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Selective Determination of Dopamine in Presence of Ascorbic Acid by using Triton X-100 Poly(Safranin) Modified Carbon Paste Electrode

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Abstract- In this, we have studied the preparation, characterization and application of a poly Safranin Triton X-100 (SFRTX-100) modified carbon paste electrode for the simultaneous determination of dopamine (DA) in presence of ascorbic acid (AA) without any interference in 0.1 M phosphate buffer solution (PBS) with a scan rate of 50 mV/s by using cyclic and differential pulse voltammetry techniques. Modified electrode was successfully applied for the determination of DA in pharmaceutical and human serum samples with good results. Bare carbon paste electrode (BCPE) and modified carbon paste electrode (MCPE) was characterized by scanned micrographs. Modified electrode exhibited a well-defined redox peak of dopamine (DA) due to electrostatic interaction of poly(SFRTX-100) monolayer. Limit of detection (LOD) and quantification (LOQ) of DA was found to be 1.78×10^{-7} M and 5.43×10^{-6} M respectively. The poly(SFRTX-100) showed excellent sensitivity, selectivity and stability which are more favorable for the detection of DA in physiological matrices.

Keywords- Dopamine, Ascorbic acid, Safranin, Triton X-100, Cyclic voltammetry, Differential pulse voltammetry

1. INTRODUCTION

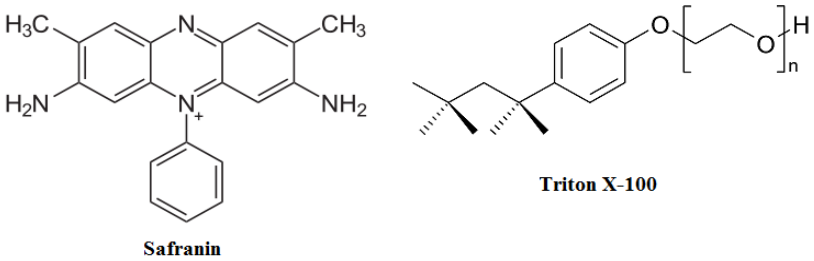
Monitoring of biologically electro active molecules like Dopamine (DA) and Ascorbic Acid (AA) has attracted the researchers due to their oxidation potentials and concentration variation levels in the body. Catecholamine neurotransmitters are a group of biogenic compounds that plays a vital role as chemical messenger that transmits nerve signals. DA is an inhibitory neurotransmitter which is produced in the "Dopaminergic neurons in the ventral tegmental area (VTA) of the mid brain", strongly associated with reward mechanisms in the brain like memory, locomotion, learning and behaviour of cognition. It is secreted in hypothalamus region that mediates the brain during physical activities like sports, eating and during sex [1]. As a hormone, it mediates the functions of central nervous system including memory, emotions and endocrine regulation. It is a small-molecule recognized by a G-protein coupled receptor which induces some biological reactions like muscle contraction, emptying of the gland etc. In the central nervous system DA ranges from 0.01 nM to 1 M [2]. Various levels of this vital chemical cause several diseases and addiction towards cocaine, opium, heroin, nicotine and alcohol could increase the levels of DA. Long-term smoking, based on the taking of hard drugs leads to high concentrations [3] and abnormal levels of DA results in neurodegenerative diseases such as Tourett'e syndrome, burning mouth syndrome [4], restless leg syndrome [5], Senile dementia, fibromyalgia [6,7] and rarely depression [8]. Depletion of DA in cerebral region is the hall mark for Parkinson's disease [9].

Ascorbic Acid (AA) is another vital anti-oxidant, water-soluble vitamin present in many fruit juices, vegetables, soft drinks, biological fluids etc. which is important in human diet. It is necessary for the formation of collagen, assists in the absorption of iron by promoting its reduction to the ferrous state and lack of it provokes increased susceptibility to many kinds of infections and slows down the rate at which wounds and fractures heal [10]. It functions as a redox cofactor and catalyst in a broad array of biochemical reactions and processes. AA is used in the treatment of common cold, mental illness, cancer, AIDS [11] and prevention of scurvy. A reliable determination of DA and AA in biological fluids has very good interest among chemists and neuro-scientists.

Now-a-days advanced sensors are used to detect biomolecules at low concentrations, which is necessary for early-stage disease detection. On the other-hand electrochemical sensors are having remarkable detection sensitivity, reproducibility, biocompatibility, facile surface renewing, rapid analysis, inexpensive, non-toxic and ease of miniaturization rather than other instrumental analytical methods [12-15]. Till now there are few reports in the modification of electrodes for the simultaneous detection of DA in the presence of AA by using conducting polymers [16], molecular imprinted polymer [17,18], redox- mediators modified electrode [19], pyrolytic graphite-electrode [20,21], electrochemically-oxidized GCE [22] and screen printed electrodes [23]. However these electrodes are problematic due to their longer-response time, limited sensitivity and poor-long term stability. Recently, the

modification of electrodes by using poly-redox/polyazine modifiers is an interesting field in the analytical chemistry. These polyazine modifiers are the powerful tools, because of film thickness, charge transport that effectively shuttle electrons between the electrode and the analyte. Therefore, these poly-redox modified electrodes (PRMEs) have advantages like absence of surface fouling, improved electro catalysis and undesirable reactions competing kinetically with the desired electrode process [24, 25]. The most important effects of any modifier is to reduce the over potential of an electrochemical reaction, which enhances the selectivity and sensitivity of the method.

Safranin [SFR] is an azonium compound with a molecular formula $C_{20}H_{19}N_4$ and chemically it is known as 2,7-diamino-1,8-dimethyl-5-phenylpheazin-5-ium-chloride (scheme-1) and it is a biological stain used in cytology and histology. It is an electro active poly aromatic cation [26], used as a redox indicator in analytical chemistry. It is also known as basic red 2 or Safranin O. Safranin modified carbon paste electrode [SFRME] was mobilized with TritonX-100 (TX-100) [SFRMETX] was used for the oxidation of DA under the coexistence of AA. The surfactant modified carbon electrode was reported by Wen et al. [27, 28]. It is a non-ionic surfactant with a molecular formula of $C_{14}H_{22}O(C_2H_4O)_n$ (Scheme 1) that has a hydrophilic polyethylene oxide chain and aromatic hydrophobic group.



Scheme 1. Structure of Safranin and Triton X-100

In the present study, a simple and rapid electrochemical method was used to study the properties of modified carbon paste electrode by using cyclic and differential pulse voltammetric techniques. The results show that the modified electrode exhibits well-defined oxidation peak without any interferences. This modified electrode was successfully applied for commercial and real sample analysis.

2. EXPERIMANTAL

2.1. Materials and Stock solution

Analytical grade Ascorbic Acid (AA), Dopamine (DA) disodium hydrogen phosphate (Na_2HPO_4), sodium hydrogen orthophosphate (NaH_2PO_4), per-chloric acid, silicon oil were procured from Himedia chemicals. Fine graphite powder (particle size <20 µm) was supplied

from Sigma-Aldrich. Dopamine stock solution (25 mM) was prepared by dissolving in perchloric acid; Phosphate buffer (pH 7.0) was prepared with 0.2 M NaH_2PO_4 and Na_2HPO_4 solution in distilled water. Chemicals were used as supplied without any further purification.

2.2. Instrumentation

All voltammetric experiments were performed with a CH-Instrument Model no. CHI 610D, Electrochemical work station connected to a personal computer was used for the electrochemical measurements and data storage. A conventional three electrode cell was employed throughout the experiments, with bare and SFR modified carbon paste electrode (homemade cavity of 3.0 mm diameter) as a working electrode, platinum wire as a counter electrode and Ag/AgCl electrode as a reference electrode.

2.3. Preparation of Bare carbon paste electrode [BCPE]

The carbon paste electrode was prepared by hand mixing of graphite powder and of silicon oil in the ratio of 70:30(w/w) in an agate mortar until homogeneous carbon paste was obtained. Then the prepared carbon paste was incorporated into 3.0 mm diameter of Teflon cavity current collector and polished using the transparent paper before application.

2.4. Fabrication of poly(SFR TX-100) modified Electrode

Optimized ratio of graphite powder and silicone oil [70:30] was grinded in an agate mortar until homogeneous carbon paste was obtained. The prepared carbon paste was incorporated into 3.0 mm diameter of Teflon cavity current collector and polished using the transparent paper for smooth surface before application. The electro-polymerization was carried out in 1 mM SFR in 0.1 M phosphate buffer solution (PBS) of pH 7.0 with a scan rate of 50 mVs^{-1}. The CPE was scanned for 15 cycles, between the potential ranges of -400 mV to 1300 mV due to which thin polymer film was successfully achieved and it was confirmed by using scanning electron microscopic analysis (SEM). After the completion of the polymerization process the electrode was washed with distilled water to remove the physically adsorbed material, was mobilized with non-ionic surfactant TX-100 and it was immersed in 0.1 M PBS with pH 7.0 for further electrochemical studies.

2.5. Electrochemical measurements

A three–electrode system was used to analyze the electrochemical properties of DA&AA in an electrolytic cell with a stock solution of DA, in 0.1 M physiological phosphate buffer solution (pH-7.0) as a supporting electrolyte. Cyclic and differential pulse voltammograms

were recorded with a particular scan rate from -40 mV to +500 mV. The same procedure was applied for further analysis of DA and AA at room temperature.

3. RESULTS AND DISCUSSION

3.1. Surface morphology of the bare CPE and poly(SFR) Electrode

Scanning Electron Microscopy can directly represent the information of the electrode surface. Scanned electron micrograph shows the surface morphology of BCPE and poly(SFR) modified carbon paste electrode in Fig. 1. Fig. 1a shows the irregular flakes of BCPE with a size of 2µM, having porous cavity and uniformity was not maintained. However after polymerization the surface of modified electrode was shown in Fig. 1b was having smooth surface without any cavities. SEM images of BCPE and poly(SFR) modified electrode showed significant difference in the surface morphology, which confirms the CPE was changed by Safranin monolayer on the electrode surface.

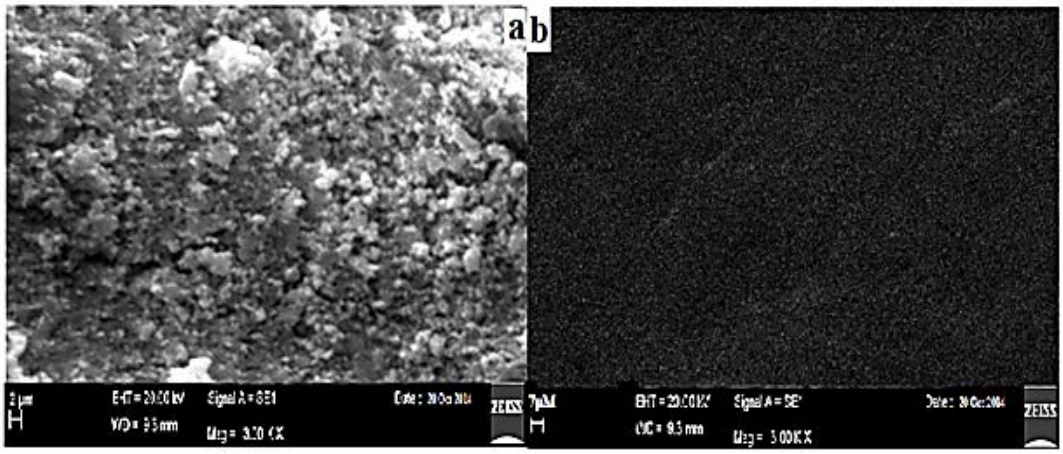


Fig. 1. (a) Shows the SEM image of BCPE; (b) the poly(SFR) film on electrode surface

3.2. Cyclic Voltammetric results of Safranin on CPE

Fig. 2a shows the consecutive electro polymerization of 1 mM SFR in 0.1 M phosphate buffer solution with pH 7.0 at unmodified carbon paste electrode. The growth of polymer film was achieved by scanning over the range of -400 mV to 1300 mV continuously for 15 cycles at a scan rate of 50 mVs^{-1}. The redox peak current was gradually increased by increasing the number of cyclic scans, indicating that the electro conductive polymer film was formed and deposited on the electrode surface.

Poly(SFRTX-100) thickness was controlled by varying the concentrations and voltammetric scans which shows the effect on oxidation peak current (I_{pa}) in neutral pH 7.0 of 1 mM DA with a scan rate of 50 mVs^{-1} as shown in Fig. 2b. However, the maximum

current response was observed for 15 cycles with an active surface area that covers the electrode surface completely. Further increment in cycles leads to thickness and decrease in redox peak current. Therefore, we selected 15 cycles as the optimum scan number for the film formation and we observed better enhancement for further studies.

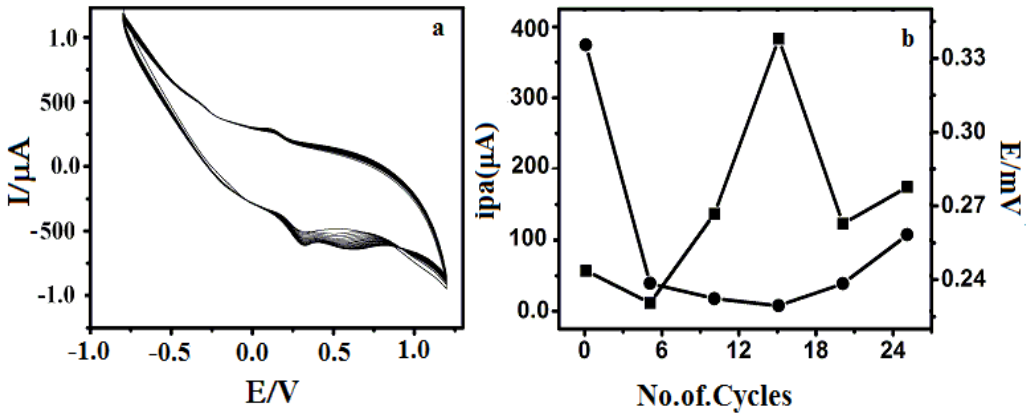
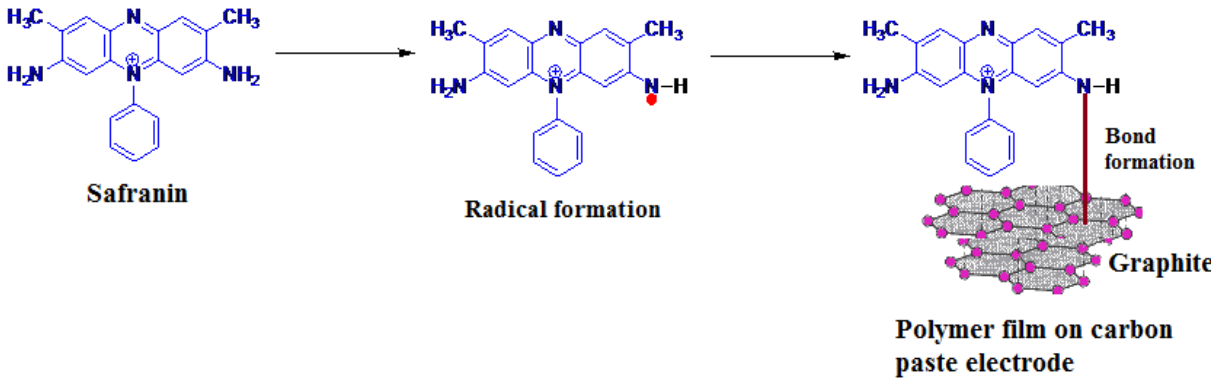


Fig. 2. (a) Cyclic voltammograms for the electro polymerization of 1 mM SFR on CPE in pH 7.0 with a scan rate of 50 mVs^{-1}; (b) Oxidation peak currents variations *vs*. potential with number of voltammetric cycles

3.3. Electrochemical properties of poly (SFR) MCPE

The electrochemical behavior of poly(SFRTX-100) was studied by using CV technique in PBS (pH 7.0) with a scan rate of 50 mVs^{-1}. The formation of polymer film on CPE was explained with plausible radical formation at 0.229 mV (Fig. 2b) which indicated the polymerization reaction. SFR undergoes oxidation process in which the amine group turns to radical formation. These radical forms a bond with aromatic group of bare and poly(SFRTX-100) was shown in Scheme 2 [29].



Scheme 2. Mechanism and polymerization of Safranin on carbon paste electrode

3.4. Electro-catalytic oxidation of DA on poly (SFRTX-100)

Electro-catalytic activity of bare and MCPE exhibited a redox wave in 1 mM DA with 0.1 M physiological PBS (pH-7) with a scan rate of 50 mVs^{-1} and is shown in Fig. 4. BCPE (solid line) showed a poor redox peak with a potential difference of 0.336 mV. Under the same condition the electro chemical response of (poly (SFRTX-100) (dotted line) showed a well-defined redox wave with a potential difference of 0.236 mV, it is due to the electro-catalytic activity of the poly (SFRTX-100). Intensive increase in anodic peak current (I_{pa}) of poly (SFR TX-100) showed a good enhancement of 2.2 folds when compared with the BCPE. These changes in cyclic voltammograms indicate that the modified electrode had a good catalytic activity for the redox reaction of DA with the transfer of equal number of electrons and protons.

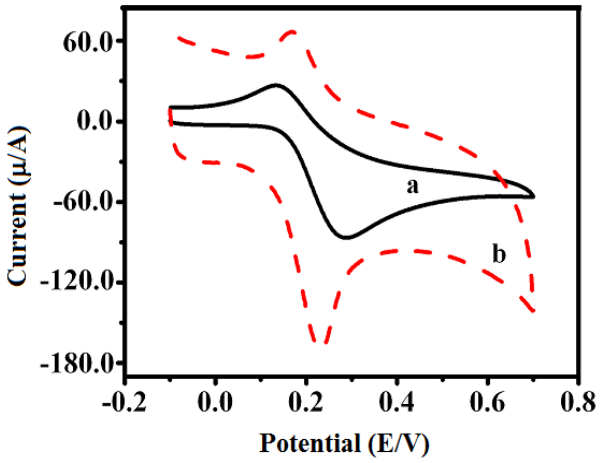


Fig. 4. Cyclic voltammograms of bare (a) and poly Safranin; (b) carbon paste electrode in 0.1 M phosphate buffer solution

3.5. Influence of Scan rate on the oxidation of DA with poly (SFR TX-100)

Scan rate was influenced to study the kinetics of an electrode reaction that influence the electrochemical response of redox peak current (1 mM DA) in 0.1 M physiological PBS with pH-7 by varying the scan rates from 0.05 to 0.5 Vs^{-1} measured by cyclic voltammetry (CV). According to Randles-Sevick's equation the anodic peak current (I_{pa}) increases linearly with the scan rate (v) as it was evident from Fig. 5. Suggesting that the electrode reaction was diffusion controlled process with a co-relation co-efficient of 0.998.

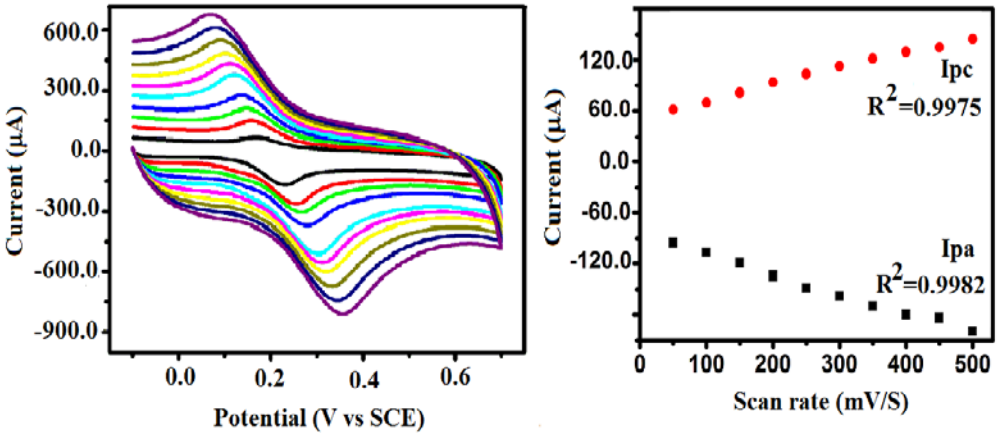


Fig. 5. Cyclic voltammograms with different scan rate in the presence of 1 mM DA with plots of anodic and cathodic linearities

3.6. pH influence on DA

Cyclic voltammograms of DA (1 mM) was studied by varying the pH from 5.5 to 8.0 using 0.1 M phosphate buffer as a supporting electrolyte at a scan rate of 50 mVs^{-1} in presence of poly (SFRTX-100) modified electrode. Increase in anodic peak current and decrease in peak potential was observed at pH 7.0 indicating that pH changed from acid to neutral and further increase in pH leads to increase in peak potential and decrease in peak currents which may be due to the participation of protons in oxidation process as shown in Fig. 6. Graph was plotted between anodic peak potentials and pH from which the calculated slope value is 51 mv/pH which is very close to the theoretical value of (59 mV/pH), which is dependent on the Nernst equation indicating that the number of electrons and protons were involved in the electrode reaction.

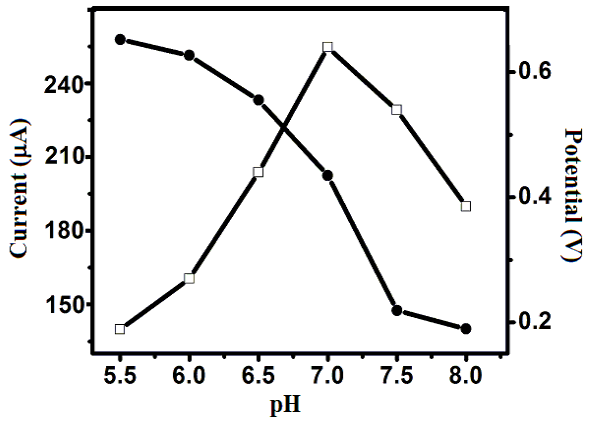
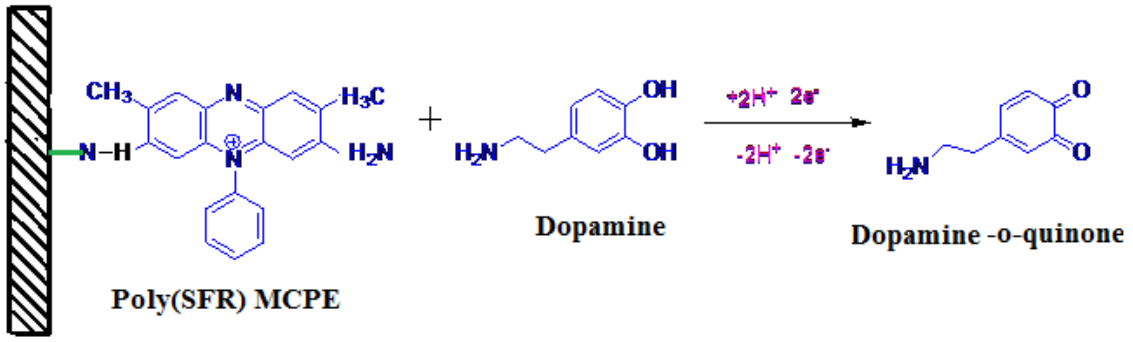


Fig. 6. Redox behaviour of DA in presence of MCPE with a pH variation from 5.5 to 8.0

pH plays a major role in the electrochemical oxidation of DA based on the proposed oxidation mechanism occurring at poly (SFRTX-100) modified CPE. The proposed oxidation mechanism for DA (Scheme 3) is believed to occur as two- proton and two-electron transfer takes place to form dopamine-o-quinone (rate-determining step). In our studies no further efforts was taken to identify the final products of DA, as it was in the literature [30].



Scheme 3. Shows the conversion of DA to DA-O-quinone in presence of Poly (SFR) MCPE

3.7. Calibration plot of DA with MCPE

In Differential pulse Voltammetry the contribution of charging current to the back ground current is a limiting factor in analytical determination. DPV technique was used to study the current sensitivity, lower quantification and detection by varying the concentrations of DA in 0.1 M physiological PBS (pH-7.0) by using poly (SFRTX-100) MCPE. Fig. 7 shows the oxidation peak current increased linearly with the concentration of DA, showed a linear relationship with a correlation co-efficient of R^2=0.99579. The detection limit (DL) and the quantification limit (QL) of DA were found to be 1.78×10^{-7} M and 5.43×10^{-6} M respectively.

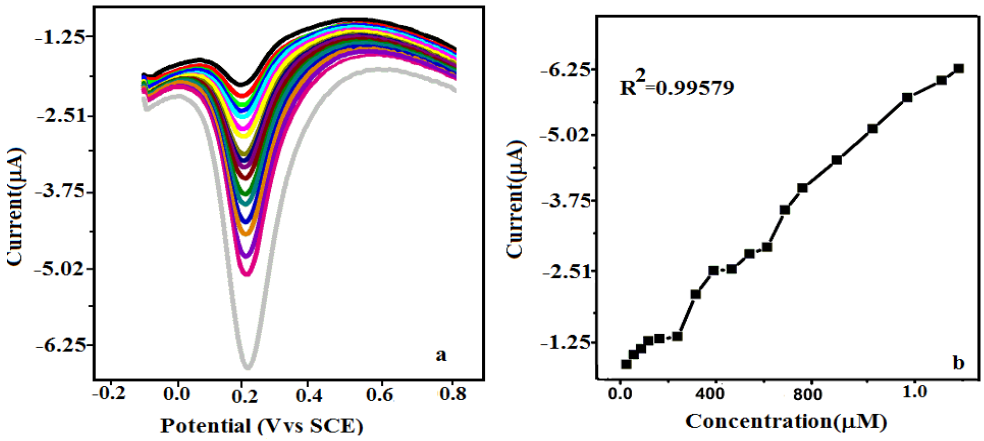


Fig. 7. (a) Differential pulse voltammograms of Dopamine; (b) Various concentrations of DA in presence of 0.1 M PBS with a pH 7.0

3.8. Interference effect on DA and AA

The interferences of DA level in blood serum are important due to the interfering compounds, such as ascorbic acid, urea and creatinine. Anti-interference ability of the sensor was investigated by using cyclic and differential pulse voltammograms in a physiological phosphate buffer solution (pH-7) with a scan rate of 50 mVs^{-1}. DA and AA was not resolved at BCPE (thick-line a) and poly (SFR) without Triton X-100 (thick-line b) due to similar peak potentials. However and poly(SFRTX-100) (thick-line c) showed resolved oxidation peaks of DA and AA with potentials of 0.089 and 0.1840 mV as shown in Fig. 8 this suggests that the oxidation peak of DA was shifted to less positive potential without the interference of AA.

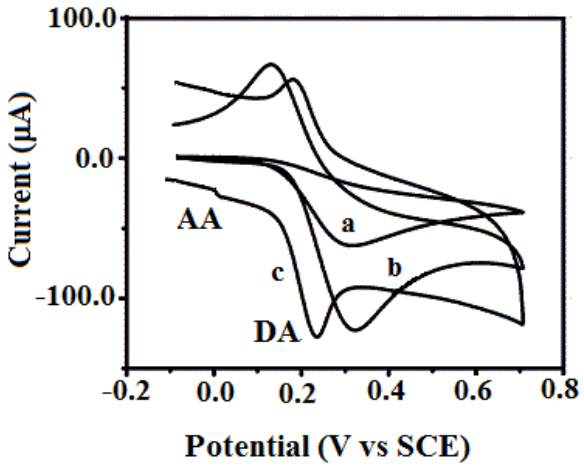


Fig. 8. Cyclic voltammograms of BCPE, poly (SFR) and poly (SFRTX-100) with a scan rate of 50 mVs^{-1} at pH 7.0

3.9. Electrochemical resolution of DA and AA

Further, the electrochemical separation study was again carried out by DPV technique to detect DA and AA due to its higher current sensitivity and better resolution than CV as shown in Fig. 9. Under the optimum conditions the concentration of DA and AA ranges from 1 mM to 6.89 mM and 1.02 mM to 8.55 mM in PBS at pH 7.0 as shown in Fig. 9. Concentration was proportional to oxidation peak currents of AA with a correlation co-efficient of 0.994 and DA with a correlation co-efficient of 0.998 as shown in Fig. 10. Therefore, the modified electrode successfully resolved the peaks without any interference. So, the fabricated simple electrode was fairly sensitive, has wider linear range and low detection limit.

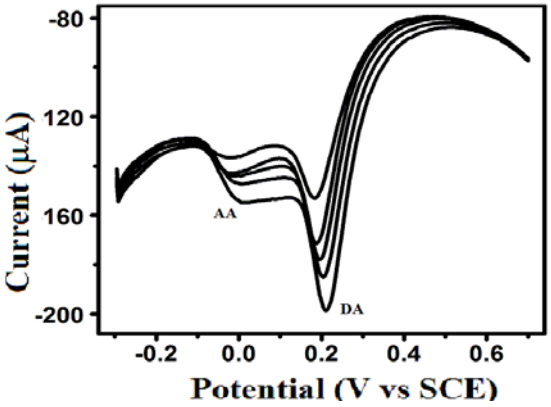


Fig. 9. Differential pulse voltammogram of AA&DA in presence of 0.1 M PBS at pH 7.0 with a scan rate of 50 mVs^{-1}

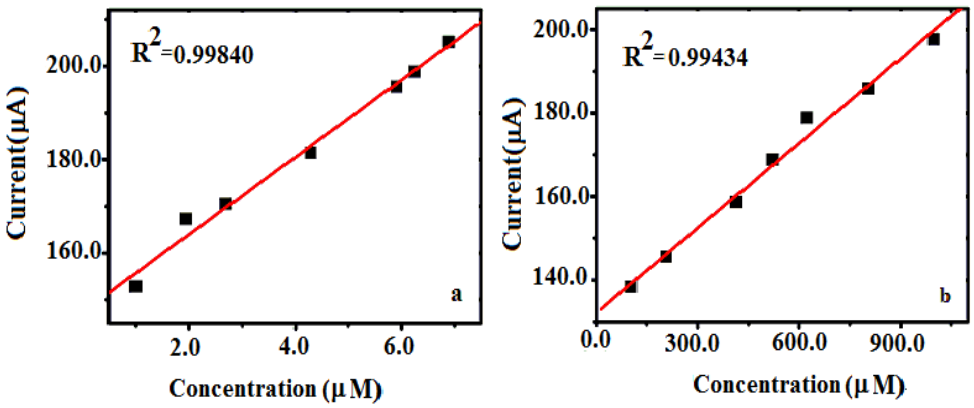


Fig. 10. Simultaneous calibration plot of AA&DA in presence of 0.1 M PBS at pH 7.0 with a scan rate of 50 mVs^{-1} by using poly(SFR) modified electrode

3.9. Stability of the electrode

Modified electrode stability was evaluated after one-week. The stability of the modified poly(SFRTX-100) was investigated in 1 mM DA with a scan rate of 50 mVs^{-1} in 0.1 M PBS at a neutral pH 7.0 by using CV technique. After 40 multiple cycles in DA with a potential range of -0.2 V to 0.6 V, in which there was no disturbance in the current was observed. From this observation, we can confirm that the developed electrochemical sensor possesses remarkable repeatability.

3.10. Sample analysis

Poly(SFRTX-100) was used to analyze the concentration of DA in Dopamine hydrochloride injection and human blood serum samples (obtained from the Health Centre,

Sri Venkateswara University, Tirupati, Andhra Pradesh, India) were analyzed by standard addition method by using DPV technique. DA injection was purchased from Neon Laboratories India Private Ltd., with a specified content of 20.0 mg/mL. Samples were diluted in 0.1 M PBS at pH 7.0 before the measurement to prevent the matrix effect of authentic samples. DA was analyzed by using a calibration plot. The recovery and relative standard deviation (RSD) were acceptable (n=5), in the range of 95-104% showing that the prepared sensor is very efficient, reliable and sensitive for the determination of DA in pharmaceutical samples.

Table 1. Determination of dopamine in human serum and dopamine hydrochloride injection (n=5)

Samples	**Spiked (mM)**	**Found (mM)**	**Recovery (%)**	**RSD (%)**
Human Serum	0.1	0.095	95%	3.5
	0.2	0.194	97%	2.55
	0.3	0.297	99%	1.69
DA-Injection	0.1	0.097	97%	2.55
	0.2	0.199	99.5%	2.5
	0.3	0.294	98%	1.56

4. CONCLUSION

The developed simple and rapid method was successfully applied to resolve DA and AA in presence of 0.1 M PBS at physiological pH 7.0 with a scan rate of 50 mVs^{-1}. Characteristics of the fabricated electrode were studied by using CV and DPV techniques. poly(SFRTX-100) modified electrode not only showed the excellent electro-catalytic activity towards the oxidation of DA, but resolved the oxidation peaks of DA and AA successfully without any interference. Therefore a modified electrode showed a well-defined redox and discrete voltammetric peaks with good sensitivity and stability. Thus, the method is cost-effective and provides a novel strategy for the detection of DA in pharmaceuticals and biological samples.

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