UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF AN ELECTROCHEMICAL APTASENSOR BASED ON NANOMATERIALS-MODIFIED ELECTRODE FOR THE DETECTION OF ZEARALENONE AND CROSS-REACTIVITY TOWARDS MASKED ZEARALENONE

FARAH ASILAH BINTI AZRI

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By

FARAH ASILAH BINTI AZRI

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

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

DEVELOPMENT OF AN ELECTROCHEMICAL APTASENSOR BASED ON NANOMATERIALS-MODIFIED ELECTRODE FOR THE DETECTION OF ZEARALENONE AND CROSS-REACTIVITY TOWARDS MASKED ZEARALENONE

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August 2021

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Zearalenone (ZEA) is a phytohormone that is primarily an estrogenic fungal metabolite. ZEA exhibits a genotoxic potential in vitro and in vivo, and therefore, the determination of ZEA in food and feedstuffs is of great importance for food safety monitoring. Masked ZEA is derivative generated through chemical transformations which catalysed by plant enzymes and commonly undetectable by conventional analytical techniques. Hence, the aim of this study is to develop a novel, sensitive, and direct aptamer-based electrochemical biosensor based on a graphene nanoplatelets-chitosan/gold nanoparticle (GNP-CS/AuNPs) modified electrode using a thiolated aptamer with methylene blue (MB) as a redox mediator, for detection of ZEA and masked ZEA. In this study, two different detection formats which are indirect competitive and direct have been employed on gold and carbon electrode, respectively. The binding affinity and selectivity of the selected aptamer were first studied and proved that the hypothesis of possible cross-reactivity towards masked ZEA, including α-zearalenol (α-ZEL), β -zearalenol (β-ZEL) and zearalenone-14-glucoside (ZEA-14-Glc) was correct. The dissociation constant (Kd) of the aptamer was obtained at 13.42 ± 2.1 nM and the conformational characterisation using circular dichroism (CD) resulted in a change in the molecular ellipticity. The indirect competitive aptasensor showed an excellent dynamic range in between 0.01 to 1000 ng/mL, with a detection limit of 0.017 ng/mL. Besides, the cross-reactivity analysis proved a high degree of cross-reaction towards the masked ZEA. The sensitivity of aptamer was further improved by truncation. Fifteen truncation variants were evaluated by computational docking for binding simulation. Aptamer Z31N had the lowest Vina score of -7.8 kcal/mol, with the most significant number of intermolecular interactions and Kd of Z31N aptamer was calculated as 11.77 ± 1.44 nM. The sensing platform was then fabricated by modifying the SPCE with GNP-CS mixture and AuNPs. The AuNPs were synthesised using the extract of Etlingera

elatior and the results showed that the AuNPs with an average size of 31 nm and zeta potential of -32.0 mV were comparable with the citrate-mediated AuNPs. The modified SPCE with 4 mg/mL of GNP in 0.1% CS and 20 µL of AuNPs showed an excellent electrical conductivity and stability. Under optimised conditions, the detection of ZEA was based on the redox probe of MB at the 3' end of the aptamer. Good response of the direct aptasensor was obtained within the range of 0.001 to 100 ng/mL with a detection limit of 0.14 pg/mL, which was lower than the indirect competitive aptasensor. This result proved that the truncation of the aptamer was efficient in enhancing the sensitivity of the aptamer as biosensing material. Moreover, the analysis in grain maize sample also showed good recovery of between 96 - 122%. It can be concluded that the developed aptasensor can be used for a total detection of ZEA in the sample as a high degree of cross-reactivity can be observed in both formats which could be advantages for mycotoxin screening.

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

PEMBANGUNAN SENSOR APTA ELEKTROKIMIA BERDASARKAN ELEKTROD MODIFIKASI NANOMATERIAL UNTUK PENGESANAN ZEARALENON DAN REAKTIVITI SILANG TERHADAP ZEARALENON TERSEMBUNYI

Oleh

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Zearalenon (ZEA) adalah hormon tumbuhan yang merupakan metabolit kulat estrogenik. ZEA mempamerkan potensi genotoksik secara in vitro dan in vivo. Oleh itu, pengesanan ZEA dalam makanan dan makanan haiwan adalah penting untuk pemantauan keselamatan makanan. ZEA tersembunyi adalah derivatif yang terhasil melalui transformasi kimia yang dimangkin oleh enzim tumbuhan dan lazimnya tidak dapat dikesan oleh teknik analisis konvensional. Oleh itu, matlamat kajian ini adalah untuk menghasilkan biosensor elektrokimia yang baru, peka dan langsung berdasarkan nanoplatelet grafin-kitosan/nanopartikel emas (GNP-CS/AuNPs) elektrod menggunakan aptamer thiol bertag metilena biru (MB) sebagai pengantara redoks, untuk pengesanan ZEA dan ZEA tersembunyi. Dalam kajian ini, dua format pengesanan yang berbeza iaitu tidak langsung dan langsung telah dibangunkan masing-masing menggunakan elektrod emas dan karbon. Affiniti lekatan dan selektiviti bagi aptamer yang terpilih telah dikaji dan membuktikan bahawa hipotesis awal mengenai kebarangkalian keaktifan silang aptamer terhadap ZEA tersembunyi termasuk α-ZEL, β-ZEL dan ZEA-14-Glc adalah benar. Pemalar pemisahan (Kd) telah diperoleh pada nilai 13.42 ± 2.1 nM dan penilaian konformasi aptamer dengan menggunakan dikromisme bulat (CD) menunjukkan perubahan pada elips molekul. Aptasensor berdaya saing secara tidak langsung menunjukkan julat dinamik yang baik antara 0.01 hingga 1000 ng/mL dengan had pengesanan pada 0.017 ng/mL. Selain itu, ujian terhadap kereaktifan silang juga membuktikan respon yang tinggi terhadap ZEA tersembunyi. Kepekaan aptamer telah dipertingkatkan dengan kaedah pemotongan. Lima belas varian pemotongan telah dinilai dengan menggunakan kaedah pengkomputeran untuk simulasi lekatan. Aptamer Z31N mempunyai skor Vina yang terendah iaitu -7.8 kcal/mol dengan bilangan interaksi molekul terbanyak dan nilai Kd bagi aptamer Z31N adalah 11.77 ± 1.44 nM. Platform pengesanan disediakan dengan mengubahsuai permukaan SPCE dengan GNP-

CS dan AuNPs. AuNPs dari Etlingera elatior dan keputusan menunujukkan bahawa AuNPs berukuran 31 nm dan mempunyai potensi zeta -32.0 mV ini adalah standing dengan AuNPs yang dihasilkan dari sitrat. SPCE yang diubahsuai dengan 4 mg/mL GNP dalam 0.1% CS dan 20 µL AuNPs telah menunjukkan pengkonduksian elektik dan kestabilan yang baik. Dalam keadaan yang optimum, pengesanan ZEA dilakukan berdasarkan prob redoks MB pada bahagian 3' akhir aptamer. Aptasensor langsung ini menghasilkan tindak balas yang baik dalam lingkungan 0.001 hingga 100 ng/mL dengan had pengesanan 0.14 pg/mL, iaitu lebih rendah daripada aptasensor berdaya saing secara tidak langsung. Hasil dapatan ini membuktikan bahawa pemotongan aptamer adalah berkesan dalam meningkatkan kepekaan atau sensitiviti aptamer sebagai bahan penderia bio. Tambahan pula, ujian dalam sampel sebenar juga menunjukkan keputusan yang baik antara julat 96 - 122%. Ia dapat disimpulkan bahawa aptasensor yang dibangunkan dapat digunakan untuk mengesan ZEA secara keseluruhan kerana tahap reaktiviti silang yang tinggi dapat diperhatikan bagi kedua-dua jenis format, di mana boleh menjadi kelebihan untuk pemeriksaan mikotosin.

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"In the name of Allah, the most beneficent, the most merciful"

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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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Date: 12 August 2021

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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°C	Degree celcius
M	Molar
mM	Millimolar
µM	Micromolar
nM	Nanomolar
L	Litre
mL	Millilitre
µL	Microlitre
mV	Millivolt
mg	Milligram
µg/mL	Microgram per millilitre
3-D	Three-dimensional
α-ZEL	α-zearalenol
β-ZEL	β-zearalenol
AF	Aflatoxin
AFB1	Aflatoxin B1
Ag/AgCl	Silver/silver chloride
AgNPs	Silver nanoparticles
ALISA	Aptamer-linked immunosorbent assay
Apt	Aptamer
AuNPs	Gold nanoparticles
BTX-2	Brevetoxin-2
BTX-3	Brevetoxin-3
CD	Circular dichroism
cDNA	Complementary strand deoxyribonucleic acid
CNT	Carbon nanotubes
CS	Chitosan
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
DPV	Differential pulse voltammetry
DLS	Dynamic light scattering

EIS	Electrochemical impedance spectroscopy
EDX	Energy dispersive X-ray
ELISA	Enzyme-linked immunosorbent assay
ELONA	Enzyme-linked oligonucleotide assay
ELASA	Enzyme-linked aptamer assay
EU	European Union
FAO	Food and Agriculture Organization
FAM	Carboxyfluorescein
FDA	U.S Food and Drug Administration
FESEM	Field emission electron microscopy
FGO	Functional graphene oxide
FMs	Fuminisins
FB1	Fumonisin B1
FRET	Fluorescence resonance energy transfer
FTIR	Fourier transform infrared spectroscopy
GNP	Graphene nanoplatelets
GC-MS	Gas chromatography mass spectroscopy
h	Hour
HPLC	High performance liquid chromatography
HRTEM	High-resolution Transmission electron microscopy
IARC	International Agency for Research on Cancer
ITC	Isothermal titration calorimetry
Kd	Dissociation constant
LAMP	Loop-mediated isothermal amplification
LC-MS	Liquid chromatography mass spectrometry
LFA	Lateral flow assay
LOD	Limit of detection
LSPR	Localised surface plasmon resonance
min	Minute
MB	Methylene blue
mPCR	Multiplex polymerase chain reaction
MoS2	Molybdenum disulphide
MWCNTs	Multiwalled carbon nanotubes
NMR	Nuclear magnetic resonance spectroscopy
OTA	Ochratoxin A

PCR	Polymerase chain reaction
PEI	Polyethyleneimine
pH	Potential of Hydrogen
PLIP	Protein-ligand Interaction profiler
QD	Quantum dots
R_{CT}	Charge transfer resistance
rpm	Revolutions per minute
RT-PCR	Real-time polymerase chain reaction
RNA	Ribonucleic acid
RSD	Relative standard deviation
sec	Seconds
SD	Standard deviation
SELEX	Systematic evolution of ligands by exponential enrichment
SH	Thiol functional group
ssDNA	Single-stranded deoxyribonucleic acid
ssRNA	Single-stranded ribonucleic acid
SPCE	Screen-printed carbon electrode
SPR	Surface plasmon resonance
SWV	Square wave voltammetry
TLC	Thin layer chromatography
TPC	Total phenolic compound
TFC	Total flavonoids content
UV	Ultra-violet
UV-Vis	Ultraviolet-Visible spectroscopy
ZEA	Zearalenone
ZEA-14-Glc	Zearalenone-14-glucoside

CHAPTER 1

INTRODUCTION

1.1 Research background

Malaysia has tropical weather throughout the year with a high temperature (28-31^0 C) and high relative humidity (79-81%). These favourable conditions promote the formation of mycotoxins in various agricultural commodities. Mycotoxins are secondary metabolites produced by fungi on crops in the field and also formed under improper storage conditions. Symptom of mycotoxicosis can be seen in the infected crops by the growth of molds or microfungi and commonly found in maize, peanut, chillies and spices. The formation of mycotoxins is influenced by various physical, chemical, and biological factors. Therefore, it has become a substantial concern for consumers, governments, and producers. This is because, mycotoxins can negatively impact livestock performance and general well-being. In addition, occurrence of these mycotoxins has a significant impact on food and feed safety, food security, as well as global trade. According to Berthiller et al. (2016), more than 300 mycotoxins have been recognised to date, with widely diverse chemical structures and varying modes of action, some of which affect the kidney, liver, immune system, and mostly carcinogenic.

Once infected by mycotoxins, plants can overcome or at least to lessen fungal attack by a variety of mechanisms, as their natural defence system against xenobiotics. As a result, masked mycotoxins can form through various means, which may include precursors, metabolites, or degradation products of the parent or free form of the mycotoxin. Besides, they may have been formed abiotically through a chemical reaction of the parent toxin with the matrix (Berthiller et al., 2009; Warth et al., 2012). Integral masked mycotoxins are less potent compared to their parent or unmodified forms. This is due to severe modifications of these toxins during detoxification reactions of plants (Berthiller et al., 2016). Despite the growing number of studies on masked mycotoxins, neither regulations limits nor recommendations have been enforced for these compounds (Dellafiora et al., 2016). This is because, the toxicological data on these masked mycotoxins are scarce, and the real risks of these compounds are still insufficient. This makes it difficult to conduct a proper risk assessment.

In 2016, Malaysian government has announced to expand the plantation of grain maize in Terengganu to overcome the problem of rising prices of these import supplies and also ensuring the guarantee of feed supply as the cheapest source of protein. Zearalenone (ZEA) and its derivatives including α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) have been found in *Fusarium*-infected maize in the field (Afsah-Hejri et al., 2013). Moreover, the Malaysia's climate promotes the formation of this fungi in the planted crops. Farm animals fed with ZEA

contaminated feed display alterations in their reproductive tract, decrease in fertility and these effects are permenant. Compared to ZEA, α-ZEL has higher estrogenic potency although its rate of absorption is lower (Fink-Gremmels & Malekinejad, 2007; Fink-Gremmels, 2008). Cytotoxic effects of α-ZEL and β-ZEL occurred because of inhibition of DNA and protein syntheses and inducing oxidative damage. Most mycotoxins are chemically stable, hence are persistent during storage and processing. The best strategy to control mycotoxins is by prevention. The mycotoxins derivaties or also known as masked mycotoxins can be easily missed in the routine analysis as they act differently in their chemical reactions to parent mycotoxins. Moreover, these mycotoxin derivatives are undetectable by conventional analytical techniques (except LC/MS-MS) because the chemical structure has been altered in the plant into less harmful compounds (Berthiller et al., 2016). Hence, there is a need of using other different alternative techniques for detection.

Conventionally, the commonly used techniques to detect these low-molecular-weight mycotoxins in agricultural commodities include high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectroscopy (GC-MS), and enzyme-linked immunosorbent assay (ELISA) techniques (Espinosa-Calderón et al., 2011; Mosiello & Lamberti, 2009; Pittet, 2005). Although these analytical methods provide accuracy, precision, sensitivity, and reproducibility, they are not applicable for on-site analysis because they require extensive sample preparation, expensive equipment, skilled operators, and may lack accuracy at low analyte concentration (He et al., 2012). Therefore, biosensors appeared as advanced tools that offering important features including simplicity, easy-to-use, relatively fast, and portability (Chauhan et al., 2016). Biosensors are devices that combine a biochemical recognition element with a physical transducer (Rasooly & Herold, 2006). The biosensor can be applied as alternative method for determination of mycotoxins, owing to their unique properties of real-time, reduce labour, and less time required for analysis with minimal cost (Farah et al., 2016). Besides that, biosensors can be made into portable instruments that can be operated on-site. This method offers a real-time detection without extensive pre-treatment of the sample and, therefore, reducing the dependency on the expensive and centralised laboratory-based methods.

The biochemical recognition material can be classified as affinity or biocatalytic materials such as antibodies, deoxyribonucleic acid (DNA), receptor proteins, enzymes, tissues, whole cells, or organs interrelating with specific analytes. The interaction between these materials and their specific analyte can then be converted by the transducer into a quantifiable electrical signal. For the past few years, antibodies are the common biological component which was used to detect the specific analyte in biosensor development. However, aptamers are the trending alternative of antibodies, especially for the detection of small molecules such as toxins, antibiotics, molecular markers, drugs, heavy metals, and ions (Ruscito & DeRosa, 2016). Aptamers are single-stranded, synthetic oligonucleotides (DNA or RNA) that are able to fold into 3-dimensional (3D) shapes and can bind non-covalently to the target molecule with high affinity (Ruscito & DeRosa, 2016). Aptamers have been widely used in the diagnostics

of various substances due to their inherent specificity and affinity. Numerous studies have been reported on the application of aptamers for the detection of mycotoxins, especially for AFB_1 and OTA (Al-Rubaye et al., 2018; Myndrul et al., 2017; Shim et al., 2014; Yang et al., 2014; Zhang & Wang, 2013). However, only a few studies can be found on ZEA detection, especially via the electrochemical transducing platform. Hence, this study explores the application of aptamer as biorecognition molecule in detecting ZEA and masked ZEA, to substitute the use of antibody.

With the nanotechnology advancement, the incorporation of nanomaterials in biosensor was proven to provide many advantages, including amplification of the signal (Azri et al., 2017), enhancement of the surface properties for better immobilisation (Artiles et al., 2011), and also to increase the electrochemical response (Yáñez-Sedeño et al., 2010). Various kinds of nanomaterials have been explored in the development of biosensor, namely gold nanoparticles (AuNPs), silver nanoparticles (AgNPs), carbon nanotubes (CNT), graphene, nanowires, and quantum dots (QD). In recent years, AuNPs have been used in numerous types of biosensors including colourimetric and electrochemical. This is due to their narrow size distribution, efficient surface modification, excellent conductivity, biocompatibility, and electrochemical properties (Rasheed & Sandhyarani, 2017). These noble metal nanoparticles are commonly produced via a chemical reduction in the presence of metal salt precursors as the reducing agents (Usman et al., 2019). However, the green synthesis approach utilising the natural materials, including plants and microorganisms in AuNPs production, is eco-friendly, and controlling the shapes with confined shape is possible. This study explores the ability of green-synthesised AuNPs as the electrode surface modifier and their potential as the electrochemical current enhancer in the development of aptamer-based biosensors.

1.2 Statements of problem and research motivation

With the current gaps in routine mycotoxin analysis and no established regulations for the emerging mycotoxins, these compounds might not be detected along the supply chain and subsequently poses a harmful threat to both humans and animals. In brief, there are only three analytical strategies to determine masked mycotoxins in food and feed (Berthiller et al., 2016). First, these compounds, along with their parent mycotoxins, can be quantified using the LC-MS/MS-based methods. Secondly, it can be detected by immunochemical techniques, provided there is cross-reactivity of antibodies towards them. Finally, these compounds may need to be hydrolysed to their parent compounds using enzymes or harsh acidic or alkaline conditions before analysis. Therefore, to overcome this issue, a sensitive biosensor is highly suggested as a promising way for a direct detection of ZEA as well as masked ZEA.

This research was motivated by the idea of an immunchemical concept in detecting mycotoxins and masked mycotoxins. In the immunoassay approach, the development of antibodies against the particular toxins via *in vivo* technique is required. Nevertheless, obtaining the antibodies that recognise only one analyte and not to a closely similar analogue can be challenging (Maragos, 2016). Hence, cross-reactivity might occur. Although this cross-reactivity is a disadvantage for the case of detecting a specific target, however, it is eventually become an advantage when measuring 'integrated' or 'summed' response (Maragos, 2016). The integrated or summed response is reffering to the detection of total mycotoxins including parent and masked mycotoxins presence in the sample. Since aptamer is also known as 'chemical antibodies,' aptamers could display high affinity and selectivity towards the targeted analyte, similar to antibodies. Therefore, it is believed that aptamers could possibly detect the total of zearalenone, which are present in the sample, including the parent mycotoxin as well as masked mycotoxins, with better binding affinity. The aptamer-analyte binding mechanism is basically based on the molecular structure of the specific analyte that is being recognised by the specific folded shape of the aptamer. Due to this basis, aptamers will possibly detect the masked mycotoxins because the molecular structures of masked ZEA are basically similar with the parent mycotoxins, just with a minor additional group.

Direct electrochemical detection of ZEA is challenging due to their low molecular weight, limited binding sites, and also requires a sample extraction procedure. Moreover, there is limited work reported on the development of an electrochemical aptamer-based biosensor for ZEA. Other than electrochemical technique, there have been several types of biosensor which successfully developed based on fluorescence (Niazi et al., 2018; Wu et al., 2017; Goud et al., 2017), colourimetric (Sun et al., 2018; Taghdisi et al., 2018) and lateral flow techniques (Wu et al., 2018). Therefore, in the present study, the aptamer was employed as the biorecognition molecule, and indirect and direct electrochemical aptasensors were developed for the detection of ZEA. An indirect competitive detection format was first designed to evaluate the feasibility of the aptamer in detecting ZEA in terms of their performance and binding affinity. The aptamer was further truncated into shorter sequence with different conformation to be applied in a direct detection format. The green synthesised AuNPs were used to modify the screen-printed electrode along with the graphene nanoplatelets (GNP) and chitosan (CS). This nanocomposite (GNP-CS/AuNPs) is believed to improve the electrochemical response in the detection of ZEA. To date, the utilisation of graphene nanoplatelets and green AuNPs for electrochemical detection of mycotoxins, especially ZEA, has not been explored. Thus, the applicability of green synthesised AuNPs is still unknown.

1.3 Aim and objectives

The aim of this study is to develop a novel, sensitive, and direct aptamer-based electrochemical biosensor based on a GNP-CS/AuNPs-modified electrode using a thiolated aptamer with MB as a redox mediator, for detection of ZEA and masked ZEA. Hence, the following specific objectives were designed to achieve this aim;

I. To develop an indirect competitive electrochemical aptasensor using a label-free aptamer for detection of ZEA and masked ZEA
II. To evaluate the affinity of ZEA aptamer by truncation at different regions of full aptamer using computational approach
III. To characterise the screen-printed carbon electrode modified with graphene nanoplatelets-chitosan/gold nanoparticles (GNP-CS/AuNPs) nanocomposite as sensor platform
IV. To develop a direct electrochemical aptasensor based on MB-tagged thiolated aptamer for detection of ZEA and masked ZEA

1.4 Novelty of research

The utilisation of aptamer as the biorecognition component to detect ZEA and masked ZEA was the first explored in electrochemical biosensor. Furthermore, the truncation and binding simulation using a computational docking approach for the improvement of the aptamer's binding affinity was employed in this study which subsequently produce a new truncated aptamer. Besides, the ability of GNP-CS/AuNPs nanocomposite as for the aptamer immobilisation platform was first studied. By using the MB redox label-aptamer, the developed aptasensor can be an alternative technique for the detection of ZEA and masked ZEA, which previously relies on the conventional techniques.

1.5 Limitation of research

Aptamers are commonly synthesised using the *in vitro* selection technique known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment). However, the facilities to perform SELEX is not yet to be established in UPM, due to its new exposure in the biosensor field. In addition, experts are scarce. Therefore, there was a limitation in this study to develop a new aptamer specifically for the target analyte. Hence, to overcome these limitations, the aptamer used in this study was based on the reported work.

1.6 Research approach

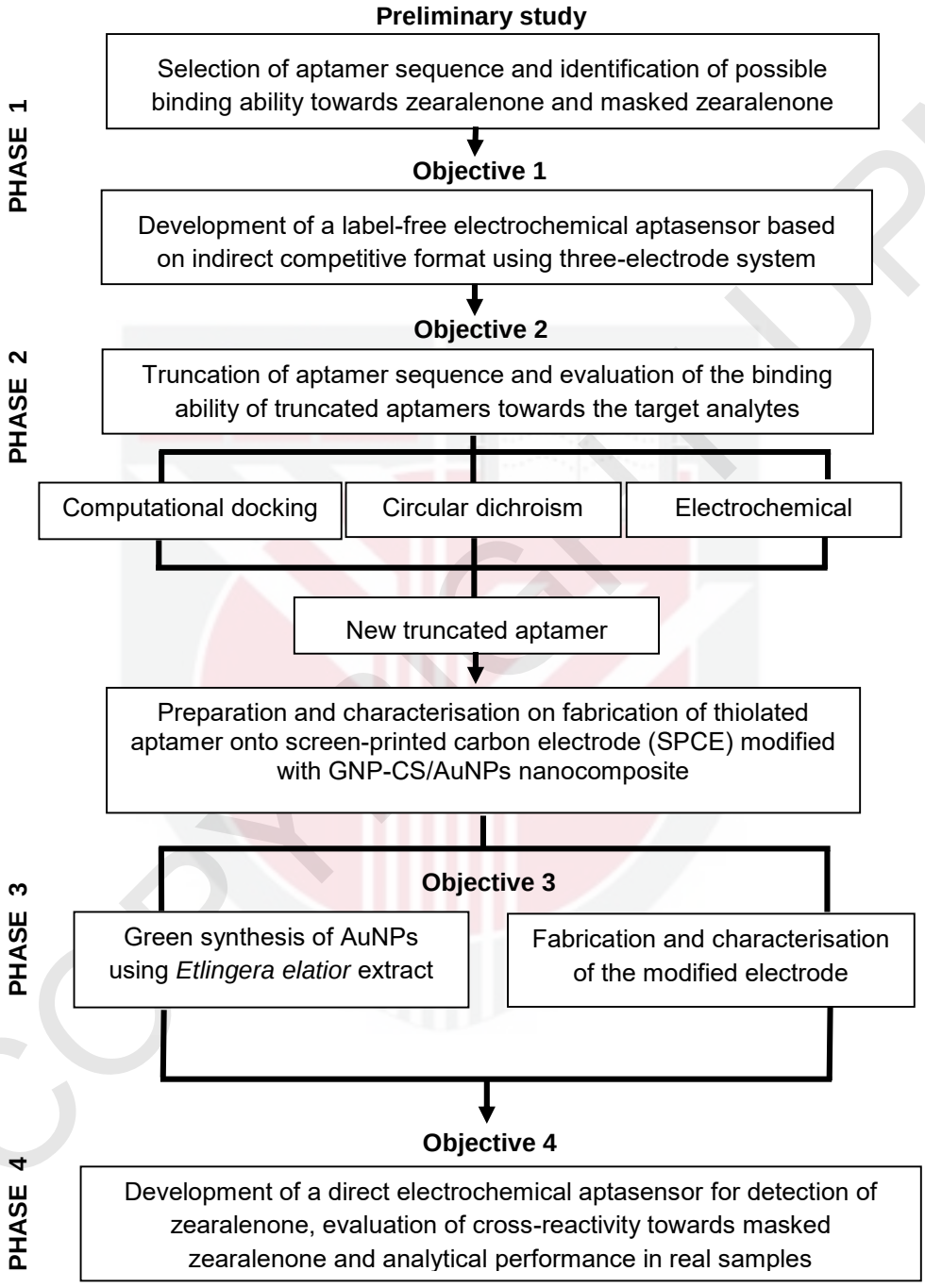

Figure 1.1: Systematic flow of the research including phase 1 until 4 throughout the aptasensor development process

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LIST OF PUBLICATIONS

From this thesis

Azri, F.A., Jinap, S., Sukor, R., Yusof, N.A., Raston, N.H.A., Nordin, N. and Jambari, N. (2019). *Etlingera elatior*-mediated synthesis of gold nanoparticles and their application as electrochemical current enhancer. *Molecules*, 24(17), 3141-3156. (Q2, IF=3.098)

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Azri, F.A., Jinap, S., Sukor, R., Yusof, N.A., Raston, N.H.A. Electrochemical aptasensor using methylene blue tagged aptamer on graphene nanoplatelets/gold nanoparticles platform for the detection of zearalenone. (*Submitted*)

Previous publications

Farah, A. A., Sukor, R., Fatimah, A. B. and Jinap, S. (2016). Application of nanomaterials in the development of biosensors for food safety and quality control. *International Food Research Journal*, *23*(5), 1849-1856.

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Co-authors publications

Norlia, M., Jinap, S., Nor-Khaizura M. A. R., Radu, S., Samsudin, N. I. P. and **Azri, F. A.** (2019). Aspergillus section Flavi and aflatoxins: Occurrence, detection and Identification in raw peanuts and peanut-based products along the supply chain. *Frontiers in Microbiology, 10*(2602), 1-17.

Mustafa, R. R., Sukor, R., Nor, S. M. M., Saari, N. and **Azri, F. A.** (2020). Enhancing extraction yield and purity of mitragynine from *Mitragyna speciose* through sequential solvent extraction and characterisation using NMR technique. *International Journal of Scientific & Technology Research*, *9*(10), 3846-3854.

List of presentations

Azri, F. A., Jinap, S., Sukor, R., Yusof, N. A., Raston, N.H.A., Eissa, S., Zourob, M. & Chinnappan, R. (2019, August). *Development of electrochemical aptasensor for detection of zearalenone in corn feed*. Paper presented at the Universiti Putra Malaysia-Kasetsart University Postgraduate Colloquium and Research Forum, 14-15 August 2019, UPM Serdang, Malaysia.

Azri, F. A., Jinap, S., Sukor, R., Yusof, N. A., Raston, N.H.A., Eissa, S., Zourob, M. & Chinnappan, R. (2019, September). *Development of electrochemical aptasensor for rapid and sensitive detection of zearalenone.* Paper presented at the 4th International Conference on Molecular Diagnostics & Biomarker Discovery, 25-26 September 2019, Equatorial Hotel, Penang, Malaysia.

List of awards

1. Top 3 Winners **–** MIFT-MEAD JOHNSON nutrition postgraduate food safety research award competition 2017.

2. Best oral presenter award at 4th International Conference of Molecular Diagnostic and Biomarker Discovery (MDBD) 2019.