



Highly sensitive electrochemical sensing of neurotransmitter dopamine from scalable UV irradiation-based nitrogen-doped reduced graphene oxide-modified electrode

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Abstract. Present study develops a facile, low-temperature and cost-effective route for the synthesis of nitrogen-doped reduced graphene oxide (N-rGO). The synthesized N-rGO was characterized using X-ray diffraction (XRD), micro-Raman and Fourier transform infrared (FTIR) spectroscopies. An electrochemical sensor using N-rGO-modified glassy carbon electrode (GCE) was fabricated for the determination of dopamine (DA), a neurotransmitter. Because the electrochemical determination and quantification of DA play a significant role in medical diagnosis, such as making soft material-based hydrogel for wound healing. Cyclic voltammetry (CV), amperometry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS)-based standard techniques were used to evaluate and establish the optimum electrochemical sensing performance, detection limit, steadiness and reliability of N-rGO/GCE sensing system to the DA detection. The DPV measurements resemble a wide linear range from 100 to 3000 μM and demonstrated a limit of detection (LOD) of 57 nM. It is evidently proved that N-rGO/GCE has great potential to be a preferable electrochemical sensing system for DA detection.

Keywords. Nitrogen-doped; reduced graphene oxide; soft material dopamine; electrochemical; sensor .

1. Introduction

Dopamine (DA), chemically called 4-(2-aminoethyl) benzene-1,2-diol, is an important biological molecule and more specifically is a hormone belonging to the family of catecholamines [1]. Dopamine and its derivatives are applied in the synthesis of various types of soft materials, such as wound healing hydrogel, making adhesives, water absorbents, actuators and soft healing hydrogels. DA is majorly involved in chemical transmission of nerve impulses, acting as a neurotransmitter, which influences the central nervous system, attention duration, cognition, emotions and neuronal plasticity [2]. DA is present in the range of 10 nM to 1 μM in brain fluids [3]. Moreover, DA is also available as an intravenous medication to increase heart rates and blood pressure [4]. Neurological disorders like Parkinson's disease, schizophrenia, epilepsy are caused due to abnormal levels of DA in the living beings [1,5]. Detection of neurological disorders in patients at an early stage is essential so as to treat them easily. Prompt diagnosis has higher chances to combat a disease before symptoms worsen. Thus, the detection and sensitivity to a particular molecule of DA are of great importance in the clinical diagnosis. Especially, at a very

low concentration of DA, the existing electrode-based system for detection is less sensitive and predictable compared to biomarkers-based detection. However, DA is highly soluble in water (~30 g per 50 ml) and thus, it is electrochemically and spectroscopically active. Therefore, it is conceivable that the detection of DA can be performed based on electrochemical and spectroscopic techniques, such as cyclic voltammetry (CV) and reflection absorption infrared spectroscopy [1]. In addition, DA can also be determined by chromatography, fluorometry, electrochemiluminescence, calorimetric and high-performance liquid chromatography (HPLC). Conversely, the electrochemical prospective method for the detection of DA is globally accepted owing to its fast detection, high electrochemical activity, reproducibility, cost-effectiveness, ease of detection, the potential for miniaturization and capability of *in-situ* detection [6].

As described, the electrochemical sensors are highly sensitive analytical tools with rapid detection [7]. The sensing of DA based on electrochemical measurements is carried out usually *via* the oxidation process and results in the formation of dopamine quinone (DAQ) [8]. Therefore, modified electrodes are used for the detection of DA as bare electrodes have poor sensitivity towards DA, particularly for the lower

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limit of detection. Recently, Xie *et al* [9], Huang *et al* [10], Yue *et al* [11], proposed a composite of SnO₂-rGO, Cu₂O-carbon dots, ZnO nanowires resulting in a limit of detection (LOD) value of 1, 0.1, 0.5 μ M, respectively. Along with, a wide variety of nanomaterials/composites reported for DA sensing suffers from cumbersome synthesis, limited sensitivity and low response signal due to slow electron-transfer rate kinetics [3].

More recently, graphene an archetypal two-dimension carbon material has sparked tremendous interests across many disciplines, including electronics applications in transparent electrodes [12], active material for energy harvesting and storage [12] and channel materials for FETs [13,14]. Furthermore, graphene has a great potential in various novel sensors, which utilize graphene's exceptional electrical properties; extremely high carrier mobility and capacity, electrochemical properties; high electron transfer rate, optical properties; excellent ability to quench fluorescence, structural properties; one-atom thickness and extremely high surface-to-volume ratio, or its mechanical properties; outstanding robustness and flexibility [15].

On the other hand, pure graphene is intrinsically rather inert [16], while graphene oxide (GO), a functional group rich, layered carbonaceous insulating material has gained significant attention for chemical and biological sensors owing to the excellent physicochemical properties [17–19]. Even then, GO containing abundant oxygen functional groups was too electrically insulated to be used in a resistance/conductance-based highly sensitive sensors [20]. Instead, reduced graphene oxide (rGO) formed after removal of attached functional groups of GO is reported to act as a conductor, with conductivity considerably higher than that of GO [21,22] and has sufficiently higher number of chemically active defect sites, which makes it a potential candidate for use in sensor-based applications [17]. However, the synthesis steps of rGO usually involve: (i) oxidation of graphite in presence of oxidizing conditions, followed by washing and centrifugation to result in GO [23]; and (ii) thermal, chemical strategies for the reduction of GO to rGO. Although, the thermal and chemical approaches for

the production of rGO suffers from several limitations, as discussed in previous reports elsewhere [24]. Additionally, doping of rGO is an active immense interest area of ongoing research, because doping generally tailors the physical and chemical properties of rGO and enables its application for various electronic and biological purposes [25,26]. In this classification, nitrogen is thought to be an excellent choice for the doping of rGO due to its comparable atomic size and availability of valence electrons to form strong bonds with carbon atom [25–27]. Here, N-doping increases the mobility of electrons and introduces chemically active sites beneficial for catalytic reactions [28,29]. However, commonly available nitrogen doping methods are based on high-temperature processes like nitrogen plasma process, chemical vapour deposition (CVD), hydrothermal/annealing, which are highly costly, resource-consuming, complicated in operational nature and moreover involves toxic reagents also [26,30].

Therefore, in the present study, we report a rapid, facile, photo-catalytic, low temperature and cost-effective approach for the synthesis of nitrogen-doped reduced graphene oxide (N-rGO). GO was synthesized using the modified Hummer's process, as reported in previous work [31]. Then, for the synthesis of N-rGO, aqueous GO was mixed with ammonia solution and the mixture was stirred followed by photo-catalytic reduction. Subsequently, the as-synthesized N-rGO was targeted to construct a highly sensitive, low-cost electrochemical sensor for the rapid detection of DA. The novelty of the present work lies in the facile and cost-effective approach to synthesize N-rGO. Also, the performance of the N-rGO/glassy carbon electrode (GCE) sensor was compared with other dopamine sensor systems and a complete description of the detection limit and the linear range are listed in table 1. It can be inferred that although the detection limit is inferior to WO₃ [6], rGO-Cu₂O [3,32], gold/cysteamine/dihydrolipoic acid [33] composites, the wide linear range is superior to other electrochemical sensor systems. Also, the present N-rGO-based sensor has a better detection limit in comparison to many metals-doped composites, which adds to its advantage.

Table 1. Comparison of LOD for various dopamine sensors.

Electrode material	pH	LOD (μ M)	Linear range (μ M)	References
rGO-Cu ₂ O	7.0	0.05	10–900	[3]
SnO ₂ /rGO	6.8	1	1–20	[9]
Cu ₂ O/carbon dots	7.2	0.1	0.01–100.5	[10]
ZnO (nanowire)	7.4	0.5	0.5–40	[11]
Fe ₃ O ₄ /rGO	7.0	0.08	0.4–160	[55]
Pt/graphene/MnO ₂	7.0	0.1	1.5–215.5	[52]
Cu ₂ O/rGO	5.6	0.01	0.1–10	[32]
WO ₃	7.0	0.024	0.1–600	[6]
Gold nanoparticles/cysteamine/ dihydrolipoic acid	7.0	0.023	0.0001–0.85	[33]
N-rGO	6.0	0.057	100–3000	Present work

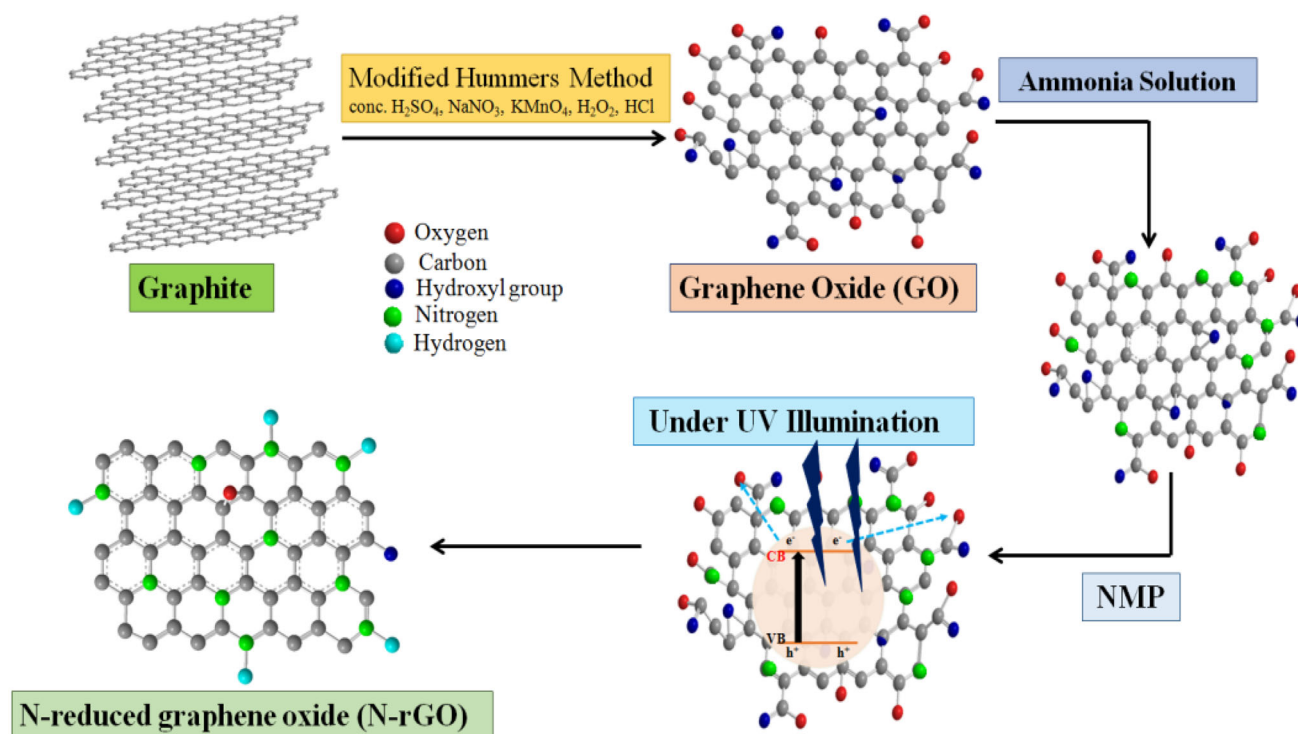


Figure 1. Schematic illustration for the synthesis of N-rGO.

2. Materials and methods

2.1 Materials

Analytical grade chemicals were used as received without further purification for the present study. Graphite powder (99.999%, particle size $< 50 \mu\text{m}$), dopamine, ethanol, sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), sodium chloride (NaCl), phosphoric acid (H_3PO_4), sodium hydroxide (NaOH) were used from Merck, India. Deionized water ($18.2 \text{ M}\Omega \text{ cm}$, Elga Make) was used throughout the experiments. The standard phosphate buffer saline solution (PBS 0.1 M) containing 0.15 M NaCl with different pH values was prepared by mixing stock solutions of Na_2HPO_4 (0.08 M) and NaH_2PO_4 (0.02 M) pH (1–10) was adjusted using H_3PO_4 and NaOH .

2.2 N-rGO synthesis procedure

GO was synthesized from parent graphite powder using the modified Hummer's method, as reported in our previous reports [31]. For the synthesis of N-rGO, aqueous GO dispersion (1 mg ml^{-1}) was mixed with an ammonia solution (~ 30 – 50 ml) and the mixture was heated at 50°C under constant stirring at 600 rpm for 8 h. Then, N-methyl-2-pyrrolidone (NMP ~ 10 – 15 ml) was added to the aqueous solution followed by UV illumination for 60 min. Three different N-rGO samples were prepared by the variations in

ammonia solution. The change in colour of the aqueous solution from yellowish-brown to black confirms the reduction of GO to N-rGO. The aqueous solution was dried in a vacuum oven at 80°C to form an N-rGO composite. Figure 1 shows a schematic illustration for the formation of the N-rGO composite.

2.3 Material characterization

The nitrogen doping was confirmed using Fourier transform infrared (FTIR) characterization technique. The FTIR measurements were acquired from Agilent Technologies Cary 600 Series FTIR spectrometer. The synthesized N-rGO was further characterized using X-ray diffraction (XRD) and Raman spectroscopy. XRD measurements were performed on Rigaku Smart Lab 9 KW rotating anode powder X-ray diffractometer using advance $\text{CuK}\alpha$ radiation ($\lambda = 1.54 \text{ \AA}$). Raman spectroscopy was performed on a LabRAM HR evolution microscopic confocal Raman spectrometer (Horiba, Japan) using green laser excitation. Scanning was performed on an extended range (800 – 2000 cm^{-1}). For FTIR spectra and XRD, the aqueous solutions were sonicated and dried in a vacuum oven at 50°C . For Raman measurements, the aqueous solutions were sonicated and drop-cast on freshly cleaned p-Si wafers. The morphological structure was analysed by the scanning electron microscope (SEM, Zeiss, Germany). Elemental composition and electronic states were analysed by X-ray photoelectron spectroscopy (XPS, Nexsa base, Thermo Fisher Scientific) in the binding energy range of 0 – 1300 eV .

Specific Brunauer–Emmett–Teller (BET) surface area and pore size were determined by the nitrogen adsorption–desorption method (Quantachrome Autosorb iQ3 system) at 77 K. The sample was degassed at 200°C for 4 h in a vacuum.

2.4 Electrochemical investigations

All electrochemical measurements such as CV, amperometry, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were carried out using CH600E (CH Instruments, USA) based conventional three-electrode assembly. Platinum wire and Ag/AgCl (1 M KCl) were used as counter and reference electrodes, respectively. The GCE with a 3 mm diameter was employed as the working electrode. The GCE was sequentially polished with alumina slurry (1.0, 0.3 and 0.05 μm) and fine emery paper to a mirror finish followed by ultrasonication to remove any adsorbed impurities. Afterwards, $\sim 40 \mu\text{l}$ of N-rGO (1 mg ml^{-1}) mixed with Nafion ($\sim 10 \mu\text{l}$) was dropcast on the clean GCE surface and dried at 80°C. Firstly, the electrocatalytic studies were carried out based on CV measurements using N-rGO/GCE and a mixture of freshly prepared DA (400 μM), PBS (0.1 M, pH 1.0–10.0) containing 0.15 M NaCl. The potential was scanned between -1 to 1 V at a scan rate of 0.1 V s^{-1} . The study was performed to understand the influence of pH on the detection of DA. Afterwards, the PBS (0.1 M, pH 6.0) containing 0.15 M NaCl was used for the determination of DA. All pH measurements were carried out using the pHep® digital pH meter (Hanna instruments).

3. Results and discussion

3.1 Characterization

Synthesis of GO, rGO and N-rGO was confirmed using FTIR spectroscopy (figure 2a). As demonstrated by FTIR spectrum, the GO sheet shows the sp^3 hybridized, absorption band peaks

for hydroxyl ($-\text{OH}$), carbonyl ($\text{C}=\text{O}$), epoxy ($\text{C}-\text{O}-\text{C}$), carboxyl ($\text{O}=\text{C}-\text{OH}$) groups and characteristic sp^2 hybridized carbon ($\text{C}=\text{C}$) peak at 3371, 1735, 1386, 1057, 956 and 1615 cm^{-1} , respectively [31]. On the other hand, the FTIR of rGO shows a characteristic sp^2 hybridized carbon ($\text{C}=\text{C}$) peak at 1650, 1570 cm^{-1} [34,35], and considerable decrease in the intensity of absorption band peaks for oxygen-containing functional groups (at about 1330 cm^{-1} due to carboxy $\text{C}-\text{O}$ stretching, and 1000 cm^{-1} due to alkoxy $\text{C}-\text{O}$ stretching). On the other hand, the FTIR for N-rGO shows new vibrational bonds such as $\text{N}-\text{H}$ (3300 cm^{-1}), $\text{C}-\text{N}$ (1300, 1051 cm^{-1}) [36]. The peak at $\sim 1630 \text{ cm}^{-1}$ corresponds to $\text{C}=\text{C}$ stretching [36].

The typical XRD patterns of GO, rGO and N-rGO were performed and results are shown in figure 2b. For GO, the XRD shows a peak at around $2\theta = \sim 10^\circ$ which corresponds to the (002) plane with interlayer spacing (d) of 0.88 nm [36]. However, in the case of rGO and N-rGO the peak at around 10° vanishes and a new peak at $2\theta = \sim 23^\circ$ corresponding to the (002) plane with $d \sim 0.4 \text{ nm}$ is observed which confirms the GO reduction [37]. The strong (002) graphitic peak at $\sim 23^\circ$ attributes to a high crystalline degree for all the doped and undoped rGO samples [36].

The Raman spectroscopy of GO, rGO and N-rGO was performed for the investigation of the graphitic nature as shown in figure 2c. The Raman spectrum of GO shows defect induced D band [38] (due to a breathing mode of A_{1g} symmetry) and G band (due to doubly degenerate phonon mode of E_{2g} symmetry) [39] at 1353 and 1597 cm^{-1} , respectively. The Raman spectroscopy of GO shows I_D/I_G ratio of ~ 0.98 . In contrast, the Raman spectroscopy of rGO shows an enhanced D band peak (with I_D/I_G of ~ 1.15) which is attributed to the decrement in the average size of the sp^2 hybridized carbon atom thereby resulting in the formation of smaller graphitic domains on reduction [40,41]. Similarly, for N-rGO, there is a further increment in $I_D/I_G \sim 1.26$ which may be attributed to the increase in defects on doping [3], and strong interaction of nitrogen with GO on reduction.

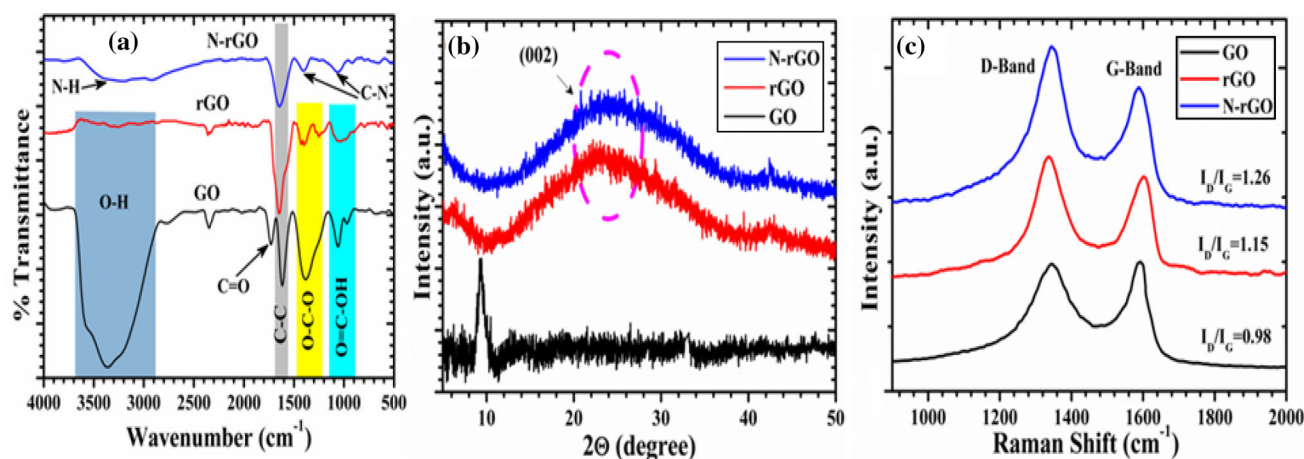


Figure 2. Material characterization of GO, rGO, N-rGO (a) FTIR absorption spectra, (b) XRD spectra and (c) Raman spectroscopy.

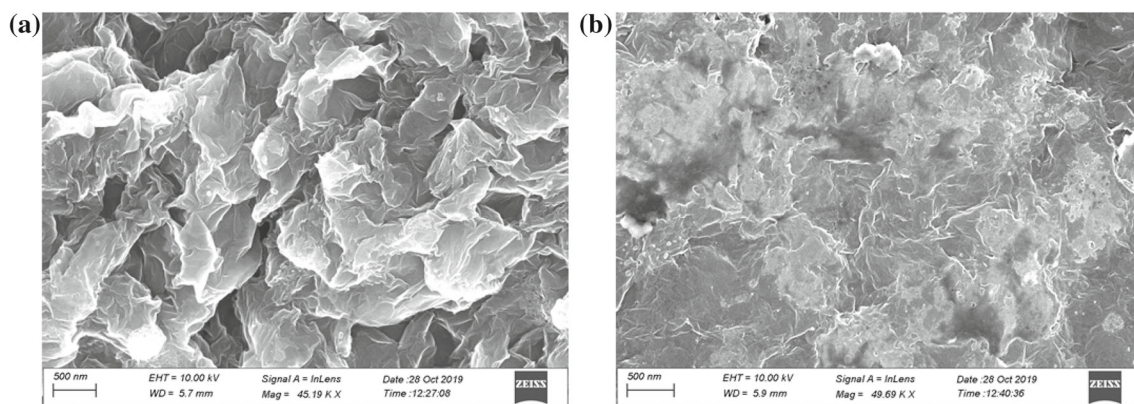


Figure 3. SEM images of (a) rGO and (b) N-rGO.

The intensity ratio of D and G bands (I_D/I_G) is used to explain the doping or defects phenomenon in graphene [26]. The variations in nitrogen doping is further analysed by Raman spectroscopy and the results are presented in figure S1 (supplementary information). The I_D/I_G ratio is increased from 1.23 to 1.33 when the nitrogen doping is increased. This indicates that the amount of nitrogen doping enhances the defects in N-rGO electrode. The surface morphological study of the as-prepared rGO and N-rGO was performed by FESEM. Figure 3a clearly shows the SEM image of rGO with disintegrated sheets and wrinkled like structure. Figure 3b shows the image of N-rGO with a rough and undulated fracture structure.

Nitrogen adsorption–desorption isotherm and pore size distribution of N-rGO are shown in figure S2a–b (supplementary information). The BET surface area and total pore volume were calculated as $13 \text{ m}^2 \text{ g}^{-1}$ and 0.0179 cc g^{-1} , respectively. The average pore size radius of $17.02 \text{ \AA}$ was measured (figure S2b, supplementary information). XPS was further performed to analyse the elemental composition and electronic states of the elements in N-rGO electrode. Figure S3a (supplementary information) shows the survey spectra of N-rGO with different amounts of nitrogen doping. Carbon, oxygen and nitrogen were clearly visualized in the survey spectra. The increase in ammonia concentration first leads to the increase in nitrogen doping in rGO followed by the decrease in nitrogen concentration as shown in figure S3b (supplementary information). The atomic percentage of nitrogen was measured as 5.39, 6.52 and 6.23% when increasing the ammonia concentration during the synthesis of N-rGO. The high-resolution deconvoluted spectra of C 1s, O 1s and N 1s are shown in figure S4a–i (supplementary information). The deconvoluted peaks of carbon were identified at 284.8, 286.1, 287.2 and 288 eV corresponding to the C=C, C–N, C–O–C and C=O bonds, respectively [42]. Also, the deconvoluted peaks of oxygen 1s spectra were found at 530.8, 532.4 and 533.3 eV corresponding to the COOH, C=O and C–O bonds, respectively. The deconvoluted peaks of nitrogen 1s spectra were identified at 398.4, 399.6 and 401.5 eV corresponds to pyridine,

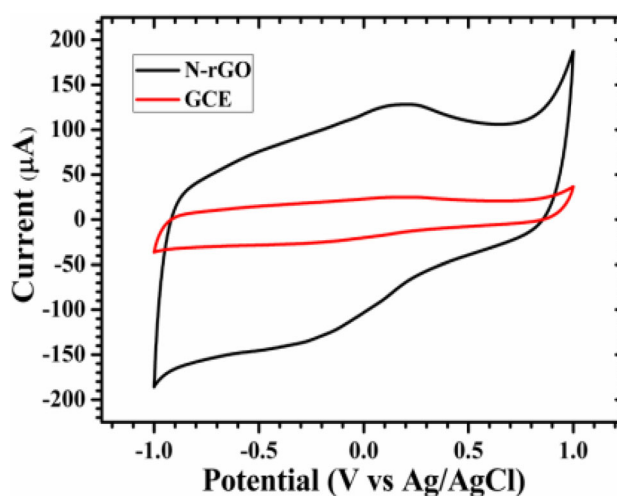


Figure 4. Cyclic voltammograms of $400 \mu\text{M}$ DA in PBS (pH 6.0) at the bare GCE and N-rGO-modified GCE.

pyrrolic and quaternary nitrogen, respectively [42]. We further checked the C–N bond percent in different N-doped rGO. It is clear from the deconvoluted C 1s peaks that the C–N bond percent was first increased from 15.9 to 31.3% followed by the decrease up to 23% when the ammonia concentration increases. Our results are analogous to the others work as reported in the literature [42,43]. Therefore, the high percentage of nitrogen-doped rGO was used as a sensing electrode material in this work.

3.2 Electrochemical characteristics of N-rGO/GCE

Application of potential to the N-rGO-modified GCE, results in the electrocatalytic oxidation of DA to DAQ. The DAQ formation is owing to the exchange of electrons between the phenyl-containing functional group present in DA and π – π stacking in rGO [44]. As a result of this interaction, the electrons are donated to N-rGO-modified GCE to produce a Faradic current [38].

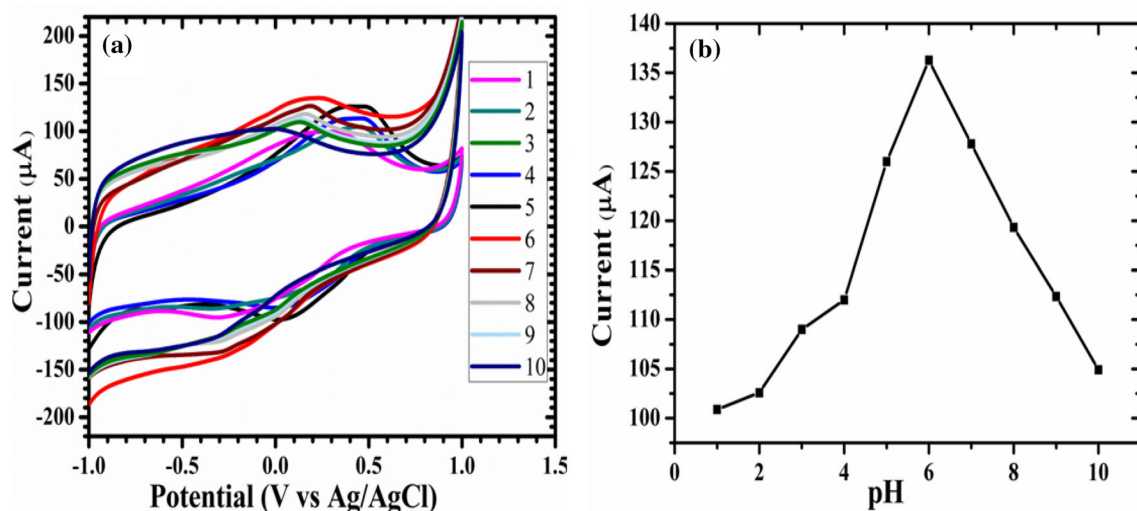


Figure 5. (a) Influence of pH on the CV of DA at the surface of N-rGO-modified electrode. (b) Current–pH curve for electrooxidation of 400 μM DA at N-rGO/GCE with a scan rate of 0.1 V s^{-1} .

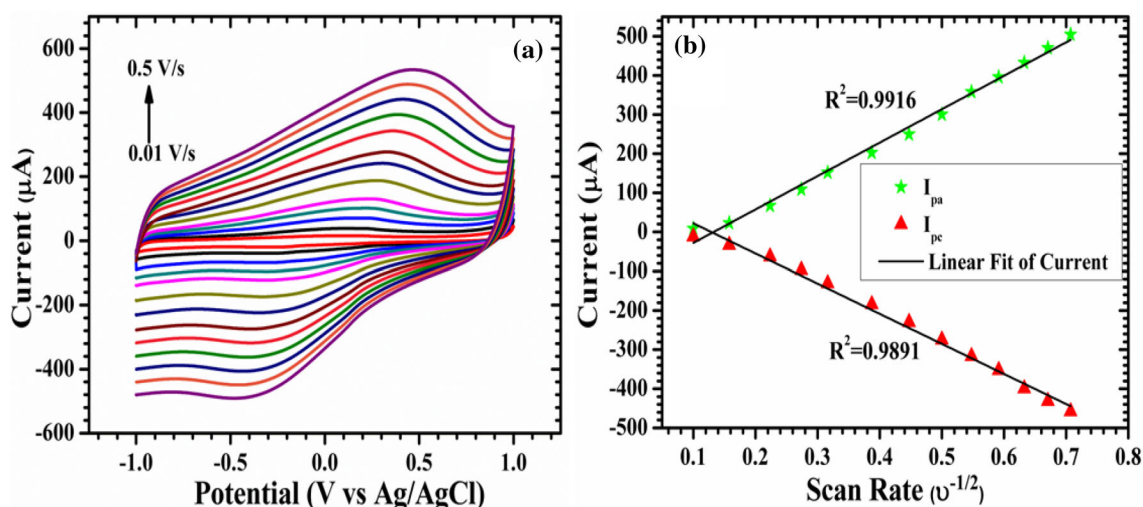


Figure 6. (a) CVs of the N-rGO electrode in 0.1 M PBS (pH = 6.5) solution containing 400 μM DA at scan rates from 0.01 to 0.5 V s^{-1} . (b) The plots of anodic and cathodic peak currents to the square root of scan rate.

The electrochemical responses of DA at the bare GCE and the N-rGO/GCE were examined using CV in a 400 μM DA solution (pH 6.0). As shown in figure 4, almost five times increase in the value of peak current was recorded for N-rGO-modified GCE in comparison to bare GCE. At the bare GCE, DA showed a relatively weak redox peak due to the sluggish electrochemical response towards DA and restricted diffusion of DA [45]. However, when the GCE surface was modified with N-rGO, a significant response in redox peak current was observed for the modified electrode due to enhanced electron transfer process. The N-rGO-modified GCE shows sensitivity towards the electrochemical redox of DA. Nitrogen atoms act as an electron donor and increase the n-type conductivity of GO due to its high electronegativity

(3.04 in the Pauling scale) compared to C (2.55 in the Pauling scale) [36]. N doping enhances the conductivity of rGO owing to the introduction of free electrons in GO [46].

The effect of pH value on the electrochemical behaviour of 400 μM DA in 0.1 M PBS at N-rGO/GCE was investigated by CV in the pH range of 1.0–10.0. Figure 5a depicts CV curves for DA oxidation at different pH and figure 5b presents the plot of peak current with respect to pH for DA oxidation. The maximum peak current was obtained at pH 6.0 (figure 5b). Hence, considering the sensitive determination for DA, the PBS of pH 6.0 was chosen as optimal pH for further experimental studies.

To investigate the reaction kinetics, figure 6a shows the CV of 400 μM DA at N-rGO/GCE-based sensor system at

different scan rates (0.01–0.50 V s⁻¹). With the increase in scan rate from 0.01–0.50 V s⁻¹, an increase in anodic peak current can be observed. It was noticed that both anodic peak (I_{pa}) and cathodic peak (I_{pc}) current increases linearly with the square root of scan rate ($v^{1/2}$) and the calibration equations are $I_{pa} (\mu A) = 854v^{1/2} (V s^{-1})^{1/2} - 113$ ($R^2 = 0.9916$) and $I_{pc} (\mu A) = -768.50v^{1/2} (V s^{-1})^{1/2} + 98.84$ ($R^2 = 0.9891$) (figure 6b). In this process, when the potential is swept from -1 to 1 V, initially DA ions move towards the N-rGO electrode surface and starts filling the pores in N-rGO/GCE electrode, resulting in the gradual increase of current. As the potential approaches more positive value, more DA ions move and this swing continuously followed as perceived from the graph for the N-rGO/GCE electrode, which further increases the current, thereby finally attains its maximum value at 0.5 V. Beyond this potential, the available pores in the N-rGO/GCE electrode are completely exhausted and no more dopamine ions can be trapped in the N-rGO/GCE, leading to a decrease in response to the current value. This shows that the electron transfer for DA at the N-rGO/GCE electrode may be a diffusion-controlled mechanism. This observation corroborates with the analogous results reported in the literature [6,44,47,48].

In addition, figure 6a also reveals that on increasing the scan rate, the redox peak potentials shifted slightly, which suggests that the reaction is a quasi-reversible electrochemical process [2]. The number of electrons participating in the electrode reaction (n) for a reversible couple is computed from the separation between the peak potentials using the relation in equation (1) [49].

$$\Delta E_p = E_{pa} - E_{pc} \cong \frac{0.059}{n} \quad (1)$$

where ΔE_p is peak potential difference, E_{pa} is anodic peak potential and E_{pc} is cathodic peak potential. The potential measured from the separation of the peaks in the present study is 0.035 V. Therefore, the number of electrons transferred was computed to be 2. Thus the oxidation of DA probably involves two electrons which are consistent with the previous reports [38,47]. To obtain the kinetic parameters of the electrochemical process E_p values were plotted vs. logarithmic of the scan rate (figure S5 in supplementary information). A linear relationship was observed when the scan rate approached above 0.15 V s⁻¹ (figure S7 in supplementary information). According to the Laviron theory, the linear slopes of E_{pa} and E_{pc} are shown in equations (2) and (3), respectively [50]

$$E_{pa} = \frac{2.3RT}{(1 - \alpha)nF}, \quad (2)$$

$$E_{pc} = \frac{-2.3RT}{\alpha nF}. \quad (3)$$

The computed electron transfer coefficient (α) was 0.55, which is consistent with the typical value of 0.5 observed for

electrochemical reactions [38,44]. The charge transfer constant k_s was evaluated using equation (4) [38,51]

$$\log k_s = \alpha \log (1 - \alpha) + (1 - \alpha) \log \alpha - \frac{\log (RT/nFv) \alpha (1 - \alpha) nF \Delta E_p}{2.3RT} \quad (4)$$

where v is the scan rate, n the number of transferred electrons, F the Faraday's constant, R the universal gas constant and T the temperature. The k_s value was calculated as 0.89 s⁻¹. The plot of the anodic peak current (figure S8 in supplementary information) against the scan rate shows a linear relationship, which was employed to determine the surface concentration of electroactive species (Γ) using equation 5 [48]. The electroactive species refer to the oxidizable electroactive species, which are participating in the reaction.

$$I_p = \frac{n^2 F^2 A \Gamma v}{4RT}, \quad (5)$$

where n represents the number of electrons involved in the reaction, A the geometric surface area of the electrode (0.07 cm²), Γ (mol cm⁻²) the surface concentration of electroactive species and v the scan rate. R , F and T have their usual meanings as mentioned earlier. The slope of the anodic peak current vs. the scan rate was found to be 0.001. From the slope, the surface concentration of electroactive species in N-rGO/GCE was found to be 3.8×10^{-9} mol cm⁻².

Chronoamperogram plots (current vs. time) for N-rGO/GCE and reference rGO/GCE were obtained at different concentrations of DA ranging from 0.1 to 3000 μ M at a fixed potential of 0.2 V, as shown in figure 7. As it can be clearly seen from figure 7a that the value of current for DA based on N-rGO/GCE rises rapidly up to 'X' (~13 s) (from 0) and afterwards it saturates, hence, the sensor approaches the 90% of steady-state current value within ~13 s. While for rGO/GCE (figure 7b), the current value increases very slowly up to 'Y' (~60 s) and thereafter saturates. Hence, the higher response for DA using N-rGO/GCE as compared to rGO/GCE, shows enhanced and rapid detection of DA using N-rGO. Therefore, further activity of DA was studied with the N-rGO/GCE. In addition, chronoamperometry response of the as-prepared N-rGO/GCE electrode was evaluated after the addition of successive concentrations of 0.5 and 1 mM DA to the 0.1 M PBS. Figure S6 (supplementary information) shows a typical chronoamperogram curve of the successive addition of DA. The N-rGO/GCE showed excellent electrocatalytic activity towards the amperometric detection of DA.

For further detection of DA, a more sensitive technique of DPV was used. DPV study is shown in figure 8a, which shows an increase in anodic peak current with the addition of DA in the range from 0.1 to 3000 μ M. Hence, by plotting a calibration graph (figure 8b), two separate linear segments corresponding to different linear dynamic ranges were observed, which has the linear regression equations as

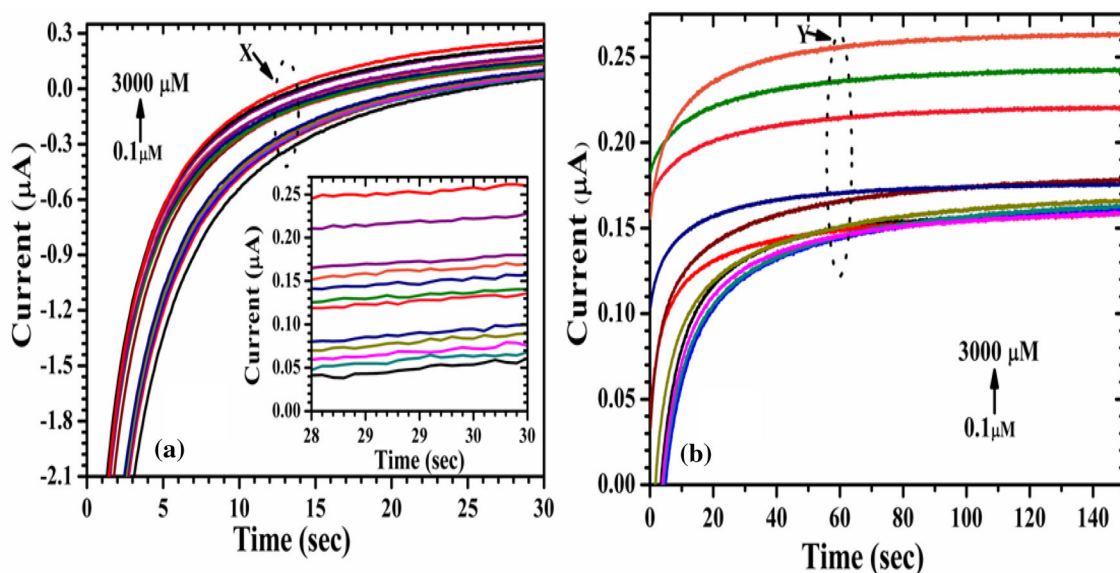


Figure 7. Amperometric response at varying concentrations (0.1, 10, 20, 30, 40, 100, 200, 400, 800, 1000, 2000, 3000 μM) of DA at (a) N-rGO-modified electrode and (b) rGO-modified electrode.

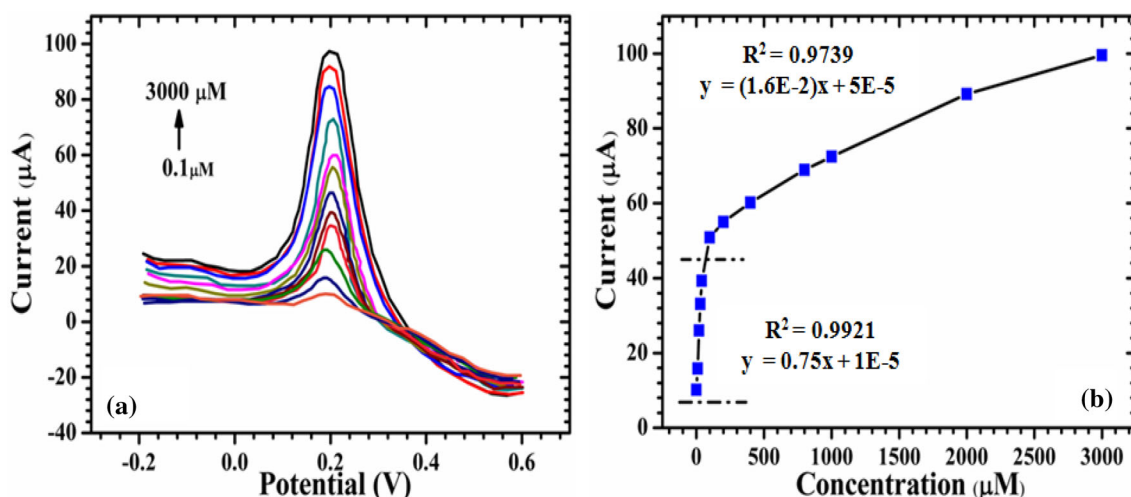


Figure 8. (a) DPV obtained for N-rGO-modified GCE during the successive addition of DA (0.1–3000 μM) into the 0.1 M PBS (pH = 6.0). (b) Calibration plot observed for the oxidation peak current vs. concentration of DA.

$I_{pa} (\mu A) = 0.75x + 1E-5$ and $I_{pa} (\mu A) = (1.66E-2)x + 5E-5$ for the concentration range of 0.1–40 and 100–3000 μM, respectively. This behaviour clearly indicates that the linearity of the sensor signal changes at the concentrations beyond 100 μM. Whereas, the observation of two linear segments in the calibration curve is also noticed and depicted by several researchers elsewhere [6,52,53]. The LOD is calculated using the formula presented in equation 6 [54]:

$$LOD = \frac{3\sigma}{m} \quad (6)$$

where σ is the standard deviation of the peak currents (five runs), m is the slope of the calibration curve. The detection limit was calculated as 57 nM, which was lower than some previous reports [9–11,52,55], and illustrated N-rGO/GCE had good sensitivity and wide linear range.

In addition, selectivity is one of the important characteristics to verify the performance of the sensor, because real samples may also contain some potentially coexisting compounds that are common in biological systems, such as ascorbic acid (AA) and L-cystine. Figure 9 shows the signal following the addition of 1.0 mM DA, 1.0 mM L-cystine, 1.0 mM AA, 0.5 mM DA and the second addition of

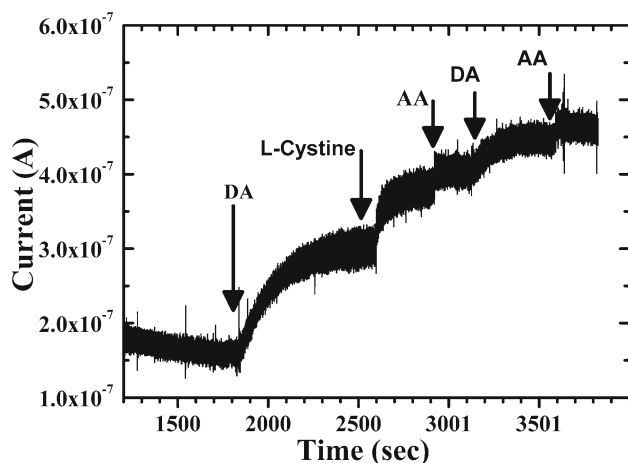


Figure 9. Amperometric responses of 1.0 mM DA, 1.0 mM L-cystine, 1.0 mM AA, 0.5 mM DA and 1.0 mM AA on N-rGO/GCE in 0.1 M PBS.

1.0 mM AA. There was no significant change in the signal in response to AA and L-cystine, indicating that no substantial interference on the detection of DA.

The performance of the N-rGO/GCE sensor was compared with other dopamine sensor systems and a complete description of the detection limit and the linear range are listed in table 1. It can be inferred that although the detection limit is inferior to WO_3 [6], rGO- Cu_2O [3,32], gold/cysteamine/dihydrolipoic acid [33] composites, the wide linear range is superior to other electrochemical sensor systems. Also, the present N-rGO-based sensor has a better detection limit in comparison to many metal-doped composites, which adds to its advantage.

EIS study was performed at a frequency range from 0.8 MHz to 0.1 Hz to estimate the capacitive property and electrode conductivity of the N-rGO-modified electrode. The impedance curve of Nyquist plots for the GO/GCE, rGO/GCE and N-rGO/GCE are shown in figure 10a. The diameter of the semicircle equals the R_{ct} (charge transfer resistance) value indicative of the electron transfer kinetics of the redox probe at the electrode/electrolyte interface. The EIS spectra illustrate a semicircle in the high-frequency region and a nearly straight line in the low-frequency region [26]. An equivalent series resistance (ESR) is represented by semicircle, which attributes towards the electrode conductivity and the charge-transfer resistance in the electrode materials, while the straight line is representative of the capacitive behaviour of the electrode. A vertical line is exhibited by an ideal capacitor. For the present study, the inset of figure 10b shows the equivalent circuit diagram for the N-rGO/GCE. It shows a series resistance (R_s) of $\sim 14.8 \Omega$, charge transfer resistance (R_{ct}) of $\sim 138.8 \Omega$, and a parallel capacitance (C_p) of 3.37 nF. W and CPE correspond to Warburg slope and constant phase element, respectively. The small semicircle of N-rGO/GCE as compared to GO/GCE and rGO/GCE is indicative of the synergistic effect between rGO and N which allows high electron transfer between electrode/electrolyte interfaces. The EIS for N-rGO/GCE exhibits a smaller semicircle indicating low charge-transfer resistance as compared with previously reported results [3,6,38]. Hence, it can be inferred that the excellent conductivity of N-rGO is due to the high graphitic N content introduced into the rGO.

3.3 Reproducibility and stability

The reproducibility of the N-rGO/GCE-based sensor for DA was evaluated by conducting experiments at various

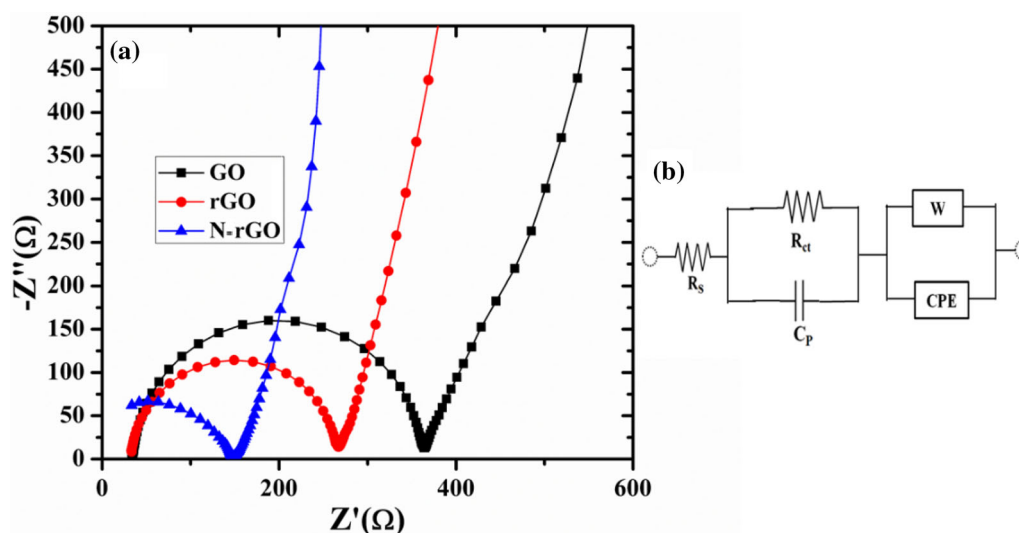


Figure 10. (a) Nyquist plot for GO, rGO and N-rGO-modified GCE in 0.1 M PBS and 400 μM DA. (b) Equivalent circuit diagram for the N-rGO/GCE.

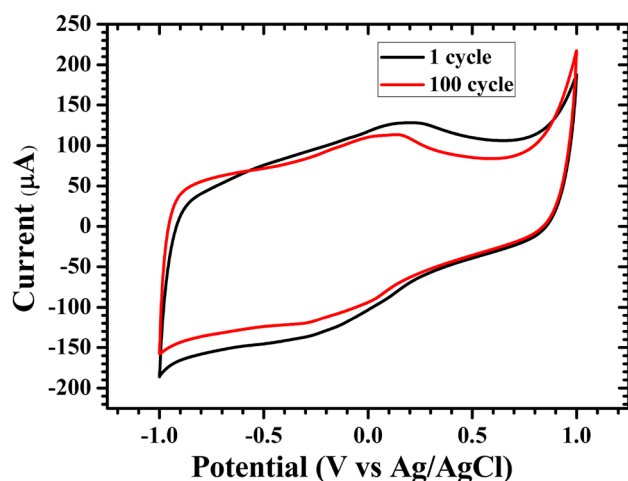


Figure 11. CV response for the 1st and 100th cycles on N-rGO/GCE from a solution of 0.1 M PBS and 400 μM DA at a scan rate of 0.1 V s^{-1} .

concentrations. The sensor showed an acceptable response with a standard deviation of 5.1% for 10 successive measurements. Additionally, the stability of the sensor towards oxidation of DA was analysed using CV measurements. Figure 11 shows the CV measurements for DA at the initial and 100th cycle in 0.1 M PBS buffer. The oxidation current retained 90% of the initial value even after the 100th cycle, which is indicative of good stability for the proposed N-rGO-modified electrode. The long term stability of the electrode was evaluated from the CV response for 400 μM in 0.1 M PBS buffer and then, the experiments were repeated with the same modified electrode after 2 weeks. The oxidation current retained 95% of the original value, signifying the stability of the N-rGO-modified sensor system. The stability is attributed to the fact that there is an efficient immobilization of N-rGO on the electrode.

4. Conclusions

In summary, the study demonstrates a low-temperature synthesis of N-rGO for electrochemical detection of DA. A simple, photo-catalytic approach was employed for the synthesis of N-rGO and used to modify GCE for electrochemical sensing of the neurotransmitter DA. The N-rGO-modified GCE showed a rapid electron transfer and higher activity towards the oxidation of DA as compared with rGO modified GCE. Under the selected conditions the N-rGO-modified GCE showed a DPV oxidation peak current with a wide linear relationship with DA concentration in the range of 100–3000 μM with the detection limit as 57 nM. The EIS study showed a low charge-transfer resistance as compared to several past reports. Thus, the electrochemical sensing of dopamine by N-rGO could further use as the synthesis of the soft conductive hydrogel, adhesives, wound

healing hydrogel, actuators and other futuristic medical applications.

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Author's contribution MS and SKS initiated the study and idea; RS prepared the working solutions and performed the experiments related to pH, DPV. KP performed the CV and EIS experiments. MS analysed and interpreted the data. RK performed the chronoamperometry and selectivity study. All the authors contributed to writing the manuscript, have read, and approved the final version of the manuscript.

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