Graphene quantum dots based fluorescent biosensor for nitrogen detection of urea fertilizer

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Abstract. A simple and fast fabrication of fluorescent biosensor based on graphene quantum dots (GQDs) for agricultural application is presented. Graphene oxide was used as a starting material for GQDs synthesis by hydrothermal method. Optical properties and morphology of the synthesized GQDs were characterized by UV-Vis, photoluminescence (PL) spectroscopy and transmission electron microscopy (TEM). The results showed that the synthesized GQDs exhibited spherical shape with their average diameter of 5.70 nm. The UV-Vis and PL spectra showed typical adsorption peak and maximum emission peak of the synthesized GQDs at 330 nm and 472 nm, respectively. To study sensing performance of fluorescent biosensor for nitrogen detection, aqueous solution of GQDs was mixed with different concentrations of nitrogen solution. According to PL spectra result, it is found that PL intensity of GQDs solution significantly increased corresponding to higher concentrations of nitrogen solution. This result suggests that fluorescent biosensor based on GQDs may be an alternative technique for primary nutrient detection in soils.

1. Introduction

Graphene quantum dots (GQDs) are zero-dimensional nanostructured materials which their size is less than 20 nm [1]. Quantum confinement phenomenon leads to unique optical and electronic properties of GQDs [2]. Furthermore, GQDs exhibit high surface area because of their size below 20 nm resulting in higher active sites. According to their unique properties, they have been experimentally and theoretically studied for different applications such as energy storage [3], drug delivery [4], light emitting diodes [5] chemical sensors [6] and biosensors [7]. Nowadays, sensor technology plays an important role within our society, offering a wide range of applications that encompass health monitoring, toxic gas detection, agriculture, and more. The sensing components of these sensors primarily rely on materials like metal oxide and semiconductors. Unfortunately, limitations of these materials are related to high operating temperature, low selectivity and high toxicity etc. GQDs have attracted research interest for fluorescent biosensors to detect different analytes because of their strong and stable photoluminescence (PL) [8, 9]. For examples, Nair *et al*. [10] reported study of fluorescent sensor fabricated with sulphur doped GQDs for ultrasensitive detection of carbamate pesticides in ppb levels. Sahub *et al*. [7] developed a biosensor using GQDs and active enzyme for monitoring

organophosphate pesticides. It should be noted that PL of GQDs can be used to explain light emission of GQDs when electrons are excited to a higher excited state by incident photons and the excited electrons return to a lower energy state [11]. However, reports on study of pristine GQDs based fluorescent biosensors for detection of three primary nutrients in fertilizers are less available.

In this study, based on PL property of GQDs, we demonstrate the synthesis and application of GQDs as fluorescent biosensor for detection of nitrogen in urea fertilizer.

2. Experimental section

2.1. GQDs synthesis

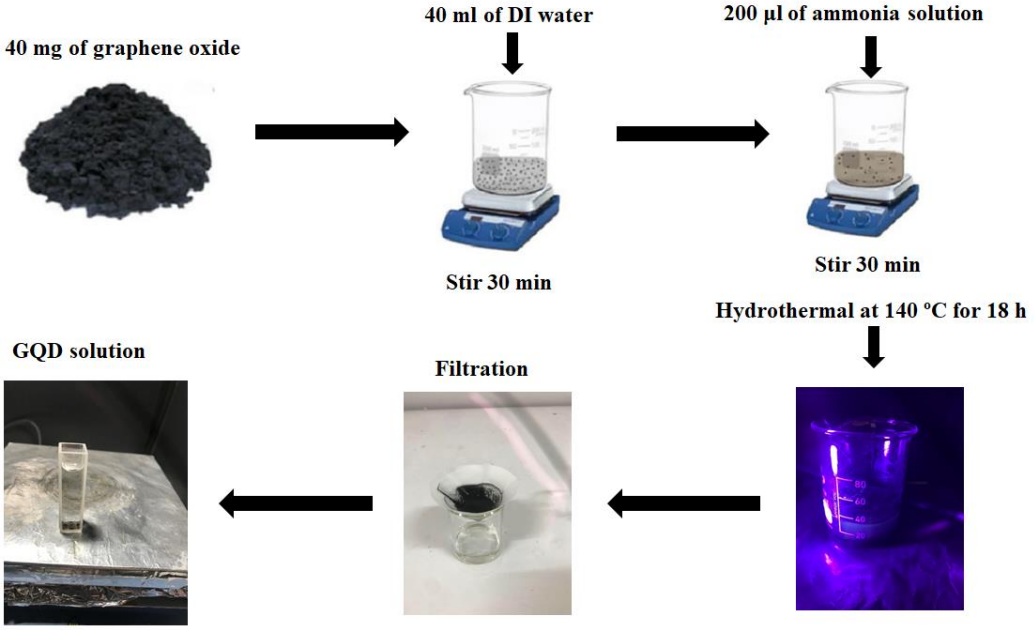

Figure 1. Step for GQDs synthesis by hydrothermal method.

A step for GQDs synthesis by the hydrothermal method is presented in figure 1. Starting, 40 mg of graphene oxide was dispersed in 40 ml of deionized (DI) water with stirring for 30 min. After that, the dispersed solution of graphene oxide was mixed with 200 µl of ammonia solution under stirring for 30 min in order to cut graphene oxide into GQDs. Then, the prepared solution was transferred into 150 ml capacity of stainless-steel autoclave and heated at 140 ºC for 18 h. After hydrothermal process, the obtained solution was filtered to remove the unreacted graphene oxide. Finally, the colorless GQDs solution was obtained.

2.2. Characterization

Optical properties of the synthesized GQDs dispersed in solution (from section 2.1) were investigated by UV-Vis spectrophotometer in the adsorption wavelength of 200-550 nm and PL spectrometer at an excitation wavelength of 380 nm. Transmission electron microscopy (TEM) was performed to study the morphology of the synthesized GQDs. Sizes and size distribution of the synthesized GQDs were measured and calculated using Image J software.

2.3. Nitrogen sensing of fluorescent biosensors based on GQDs

Urea 46-0-0 fertilizer was weighed and dissolved in 100 ml of DI water to prepare four different concentrations of nitrogen solution; 2,000, 6,000, 12,500 and 30,000 ppm. Then, each concentration of nitrogen solution was added into the GQDs solution used as fluorescent biosensors under stirring for 5 min. To study sensing response of the fluorescent biosensors towards nitrogen, PL spectra of the

GQDs solution mixed with four concentrations of nitrogen solution were recorded with an excitation wavelength of 380 nm.

3. Results and discussion

3.1. GQDs morphology

Figure 2 shows TEM image and size distribution of the synthesized GQDs. As presented in figure 2a, the synthesized GQDs displayed spherical shape resulted from OH^- produced from ammonia used as a reducing agent. It is attributed that defect sites of graphene oxide are reacted by the OH^- leading to cutting of graphene oxide into small fragments (GQDs) [2]. Size measurement using Image J software revealed that the synthesized GQDs diameter was in the range of ~2 to 10 nm and their average diameter was 5.70 nm as illustrated in figure 2b.

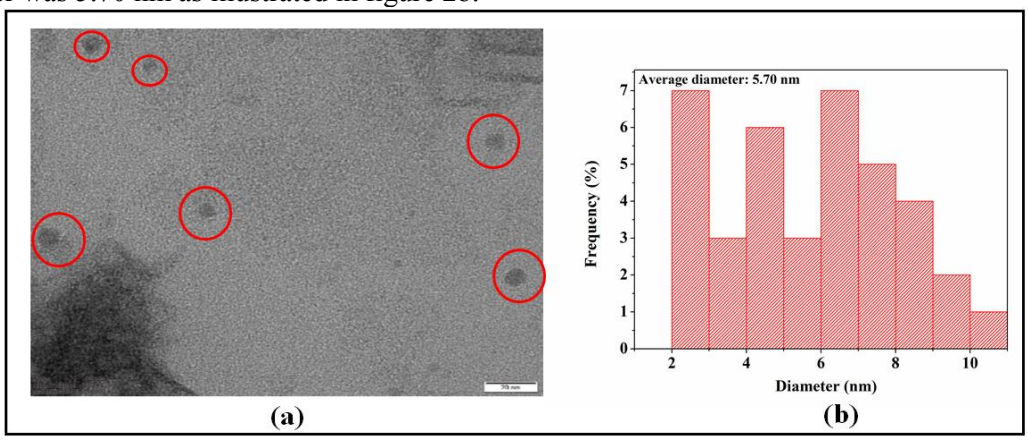

Figure 2. (a) TEM image and (b) size distribution of the synthesized GQDs.

3.2. Optical properties

UV-Vis and PL spectra of the synthesized GQDs are presented in figure 3. The UV-Vis result as displayed in figure 3a shows that the typical adsorption peak was observed at 330 nm, which is assigned to n-π^* electron transition of sp^2 carbon bonds [8, 9], indicating graphene structure of GQDs. To study light emission of the synthesized GQDs, PL emission spectrum was obtained as presented in figure 3b. The inset of figure 3b depicts photographs of the synthesized GQDs solution under visible light and 395 nm-UV light. The emission peak was at 472 nm corresponding to green fluorescence.

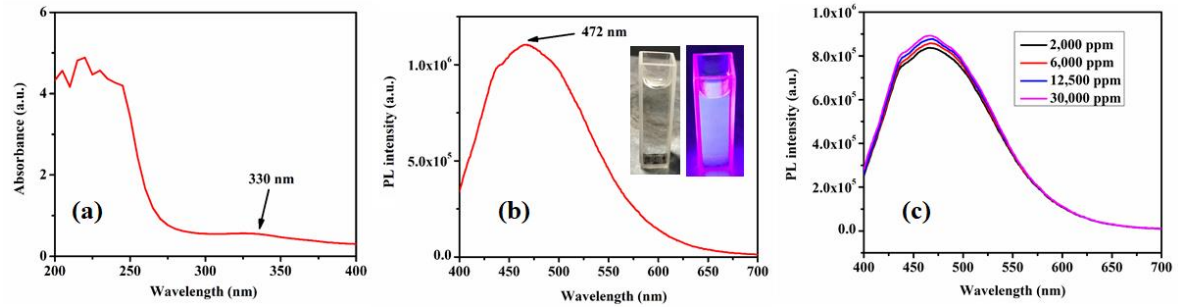

Figure 3. (a) UV-vis, (b) PL spectra of the synthesized GQDs and (c) PL of fluorescent biosensor.

3.3. Nitrogen sensing

PL spectra of the synthesized GQDs solution in the presence of different concentrations of nitrogen solution are displayed in figure 3c. It is found that the PL intensity gradually increased with increasing nitrogen concentrations at 472 nm. The increased PL intensity is attributed to additional surface energy state and more active sites resulted from more nitrogen atoms decorating on the GQDs surface

[12]. This effect promotes radiative recombination leading to more fluorescence. Generally, PL light is emitted through the radiative recombination by the excited electrons [9].

4. Conclusions

In summary, GQDs based fluorescent biosensors for nitrogen detection in urea fertilizers were studied. The GQDs were synthesized by hydrothermal method. The TEM results showed that the synthesized GQDs displayed spherical shape with an average size of 5.70 nm. For optical properties, UV-Vis characterization revealed the adsorption peak of the synthesized GQDs at 330 nm which confirmed graphene like-structure. In order to study light emission, PL spectrum showed the emission peak at 472 nm corresponding to green fluorescence. To investigate the performance of GQDs as fluorescent biosensor for nitrogen detection, the synthesized GQDs solution was mixed with four concentrations of nitrogen solution. According to the PL result, PL intensity gradually increased corresponding to higher concentrations of nitrogen solution. Based on the results, GQDs based fluorescent biosensors for nitrogen detection can be used as an alternative technique for nitrogen detection in fertilizers.

Acknowledgement

The authors gratefully acknowledge financial support from a research fund for development of science, research and innovation in 2023, Phetchabun Rajabhat university.

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