

DETERMINATION OF BISPHENOL A IN PLASTIC PACKAGING
BY UV-VIS SPECTROPHOTOMETRIC METHOD

by

NOOR SHAZWANI BINTI ANUAR MAHAYUDIN

Dissertation submitted in partial fulfillment
of the requirements for the degree
of Bachelor of Science (Honours) (Forensic Science)

JUNE 2017

ACKNOWLEDGEMENT

I am grateful for this opportunity to acknowledge the people who had made this research and thesis possible who had given me the confidence and encouragement throughout those countless hard working days and nights.

Firstly, I would like to express my personal and sincere gratitude to my supervisor Dr. Noor Zuhartini Md Muslim for her kind encouragement, understanding, detailed and constructive comments and believing on me to complete this research. Special thanks to course coordinator, Dr. Nur Haslindawaty Abd Rashid for her influential support and guide throughout this work.

I am also deeply grateful to laboratory technical staff in forensic lab, analytical lab and UPMS who gave full co-operation as I need their support. Also my warm and sincere thanks are dedicated to all lecturers and staffs and who has involved in the production of this project. Your helps and cooperation will never be forgotten.

In addition, I owe my loving thanks to my parents Anuar Mahayudin Mokhtar, Noorma Yusoff and all my family members for their continued love, supportive, understanding and motivation. Special appreciation goes to my colleagues for helping me throughout the development of the research and moral support, especially Saiful Ammar Zahrin, Siti Nabila Husna Mohd Yunos and Mardhiah Abdul Aziz. May Allah bless and reward them for their sincere and contribution in the way of knowledge.

TABLE OF CONTENTS

	Page
Acknowledgement	iv
Table of contents	v
List of figures	viii
List of tables	ix
List of abbreviations	x
List of symbols	xii
Abstrak	xiii
Abstract	xiv
Chapter 1	1
Introduction	1
1.1 Bisphenol A	1
1.2 Uses of BPA	2
1.3 Health risks linked to BPA	5
1.4 Problem statement	6
1.5 Objectives	7
1.5.1 General objective	7
1.5.2 Specific objectives	7
1.6 Significance of the study	7
Chapter 2	9
Literature review	9
2.1 High Performance Liquid Chromatography	9
2.2 Chromatography-Mass spectrophotometry	11

2.3 Enzyme Linked Immunosorbent Assays (ELISA)	12
2.4 Electrochemical sensor	13
2.5 Ultraviolet-Visible spectrophotometric (UV-Vis)	14
Chapter 3	16
Methodology	16
3.1 Reagents, chemicals and apparatus	16
3.2 Preparation of BPA standard solution and series of working solution	16
3.3 Determination of maximum absorption scanning and stability test of BPA	18
3.4 Calibration curve and LOD	18
3.5 Sample preparation	19
3.6 Sample analysis by UV-Vis spectrophotometer	21
3.7 Repeatability and reproducibility	21
Chapter 4	22
Result and discussion	22
4.1 Selection of wavelength	22
4.2 Stability test of BPA	23
4.3 Calibration curve and LOD	24
4.4 Determination the trace amount of BPA	27
4.5 Trace amount of BPA in HM type of plastic packaging	34
4.6 Repeatability and reproducibility	35
Chapter 5	37
Conclusion	37
5.1 Conclusion	37
5.2 Limitation	38

5.3 Future work	38
References	40
Appendices	44

LIST OF FIGURES

	Page
Figure 1.1 Derivation of BPA from phenol and acetone	1
Figure 4.1 The spectrum of scanning the maximum absorption wavelength of BPA	23
Figure 4.2 Error bar of average absorbance against time in stability test	24
Figure 4.3 Calibration curve of absorbance intensity against concentration of BPA	25
Figure 4.4 Graph of concentration of BPA leached from sample in different temperature	31
Figure 4.5 Graph of concentration of BPA leach from each sample in different temperature	33
Figure 4.6 Graph of concentration of BPA leach from HM type sample in different temperature	34
Figure 4.7 Graph of RSD in different temperature for sample A at three trial	36

LIST OF TABLES

	Page
Table 1.1 The plastics types of material and their properties	4
Table 3.1 The appropriate amount of reagents and chemicals for preparation of BPA working solution	17
Table 3.2 List of plastic packaging samples	19
Table 4.1 The average of the absorbance in the stability test	24
Table 4.2 The absorbance value of BPA in different concentration	25
Table 4.3 The absorbance of the blank sample	26
Table 4.4 The results of the concentration of BPA leach out from samples in different temperature	28

LIST OF ABBREVIATIONS

Abs	:	absorbance
BPA	:	bisphenol A
BPF	:	bisphenol F
BPS	:	bisphenol S
BSA	:	bovine serum albumin
DI	:	deionised distilled water
ELISA	:	enzyme-linked immunosorbent assay
g	:	gram
GC	:	gas chromatography
HD	:	high density
HM	:	high molecular
HPLC	:	high performance liquid chromatography
LC	:	liquid chromatography
LOD	:	limit of detection
Min	:	minute
MS	:	mass spectrometer
NOAEL	:	no-observed-adverse-effect level
ODS	:	octadecyl
PA	:	polyamide
PC	:	polycarbonate
PE	:	polyethylene
PET	:	polyethylene terephthalate
PP	:	polypropylene

PS	:	polystyrene
PVC	:	polyvinylchloride
RT	:	room temperature
SAESC	:	sonication assisted extraction in small column
SD	:	standard deviation
UV-Vis	:	ultraviolet-visible

LIST OF SYMBOLS

>	:	more than
%	:	percentage
$\lambda_{\max}$	:	maximum wavelength
°C	:	degree celsius
cm ²	:	centimeter square
°F	:	fahrenheit
mg	:	milligram
mg/kg bw/day	:	milligram per kilogram body weight per day
mM	:	millimolar
ngg ⁻¹	:	nanogram per gram
ngL ⁻¹	:	nanogram per liter
ngmL ⁻¹	:	nanogram per milliliter
nm	:	nanometer
ppb	:	part per billion
R ²	:	correlation coefficient
v/v	:	volume per volume
w/w	:	weight per weight

ABSTRAK

Bisphenol A (BPA) adalah bahan kimia yang digunakan secara meluas dalam pengeluaran plastik dan ia merupakan estrogenik lemah yang boleh memberi potensi kemusnahan endokrin kepada tubuh manusia. Dalam kajian ini, kehadiran BPA telah dikesan di dalam lima sampel plastik yang berlainan jenis dan jenama yang dibeli dari sebuah pasar raya tempatan. Kepekatan BPA larut lesap pada suhu yang berbeza diukur menggunakan kaedah yang mudah, sensitif dan cepat iaitu spektrofotometri Sinar Tampak (UV-Vis). Panjang gelombang telah ditetapkan pada 276 nm. Lengkung linear piawai telah diperolehi dalam julat 0.07 hingga 0.35 mM dengan regresi 0.992 dan had pengesanan 0.0002 mM. Berdasarkan keputusan, sampel jenama 1 jenis HM mempunyai kepekatan kebocoran BPA yang paling rendah apabila sampel diuji pada suhu 0 °C dengan pengesanan $1.148 \times 10^{-7} \pm 0.236 \times 10^{-3} \text{ g/cm}^2$, manakala sampel jenis HD mempunyai nilai yang paling tinggi kepekatan BPA dengan nilai ukuran $6.861 \times 10^{-7} \pm 2.915 \times 10^{-3} \text{ g/cm}^2$ pada suhu 70 °C. Walaupun kehadiran BPA dikesan di dalam semua jenis sampel plastik pada setiap suhu yang berbeza, walaubagaimanapun berdasarkan kajian, ia masih selamat untuk digunakan kerana semua nilai kepekatan BPA yang dikesan adalah masih di bawah had NOAEL (5 mg/kg bw/hari).

ABSTRACT

Bisphenol A (BPA) is a widely used chemical in the plastic production and it is well known as weak estrogenic character which can give potential endocrine disruptor to human body. In this study, trace amounts of BPA have been detected in five different type and brands of plastic packaging sample purchased from a local supermarkets. The concentration of BPA leaching at different temperature was measured using a simple, sensitive and rapid UV-Vis spectrophotometric method. The detector wavelength was set at 276 nm. Linear standard curve was obtained over the range from 0.07 to 0.35 mM with regression coefficient of 0.992 and detection limit of 0.0002 mM. Based on the result, brand 1 of HM type has the lowest concentration of BPA leach out from sample when it immersed in 0 °C with the detection of $1.148 \times 10^{-7} \pm 0.236 \times 10^{-3} \text{ g/cm}^2$, while HD type sample has the highest value of concentration of BPA leaching with measurement value of $6.861 \times 10^{-7} \pm 2.915 \times 10^{-3} \text{ g/cm}^2$ when it immersed in 70 °C. Although the concentration of BPA leached out from all types of plastic sample in every different temperature were detected, though as the part of research concern, it is still safe to use because all the values of concentration BPA detected in this study was still under the NOAEL range (5 mg/kg bw/day).

CHAPTER 1

INTRODUCTION

1.1 Bisphenol A

Bisphenol A often known as BPA is an organic compound that has been used to make polycarbonate plastics (PC), epoxy resins, polysulphones (PS) and polycrylate resins (Letcher *et al.*, 2005; Joskow *et al.*, 2006). Other common name for BPA are 2,2-Bis(4-hydroxyphenyl)propane and 4,4'-dihydroxy-2,2-diphenylpropane. BPA is colorless solid, having molecular weight of 228.29 g/mol and having mild phenolic odor (National Center for Biotechnology Information, 2017). It has chemical formula of $(\text{CH}_3)_2\text{C}(\text{C}_6\text{H}_4\text{OH})_2$ which derived from condensation of phenols with an acetone catalyzed by an ion exchange resin (Leena *et al.*, 2016). The illustration was presented in Figure 1.1 (Shimizu, 2008).

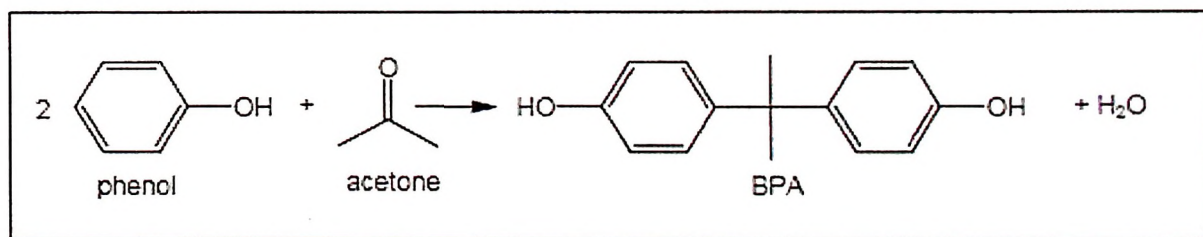


Figure 1.1 Derivation of BPA from phenol and acetone

A review by Hammad *et al.* (2015) stated that BPA was first synthesized by Dianin, a Russian chemist in 1891 and has been commercially use as industrial chemical since 1957. About eight billion pounds of BPA are used by manufacturers yearly in production of PC and epoxy resins for manufacturing of many valuable products.

1.2 Uses of BPA

BPA is a monomer and plasticizer employed to make PC and epoxy resins. These BPA-based plastic is hard, clear and rigid thermoplastic and have been widely made into a variety of common consumer goods (Grumetto *et al.*, 2008). BPA is preferred as color developers in carbonless copy and thermal point of sale receipt paper (Hammad *et al.*, 2015). The BPA is used for thermal paper coatings because of its stability and heat-resistance properties. This allows inkless printing for receipts from cash registers. BPA can be absorbed through the skin, thus people who often get in contact with BPA coated receipts do have a higher level of BPA in their bodies than people with average contact.

The BPA-derived plastic of PC is used to make food container, baby bottles, reusable bottles, tableware, compact disks and eyeglass lenses. In the other hand, epoxy resins containing BPA are generally used as protective coating on metal equipment such as beverage cans and water pipes to protect the food from direct contact with the metal thus helps to extend the shelf life of food (Ginsberg *et al.*, 2009; Beronius *et al.*, 2009; Grumetto *et al.*, 2008).

This study focused on the BPA leach from the uses of plastic packaging. The information about the plastic packaging is explained thoroughly in this section. Plastic is a processable materials based on polymer and their products are classified and coded based on their types of material. The plastics identification code such as polyethylene terephthalate (PET), polyvinylchloride (PVC), polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyamide (PA) were designed to be easy to read. These plastic

have been progressively used as packaging materials because of their large availability at relatively low cost and because of their good mechanical performance such as tensile and tear strength. Plastics also have tensile strength of heat seals, good barrier to oxygen, carbon dioxide, anhydride and aroma compound (Ebottles, 2015).

In this study, PP and PE types of plastic were used as samples because these two types of plastics packaging are commercially available at many supermarket. PP packaging mostly used as food pouches and bags for snack foods. PP originally is translucent, so colorants and additives are applied to all commercially produce PP resins to protect the polymer during processing and to enhance end use performance.

While, PE tends to be translucent and compatible with foods and most household chemicals. It also commonly used in some industrial and carry-out bags. Although PP and PE are seem similar as far as physical properties, however they have their own pros and cons. Table 1.1 shows the properties summary of PP and PE types of plastic (British Plastics Federation, 2017; Porex, 2017).

Table 1.1 The plastic types of material and their properties

Types of Material	Properties
Polypropylene (PP)	• Semi-rigid • Translucent • Tough • Good chemical resistance (highly resistant to most acids and alkalis) • Resistant to most organic solvents below 176°F (80°C) • Good fatigue resistance • Good heat resistance (can be subjected to steam sterilization)
Polyethylene (PE) High Density (HD) High Molecular (HM)	Most commonly used plastic in the world • Strong • Lightweight • Tough • Suitable for use in continuous service at temperatures up to 180°F (82°C) and intermittently at 240°F (116°C) • High tensile strength (if not stressed, stable at 212°F (100°C) in continuous service) • Excellent impact resistance • Low moisture absorption • Chemical and corrosion resistance • Embodies all the characteristics of high-density polyethylene (HDPE) • With the added traits of being resistant to concentrated acids and alkalis as well as numerous organic solvents • Has the same temperature limitations as HDPE

BPA is also found in variety of other products including dental fillings, paints, adhesives, flame retardants, electrical and electronic products, toys, playground and sporting equipment (National Center for Biotechnology Information, 2017).

1.3 Health Risks Linked to BPA

For past several decades, BPA was accepted by everyone as a non-toxic compound to humans and animals (Staples *et al.*, 1998; Kuo *et al.*, 2004; Coleman *et al.*, 2003; Lopez--Cervantes *et al.*, 2003; Liu *et al.*, 2004). However, at present many people are concerned about BPA because human exposure to BPA is widespread. Although BPA is advantageous for industrial use, on the other side, BPA and its derivatives can gives highly risk to health (Biles *et al.*,1997).

The article of Scientific American (2017) stated that in recent years, dozens of scientists around the world have linked BPA to a number of concerns in human health particularly in pregnant women, fetuses and young children, but also in adults. Very young and unborn humans are more susceptible to BPA exposure and its effects than adults because they cannot eliminate xenobiotics, a foreign substance so well.

According to Rubin (2011) BPA is an endocrine disruptor that has been shown to cause adverse effects in animal study. The disruptor mimic or partly mimic naturally occurring hormones structure like estrogen in the body and function similarly as natural hormone by having ability to bind to and activate the estrogen receptor. By having such that interference in the body, a person may elicit unwanted estrogenic effects such as

hormone imbalance, reduced fertility, altered development, heart disease, cancer, obesity and diabetes (Kavlock, 1999; O'Connor *et al.*, 2003; Vom-Saal *et al.*, 2008).

The entry routes of BPA into the human body can be passes through ingestion, dermal and inhalation. However, the primary exposure pathway to BPA is from ingestion. The BPA leaching will be transfer to food and drinks and consuming that contaminated food will cause negative effect to health (Hu *et al.*, 2006; Kavlock, 1999). BPA actively leached from the food container especially when the container are being mechanically cleaned with harsh, use of detergents, store at elevated temperature and use of acidic liquids (Coulier *et al.*, 2010; Carvalho *et al.*, 2015; Lane *et al.*, 2015).

1.4 Problem Statement

Nowadays, people commonly buy food at the food stall where the food was wrapped or packaged with the plastic. From the literature, plastic is made up from BPA and this compound can leach from the plastic due to the breakdown of polymer upon heating and cooling (Poorahong *et al.*, 2011). Even in small doses BPA can cause significant changes in metabolism and affect the reproductive and nervous systems (National Institute of Environmental Health Sciences, 2010). Concerning about the harmful effects of BPA to humans, therefore it is necessary to conduct an experiment to further clarify whether at certain temperature, BPA can be leached out from plastic packaging and whether type and brand of plastic packaging effect the BPA leaching.

1.5 Objectives

1.5.1 General Objective

- i. The purpose of this research is to analyse and determine the trace amounts of BPA leach out from plastic packaging using UV-Vis spectrophotometric method.

1.5.2 Specific Objectives

- i. To measure the concentration of BPA leach out from plastic packaging at four different temperatures which were 0°C, 4°C, room temperature (RT) and 70°C.
- ii. To determine the concentration of BPA leach out from plastic packaging for five different type and different brand of plastic packaging (PP, HD, HM (Brand 1), HM (Brand 2), and HM (Brand 3)).
- iii. To identify whether similar type (HM) with different brands (Brand 1, Brand 2 and Brand 3) of plastic packaging having similar concentration of BPA leach out from the plastics.

1.6 Significance of the Study

Plastic is an important source for human exposure to BPA. Public may not concern the facts that BPA can migrated out from the plastic when in contact with food under certain temperatures. The BPA migrated will contaminate the food and ingestion of BPA might lead to poor health.

As plastic packaging is commonly used for taking away food from a stall therefore this research could have important implications especially to the food vendors and also public to know whether at certain temperature BPA will leach out from plastic packaging. Hopefully this will be a significant information for the food vendors and public awareness to reduce the uses of plastic and this could be a strong encouragement for them to elevate the uses of biodegradable plastic campaign.

CHAPTER 2

LITERATURE REVIEW

Nowadays, usage and human exposure to BPA has increase the public concern to the fact of the BPA. In order to determine the trace amount of BPA in complex matrices, multiple research works using sensitive analytical methods have been demonstrated.

To date, the analysis of BPA has mainly been accomplished by high performance liquid chromatography (HPLC) (Sun *et al.*, 2000; Kuroda *et al.*, 2003; Sun *et al.*, 2004; Tanigawa *et al.*, 2011), gas chromatography (GC) and liquid chromatography (LC) with either GC or LC coupled to mass spectrometry (MS) (Mozaz *et al.*, 2004; Sanchez-Brunete *et al.*, 2009), enzyme-linked immunosorbent assay (ELISA) (Kim *et al.*, 2007), electrochemical sensor using voltammetry (Huang *et al.*, 2011; Li *et al.*, 2011) and ultraviolet-visible spectrophotometric (UV-Vis) (Zhuang *et al.*, 2014).

2.1 High Performance Liquid Chromatography (HPLC)

A highly sensitive and selective HPLC method with peroxyoxalate chemiluminescence detection was developed by Sun *et al.* (2000) for detection of BPA leached out from baby bottle samples. In this paper, they introduced highly sensitive and selective method for the determination of BPA at sub-ppb level. Two polycarbonate plastic baby bottle (sample 1 and 2) and one glass baby bottle (sample 3)) in contact with hot water at 95°C were used as the samples while simple solid-phase extraction technique was used to remove excess of BPA derivative. The separation was run on an octadecyl (ODS) column with a mixture of imidazole-HNO buffer:acetonitrile (17:83 v/v) as a mobile

phase. Bis[2-(3,6,9-trioxadecanyloxycarbonyl)- 4- nitrophenyl]oxalate (0.6 mM) and hydrogen peroxide (25.0 mM) dissolved in acetonitrile was used as a chemiluminescence reagent solution. In this study, the linear standard curve was obtained over the range from 0.57 to 22.8 ppb with regression of 0.996 and detection limit of 0.38 ppb. During the first use of the bottles, BPA was found to migrate at 0.59 and 0.75 ppb from sample 1 and 2 respectively, while none appeared to migrate from the glass bottle. However, there is no significant change was observed after the bottles were washed with a brush to create scratches on inner surface.

In year 2003, Kuroda *et al.* had conducted a study using a sensitive column-switching HPLC method with fluorescence detection to determine a trace amount of BPA in several biological fluids (blood serum and ascitic fluid) in human maternal. A column-switching technique was use in removing the excess fluorescent labeling reagent (4-(4,5-Diphenyl-1H-imidazol-2-yl)benzoyl chloride (DIB-Cl)) and the pretreatment of samples were done by liquid-liquid extraction with chloroform. The method enabled highly sensitive detection of BPA in serum and ascetic fluid samples with detection limit as low as 0.004 ppb. BPA of the both samples could be determined in the concentration range of 0.1 to 7.0 ppb with regression of 0.785. The mean concentration of BPA in blood serum and ascitic fluid from healthy pregnant women were 0.469 and 0.629 ppb respectively. Other than healthy maternal subject, Kuroda also determined BPA levels blood serum and ascetic fluid obtained from the sterility patients by the method and the results obtained has no significant to the healthy maternal samples.

Another study involving fluorescent detection was carried out by Sun *et al.* (2004). The study had similar steps as Kuroda *et al.* (2003) with different only at sample clean up by

using a two step liquid–liquid extraction. Sun *et al.* (2004) had developed the method for the determination of BPA in human breast milk samples. The separation of DIB-BPA from endogenous materials in milk was carried out on two C18 columns and the fluorescence intensity was monitored at 475 nm. The linearity was good with 0.994 in the concentration range of 0.2–5.0 ngmL⁻¹ in breast milk and its limit of detection was 0.11 ngmL⁻¹. The mean concentration value of breast milk samples was 0.61 ngmL⁻¹ with no correlation to the lipid content of milk samples. By the proposed method, the concentrations of BPA in milk samples were determined to be in the range of 0.28 to 0.97 ngmL⁻¹. To compare, the two steps liquid-liquid extraction method resolves better than single step of sample clean up method proposed by Kuroda *et al.* (2003).

2.2 Chromatography-Mass Spectrophotometry

Previous study by Mozaz *et al.* (2004) had focused on monitoring BPA in natural waters and drinking water treatment plants by solid phase extraction liquid chromatography mass-spectrometry. The aimed of this research is to study the occurrence of the selected pollutants in surface and groundwater samples from the river and the aquifer that used for abstraction of drinking water. Mozaz also evaluate the removal efficiency of the different water treatments stages which are sand filtration, ozonation, activated carbon filtration and post-chlorination. These treatments take a long time consuming monthly from February to August of 2002. It was found that the most of the compounds were completely removed during the water treatment, especially after activated carbon filtration. Mozaz stated that BPA was detected in all surface water samples and no significant change observed. It shows that the degradation rate of BPA in groundwater is very low even the pollution of the groundwater is persistent. The method is suitable

for water treatment due to having good linearity ($R^2=0.999$) with detection limit of 6.30 ngL⁻¹.

In the other hand, Sanchez-Brunete *et al.* (2009) had studied on determination in agricultural and industrial soil samples of BPA by gas chromatography mass spectrometry through isotope dilution analysis using stable isotopes. The agricultural samples were extracted from soil by sonication assisted extraction in small columns (SAESC) whereas a simultaneous clean-up on an acidified florisil–anhydrous sodium sulfate mixture was necessary to remove interferences from industrial samples. The response obtained showed good recoveries with linear over the range of 5 to 300 ngmL⁻¹ with correlation coefficients of 0.999. BPA was detected in all samples at concentrations from 0.7 ngg⁻¹ to 4.6 ngg⁻¹ in agricultural soils and from 1.1 ngg⁻¹ to 44.5 ngg⁻¹ in industrial soils. Accurate quantification was achieved by isotope dilution analysis using stable isotopes as internal standards.

2.3 Enzyme Linked Immunosorbent Assays (ELISA)

A sensitive and reliable quantification method for BPA based on modified competitive ELISA method was proposed by Kim *et al.* (2007). The method was based on bovine serum albumin (BSA) to produce polyclonal antibody against bisphenolic structure and a modified competitive ELISA method for quantification of BPA. BSA, a carrier protein and 4,4-Bis(4-hydroxylphenyl) valeric acid (BHPVA), a reagent to make antigen were used in this study. This system is based on BHPVA–BSA for polyclonal antibody production against bisphenolic structure and BHPVA-horseradish peroxidase (HRP) is used for determination of BPA substituting detection antibody in competitive reaction.

The detectable concentration found to be between 2 and 1000 ngmL⁻¹ with recovery range between 96.3% and 107.2%. In this study, the modified competitive ELISA method has proven to be a very useful tool for quantification of BPA without the unexpected interaction of BSA and anti-BSA polyclonal antibody.

2.4 Electrochemical Sensor

A sensitive electrochemical sensor was applied for detection of BPA in plastic products based on molecularly imprinted polymers (MIP) and gold nanoparticles (NG) (Huang *et al.*, 2011). In this study, Huang concluded that the combination of the nanoparticles with MIP offered a strong specific binding activity toward BPA to enhance the sensitivity of the imprinted sensor. It also provided a broad linear range, remarkable stability and quantitatively repeatable analytical performance. The linearity of this method was between 8.0 to 6.0 μmolL^{-1} with a detection limit of 0.14 μmolL^{-1} and the average recovery value was 95.6%. Huang believed that the MIP–NG modified electrochemical sensor will have promising application for BPA detection, not only exhibited strong specific binding activity toward BPA.

Another study involving electrochemical sensor was done by Li *et al.*, (2011) in determination of BPA in food package by a glassy carbon electrode modified with carboxylated multi-walled carbon nanotubes. Li found that the trace levels of BPA can be determined over a concentration range that is linear from 10 nM to 104 nM with the correlation coefficient of 0.9983 and the detection limit of 5.0 nM. The recovery of this method was excellent in which the recoveries of BPA were in the range of 98.4% to 102.8%.

2.5 Ultraviolet-Visible Spectrophotometric (UV-Vis)

Zhuang *et al.*, 2013 had studied a new spectrophotometric method for sensitive determination of BPA based on a diazotization-coupling reaction. The methods were applied to determine the leached out of BPA in hot water in contact with commercially available table-water bottle samples. According to Krishnan *et al.* (1993) an azo-compound was form in $\text{NH}_3\text{--NH}_4\text{Cl}$ buffer. Since BPA is colorless, clenbuterol was used to diazotized with sodium nitrite, then coupled with BPA which showed maximum absorption at 410 nm. The determination linear range of BPA under optimum condition was 0.24 to 8.4 $\mu\text{g mL}^{-1}$, correlation coefficient of 0.9905 and detection limit of this method was 0.15 $\mu\text{g mL}^{-1}$. The method showed no serious interference in samples by having the recoveries between 95.6% and 109.1%. These results demonstrate the spectrophotometric method offers potential advantages of simplicity, rapidity and sensitivity determination.

To conclude all of the information above, a chromatographic determination affords highly reliable results. Among several chromatographic approaches, HPLC system has its obvious benefits over other methods such as GC. HPLC analysis requires simple pretreatment because the target compounds are already contained in liquid phase whereas GC would require a complicated vaporizing procedure for sample pretreatment (Tanigawa *et al.*, 2011) especially the effective removal of contaminants contributes to sensitive detection with commonly used detectors. The HPLC methods are simple, rapid and sensitive. The discovery of these methods will enlarge the range of the practical application for the BPA detection. ELISA has been employed because of the ease of use, relatively simple protocol and fairly good sensitivity despite relatively low

reproducibility. The voltammetric electrochemical sensor method for trace determination of some chemical substances has advantages of high sensitivity, sufficient accuracy and simple procedures. For spectrophotometric, photomultipliers are very sensitive to UV and visible radiation. They had fast response times which gave advantages of rapid, high sensitivity with better accuracy to the method.

In this study, the UV-VIS spectrophotometric method was chosen to determine the trace amount of BPA leached out from several types of plastic packaging at different temperatures.

CHAPTER 3

METHODOLOGY

3.1 Reagents, Chemicals and Apparatus

BPA and sodium nitrite (NaNO_2) were purchased from Sigma Aldrich in Missouri, United States. Clenbuterol with purity solutions brands was purchased from Los Angeles, United States. Ethanol, hydrochloric acid (HCl) and ammonia (NH_3) were purchased from HmbG® Chemicals in Darmstadt, Germany. Deionized distilled water (DI) was used in the entire experiments.

A Cary 100 model of ultraviolet-visible spectrophotometer (UV-Vis) was used for making absorbance measurements and recording spectra. An ERLA hot plate EMS HP-7000 was used to control the reaction temperature. A Shimadzu brand of electronic balance from Kyota, Japan was used to measuring the weight of the chemicals and samples. All the apparatus used were made up from glassware to minimize the positive-false of BPA leached out from plastic samples.

3.2 Preparation of BPA Standard Solution and Series of Working Solution

BPA standard solution was prepared by dissolving 60 mg of BPA in 0.5 mL of ethanol in a 25 mL beaker. The mixture solution was stirred using glass rod to dissolve the BPA and transferred to a 50 mL volumetric flask. The solution was then diluted to the mark with DI and was transferred to amber bottle and keep at 4°C.

Serial dilutions of working solution were prepared by further dilution with DI and derivative reagent to yield solutions with final concentration of 0.07 mM, 0.14 mM, 0.21 mM, 0.28 mM and 0.35 mM. The detailed procedures how to prepare the solution were explained as followed. For 0.07 mM, firstly, 0.60 mL of 0.10 mg/mL of clenbuterol solution was placed into a 10.0 mL volumetric flask, then 0.40 mL of 0.10 M HCl solution and 0.60 mL of 0.20 mg/mL NaNO₂ were added. The mixture was swirled and stored in a fridge at 4 °C for 10 minutes. Then, 0.14 mL from the standard BPA solution was added to the mixtures and shake well before 1.4 mL of 8.8 mg/mL of NH₃ was added. Lastly, the mixture was diluted to the mark with DI and mixed well. The same procedure was performed for the other concentration except for the volume of BPA from the standard solution. The solution was added according to desired concentration of working solution (refer to Table 3.1). For the blank reagent, DI was used instead of standard BPA.

Table 3.1 The appropriate amount of reagents and chemicals for preparation of BPA working solution

Concentration of working solutions (mM)	0.10 mg/mL of Clenbuterol (mL)	0.10 M of HCl (mL)	0.20 mg/mL of NaNO ₂ (mL)	5.0 mM of standard BPA (mL)	8.8 mg/mL of NH ₃ (mL)
0.07	0.6	0.4	0.6	0.14	1.4
0.14	0.6	0.4	0.6	0.28	1.4
0.21	0.6	0.4	0.6	0.42	1.4
0.28	0.6	0.4	0.6	0.56	1.4
0.35	0.6	0.4	0.6	0.70	1.4

3.3 Determination of Maximum Absorbance Wavelength and Stability Test of BPA

After the series of working solution were prepared, the maximum wavelength absorbance ($\lambda_{\max}$) for the BPA measurement was identified by scanning the serial dilution solutions of standard BPA.

Then, stability test of standard BPA was run by using 0.21 mM. The absorbance of the BPA was measured for one hour with 10 minutes interval. The test was done in triplicate using the same concentration of solution. The graph was plotted using excel and the average with standard deviation (SD) value were then calculated to formed an error bar for reliability identification purposes.

3.4 Calibration Curve and LOD

The absorbance of the serial working solution that prepared in 3.2 were taken and plotted to see whether it was linear or not. Then, the LOD was identified.

Five readings of blank sample were measured to identify the lowest analyte concentration that can be detected. The LOD was determined by using the equation below (Morano *et al.*, 1997) :

$$\text{LOD} = 3.3 \left(\frac{\text{SD}}{\text{slope}} \right)$$

Where :

SD = Standard deviation of the blank

Slope = The slope of the standard calibration curve

3.5 Sample Preparation

Five brands and types of common used plastic packaging were purchased from a supermarket in Kubang Kerian, Kelantan as samples (refer to Table 3.2).

Table 3.2 List of plastic packaging samples

No.	Sample	Type and brand of sample
1.	Sample A 	Polypropylene (PP) type
2.	Sample B 	High Density (HD) type

3.	Sample C 	High Molecular (HM) type (Brand 1)
4.	Sample D 	High Molecular (HM) type (Brand 2)
5.	Sample E 	High Molecular (HM) type (Brand 3)

The sampling process was done by cutting the samples into 1 cm² of several pieces. About 10 pieces of the sample were placed into four different 20 mL of amber bottle. The bottles were label as A, B, C and D for representing 0°C, 4°C, RT (24 °C) and 70°C respectively. 10 mL of DI was added into each of the amber bottle using a pipette except for 70 °C.

For 70 °C, DI was heated first to reach 70 °C before the adding of sample then it was left at room temperature. For 0°C and 4°C samples, they were stored in a refrigerator overnight. While for RT, the samples were let to stand for one hour before analyzing the sample. These steps were repeated in triplicate for each of the samples type.

3.6 Sample Analysis by UV-Vis Spectrophotometer

The preparation of sample derivative was followed the same procedure as 3.2 except for the standard BPA was replaced by 0.7 mL of the sample. The absorbances of samples were measured and the concentrations of BPA leached out from the samples were calculated from the calibration curve.

3.7 Repeatability and Reproducibility

Repeatability and reproducibility were utilized to measure precision. It was performed in order to confirm the finding of the results because every measurement may give variation of finding. In this study, the experiments were performed in every type of samples and also at every different temperature.

For repeatability study, each sample was measured three times from a bottle of sample at same conditions, using same instrument and the repetition was done over a short period of time. While for reproducibility, the measurement was identify by examined at the ability of the instrument to consistently reproduce the same measurement of the same sample in three different bottles under the same conditions.

CHAPTER 4

RESULTS AND DISCUSSION

Various of analytical analysis (λ_{max} , stability of BPA, calibration curve, LOD, repeatability and reproducibility) had been carried out in this study in order to determine the trace amount of BPA leaching from plastic packaging in four different temperature.

4.1 Selection of Maximum Absorbance Wavelength (λ_{max})

The λ_{max} was scanned using standard solution in UV-Vis spectrophotometer between 200 nm to 350 nm on spectrum mode using diluents as a blank. Based on the spectrum in Figure 4.1, it showed that most of the peak absorbed maximum intensity at wavelength 276 nm. Hence, the λ_{max} was chosen at 276 nm.

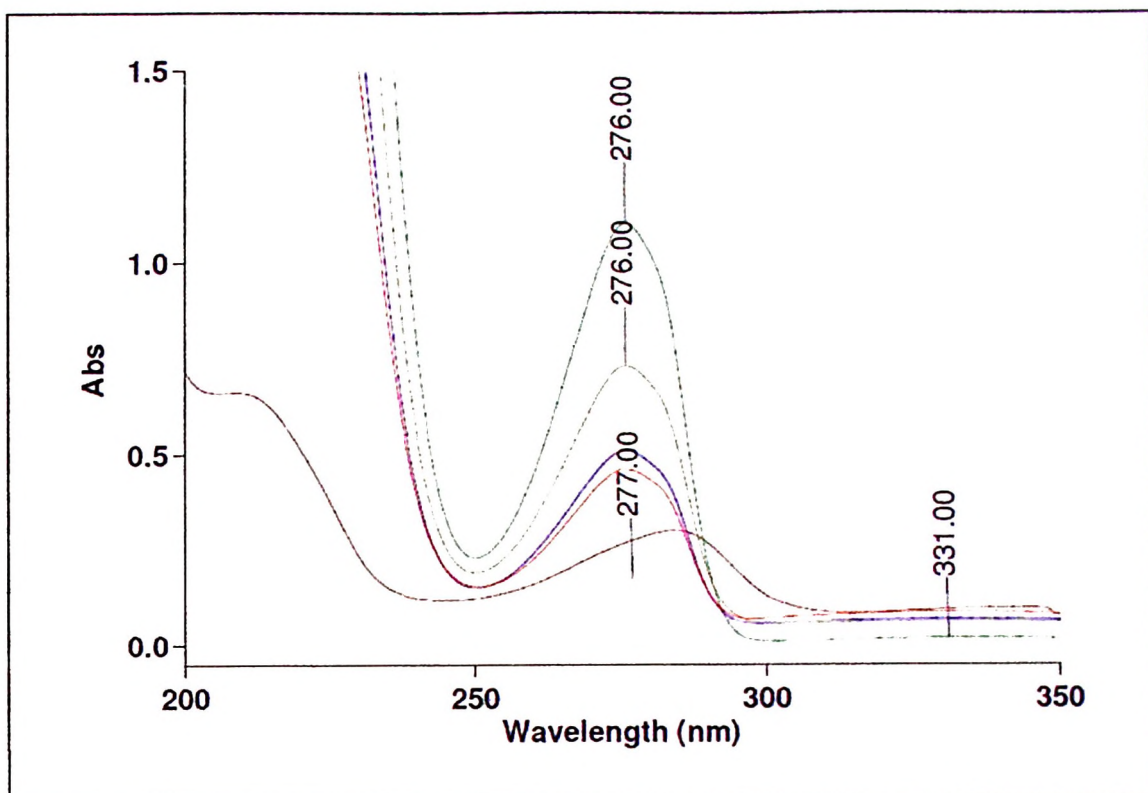


Figure 4.1 The spectrum of scanning the maximum absorption wavelength of BPA

4.2 Stability Test of BPA

The stability test of BPA was determined by measuring the absorbance of BPA solution after it was treated with derivative reagent. The test was done in triplicate and the reading was taken for each ten minutes within an hour. A graph was plotted based on the average of the absorbance value in Table 4.1. Based on the graph in Figure 4.2, the curve keep rising over time, therefore to be consistent, the analysis was run immediately after the sample was treated with derivative reagents.

Table 4.1 The average of the absorbance in the stability test

Time (min)	Average absorbance $\pm$ SD
0	0.5787 ± 0.02550
5	0.6044 ± 0.02488
15	0.6191 ± 0.02178
25	0.6334 ± 0.01945
35	0.6486 ± 0.02482
45	0.6677 ± 0.02825
55	0.6854 ± 0.03398
60	0.6983 ± 0.03605

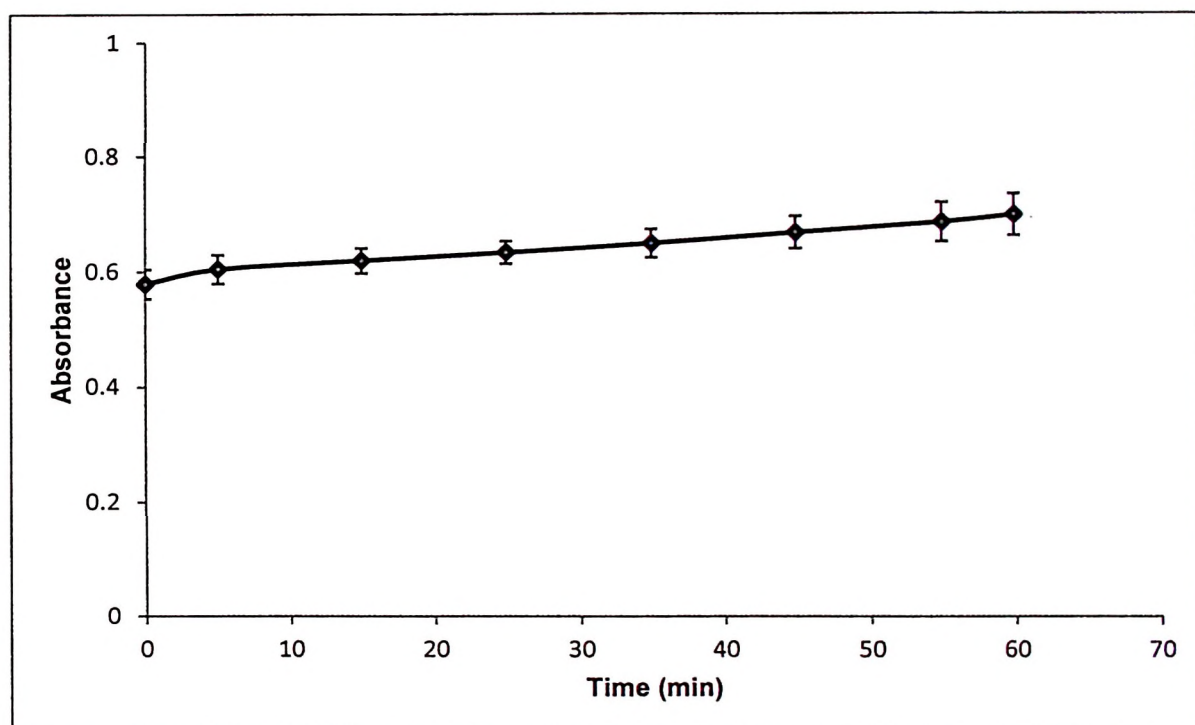


Figure 4.2 Error bar of average absorbance against time in stability test

4.3 Calibration Curve and LOD

A calibration curve of absorbance against concentration in Figure 4.3 was plotted based on the value in Table 4.2 using Microsoft Excel 2007. The linear regression equation of the graph obtained was $y = 2.6977x + 0.002$. The calibration curve has good linearity with the correlation coefficient, $R^2 = 0.9928$.