Fabrication of disposable electrochemical dopamine sensor using photoluminescent graphene oxide

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Abstract. Graphene oxide (GO) with photoluminescent property was prepared by modifiedHummer’s method. Spectroscopic and morphological studies were carried out using photoluminescence spectroscopy, UV-Vis absorption spectroscopy, FT-IR, XRD and FE-SEM. Electrochemical dopamine sensor was fabricated by drop casting GO onto screen printed carbon electrodes (SPCE). Cyclic voltammogram, differential pulse voltammogram and amperometry were performed to test the fabricated sensor. The sensor exhibits wide detection range of 12.5 μM to 1 mM dopamine concentrations in 0.1 M PBS of pH 7.4. The major interferents such as ascorbic acid shown negligible current response compared to dopamine.

Keywords: Graphene oxide, photoluminescence, dopamine, biosensor.

1. Introduction
Dopamine, a major catecholamine neurotransmitter and neuromodulator present in both CNS and peripheral circulation[1]. It is involved in important cerebral and other mesenteric organ functions. Dopamine is also an essential signaling molecule in the regulation of gastric acid secretion[2], gut-brain connection[3], neuroimmune interaction of autoimmune disorders, tumor immunity[4], and neuroinflammation. Dopamine dysregulation has been linked to neurological conditions like Parkinson’s disorder, autism, depression, schizophrenia, duodenal ulcer, drug abuse, and addiction[5][6].

The detection of abnormal dopamine level plays a pivotal role in the investigation and effective treatment. Selective determination of dopamine is essential as the interferents like ascorbic acid, uric acid are present in higher concentration in circulation with redox ability which makes the detection difficult. Despite the other expensive time-consuming detection methods like chromatographic and colorimetric assays[7][8], electrochemical sensors[9] have proved to be the promising approach for non-enzymatic method of determination of neurotransmitters. Biosensors as Point of care testing (PoCT)[10] or lab on a chip device[11] are considered as one of the best methods for speedy and continuous bedside monitoring of dopamine levels due to its excellent redox property[12].

Graphene oxide, a honey comb structured carbon nanomaterial with defective functional groups on their planes and edges exhibits unique electrochemical and mechanical properties[13][14]. Owing to its importance, the graphene oxide modified sensors have been developed for the determination of
various biomolecules including dopamine since they are rapid, reliable and accurate[15][16][17]. Negative charges developed on graphene oxide surface due to its functional groups have the great affinity for positively charged aromatic rings of dopamine. This mechanism enhances the sensing ability of graphene oxide modified screen-printed carbon electrodes towards dopamine[18]. The photoluminescent graphene oxide synthesized in this work shows good electro-catalytic and photoluminescence property[19]. The long duration ultra-sonication and longer storage time of centrifuged supernatant have shown to have a great impact on their electrochemical and fluorescence property[20][21].

Studies have reported that the origin of photoluminescence or fluorescence in graphene oxide [22] may be pH or size dependent or due to the presence of oxidative debris and hydroxyl functional groups in the solution. Extended ultra-sonication time have impact on the exfoliation to produce large amount of oxidative debris which resembles quantum dots [23][24]. The band gap difference, disorder induced states and the presence of oxygen functional group have been implemented in tunable optical properties as reported in[25][26].

In this work, graphene oxide modified screen-printed carbon electrodes were prepared and their electrochemical behaviour for dopamine sensing was studied. The sensor developed shows detection range of dopamine from 12.5 μM to 1mM concentrations.

2. Experiments

2.1 Chemicals and Instruments

Graphite powder (< 20 µm), dopamine (DA), L-ascorbic acid (AA), uric acid (UA), β-D-(+)-glucose were purchased from Sigma Aldrich. KMnO₄, N,N' dimethylformamide (DMF), hydrochloric acid (HCl), hydrogen peroxide (H₂O₂), potassium chloride (KCl) and sodium chloride (NaCl) were obtained from Finar chemicals (India). KH₂PO₄, Na₂HPO₄, KCl, and NaCl were used in 0.1 M phosphate buffer saline of pH 7.4 preparation. Biosensor grade carbon inks (BQ242) were purchased from DuPont Ltd., Singapore. Deionised water was used throughout the experiment.

All electrochemical experiments were carried out using CHI660C electrochemical workstation (CH Instrument, TX, USA). A screen printed three electrode system was used for the fabrication of the sensor. Surface morphology was studied using FE-SEM FEI Quanta 250 field emission scanning electron microscope (FESEM, Zeiss, Germany). Photoluminescence Spectroscopy was performed with RF-6000 spectrofluorophotometer (Shimadzu). UV-Visible absorption spectroscopy was done using Shimadzu. Rigaku Miniflex 600 diffractometer was used for X-ray diffraction measurement. FT-IR was performed with Nicolet iS10 FTIR spectrometer from Thermo Fisher scientific.

2.2 Synthesis of graphene oxide

Graphitic oxide was prepared from graphite powder by modified Hummer’s method. Briefly, 2 g of graphite powder was dispersed in 50 mL of concentrated sulphuric acid and 6 g of potassium permanganate (KMnO₄) was added gradually under stirring, at low temperature. The reaction mixture was heated for 2 hours under stirring and the temperature was maintained at 50 °C. Then the mixture was cooled to room temperature and poured into 400 mL ice water and stirred. To this, 10 mL of hydrogen peroxide was added. Yellow effervescence was observed which confirms the presence of graphite oxide. The reaction mixture was stirred overnight at room temperature. This mixture was centrifuged several times at 12,000 rpm for 45 minutes with 10% hydrochloric acid (HCl) and water, to remove the residues. The removal of chloride ion was confirmed by silver nitrate test. The sediments were dried at 70 °C. 0.1 gram of graphite oxide powder was dispersed in 200 mL distilled water and ultrasonicated for long duration of 8 hours for exfoliation. This mixture was centrifuged to obtain as-prepared graphene oxide and the supernatant, rich in oxidative debris. The supernatant was
stored in dark at room temperature for longer time duration. The stored suspension was ultrasonicated for 30 minutes and washed thrice by centrifugation at 12,000 rpm for 45 minutes. The sediment was dried at 70 °C [27][21].

2.3 Fabrication of the sensor
The disposable screen-printed sensor strip was fabricated on a pre-treated polyethylene terephthalate (PET) substrate. The carbon inks were printed and baked at 130 °C for 20 minutes. GO was dispersed in DMF and sonicated for 30 minutes for uniform dispersion. 5 µL of this suspension was drop casted onto the working electrode of screen-printed carbon electrodes (area 3.14 mm²) and dried at 70 °C for 5 minutes. Ag/AgCl was used as a reference electrode, carbon as a counter and working electrode. 0.1 M PBS of pH 7.4 which resembles the serum condition was used as the electrolyte.

2.4 Electrochemical measurement
The electrochemical behavior of the sensor was studied by cyclic voltammetry and differential pulse voltammetry at a potential window range -0.3 to +0.6 V, scan rate at 50 mV/s and -0.1 V to +0.4 V respectively. Amperometric study was carried out at a potential +0.25 V in 0.1 M PBS pH 7.4. The interference study was carried out with ascorbic acid and glucose at their physiological range in 0.1 M PBS pH 7.4. The anodic peak currents were calculated for the measurement of electro-oxidation of dopamine.

3. Result and discussion
3.1 Spectroscopic characterization of graphene oxide:
The solution of GO in DMF observed under long UV wavelength showed bright yellow fluorescence and pinkish orange photoluminescence in visible light (Figure 1). Photoluminescence studies were carried out with GO dispersed in DMF (Figure 2). PL spectrum displays an emission peak at 535 nm which matches with previous reports[28]. The origin of photoluminescence in this work has believed to be size dependent and due to the presence of oxidative debris in the supernatant but the exact mechanism is unknown[20].

Figure 1. Photoluminescence image of GO dispersed in DMF viewed under long UV light and in visible light.
Figure 2. Photoluminescence spectra for GO dispersed DMF.

UV-Visible absorption spectra in Figure 3 depicts the excitation wavelength of graphene oxide in stored suspension (A); dried GO after centrifugation (B). The graph A shows two peaks at 231 nm and 320 nm[29]. These peaks represent $\pi$-$\pi^*$ excitation of aromatic C=C groups in sp$^2$ hybrid region and n-$\pi^*$ excitation of carboxylic moieties (C=O) in sp$^3$ hybrid regions. In graph B, two peaks were observed at 366 nm and 531 nm which indicates –OH rich GO[30] with yellow fluorescence at long UV wavelength and pinkish orange photoluminescence at visible region (refer Figure 1).

Figure 3. UV-Visible absorption spectra for graphene oxide in stored suspension (A); after centrifugation (B).

X-Ray diffraction (Figure 4A) studies shows diffraction peak at $20 = 12^\circ$ indicating the purity of GO with the intercalation efficiency and degree of oxidation graphene oxide sheets[30]. The result obtained was different from that of usual as-prepared GO which has intercalation spacing at $20 = 26.3^\circ$[31]. Fourier Transform-Infrared spectra (figure 4B) displays characteristic peaks for different functional groups at 3473.53 cm$^{-1}$, 1651.69 cm$^{-1}$ which corresponds to the presence of O-H and C=O stretching.
3.2 Morphological characterization
Morphological studies were carried out with FE-SEM shows sheets with wrinkles which are the characteristic morphology of graphene oxide. This structure is due to the presence of defective functional groups on the surface and edges [31] (Figure 5).

3.3 Electrochemical detection of DA
Cyclic voltammogram obtained on the bare and modified in 0.1M PBS solution containing 100 µM dopamine is shown in figure 6. The oxidation of dopamine occurred at a potential of + 0.33 V on the bare electrode (figure 6A). On the modified electrode, the oxidation peak shifted to a lower potential of +0.21 V with an enhanced current (figure 6B), thus making it ideal for dopamine sensing [32]. Further, from the CVs it is obvious that the response current on the GO modified electrode is about double than that on the unmodified electrode. The results shows better electro-oxidation of dopamine at low potential compared with other GO based dopamine sensors[33][34].
Figure 6. CV response to 100 µM dopamine on bare/SPCE (A) and GO/SPCE (B) performed in 0.1M PBS pH 7.4.

Cyclic voltammogram on GO/SPCE (figure 7A) depicts the linear increase in current response for increasing dopamine concentrations. The anodic peak represents the electro-oxidation of dopamine to dopamine-o-quinone through two electron transfer mechanism. CV of GO/SPCE exhibited two linear response range from 12.5 µM to 200 µM with regression equation \( I_p (\mu A) = 0.3943 + 0.015C (\mu M) \) and 300 µM to 1000 µM with regression equation \( I_p (\mu A) = 2.63 + 0.0065C (\mu M) \) (Figure 7 B and C). The sensitivity was calculated as 0.477 \( \mu A/\mu M/cm^2 \) for 12.5 µM to 200 µM range and 0.207 \( \mu A/\mu M/cm^2 \) for 300 µM to 1000 µM range.

Figure 7. Cyclic Voltammogram at a scan rate of 50 mV/s in 0.1 M PBS with increasing dopamine concentrations, A) 12.5, 25, 50, 100, 300, 500, 700, 900 µM, 1mM (a to j). Calibration curve of GO modified SPCE for different dopamine concentration B) 12.5 to 200 µM, B) 300 to 1000 µM.
Differential pulse voltammogram response (figure 8 A) for increasing dopamine concentrations were carried out at potential window -0.1V to +0.4V in 0.1M PBS pH 7.4. The results display electro-oxidation at +0.15 V and larger peak separation with increase in current. Amperometric experiments (steady state current) were carried out on GO/SPCE electrodes at +0.15V, +0.25 V, +0.3 V in 0.1 M PBS pH 7.4 and found that the results obtained at 0.25 V was better, which is shown in figure 8 B.

3.4 Effect of interferents

Figure 9. DPV response for interfering species A) dopamine 100 µM; B) ascorbic acid 125 µM; C) glucose 6 mM in 0.1 M PBS pH 7.4 (a – buffer).
Selectivity of the GO/SPCE sensor towards the dopamine detection and other interfering molecules like ascorbic acid and glucose in physiological concentration was tested by differential pulse voltammetry. From figure 9 it is evident that dopamine shows greater response than other interferents. Keeping dopamine concentration as 100% the percentage of response for other interference was calculated and presented in table 1.

Various graphene oxide modified electrochemical dopamine sensors were compared with the sensor developed in this work in table 2. The sensor developed with photoluminescent graphene oxide shows wide detection range with high sensitivity in 0.1 M PBS pH 7.4.

Table 1. Current response for dopamine and other interfering molecules at their physiological range.

| S. No. | Analyte Tested | Concentration | Current response % |
|--------|----------------|---------------|--------------------|
| 1      | Dopamine       | 100 µM        | 100                |
| 2      | Ascorbic acid  | 125 µM        | 12                 |
| 3      | Glucose        | 6 mM          | 1                  |

Table 2. Comparison with other graphene oxide based dopamine sensor.

| Electrode material | Method | Potential (V) | pH | Linear range of detection (µM) | Sensitivity (µA/µM/cm²) | Ref |
|--------------------|--------|---------------|----|--------------------------------|-------------------------|-----|
| ERGO/GCE           | DPV    | + 0.2 V       | 7  | 0.5 – 60                       | 0.482                   | [35]|
| GO/GCE             | CV     | + 0.5 V       | 5  | 1 – 15                         | -                       | [18]|
| pGO-GNP            | CV     | + 0.3 V       | 7.4| 0.1 – 30                       | -                       | [34]|
| RGO/AuNP/GCE       | DPV    | + 0.2 V       | 7.4| 0.14 - 700                     | 0.27                    | [36]|
| RGO/AgNPs          | LSV    | + 0.5 V       | 3.5| 10 - 800                       | 0.39                    | [37]|
| GO/SPCE            | CV     | + 0.2 V       | 7.4| 12.5 - 200                     | 0.477                   | Present |
|                    |        |               |    | 300 - 1000                     | 0.207                   | Work |

4. Conclusion

Fabrication of disposable electrochemical dopamine sensor was carried out using photoluminescent graphene oxide. The electro-oxidation of dopamine on GO/SPCE was found to have a wide detection range from 12.5 µM to 1mM with increased current response at low potential. The sensor shows sensitivity of 0.477 µA/µM/cm² for 12.5 µM to 200 µM and 0.207 µA/µM/cm² for 300 µM to 1000 µM. This disposable sensor is highly selective to dopamine in the presence of other redox interferents.

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