SYNTHESIS, CHARACTERIZATION AND SIMULATION OF FLUORESCENT GRAPHENE QUANTUM DOTS FOR THREE-DIMENSIONAL CELL IMAGING

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ABSTRACT

In the search for staining dyes for three-dimensional cell imaging, graphene quantum dots (GQDs) that are nanometers in scale, photoluminescent (PL), and dispersible in water may be investigated. The doping of graphene-based materials with nitrogen heteroatoms has demonstrated the ability to control the optical, electrical and opto-electronic properties of nitrogen-doped GQDs (N-GQDs). However, the characteristics of the energy-state and the properties of the N-GQDs are still unclear and require more study. This thesis designed and synthesised nanometer-sized GQDs using the hydrothermal technique. In addition, theoretical model based from experimental results of N-GQDs was execute by electronic structure calculations via the density function theory (DFT) using the GAUSSIAN 09 and GAMESS software. The sterilised N-GQDs after hydrothermal synthesis produce a high-crystalline form of N-GQDs with aspect (100) with lattice distance of 0.21 nm. The synthesis also produced one-layer or multiple-layer N-GQDs with an average diameter of 3.2 nm. The N-GQDs also highly soluble in water. It also exhibited high fluorescence emission ranging from 500 to 600 nm with green-coloured PL with highest peak at 525 nm contributed to electronic energy gap of 3.38 eV. In terms of bio-compatibility, the study shows cell viability ranging from 80%–90%, non-toxic. Moreover, this is the first study using GQDs for 3D cell imaging to display stained 3D cells with scattered individual cells in a multi-layered structure. For the DFT simulations, calculation shows that the range of electronic energy from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) of the GQDs depended on graphitic nitrogen doping and edge functionality. In conclusion, this research shows a novel optoelectronic properties extract from the energy levels of N-GQDs derived from experimental and theoretical calculations for 3D cell imaging applications.

ABSTRAK

Di dalam pencarian pewarna untuk pengimejan sel tiga dimensi, titik kuantum grafen (GQD) yang berskala nanometer, *photoluminescent* (PL) dan kebolehlarutan di dalam air akan dikaji. Mendopan grafen dengan heteroatom nitrogen (N-GQDs) telah menunjukkan keupayaan untuk mengawal sifat optik, elektrik dan opto-elektronik bahan tersebut. Walaubagaimanapun, ciri-ciri tenaga dan sifat-sifat N-GQD masih tidak jelas dan memerlukan lebih banyak kajian. Tesis ini merekabentuk dan mensintesis GQD bersaiz nanometer dengan menggunakan teknik hidroterma. Disamping itu, dengan menggunakan model N-GQDs berdasarkan keputusan eksperimen, pengiraan struktur elektronik telah dikira menggunakan teori fungsi ketumpatan (DFT) melalui perisian GAUSSIAN 09 dan GAMESS. N-GQD yang telah disteril selepas sintesis hidroterma menghasilkan bentuk N-GQD yang berhablur tinggi dengan aspek (100) dengan jarak kekisi 0.21 nm. Sintesis juga menghasilkan satu lapisan atau berbilang lapisan N-GQD dengan purata diameter 3.2 nm. N-GQD ini juga sangat larut dalam air. Ia juga mempamerkan pelepasan pendarfluor yang tinggi iaitu antara 500 hingga 600 nm dengan PL di puncak tertinggi pada 525 nm yang berwarna hijau, menyumbang kepada jurang tenaga elektronik sebanyak 3.38 eV. Dari segi keserasian bio, kajian menunjukkan daya maju sel antara 80%–90%, iaitu tidak toksik. Selain itu, ini adalah kajian pertama menggunakan GQD untuk pengimejan sel 3D untuk memaparkan sel 3D berwarna dengan sel individu bertaburan dalam struktur berbilang lapisan. Untuk simulasi DFT, pengiraan menunjukkan bahawa julat tenaga elektronik daripada orbital molekul yang diduduki tertinggi (HOMO) kepada orbital molekul tidak diduduki terendah (LUMO) GQD bergantung kepada pendopan nitrogen di dalam grafen dan kefungsian tepi grafen. Kesimpulannya, penyelidikan ini menunjukkan penemuan baharu pada sifat optoelektronik daripada tahap tenaga N-GQD yang diperoleh daripada pengiraan eksperimen dan teori untuk aplikasi pengimejan sel 3D.

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LIST OF SYMBOLS AND ABBREVIATIONS

°	-	Degree
α	-	Absorption coefficient
cm^{-1}	-	Reciprocal centimeter (wavenumber)
C	-	Celcius
π	-	22/7
λ	-	Wavelength
μ	-	Micro
h	-	Planck constant
v	-	Light frequency
ml	-	Milliliter
nm	-	Nanometer
μl	-	Microliter
Eg	-	Band gap energy
eV	-	electron Volt
AFM	-	Atomic Force Microscopy
CdS	-	Cadmium Sulfide
CdSe	-	Cadmium Selenide
CO_2	-	Carbon Dioxide
CNT	-	Carbon Nanotube
Da	-	Dalton
DFT	-	Density Functional Theory
ECM	-	Extra Cellular Matrix
FESEM	-	Field Emission Scanning Electron Microscopy
FTIR	-	Fourier Transform Infrared Spectroscopy
GO	-	Graphene Oxide
GQDs	-	Graphene Quantum Dots
H_2SO_4	-	Sulphuric Acid

HCL	-	Hydrogen Chloride
HF	-	Hartree Focks
HOMO	-	Highest Occupied Molecules Orbitals
HRTEM	-	High-Resolution Transmission Electron Microscope
IR	-	Infrared
LUMO	-	Lowest Unoccupied Molecular Orbitals
N_2	-	Nitrogen
$NaNO_3$	-	Sodium Nitrate
NH_2	-	Amino
O_2	-	Oxygen
PL	-	Photoluminescence
PLE	-	Photoluminescence Excitation
QD	-	Quantum Dots
SCF	-	Self consistent field
Si	-	Silicon
STEM	-	Scanning Transmission Electron Microscope
T	-	Temperature
TEM	-	Transmission Electron Microscope
TGA	-	Thermogravimetric
UV	-	Ultra-violet
XRD	-	X-ray Diffraction
XPS	-	X-Ray Photoelectron Spectroscopy

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The development of many low-cost optoelectronic devices, such as fluorophores and biological labelling sensors, is derived from the fundamentals of luminescent nanomaterials [1]. Luminescent nanomaterials with tunable properties and controllable emission wavelength are still gaining research attention. Generally, semiconductor quantum dots, gold nanodots, silicon nanoparticles, and carbon-based nanomaterials are recognised as new efficient emitters [1–4]. Among these nanoparticles, quantum dots (QDs) are known to have the brightest fluorescence. Moreover, QDs have been defined as a class of semiconductor nanoparticles composed of elements from the II–VI or III–V periodic groups with diameters ranging from 1 to 10 nm [5]. Based on quantum physics, nanometer-sized QDs possess optical and electrical properties that differ from those of macroparticles. Therefore, various compositions and sizes of QDs have been developed for clinical applications.

The fluorescence of QDs is caused by light absorption, which enables electrons to be excited from the valence band to the conduction band, leaving holes behind. Light can be emitted when an electron and a hole bond together to produce exciton energy. Figure 1.1 illustrates the excitation process of an electron-hole pair. Bandgap energy is calculated as the sum of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). When a particle has a radius larger than its own Bohr radius, the exciton can move freely without any boundary. However, the size of a QD is smaller than its own Bohr radius, and hence the exciton is confined in a space, leading to the quantum confinement effect. When an electron and a hole are attracted to each other due to the electrical Coulomb attraction, the exciton of photons occurs, leading to the excitation of the electron from the valence band to the conduction band.

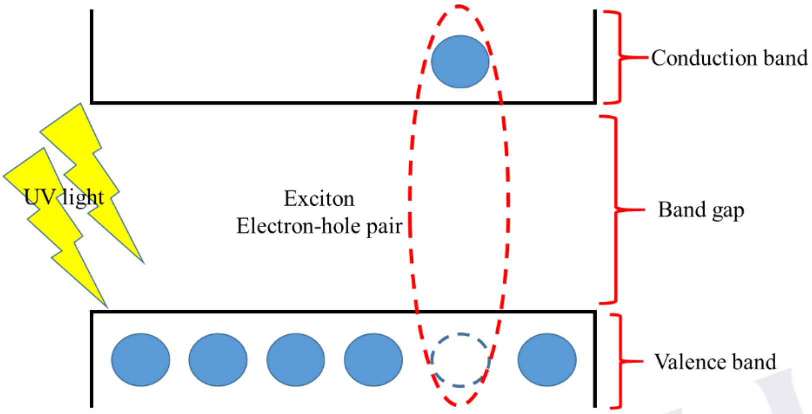

Figure 1.1: Excited electron and hole after UV light was given, leading to total energy as bandgap energy

A Bohr radius in Gaussian unit is given by:

$$a_o = \frac{h^2}{m_e e^2} \tag{1.1}$$

where a_o is the Bohr radius, h^2 is the reduced Plank's constant, and m_e is the electron rest mass. For instance, the Bohr radius of the hydrogen atom is approximately 0.53 Å [6]. The light emission of quantum dots occurs due to the quantum confinement effect. This event is a result of excitons taking quantised energy levels and being confined in space, and the free movement of excitons in all directions is restricted [7]. Therefore, QDs have larger bandgaps with sharp absorbance peaks and high photoluminescence (Figure 1.2). The confinement of energy depends on the quantum dots' size, which can be tuned during the synthesis process. Powerful inorganic fluorescent probes, such as semiconductor QDs, have exceptional long-term resistance to photobleaching [8].

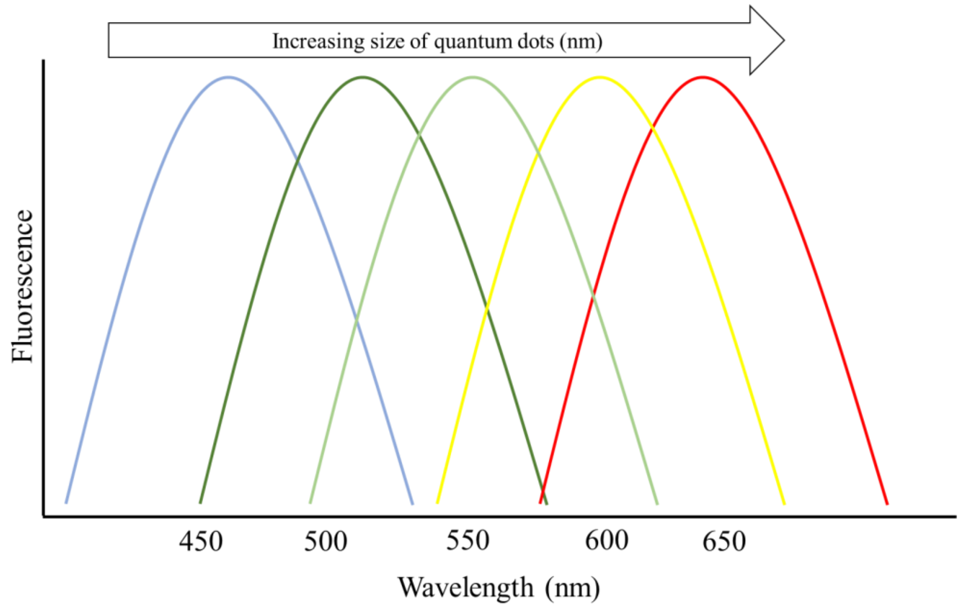

Figure 1.2: Emission spectra of quantum dots relative to increasing size of QDs

Carbon-based nanomaterials or carbon dots have advantages such as providing more stable emission, lower environmental impact, and lower toxicity compared with those of other semiconductor nanomaterials, such as cadmium selenide and cadmium telluride quantum dots. The size-tunable property of QDs is also important for wide applications of fluorescent quantum dots and related nanostructures.

1.2 Problem Statement

In numerous biomedical optical imaging experiments, cadmium selenide (CdSe) or cadmium sulphide (CdS) and their core shell nanoparticles from semiconductor quantum dots (QDs) have been employed [9], [10]. However, the QDs of cadmium telluride are toxic to cells [11]. Nevertheless, due to the size of QDs being larger than that of biomolecules, QDs have the potential of influencing the dynamics and functions of the molecules of interest, as well as constructing artificial clusters and interacting with a wide range of targets [12]. To fill up this research gap, the current work is to synthesize QDs in a few nanometer scale. In addition, the synthesis of QDs suffers from high complexity, low quantities, and high material expenses. Finding fluorophores that can provide photostability is crucial for bio-imaging. To date, researchers have shown interest in carbon-based nanomaterials, such as graphene quantum dots (GQDs), where the application of GQDs in biomedical sciences is still in the early stages [13].

Despite recent breakthroughs in GQD synthesis, the development of environmentally friendly techniques for the synthesis of graphene is now crucial for practical applications. [14] Conventional GQD synthesis processes have environmental concerns, such as hazardous compounds employed as cutting agents [15]. These technologies frequently have severe negative consequences on the environment and human health, limiting the practical applications of GQDs. Several initiatives have been undertaken in recent years to solve this issue, including the creation of non-toxic chemical cutting agents, alternate reduction pathways for graphene oxide (GO), and direct exfoliation of natural or synthetic graphite [15]. For instance, hydrothermal synthesis is an environmentally friendly technique that can also generate small particle sizes of GQDs.

Nitrogen-doped GQDs (N-GQDs) have received a lot of attention due to their optical features, electrocatalytic ability, and biocompatibility [16]. However, the energy-state characteristics and the properties of N-GQDs remain unclear and need more studies in order to develop N-GQDs for various applications. Doping graphene-based materials with heteroatoms has demonstrated the capacity to control the optical, electrical, and optoelectronic properties of GQDs. Hence, the tuning of optical properties via the electronic structure of GQDs can be further studied.

To date, researchers have engineered two-dimensional (2D) cells into microtissues [17] or three-dimensional (3D) cells that mimic the in-vivo environment of cells being surrounded by other cells and the extracellular matrix (ECM). There are limited studies on drug delivery that used 2D cells due to the irrelevant physiological properties of a cell culture in a plastic vessel, such as proliferation, in-vivo protein expression, cell morphology, and gene [17]. For the application of nanomaterials in biomedical engineering, most of the current investigations on nanotherapeutics are still performed either using 2D cell cultures or in-vivo models. Nanomaterials' application in 2D cell models is not representative and far from in-vivo models due to the oversimplicity of 2D cell models. On the other hand, the use of in-vivo models requires stringent bioethics when handling animals. Moreover, as compared with cells in a 3D environment, 2D monolayer cell cultures present a less significant barrier for transport and lower cell binding. Hence, a 3D cell model that mimics in-vivo tissues presents an improved model for investigating the interaction of fluorescent carbon quantum dots with multilayered cells.

The development of 3D cells into scaffolds of microtissues might be beneficial for research on drug delivery, tissue implants, and cancer treatment and medication. The interaction of GQDs with a 3D cell model is rarely reported, and this study is the first study to apply GQDs in a 3D cell culture model. Cells do not require an external scaffold for aggregation, since they create the ECM, which, in turn, improves intercellular adhesion [18]. Hence, a 3D cell model can serve as a tissue-mimicking model for the study of GQDs. This research provided evidence that the GQDs produced in this work are suitable to be applied in 2D and 3D cell studies and may even be applied in the future as an anti-cancer drug carrier with a fluorescence property for cell tracking.

1.3 Objective

The aim of this study is to synthesise and investigate the properties of fluorescent GQDs. The following are the objectives that needed to be achieved:

a) To study the physical properties of graphene oxide synthesised using modified Hummer's method prior to producing the GQDs, as well as to quantise the energy levels.
b) To optimise nitrogen-doped GQDs synthesis using the hydrothermal technique and investigate the structural, morphological and optical characterisation of the N-GQDs.
c) To investigate the microencapsulated 3D cells doped with N-GQDs for fluorescence imaging of cells.
d) To compare the defects on the electronic energy structure of GQDs from experimental synthesis of nitrogen doping GQDs and theoretical calculations.

1.4 Scope

In this study, the approach to synthesise GQDs with fluorescence properties was:-

(a) By using a precursor, which was graphene oxide.

(b) The characterisations of the physical properties of graphene oxide were via thermal, Raman spectra, and XRD analyses.

(c) Its chemical elements were characterised using FTIR and XPS.

(d) The structural properties of graphene oxide were characterised using TEM, FESEM, and AFM analyses.

(e) Theoretical calculations of the electronic structure of GQDs employed the density functional theory (DFT) via GAUSSIAN 09 and GAMESS software and the results were studied.

1.5 Research Contribution

This study's main originality lies in the following:

a) An enhancement of energy gap (3.38 eV) from nitrogen doping to the energy levels of the graphene layer using hydrothermal method which used to produce nano-sized graphene sheets provides an effective means of tuning the optical properties of N-GQDs.

b) The elucidation of the effects of nitrogen doping, size, and edge termination of GQDs from experimental and theoretical calculation for fluorescent enhancement.

c) A novel contribution to bio-imaging modality via detailed properties of graphene nanosheets for engineering GQDs for application in 3D cell imaging.

1.6 Outline of Thesis

This thesis comprised five chapters, with the contents of each chapter briefly summarised as follows.

In Chapters 1, introduction on this research were discussed. The objective, scope and novelty of this research were highlighted.

Chapters 2 present the background of this study such as introduction on graphene oxide, graphene, GQDs and previous GQDs synthesis techniques. Some applications of GQDs are also explained in this chapter. The purpose of this work is defined.

In Chapter 3, the characterisations of GQDs and the parameters used to determine quantisation are described. The methods for the physical characterisations of GQDs are explained. The contribution of electronic and quantum states is briefly described. The effects of the nanostructure on the electronic state and the possibility to improve the efficiency of GQDs are also discussed.

In Chapter 4, the results are revealed and a detailed discussion is presented on the effect of hydrothermal synthesis on the energy levels of the nitrogen-doped GQDs. The biocompatibility of the GQDs with HeLa cells and the bio-imaging of the GQDs on HeLa cells were studied. Furthermore, the toxicity and imaging of microencapsulated cells doped with the N-GQDs are also discussed in this chapter. The N-GQDs were tested on 3D cells, instead of 2D cells, for the bio-imaging application.

Chapter 5 explained about the theoretical calculations of the GQDs performed using the GAUSSIAN 09 and GAMESS software. The measurement results of the nitrogen-doped GQDs' bandgap output efficiency are also presented. The comparison between the measured and theoretical HOMO and LUMO electronic transitions of the GQDs are briefly discussed in this chapter. The hydrothermal synthesis of GQDs from the GO precursor and the effects of heteroatom doping on the GQDs are discussed. The quantisation of energy gap was studied via the physical and photoelectronic properties of the nitrogen-doped GQDs.

Finally, in Chapter 6, the summary of the simulations of the GQDs' electronic structure, the characterisations of the GQDs, and the imaging of 3D cells using the GQDs is explained. Future works in bio-imaging applications are also presented.

CHAPTER 2

LITERATURE REVIEW

In this chapter, the techniques used to produce graphene are described. Graphene has attracted the attention of numerous researchers to study the use of this material in many applications. However, this study focused on the fluorescence properties of graphene.

2.1 Graphene and Graphene Oxide

Scientist R. Wallace [19] discovered the characteristics of graphene in 1947, and Hoffman et al. produced pure graphene from graphene oxide via hydrazine reduction in 1963. After Geim and Novoselov's work on separating graphene from highly oriented pyrolytic graphite (HOPG) and researching graphene's beneficial features, graphene has drawn a lot of attention, such as research on fluorescence emission[3]. Graphene is a one-atom-thick planar structure of carbon atoms arranged in a honeycomb crystal lattice, and it is a semiconductor with a zero bandgap and with excitons having an infinite Bohr diameter. Theoretically, by varying the size of benzene in graphene's structure, the bandgap of 0 eV can be modified. In other materials, due to the low temperature dependence of electron mobility, electron transport is constrained by defect scattering rather than phonon scattering [20]. Thus, confinement can be observed in any fragment. The study of the optical properties of graphene typically starts with the investigation of the optical properties of graphite intercalation compounds.

Due to the properties of quantum dots, such as long-term photobleaching resistance, adjustable photoluminescence, and minimal cytotoxicity, graphene has

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APPENDIX C

LIST OF PUBLICATIONS

Journal Paper

1) Wan Ibtisam Wan Omar, Yamaoka O, Soon CF, M.K. Ahmad, M. Shimomura. Quantization of Energy Gap of Nitrogen Doped Graphene Quantum Dots. J Adv Res Fluid Mech Therm Sci 2019; 2: 329–335. (Scopus)
2) Wan Ibtisam Wan Omar, Soon CF, M.K. Ahmad, M. Shimomura. Hydrothermal Synthesis of Biocompatible Nitrogen Doped Graphene Quantum Dots. Energy & Environment SAGE Publishing. (Impact factor: 2.945)

International/National Conference

1) Wan Ibtisam Wan Omar, Okei Yamaoka, Chin Fhong Soon, Mohd Khairul Ahmad, Masaru Shimomura, Quantization of Energy Gap of Nitrogen Doped Graphene Quantum Dots. The 6th International Conference of Applied Science and Technology (ICAST 2019), Kyoto, Japan.
2) Wan Ibtisam Wan Omar, Chin Fhong Soon, Mohd Khairul Ahmad, Masaru Shimomura, Hydrothermal Synthesis of Biocompatible Nitrogen Doped Graphene Quantum Dots, BIOSES 2019 2nd Seminar on Biological Security and Sustainability, Universiti Malaysia Terengganu.[e-Poster presentation]
3) Wan Ibtisam Wan Omar, Chin Fhong Soon, Mohd Khairul Ahmad and Masaru Shimomura, Tunable Luminescence of Biocompatible Nitrogen-doped Graphene Quantum Dots, The 6th International Symposium in Shizuoka University 2020.[Poster presentation]
4) Wan Ibtisam Wan Omar, Okei Yamaoka, Chin Fhong Soon, Mohd Khairul Ahmad, Masaru Shimomura, Quantization of Energy Gap to the Tunable Photoluminescence of N-doped Graphene Quantum Dots. The 67th JSAP Spring Meeting 2020 in Sophia University.[Poster presentation]

APPENDIX D

VITA

Wan Ibtisam Binti Haji Wan Omar was born in Kota Bharu, Kelantan in 1989. She also had received her early education there. She then graduated for her bachelor's degree in 2012 and Master's degree in Electrical and Electronics Engineering from the Universiti Tun Hussein Onn Malaysia (UTHM) in 2016. She then received her PhD in Optoelectronics and Nanostructure Science from Shizuoka University, Japan in 2020 under Double Degree Program between UTHM and Shizuoka University. At present, she is working on the following research topics: Nanomaterials for Bio-application.