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NAKED EYE DETECTION OF MERCURY (II) ION USING HORSERADISH PEROXIDASE INHIBITIVE ASSAY

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PENGESAHAN

Dengan ini adalah disahkan bahawa tesis projek yang bertajuk "Naked eye detection of mercury (II) ion using horseradish peroxidase inhibitive assay" telah disiapkan serta dikemukakan kepada Jabatan Biokimia oleh Nur Khaliesah Binti Jamadon (161563) sebagai syarat untuk kursus BCH 4999 Projek.

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ABSTRACT

Urbanization and industrialization process have caused the elevation of heavy metals in the environment including mercury. The current determination of the heavy metal ions primarily depends on sophisticated instruments such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry, anodic stripping voltammetry, x-ray fluorescence spectrometry, and microprobes. The techniques used in determining the level of heavy metals are accurate but the process involved is time-consuming, expensive, and require highly trained personnel to operate. Therefore, it is necessary to develop simple and rapid detection for monitoring the heavy metal ions through the naked eye detection in an inhibitive horseradish peroxidase enzyme assay. In this study, 4-aminoantipyrine and phenol are the chromogens used. When they react with hydrogen peroxide, in the presence of horseradish peroxidase, it will form a red coloured product which is visible by our naked eye. A colourless product on the other hand will be formed indicating the presence of heavy metals. This naked eye detection shows good selectivity for mercury (II) ions (Hg^{2+}) over other metal ions as the distinctive colour of Hg^{2+} can be observed at as low as 0.5 ppm. IC_{50} of Hg^{2+} shows the lowest value which is 0.290 ppm compared to the other metal ions tested. This study also investigated the mechanism of Hg^{2+} inhibition towards HRP by using dynamic light scattering and molecular dynamic simulation. The naked eye detection can also qualitatively be determined by using application downloaded via the smartphone namely Color Assist which shows that the naked eye detection does not require expensive instruments to run through. Thus, this naked eye detection is projected to be applied to the environmental for its low cost, easier preparation, and rapid.

ABSTRAK

Proses urbanisasi dan industrialisasi telah menyebabkan peningkatan terhadap tahap logam berat di alam sekitar termasuklah merkuri. Kebiasaanya, penentuan semasa logam berat sangat bergantung kepada alatan yang sofistikated seperti atomic absorption spectrometry, inductively coupled plasma mass spectrometry, anodic stripping voltammetry, x-ray fluorescence spectrometry, dan microprobes. Teknik-teknik yang digunakan dalam menentukan tahap logam berat ini sangatlah tepat tetapi penglibatan proses tersebut mengambil masa, mahal, dan memerlukan seseorang yang terlatih untuk menggunakan mesin tersebut. Oleh itu, adalah menjadi keperluan untuk mereka pengesanan yang sederhana dan cepat dalam pengawalan logam berat melalui pengesanan mata kasar daripada assay perencatan enzim horseradish peroxidase (HRP). Dalam kajian ini, 4-aminoantipyrine dan phenol adalah kromogen yang digunakan. Apabila mereka bertindak balas dengan hydrogen peroxide (H_2O_2), dengan kehadiran HRP, tindak balas ini akan membentuk produk yang berwarna merah yang dapat dikesan oleh mata kasar. Produk tanpa warna akan terbentuk untuk menunjukkan kehadiran logam berat. Pengesanan mata kasar ini menunjukkan kecenderungan kepada merkuri (II) ion (Hg^{2+}) berbanding dengan logam berat yang lain kerana pembentukan warna yang terbentuk oleh Hg^{2+} boleh dilihat pada tahap serendah 0.5 ppm. IC_{50} yang ditunjukkan juga sangat rendah iaitu 0.290 ppm berbanding dengan logam berat lain yang diuji. Kajian ini juga telah mengkaji mekanisma perencatan yang dilakukan Hg^{2+} terhadap HRP dengan menggunakan dynamic light scattering dan simulasi dinamika molekular. Pengesanan dengan mata kasar juga boleh ditentukan melalui applikasi yang dimuat turun melalui telefon pintar yang bernama Color Assist di mana ia menunjukkan bahawa pengesanan mata kasar tidak memerlukan instrumen yang mahal untuk dijalankan. Dengan itu, pengesanan mata kasar dijangkakan dapat digunakan di alam sekitar disebabkan oleh kos yang rendah, penyediaan yang mudah serta pantas.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
Å	Amstrong
°C	Degree celcius
4- AAP	4-aminoantipyrine
e.g.,	Example
et al,.	And friends
AChE	Acetylcholinesterase
Ag^{2+}	Silver ion
As^{5+}	Arsenic ion
Cd^{2+}	Cadmium ion
Co^{2+}	Cobalt ion
Cr^{3+}	Chromium ion
Cu^{2+}	Copper ion
DLS	Dynamic light scattering
g	Gram
H_2O_2	Hydrogen peroxide
Hg^{2+}	Mercury ion
HRP	Horseradish peroxidase
IC_{50}	Inhibitory concentration 50
ID	Identification
K	Kelvin
M	Molar
MD	Molecular dynamics
mg	Milligram

mL	Milliliter
mM	Millimolar
Ni^{2+}	Nickel ion
nm	Nanometer
ns	Nanoseconds
Pb^{2+}	Lead ion
PBS	Phosphate buffer saline
PDB	Protein Data Bank
ppm	Part per million
ps	Picoseconds
R_g	Radius of gyration
Zn^{2+}	Zinc ion

CHAPTER 1

INTRODUCTION

Heavy metals are regarded as one of the most serious pollutants affecting environment worldwide. Industrial activities such as mining, production of lead acid batteries and agricultural fertilizers as well as vehicle emission are among the causes that can influence the accumulation of heavy metals in the environment (Peñuelas and Filella, 2002). Heavy metals are now becoming a major concern to mankind as it poses health hazard to mankind (Järup, 2003). Despite the prevailing danger arising from heavy metals, there is a lack of monitoring of these pollutants in the environment.

Conventional analytical techniques are often used to monitor the level of heavy metals in the environment. The analysis includes atomic absorption spectrometry, inductively coupled plasma mass spectrometry, anodic stripping voltammetry, x-ray fluorescence spectrometry, and microprobes (Liu *et al.,* 1999; Bannon and Chisolm, 2001; Eksperiandovaer *et al.,* 2002; Arai *et al.,* 2003). Even though these techniques provide accuracy and precision in determining the heavy metals level, these techniques in general have certain limitations in a way that they are costly, time-consuming especially in preparing sample, require expensive apparatus and trained personnel to operate. Due to high toxicity caused by heavy metals, there is an obvious need to develop sensitive and rapid determination methods for detection of heavy metals in the environment. One avenue to be considered is through the development of a biosensor.

Biosensors have received much attention globally due to its rapidness, simplicity and selectivity. Biosensors are analytical devices which tightly combine

biorecognition elements and physical transducers for detection of the target compounds (Amine *et al.,* 2006). There are many types of biorecognition elements that can be used in biosensors. Among them are enzymes, antibodies, microorganisms, and nucleic acids. Over the years, many enzyme based biosensors are used to detect the presence of heavy metals in the environment due to its strong interaction with heavy metals (Gayet *et al.,* 1993). In general, this type of biosensors are usually involved inhibitive reaction towards the enzyme itself but to date not much has been reported that could provide a simple and rapid detection for indication.

A colorimetric sensor is one attractive approach to provide rapid detection of heavy metals via enzyme based biosensors for its easy preparation as well as detection (Miyaji *et al.,* 2000; Kim *et al.,* 2013). This biosensors system provides a signal in the form of colored product when the enzyme reacts actively with its respective substrate as well as the indicator used. However, upon the presence of heavy metals, the enzyme will be potentially inhibited and that will decrease the enzymatic activities. This in turn could affect the intensity of the colored product formed. Thus, heavy metals detection can be easily determined by the naked eye as the changes in colored product formed can act as indicator to monitor the level of heavy metals in the environment.

Hence, this study involves the following objectives:

1.1 To study the influence of heavy metals to horseradish peroxidase enzyme activity.

1.2 To develop a naked eye detection for rapid determination of metal ions by using appropriate colorimetric indicators

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