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Carboxyl functionalized graphene oxide based electrochemical sensor for detection of dopamine in presence of ascorbic acid, uric acid and synthetic cerebrospinal fluid

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Dopamine is an important neurotransmitter and plays a vital role in the proper functioning of human metabolism. Therefore, fabrication of a sensitive sensor for the detection of dopamine is crucial. The work reports detection of dopamine in presence of interfering ascorbic acid, uric acid, Na^+, K^+, Ca^{2+}, urea and glucose. The linear range for the detection of dopamine is observed from 1.07×10^{-4} M to 2.19×10^{-4} M in presence of 1.00×10^{-3} M ascorbic acid and 1.00×10^{-3} M uric acid. The detection limit is estimated to be 1.46×10^{-8} M.

Keywords: Ascorbic acid, Cyclic Voltammetry, Dopamine, Electrochemical Impedance Spectroscopy, Cerebrospinal Fluid, Uric acid

Graphite is an allotrope of carbon with an important property of electrical conduction. It can be easily oxidized to graphite oxide (GO) using different oxidising agents. The first method to be demonstrated was by Brodie in 1859, in which potassium chlorate was added to graphite slurry in fuming nitric acid[1-3]. The second method was given by Staudenmaier in 1898, in which he added concentrated sulphuric acid to fuming nitric acid to a graphite slurry and followed it with gradual addition of potassium chlorate[1-3]. In 1958, Hummer developed a method in which he used potassium permanganate and sodium nitrate in presence of concentrated sulphuric acid[1-3]. Many methods have been developed so far for the better preparation of GO.

GO has a structure similar to that of graphite, but the plane of C-atoms is functionalized with oxygen-containing groups, such as, hydroxyl, epoxide and carboxyl[1,4]. These groups increase the interlayer distance between any two planes, and makes GO hydrophilic in nature[1].Ultrasonication of GO transforms it into a material with a few sheets of GO, which is then called graphene oxide[1]. Graphene oxide is also hydrophilic in nature[1,3], and hence gets dispersed in many solvents[1].

GO can be further functionalized with strong oxidising agents. Carboxyl groups are added in this manner, which makes the material more hydrophilic[4]. Also, with the help of these carboxyl functionalization, different moieties can strongly bind to the GO-COOH(CGO) material[4].

Dopamine (DA) is an important neurotransmitter[5-7] belonging to the family of catecholamines[8-11]. It is present in the central nervous system[6,8,9,12]. It can exist in three forms, viz. zwitterionic, protonated cationic and as deprotonated anion[5]. It is involved in a number of physiological processes of the renal, cardiovascular, central nervous and the hormonal system[9,11,13-15]. It is involved in the coordination and control of our cognition, emotion, movement, hormone secretion[8], metabolism and pressure regulation[16]. Any abnormalities in the neurotransmission of DA causes medical conditions, such as fibromyalgia, burning mouth syndrome, and restless leg syndrome[16]. Abnormal levels of DA in a person's body causes neurological conditions, such as Parkinson's disease, schizophrenia, autism, attention deficit hyperactivity disorder (ADHD), Alzheimer, Huntington's chorea, drug addiction, HIV infection[14,16-20].

DA is found to be present in the human body in concentration level of 10^{-7}–10^{-3} mol L^{-1}[8]. It also co-exists with a higher concentration of ascorbic acid (AA) and uric acid (UA) in the extracellular fluids of the central nervous system and in mammalian blood serum[8,11-13,15,17]. Also, the anodic potential of DA overlaps with the respective anodic potentials of AA and UA[7,8,11-13,15]. This makes the selective detection of DA somewhat difficult. A major part of the research

involving the detection of DA in the presence of AA and UA have been done in the field of electrochemistry owing to their electro-active nature. Electrochemical methods are also known to possess advantages of real-time detection, ease of operation, high sensitivity, better stability, low cost, and production of rapid results[9,10,18,20]. But, the detection of DA in the presence of higher concentration of AA and UA requires chemically modified electrodes because the unmodified electrodes have known to produce foul results because of the overlapping anodic potentials of the three species[18]. This has been a challenge, although many good results have been published in the literature.

In this work, we report the fabrication of two modified electrodes using GO and CGO, and compare between their sensitivity towards the detection of DA in the presence of AA, UA and synthetic cerebrospinal fluid. CGO proves to be a better electrode modifying agent for the detection of DA. The detection limit is calculated to be 1.46×10^{-8} M in aqueous medium and 1.32×10^{-10} M in synthetic cerebrospinal fluid.

Experimental Details

Graphite rods of used Eveready batteries (1.5 V) were taken for the preparation of GO. $NaNO_3$, $KMnO_4$, 30% H_2O_2, H_2SO_4, HCl, CH_3CN, NaOH pellets, styrene, $CaCl_2$, NaCl, KCl, $NaHCO_3$, urea and glucose were purchased from Merck. L-ascorbic acid and uric acid were purchased from Loba Chemie, and dopamine was purchased from Alfa Aesar. Double-distilled water from quartz double distillation plant was used throughout the work.

The electrode modifying agent was characterised by UV-visible absorption spectroscopy using UV-1800 SHIMADZU UV-spectrophotometer. Fourier transform infra-red (FTIR) spectroscopy was recorded by using SHIMADZU IR AFFINITY. Thermogravimetric analysis (TGA) was done using METTLER TOLEDO TGA/DSC 1 STAR Instrument. Powder X-ray diffraction (PXRD) was done using BRUKER ULTIMA IV X-RAY Diffractometer. Scanning Electron Microscopy (SEM) was done using ZEISS FESEM SIGMA 300. All the electrochemical measurements were carried out on a CHI 660D CH Instrument electrochemical analyser (USA) consisting of a three-electrode system: a bare GCE or a surface modified GCE as the working electrode, a Ag/AgCl (3 M NaCl) as the auxiliary electrode (AE), a Pt wire as the reference electrode (RE) and $NaNO_3$ as a supporting electrolyte (SE).

Preparation of GO and CGO

GO was synthesized using the modified Hummer's method[21]. It was further carboxyl functionalized to prepare CGO in concentrated sulphuric acid and concentrated nitric acid mixture under microwave radiation at 450 W for 10 min[22]. The dispersion was oven heated at 100 °C for 12 h and then centrifuged. The product obtained was washed with distilled water and then dried.

Preparation of GO modified GCE and CGO modified GCE

1 mg GO was dispersed in 5.0 mL acetonitrile to which 10 µL styrene was added. The dispersion was stirred for about 1 h, then 10 µL were pipetted out and casted on a cleaned GCE, as reported[23], and dried under N_2 environment. This process was repeated until the surface of the electrode was completely covered. The modified electrode shall be referred to as GO/GCE from now onwards. In the same manner, CGO modified GCE was prepared, and shall be referred to as CGO/GCE from now onwards.

Results and Discussion

GO and CGO were characterised using UV-visible absorption spectroscopy (Fig. S1), FT-IR spectroscopy (Fig. S2), PXRD (Fig. S3) and FESEM (Fig. S5). Their thermal stability was analysed using TGA (Fig. S4).

Influence of GO and CGO on the cyclic voltammetry of DA

The cyclic voltammogram of DA shows well-defined reversible redox peak at GO/GCE at + 0.100 V (E_R) and + 0.159 V (E_O) with a formal redox potential (E´) of + 0.130 V (Fig. 1(a)). The separation in the peak potential ($\Delta E = E_R-E_O$) is -0.059 V. The cyclic voltammogram of DA at CGO/GCE also shows a reversible redox peak at + 0.349 V (E_R) and + 0.408 V (E_O) with a formal redox potential (E´) of + 0.378 V (Fig. 1(b)). The separation in the peak potential (ΔE) is – 0.059 V. The reversible redox peak can be assigned to the oxidation of DA to dopamine quinone (DAQ) and the reduction of DAQ back to DA[18].

Influence of AA and UA on the cyclic voltammogram of DA

AA and UA do not interfere in the detection of DA at both the modified working electrodes. The peaks for the three species are well separated for detection.A reversible redox peak is observed for DA in the cyclic voltammogram at GO/GCE at + 0.413 V (E_R) and + 0.554 V (E_O), with a formal redox potential (E´) +0.484 V. The separation in the peak potential (ΔE) is -0.141 V (Fig. 2(a)). A reversible redox peak for DA is also observed in the cyclic voltammogram at CGO/GCE

with an increase in the peak current intensity at +0.489 V (E_R) and +0.551 V (E_R). The formal redox potential (E′) is +0.520 V and separation in the peak potential (ΔE) is –0.062 V (Fig. 2(b)). A difference in the separation in the peak potential is observed for the two modified electrodes. Since CGO is negatively charged carboxylate functionalized, CGO/GCE modified electrode oxidises the reduced DA species faster during the potential sweep and at a lower potential. This results in a lower ΔE value for CGO/GCE.

Limit of detection

The limit of detection (LOD) of DA at CGO/GCE is calculated as per the reported procedure[24] from:

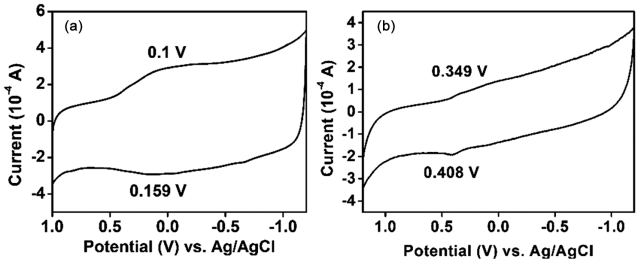

Fig. 1 — Cyclic voltammogram of 2.2×10^{-4} M aqueous DA (a) at GO/GCE working electrode and (b) at CGO/GCE working electrode. RE: Ag/AgCl, AE: Pt wire, SE: $NaNO_3$, Scan rate: 0.1 Vs^{-1}

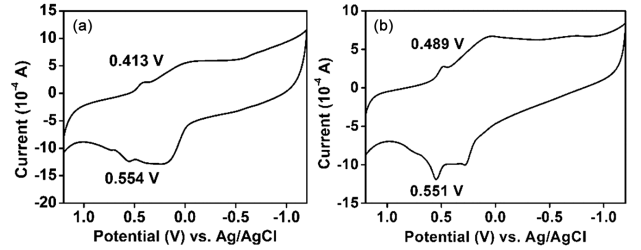

Fig. 2 — Cyclic voltammogram of 2.2×10^{-4} M aqueous DA in the presence of a mixture of 1.00×10^{-2} M AA and 1.00×10^{-3} M UA at (a) GO/GCE and (b) CGO/ GCE working electrodes. RE: Ag/AgCl, AE: Pt wire, SE: $NaNO_3$. Scan rate: 0.1 Vs^{-1}. The selectivity of CGO/GCE is better than of GO/GCE towards DA

LOD = (3×S.D.)/Slope

$$\text{Here, } S.D. = \frac{1}{(n-2)}\sum_{j=0}^{n}(i_j - I_j)^2 \quad \text{... (1),}$$

Where, n is the number of readings, i_j is the experimental value of reading 'j', I_j is calculated value of reading 'j' obtained from the regression line equation y = 0.80324x +106.52606. The LOD is calculated to be 1.46×10^{-8} M.

The limit of detection and linear ranges from some reported literatures have been compared and given in Table 1. The data obtained from the present work is better than or comparable to the ones given in the table.

Effect of scan rate on the cyclic voltammogram of DA in the presence of AA and UA

The effect of scan rate was studied at different scan rates from 0.05 mV/s to 0.5 mV/s using cyclic voltammetry. It is observed that with the increase of scan rate, the anodic and cathodic peak current intensity increases due to heterogenous kinetics (Fig. 3(b))[32]. Shift in the oxidation and reduction potential towards the positive and in the negative directions are also observed respectively, which is a result of diffusion-controlled process[25] (Fig. 3(a)).

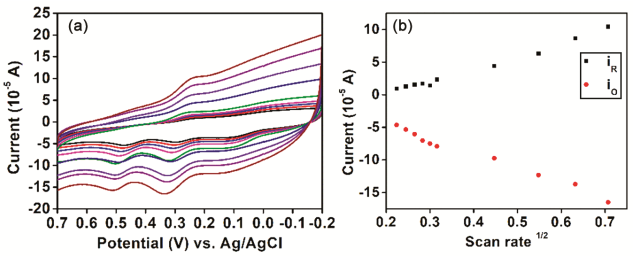

Fig. 3 — (a) Scan rate dependence on the cyclic voltammogram of 2.2×10^{-4} M aqueous DA in the presence of a mixture of 1.00×10^{-2} M AA and 1.00×10^{-3} M UA at CGO/GCE; (b) Current *vs.* $(\text{scan rate})^{1/2}$ plot

Table 1 — Comparison of different reported electrochemical sensors for the detection of DA

Modified electrode	Limit of detection	Linear range	Ref.
RGO/Cu-TECP/GCE	5.8×10^{-6} M	2×10^{-6} M – 2×10^{-4} M	9
OPPy/ERGO/GCE	5.0×10^{-7} M	2×10^{-6} M – 1.6×10^{-4} M	12
GO/$TmPO_4$/GCE	7.85×10^{-7} M	2×10^{-6} M – 2×10^{-5} M	15
GO/CPE	1.5×10^{-8} M	7×10^{-8} M – 7×10^{-5} M	25
GPU	6.4×10^{-8} M	—	26
Graphene/GCE	2.64×10^{-6} M	4×10^{-6} M – 1×10^{-4} M	27
FGGE	2.5×10^{-7} M	5×10^{-7} M – 5×10^{-5} M	28
Graphene modified electrode	5.0×10^{-7} M	2.5×10^{-6} M – 1×10^{-4} M	29
GO/GCE	2.7×10^{-7} M	1×10^{-6} M – 1.5×10^{-5} M	30
RGO/GCE	1×10^{-7} M	1×10^{-7} M – 4×10^{-4} M	31

Diffusion co-efficient

The effective surface area (A_{eff}) of CGO/GCE working electrode is firstly calculated using the reported method[33] from the Randles-Sevcik equation for a reversible redox reaction

$$i_R = (2.69 \times 10^5) \times n^{\frac{2}{3}} \times A_{eff} \times D^{\frac{1}{2}} \times \nu^{\frac{1}{2}} \times C_{bulk} \quad \ldots (2)$$

Here, n is the number of electrons transferred in the redox reaction, D is diffusion co-efficient and $C_{bulk}$is the bulk concentration of 4 mM $K_4[Fe(CN)_6]$ in 1 M KCl and ν is the scan rate. The slope of i_R *vs.* $\nu^{1/2}$ plot is 8.61957 $\times 10^{-4}$ A $V^{-1/2}$ $s^{1/2}$, the value of D is 6.3×10^{-10} m^2 s^{-1}(Ref.34). Therefore, A_{eff} is calculated to be 0.319 cm^2. Again, using the same method and putting the value of A_{eff} in the Randles-Sevcik equation, the value of D is calculated for DA in the presence of AA and UA at CGO/GCE calculated to be 4.26×10^{-9} m^2 s^{-1}.

Electrochemical impedance spectroscopic analysis of CGO/GCE

The EIS measurements of interaction between DA and CGO/GC working electrodein the presence of interfering species AA and UA were executed at E_{DC} = 2.0 V. From the EIS Nyquist plots it is observed that charge transfer resistance (R_{CT}) decreases with increase of different added concentration of DA (Fig. 4(a)). The difference in charge transfer resistance (ΔR_{CT}) was found to increase linearly with increase in added concentration of DA (Fig.4(b)).

Determination of DA in synthetic cerebrospinal fluid

The synthetic cerebrospinal fluid (SCF) is prepared by mixing 84 mg NaCl, 2.8 mg KCl, 3.2 mg $CaCl_2$, 8 mg glucose, 16 mg $NaHCO_3$ and 0.08 mg urea in 10 mL aqueous solution[26]. The determination of DA in SCF was done to evaluate the selectivity of both the modified electrodes towards DA with and without the presence of AA and UA. A quasi-reversible redox peak is observed due to DA at both the modified electrodes (Fig. 5(a)), whereas a clean cyclic voltammogram is obtained for SCF. The redox peak obtained at CGO/GCE (blue curve) is associated with greater current. Again, AA and UA also do not interfere in the sensitivity of the modified CGO/GCE working electrode (Fig. 5(b)).The LOD of DA in SCF in presence of AA and UA is calculated to be 1.32×10^{-10} M.

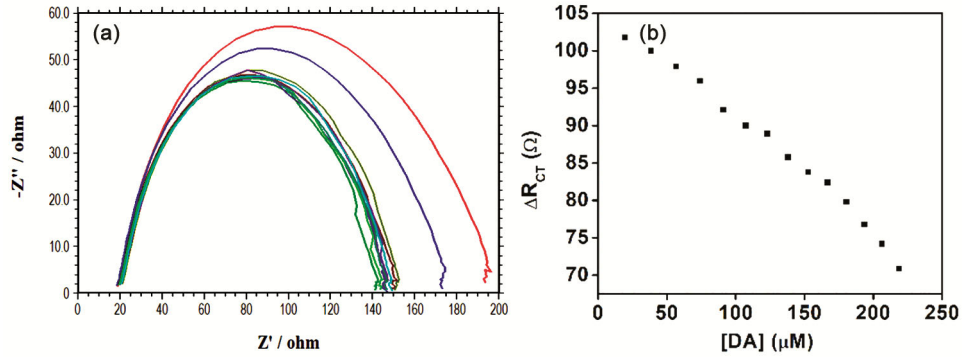

Fig. 4 — (a) EIS Nyquist plot of CGO/GCE at different added concentration of DA; (b) Plot of ΔR_{CT} *vs* different added concentration of DA

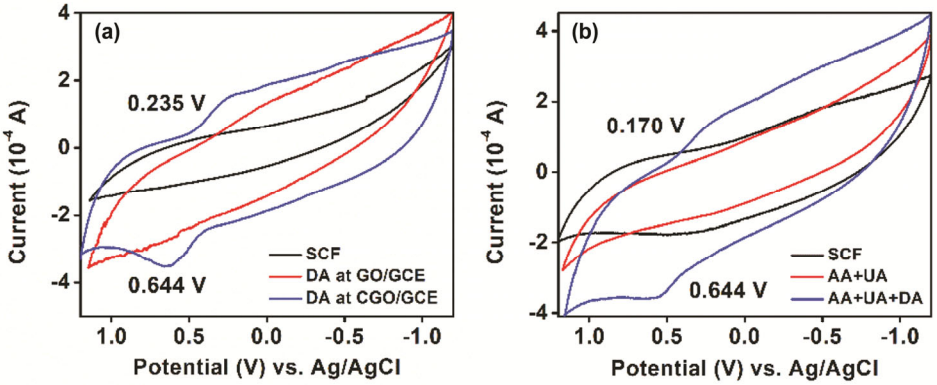

Fig. 5 — (a) Cyclic voltammogram of SCF at GO/GCE (black), cyclic voltammogram of DA in SCF at GO/GCE (red), and at CGO/GCE (blue). (b) Cyclic voltammogram of SCF (black), a mixture of AA and UA in SCF (red), and DA in presence of AA, UA and SCF (blue) at CGO/GCE

Conclusions

We have reported that CGO/GCE act as a sensitive dopamine sensor using cyclic voltammetry and electrochemical impedance spectroscopy. Ascorbic acid, uric acid, urea, glucose and ions, such as, Na^+, K^+ and Ca^{2+} do not interfere in the detection of dopamine. The modified CGO/GCE electrode can also detect dopamine in synthetic cerebrospinal fluid. Also, graphite rods from used batteries have been successfully utilized in the preparation of the material.

Supplementary Data

Supplementary information is available in the website http://nopr.niscair.res.in/ handle/123456789/ 58776.

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