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Full Paper

Poly(6-phenyl-1,3,5-triazine-2,4-diamine) Modified Carbon Paste Electrode for Uric Acid in Presence of Dopamine: A Voltammetric Study

K. Chetankumar, and B. E. Kumara Swamy*

Department of P.G Studies and Research in Industrial Chemistry, Kuvempu University, Jnanasahyadri, Shankaraghatta-577451, Shivamogga (D), Karnataka, India

*Corresponding Author, Tel.: +91-8282-256225

E-Mail: bek@kuvempu.ac.in

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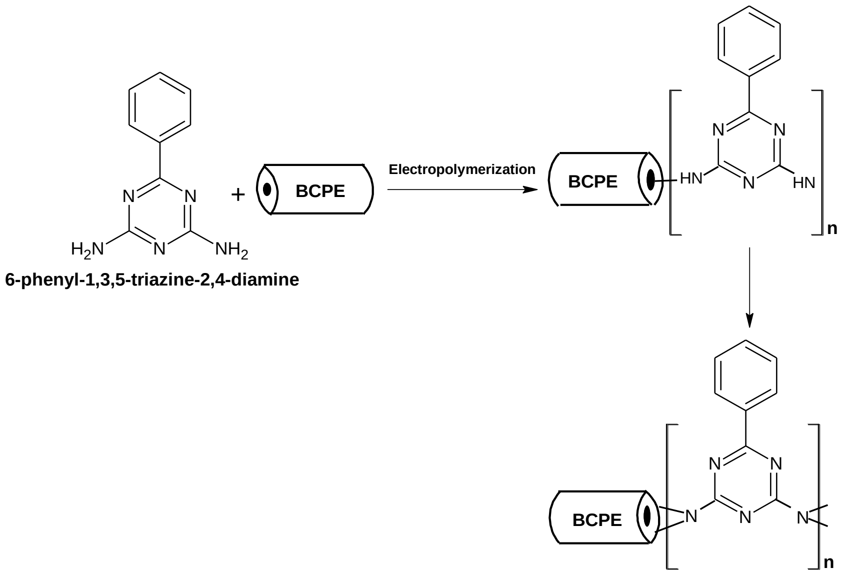
Abstract- The electrode was fabricated by electropolymerization of 6-phenyl-1,3,5-triazine-2,4-diamine as a thin film on the surface of unmodified carbon paste electrode (UCPE) by using cyclic voltammetry. Superficial morphology of UCPE and improved electrode was traced by scanning electron microscopy (SEM). The constructed improved electrode shows admirable electrocatalytic movement for the analysis of dopamine (DA) and uric acid (UA) in 0.2 M phosphate buffer (PBS of pH7.4), which is attributed to increased active surface area. The fabricated electrode process was found to be diffusion controlled and it shows linear range from 10 to 90 µM for DA and 10 to 70 µM for UA and lower limit of detection (LOD) for DA and UA are established at 0.25 and 0.37 µM separately. The fabricated electrode was applied in analytical application in dopamine injection samples.

Keywords- 6-phenyl-1,3,5-triazine-2,4-diamine; Cyclic voltammetry; Electrocatalytic activity; Dopamine; Biosensor

1. INTRODUCTION

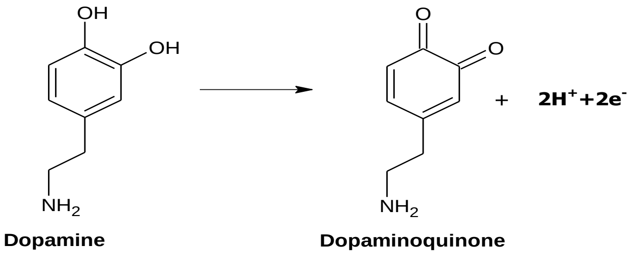
In latest year expansion and construction of electrochemical sensor have accomplished additional attention in the field of electroanalytical chemistry. On the other hand, analytical systems that can afford selective, sensitive, simple and cost-effective revealing of DA are exceedingly desired [1]. Generally, electrochemical approaches can recommend all these benefits. The neurotransmitters are the utmost proper classes for electrochemical detections because their response potentials usually occur in the potential windows of carbon and metal electrodes in physiological buffers [2,3]. The selection category of working electrode material plays a substantial influence to the practical consequence of the established sensor. However, the carbon electrodes are frequently used for electrochemical analysis of oxidizable compounds because of their less sensitivity, selectivity, and anodic oxidation intersection in the simultaneous resolution of particles in a sample matrix [4-6]. There are numerous sophisticated ways for quantification of DA and UA including UV-visible spectroscopy, fluorimetry, chemiluminescence, capillary electrophoresis, and electrochemical methods. Amongst, the electrochemical analysis is a fascinating routine owing to its ease, sensitivity, speedy reaction, low cost, flexibility and its ecological friendly nature [7-9].

Dopamine (DA) was an important monoamine neurotransmitter and plays critical roles in many biological roles in the bodies of animals. Among the catecholamines DA has fascinated much activity. DA can control our emotions and action-selection, being closely related to drug addiction, such as nicotine and ethanol. DA has fascinated a lot of considerations in modern years due to its indicator of numerous diseases, such as schizophrenia, Parkinson's and Huntington's diseases [10-15]. Thus, DA level determination in body fluid is very imperative significance. UA remained the major product of purine metabolism in the hominoid body. UA was an antioxidant that was existing in biological fluids identical as blood serum and urine. Persons with illnesses like renal failure, gout and leukemia partake a high level of UA in their body liquids. It has been revealed that little quantity of UA level in serum and cerebrospinal fluid was a biomarker of Parkinson's disease. So, the level of UA affords the valuable clinical evidence for human health. Frequently, DA and UA can be oxidized almost at the same potential upon traditional electrodes. Consequently, the expansion of fabricated electrodes for the concurrent resolves of them with sensitivity as well as selectivity was extremely desirable for diagnostic and analytical claims [16-22]. Some polymer films have been used for the quantification of UA and DA, they could distinct the redox peaks of DA and UA such as Sunset yellow, Rhodamine B, Naphthol green B, l-arginine, O-phenylenediamine, Diphenylamine [23-28] as monomers for the reform of CPE by electrochemical polymerization method. 6-phenyl-1,3,5,-triazine-2,4-diamine (benzoguanamine) is an organic compound used in the manufacturing of melamine resins. It was a modifier and an intermediary in the pharmacological industry and utilized as a raw constituent for guanamine resins. It was also applied as a cross-associating agent of alkyd and acrylic resin to growth thermoset assets. Scheme 1 shows the possible electropolymerization of 6-phenyl-1,3,5, -triazine-2,4-diamine on the surface of CPE.

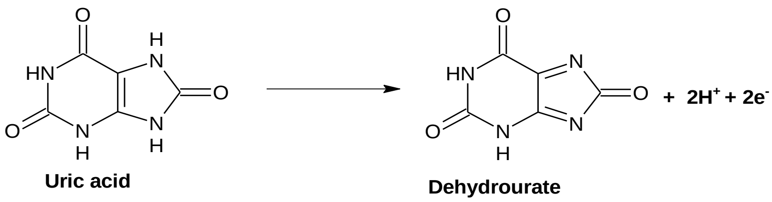


Scheme 1. Graphical electropolymerization illustration of 6-phenyl-1,3,5-triazine-2,4-diamine on the surface CPE

The existing analysis states the modification of UCPE by electropolymerization of 6-phenyl-1,3,5, -triazine-2,4-diamine film using CV technique and used in the analysis of DA and UA using PBS. The redox response of DA and UA displayed in Scheme 2 and 3.



Scheme 2. Oxidation reaction of DA



Scheme 3. Oxidation reaction of UA

2. EXPERIMENTAL UNIT

2.1. Reagents

DA and UA were obtained from Himedia chemicals and used without further purification. Stock solutions of DA was prepared in 0.1 M perchloric acid and UA was prepared in 0.1 M

NaOH. 6-phenyl-1,3,5-triazine-2,4-diamine, Perchloric acid, Sodium hydroxide, Disodium hydrogen phosphate, Sodium dihydrogen orthophosphate were obtained from Sigma Aldrich. Pure graphite powder (GP, 50 µM particle size), silicone oil (SO) from Merck and fluka were used for the preparation of UCPE.

2.2. Instrumentation

Electrochemical experiment was conducted by using a CH Instrument-660 (model CHI-660c) with a conventional three-electrode. Such as, platinum wire as counter electrode, saturated calomel electrode (SCE) as a reference electrode, UCPE and fabricated electrode were used as working electrode. The superficial surface distinctive of UCPE and fabricated electrode was performed by SEM using a ZEISS Ultra-55.

2.3. Fabrication of bare and modified electrodes

The UCPE was prepared via mechanochemical method in the ratio 70:30 (w/w) GP and SO in a mortar ground up to thirty minutes to yield a homogenous mixture and attained paste was packed into homemade Teflon cavity. Copper wire was connected at the end tube for the electrical contact.

Electropolymerization manner was practically applied for the preparation of modified electrode using solution of 6-phenyl-1,3,5-triazine-2,4-diamine (1 mM) on the surface of UCPE using NaOH (0.1 M). Electrochemical polymerization was achieved by repetitive 15 consecutive cycles intermediate in the potential ranges -0.4 to 0.9 V with scan rate 50 mV/s.

3. RESULTS AND DISCUSSION

3.1. Electropolymerization on the surface of UCPE

Electropolymerization of 6-phenyl-1,3,5-triazine-2,4-diamine was carried on the surface of UCPE using CV. Figure 1a shows the CVs obtained for 1 mM of 6-phenyl-1,3,5-triazine-2,4-diamine on UCPE in the existence of 0.1 M NaOH with scan rate 50 mV/s for fifteen multiple cycles and the polymer deposition was accomplished in-between the potential -0.4 V to 0.9 V. During electropolymerization, the CVs gradually increases this indicating poly (6-phenyl-1,3,5-triazine-2,4-diamine) film was positioned on the surface of the UCPE. The polymeric film thickness changes the electrocatalytic property of CPE [29]. Therefore, coating of targeted compound was carried out by altering the number of cycles on UCPE (5 to 25 multiple cycles) and conforming the electrocatalytic activity towards the analysis of DA. Figure 1b confirms the effect of multiple cycles during polymerization on the surface of CPE. In those 15 cycles showed magnificent current enhancement with good sensitivity. Therefore 15 multiple cycles were optimum for further investigations. Afterward, the electropolymerized electrode was rinsed in double distilled water to remove unreacted modifier and used for further electrochemical investigations.

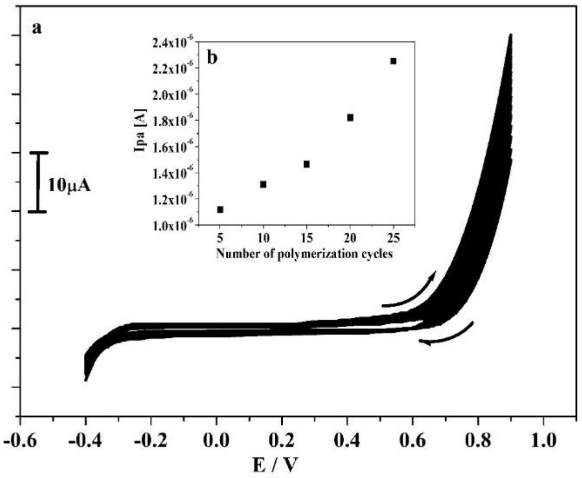


Figure 1. (a) Electropolymerization of 6-phenyl-1,3,5-triazine-2,4-diamine (1.0 mM) on the surface of UCPE in the existence of 0.1 M NaOH (supportive electrolyte) with scan rate 50 mV/s at 15 multiple cycles; (b) Graph of Ipa vs. a number of varied polymerization cycles

3.2. Surface morphology of unmodified and modified electrodes

SEM images of UCPE and fabricated electrode were shown in Figure 2a and 2b. The irregular formed graphite flakes were appeared with less surface area at UCPE [30] and after fabrication the pore size was enlarged and electrode surface of covered with a thin film of 6-phenyl-1,3,5-triazine-2,4-diamine with uniform arrangement [31] and which leads to improves the electroactive surface area and electrocatalytic activity of fabricated electrode [32].

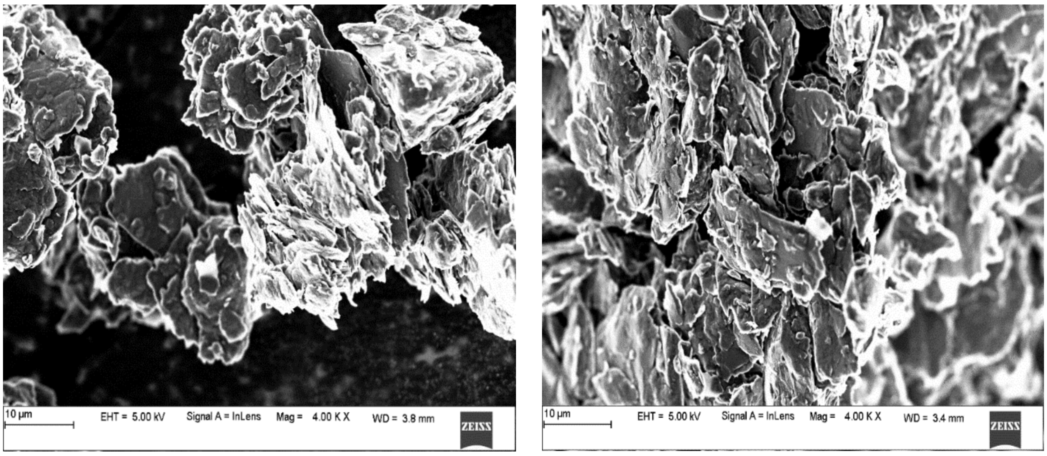


Figure 2. SEM images (a) UCPE; (b) poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE

3.3. Electrochemical study of DA

Cyclic voltammetry was utilized for the analysis of DA at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE. Figure 3 illustrate the CVs obtained for DA (10 µM) in the existence 0.2 M PBS with scan rate of 50 mV/s for both UCPE (dashed line) and MCPE (hard line). At UCPE, DA shows the low current signal with less voltammetric response was observed and difference in peak potential (ΔE_p) was gotten at 0.058 V. However, in the identical condition the fabricated CPE shows magnificent increment in current signals and the ΔE_p value was found at 0.031 V. The modified electrode having less ΔE_p value compared to UCPE, this suggested the fabricated electrode have speeder in electron transmission rate. From the above result, the poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE has a well affinity towards the electrochemical analysis of DA.

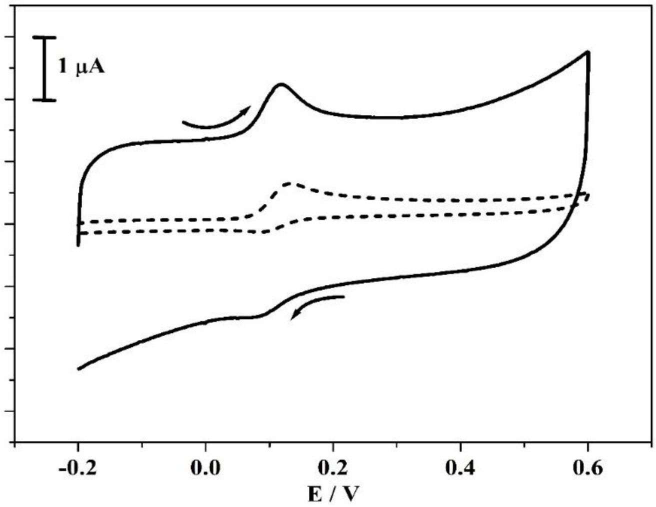


Figure 3. CVs of DA (10 µM) at unmodified CPE (dashed line) and poly(6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE (solid line) with scan rate 50 mV/s using 0.2 M PBS (pH 7.4)

3.4. Impact of scan rate on DA

The effect of sweep rate on fabricated electrode provides beneficial information about the electrode kinetics process. Figure 4a illustartes the effect of applied scan rates on DA (10 µM) in the occurrence of 0.2 M PBS at fabricated electrode. From this obtained data it was determined that the resultant I_{pa} and I_{pc} gradually enhanced with increasing the scan rates (60-160 mV/s) with tiny shifting in their redox peak potential towards $+^{ve}$ and $-^{ve}$ direction respectively. Figure 4b displayed the correlation of I_{pa} *vs.* scan rates (ν), which gives fine linearity with correlation co-efficient value (R^2) was gotten at 0.973. Moreover, the plot of Ipa vs. square root of scan rates ($\nu^{1/2}$) was also examined described in Figure 4c and R^2 value was originated at 0.969. Therefore, the attained result suggested that the designed electrode kinetics was diffusion controlled.

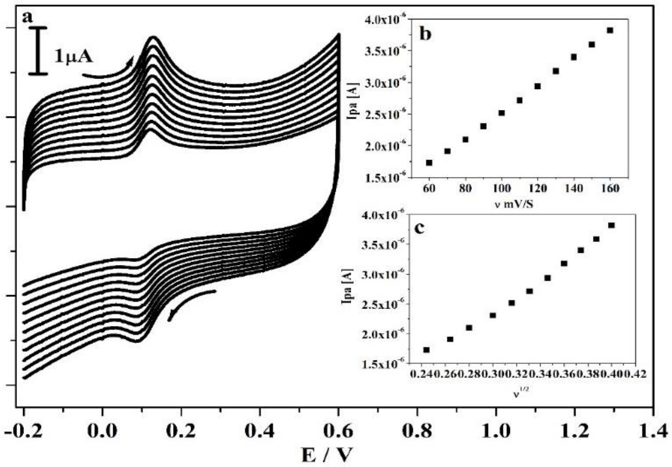


Figure 4. (a) CVs of DA (10 µM) at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE with different scan rates (60-160 mV/s) using 0.2 M PBS (pH 7.4); (b) Graph of Ipa vs. scan rates; (c) Graph of Ipa vs. square root of scan rates

3.5. Impact of DA concentration at fabricated electrode

Figure 5a depicts the increasing concentration of DA from 10-90 µM at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE in the presence of 0.2 M PBS at pH 7.4 with scan rate 50 mV/s. Obtained result was evidence for the as the concentration of DA increases redox peak current (I_{pa} and I_{pc}) also enlarges linearly with a minute shifting in their E_{pa} and E_{pc}. The graph of I_{pa} vs. varied concentrations of DA was plotted as elucidated in Figure 5b and it implies good linearity with R^2=0.989. The obtained LOD and LOQ for DA was 0.25 and 0.83 µM calculated using the formulas LOD = 3S/M and LOQ = 10S/M [33-35]. The gottenl LOD value was compared with formerly reported literature was shown in Table 1.

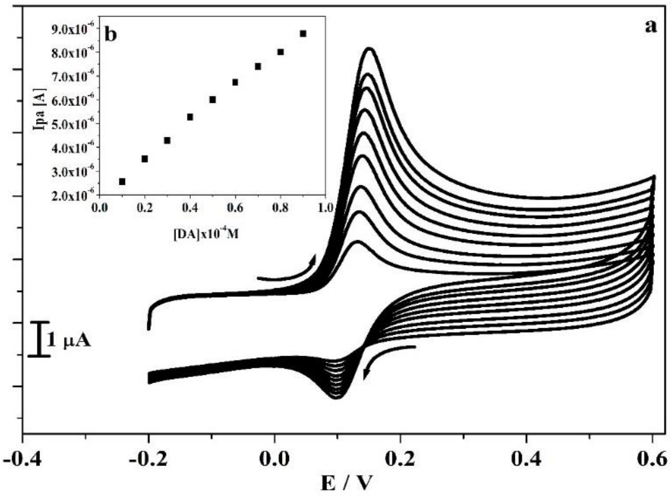


Figure 5. (a) CVs of DA at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE with varied concentrations (10-90 µM) using 0.2 M PBS (pH 7.4) with scan rate 50 mV/s; (b) Graph of I_{pa} *vs.* varied concentration of DA

Table 1. LOD comparison of poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE with other reported electrodes

Modified electrodes	**Detection limit (µM)**		**Reference**
	DA	**UA**	
ERGO/GCE	0.5	0.5	[41]
MWCNT/GCE	1	1	[42]
Au-SiO_2/GCE	1.98	2.58	[43]
CuO nano-rice/GCE	0.42	1.2	[44]
Au-GQDs-Nafion	0.84	___	[45]
PtNCs-MWCNTs-GNPs	0.500	___	[46]
NPG electrode	___	9.0	[47]
SDS/R-GO/MCPE	0.26	0.35	[48]
poly(6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE	0.25	0.37	This work

3.6. Influence of pH

The impact of acidity on the I_p response at fabricated CPE was carefully investigated within a series of 0.2 M PBS solutions using cyclic voltammetry. Figure 6a reveals the CVs verified for 10 µM DA at different pH (6.2 to 7.8) with scan rate of 50 mV/s.

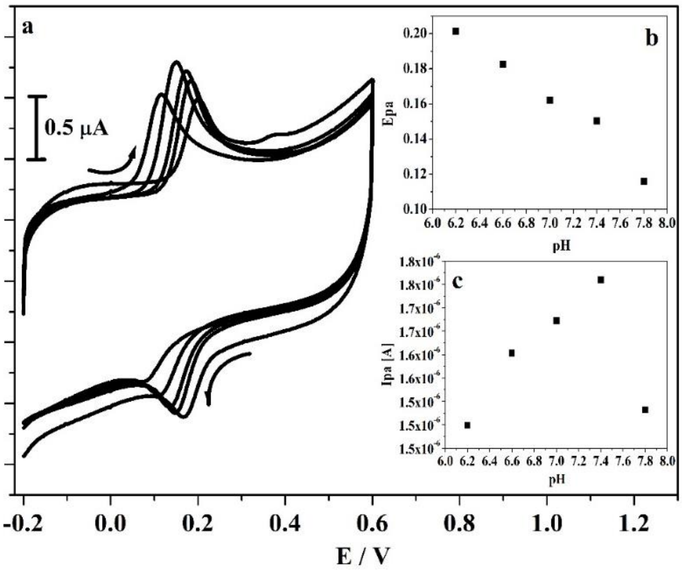


Figure 6. (a) CVs of DA (10 µM) in the presence of different pH (6.2, 6.6, 7.0, 7.4, 7.8); (b) Graph of E_{pa} *vs.* pH. (c) Graph of I_{pa} *vs.* pH

As the pH raised redox peak potential shifted to negative direction, this result indicates the proton was directly partaken in the electrochemical process [36-37]. The graph of E_{pa} *vs.* pH elucidated in Figure 6b and it gives regression equations are $E_p(V)=0.462-0.051$ pH ($R^2=0.985$) and the slope value 0.051 was near to the Nernstian theoretical value. This suggest that equal quantity of electron and proton ($2e^-/2H^+$) takes place in the electrode reaction. Figure 6c implies the plot of I_{pa} *vs.* pH and maximum peak currents were observed at pH 7.4. So, pH 7.4 was optimum for other subsequent measurements.

3.7. Electrochemical oxidation of UA

The electrochemical response of UA on the unmodified CPE and fabricated CPE have been studied by using cyclic voltammetry. Figure 7 shows the CVs verified for 10 µM UA at UCPE (dashed line) and fabricated CPE (hard line) in 0.2 M PBS with the scan rate 50 mV/s. It is noticed that obtained voltammograms at unmodified CPE was confirms the poor electrochemical reaction with less sensitivity. Though, at fabricated electrode observed the well-defined with enhanced in oxidation peak current than the UCPE. So, the designed electrode has upgraded in their electron transfer kinetics. Therefore, fabricated electrode act as good electrochemical sensor for examine of UA. Oxidation mechanism for UA was shown in Scheme 3 [38,39].

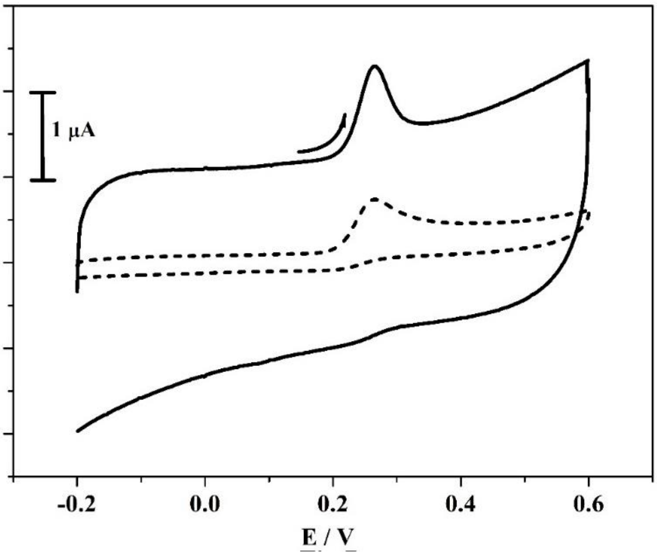


Figure 7. CVs of UA (10 µM) at unmodified CPE (dashed line) and poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE (solid line) with scan rate 50 mV/s using 0.2 M PBS (pH 7.4)

3.8. Impact of scan rate on UA at fabricated electrode

The effect of scan rates studies was examined to investigate the mechanism of electrode process at fabricated CPE. Figure 8a depicts the acquired CVs for 10 µM UA in the occurrence of 0.2 M PBS. As the scan rate increases (60-160 mV/s) the oxidation I_p of UA enlarged linearly. Figure 8b demonstrates the connection between I_{pa} *vs.* scan rate and Figure 8c portrays the graph of I_{pa} *vs.* $\nu^{1/2}$ was plotted in the range 60-160 mV/s, the resultant graphs provide fine linearity with R^2=0.971 and 0.983. Therefore, fabricated CPE clearly suggested that the electrode process was diffusion controlled for UA.

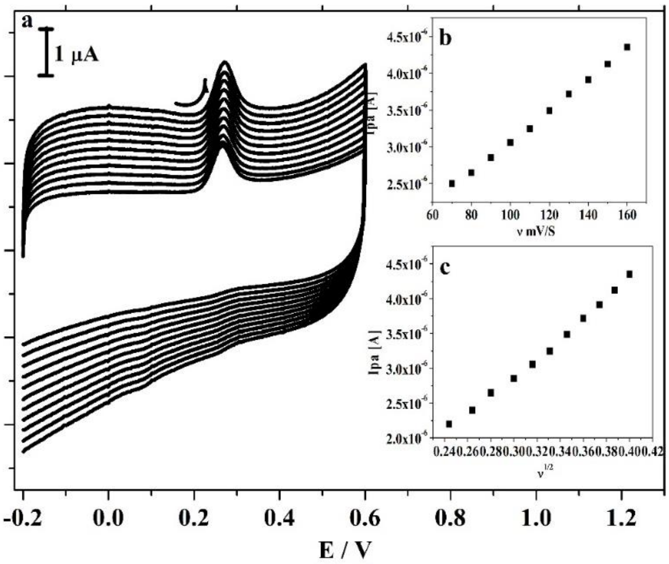


Figure 8. (a) CVs of UA (10 µM at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE with different scan rates (60-160 mV/s) using 0.2 M PBS (pH 7.4); (b) Graph of I_{pa} *vs.* scan rates; (c) Graph of I_{pa} *vs.* square root of scan rates

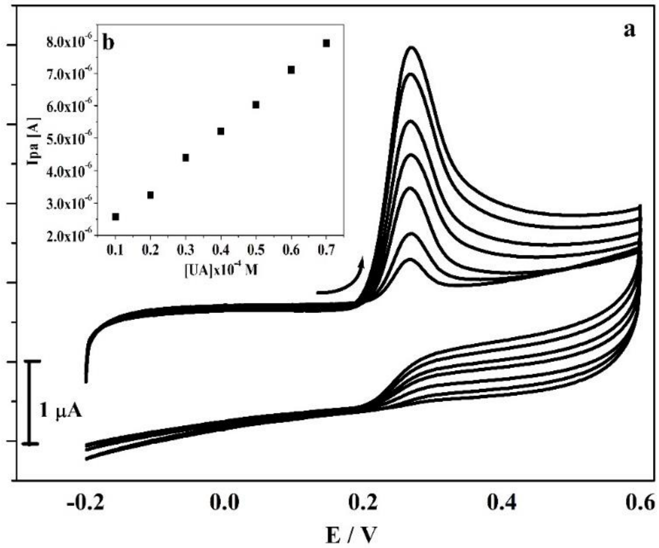


Figure 9. (a) CVs of UA at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE with varied concentrations (10-70 µM) using 0.2 M PBS (pH 7.4) with scan rate 50 mV/s; (b) Graph of I_{pa} *vs.* varied concentration of UA

3.9. Impact of UA concentration at designed electrode

CV was applied for the study of concentrations variation of UA at fabricated CPE. Figure 9a illustrated the influence of increasing concentrations of UA in 0.2 M PBS with scan rate 50 mV/s. The oxidation I_p was increased linearly as the concentration of UA increased from 10-70 μM. The graph of I_{pa} *vs.* analyte variation concentration was elucidate in Figure 9b and that calibration curves contributes fine linearity with correlation R^2=0.972. The LOD and LOQ was gotten at 0.37 and 1.22 μM correspondingly.

3.10. Simultaneous analysis of DA and UA

The concurrent detection of DA and UA was important because they are coexisting in extra cellular fluid of the mammalian central nervous system and concentration of DA was predominant. In demands to determination of these biomolecules CV technique was employed at fabricated CPE. Figure 10 reveals the recoded voltammograms for mixture of DA and UA (10 μM) at unmodified CPE (dashed line) and modified CPE (hard line) in 0.2 M PBS of pH 7.4 with scan rate 50 mV/s. The obtained voltammograms at MCPE demonstrates superior enhancement in peak current with oxidation peaks of two antioxidant species were clearly separated and shows good sensitivity compare to unmodified CPE and I_p located at 0.257 and 0.12 V individually. The above result implies the fabricated CPE was efficiently engaged for quantitative detection of DA and UA.

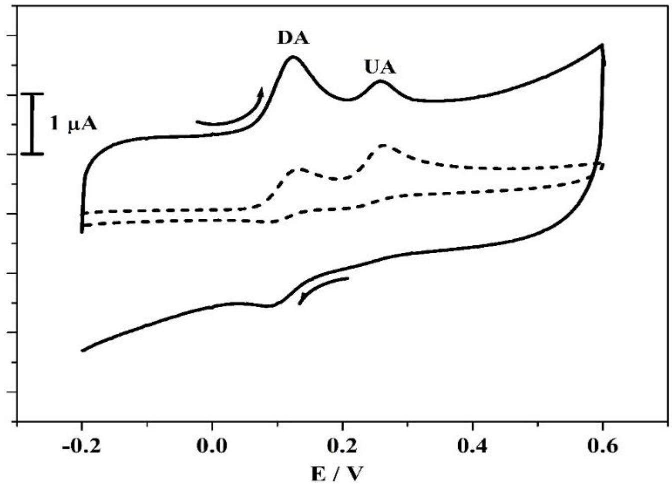


Figure 10. CVs for mixture of DA and UA (10 μM) at unmodified CPE (dashed line) and poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE (solid line) using 0.2 M PBS (pH 7.4) with scan rate of 50 mV/s

3.11. Interference study of DA and UA

The interference study was carried out in the mixture of DA and UA at fabricated CPE when concentration of one analyte was kept constant whereas the other species is changed.

Figure 11a illustrates the peak current of UA (50 µM) was kept constant, where DA was proportional to its concentration which was increased from 50-450 µM. Similarly, from the Figure 11b it can be seen that the concentration of UA was varied from 50-450 µM while the concentration DA was kept constant (50 µM). From the above obtained experimental result there was no change in peak current and peak potentials of constant analyte. So, this results clearly indicates the fabricated electrode possessed excellent selectivity towards the determination DA and UA they can have independently in the mixed solution.

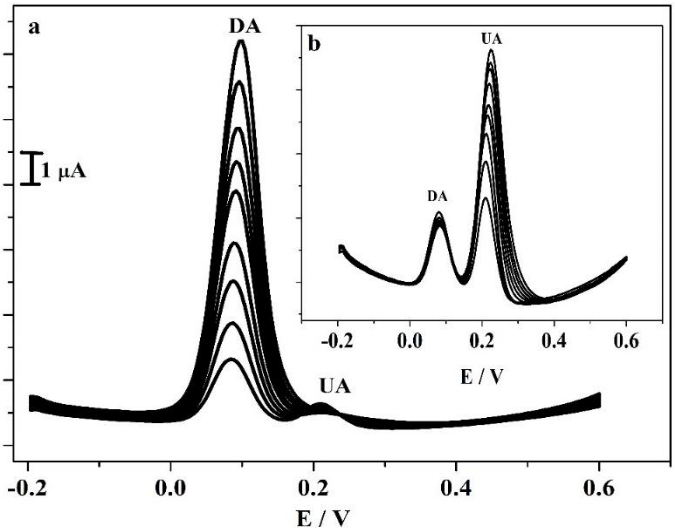


Figure 11. (a) DPVs of DA with different concentration (50-450 µM) in presence of UA (50 µM) at MCPE; (b) DPVs of UA with different concentration (50-450 µM) in the presence of DA (50 µM) at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE

3.12. Analytical application

Recovery tests were performed to investigate the analytical efficiency and reliability of the fabricated electrode in commercial dopamine hydrochloride injection sample was studied by standard addition method [40] using CV technique. The obtained results were shown in Table 2 and it shown in between 95%-98.66% recovery for DA at three different concentrations in injection sample using the modified electrode with adequate recovery.

Table 2. Determination of DA in injection sample at poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE

Sample	DA added (µM)	Found (µM)	Recovery (%)
1	5	4.75	95.00
2	15	14.8	98.66
3	25	24.6	98.40

4. CONCLUSION

In this present work, successfully prepared the poly (6-phenyl-1,3,5-triazine-2,4-diamine)/MCPE using cyclic voltammetry, which was used for the detection of DA and UA. The fabricated electrode exhibited sufficient electrocatalytic activity for the simultaneous and individual analysis of DA and UA. The modified electrode gave low detection limit at 0.25 and 0.37 μM respectively for individual analysis. The electrode kinetics was established diffusion controlled at fabricated CPE. The prepared CPE was used to determination of DA in injection samples and these outcomes shows the developed electrode have exhibits excellent sensitivity, selectivity and good reliability for the recognition of these analytes in biological samples.

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