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Covalently Anchored p-Aminobenzene Sulfonate Multilayer on a Graphite Pencil Lead Electrode: A Highly Selective Electrochemical Sensor for Dopamine

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Abstract: An electrochemical sensor for dopamine (DA) has been developed based on the electrografting of 4-aminobenzene sulfonic acid (4-ABSA) onto the graphite pencil lead electrode (GPLE). The process of covalent anchoring and presence of 4-ABSA on the GPLE was studied using cyclic voltammetry and electrochemical impedance spectroscopy. Electrochemical behaviour of the sensor towards DA, ascorbic acid (AA), and uric acid (UA) was studied in detail in phosphate buffer of pH 7. After optimizing the various parameters that influence the differential pulse voltammetric (DPV) signal for DA, the sensor exhibited a linear response over the 0.5 – 10 $\mu mol \cdot L^{-1}$concentration range with a limit of detection, 0.095 $\mu mol \cdot L^{-1}$ (at an S/N of 3). The sensor can selectively quantify DA even in the presence of 1 $mmol \cdot L^{-1}$ AA. Distinct DPV signals were obtained for DA (at 0.191 mV vs. Ag/AgCl) and for UA (at 0.343 mV vs. Ag/AgCl). The sensor is highly selective, sensitive and stable. It was applied to the quantification of DA in injections and urine. Recovery studies were done by spiking both the real samples with a known quantity of DA.

Keywords: 4-Amino benzene sulphonic acid · multilayer · dopamine · impedance · DPV

1 Introduction

Dopamine (DA) is one of the important catecholamine family neurotransmitters. As a powerful neuromodulator, it affects various physiological aspects such as brain circuitry, neuronal plasticity, organization and control of stress response. It also affects the cardiovascular and renal systems. Drop in the concentration of DA level may lead to the neurological diseases such as schizophrenia, Parkinson's, epilepsy [1–3]. Medical studies infer that the accurate quantification of DA at low levels in biological systems will help a lot in the diagnosis of diseases [4]. One of the challenges to develop an electrochemical sensor for DA is to overcome the interference of coexisting compounds in the biological systems. Particularly AA is the major interferent because of the overlapping of its oxidation potential with that of DA [5–7].

A Wide range of analytical methods is reported for the detection of DA including colorimetric, fluorometric, electrochemical, electrophoresis, etc [8–10]. Among them, electrochemical determination of DA is a simple, sensitive, rapid and cost-effective method. Electrochemical methods developed for the quantification of dopamine were recently reviwed by giving much emphasis on the materials used for the modification of electrodes [11]. The various strategies such as self-assembled monolayers, covalent modification, perm-selective membranes, and electrograting of conducting polymer films have been reported to develop electrochemical sensors [12]. Among them, polymer film modified electrodes based on electrografting method was extensively used due to higher active sites, greater electron transfer kinetics, well resolved analytical signals and facile process for electrode modification [8, 13, 14]. Polyaniline nano-networks electrografted onto glassy carbon electrode (GCE) and used for the simultaneous determination of AA and UA [15]. Poly-1,5-diaminonaphthalene was decorated with cibacron blue on the GCE to obtain sufficient negative charge in the form of sulfonate groups, which resulted in the highly selective and sensitive method for DA quantification [16]. rGO composite of sulfonated calixerene was also used for the selective determination of DA[17]. 4-ABSA has been often used to modify GCE to selectively determine analytes such as DA, UA or AA under different optimized conditions [18, 19]. Li et al. reported the nafion-sulfonated-graphene modified GCE for selective determi-

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nation of DA in presence of AA and UA [20]. However, the presence of nafion on the electrode surface may hinder the diffusion of analyte and leads to slow response [21, 22]. Surface modification of GCE is an easy process, but the cleaning of the modified surface to regain the fresh and clean surface is difficult. Particularly when DA is oxidized on GCE, polymer product will irreversibly adsorb on its surface. Hence, there is a need for the alternative carbon substrate, that should be similar to GCE in surface robustness and conductivity but that should be surface renewable and cheaper. Graphite pencil is one of the competent materials for the disposable electrodes and seems to be a better alternative to GCE. Aoki et al. have pioneered the usage of composite pencil graphite (CPG) lead as a working electrode [23]. Ghani's group has been reported a substantial number of articles based on CPG leads (especially 2B pencil) as a substrate for working electrodes in either rod or paste form for electrochemical sensing of various analytes [24, 25].

The main objective of this work is to develop an electrochemical sensor which is highly selective towards DA in presence of large excess, at least 1000 fold, of AA by adopting much simpler electrode modification procedure. In this report we made an attempt to use the graphite pencil lead (GPL) as a working electrode. 4-ABSA molecules were electrografted onto the GPL electrode (GPLE) and it was electrochemically characterized using cyclic voltammetry. Electrochemical response of the 4-ABSA electrografted GPLE (4-ABSA/GPLE) towards DA, AA and UA was studied. And the various parameters which influence differential pulse voltammetric determination of DA were optimized. Developed electrochemical sensor was applied for the determination of DA in injections and urine samples.

2 Experimental

2.1 Materials

4-aminobenzene sulfonic acid, DA, AA, UA procured were of Sigma-Aldrich make and used as received without any treatment. Graphite pencil leads used of Grafo (India) make. Buffers of pH 6 to 8 were prepared using 0.01 M K_2HPO_4 and 0.01 M KH_2PO_4solutions. Acidic buffers (pH = 3 to 5) were prepared using 0.01 M acetic acid and 0.01 M sodium acetate solutions. Unless otherwise stated water used throughout the experiments was double distilled water. Dopacef (dopamine hydrochloride injection USP 200 mg 5 mL^{-1})

2.2 Instrumentations

Electrochemical experiments were carried out using CHI660D electrochemical workstation (CHI instruments, Inc. USA) with a conventional three-electrode system. Graphite pencil leads of 2 $mmol{\cdot}L^{-1}$ has been used as a working electrode. Platinum wire was used as a counter electrode and saturated Ag/AgCl electrode as reference electrode.

2.3 Electrode Preparation

Commercially procured graphite pencil leads (GPLs) were of 8.8 cm length and 2 mm diameter with a flat surface at one end (face of GPLs) and the another end has sharp finishing. GPLs were sonicated for 5 minutes each, first in ethyl alcohol and then in water by immersing the face of the GPLs up to 4 cm. Then the face was gently polished first on bare polishing cloth till it gains mirror-like finishing and then with 0.05 μm alumina slurry. Again the prior mentioned sonication procedure was repeated, and then the face was polished on bare polishing cloth once again to regain the mirror like luster. Nearly 3 cm length from the face was then compactly covered with Teflon tape leaving the face uncovered. Resulting GPLs were then used as working electrode in further experiments. The inspiration to prepare the electrode was derived from the reported literature [26].

2.4 Covalent Anchoring of 4-Aminobenzene Sulfonic Acid onto Graphite Pencil Lead Electrode

Electrochemical modification of GPLE with 4-ABSA was carried out by following the procedure detailed by Li et al. [27]. Thirteen continues potential cycles were run in a 0.1 M KCl solution containing 5 $mmol{\cdot}L^{-1}$ 4-ABSA with a potential window of +0.5 to +1.5 V at a 10 mVs^{-1} scan rate. After the completion of electrochemical modification procedure, 4-ABSA covalently modified GPLE (4-ABSA/GPLE) face was dipped in water and stirred for 10 minutes to remove the physically adsorbed 4-ABSA from the electrode surface.

2.5 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) measurements were performed in the presence of 5 $mmol{\cdot}L^{-1}$ $[Fe(CN)_6]^{3-}$ + 5 $mmol{\cdot}L^{-1}$ $[Fe(CN)_6]^{2-}$ + 0.1 M KCl solution as a redox probe. Impedance measurements were performed in the frequency range from 0.1 Hz to 100 kHz using signal amplitude of 10 mV. EIS measurements were performed with the same instrument that was used for CV studies.

3 Results and Discussion

3.1 Oxidative Electrografting of 4-Aminobenzene Sulfonic Acid Onto Graphite Pencil Lead Electrode

Figure 1 depicts the thirteen continues cyclic voltammograms performed to result the covalent modification of 4-ABSA onto GPLE. Distinct and irreversible anodic peak was observed at a 0.98 V potential, which indicates the irreversible oxidation of 4-ABSA on GPLE. This electrochemical reaction involves the irreversible one-electron

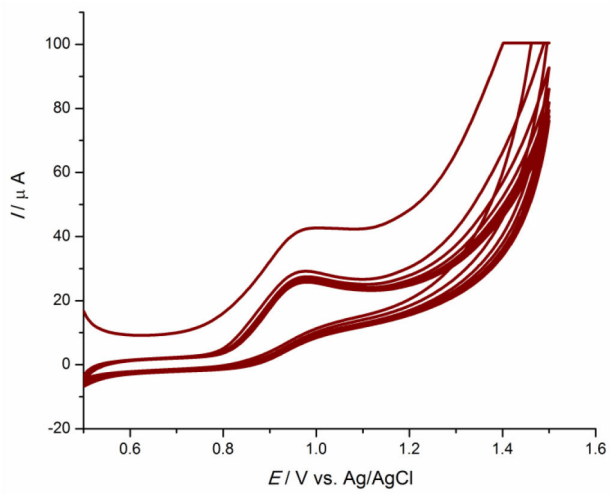
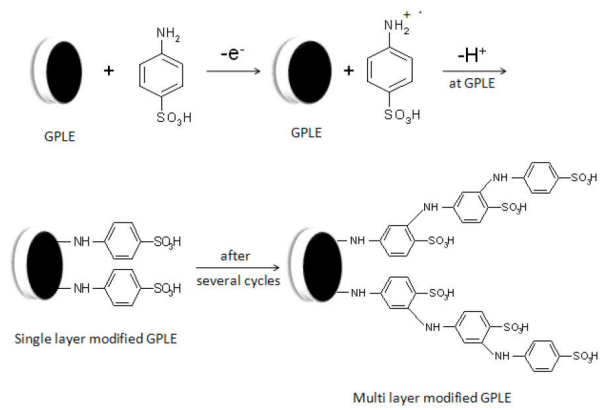


Fig. 1. Thirteen continuous CVs in 0.1 M KCl containing 5 mmol·L^{-1} 4-aminobenzene sulfonic acid at a 10 mVs^{-1}scan rate of 10 mVs^{-1}. .

oxidation of amine group to its cation radical [28], that further forms the covalent bond with the GPLE surface through NH group (Scheme 1). Decrease in the anodic peak current was observed in the next cycles. This can be explained due to the negatively charged 4-ABSA molecules covalently anchored onto GPLE surface during the first cycle will repel the 4-ABSA molecules present in the electrolyte solution [27].



Scheme 1. Representation of oxidative electrografting of 4-aminobenzene sulfonic acid onto graphite pencil lead electrode in aqueous 0.1 M KCl solution.

3.2 Electrochemical Characterization of 4-Aminobenzene Sulfonic Acid/Graphite Pencil Lead Electrode

Cyclic voltammetry was used to confirm the presence of 4-ABSA organic layers on the GPLE. CVs were recorded for benchmark redox couple $[Fe(CN)_6]^{3-}$ / $[Fe(CN)_6]^{2-}$ on bare GPLE and 4-ABSA/GPLE with a scan rate of 50 mVs^{-1}. CV was recorded for $[Fe(CN)_6]^{3-}$ on bare GPLE, it was obvious that considerable Faradic current was observed in 1st and 2nd segments due to the facile redox reaction. Similarly CV was recorded for $[Fe(CN)_6]^{3-}$ on 4-ABSA/GPLE, in this case considerable decrease in the Faradic current was observed (Supplementary information 1). This was due to the presence of the negative charge i.e. SO_3^- on the 4-ABSA/GPLE, which hinders the $[Fe(CN)_6]^{3-}$ from the electrode surface and in turn blocks the charge transfer. Non-Faradic current was also changed in case of modified electrode due to the presence of organic layer on it.

3.3 EIS Investigations of 4-Aminobenzene Sulfonic Acid/Graphite Pencil Lead Electrode

To make sure the presence of covalently anchored 4-ABSA on the GPLE surface electrochemical impedance spectroscopy studies were done. Surface, interface properties of bare and 4-ABSA/GPL electrodes will be different; hence the microscopic areas of both the electrodes can be competently characterized using EIS [29, 30]. EIS studies were performed by employing the alternating current (AC) impedance method. Surface areas of bare and 4-ABSA/GPL electrodes were same i.e. 2 mm. Figure 2 shows the EIS results of 4-ABSA/GPLE in a redox probe $[Fe(CN)6]^{3-/4-}$ (5 mmol·L^{-1}) of different pH buffers. EIS of 4-ABSA/GPLE consists of semi-circle and linear parts when the pH of the solution was 3 and 6. Diameter of the semicircle was considerably increased in case of pH 6 (R_{ct} = 1500 Ω) compared to pH 3 (R_{ct} = 500 Ω). Diameter of the semicircle was still increased to greater extent (R_{ct} = 3000 Ω) and linear part was almost disappeared for the spectrum recorded in the same redox probe solution

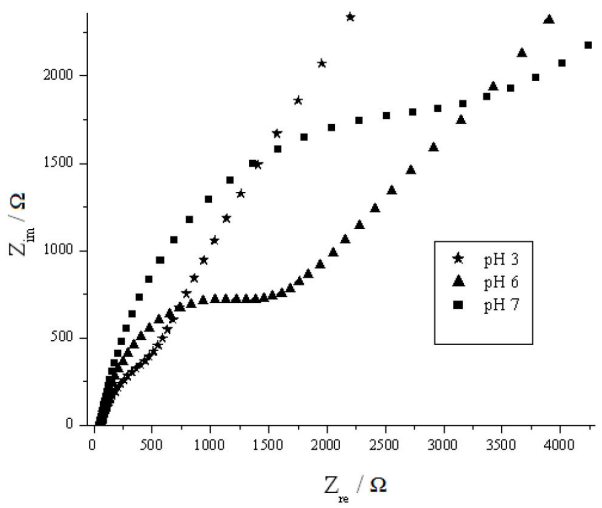


Fig. 2. Complex impedance plots of a 4-aminobenzene sulfonic acid/graphite pencil lead electrode in a 5 mmol·L^{-1} hexacyanoferrate (III/IV) prepared using acetate and phosphate buffers of different pH: 3, 6, 7.

of pH 7. Increase in diameter of the semicircle with increase in pH of the solution was observed, that is due to the increase in opposition to the electron transfer. The above results can be defended on the basis of surface pK_a of the 4-ABSA/GPLE that is estimated to be 3.2 [27]. EIS recorded for bare GPLE in $[Fe(CN)_6]^{3-/4-}$ (5 mmol·L^{-1}) of pH 7 consists of very small semicircle at high frequency and linear plot at low frequency with 45° inclination (Supplementary information 2). Increase in the R_{ct} values of modified electrode with increase in pH confirms the successful attachment of 4-ABSA onto the GPLE.

3.4 Effect of 4-Aminobenzene Sulfonic Acid Layer Thickness

The experiments were conducted to understand the effect of 4-ABSA film thickness on the determination of DA in the presence of large excess of AA. Hence, films of different thickness were grafted on the electrode by recording the different number of cyclic voltammograms for 5 mmol·L^{-1} 4-ABSA in a 0.1 M KCl as described above. With the increase in film thickness, interference of 1 mM AA signal in determining 6 μM DA was decreased. Film obtained by recording the 13 cyclic voltammograms was found to be completely suppressed the signal due to 1 mM ascorbic acid by retaining the good response to dopamine. Further increase in the film thickness resulted in the increase in background current without further improvement in the electrochemical response (Supplementary information 3a-e). Hence, film thickness obtained as a result of 13 cyclic voltammograms was used in all further studies.

3.5 Electrochemical Behaviour of AA and DA on Bare and 4-Aminobenzene Sulfonic Acid/Graphite Pencil Lead Electrode

Figure 3 shows the CVs recorded in the presence of DA, AA, a mixture of AA and DA on bare GPLE in phosphate buffer of pH 7. Signal was not observed when voltammogram was recorded in presence of 6 μmol·L^{-1}DA. Significant anodic peak with increase in current of nearly 5 μA was observed upon the addition of 1mmol·L^{-1}AA that indicates the facile oxidation of AA. Further increase in the anodic peak current was observed for the mixture of 1 mmol·L^{-1} AA and 20 μmol·L^{-1} DA. On the other hand, anodic peak due to the electrochemical oxidation of 1 mmol·L^{-1} AA was completely suppressed on 4-ABSA/GPLE (Figure 4). This can be explained on the basis of pKa values of AA and 4-ABSA. The surface pKa of the 4-ABSA/GPLE is about 3.26 [15]. The modified electrode surface will get a sufficient negative charge due to the deprotonation of the sulfonic acid groups when the pH of the solution is greater than its pKa value. Whereas the pKa value of AA is 4.10, which will exists as a negatively charged species in the pH greater than its pKa value. Since the electrochemical experiments were carried out at pH 7, there will be a strong repulsion between

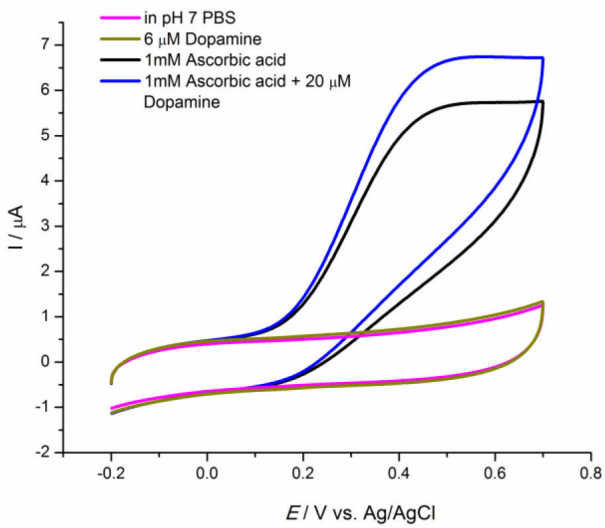


Fig. 3. Cyclic voltammograms recorded on bare graphite pencil lead electrode in pH 7 phosphate buffer for 6 μmol·L^{-1} DA, 1 mmol·L^{-1} AA, a mixture of 1 mmol·L^{-1} AA and 20 μmol·L^{-1} DA at a scan rate of 50 mVs^{-1}.

negatively charged deprotonated sulfonic acid groups and ascorbate ions; hence the electrochemical signal for the AA was completely suppressed. When 6 μmol·L^{-1} DA was electrochemically reacted on 4-ABSA/GPLE one set of well resolved redox peaks were observed at E_{pa} = 0.21 V and E_{pc} = 0.15 V potentials (Figure 4) with a peak potential difference of 0.18 V. This indicates a facile electron transfer between the DA and modified electrode. Presence of 1 mmol·L^{-1} AA was not at all interfered in the electrochemical determination of DA on 4-ABSA/GPLE is also depicted in Figure 4.

3.6 Effect of Scan Rate

CVs were recorded at various scan rates in the presence of 6 μmol·L^{-1} DA in phosphate buffer of pH 7 (figure not shown). Anodic and cathodic peak currents of resulted CVs were plotted against scan rates (Supplementary information 4). Linear correlation between the peak current and scan rate was observed. This indicates that the redox reaction of DA on 4-ABSA/GPLE is surface confined. This can be explained due to the strong electrostatic interaction between the highly negatively charged 4-ABSA/GPLE and positively charged DA under the experimental conditions.

3.7 Effect of pH

The effect of pH on the peak current and peak potential of DA at 4-ABSA/GPLE was studied by recording the CVs in the buffer of different pH i.e. from 3 to 8. Negative shift in the peak potential of DA was observed with the increase in pH. This indicates the involvement of protons transfer in the redox reaction. Involvement of equal

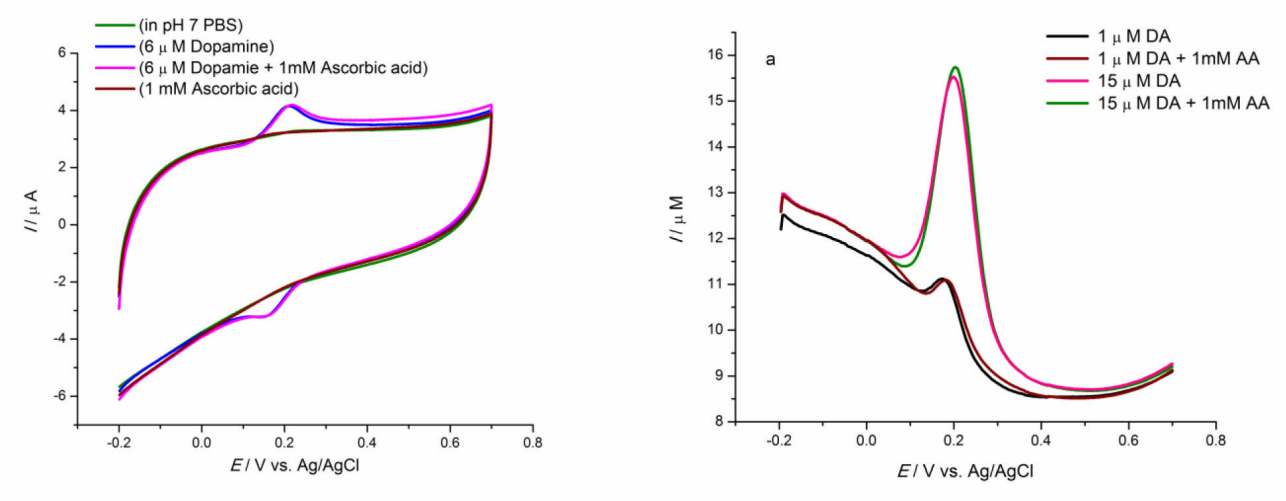


Fig. 4. Cyclic voltammograms recorded on 4-aminobenzene sulfonic acid/graphite pencil lead electrode in phosphate buffer of pH 7 for 1 mmol·L^{-1} AA, 6 μmol·L^{-1} DA, a mixture of 6 μmol·L^{-1} DA and 1 mmol·L^{-1} AA at a scan rate of 50 mVs^{-1}.

number of electrons and protons was understood by the plot of anodic peak potential versus pH (not shown) that exhibited linearity in the pH range 3 – 7 with a slope -51.6 mVpH^{-1} [20,31]. With the increase in pH, anodic peak currents of DA and AA were increased and decreased respectively. pH 7 was found to be optimum, where current signal for DA was greater with almost no signal for AA (Supplementary information 5). Hence pH 7 was used in all further experiments.

3.8 Effect of Accumulation Time

From the scan rate studies it was clear that, DA will adsorb at the negatively charged surface of the 4-ABSA/GPLE. Hence, accumulation time will definitely affects the anodic and cathodic peak currents. To examine this, CVs were recorded for 20 μmol·L^{-1} DA at different accumulation times such as 1, 2, 3, 4, 5 etc…. Peak currents were increased considerably with increase in accumulation time (Supplementary information 6) up to 4 minutes. After that peak currents were remained same (CVs not shown), that indicates the surface accumulation of DA was complete at 4 minutes.

3.9 Determination of DA Using Differential Pulse Voltammetry

Sensitivity of the method can be improved by adopting the differential pulse voltammetry (DPV) for the quantification measurements over CV [32]. From the CV studies it was clear that, DA accumulate on 4-ABSA/GPLE surface with time. Instead of accumulating the DA in a quiet solution, process was speeded up by stirring the solution present in an electrochemical cell. Hence before constructing the calibration graph for the quantification of DA using DPV, accumulation time and stirring speed were optimized. Differential pulse voltammograms (DPVGs) were recorded for 4 μmol·L^{-1} DA in phosphate buffer of pH 7 with 600 rpm for different accumulation times such as 30, 60, 120, 180, 240, 300 seconds. Peak current was increased with increase in time from 30 to 180 s, thereafter it remain almost unchanged (Supplementary information 7). Hence 180 s was fixed as the optimized time for the accumulation and the same time was used in all further DPV experiments. Then, stirring speed was optimized by recording the DPVGs for 4 μmol·L^{-1} DA with 180 s accumulation time at varied rpm of magnetic bit. Peak current was increased with increase in rpm from 0 to 600, there after it remained constant (Supplementary information 8). Hence, all further DPV experiments were done with 600 rpm.

4 Calibration Curve

Calibration curve was constructed by recording the DPVGs in presence of various concentrations of DA under the optimized conditions in 10 mL phosphate buffer of pH 7 (Figure 4). Varied volumes of 2 mmol·L^{-1} DA stock solution were added by standard addition method. Each time after the addition of DA solution, it was stirred for 3 min at 600 rpm then the DPVG was recorded. Peak currents of the recorded DPVGs were drawn against the added DA concentration (Supplementary information 9), correlation between them was obtained from 0.5 to 10 μmol·L^{-1} DA. Deviation from the linearity was observed at concentrations greater than 10 μmol·L^{-1} DA due to the drop in sensitivity of the electrode, that can be explained due to the small surface area of the electrode [33]. The linear regression equation for this plot can be expressed as i_{pa} = 10.786 + 0.42 X (μmol·L^{-1}) with a correlation coefficient r = 0.99. The detection limit (S/N = 3) was found to be 0.095 μmol·L^{-1}.

DPVGs were again recorded for same concentrations (0.5, 1, 2, 3……..15 μmol·L^{-1}) of DA but in the presence of

1 mmol·L^{-1} AA (Supplementary information 10a). Peak currents were plotted against the concentrations of DA (Supplementary information 10b), the correlation between the peak current and DA concentration was observed in the range 0.5 to 10 μmol·L^{-1}(Supplementary information 10b inset) and thereafter deviation was observed. Linear regression equation for this plot can be expressed as $i_{pa} = 10.73 + 0.418$ X (μmol·L^{-1}) with r = 0.99. In presence of 1 mmol·L^{-1} AA, neither the separate peak was observed in addition to that DA nor the anodic peak current of DA was noticeably altered (Figure 5a). DPVGs were recorded for the mixture of DA, AA and UA at different concentrations. The separate peak for UA was observed at about 150 mV away from the DA oxidation potential. Resolved peaks were observed for DA and UA at all the measured concentrations (Figure 5b). Hence, it can be stated that developed electrochemical sensor completely rejects the 1 mmol·L^{-1}AA and selectively determines DA. 4-ABSA/GPLE can be applied for the quantification of DA in presence of 1000 fold excess of AA in real biological and pharmaceutical samples.

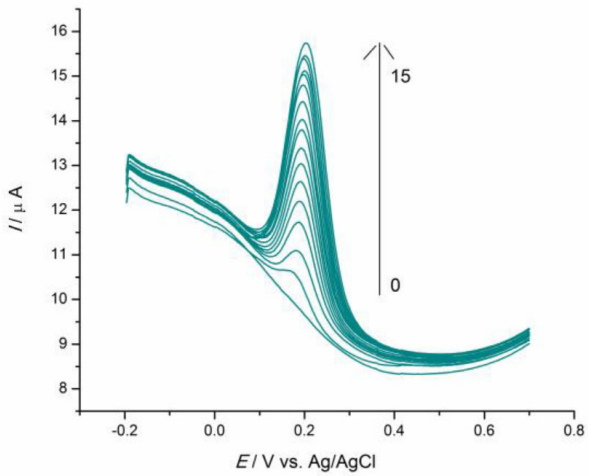


Fig. 5. Differential pulse voltammograms recorded for 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 μmol·L^{-1} DA in phosphate buffer of pH 7 after 180 s stirring with 600 rpm.

4.1 Stability and Reproducibility

The standard deviation of 3.5% was obtained for the 10 repetitive measurements of 6 μmol·L^{-1} DA under the optimized conditions indicates the good reproducibility. Developed sensor was stored under the ambient conditions for 20 days and then the electrochemical response for 6 μmol·L^{-1}. DA was measured under the optimized conditions displayed the standard deviation of 3.9 %. Developed sensor exhibited the good stability and reproducibility.

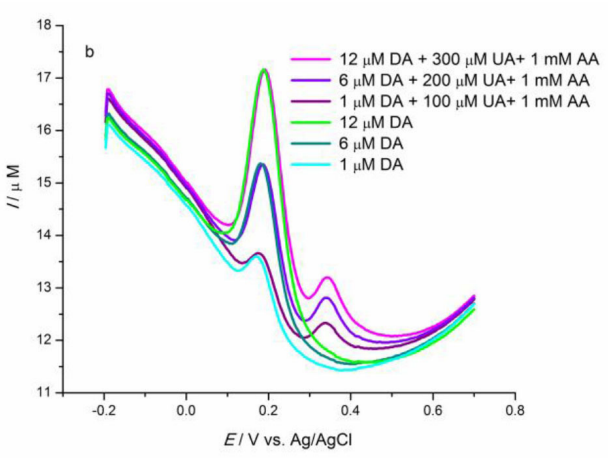


Fig. 6. (a) Overlay of the differential pulse voltammograms recorded for 1, 15 μmol·L^{-1} DA in the absence and presence of 1 mmol·L^{-1} AA (b) Overlay of the differential pulse voltammograms recorded for 1, 6, 12 μmol·L^{-1} DA in the absence, presence of various concentrations (100, 200, 300 μmol·L^{-1}) of uric acid and 1 mmol·L^{-1} AA under the optimized conditions.

4.2 Interference Study

Interference of various species generally present in urine samples along with DA were studied by recording the amperogram for 6 μmol·L^{-1} DA in phosphate buffer of pH 7 under stirring with 300 rpm speed. The concentration of species which caused more than 5% deviation in the DA signal was considered as an interferent. AA (1mM), UA (400 μmol·L^{-1}), glucose (100 μmol·L^{-1}), citric acid (150 μmol·L^{-1}) and $CaCl_2$ (200 μmol·L^{-1}) did not found to interfered in the quantification of DA

Comparison of the analytical figures of merit, working pH and the ease of preparation of the developed electrochemical sensor with that of reported methods has been summarized in Table 1. It can be observed that the electrode preparation procedure is complicated and time-consuming in case of RGO/Pd-NPs and Nafion/sulfonated-graphene/GCE sensors. Though the linear range, limit of detection and electrode preparation procedure of the other methods listed in Table 1 are appreciable, interference limit of AA is less than 100 fold excess. This may pose a serious problem when it comes to real sample analysis. It is evident from the comparison that, proposed 4-ABSA/GPLE provides the simple, sensitive and highly selective determination of DA in the presence of 1000 fold excess of AA. These advantages make the proposed sensor suitable for real sample analysis.

4.3 Sample Analysis

In order to examine the applicability of the 4-ABSA/GPLE for quantifying the DA in real sample matrices, the analyte was quantified in DA hydrochloride injection. A known volume of injection solutions was diluted with phosphate buffer of pH 7. Urine samples collected from

Table 1. Comparison of the important facts of various electrochemical systems developed for the determination of DA.

Material / electrode	pH	Linear range (μmol·L^{-1})	Detection limit (nmol·L^{-1})	Interference limit for AA (fold excess)	Working voltages (V) for AA	DA	UA	Electrode preparation procedure	Reference
Polychromotrope 2B/GCE	5	2.0-80.0	300	80	0.17	0.35	0.51	straightforward	34
RGO/Pd-NPs	7	1-150	233	—	NG	0.23	NG	complicated	35
Flower-like Au nanostructures/gold electrode	7.4	1-150	200	100	-0.03	0.16	ND	straightforward	36
porous gold nanosheets/GCE	7	2-298	280	10	ND	0.18	ND	straightforward	37
Poly(sulfosalicylic acid)/GCE	7.4	0.5-22.0, 22.0-110.0	5	20	0.02	0.14	ND	straightforward	38
PEDOT/DNA/CPE	5	0.25-66	74	1	ND	0.30	ND	straightforward	39
Methoxypolyethylene glycols/GCE	5	1.0-140.0	46.8	500	0.04	0.22	ND	straightforward	23
Nafion/sulfonated-graphene/GCE	7	0.2-20.0	20.0	1000	-0.08	0.14	ND	needs more than 12 h	12
4-ABSA/GPLE	7	0.5-10.0	95	1000	NS	0.19	0.34	straightforward	This work

ND-not done, NG-not given, NS-no signal

healthy persons were diluted with phosphate buffer of pH 7 to known volume, no pre-treatment was done.Then the analysis of each sample was done under optimized condition by following a standard addition method. Recovery studies were done by adding the known concentrations of DA to the prepared samples and then by quantifying it. Obtained results are summarized in Table 2, the recovery of the added DA was found to be 98.2 % and 100.7 % with a low relative standard deviation.This indicates the potential applicability of 4-ABSA/GPLE for the quantification of DA in real sample matrices.

Table 2. Results of determination of DA in injection and urine samples using 4-aminobenzene sulfonic acid /graphite pencil lead electrode (n = 5)

Sample	Added/ μmol·L^{-1}	Detected/ μmol·L^{-1}	RSD/ %	Recovery / %
Injection	00.00	5	3.15	–
	4.00	8.84	3.27	98.2
Urine 1	00.00	6.30	3.7	–
Urine 2	00.00	4.26	3.65	–
	4.00	8.32	3.72	100.7

5 Conclusions

Very simple and efficient methodology has been developed to covalently modify the good conducting, easily available and inexpensive GPL. Covalent binding of 4-ABSA onto the GPLE surface was well understood by CV and EIS studies. Electrochemical behaviour of AA, DA on bare and 4-ABSA/GPL electrodes were studied using CV, these results confirmed that the modified electrode completely eliminates the interference of 1 mmol·L^{-1}AA. Even in the case of DPV studies, neither the peak was observed for 1mmol·L^{-1} AA in addition to thatof DA nor the peak current of DA was altered noticeably. Hence using 4-ABSA/GPLE, DA can be selectively quantified in the range 0.5 to 10 μmol·L^{-1} even in presence of nearly 1000 folds excess of AA. UA was also not interfered since it exhibits a separate peak at 0.191 mV vs. Ag/AgCl that was 150 mV away from the DA signal. The narrow calibration range can be improved just by making use of GPL of greater diameter. Recovery and real sample analysis results infer that the sensor can be competently used to quantify DA. Hence this article describes a simple and selective method for the determination of dopamine in the presence of a large excess of ascorbic acid (but not other neurotransmitters). The method is applicable to sample matrices such as urine or injections, but probably not to whole blood where other (positively charged) neurotransmitters are present at pH 7 and likely to interfere.

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