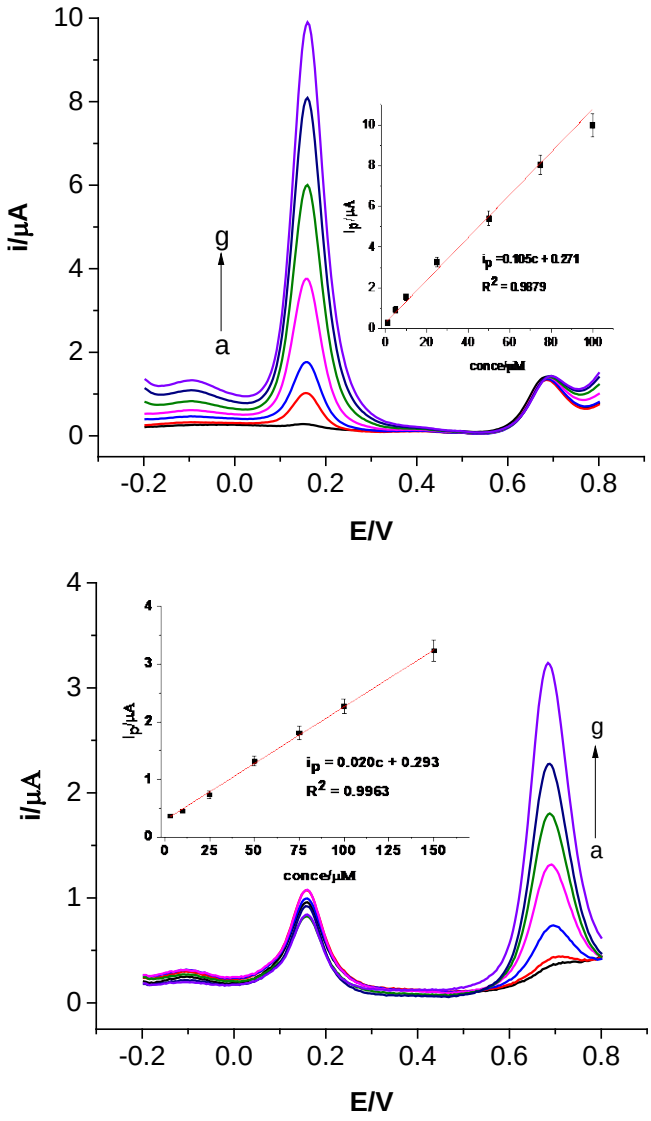


Fig. 6. (A) Anodic stripping voltammetry peaks at the PEDOT modified GCE in the presence of PAM-2 (50 μM) for different concentrations of DA: (a) 1, (b) 5, (c) 10, (d) 25, (e) 50, (f) 75 and (g) 100 μM. (B) The peak currents of the modified electrode in the presence of DA (5.0 μM) for different concentrations of PAM-2 (from a to g): (a) 3, (b) 10, (c) 25, (d) 50, (e) 75, (f) 100 and (g) 150 μM. (Background subtracted and the inset (A) and (B) are calibration graphs of anodic peak currents versus concentrations of DA and PAM-2, respectively)

Similarly, the anodic peak currents of PAM-2 varied linearly with its concentration (3.0–150 μM) in the presence of 5.0 μM DA (inset of Figure 6B). The linear regression equation was found to be i_{pa}=0.293+0.020c), with a correlation coefficient (R^2) of 0.9963. The relative standard deviation (RSD) of five successive determinations of 50 μM DA and 75 μM PAM-2 at the modified electrode were found to be 1.56% and 0.12%, respectively. Moreover, the RSDs for the detection of the analytes using three individual PEDOT-modified GCEs were

found to be 2.69% for 50 µM DA and 0.45% for 75 µM PAM-2. Hence, the results indicate that the PEDOT modified electrodes exhibit a very good repeatability and reproducibility.

The analytical performance of the modified GCE for the determination of PAM-2 and DA was compared with similar reports in the literature (Table 1). The detection limit of the modified electrode for PAM-2 was found to be 1.91×10^{-7} M based on signal to noise ratio of 3. The obtained detection limit is nearly the same as the carbon nanotube modified GCE (3.0×10^{-7} M) [11] and zeolite modified electrode (1.61×10^{-7} M) [12].

The modified electrode shows better detection limit (3.0×10^{-8} M) compared to most of the modified electrodes except for the Nafion/carbon nanotubes coated poly(3-methylthiophene) modified electrode [17] and RGO–TiN nanocomposite/GCE [19]. Since this modified electrode contains a ternary system with narrow linear range, the PEDOT/GCE is better in terms of simplicity of the preparation of the electrode and cost of materials as well as a wider linear range (Table 1).

Table 1. Analytical performance of the modified electrode for PAM-2 and DA determination in comparison with similar works in literature

Analyte	Modified electrode	Linear range(µM)	Detection limit (µM)	Ref.
PAM-2	Fe^{3+}Y/GCE	0.5–100	0.16	[12]
	p-TSA/GCE	0.1–1000	0.03	[10]
	CNT/GCE	1-1000	0.30	[11]
	MWCNT/Au	0.8 - 10	0.1	[13]
	PEDOT/GCE	5–150	0.19	This work
DA	β-cyclodextrin/GCE	10–170	1.5	[14]
	Fe_3O_4/rGO/GCE	0.5 – 100	0.12	[15]
	Nafion/NTs/P3MT	1–6	0.005	[17]
	SnO_2/CHIT nanocomposite/GCE	1 - 18	0.89	[18]
	RGO–TiN nanocomposite/GCE	0.1 to 80	0.01	[19]
	ICDACH/GCE	11.8- 90	0.19	[20]
	Poly-AHMP/GCE	2.5 - 25	0.20	[21]
	Ni/C	1- 55	0.05	[22]
	PEDOT/GCE	0.5–100	0.03	This work

3.5. Interference study

Potential interfering substances that could exist in blood serum samples such as ascorbic acid, uric acid and glucose were tested by running anodic stripping square wave voltammetry. Ascorbic acid and glucose did not show any response in the potential window, indicating that the modified electrode can be used without any interference from ascorbic acid and glucose. On the other hand, uric acid (UA) gave a distinct oxidation peak at 290 mV which do not overlap with the peak potentials of both PAM-2 and DA (Figure 7). Thus, there is no interference of UA for the determination PAM-2 and DA at the PEDOT modified electrode.

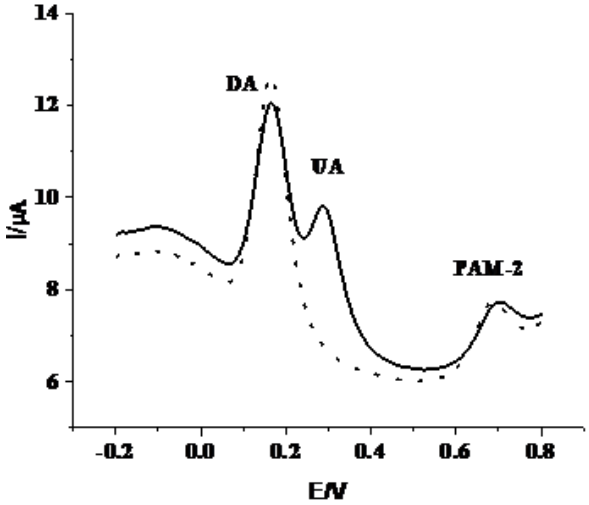


Fig. 7. Voltammogram of the mixture solution of containing 60 μM of DA and 60 μM of PAM-2 without UA (broken line) and with UA (solid line)

3.6. Analytical application

To further validate the use of PEDOT modified electrode, recovery tests were performed by using multiple standard additions. The electrochemical responses of the analytes at the modified electrode in the serum sample and 0.1 M PBS were shown in Figure 8 (A and B), respectively. The sensitivities of the plots of the anodic stripping peak current of DA *versus* the volume of the DA added were found to be 17.01 μA/mL in serum sample (inset Figure 8A) and 17.11 μA/mL in PBS (inset Figure 8B). Similarly, the sensitivities of the plots for the anodic stripping peak current of PAM-2 *versus* the volume of PAM-2 added were found to be 1.608 μA/mL and 1.573 μA/mL in serum and PBS samples, respectively.

The sensitivities for both PAM-2 and DA in the PBS buffer and the serum sample are nearly the same, suggesting that the matrix effect serum samples are negligible. Moreover, the recovery found from the plots for PAM-2 and DA was 95.2% and 101.6%, respectively, showing that the values are within the acceptable recovery ranges.

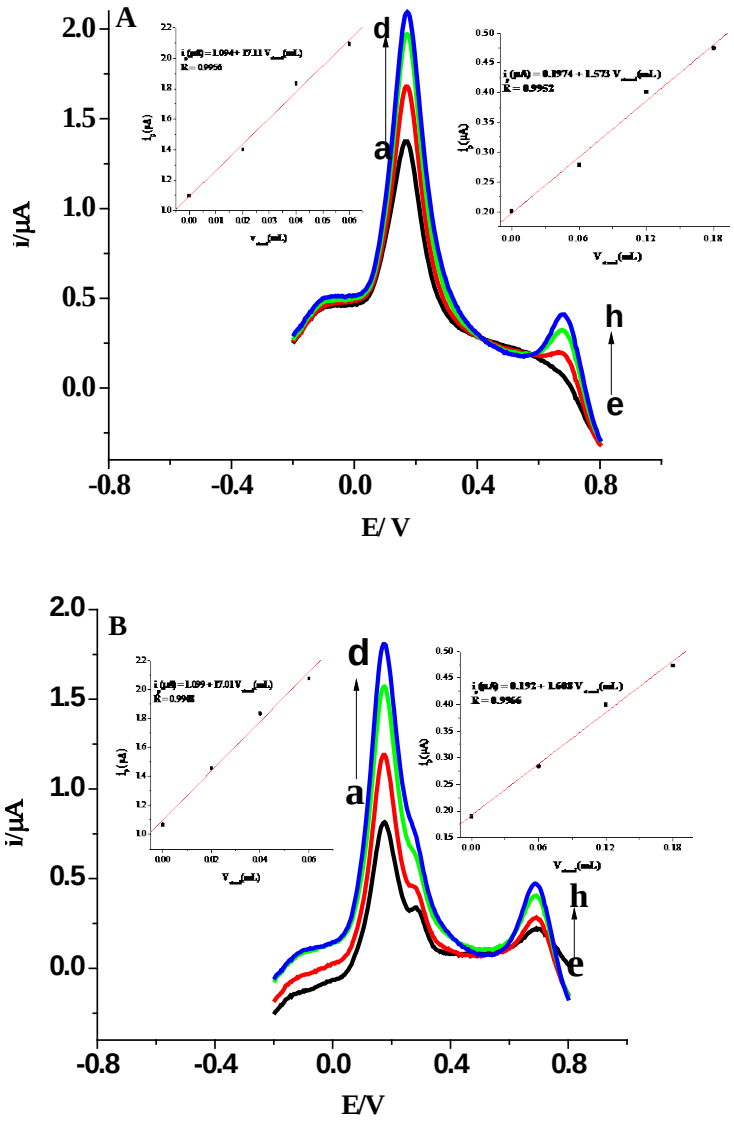


Fig. 8. Anodic stripping voltammetry responses for: 8 μM DA and 10 μM PAM-2 in 25 mL PBS (A) and human blood serum sample (B) and each sample was spiked with20, 40, 60 μL of 5.0 mM DA, and 60 μL, 120 μL, 180 μL of 5.0 mM PAM-2. (The insets are the corresponding plots of anodic peak current of DA and PAM-2 versus the volume of samples)

4. CONCLUSION

The present study demonstrated the simultaneous determination of pyridine-2-aldoxime methochloride and dopamine using PEDOT modified electrode. The modified electrode is tested for the determination of the analytes in blood serum sample without the interference coexisting species. The study has showed high sensitivity and selectivity for the detection of PAM-2 and DA. Furthermore, the stability and high conductivity of the PEDOT-modified electrode make it attractive for the development of a simple and robust electrochemical device.

Acknowledgements

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