DETERMINATION OF Pb(II), Cu(II) AND Ni(II) IN WATER BY DIRECT MEASUREMENT USING UV/VIS SPECTROPHOTOMETER

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by

TAN CHUN HO

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LIST OF ABBREVATIONS

Symbol / Abbreviation	Caption
A	Absorbance
A	Absorbance Matrix
AAS	Atomic Absorption Spectroscopy
b	Path length
C#	C-Sharp
CCD	Charge-Coupled Device
CLS	Classical Least Squares
D	Dark intensity
E	Energy
f	Frequency
F	Focal length
h	Planks constant
I	Intensity
IC	Ion Chromatography
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
k	Proportional constant
mg/day	milligram per day
mg/L	milligram per litter
MLS	Multiple Least Square
ms	millisecond
nm	nanometer
PAN	1-(2-pyridylazo)-2-naphthol
PAR	4-(2-pyridylazo)resorcinol

PCA	Principle Component Analysis
ppb	parts per billion
ppm	parts per million
R	Reference intensity
R^2	R-Square
RMSE	Root Mean Square Error
S	Sample intensity
T	Transmittance
UV	Ultraviolet
VIS	Visible
WHO	World Health Organization
XRF	X-ray Fluorescence
λ	Wavelength
σ	Standard deviation
ε	Extinction coefficient
$\boldsymbol{\varepsilon}$	Extinction coefficient Matrix
ρ	Concentration
$\boldsymbol{\rho}$	Concentration Matrix
µg/kg	microgram per kilogram
µg/L	microgram per litter
µmol/L	micromol per liter

LIST OF PUBLICATIONS AND CONFERENCE PAPERS

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2.	Ho, T. C., Jafri, M. Z. M., San, L. H., & Chong, M. Y. (2012, July). Detection of heavy metal ions in aqueous solution using fiber optic sensor. In *Computer and Communication Engineering (ICCCE), 2012 International Conference on* (pp. 267-270). IEEE.

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4.	Tan, C. H., Moo, Y. C., Jafri, M. M., & Lim, H. S. (2014, May). UV spectroscopy determination of aqueous lead and copper ions in water. In *SPIE Photonics Europe* (pp. 91410N-91410N). International Society for Optics and Photonics.

PENENTUAN Pb(II), Cu(II) DAN Ni(II) DALAM AIR DENGAN MENGGUNAKAN UV/VIS SPEKTROFOTOMETER

ABSTRAK

Logam berat merupakan unsur logam dengan ketumpatan yang tinggi dan kebanyakannya bersifat toksik pada kepekatan yang rendah. Selain itu, kepekatan logam berat dalam organisma akan semakin meningkat dengan masa disebabkan oleh sifat bioakumulasi daripada logam berat dan tidak dapat diuraikan. Teknik terkini untuk menentukan logam berat dalam air adalah melalui AAS, IC, ICP-AES, ICP-MS, XRF dan elektrokimia. Teknik-teknik ini dapat memberi ketepatan yang tinggi dalam pengukuran tetapi memerlukan kos penyelenggaraan yang tinggi dan prosedur penyediaan yang rumit. Dalam penyelidikan ini, analisis kuantitatif terhadap ion Pb^{2+}, Cu^{2+} dan Ni^{2+} dalam larutan akueus telah berjaya dijalankan dengan menggunakan UV/VIS spektroskopi tanpa reagen kimia tambahan. Penyelidikan bermula dengan mengenalpasti panjang gelombang yang berkesan untuk penyerapan dan kemudiannya disahkan dengan bilangan sampel yang banyak. Daripada penyelidikan ini, panjang gelombang berkesan untuk penyerapan di dalam julat UV bagi Pb^{2+} dan Cu^{2+} adalah daripada 200 nm hingga 230 nm dan Cu^{2+} dan Ni^{2+} daripada 600 nm hingga 800 nm. Selain itu Ni^{2+} mempunyai penyerapah yang tinggi pada panjang gelombang 400 nm. Disebabkan oleh masalah pertindihan spektra, analisis berbilang akan digunakan untuk mengukur kepekatan daripada campuran akueus. Analisis berbilang ini merangkumi 50 pekali kepupusan yang diekstrak daripada 50 gelombang cahaya yang berbeze. Akhir sekali, semua persamaan terhasil akan dimasukkan ke dalam pengaturcaraan C# yang mana akan membantu untuk melaksanakan pengiraan yang rumit. Pengaturcaan C# ini dapat mengesan dan

mengecualikan titk terpencil, pengiraan sisihan piawai dan analisis berbilang. Ketepatan penyelidikan ini ialah 0.178 mg/L, 0.243 mg/L dan 1.815 mg/L bagi Pb^{2+}, Cu^{2+}, and Ni^{2+} dalam larutan akueus dengan had limit lebih kurang dari 0.2 mg/L sampai 10 mg/L bagi Pb^{2+} dan Cu^{2+} dan 2 mg/L sampai 100 mg/L bagi Ni^{2+}.

DETERMINATION OF Pb(II), Cu(II) AND Ni(II) IN WATER BY DIRECT MEASUREMENT USING UV/VIS SPECTROPHOTOMETER

ABSTRACT

Heavy metal are metallic element with relatively high density and mostly toxic at low concentration. Heavy metal does not degrade and tends to bioaccumulate in organism over time. Current technique to determine heavy metals in water mostly via AAS, IC, ICP-AES, ICP-MS, XRF and electrochemical method,these techniques provide high precision in measurement but required high maintenance cost and complicated preparation. In this research, quantitative analysis of Pb^{2+}, Cu^{2+} and Ni^{2+} ions in aqueous solution was carried out successfully using UV/VIS spectroscopy without additional chemical reagent. The research begins with identifying the effective absorption wavelength and was later verified using large amount of samples. From this research, the effective wavelength within UV range for Pb^{2+} and Cu^{2+} is roughly from 200 nm to 230 nm and both the Cu^{2+} and Ni^{2+} have absorbance from wavelength 600 nm to 800 nm. Also, the Ni^{2+} has high absorbance at wavelength 400 nm too. Furthermore, multicomponent analysis is used in order to calculate the concentration with overlapped spectra caused by mixture of Pb^{2+} and Cu^{2+} aqueous solution. There are total number of 50 extinction coefficient which correspond to 50 different wavelength from 201.20 nm to 223.39 nm are used to construct the multicomponent analysis equation. Finally, all regression model and multicomponent analysis equation obtained was compiled into a C# program which help to perform complex calculation. This program created for the removal of outliers, identification of ions, calculation of concentration, and root mean square error (RMSE) calculation. In this research, the accuracy of this detection method

is0.178 mg/L, 0.243 mg/L, and 1.815 mg/L for Pb^{2+}, Cu^{2+}, and Ni^{2+} in aqueous solution with a detection limit of roughly 0.2 mg/L to 10 mg/L for Pb^{2+} and Cu^{2+} and 2 mg/L to 100 mg/L for Ni^{2+}.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Spectroscopy is an experimental method that involves the study of absorption, scattering and emission of electromagnetic radiation by atoms or molecules. Recently, spectroscopy is widely used in many areas such as environment study, health care, food quality control and pollution monitoring.

Nowadays, detection of heavy metal ions in water can be done using various methods. One of the easiest method is chemical reagent method such as PAR (4-(2-pyridylazo)resorcinol) which will change color when react with heavy metal ions. More precise detection method is carried out using instrument such as inductively coupled plasma - atomic emission spectroscopy (ICP-AES), inductively coupled plasma - mass spectrometry (ICP-MS), atomic absorption spectrophotometer (AAS), and X-ray fluorescence spectrometry (XRF). However, those techniques require toxic chemical reagent, expensive equipment and trained operator.

Thus, in this project, ultraviolet / visible (UV/VIS) spectroscopy method was introduce for the detection of nickel (II) (Ni^{2+}), copper (II) (Cu^{2+}), and lead (II) (Pb^{2+}) ions in water without any chemical reagent. In addition other heavy metal ions such as Al^{3+}, Ca^{2+}, Mg^{2+}, and Zn^{2+} was tested using UV/VIS spectroscopy too.

1.2 Problems Statement

Heavy metal ions that exist in our daily drinking water and tap water are highly dangerous to our health. Due to the bioaccumulation properties of heavy metal ions, drinking that water in long term period will seriously affect our health. Detection of heavy metal ions is important to avoid consuming the harmful ions unconsciously. However, monitoring the water quality everyday is not simple and time consuming due to the need of sample preparation. Besides that, some of chemical reagent that required for heavy metal detection was highly toxic, not environmental friendly and may require complicated preparation. Recent years, UV/VIS spectroscopy was proven useful and it is one of the non-destructive method used in many application such as measurement of sugar, glucose, and fruit quality.

1.3 Scope of Study

This research is mainly focus on study of UV/VIS absorbance spectroscopy on heavy metal ions (Cu^{2+}, Ni^{2+}, and Pb^{2+}) ions in aqueous solution to determine the effective wavelength of it. The concentrations of these three heavy metal elements will be calculated via simple linear regression equation and multiple linear regression method. At final stage of this study, multicomponent and C# programming will be used to aid the calculation of concentration in mixture solution and single element solution.

1.4 Research Objectives

The objectives of this study are as follow:

1.   To determine the effective absorption wavelengths for Cu^{2+}, Ni^{2+}, and Pb^{2+} ions for direct detection using UV/VIS spectroscopy in aqueous solution.

2. To validate the regression equation for determination by effect of different anion and inference ions.

3. To develop a detection algorithm using C# programing which include all the validated regression model and multicomponent analysis to help calculation of Cu^{2+}, Ni^{2+}, and Pb^{2+} concentration.

1.5 Novelty of This Study

By studying the UV Spectrum of Cu^{2+}, Ni^{2+}, and Pb^{2+} ions in water provides alternative way of Cu^{2+}, Ni^{2+}, and Pb^{2+} detection based on Beers-Lambert law and multicomponent analysis. In this research, multicomponent analysis is used to analyze and overcome spectra overlapping issue from different ions. Besides that, additional toxic chemical reagent and sample preparation are not required using this method. The concentration of the sample can be calculated easily within minutes using this method.

1.6 Structure of the Thesis

This thesis consists of five chapters. First chapter provides an overview of this study, problems statements, scope of study, research objectives and novelty of this study. Literature reviews will be covered in chapter two which include some current works by researcher and basic theory such as heavy metal, Beer-Lambert Law, and multicomponent analysis. Chapter three will describe about the instruments and method used in this study. Chapter four presents all the results of this research with discussion and how the result being analyzed. Chapter five summarizes all the output and result of this research with recommendation for future study. Finally, the full C# programming code will be included inside appendix.

CHAPTER 2

THEORY AND LITERATURE REVIEW

2.1 Introduction

This chapter introduces some basic theories and information about heavy metal elements, spectroscopy, and common detection method for heavy metal. In addition, a basic theory of absorbance spectroscopy and multicomponent analysis will be included in this chapter too.

2.2 Heavy Metal

Most of the heavy metals element are transition element in periodic. Common heavy metal element such as zinc, magnesium, calcium, and copper are essential element that are required by our body's metabolism. However, most of the heavy metals are extremely harmful to our health such as aluminum, lead, and nickel. Heavy metal element are well known for their unique bioaccumulation characteristic. Bioaccumulation characteristic is the main reason that causes heavy metal and highly toxic in long term period. Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is exaggerated. Thus it caused severe health effect when its concentration accumulated exceed certain level. In addition, they are not degradable into other harmless product (Yu, 2005).

2.2.1 Lead (Pb)

Lead, Pb, is a metal with atomic number 82 and molar mass 207.2 with its ions exist in Pb^{2+}. Lead is not required by our body and it is highly toxic in long term exposure or intake. Lead is widely used in industrial area and daily life such as lead acid

battery, plastic stabilizers, ammunition, paint, and etc. In the old days, lead is widely used in water distribution system as lead pipe and it was banned after the discovery of health problems caused by it, but lead pipe may still exist in older water distribution system nowadays. Our body are able to excrete lead if intake is less than 4 µg/kg of body weight per day. Lead are carcinogenic metal and very poisonous, it was recognized that it has caused several health issue such as kidney damage, loss of memory, hallucinations, lung cancer, stomach cancer, and reproductive problems (Muhammad, 2011; WHO, 2013). Furthermore, lead easily deposits in reproductive system, kidney, blood, brain, and nervous system and cause irreversible damage (Tan and Nguyen, 2010). According to Ministry of Health Malaysia, the drinking water quality standard is 0.05 mg/L and 0.01 mg/L for raw water and treated water (Ministry of Health Malaysia, 2010).

2.2.2 Copper (Cu)

Copper, Cu is a transition element with atomic number 29 and molar mass 63.546 with its ions commonly exist in cuprous (Cu^+) and cupric (Cu^{2+}) form. Copper can be found in most of the water such as underground water, surface water, tap water and drinking water. The daily intake of copper for an adult usually ranged from 1 to 5 mg per day. Copper is an essential element for human in order to maintain enzyme system in body and plays an important role in center nervous system development. However, only less than 3% of copper intake are excreted through urine per day thus excessive copper intake for long period of time will caused several health issue (WHO, 2013). Recently, there are lots of studies on the effect of excessive copper intake by human. According to Desai and Kaler, some common neurological disease such as Alzheimer disease and Parkinson disease are suspect to be related to the

excessive copper in body. Besides that, copper also suspected for the production of free radical in our body which will cause mitochondrial damage, DNA breakage, and neuronal injury (Desai and Kaler, 2008). According to Ministry of Health Malaysia, the drinking water quality standard is 1.00 mg/L for raw water and treated water (Ministry of Health Malaysia, 2010).

2.2.3 Nickel (Ni)

Nickel, Ni is a transition element with atomic number 28 and molar mass 58.69 with four oxidation state +1, +2, +3 and +4. Nickel is not required by our body and according to WHO, dietary intake of nickel should not exceed 2mg/day. Nickel may exist in underground water, tap water and drinking water. Nickel intoxication will cause symptoms such as nausea, vomiting, headache and weakness. There are a lot of carcinogenicity studies about nickel, however, there is lack of evidence to prove the carcinogenic risk from oral exposure to nickel (WHO, 2013). According to Ministry of Health Malaysia, drinking water quality standard is 0.02 mg/L for treated water and not specific standard for raw water (Ministry of Health Malaysia, 2010).

2.3 UV/VIS Absorbance Spectroscopy

In analytical laboratory, spectroscopy method is widely used as the tool for determination of chemical structure or chemical elements for an unknown gas, liquid or solid. Different wavelengths will require different kind of spectroscopy method. Figure 2.1 shows the electromagnetic spectrum. The energy of the electromagnetic radiation is defined as E = hf, where E is energy (J), h is planks constant (6.62 x 10^{-34} Js) and f is frequency of the electromagnetic wave (Hz). In this study, heavy metal aqueous solution will be tested using UV/VIS absorbance spectroscopy with

wavelength from 200 nm to 800 nm. The absorbance theory will be further explained in section below.

Depending on the frequency of incident electromagnetic wave, molecules will absorb its energy and experience rotational, vibrational or translational motion or ionization of the molecules. The molecules will then go to ground state from excited state by giving off the energy in electromagnetic radiation. The absorption and emission can be then recorded in spectra form so that qualitative and quantitative information can be obtained through spectra analysis.

The UV/VIS absorbance is cause by excitation of electrons and there are three types of electronic transitions (Lobnik, 2004). One of them is electronic transition between σ bonding, π bonding and n bonding to σ* antibonding and π* antibonding. Second type of transition involving charge-transfer electrons and third type of transitions involving d and f electrons.
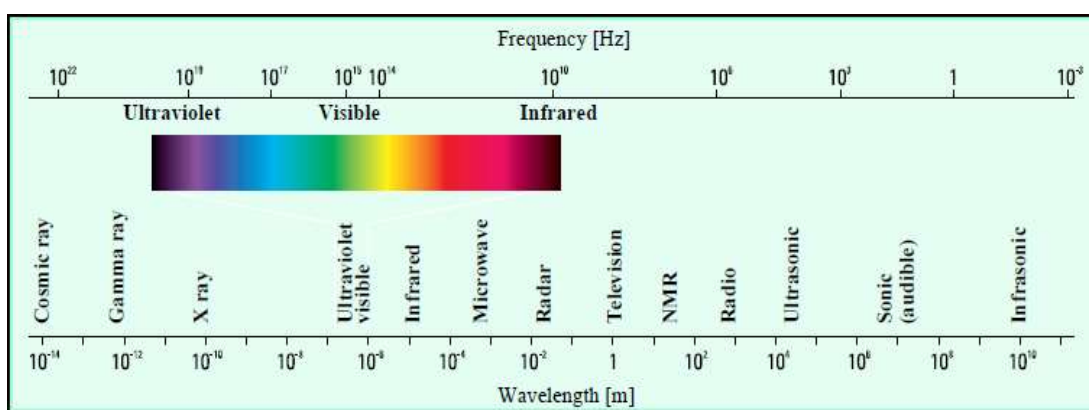
(Source: Owen, 2000).

Figure 2.1 Electromagnetic spectrum.

For inorganic element or ions such as transition metal, the absorption of UV/VIS is mostly due to d to d electronic transition. Because of this phenomena, some of the transition elements will have color in their ions form. Beer-Lambert Law can be use to perform quantitative analysis with UV/VIS absorbance spectroscopy.

2.3.1 Beer-Lambert Law

Lambert's law states that each equal thickness of light absorbing medium will absorb equal fraction of radiant energy. The law also states that the proportion of absorbed light is independent to the intensity of incident light. Lambert's law can be expressed by Equation 2.1 where I is the intensity of transmitted light, I_o is the intensity of incident light and T is the transmittance which can also expressed as percentage by multiplying T with 100.

$$T = \frac{I}{I_0} \tag{2.1}$$

Beer's Law states that the total absorbance of a solution is equal to the sum of the absorbance of every individual element in the solutions. Thus, this makes the quantitative analysis possible to estimate the element present in the solutions. In addition, Beer's Law also state that absorbance is proportional to the thickness and concentration of the absorbing medium. Beer's Law can be expressed by Equation 2.2 where A is the absorbance, k is a proportional constant, b is the path length and ρ is the concentration of the sample.

$$A = kb\rho \tag{2.2}$$

The combination of both laws are known as the Beer-Lambert Law which describes the relationship of both transmittance and absorbance. Equation 2.3 and Equation 2.4 show the relationship between transmittance and absorbance, where ε is the extinction coefficient. Extinction includes all the light lost due to scattering, absorption and reflection by the particle in the sample (Owen, 2000; Hardesty and Attili, 2010). Figure 2.2 shows how Beer-Lambert Law works.

$$T = e^{-\varepsilon b} \tag{2.3}$$

$$A = \log\left(\frac{1}{T}\right) = \varepsilon b \rho \tag{2.4}$$
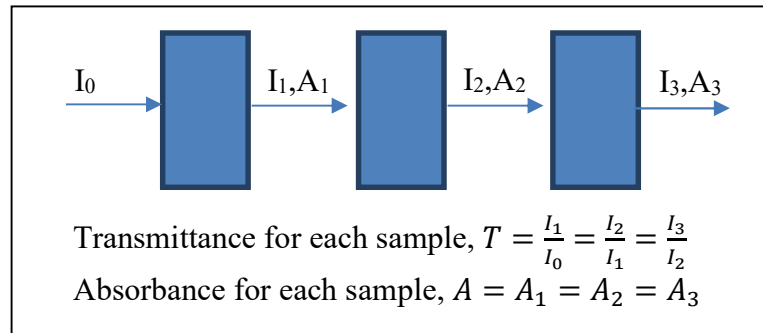
Figure 2.2 Beer-Lambert Law.

2.4 Multicomponent Analysis

Multicomponent analysis is a common analyze technique that used for UV-VIS spectroscopy. With this technique, concentration for the mixture of sample can be calculated. There are other statistical method such as principle component analysis (PCA) and multiple least squares (MLS) which offer some advantages over this technique but the calibration process will be much more complicated (da Silva Soares, 2013). This technique can be performed if the simple linear regression equation for each samples is correct. The result accuracy will be improved with the

increasing number of equation for each samples. However, the result might deviate if there is an unknown sample which can interfere the related spectrum.

In this study, classical least squares (CLS) calibration method was used to determine the concentration of copper and lead ions with its total spectrum of the mixture. The mathematical procedure is shown as below. According to Beer's Law, the total absorbance is the sum of absorbance from each element in the solution.

$$A = A_{\rho 1} + A_{\rho 2} + A_{\rho 3} + \cdots + A_{\rho n}$$

$$A_{\lambda 1} = \varepsilon_{\lambda 1,\rho 1}\rho_1 + \varepsilon_{\lambda 1,\rho 2}\rho_2 + \varepsilon_{\lambda 1,\rho 3}\rho_3 + \cdots + \varepsilon_{\lambda 1,\rho n}\rho_n$$

$$A_{\lambda 2} = \varepsilon_{\lambda 2,\rho 1}\rho_1 + \varepsilon_{\lambda 2,\rho 2}\rho_2 + \varepsilon_{\lambda 2,\rho 3}\rho_3 + \cdots + \varepsilon_{\lambda 2,Cn}\rho_n$$

$$\vdots$$

$$A_{\lambda N} = \lambda_{\lambda N,\rho 1}\rho_1 + \varepsilon_{\lambda N,\rho 2}\rho_2 + \varepsilon_{\lambda N,\rho 3}\rho_3 + \cdots + \varepsilon_{\lambda N,\rho n}\rho_n$$

By combining all the equations above, this set of equation is then written in matrix form and can be expressed in Equation 2.5, where A is the absorbance value on wavelength N, ρ is the concentration of species n and ε is the extinction coefficient for specific wavelength, λ.

$$\begin{bmatrix} A_1 \\ A_2 \\ . \\ . \\ . \\ A_N \end{bmatrix} = \begin{bmatrix} \varepsilon_{\lambda 1,\rho 1} & \varepsilon_{\lambda 2,\rho 1} & \cdots & \varepsilon_{\lambda N,\rho 1} \\ \varepsilon_{\lambda 1,\rho 2} & \varepsilon_{\lambda 2,\rho 2} & \cdots & \varepsilon_{\lambda N,\rho 2} \\ . & . & \cdots & . \\ . & . & \cdots & . \\ . & . & \cdots & . \\ \varepsilon_{\lambda 1,\rho n} & \varepsilon_{\lambda 2,\rho n} & \cdots & \varepsilon_{\lambda N,\rho n} \end{bmatrix} \begin{bmatrix} \rho_1 \\ \rho_2 \\ . \\ . \\ . \\ \rho_n \end{bmatrix} \quad (2.5)$$

$$\mathbf{A} = \boldsymbol{\varepsilon}\,\rho \quad (2.6)$$

From Equation 2.6, **A** is the a N-length matrix with the total measured signal, $\mathcal{E}$ is the N x n matrix with collection of extinction coefficient for chosen wavelength and ρ is the n-length matrix with concentration of sample n. The concentration, ρ can be easily calculated by multiplying both side with inverse matrix of $\mathcal{E}$ which would obtain the equation $\rho = \mathcal{E}^{-1}\mathbf{A}$. However, this only can apply when number of wavelength is equal to number of sample due to the fact that inverse matrix only exist for square matrix.

In real experiment, most of the spectra obtain would subjected to random noise and its accuracy can be improve by increasing the number of chosen wavelength. Besides that, number of chosen wavelength are not always equal to number of sample therefore it cannot be solved using inverse matrix method. To solve this problems, multiplying both side of the equation by $(\mathcal{E}^{T}\mathcal{E})^{-1}\mathcal{E}^{T}$ where $\mathcal{E}^{T}$ is the transformation matrix of $\mathcal{E}$ and equation below is obtained:

$$(\mathcal{E}^{T}\mathcal{E})^{-1}\mathcal{E}^{T}\mathbf{A} = (\mathcal{E}^{T}\mathcal{E})^{-1}\mathcal{E}^{T}\mathcal{E}\,\rho = (\mathcal{E}^{T}\mathcal{E})^{-1}(\mathcal{E}^{T}\mathcal{E})\,\rho$$

Since the $(\mathcal{E}^{T}\mathcal{E})^{-1}(\mathcal{E}^{T}\mathcal{E})$ is a unit matrix and $\mathcal{E}^{T}\mathcal{E}$ is a n x n square matrix, thus concentration of sample can be estimated using Equation 2.7.

$$\rho = (\mathcal{E}^{T}\mathcal{E})^{-1}\mathcal{E}^{T}\mathbf{A} \tag{2.7}$$

2.5 Recent Work on Spectroscopy and Heavy Metal Detection Method

Normally, analysis of heavy metal in environment is done through ion chromatography (IC), inductively coupled plasma atomic emission spectrometry

(ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS). Ion chromatography is the combination of chromatographic technique ions separation and a detection technique to perform qualitative and quantitative analysis on the ions. The most common ions separation technique is anion exchange and cation exchange. The most common detection technique for ionic near neutrality usually done using conductivity. However, amperometric, optical, ICP, or mass spectrometric methods may be preferred in some cases (Statler, 1997). Ion chromatography method was used by Michalski (2003) for the determination of trace levels of chromium(VI) in water and was able to determine the Cr(VI) on µg/L level in different water sample. In year 2011, Srijaranai used 1-(2-pyridylazo)-2-naphthol (PAN) as the post column reagent for ion exchange chromatography to determine the heavy metals in environmental samples.

AAS/AES is basically based on theory of energy level of the atoms. When the valence electrons of an atom is being excited and moved up energy level due to irradiation, it will create an absorption spectrum. The excited electron will then goes back to ground state from excited state by emission of light. The light was usually in visible or ultraviolet spectrum. Since the energy level is quantized and only certain wavelength is permitted for the excitation, each atom will have its own unique spectrum. Moreover, by increasing the population of atoms, absorption and emission will be increased correspond to it. Thus, both the atomic absorption and emission spectrum is then can be used to analyze and calculate sample's concentration. AAS/AES mostly comprise of atomizer which responsible to atomize the sample, radiation source that will irradiates the atomized sample and spectrophotometer

which required to detect the absorption and emission spectrum (Rutledge and Barron, 2011).

AAS was widely used by researchers to determine heavy metal in various way. Kojuncu *et al* (2004) used AAS for the Cd, Cu, Fe, Ni, Pb, Zn, and Ti traces in seawater with flotation separation technique. In the same paper, Kojunch's group also used ICP-AES for the result comparison and the recovery percentage was ranged from 90.8 % to 108.2 %. AAS was widely used for case study of heavy metal in water by researchers. Heavy metal in tap water was analyzed using AAS for a case study in Bandar Sunway residential area by Nalatambi (2009) and another case study about the heavy metal in ground water was done by Momodu and Anyakora (2009) at Lagos. In addition, AAS also used on many case study on heavy metal in environment such as mechanic workshops and aquatic environment (Hamed, 2004; Abii, 2012).

ICP-AES is based on same theory as AES except that this utilizes a plasma as the atomization and excitation source. Plasma is an electrically neutral but highly ionized gas that consists of ions, electrons, and atoms which all bonds are broken. In addition, the plasma commonly operate with argon or helium gas which make it impossible to combust. ICP-AES was able to detect concentration of numerous metal of a solution in ppb depending on types of element (Manning and Grow, 1997). ICP-AES was used for simultaneous determination of K, Na, Ca and Mg in Daqing oil field water by Yan in year 2011.

ICP-MS too atomized the sample into plasma form. Once the ions of the sample is produced in plasma, they will then transported to mass spectrophotometer via interface region. After that, the ions will then directed into a vacuum chamber by a series of electrostatic lenses called ions optic. Ions optic is responsible to focus the ions beam of the sample into mass separation device and finally the ions will arrived at the ions detector for data collection. The element are identified by their mass to charge ratio (m/e) and the intensity of peak is directly proportional to the amount of the corresponding elements in sample (Ammann, 2007; Batsala $et\ al$, 2012). At Transylvania, Voica $et\ al$ (2011) carried out a study about the surface water at Cluj and Salaj district by using ICP-MS which allow him to detect heavy metal in the level of μg/L. In year 2013, ICP-MS was used for assessment of surface water in Subarnarekha River, India. ICP-MS was used to determine the concentration of heavy metal for seasonal fluctuation, source apportionment and heavy metal pollution indexing (Giri and Singh, 2013). The detection limit of ICP-MS, ICP-AES and AAS technique is roughly shown in Figure 2.3.
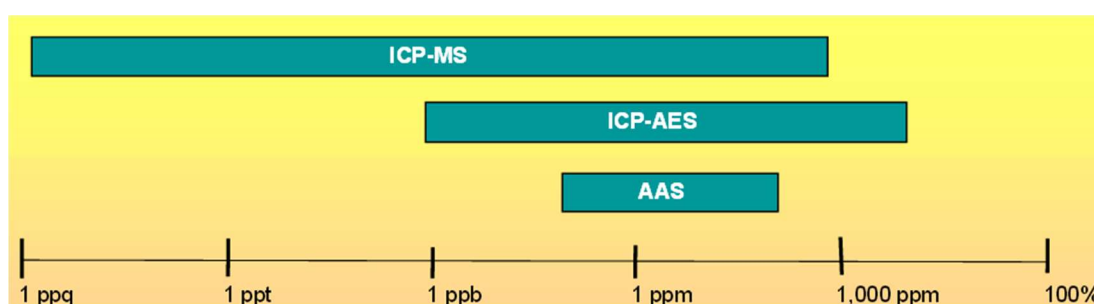
(Source: Gennaro)

Figure 2.3 Detection limit of ICP-MS, ICP-AES, and AAS.

Besides equipment like AAS, ICP-AES, ICP-MS and XRF, colorimetric reagent such as 4-(2-Pyridylazo)resorcinol (PAR) is used for heavy metal detection.

Initially PAR solution is orange in color and it will react with different heavy metal ions to produce different color of solution. However, it is a non-selective reagent. PAR is able to form complex with Cu, Pb, Zn, Ni, Co, and Cd which will have peak absorbance near 500 nm region (Ren and Gao, O'Toole and Diamond, 2008). According to Engström et al (1998), PAR was used for assessment of a screening method for metals in seawater. The heavy metal concentration is ranged from 2 μmol^{-1} to 20 μmol^{-1} with up to four heavy metal elements (Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}). The root mean square error (RMSE) of the research is from 0.3 $\mu mol-1$ to 0.9 $\mu mol-1$ depending on pH value and types of heavy metal ions. With principle component analysis (PCA), the researcher was able to predict weather the sample is polluted by metals, Ca^{2+} and Mg^{2+} even with Cl^-, Ca^{2+} and Mg^{2+} as the interference ions. In year 2003, determination of total metal pollutant in water is carried out using a special flow cuvette with spectra measurement by diode array spectrophotometer and CCD detector (Chudy et al, 2003). Besides that, there is another researcher used PAR method for colorimetric determination of copper in pure form (Mir, 2011). Mir was able to obtain linearity from 0.05 µg to 1 µg of copper with peak absorption at 450 nm.

Besides than using the PAR reagent directly to the sample, there are others that used the immobilized PAR for heavy metal detection. In year 2010, Li et al used PAR immobilized colorimetric fiber for heavy metal ion detection and absorption. The color colorimetric fiber will change into orange-red to dark-brown in the presence of heavy metal ions (Hg^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Cu^{2+}) and these fiber are capable of detecting the 1 µmol/L concentration of heavy metal. However, quantitative analysis using this method seems impossible. In year 2011, same group

of researcher have presented a new improved method by immobilizing PAR on the functionalized hexagonal mesoporous silica (HMS) via N-trimethoxysilylpropyl-N,N,N-trimethylammonium chloride (TMAC) (Liu *et al*, 2011). This solid state sensor was able to change color from yellow to red in respond to heavy metal ions and it was able to detect low concentration Cu^{2+} as low as 40 ppb at pH 12. In addition, at pH 2, the sensor can selectively respond to Cu^{2+} only.

Another chemical reagent, 1-(2-pyridylazo)-2-naphthol (PAN) which will also form complex with metal ions and able to study through UV/VIS spectroscopy, Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy (Safari *et al*, 2011; Szabó *et al*, 2012). Complex of PAN with metal ions will show peak absorbance near 500 nm which is similar to PAR reagent (Vuković *et al*, 2012).

One of the recent trends in heavy metal is electrochemical method. Among the different electrochemical techniques voltammetric and potentiometric techniques are the most reported for heavy metals detection (Bakker and Qin, 2006; Privett *et al*, 2008). Voltammetry involves the perturbation of the initial zero-current condition of an electrochemical cell by applying either time-constant or time-varying potential (ramp) to an electrode surface and the further measurement of the resulting current. Among the different known voltammetric techniques, stripping techniques (i.e. anodic stripping voltammetry, ASV) are the most used for trace heavy metal detection due to the high selectivity and sensitivity they present coming from the combination of the separation, pre-concentration and determination steps in one single process (Wang, 2008).

In potentiometry, information on the sample composition is obtained through the potential change/s appearing and indicated at the two electrodes. Two main devices fall within the category of potentiometric sensors: ion selective electrodes (ISEs), the chemical sensors with longest history, and field-effect transistors (FETs). They combine the fundamental membrane science with fundamental host–guest chemistry. Several ISEs have been developed for the detection of ultra-low activities of heavy metal ions (Bobacka *et al*, 2008).

UV/VIS spectroscopy was also widely used as a tools to study and determination of ions in aqueous solution. UV/VIS Spectroscopy is about the analysis of interaction between sample and radiated energy described by Beer-Lambert Law. Most of the time, spectroscopy method encounter overlapping problems from different element's spectra. To overcome this problems, spectroscopic multicomponent analysis is introduced. In this technique, different wavelength of light will be radiate through the sample so that the concentration of the sample can be calculated through multiple regression model (da Silva Soares *et al*, 2013).

In year 2010, Alula *et al* (2010) used VIS spectroscopy with chemometric method for determination of iron (II) and copper (II) ions in tablets. In the research, they used 8-hydroxyquinoline as the chemometric reagent with 10 mg of iron and 1 mg of copper tablet were used for the test. Hydrochloric acid, ethanol and demineralized water were used as solvent. The absorption spectra was then recorded within wavelength 350 nm to 600 nm and spectra were digitized each 1.0 nm. Furthermore, the analysis method used by this paper was classical least squares with first derivative spectra.

Transmittance of heavy metal ions in aqueous solution was studied by another researcher, Chávez *et al* (2011). The heavy metal ions tested by this researcher are cadmium, zinc, lead, copper, and manganese with concentration from 100 ppm to 10000 ppm. The transmittance test was performed using UV spectroscopy with wavelength 200 nm to 400 nm. The test however did not differentiate types of ions in the solution. In the paper, they observed that heavy metal are able to be detected using UV light but the spectra are almost with similar pattern. There are other methods such as dispersive liquid-liquid microextraction with combination of UV/VIS spectrophotometry which is introduced recently for determination of cadmium and copper in rice, food and water (Wen *et al*, 2011a; Wen *et al*, 2011b; Wen *et al*, 2011c).

Finally, spectroscopy method not only useful on determine the heavy metal ions, but it can be used to do qualitative and quantitative analysis on other ions in water such as nitrate ions, and nitrites ions (Krishnan and Guha, 1934; Gvozdić *et al*, 2009). Besides that, spectroscopy on sucrose, glucose, fructose, acidity and even fruits quality has been carried out by a group of researcher (Omar *et al*, 2011; Omar *et al*, 2012a; Omar *et al*, 2012b; Omar, 2013).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter describes the research design, sample preparation and preliminary experiment used to study the spectrum of both copper and lead ions by UV Spectroscopy. The data, equation obtained and further data analysis will be included in next chapter.

3.2 Research Design

This research is mainly focus on spectroscopy on Pb^{2+}, Cu^{2+} and Ni^{2+} ions in aqueous solution. Sample solution consists of heavy metal ions (Al^{3+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+}) is prepared and tested using UV-VIS spectroscopy. In preliminary study, absorbance wavelength of the sample is studied and further investigation is carried out. Using the gathered data, regression equation for the corresponding absorbance wavelength is generated using Minitab 16. Besides that, the regression equations is then validated through three different test.

In the final stage of this study, multicomponent analysis is used to calculate the concentration of ions in mixture solution and tap water. Besides that, C# program which included all linear regression equation and multicomponent analysis equation is created to help the calculation of ions concentration. Figure 3.1 shows the overall research flow in this study.

```
                    ┌─────────────────────────────┐
                    │      Sample preparation     │
                    └─────────────────────────────┘
                                  │
                                  ▼
              ┌───────────────────────────────────────┐
      ┌──────▶│   Determine the effective absorption   │
      │        │               wavelength               │
      │        └───────────────────────────────────────┘
      │                          │
      │                          ▼
      │        ┌───────────────────────────────────────┐
      │        │      Construct regression model        │
Failed│        │   equation using 50 samples of         │
      │        │         Pb(II), Cu(II) and Ni(II)      │
      │        └───────────────────────────────────────┘
      │                          │
      │                          ▼
      │   ┌──────────────┬──────────────┬──────────────┐
      │   │Validation test│Validation test│Validation test│
      └───┤using 50 samples│using 50 samples│through the effect│
          │from different │contain differ │of interference│
          └──────────────┴──────────────┴──────────────┘
                                  │ Passed
                                  ▼
      ┌──────▶┌───────────────────────────────────────┐
      │        │        Multicomponent Analysis         │
      │        └───────────────────────────────────────┘
      │                          │
      │                          ▼
      │        ┌───────────────────────────────────────┐
Failed│        │   Compiling all regression model and   │
      │        │    multicomponent analysis into C#     │
      │        └───────────────────────────────────────┘
      │                          │
      │                          ▼
      │        ┌───────────────────────────────────────┐
      └────────┤       Validation test for the          │
               │       multicomponent analysis          │
               └───────────────────────────────────────┘
                                  │ Passed
                                  ▼
               ┌───────────────────────────────────────┐
               │     Test on Real Sample (Tap Water)    │
               └───────────────────────────────────────┘
```

Figure 3.1 Research work flow.

3.3 Sample Preparation

All test samples was prepared using corresponding cation salt with 1L of distilled water and was then diluted into required number of samples. In this study, Pb^{2+} solution was prepared using lead (II) chloride ($PbCl_2$) and lead (II) acetate ($Pb(COOCH_3)_2$). Cu^{2+} and Ni^{2+} solution was prepared using its chloride and sulfate salt which is copper (II) chloride ($CuCl_2$), copper (II) sulfate ($CuSO_4$), nickel (II)

chloride ($NiCl_2$) and nickel (II) sulfate ($NiSO_4$). Besides that, other heavy metal ions (Al^{3+}, Ca^{2+}, Mg^{2+}, and Zn^{2+}) was prepared using corresponding chloride salt. In addition, every sample was 10 ml volume and was prepared using micropipette and the salt was weighted using AB204 analytical balance. The reason all these heavy metal is selected is because they are commonly exist in water, it has crucial health effect on human.

The prepared Pb^{2+}, Cu^{2+} and Ni^{2+} solution is in different color by default. The Cu^{2+} solution is blue in color, Ni^{2+} solution is light green in color and Pb^{2+} is completely colorless. The solubility of each chosen salt also taking into account in this research. The solubility of each chosen salt is shown in Table 3.1 below, and effect of ph is neglected since distilled water is used as base solvent in this research.

Table 3.1 Solubility table for selected salt in this research

Salt	Solubility in water at 20 ºC (g/100g of water)	Color of solution
$PbCl_2$	1.08	Colorless
$Pb(COOCH_3)_2$	44.3	Colorless
$CuCl_2$	73	Blue
$CuSO_4$	32	Blue
$NiCl_2$	66.8	Light Green
$NiSO_4$	44.4	Light Green

(Source: Wikipedia, 2015)

In addition, tap water from Universiti Sains Malaysia (USM) and Island Plaza was collected and used as real sample for this study. There are at least 50 test samples prepared for every test measurement carried out in this research. All samples will be tested using UV/VIS spectroscopy immediately after sample preparation. Concentration of the sample are depending on type of measurement and method used. Further detail about sample concentration will be described in chapter 4.

3.4 Experiment Setup

Experiment of this research uses JAZ spectrophotometer, light source, fiber optic, cuvette holder and a quartz cuvette. Quartz cuvette is chosen due to its low UV/VIS absorption properties which very suitable for UV/VIS spectroscopy. Software used for the experiment and data processing are Ocean Optics SpectraSuite, Minitab 16, and Microsoft Visual Studio 2012. Spectroscopy method was chosen due to its result can be reproduced easily with correct regression equation. The entire experiment is carried out at air-conditioned dark room with fixed temperature.

3.4.1 Spectroscopy

The spectroscopy system used include a CCD array spectrophotometer where each pixel on the CCD array will respond to the wavelengths of light that strikes on it. Table 3.1 shows the specification of the JAZ spectrophotometer and Figure 3.2 shows the top view of JAZ spectrophotometer.

Figure 3.2 Control panel of JAZ Spectrophotometer.

Table 3.2 Specification of JAZ Spectrophotometer.

Specification	JAZ Spectrophotometer
Detector	Sony ILX511 linear silicon CCD array
Detector range	200 nm – 1100 nm Channel 0: 200 nm - 850 nm Channel 1: 650 nm - 1100 nm
Gratings	Grating 1: efficient wavelength at 200 nm - 527 nm Grating 14: efficient wavelength at 650 nm - 1100 nm
Slits	25 μm
Pixels	2048
Pixel Size	14 μm x 200 μm
Pixel well depth	~62500 electrons
Optical resolution	~0.3 nm - 10.0 nm (FWHM)

(Source: OceanOptics, 2008)

Before any measurement, JAZ spectrophotometer is configured via Ocean Optics SpectraSuite. The important configurations of it are integration time, scan to average, boxcar width. Figure 3.3 shows the sample of measurement. Integration time is referring to the time taken for detector to collect or accumulate the signal from the incoming photon. With larger integration time, the overall signal can be amplified and peak for the spectrum can be more significant. However, higher integration time will further amplify the noise signal as well. To lower the signal to noise ratio of the measurement, scan to average function will be required. The scan to average function is meant to calculate the average of accumulated measurement after specified number of scans. Therefore, higher the scan to average will improve signal to noise ratio. Boxcar width function is a technique to produces a smoother spectrum by averaging the adjacent data point. The signal to noise ratio can be further improved by using higher boxcar value. However, higher the boxcar width

value will produce a smoother spectrum but the resolution of overall spectrum will dropped.
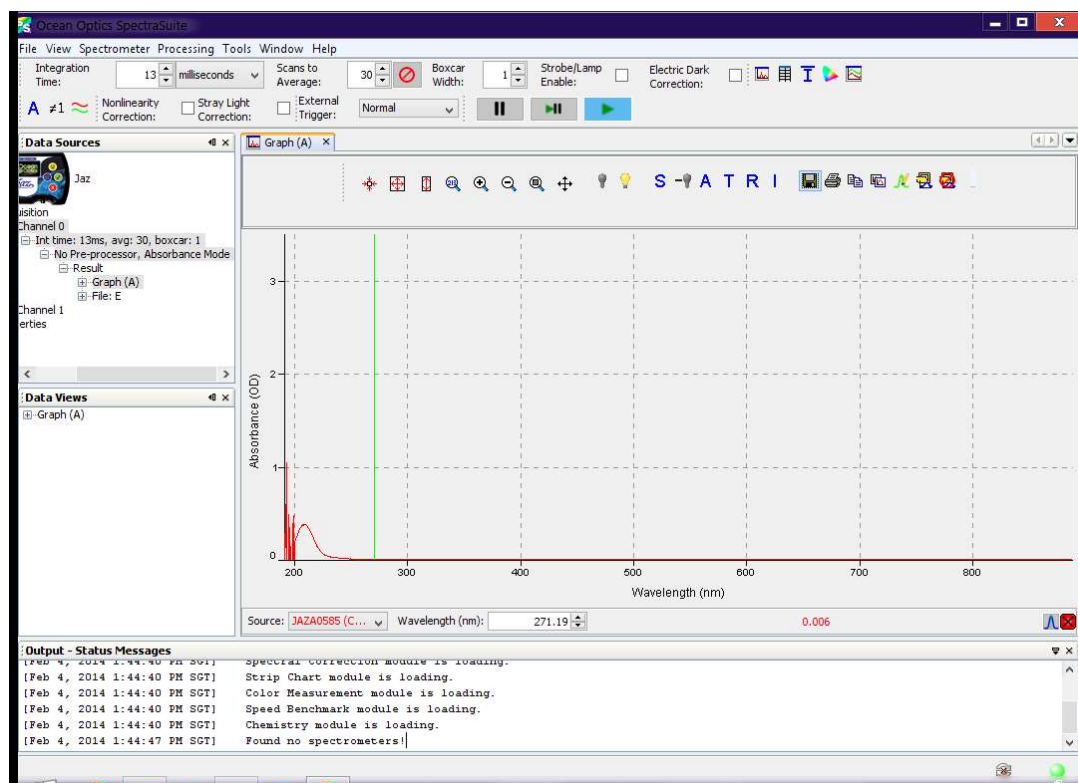

Figure 3.3 Screenshot of OceanOptic SpectraSuite.

Throughout the whole experiment, integration time was set to 13 ms, with value of 30 for scans to average and boxcar width value 1. Integration time was chosen based on maximum counts that can be received by the system without exceed saturation limit. The counts are the raw data produced by spectrophotometer and later can be further convert to transmittance, absorbance and reflectance using SpectraSuite software.