Detection of nitrite and nitrate ions in water by graphene oxide as a potential fluorescence sensor

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Abstract. In this study, graphene oxide (GO) was used as a new fluorescence sensor for detection of nitrite (NO₂⁻) and nitrate (NO₃⁻) ions. The GO was synthesized via an improved Hummers’ method, and the properties of GO were examined by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and transmission electron microscope (TEM). The XRD pattern showed the presence of (002) plane at 2θ of 9.5° while the FTIR spectrum showed the presence of C-OH, C=O, C=C, C-O chemical bonds in the GO. The layer structure of the GO was confirmed from the TEM image. The sensing performance of the GO for NO₂⁻ and NO₃⁻ was evaluated by monitoring the emission sites of the GO at 567 nm, which was corresponded to the oxygen functional groups. Fluorescence quenching was observed, suggesting that the GO interacted well with both NO₂⁻ and NO₃⁻ ions. The linear Stern-Volmer plots were obtained in the concentration range of 1-10 mM, indicating the potential ability of the GO as the fluorescence sensor. The quenching constants for the detections of NO₂⁻ and NO₃⁻ were 4.8 × 10⁻² and 1.2 × 10⁻² mM⁻¹, respectively, suggesting that the GO has greater sensitivity towards the NO₂⁻ than the NO₃⁻ ion.

1. Introduction
The negatively charged nitrogen oxide compounds, NO₂⁻ and NO₃⁻ ions can exist together in the groundwater of agricultural land due to the excessive use of fertilizers. The presence of high level of toxic NO₂⁻ and NO₃⁻ ions in the lake results in a groundwater contamination. This contamination has been identified to bring the occurrences of methemoglobinemia (blue baby syndrome) in infants, which can be fatal for the affected infants. On the other hand, it also leads to eutrophication and algae bloom in the lake of agriculture land [1].

Due to these negative effects, various techniques have been employed for the detection of NO₂⁻ and NO₃⁻ ions, such as high-performance liquid chromatography (HPLC), ion chromatography (IC) and Griess colorimetric assay. However, these techniques come with drawbacks. For HPLC method, several tedious purification steps are required to remove the interfering such as chloride [2] and biogenic amines [3]. While, for the IC, it needs extensive maintenance and sample pre-treatment prior to sample measurement [4]. For Griess assay, it is a lack of sensitivity as all the NO₃⁻ ions are converted to NO₂⁻ which later are transformed to colored azo dye [5]. Therefore, the sensitive and
simple detection technique is highly required. In this study, fluorescence spectroscopy was used as a sensitive and simple detection method for the NO$_2^-$ and NO$_3^-$ ions.

On the other hand, the two-dimensional single-atom-thick graphene oxide (GO) has been one of the most investigated materials in the recent years due to its superior electronic, optical, thermal, and mechanical properties [6-10]. Besides, GO also has a large surface to volume ratio, good chemical stability, biocompatibility, facile surface modification and low manufacturing cost, which make it an interesting material in biotechnology and biosensor [7-9]. In this work, the GO was synthesized using an improved Hummers’ method and used as a new potential fluorescence sensor for detection of the NO$_2^-$ and the NO$_3^-$ ions.

2. Experimental

2.1. Materials

All chemicals were purchased and used without further purification. Deionized distilled water was used throughout the experiments. Sodium nitrite (Merck, 99.5%), sodium nitrate (Merck, 99.5%), graphite flakes (Merck), potassium permanganate (Fisher Scientific, ≥ 99%), sulphuric acid (Fisher Scientific, >95%), phosphoric acid (Merck, 85%), hydrochloric acid (QRèc, 37%), hydrogen peroxide (Fisher Scientific, >30%), methanol (Fisher Scientific, 99.99%), and absolute ethanol (HmbG, 99.98%) were used in this study.

2.2. Methods

2.2.1. Synthesis of GO. The GO was synthesized through an improved Hummers’ method, similar to the reported literature [11]. A mixture of concentrated sulfuric and phosphoric acids in the volume ratio of 9:1 was added to a mixture of graphite flake and potassium permanganate. Then, the mixture solution was put under 50 °C with stirring for 24 h in a water bath for homogeneous heating. The solution was subsequently cooled to room temperature and poured onto ice. Deionized water was added to the mixture, followed by dropwise addition of hydrogen peroxide. The mixture was centrifuged and washed with hydrochloric acid. Deionized water was then used to wash the solid until neutral. Lastly, the obtained solid was ultrasonicated in methanol for 1 h and vacuum-dried overnight at room temperature. The obtained product was ground to get the GO powder.

2.2.2. Characterizations. The prepared GO was characterized using the following instruments. The structural property of GO was investigated by X-Ray diffractometer (XRD, Bruker Advance D8 diffractometer) with Cu Ka radiation ($\lambda = 1.5406$ Å). The functional group of the GO was determined by Fourier transform infrared spectrometer (FTIR, Thermo Scientific Nicolet iS50) with potassium bromide as the reference sample. The morphology of the GO was examined by transmission electron microscope (TEM, JEM-2100) at an accelerating voltage of 200 kV. Prior to the TEM measurement, the sample was dispersed in absolute ethanol and dropped on a carbon-coated copper grid. The fluorescence property of the GO was investigated by using a fluorescence spectroscopy (JASCO, FP-8500).

2.2.3. Sensing capability tests. The sensing capability of the GO for the detection of NO$_2^-$ and NO$_3^-$ ions was studied using the fluorescence spectroscopy (JASCO, FP-8500) at room temperature. The prepared GO was dispersed in deionized water (100 ppm, 250 mL) by ultrasonication for 30 min. The emission spectrum of GO solution (3 mL) was then monitored at an excitation wavelength of 367 nm. Certain amounts of analytes (NO$_2^-$ and NO$_3^-$ ions) were then added to the GO solution with various
concentrations (1-10 mM). The interactions between the GO and the analytes were then studied by monitoring the quenching of the emission intensity at a wavelength of 567 nm.

3. Results and discussion

3.1. Structure of the GO

The structural property of the obtained GO was investigated by the XRD. As shown in Figure 1, the prepared GO exhibited a sharp basal diffraction peak (002) at $2\theta$ of 9.50°, with $d$-spacing of 0.93 nm. Here, the obtained interspacing could be attributed to its oxygenated functional groups intercalated in the interlayer of the GO during the oxidation process of graphite flake. The obtained result agrees well with the reported literature [11], where it indicated the presence of the (002) plane in the prepared GO.

![XRD pattern of the prepared GO.](image1)

3.2. Functional groups of the GO

The functional groups of the prepared GO were determined by an FTIR spectroscopy. As shown in Figure 2, the prepared GO showed the presence of O-H stretching vibration of hydroxyl at 3416 cm$^{-1}$, C=O stretching vibration at 1729 cm$^{-1}$, C=C bonds at 1623 cm$^{-1}$, C-O stretching vibration for carboxylic acid and epoxide at 1396 cm$^{-1}$ and 1249 cm$^{-1}$, respectively, and C-OH of alcohol at 1056 cm$^{-1}$, as has been also reported elsewhere [11-13]. The presence of the mentioned functional groups demonstrated that the graphite flakes has been oxidized to GO.

3.3. Morphology of the GO

The morphology of the prepared GO was examined by the TEM under different magnifications. As can be observed in Figure 3 (a) and (b), when the GO was exfoliated in water under ultrasonic treatment, it was exfoliated into monolayer or very thin layers, where the typical wrinkle morphology of GO could be observed [6, 10].

![Figure 1. XRD pattern of the prepared GO.](image2)
3.4. Sensing capability of the GO

The sensing capability of the prepared GO for \( \text{NO}_2^- \) and \( \text{NO}_3^- \) ions was evaluated using the fluorescence spectroscopy, where the implemented concentrations of the analyte ions were in the range of 1-10 mM. It was confirmed that the GO gave maximum excitation peak at 367 nm. When the GO was excited at 367 nm, three emission peaks were observed at 422, 468, and 567 nm. The peak at 422 nm can be assigned to the presence of crystalline graphene [14], while emission peaks at 468 and 567 nm can be assigned to the \( \pi \) to \( \pi^* \) transition in the GO and the oxygen functional groups such as CO, C=O, and O=C-OH [12].

The emission intensity of the GO was monitored in the absence and presence of the analytes. The emission peaks at 422 and 468 nm did not give clear trends in the interactions with the analytes, and only emission peak monitored at 567 nm showed the trend of quenching, suggesting that the oxygen functional groups in the GO would be the possible sites to interact with the analytes. As shown in Figure 4 (a), the emission intensity of the GO at 567 nm decreased with the increase of the \( \text{NO}_2^- \) concentration from 1 to 10 mM. The same phenomenon was observed when using the \( \text{NO}_3^- \) ions as the analyte (Figure 4 (b)). The observed quenching strongly indicated that there was a certain interaction between \( \text{NO}_2^- \) and \( \text{NO}_3^- \) ions with the emission sites of the GO.
The Stern-Volmer plot was used to investigate further the sensitivity of the prepared GO towards the detection of the NO$_2^-$ and the NO$_3^-$ ions. The Stern-Volmer equation is depicted in Equation (1),

$$I/I_0 = K_{sv} [Q] + 1$$  \hspace{1cm} (1)

where $I_0$ and $I$ are fluorescence intensities in the absence and presence of analyte ion, $[Q]$ is the concentration of analyte ion, and $K_{sv}$ is the Stern-Volmer quenching constant. The obtained gradient in the plot indicated the sensitivity of the sensor to the analyte. As can be observed in Figure 5, the GO showed ca. 4 times higher $K_{sv}$ value to NO$_2^-$ ($K_{sv} = 4.8 \times 10^{-2}$ mM$^{-1}$) than the NO$_3^-$ ions ($K_{sv} = 1.2 \times 10^{-2}$ mM$^{-1}$), suggesting that the GO was more sensitive for detection of the NO$_2^-$ than the NO$_3^-$ ions. The higher sensitivity of the GO towards the NO$_2^-$ would be possibly caused by the fact that the NO$_2^-$ is smaller than NO$_3^-$, thus, the NO$_2^-$ would be interacted with the emission sites easier than the NO$_3^-$.

Moreover, the NO$_2^-$ has higher polarity compared to NO$_3^-$. These factors caused the NO$_2^-$ would have more interactions with the oxygen functional groups of the GO.

**Figure 4.** Emission spectra of the GO in the absence and presence of (a) NO$_2^-$ and (b) NO$_3^-$ ions monitored at excitation wavelength of 367 nm.

**Figure 5.** Linear Stern-Volmer plots of the GO for the detection of the NO$_2^-$ ($\bullet$) and the NO$_3^-$ ions ($\circ$).
4. Conclusions
The characterization results from XRD and FTIR showed that GO has been successfully synthesized via the improved Hummers’ method. The TEM image showed the layered wrinkle surface of the synthesized GO. The fluorescence results showed that the emission intensity of the GO was quenched linearly with the NO$_2^-$ and the NO$_3^-$ ions in the range concentrations of 1-10 mM. This study demonstrated that the GO was able to act as the potential fluorescence sensor for the detection of the NO$_2^-$ and the NO$_3^-$ ions. The prepared GO was shown to give a greater sensing sensitivity towards the NO$_2^-$ than the NO$_3^-$ ions.

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