Research Report

การสังเคราะห์หมุดควอนตัมกราฟีนสำหรับไบโอเซนเซอร์เพื่อตรวจสอบคุณภาพดินเพาะปลูกในจังหวัดเพชรบูรณ์

Synthesis of Graphene Quantum Dots (GQDs) as Biosensors Used for Applications in Agricultural Soil Quality in Phetchabun

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Full Research Report

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Research Scholarship Support for Development of Science, Research and Innovation

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ชื่องานวิจัย การสังเคราะห์หมุดควอนตัมกราฟีนสำหรับไบโอเซนเซอร์เพื่อตรวจสอบคุณภาพดินเพาะปลูกในจังหวัดเพชรบูรณ์

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มหาวิทยาลัยราชภัฏเพชรบูรณ์ **ปีเสร็จวิจัย** 2566

บทคัดย่อ

งานวิจัยนี้นำเสนอการผลิตไบโอเซนเซอร์โดยใช้หมุดควอนตัมกราฟีนสำหรับตรวจสอบแร่ธาตุไนโตรเจน การสังเคราะห์หมุดควอนตัมกราฟีนเริ่มต้นจากกราฟีนออกไซด์ด้วยวิธีไฮโดรเทอร์มอล (Hydrothermal) หลังจากนั้นศึกษาคุณลักษณะสัณฐาน (Morphology) และสมบัติทางแสง (Optical Property) ของหมุดควอนตัมกราฟีนด้วยเทคนิค Transmission Electron Microscopy (TEM) UV-Vis Spectroscopy และ Photoluminescence Spectroscopy (PL) จากผลการทดลองพบว่า หมุดควอนตัมกราฟีนมีรูปร่างทรงกลมที่มีขนาดเส้นผ่านศูนย์กลางเฉลี่ย 5.70 นาโนเมตร และแสดงพีคการดูดกลืนแสงและเปล่งแสงที่ 330 และ 472 นาโนเมตร ตามลำดับ สำหรับการศึกษาไบโอเซนเซอร์เพื่อตรวจสอบแร่ธาตุไนโตรเจน โครงการวิจัยนี้ใช้ปุ๋ยยูเรียสูตร 46-0-0 โดยทำเป็นสารละลายและผสมลงในของเหลว Suspension ที่มีหมุดควอนตัมกราฟีน (ใช้เป็นไบโอเซนเซอร์) โดยใช้ความเข้มข้นของสารละลายยูเรีย 4 ค่า จากผลการวัดการเปล่งแสงพบว่า ความเข้มของการเปล่งแสงของหมุดควอนตัมกราฟีนเพิ่มขึ้นตามปริมาณความเข้มข้นของสารละลายยูเรียที่เพิ่มขึ้นอย่างมีนัยสำคัญ ผลการทดลองนี้บ่งชี้ว่า ไบโอเซนเซอร์ที่ใช้หมุดควอนตัมกราฟีนอาจเป็นวิธีทางเลือกสำหรับตรวจวัดแร่ธาตุไนโตรเจนในดินเพาะปลูกได้ในอนาคตอันใกล้

คำสำคัญ: หมุดควอนตัมกราฟีน ไบโอเซนเซอร์ ตรวจสอบไนโตรเจน

Research Title Synthesis of Graphene Quantum Dots (GQDs) as Biosensors Used for Applications in Agricultural Soil Quality in Phetchabun

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ABSTRACT

A simple fabrication of biosensors based on graphene quantum dots (GQDs) for detecting nitrogen 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 biosensors, nitrogen of 46-0-0 urea fertilizer was used and GQD suspension was mixed with four concentrations of 46-0-0 urea solution. According to PL spectra result, it is found that PL intensity of GQD suspension significantly increased corresponding to higher concentrations of urea solution. This result suggests that fluorescent biosensor based on GQDs may be an alternative technique for nitrogen detection in soils in near future.

Keywords: graphene quantum dot, biosensor, nitrogen detection

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Kriengkri Timsorn

April 16, 2024

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance

Soil fertility is very important to grow plants because it provides primary nutrients. The primary nutrients in soils are nitrogen, phosphorus, potassium, calcium and magnesium (Khalofah, Ghramh, Al-Qthanin and L'taief. 2022) etc. Frequently, we need to supplement soil nutrients by adding fertilizers, manure or compost to increase agricultural production. Nowadays, global warming and climate change affect soil fertility and cultivation. For this serious change, knowing nutrient concentration in soil before cultivation is very crucial for farmers. To measure nutrient concentration in soils, qualitative methods based chemical approaches including mass spectrometry (Jorge, Mata and Antonio. 2016), high performance liquid chromatography (Linskens and Jackson. 2012) and ion selective microelectrodes (Miller and Smith. 2012) etc. have been performed. These methods show high accuracy and sensitivity but their limitation is related to time-consuming, high cost and needs well-trained technicians. In recent decades, 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 (Timsorn and Wongchoosuk. 2019). Unfortunately, these materials still have some disadvantages such as high operating temperature, low selectivity and high toxicity.

For development of sensor performance to be used in different applications, nanomaterials, such as carbon nanotubes, graphene, nanoparticles, have been extensively studied (Cadena, Riu and Rius. 2007). Graphene quantum dots (GQDs) are zero-dimensional nanomaterials with diameter sizes below 20 nm. Due to quantum confinement effects, the GQDs exhibit a non-zero bandgap, unique optical properties, high surface to volume ratio and tunable electronic properties (Bacon, Bradley and Nann. 2014). According to their unique properties, they have been experimentally and theoretically studied for various applications, such as energy storage, drug delivery, light emitting diodes, chemical sensors and biosensors. The GQDs have attracted research interest for fluorescent biosensors to detect different analytes because of their strong and stable photoluminescence (PL) (Abbas, Tabish, Bull, Lim and Phan. 2020). A change in PL property of GQDs resulting from different concentrations of analytes can be used to identify type and amount of the analytes. The change is mainly a principle of fluorescent biosensors (Chung, Revia and Zhang. 2019).

GQD based fluorescent biosensors are widely applied to agricultural area. It is attributed that GQDs based fluorescent biosensors can be used to identify primary nutrients including nitrogen, phosphorus, potassium in soils. Therefore, this research is focused on synthesis of GQDs using the hydrothermal technique and fabrication of GQD based fluorescent biosensors to detect nitrogen of urea fertilizers. We hope that this research will be useful for sensor fabrication used in detecting nutrients in soils in the near future.

1.2 Objectives

1.2.1 To synthesize GQDs by a hydrothermal method

1.2.2 To fabricate GQDs based fluorescent biosensors for nitrogen detection

1.3 Scope of Research

This research will synthesize GQDs by the hydrothermal method and the synthesized GQDs will be used to fabricate fluorescent biosensors for nitrogen detection. Nitrogen is obtained from 46-0-0 urea fertilizer, which represents nitrogen in soils.

CHAPTER 2

LITERATURE REVIEW

2.1 Graphene Quantum Dots (GQDs)

GQDs are nanoscale particles of graphene with a diameter less than 20 nm and consist of one atomic layer of carbon atoms which are closely stacked. Each carbon atom is connected to three carbon atoms via sp^2 hybridization as shown in figure 1. Three of four valence electrons are paired with the valence electrons of surrounding three carbon atoms to form δ-bonds and unpaired π-electrons work together to form large π bonds (Geim and MacDonald. 2007). They exhibit excellent chemical, structural, electrical and tunable optical properties of photoluminescence and electrochemiluminescence (Elvati, Baumeister and Violi. 2017). Moreover, the existence of a band gap of GQDs gives them ability to produce electron-holes pair, which can produce photoluminescence (Yan, Li, Cui, Wei, Tajima and Li. 2011). Due to the good conductivity, photoluminescence ability, and fluorescence properties of GQDs, it can be used to be sensing parts or probes to identify various targets in sensor field.

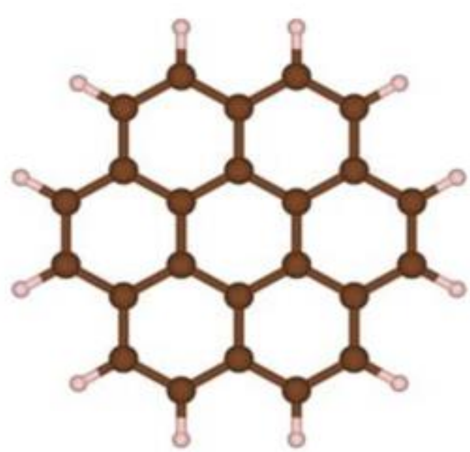

Figure 2-1 GQD structure.

2.2 Synthesis Methods of GQDs

The methods for GQDs synthesis can be generally divided into top-down and bottom-up processes as presented in figure 2-2 (Sweetman, Hickey, Brooks, Hayball and Plush. 2019). As the bottom-up methods, synthesis of GQDs requires complex reaction steps and specific organic materials, making it difficult to optimize the conditions. Therefore, it is preferred to use the top-down approach, which is to cut large blocks of carbon materials into small pieces. The raw materials needed for this method are abundant carbon materials, which are cheap and easy to obtain, also the method is relatively straightforward and easy to synthesize GQDs.

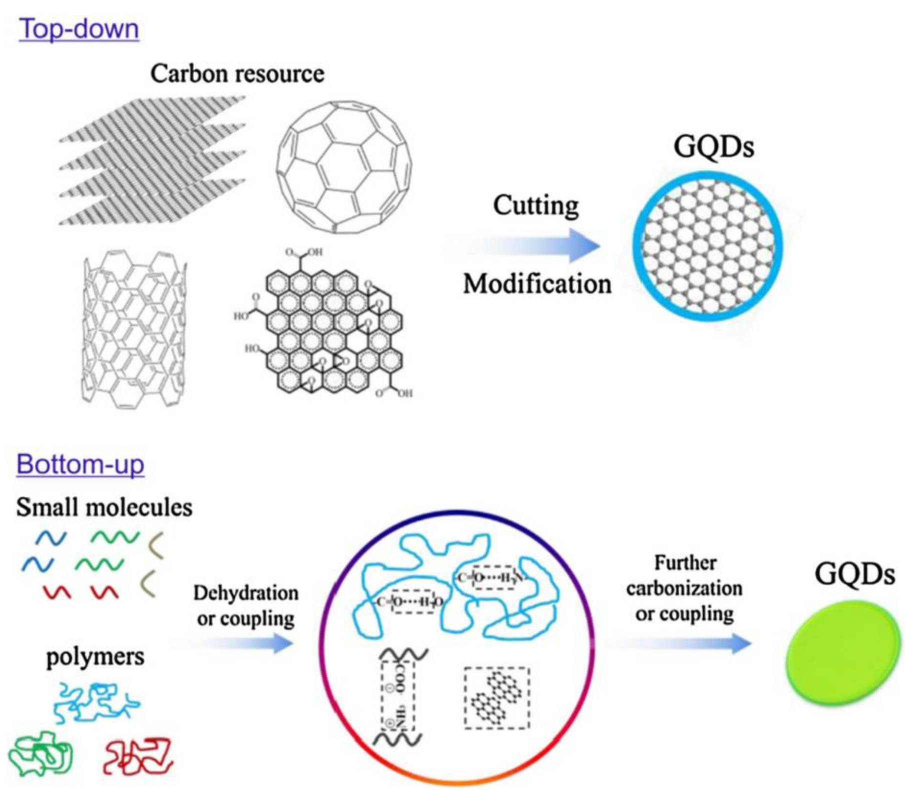

Figure 2-2 Top-down and bottom-up processes for GQD synthesis.

2.3 Hydrothermal Method for GQD Synthesis

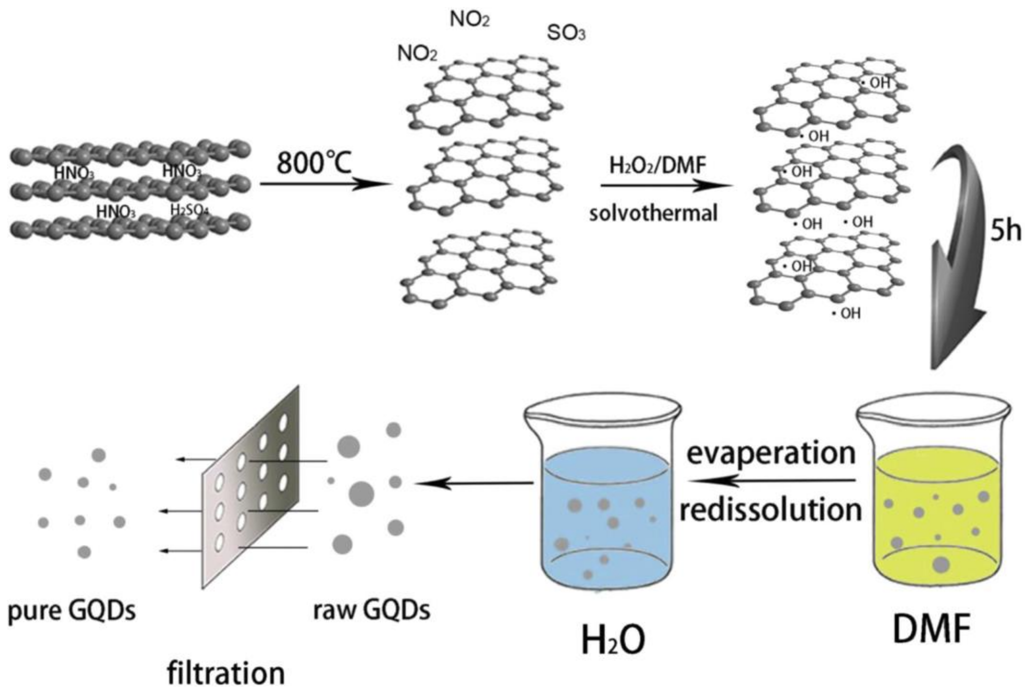

Figure 2-3 Hydrothermal method of GQD synthesis.

Hydrothermal method based on top-down approach is simple and rapid method for GQD synthesis. GQDs can be finally obtained using a variety of macromolecular or small molecular substances as the starting materials under high temperature and pressure (Wang, Jin, Duan and Jia. 2017). The mechanism of the hydrothermal method is to oxidize the carbon source material, generate a large number of oxygen-containing functional groups on its surface, further oxidize to carbonyl pairs at room temperature, and then add NaOH, NH3 • H2O, and other alkaline substances. Due to the instability of carbonyl pairs, carbon atoms on epoxy bonds can be removed under the action of hydrothermal action, thereby breaking up to obtain GQDs. The method is mainly divided into three stages: (1) oxidation of graphene in a mixture of concentrated sulfuric acid and concentrated nitric acid; (2) oxygen-containing functional groups such as epoxy groups are introduced on the oxidized graphene sheet, and these oxygen-containing functional groups tend to be arranged in a straight line on the carbon skeleton; (3) the oxidized graphene is subjected to hydrothermal treatment under weak alkaline (pH = 8) conditions to remove oxygen-containing groups, resulting in sheet rupture, the formation of GQDs, and finally filtration and purification. Figure 2-3 shows an example of hydrothermal methods of GQD synthesis.

2.4 Photoluminescence (PL)

Figure 2-4 PL emission of GQDs.

PL describes the phenomenon of a molecule absorbing the energy of an incident photon through the excitation of an electron to a higher energy level and the subsequent emission of a photon when the excited electron relaxes. As such, the bandgap of GQDs determines the operative wavelengths that may participate in the PL spectra of GQDs. In fact, a perfect zero-energy bandgap form of graphene would not exhibit PL. Only by varying parameters such as adding surface groups, altering the concentration of dopants, or engineering the physical dimensions of GQDs can be characterized with a nonzero bandgap and thereby exhibit PL. These parameters dictate the two main mechanisms by which PL in GQDs is controlled: 1) quantum confinement controlled by the size of the GQDs and 2) influencing the bandgap of a GQD due to the difference in energy levels of electrons associated with the sp^2 carbons of graphene and the electrons associated with surface groups, dopants, and edge states. Light emission of GQDs is in the range from ultraviolet, blue, green, orange, red and NIR as shown in figure 2-4 (Chung, Revia and Zhang. 2019).

2.5 Biosensors

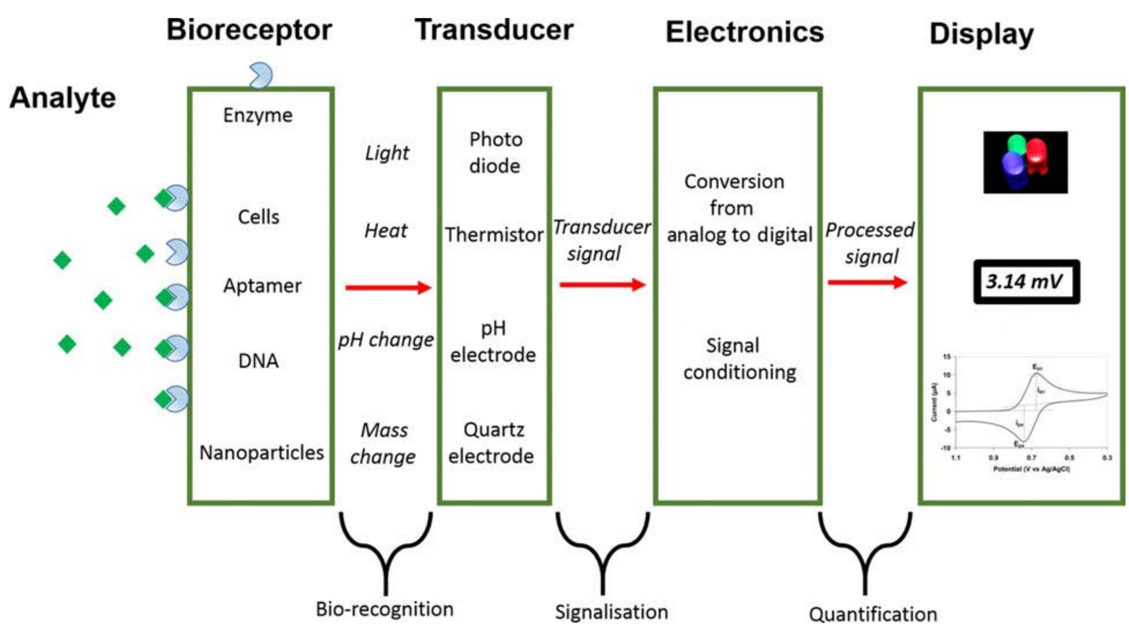

Figure 2-5 Principle of a biosensor.

A biosensor is a device that measures biological or chemical reactions by generating signals which are proportional to the concentration of an analyte in the reaction. Principle of a biosensor is presented in figure 2-5. It consists of five main components as follows:

1. Analyte: A substance of interest that needs detection. For instance, glucose is an analyte in a biosensor designed to detect glucose.

2. Bioreceptor: A molecule that specifically recognizes the analyte is known as a bioreceptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bioreceptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bioreceptor with the analyte is termed bio-recognition.

3. Transducer: The transducer is an element that converts one form of energy into another. In a biosensor, the role of the transducer is to convert the bio-recognition event into a measurable signal. This process of energy conversion is known as signalization. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte–bioreceptor interactions.

4. Electronics: This is the part of a biosensor that processes the transduced signal and prepares it for display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the digital form. The processed signals are then quantified by the display unit of the biosensor.

5. Display: The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user (Bhalla, Jolly, Formisano and Estrela. 2016).

2.6 Research on GQD Based Biosensors

Sahub, Tuntulani, Nhujak and Tomapatanaget. 2018 researched on a pesticide sensor based on GQDs and active enzyme for monitoring organophosphate pesticides. They reported that PL of GODs in the presence of organophosphate was observed at 467 nm. A change in PL of the GQD based biosensor corresponded to the amount of pesticide. The detection limit of the biosensor was 0.172 ppm. The authors concluded that the developed biosensor based on GQDs and active enzyme offered the promising determination of organophosphate pesticides in food, water and environment with low cost, less toxic to environment.

Tabish, Hayat, Abbas and Narayan. 2022 fabricated GQDs based electrochemical biosensors for early detection of acute myocardial infarction (AMI). They reported that combining GQDs with electrochemical biosensors improved sensitivity, specificity and selectivity of biomarkers associated with AMI. GQD properties such as large specific surface area, surface to volume ratio, biocompatibility and minimal toxicity offer excellent performance of the electrochemical biosensors for cardiac diagnostics.

Kalkal, Pradhan, Kadian, Manik and Packirisamy. 2020 demonstrated the fabrication and applications of fluorescent turn-on biosensor for ultrasensitive detection of small cell lung cancer biomarker. GQDs were synthesized by a hydrothermal method and used as energy donor. Gold nanoparticles were used as energy acceptor. The results showed that the fluorescent biosensor exhibited fast response time (16 min), broader linear detection range and remarkably low detection limit.

CHAPTER 3

METHODOLOGY

3.1 Synthesis of GQDs

In this research work, GQDs were synthesized by a hydrothermal method. Figure 3-1 shows synthesis steps of GQDs. Starting, 40 mg of graphene oxide were 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 at room temperature in order to cut graphene oxide into GQDs. Then, the prepared solution transferred to 150 ml capacity of stainless-steel autoclave was heated at 140 °C for 18 h. After hydrothermal process, the obtained solution was filtered to remove the unreacted graphene oxide. Finally, the colorless GQDs solution was obtained.

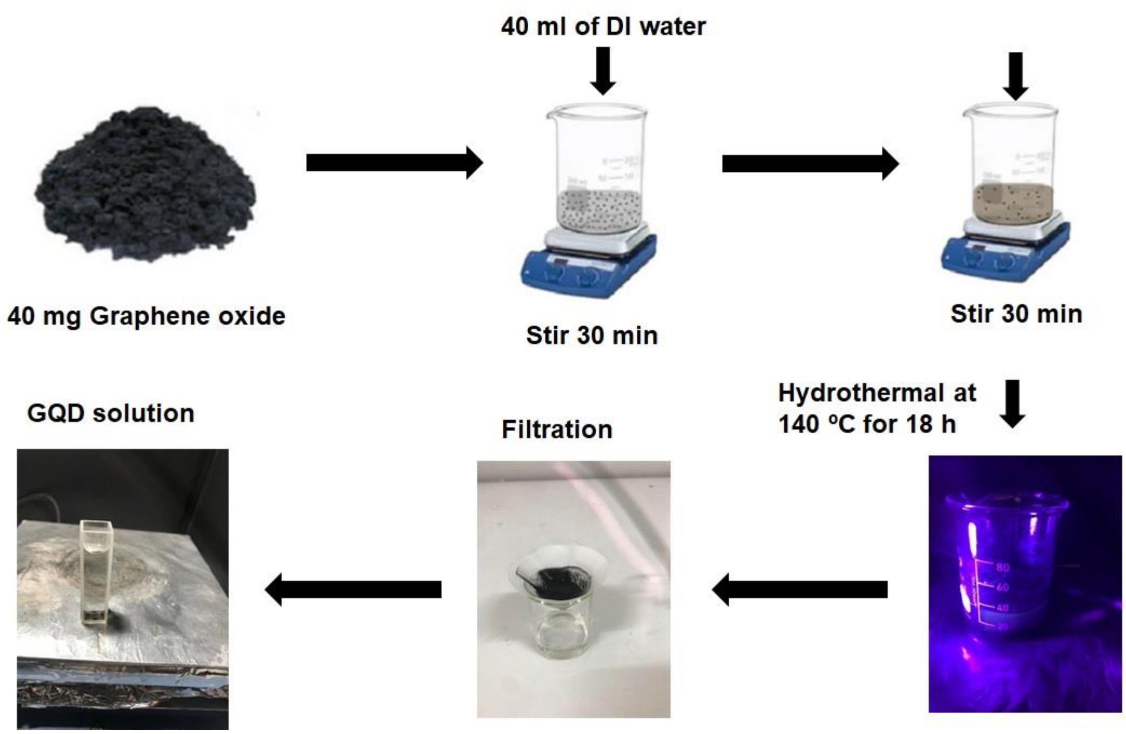

Figure 3-1 Synthesis diagram of GQDs.

3.2 Characterization

After synthesis process, optical properties of the synthesized GQDs dispersed in solution (from section 3.1) 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.

3.3 Nitrogen Sensing of GQDs Based Fluorescent Biosensors

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 as displayed in figure 3-2. Then, each concentration of nitrogen solution was added to the GQDs solution, used as fluorescent biosensors, under stirring for 5 min. To study sensitivity 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.

Figure 3-2 Preparation of urea 46-0-0 fertilizer dissolved in DI water.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Morphology of The Synthesized GQDs

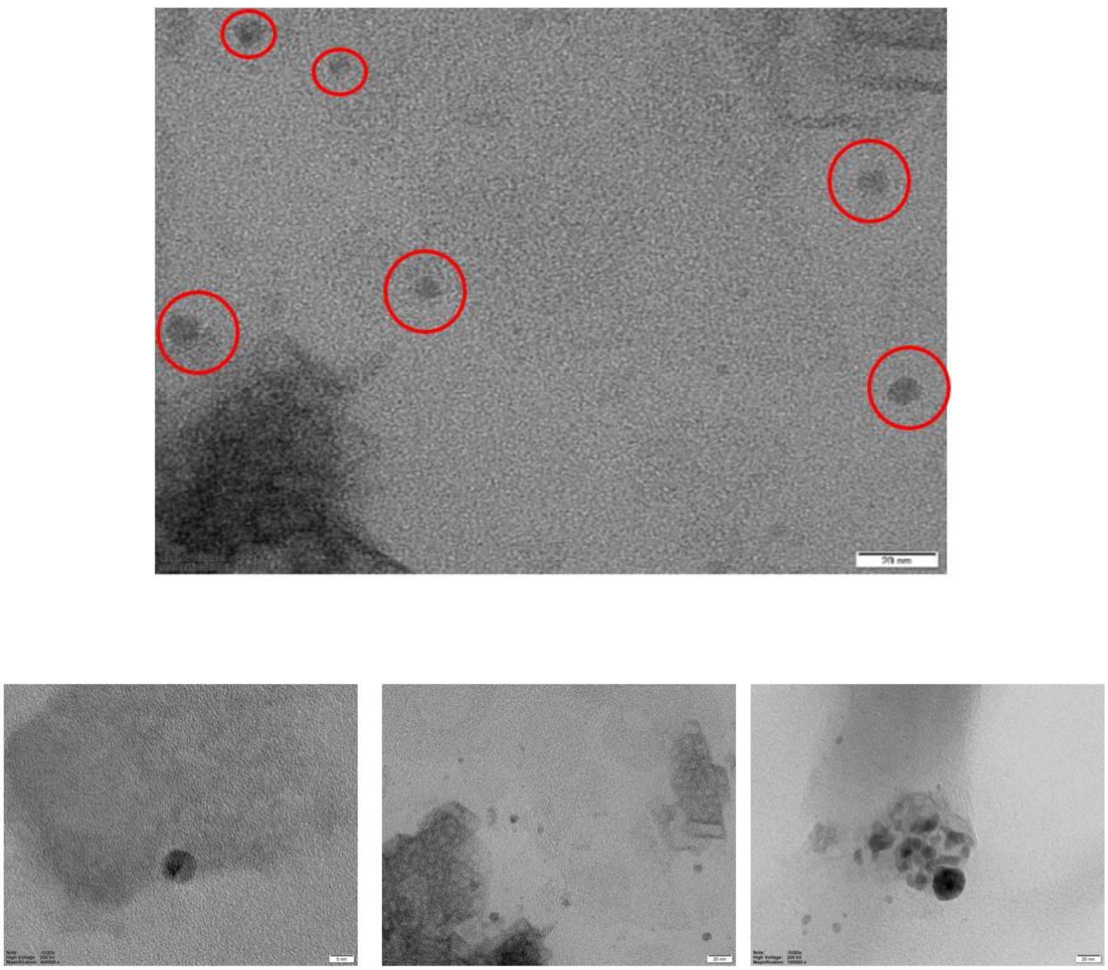

Figure 4-1 TEM images of the synthesized GQDs.

The structural morphologies of the synthesized GQDs were characterized by TEM as presented in figure 4-1. The synthesized GQDs showed spherical shape resulted from OH^-

generated 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). To measure size of GQDs, Image J software was used. It is found that the synthesized GQDs diameter was in the range of ~2 to ~10 nm and their average diameter was 5.70 nm and size distribution was illustrated in figure 4-2.

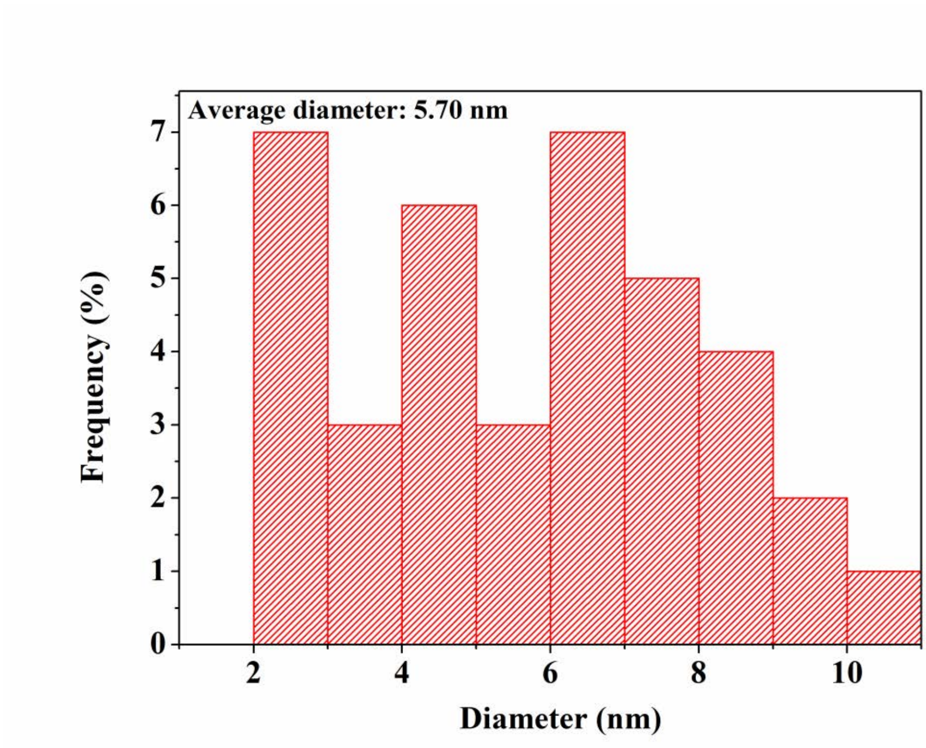

Figure 4-2 Size distribution of the synthesized GQDs.

4.2 Optical Property

The optical property of the synthesized GQDs was studied using UV-Vis and PL spectroscopies. From UV-Vis result as shown in figure 4-3, it can be seen that the typical adsorption peak was observed at 330 nm, which is assigned to n-π^* electron transition of sp^2 carbon bonds, indicating graphene structure of GQDs. Figure 4-4 displays PL spectrum of the synthesized GQDs. 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.

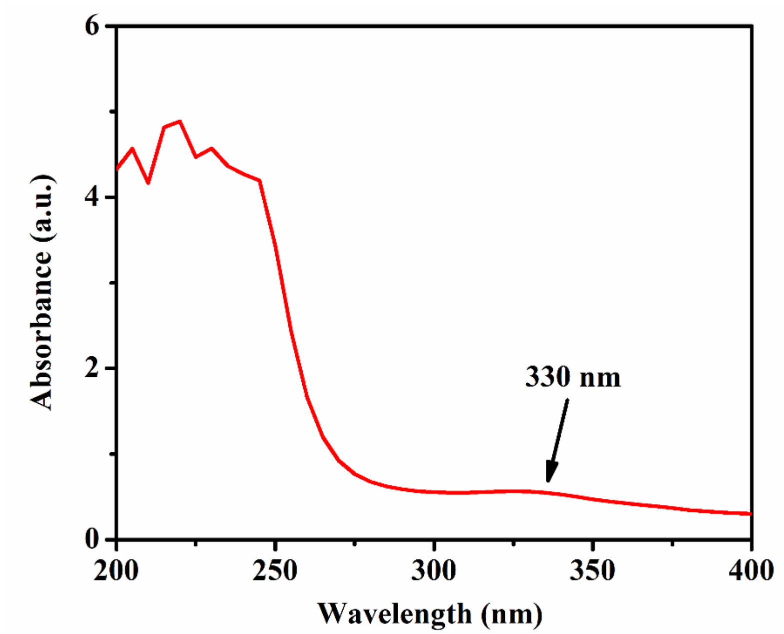

Figure 4-3 UV-Vis spectrum of the synthesized GQDs.

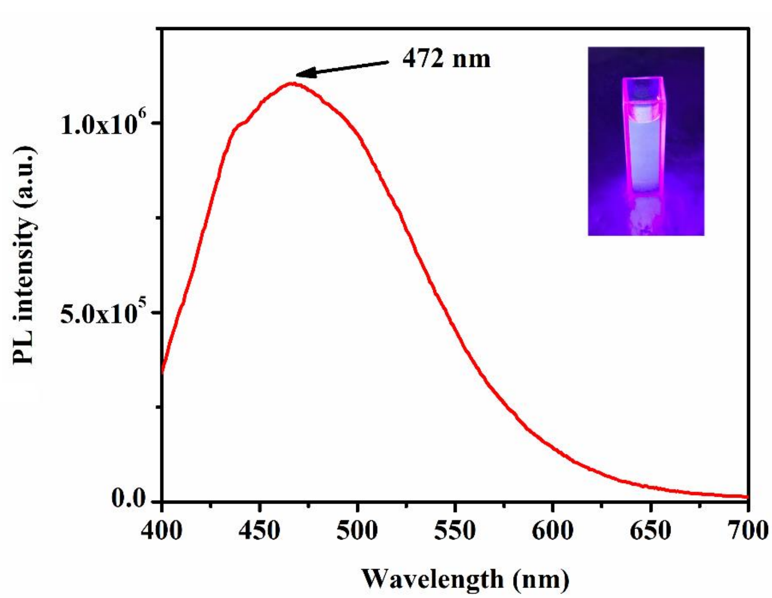

Figure 4-4 PL spectrum of the synthesized GQDs.

It can be clearly observed that the emission peak was at 472 nm corresponding to green fluorescence. The inset of figure 4-4 depicts photographs of the synthesized GQDs solution under 395 nm-UV light.

4.3 Nitrogen Sensing

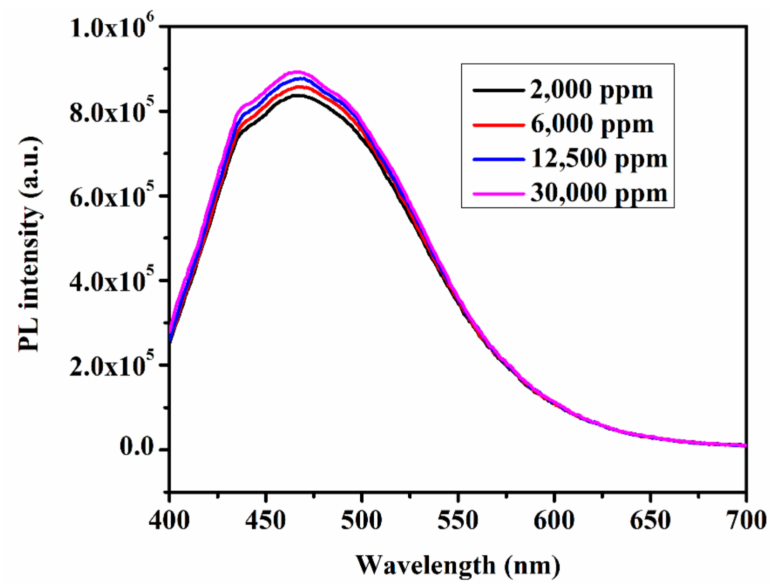

Figure 4-5 PL spectra of GQD based biosensors.

The result of nitrogen detection using GQD based biosensors, fluorescent biosensors, was obtained by PL spectroscopy. Figure 4-5 shows the PL spectra of the synthesized GQDs solution in the presence of different concentrations of nitrogen solution. It is found that the PL intensity gradually increased with an increase in 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. This effect promotes radiative recombination leading to more fluorescence. Generally, PL light is emitted through the radiative recombination by the excited electrons.

CHAPTER 5
CONCLUSION

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 exhibited spherical shape with an average diameter 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 or soils.

We hope that this research will be useful in area of sensor research for qualitative evaluation of cultivation soils in the near future.

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5. Academic Field of Specialty

   Electronic senses, i.e. electronic nose etc.

   Gas sensors

   Nanostructure materials, hybrid materials and graphene etc.

   Smart instruments and systems

   SCC-DFTB calculation
6. Publications

   1. K. Timsorn, and C. Wongchoosuk. 2020. "Adsorption of NO_2 HCN, HCHO and CO on pristine and amine functionalized boron nitride nanotubes by self-consistent charge density functional tight-binding method", *Materials Research Express* 7: 055005.

2. K. Timsorn, and C. Wongchoosuk. 2019. “Inkjet printing of room-temperature gas sensors for identification of formalin contamination in squids”, *Journal of Materials Science: Materials in Electronics* 30: 4782-4791.

3. K. Timsorn, Y. Lorjaroenphon and C. Wongchoosuk. 2017. “Identification of adulteration in uncooked Jasmine rice by a portable low-cost artificial olfactory system”. *Measurement* 108: 67-76.

4. P. Traiwatcharanon, K. Timsorn and C. Wongchoosuk. “Flexible room temperature resistive humidity sensor based on silver nanoparticles”. *Materials Research Express* 4 (2017): 1-10.

5. K. Timsorn, T. Thoopboochagorn, N. Lertwattanasakul and C. Wongchoosuk. 2016. “Evaluation of bacterial population on chicken meats using a briefcase electronic nose”, *Biosystems Engineering* 151: 116-125.

6. P. Traiwatcharanon, K. Timsorn and C. Wongchoosuk. 2016. “Effect of pH on the green synthesis of silver nanapaticles through reduction with Pistia stratiotes L. extract”, *Advanced Materials Research* 1131: 223-226.

7. K. Timsorn, C. Wongchoosuk, P. Wattuya, S. Promdaen and S. Sittichat. 2014. “Discrimination of chicken freshness

using electronic nose combined with PCA and ANN", *IEEE*: 1-4.

7. Awards

รางวัลผลงานประดิษฐ์คิดค้น ประจำปี ๒๕๖๑ รางวัลประกาศเกียรติคุณ เรื่อง ปืนตรวจวัดการปนเปื้อนสารฟอร์มาลีนในอาหารแบบพกพาได้ จากสำนักงานคณะกรรมการวิจัยแห่งชาติ (วช)