IMAGE DIGITIZATION COLORIMETRIC DETECTION OF ACEPHATE BASED ON ACEPHATE-THIOLATED ACEPHATE BINDING APTAMER-GOLD NANOPARTICLE COMPLEX

MOHD JUNAEDY OSMAN

DOCTOR OF PHILOSOPHY
(CHEMISTRY)

UNIVERSITI PERTAHANAN NASIONAL MALAYSIA

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Thesis submitted to the Centre for Graduate Studies, Universiti Pertahanan Nasional Malaysia, in fulfilment of the requirements for the Degree of Doctor of Philosophy (Chemistry)

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ABSTRACT

For years, organophosphates (OPs) compounds are used for pest control in agriculture. However, uncontrolled usage of OPs causes genotoxic, teratogenic as well as other environmental and ecological adverse impacts. The detection of OPs using conventional methods gives good results but existed with several limitations such as bulky, time-consuming analysis and expensive. A colorimetric detection method receives an attention as it can be modified to overcome these limitations due to its simplicity. In this study, colorimetric detection of Ac was conducted by surface functionalisation of gold nanoparticles with thiolated acephate binding aptamer (TABA). Preparation of gold nanoparticles (AuNPs) was conducted using two heating techniques. Preparation of AuNPs using microwave radiation technique resulted in smaller particles size (of 10 $\pm$ 3nm) compared to that of the conventional heating technique (of 16 $\pm$ 4nm). Ultra-violet visible (UV-Vis) analysis showed the existent of surface plasmon resonance phenomenon for both prepared AuNPs whereas Fourier transformed infra-red spectrometer (FTIR) analysis suggested there was a physical interaction at the surface of AuNPs. Study on the effect of AuNPs to Ac mole concentration ratio revealed that the best ratio for the detection was 1:35. In addition, better detection of Ac was also achieved using smaller particles size AuNPs. Assessments of the distinct colour change (from red to dark purple) of the complex were carried out by comparing the value of red fragment of the digitized images. Analyses of high-resolution transmission electron microscope (HRTEM), UV-Vis and FTIR spectroscopy confirmed that aggregation of particles complex occurred. A study on the effect of ions on TABA towards the detection of Ac revealed that detection

capability towards Ac was enhanced with the introduction of Mg^{2+}. Optimised sensor parameters obtained from central composite design response surface methodology (CCD-RSM) were validated and good lowest limit of detection which was 11 ppb, was achieved. In addition, selectivity and reproducibility of the optimized method for the real sample analysis was good. Based on the results of this study, it can be concluded that the proposed image digitization colorimetric detection method produced a rapid, sensitive, and selective detection towards Ac.

ABSTRAK

Pengunaan sebatian organofosfat (OPs) untuk kawalan haiwan perosak dalam pertanian telah bertahun-tahun diamalkan. Walau bagaimanapun, penggunaan OPs yang tidak terkawal menyebabkan genotoksik, teratogenik serta kesan buruk yang lain ke atas persekitaran dan ekologi. Pengesanan OPs menggunakan kaedah konvensional memberikan hasil yang baik tetapi terdapat beberapa batasan seperti memerlukan saiz sampel yang besar, masa analisis yang lama dan peruntukan kewangan yang besar. Kaedah pengesanan kolorimetrik diberi tumpuan bagi mengatasi masalah yang disebut di atas kerana keringkasannya. Dalam kajian ini, pengesanan kolorimetrik terhadap Ac dilakukan dengan mengfungsikan permukaan nanopartikel emas dengan aptamer pengikat asefat tertiol (TABA). Penyediaan nanopartikel emas (AuNPs) dilakukan dengan menggunakan dua teknik pemanasan. Penyediaan AuNPs menggunakan teknik radiasi gelombang mikro yang menghasilkan zarah yang lebih kecil (iaitu 10 $\pm$ 3nm) berbanding dengan hasil dari penyediaan yang menggunakan teknik pemanasan termal (iaitu 16 $\pm$ 4nm). Analisis ultra-ungu nampak (UV-Vis) menunjukkan fenomena resonan plasmon permukaan untuk kedua-dua AuNPs dan analisis spektrometer inframerah transfomasi Fourier (FTIR) mencadangkan interaksi fizikal permukaan AuNPs. Kajian mengenai kesan nisbah molar kepekatan AuNPs ke atas Ac menunjukkan bahawa nisbah terbaik untuk pengesanan adalah 1:35. Disamping itu, pengesanan Ac yang lebih baik diperolehi dengan menggunakan AuNPs yang mempunyai zarah yang lebih kecil. Penilaian perubahan warna (dari merah ke ungu gelap) dilakukan dengan membandingkan nilai pecahan warna merah dari pendigitan gambar. Analisis mikroskop elektron transmisi resolusi tinggi (HRTEM), UV-Vis dan

FTIR mengesahkan bahawa pengumpalan zarah kompleks berlaku. Kajian mengenai pengaruh ion ke atas TABA bagi pengesanan Ac menunjukkan bahawa keupayaan pengesanan terhadap Ac dipertingkatkan dengan penambahan Mg^{2+}. Parameter sensor yang optimum diperoleh malalui kaedah gerak balas permukaan rekabentuk komposit pusat (CCD-RSM) dengan had pengesanan 11 ppb. Kepemilihan dan keboleh ulangan kaedah terhadap pengesanan Ac untuk analisis sampel sebenar adalah baik. Hasil-hasil kajian ini memberi kesimpulan bahawa kaedah pengesanan kolorimetrik yang dicadangkan adalah pantas, peka dan selektif terhadap Ac.

APPROVAL

The Examination Committee has met on **5 October 2021** to conduct the final examination of **Mohd Junaedy bin Osman** on his degree thesis entitled **Image digitization colorimetric detection of acephate based on acephate-thiolated acephate binding aptamer-gold nanoparticle complex .**

The committee recommends that the student be awarded the of Doctor of Philosophy (Chemistry).

Members of the Examination Committee were as follows.

Prof. Dr. Azizi Miskon
Faculty of Engineering
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PM. Dr. Noor Azilah Mohd Kasim
Center for Defence Foundation Studies
Universiti Pertahanan Nasional Malaysia
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Faculty of Science
Universiti Putra Malaysia
(External Examiner)

Prof. Dr. Sharifah Mohamad
Faculty of Science
Universiti Malaya
(External Examiner)

APPROVAL

This thesis was submitted to the Senate of Universiti Pertahanan Nasional Malaysia and has been accepted as fulfilment of the requirements for the degree of **Doctor of Philosophy (Chemistry)**. The members of the Supervisory Committee were as follows.

Prof. Emeritus Dato' Dr. Wan Md Zin bin Wan Yunus
Faculty of Defence Science and Technology
Universiti Pertahanan Nasional Malaysia
(Main Supervisor)

Prof. Dr. Ong Keat Khim
Center for Defence Foundation Studies
Universiti Pertahanan Nasional Malaysia
(Co-Supervisor)

Dr. Jahwarhar Izuan bin Abd Rashid
Center for Defence Foundation Studies
Universiti Pertahanan Nasional Malaysia
(Co-Supervisor)

UNIVERSITI PERTAHANAN NASIONAL MALAYSIA

DECLARATION OF THESIS

Student's full name : Mohd Junaedy bin Osman

Date of birth : 01 Mar 1986

Title : Image digitization colorimetric detection of acephate based on acephate-thiolated acephate binding aptamer-gold nanoparticle complex

Academic session : 2017/2018

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Ac	Acephate
AChE	Acetylcholinesterase enzyme
Ac-TABA-AuNPs	Acephate reacted thiolated acephate binding aptamer gold nanoparticles complex
ANOVA	Analysis of variance
As	Asymmetric stretching
AuNPs	Gold nanoparticles suspension
BChE	Butyrylcholinesterase
CA	Chromosomal aberrations
C.V.	Coefficient of variant
DNA Apt	Deoxyribonucleic acid aptamer
FTIR	Fourier transformed infra-red spectrometer
GABA	Gamma-Aminobutyric acid
GC	Gas chromatography
GQ	Guanine quadruplex
H_o	Null hypothesis which suggests that no statistical relationship and significance exists in a set of given single observed variable, between two sets of observed data and measured phenomena.
HAuNPs	Gold nanoparticles prepared via conventional heating method
HRTEM	High resolution transmission electron microscope
IC	Ion chromatography

ImageJ	ImageJ software
IMS	Ion mobility spectrometry
LC	Liquid chromatography
LMCT	Ligand to metal charge transfer
LOD	Limit of detection
LOQ	Limits of quantification
mAChR	Muscarinic receptors
MAuNPs	Gold nanoparticles prepared via microwave heating method
mM	Unit of concentration millimolar
MS	Mass spectrometry
OPs	Organophosphates
OPFR	Organophosphate flame retardants and plasticisers
ppb	Unit of concentration part per billion
ppm	Unit of concentration part per million
R^2	Coefficient of determination
RGB	Red, green and blue colour components
ROS	Reactive oxygen species
RSD	Relative standard deviation
RSM	Response surface methodology
RVs	Digitized red values
S	Symmetric stretching
SD	Standard deviation
SERS	Surface enhanced Raman spectroscopy
SPR	Surface plasmon resonance

TABA	Thiolated acephate binding aptamer
TABA-AuNPs	Thiolated acephate binding aptamer gold nanoparticles suspension
t-Test	Statistical test that is used to compare the means of two groups
UV-Vis	Ultraviolet-visible spectrophotometer

CHAPTER 1

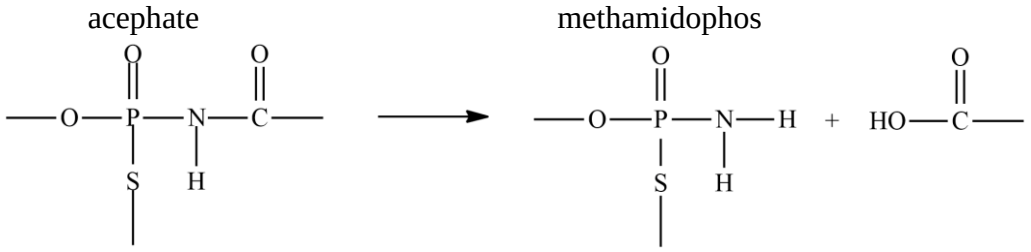
INTRODUCTION

1.1 Overview

Organophosphates (OPs) are a group of compounds that are widely used as pesticides and insecticides in an agriculture sector. Excessive application of pesticides leads to the severe environmental issues that will affect humans' and animals' health. Like the OPs that are used as chemical warfare agents, these OPs possess the same mechanism of action which causes toxicity by inhibiting the activity of acetylcholinesterase enzyme (AChE) in neuromuscular junctions and in blood (V. Kumar et al., 2015; Spassova et al., 2000). The OPs bind with the hydroxyl group of the serine unit of the AChE to inhibit acetylcholinesterase activity, leading to acetylcholine accumulation in the synaptic junctions and finally causing death (Colovic et al., 2013; Sulaiman et al., 2020).

Acephate (Ac) is the most common and efficient of an OP that is used for pest control in agriculture (Lin et al., 2020). Degradation of Ac by bacteria in the presence of oxygen produces an extremely hazardous OP, methamidophos (Han et al., 2009; Luo et al., 2016) (Eq. 1). Ac is categorized as a class II-"moderately hazardous" pesticide, but methamidophos is characterized as a class IV- "highly toxic" pesticide

(Huang et al., 2018; Luo et al., 2016; WHO, 2019). Ac and its metabolite are highly water-soluble and can easily contaminate groundwater and soil, therefore, are easily absorbed by plants and accumulated in their edible parts (Rawtani et al., 2018; Saini et al., 2017). Equation 1 shows the chemical equation of Ac that undergoes hydrolysis reaction to form methamidophos.



(Eq. 1)

Conventional detection techniques are widely used to detect OPs due to their sensitivity which can be up to part per trillion. However, they are bulky and expensive instrumentations. These techniques include a single or combination of the following techniques: gas chromatography (GC) (Cinelli et al., 2014; Ghavidel et al., 2014; Martel et al., 2018), liquid chromatography (LC) (Akoijam et al., 2018; Han et al., 2017; Mahajan & Chatterjee, 2018), ion chromatography (IC) (Weber et al., 2016) , mass spectrometry (MS) (Francis et al., 2009) and surface enhanced Raman spectroscopy (SERS) (Clauson et al., 2015; Fan et al., 2014; Kim et al., 2015). These techniques require competent operators to operate and analyse the data, therefore, expensive. In addition, time consuming in term of sample preparation and data collection and finally difficult to be adapted for in-situ and real time detection analyses (Bala et al., 2017a; P. Kumar et al., 2015; Peng et al., 2013; Songa & Okonkwo, 2016).

Colorimetric detection methods that uses metal nanoparticles as a sensor array offer a fast, an economical and relatively high sensitivity detection, has been proposed to be a promising method to be exploited to overcome the conventional technique limitations (Bala et al., 2015a; Wang et al., 2016a).

Gold nanoparticles (AuNPs) based colorimetric were most studied for detection of OPs. AuNPs or colloidal gold, usually with particle size between 1 nm to 100 nm have unique characteristics that make it beneficial for colorimetric method (G. Liu et al., 2018). AuNPs showed distinct colour changes that enable easy readout resulted from induced aggregation depending on their size and shape (Dheyab et al., 2021; Du et al., 2014). In addition, the aggregation corresponds with surface plasmon band shift in the visible region that easily noticeable by ultra-violet visible spectrophotometer (Govindaraju et al., 2015; Kim et al., 2019; Yeh et al., 2014; Yue et al., 2016). Several advantages of AuNPs an ideal nanoprobe for sensing include high stability (MA et al., 2018), easy preparation, surface modification and functionalisation (Jamkhande et al., 2019), high molar extinction coefficient (Amanulla et al., 2017; Xi et al., 2017), good biocompatibility (Lee et al., 2018) and low toxicity (Capek, 2017; Enea et al., 2020). Nevertheless, AuNPs based colorimetric detection rests on the lack of selectivity towards analyte. Recent research discussed the OPs specific aptamer (Apt) that acts as the recognition element (Bala et al., 2015a; D.-L. Liu et al., 2019; Wang et al., 2016a, 2016b).

Apt base AuNPs have been studied for a development an efficient gold nanoparticle-based sensor for detection of OPs. Detection selectivity was enhanced by aptamer coil formation which trapped the specific analyte in it (Bala et al., 2017b,

2015a). Several studies induced metal ion to the aptamer-based sensor to further enhance detection capability (Yuan et al., 2016; Zhengbo et al., 2014). Formation of stable guanine quadruplex (GQ) structure reduced nonspecific binding with analytes and later improved detection and selectivity of aptamer based AuNPs sensor (Kumar, 2020; Phopin & Tantimongcolwat, 2020).

Colorimetric detection using AuNPs based sensor produced distinct colour change from red to purple. Several studies implied naked eyes readout in their colorimetric identification (Bai et al., 2015; Bala et al., 2015b; Fahimi-Kashani & Hormozi-Nezhad, 2016; Li et al., 2011; Xu et al., 2011). However, there were some drawbacks of naked eyes readout, such as the existence of subjectivity error which mainly influence by the observer's inconsistencies from manual interpretation (Fan et al., 2021; Sankar et al., 2020) and unreliable as it only give qualitative information (Dutta & Nath, 2017). The use of image processing technique could replace naked eyes read out as it can eliminate subjective error and were able to precisely measure and quantify from the resulting colour change of AuNPs sensor.

In this study, rapid, sensitive, and selective apt based gold nanoparticles (AuNPs) sensor assisted with image processing technique for colorimetric detection of Ac were established. AuNPs suspension were prepared via two different reduction techniques. The sensitivity of detection towards Ac using prepared AuNPs suspension were compared to determine most sensitive suspension for the analysis. The optimum detection parameters were identified and validated. Finally, the selectivity and employability of the optimised detection parameter were tested. Image processing

technique was employed for better quantification and precision of colorimetric detection towards Ac.

1.2 Problem Statement and Research Motivation

The techniques used to accurately determine OPs are usually bulky, time consuming and costly as they used instruments to determine the concentrations of OP pesticides and nerve agents. The gold nanoparticle (AuNP) colourimetric assay is utilised for rapid detection method because it is easy to operate and obtain the results. The AuNPs colour change to dark purple upon aggregation can be used in colourimetric sensing and observed by naked eye even for the concentration that is as low as a few nM levels, thus the AuNPs-based colourimetric method enable highly sensitive and simple detection. This approach has been applied for screening the enzyme activity and measuring the concentrations of nucleic acid, proteins, metal ions and other small molecules. However, the results interpretation of AuNPs-based colourimetric method by naked eye is less accurate as it is operator dependent. The accuracy of the colourimetric method can be improved by incoporating the image processing technique. In addition, to improve the selectivity of the detection, DNA Apt can be utilised for specific detection of OPs. Apt is DNA or RNA sequence selected in vitro from oligonucleotides as specific target molecules. They exhibit competitive advantages such as remarkable target specificity, ease of synthesis, tight-binding capability, chemical stability, and chemical modification flexibility for labelling or favorable immobilisation.