UNIVERSITI PUTRA MALAYSIA

FLUOROMETRIC GENE ASSAY FOR DETERMINATION OF Escherichia coli O157:H7 USING GRAPHENE QUANTUM DOTS AND CARBON DOTS WITH GOLD AND SILVER NANOPARTICLES

SURIA BINTI MOHD SAAD

ITMA 2020 16

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By

SURIA BINTI MOHD SAAD

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2020

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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September 2020

Chairman : Jaafar Abdullah, PhD
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Escherichia coli (*E. coli*) O157:H7 is considered as harmful bacteria which can result in severe infections to human. Current detection methods are prolonged and inefficient due to extensive sample preparation and lengthy test procedures. Thus, there is a requirement for simplicity of techniques which are capable of sensing *E. coli* O157:H7 at high sensitivity, specific, less toxicity and agile. A fluorescence quenching assay involving graphene quantum dots (GQDs) and carbon dots (CDs) with gold (AuNPs) and silver (AgNPs) nanoparticles for the determination of *E. coli* O157:H7 have been explored. GQDs and CDs act as the fluorophore, while AuNPs and AgNPs as the quencher. Short target oligos (20 bp) have been utilized to establish distance between fluorophore and quencher in close proximity. Then, the fluorophore and quencher were complexing adjacently to trigger the fluorescence quenching mechanism following the target oligos co-hybridization. The complex immediately returns to the ground state by absorption of light without the release of photons. Several essential parameters such as reaction time and wavelength maximum of emission have been optimized to enhance the efficiency of fluorescence quenching. An excitation/emission wavelength of 400 nm/530 nm and 340 nm/450 nm were used for GQDs and CDs, respectively. The net intensity fluorescence quenching of GQDs and CDs was enhanced proportionally with the increment concentrations of target oligos. A linear correlation between the fluorescence quenching of GQDs/CDs and the logarithm concentration of target oligos in the series of 0.1 nM to 150 nM (GQDs-AuNPs), 0.01 nM to 200 nM (CDs-AuNPs) and 0.001 nM to 200 nM (CDs-AgNPs) (slope = 42.74, R^2 = 0.991; slope = 675.6, R^2 = 0.992; slope = 217.6, R^2 = 0.977) and the detection limit (LOD) of 1.10 ± 0.58 nM, 1.00 ± 0.71 nM and 1.01 ± 0.71 nM, respectively. The proposed method was utilized for verification of selectivity and specificity towards different oligonucleotide sequence and bacteria strain with satisfactory results. The practicability of the assay was also verified by evaluating the amplicon (*fliC* gene, 381 bp) of genomic DNA isolated from food samples spiked with *E. coli* O157:H7. It is noteworthy that the determined t-value is less than the critical t-value ($t_{calc.}$ < 2.78) indicating that the developed method and real time PCR method are comparable and in good agreement.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

UJIAN GEN FLUOROMETRIK UNTUK PENENTUAN *Escherichia coli* O157:H7 MENGGUNAKAN TITIK KUANTUM GRAFIN DAN TITIK KARBON DENGAN NANOPARTIKEL EMAS DAN PERAK

Oleh

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Escherichia coli (*E. coli*) O157:H7 dianggap sebagai bakteria berbahaya yang boleh menyebabkan penyakit serius kepada manusia. Kaedah pengesanan semasa adalah panjang dan tidak cekap kerana penyediaan sampel yang banyak dan prosedur ujian yang panjang. Oleh itu, terdapat keperluan untuk kesederhanaan kaedah yang mampu mengesan *E. coli* O157:H7 pada kepekaan tinggi, spesifik, kurang toksik dan tangkas. Ujian pelindapkejutan pendarfluor yang melibatkan titik kuantum grafin (GQDs) dan titik karbon (CDs) dengan nanopartikel emas (AuNPs) dan perak (AgNPs) untuk penentuan *E. coli* O157:H7 telah diterokai. GQDs dan CDs bertindak sebagai fluorofor, sementara AuNPs dan AgNPs sebagai pelindapkejut. Oligos sasaran pendek (20 bp) telah digunakan untuk menghasilkan jarak di antara fluorofor dan pelindapkejut berdekatan. Kemudian, fluorofor dan pelindapkejut telah berinteraksi secara berdekatan untuk mencetuskan mekanisme pelindapkejutan pendarfluor diikuti penghibridan bersama oligos sasaran. Kompleks segera kembali ke keadaan dasar dengan penyerapan cahaya tanpa pelepasan foton. Beberapa parameter penting seperti masa tindak balas dan panjang gelombang pelepasan maksimum telah dioptimumkan untuk meningkatkan kecekapan pelindapkejutan pendarfluor. Pada panjang gelombang pengujaan/pelepasan 400 nm/530 nm dan 340 nm/450 nm bagi GQDs dan CDs, masing-masing. Pelindapkejutan pendarfluor bersih GQDs dan CDs meningkat secara berkadaran dengan peningkatan kepekatan oligos sasaran. Hubungan korelasi linear di antara pelindapkejutan pendarfluor GQDs/CDs dan kepekatan logaritma oligos sasaran dalam julat 0.1 nM hingga 150 nM (GQDs-AuNPs), 0.01 nM hingga 200 nM (CDs-AuNPs) dan 0.001 nM hingga 200 nM (CDs-AgNPs) (kecerunan = 42.74, R^2 = 0.991; kecerunan = 675.6, R^2 = 0.992; kecerunan = 217.6, R^2 = 0.977) dan had pengesanan (LOD) 1.10 ± 0.58 nM, 1.00 ± 0.71 nM dan 1.01 ± 0.71 nM, masing-masing. Kaedah yang dicadangkan telah digunakan untuk pengesahan kepilihan dan kespesifikan terhadap urutan oligonukleotida dan baka bakteria yang berbeza dengan keputusan yang memuaskan. Kebolehlaksanaan ujian juga disahkan dengan menilai amplikon (gen *fliC*, 381 bp) DNA genomik yang diasingkan dari sampel makanan disuntik dengan *E. coli* O157:H7. Diperhatikan bahawa nilai-t yang dikira adalah kurang daripada nilai-t kritikal ($t_{calc.}$ < 2.78) yang menunjukkan bahawa kaedah yang dibangunkan dan kaedah PCR masa nyata berada dalam persetujuan yang baik dan setanding.

ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah the Almighty – by His grace, I have made this accomplishment in completing pursue of my PhD degree.

I am very grateful to Associate Professor Dr. Jaafar Abdullah as my supervisor for his guidance, suggestions, attention, criticisms and support throughout the course of this study. A particular debt of gratitude is owed to my co-supervisors Professor Suraya Abdul Rashid, Associate Professor Dr. Yap Wing Fen, Dr. Faridah Salam and Dr. Lau Han Yih for their critical readings, comments and cooperation.

I would like to extend my sincere thanks to my parents Mohd Saad Suboh (ayah) and Hasnah Othman (mak) for raising me and providing me with good education (and funding as well) until I become what I am now. For my siblings, I hope I make you all proud. This thesis also is dedicated to my in-laws, father-in-law (ayah) and mother-in-law (mama) for their prayer and help throughout the time. For my family, I would not be this strong without all their supports.

I am heartfelt thanks to my husband Syahren Zamzuri for his unconditional love, patience and support throughout my PhD journey. Also, to my children, Saif Fathi, Safiya Huda and Saif Fazil (whom was born at the beginning of my PhD struggle) for their smiles which encouraged me to complete this study efficiently. I hope all of you will treasure the meaning, value and pride of having good knowledge. Ignorance is certainly not bliss in these modern challenging days, kids.

Additionally, I would like to acknowledge the people in the biosensor group at laboratory 103 for their assistance in my research and generously sharing their knowledge with me.

With that, I present to you this dissertation.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

GQDs	Graphene quantum dots
CDs	Carbon dots
AuNPs	Gold nanoparticles
AgNPs	Silver nanoparticles
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
QDs	Quantum dots
E. coli	*Escherichia coli*
MOH	Ministry of Health
IHR	International Health Regulations
SPS	Sanitary and Phytosanitary Measures
US	United State
CDC	Centers for Disease Control
EIA	Enzyme immuno assay
FRET	Förster resonance energy transfer
IFE	Inner filter effect
SET	surface energy transfer
DET	Dexter energy transfer
PET	Photoinduced electron transfer
NSET	Nanometal surface energy transfer
Ksv	Stern-Volmer constant
K_D	Dynamic quenching constant
Ks	Static quenching constant
NMs	Nanomaterials
AuNCs@GSH	Gold nanocluster capped by glutathione
SQE	Static quenching effect
RET	Resonance energy transfer
ET	Electron transfer
AIEQ	Aggregation induced emission quenching
AIEE	Aggregation induced emission enhancement
G-LEDs	Green light emitting diodes

CNTs	Carbon nanotubes
LPS	Lipopolysaccharide
HUS	Haemolytic-uremic syndrome
NIID	National Institute of Infectious Diseases
NESID	National Epidemiological Surveillance of Infectious Diseases
EHEC	Enterohemorrhagic *Escherichia coli*
ESBL	Extended-spectrum β-lactamase
QREC	Quinolone resistant *Escherichia coli*
CPRC	Crisis Preparedness and Response Centre
DOE	Department of Environment
WHO	World Health Organization
MPN	Most-Probable-Number
HRTEM	High-resolution transmission electron microscope
EDX	Energy-dispersive X-ray
FTIR	Fourier-transform infrared spectroscopy
cm^{-1}	Wavenumber
$\sum$	Quenching efficiency
F_D	Fluorescence intensity of the donor
D	Donor
A	Acceptor
F_0	Fluorescence intensities before (0 hour) quenching in the presence of the acceptor
F_q	Fluorescence intensities after (2 hours/30 min/20 min) quenching in the presence of the acceptor
Sa	Standard deviation of the blank measurement
b	The slope of the calibration plot.
NMR	Nuclear magnetic resonance
EDC/NHS	Carbodiimide/N-hydroxysuccinimide
PL	Photoluminescence
F^o	Fluorescence intensities without of the quencher concentration
F	Fluorescence intensities with of the quencher concentration
LOD	Limit of detection

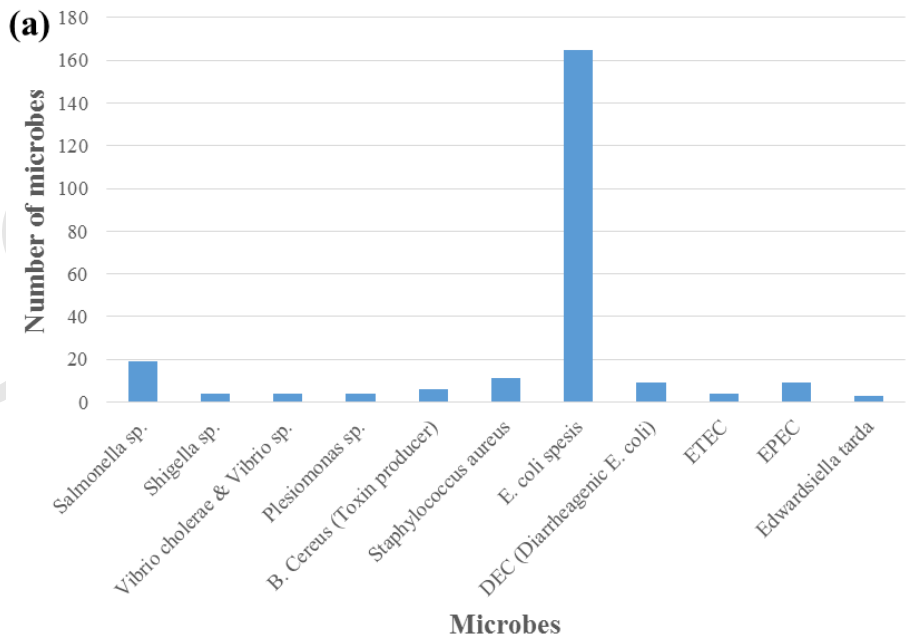
$\Delta\varepsilon$	The change in absorption coefficient
Ka	Association constant
[Q]	The concentration of quencher
K_A	The equilibrium binding constant
n	The binding sites

CHAPTER 1

INTRODUCTION

1.1 Background of study

Escherichia coli (*E. coli*) serotypes are the common bacteria that usually reside in the lower intestine of human digestive tract and warm-blooded animals. However, there are strains that are harmful to humans with a high toxicity level such as *E. coli* O157:H7 (Zhang et al. 2016) and can be categorized as microorganism with the potential for foodborne diseases burden. Generally, the food safety and outbreak of *E. coli* infection specifically in Malaysia was not a real issue and the community have low awareness on the risk and effect from its infection (New et al. 2017). Trend of food poisoning incidence in Malaysia is shown in increasing every year (Ismail et al. 2018). According to statistic prepared by Ministry of Health (MOH) in 2016, a total of 1063 premises inspection that has performed in the country such as school canteens and cafeterias, about 18 premises (2.1%) were forced to shut down their operations due to a violation of Section 11 of the Food Act 1983 for an offense of unlawful premises (Ministry of Health 2016). In 2013, approximately 14,202 cases of food poisoning reported with a total of 12 cases of death (A'aisah 2014). Figure 1.1a show that the most commonly encountered pathogen is *Escherichia coli* species followed by *Salmonella* spp., with the school canteen and cafeteria are the most commonly sector encountered with these pathogens in Malaysia (Figure 1.1b) (A'aisah 2014).



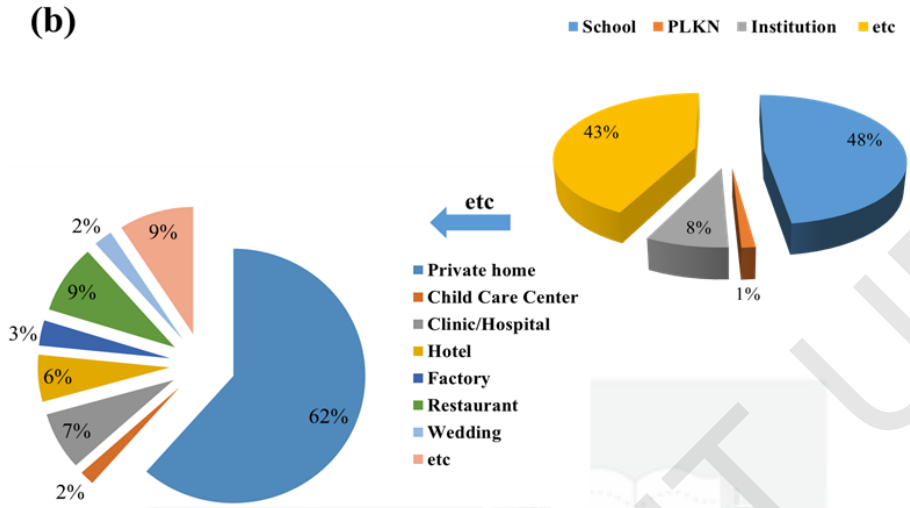


Figure 1.1: (a) Pathogen that was isolated (Lab-based surveillance - Acute Diarrhoeal Disease Project 2013) and (b) food poisoning episode and cases in Malaysia (Source: A'aisah 2014)

In addition to the crisis of foodborne illness, deprivations in demand due to microbial contamination and spoilage in agricultural and food products typically have considerable economic consequences to the producer countries (Beutin & Martin 2012). In 2011, the European outbreaks have become one of the biggest *E. coli* epidemics in history and have damaged trade and triggered trade restrictions (EN SANCO/13004/2011 2011). This is because the contaminated agricultural and food products are in violation of EU law, the International Health Regulations (IHR, 2005) and the Sanitary and Phytosanitary Measures (SPS) Agreement (Fidler 2011). Recently, Ministry of Health (MOH), Malaysia has advised the companies involved in the imported of romaine lettuce to withdraw the vegetable from the market. Consumers also were advised to stop eating the vegetables and to get rid of any they've purchased. This was in correlation with the report released by the Centres for Disease Control (CDC) and Prevention about 32 peoples in 11 states of United State (US) have reported *E. coli* infections which linked to romaine lettuce that occurred on October 2018 (Ministry of Health 2018).

Biosensors have been known as reputable options for environmental monitoring, food monitoring and clinical diagnostic because of their user friendly, mobile, direct, sensitive, affordable and capable for precise, real-time detection (Paniel et al. 2013). These technologies appear with distinctive competencies for on-site (Li et al. 2018) and real-time (Singh et al. 2018) analysis. Real-time detection of pathogenic contaminants is essential because it offers instant interactive information on the sample being tested and allows food servicers to take corrective actions prior to the product is sold to consumers (Moerman 2018).

Currently, the advancement of biosensors for microbial identification and detection assures a rapid, simple and sensitive to carry out (Singh et al. 2014) (Hu et al. 2015). DNA-based biosensors offers high-sensitivity, cost-effective, faster and simpler compared with conventional enzyme immuno assay (EIA) (Khang et al. 2016). Over the past fifteen years, several numbers of reports utilizing biosensor techniques for the determination of *E. coli* O157:H7 have been established. Among all the established biosensor methods, optical biosensors are still the most attractive transduction method to be studied in the determination of *E. coli* O157:H7 (Xu, Wang & Li 2017). Optical sensors have been recognized as one of the techniques used to detect target elements due to their excellent performance for various samples analysis, easy operation and rapid detection (Saleviter et al. 2019). In this study, major virulence genes such as shiga toxin 1 (*stx1*) and shiga toxin 2 (*stx2*) were not selected as detection targets. This is due to the common Shiga toxin-producing *E. coli* (STEC) serotypes such as strains O26, O45, O103, O111, O121 and O145 also produce *stx1* and *stx2* (Guy et al. 2014). Therefore, *E. coli* O157:H7 was identified as STEC serotype O157:H7 with serotype-specific *fliC* gene (flagellin protein) in the developed sensing system to ensure detection specificity in this study.

1.2 Problem statement

E. coli O157:H7 is considered as dangerous bacteria which can cause serious illness to human. Negligence on prevention measures and provides early treatment could cause gastroenteritis symptoms such as vomiting, fever, nausea and in worst cases can lead to death.

Various techniques have been proposed for the determination of *E. coli* spp. Conventional culture techniques for the determination of *E. coli* O157:H7 in food such as microbiological methods, involve culture on agar and followed by colony counting, which is laborious, time-consuming and inefficient (Paniel et al. 2013). The utilization of polymerase chain reaction (PCR) followed by gel electrophoresis is even faster, selective and sensitive (Afendy & Son 2015) than conventional methods, but requires agarose gel electrophoresis in the DNA detection process, which is rather troublesome and carcinogenic (Nakano, Ding & Suehiro 2017). Real-time PCR, on the other hand, involves expensive reagents, the use of optical detection equipment and also should be performed by skilled personnel (Pang et al. 2017). In addition, dealing with DNA as a target analyte mostly struggling for target amplification using various strategies such as isothermal amplification which is time consuming and troublesome (Donmez et al. 2019). Fluorescence-based biosensor, on the other hand, often have problems with sensor performance and response, which are not sensitive enough (Saleviter et al. 2019).

Thus, there is an urgent demand for simplicity of techniques which are capable to detect *E. coli* O157:H7 at high sensitivity, specific, less toxicity, stable and agile. These are due to the effort of testing for *E. coli* O157:H7 which can happen anywhere and sometimes require a tool that is user friendly and can provide with an accurate and instant result. There are very limited reliable process monitoring

and control techniques available in the market to ensure food safety and public health concern. At the moment, there is no product able to comply with all these criteria. Therefore, if genomic DNA from bacterial isolates and cultures can be quantitatively and immediately quantified, ratiometric fluorescence DNA detection methods can offer a practical substitute to conventional PCR and real time PCR. To date the combination of graphene quantum dots (GQDs) and carbon dots (CDs) with gold (AuNPs) and silver (AgNPs) as a novel nanomaterial platform for DNA-based sensor for the detection of *E. coli* O157:H7 has not been explored. Therefore, the feasibility of these nanomaterials for DNA probes immobilization and hybridization has not been proven. In addition, the use of DNA as a sensing probe in the fabrication of fluorescence quenching biosensor can enhance the specificity and sensitivity of *E. coli* O157:H7 detection that cannot be achieved with conventional immuno-based technique.

This study discusses the development of specific, sensitive and reliable optical sensor for *E. coli* O157:H7 detection based on DNA platform which utilized a fluorescence quenching assay format as the principle of detection. Three types DNA-sensor were focused on optical method that using a pair of fluorophore and quencher of fluorescence quenching: i) graphene quantum dots-gold nanoparticles (GQDs-AuNPs), ii) carbon dots-gold nanoparticles (CDs-AuNPs) and iii) carbon dots-silver nanoparticles (CDs-AgNPs) DNA-sensor.

All of the DNA-sensors were characterized with an appropriate surface chemistry in fluorescence microplate reader for co-hybridization of target oligos to provide stable assay conditions. The appliance of AuNPs or AgNPs for signal quenching in all DNA-sensor approaches was also assessed to enhance sensitivity of the assay system. The fluorescence quenching of DNA-based sensor were initially utilized to determine a short fragments of *fliC* gene of *E. coli* O157:H7 in real samples analysis, which food samples were experimentally spiked and compared with an established real time PCR method. Then the developed system finally was tested on genomic DNA as a target analyte in real sample analysis and also compared with real time PCR.

1.3 Objective of the study

This study was intended to design a specific and sensitive DNA-based sensor technique using fluorescence quenching mechanism to detect *fliC* gene of *E. coli* O157:H7 for apply in food safety monitoring. The following specific objectives are outlined to achieve the goal of this study:

I. To design and conjugate the DNA probe with graphene quantum dots (GQDs) or carbon dots (CDs) and gold (AuNPs) or silver (AgNPs) nanoparticles for the determination of *fliC* gene of *E. coli* O157:H7.

II. To optimize and characterize the conjugated amine oligos with GQDs or CDs and thiol oligos with AuNPs or AgNPs and to verify the *fliC* gene of

E. coli O157:H7 using the GQDs-AuNPs, CDs-AuNPs and CDs-AgNPs fluorescence quenching DNA-sensor.

III. To study the performance (sensitivity, selectivity and specificity) of the developed sensing system towards *fliC* gene of *E. coli* O157:H7.

IV. To validate the developed DNA-based biosensor with commercial real time PCR kit method for detection of *E. coli* O157:H7 in real samples analysis.

The flow chart below demonstrates the relationships between different parts of the study (Figure 1.2).

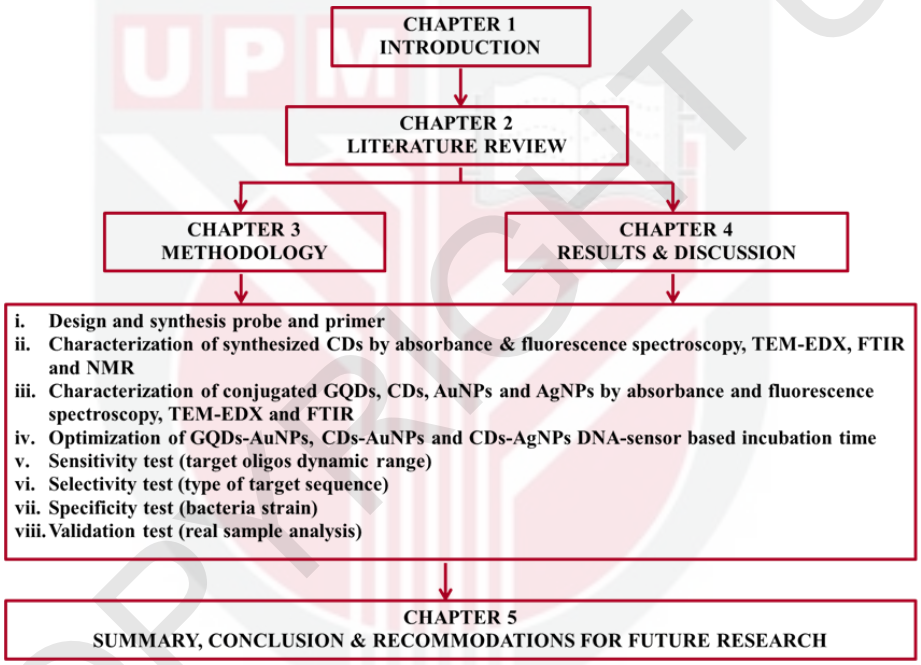


Figure 1.2: Flow chart displaying the relationships between different parts of the study in the development of fluorometric gene assay for *E. coli* O157:H7 determination

1.4 Scope and limitation of study

Fluorescence quenching DNA-sensor is an optical method that measures the fluorescence intensity changes which rely on the spatial distance between the fluorophore and quencher. Thus, for fluorescence quenching to occur it relies on incubation times for co-hybridization of target oligos with reporter oligos on fluorophore and next with quencher oligos on quencher consecutively to form stable complex structures. However, during the incubation process before and after the

adding of quencher (AuNPs- or AgNPs-thiol modified) many possibilities could take place in the sample mixture preparation. Even though it was from the same replicates it can be varied in term of fluorescence intensity reading. Therefore, the samples need to be prepared at least in five replicates in intention to calculate the quenching efficiency and to plot a linear graph. It is also difficult to obtain consistent results and reproducible techniques between replicates and batches in the development of this fluorescence quenching DNA-sensor. This is because it is a kinetic study in which the progress of fluorescence quenching depends on the incubation time. The system requires a lengthy tracking time of at least 2 hours and 30 minutes and not stable for analysis of sample that have long target sequence. This is due to the steric hindrance of long target sequences might block the co-hybridization sequence of quencher probes on AuNPs. The length, type (RNA or DNA) and secondary structure of the target mainly affect the efficiency of probe-target duplex formation, hybridization and specificity (Liu, Guo & Wu 2007).

Another limitation of this study is including the tedious sample preparation. As we know dealing with bacteria as targeted pathogen and DNA as a target analyte, the bacteria and DNA required being cultured and extracted to amplify and get its colonies and their total genome, respectively. Although this system can be used for *fliC* gene detection but there are some limitations. The developed system is still in preliminary study and is not ready for on-site application as samples require to be processed with simple sample preparation in the laboratory and instruments are used such as thermal cycler for PCR and fluorescence microplate reader for fluorescence assay. In the future, the developed system could be used in combination with flocculation assay that has been established (Wee et al. 2015) and portable fluorescence reader for on-site DNA amplification and fluorescence assay. In this study, the developed system was also tested on genomic DNA in real sample analysis and the results were comparable to real time PCR. This indicates that in the future, the developed system has the potential to be tested directly on genomic DNA without time-consuming pre-detection amplification and eventually avoids the PCR procedure.

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APPENDICES

APPENDIX A1

Data on Stern-Volmer plot for both CDs-AgNPs and CDs-AuNPs fluorometric assay were summarized in Table A1a and Table A1b, respectively.

Table A1a: Data on Stern-Volmer plot of CDs-AgNPs fluorometric assay

[AgNPs] µM	F°	F	F°/F
0.05	5845	5573	1.049
0.10	5845	5340	1.095
0.15	5845	5185	1.127
0.20	5845	4991	1.171
0.25	5845	4960	1.178
0.30	5845	4759	1.228
0.40	5845	4475	1.306
0.45	5845	4489	1.302

Table A1b: Data on Stern-Volmer plot of CDs-AuNPs fluorometric assay

[AuNPs] µM	F°	F	F°/F
0.05	5845	5320	1.099
0.10	5845	4947	1.182
0.20	5845	4227	1.383
0.25	5845	4141	1.412
0.30	5845	3773	1.549
0.40	5845	3633	1.609

APPENDIX A2

Data on Benesi-Hildebrand plot for both CDs-AgNPs and CDs-AuNPs fluorometric assay were summarized in Table A2a and Table A2b, respectively.

Table A2a: Data on Benesi-Hildebrand plot of CDs-AgNPs fluorometric assay

[AgNPs] µM	F°	F	F° – F	1/F° – F	1/[AgNPs]
0.05	5845	5553	292	0.003425	20.0000
0.10	5845	5297	548	0.001825	10.0000
0.15	5845	5192	653	0.001531	6.6667
0.20	5845	4989	856	0.001168	5.0000
0.25	5845	5017	828	0.001208	4.0000
0.30	5845	4692	1153	0.000867	3.3333
0.35	5845	4418	1427	0.000701	2.8571
0.40	5845	4458	1387	0.000721	2.5000
0.45	5845	4488	1357	0.000737	2.2222
0.50	5845	4533	1312	0.000762	2.0000

Table A2b: Data on Benesi-Hildebrand plot of CDs-AuNPs fluorometric assay

[AuNPs] µM	F°	F	F° – F	1/F° – F	1/[AuNPs]
0.05	5845	5320	525	0.001905	20.0000
0.10	5845	4947	898	0.001114	10.0000
0.20	5845	4227	1618	0.000618	5.0000
0.25	5845	4141	1704	0.000587	4.0000
0.30	5845	3773	2072	0.000483	3.3333
0.40	5845	3633	2212	0.000452	2.5000

APPENDIX A3

Data on log [(F^{o} − F)/F] vs log [Q] plot for both CDs-AgNPs and CDs-AuNPs fluorometric assay were summarized in Table A3a and Table A3b, respectively.

Table A3a: Data on plot of log[(F° − F/F] vs log [AgNPs] of CDs-AgNPs fluorometric assay

[AgNPs] µM	F°	F	F° – F	F° – F/F	Log [(F° – F) /F]	Log [AgNPs]
0.05	5845	5573	272	0.04881	-1.31152	-1.30103
0.10	5845	5340	505	0.09457	-1.02425	-1.00000
0.15	5845	5185	660	0.12729	-0.89521	-0.82391
0.20	5845	4991	854	0.17111	-0.76673	-0.69897
0.25	5845	4960	885	0.17843	-0.74854	-0.60206
0.30	5845	4759	1086	0.22820	-0.64169	-0.52288
0.35	5845	4458	1387	0.31113	-0.50706	-0.45593
0.40	5845	4475	1370	0.30615	-0.51407	-0.39794
0.45	5845	4489	1356	0.30207	-0.51989	-0.34679
0.50	5845	4599	1246	0.27093	-0.56715	-0.30103

Table A3b: Data on plot of log[(F° − F)/F] vs log [AuNPs] of CDs-AuNPs fluorometric assay

[AuNPs] µM	F	F°	F° – F	F° – F/F	Log[(F° −F) /F]	Log [AuNPs]
0.05	5320	5845	525	0.09868421	-1.00575233	-1.30103
0.10	4947	5845	898	0.18152416	-0.74106557	-1
0.20	4227	5845	1618	0.38277738	-0.41705373	-0.69897
0.25	4141	5845	1704	0.41149481	-0.38563564	-0.60206
0.30	3773	5845	2072	0.54916512	-0.26029705	-0.52288
0.40	3633	5845	2212	0.6088632	-0.21548028	-0.39794

BIODATA OF STUDENT

Suria Mohd Saad was born on 1st November 1980 in Alor Gajah, Melaka. She received her first degree in Biomedical Sciences in 2002 from the University Putra Malaysia. She earned several academic awards during her undergraduate study, namely, Crystal Award in 2000 and Bumi Sains Award in 2001. She then pursued her post-graduate studies, Master of Science in the field of Molecular Biology and completed in 2006, from the University Putra Malaysia.

She is currently a PhD student in the field of Sensor Technology in the Institute of Advanced Technology (ITMA), Universiti Putra Malaysia (UPM). Her study focusses on fluorometric gene assay for determination of *Escherichia coli* O157:H7 using graphene quantum dots (GQDs) and carbon dots (CDs) with gold (AuNPs) and silver (AgNPs). During her studies, she has successfully published two articles in the high impact journal (Q1) by a reputable publisher, Springer and Elsevier, respectively.

She has been a researcher in Biodiagnostic-Biosensor Programme of Biotechnology and Nanotechnology Research Centre, MARDI since 2005. During her career as a Research Officer, she was as a project leader of Science Fund, MOSTI and has published 2 articles with this study. She also acted as a project leader under the Wang Rezab MARDI (WRM) project of MARDI and successfully published one article through this study.

In addition, she was co-researcher for several research projects and published one article as co-author. Briefly her researches focus on detection of food pathogen, especially *Escherichia coli* O157:H7 using a molecular biology approach. She also gained experience in immuno-based assay for detection of *Escherichia coli* O157:H7 through WRM research project.

Her current research is concerned with sensor technology and molecular biology. She was awarded with 5 innovation awards and published more than 10 articles in international journals and proceeding.

LIST OF PUBLICATIONS

Journal Publication

1) **Saad, S.M.,** Abdullah, J., Rashid, S.A., W.F. Yap, Salam, F. and Lau, H.Y. 2019. A fluorescence quenching based gene assay for *Escherichia coli* O157:H7 using graphene quantum dots and gold nanoparticles. *Microchimica Acta* 186: 804. https://doi.org/10.1007/s00604-019-3913-8

2) **Saad, S.M.,** Abdullah, J., Rashid, S.A., W.F. Yap, Salam, F. and Lau, H.Y. 2020. A carbon dots based fluorescence sensing for the determination of *Escherichia coli* O157:H7. *Measurement* 160: 107845. https://doi.org/10.1016/j.measurement.2020.107845

Media Publication

1) **Suria Mohd Saad**. 2016. *E. coli* O157:H7 detection kit. *Scientia MARDI*, Vol 008, September 2016, muka surat 9.

Conference

1) **M.S. Suria**, A. Jaafar, A.R. Suraya, W.F. Yap, S. Faridah, and H.Y. Lau. 2019. The perfect graphene quantum dots-based FRET-induced quenching DNA-sensor for the detection of *Escherichia coli* O157:H7. *The 6th International Conference on Bio-Sensing Technology*. 16-19 Jun 2019, Renaissance Hotel, Kuala Lumpur.