ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Graduate Project (Chem.774)

Voltammetric Determination of Stability Constants of Lead Complexes with Vitamin C

By

Tibeb Melesse

Advisor: Prof. Theodros Solomon

In Partial Fulfillment of the Requirements for Master of Science Degree in Chemistry

---

JULY 2008

July 17, 2008

To: Dr. Ashebir Fiseha
Head, Department of Chemistry

Subject: **Tibeb Melesse's Final M. Sc. Project**

This is to confirm that Tibeb Melesse has incorporated the comments of the examining board in the final version of his M. Sc. Project.

Sincerely Yours,

Prof. Theodros Solomon

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY

Graduate Project (Chem.774)

Voltammetric Determination of Stability Constants of Lead Complexes with Vitamin C

Submitted by	**Signature**
Tibeb Melesse	_____

Approved by the Examining Board:	**Signature**
Prof. Theodros Solomon Advisor	_____
Dr. Mesfin Redi Examiner	_____
Dr. Shimelis Admassie Examiner	_____
Dr. Ahmed Mustefa Examiner	_____

Declaration

I the Undersigned confirm that the results reported in this work were obtained by research carried out by me under the supervision of my Advisor in the Faculty of Science, Department of Chemistry, Addis Ababa University in the academic year 2007 - 2008.

Name: _____ Signature: _____

This project work has been submitted for examination with my approval as university advisor.

Advisor

Prof. Theodros Solomon

_____

Place and date of submission: School of Graduate Studies
Addis Ababa University

Acknowledgement

I would like to express my sincere feelings of gratitude and appreciation to my advisor Prof. Theodros Solomon for their day to day follow up, valuable suggestions, advice, encouragement and ultimate help in the project work.

My special heartfelt appreciation and gratitude is to my wife Atsede Tesfa for she has shouldered the whole family responsibility and encouraging and assisting me to complete two year graduated programs. My mother and my brothers especially Esubalew Melesse and Yeshiwas Abitae are appreciated for their unchanging continuous support.

I am also grateful to acknowledge my friends Shibru Abadima, Fantaye Godifaye and Temesgen Worku and all staff members of Assendabo Senior Secondary School for giving me moral and encouragement to complete this study and Omo Nada Woreada of Jimma Zone also acknowledge for giving me the opportunity to join this program.

I am thankful to all members of chemistry department staffs, AAU, and all chemistry post graduated students for countless support they provide me.

At last, I would like to say that thank God, who has helped me begin and complete, my study peacefully and successfully.

TABLE OF CONTENTS **PAGES**

List of Figures

List of Abbreviations and Symbols

SYMBOL	NAME
CV	Cyclic Voltammetry
SWV	Square-Wave Voltammetry
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPP	Differential Pulse Polarography
ASV-DC	Anoding Stripping Voltammetry of Direct Current
CSV	Cathodic Stripping Voltammetry
DCP	Direct Current Polarography
SCE	Saturated Calomel Electrode
Ag/AgCl	Silver/Silver Chloride
DME	Dropping Mercury Electrode
HMDE	Hanging Mercury Dropping Electrode
SMDE	Static Mercury Dropping Electrode
i_{pa}	Anodic peak current
i_{pc}	Cathodic peak current
E_{pa}	Anodic peak potential
E_{pc}	Cathodic potential peak
SE	Start Potential
EE	End Potential

UK	United Kingdom
EC	European Community
WHO	World Healthy Organization
ROS	Reactive Oxygen Species
RDA	Recommended Dietary Allowance
DOM	Dissolved Organic Matter
HPLC	High Performance Liquid Chromatography
AAS	Atomic Absorption Spectrometry
AES	Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
XRF	X-ray Fluorescence
TLS	Thermal Lens Spectrometry
PC	Acer Computer
BAS	Bioanalytical Systems
CB	Calcein-blue (8-[*N*, *N*-bis (carboxyl-methyl) aminomethyl]-4-methylumbelliferone)
DEDC	Diethanoldithiocarbamate
TEL	Tetraethyl Lead
TAPSO	3-[N-Trios (hydroxymethyl) methylamine]-2- hydroxypropanesulfonic acid)

Abstract

The electrochemical behaviour of the complex between L-ascorbic acid (AA) and Pb (II) was studied by using Square-wave voltammetry (SWV) and Cyclic voltammetry (CV). L-ascorbic acid forms a 1:1 complex with Pb (II) in 0.1 M sodium perchlorate at pH 5.0. It was found that the reduction processes of Pb (II)-ascorbic acid complex was irreversible. The stability constant of the Pb (II) - ascorbate complexes were evaluated with the De Ford-Hume procedure at different ligand concentrations using square wave voltammetry (SWV). The logarithm values of stability constant of 1:1 Pb (II)-ascorbic acid complex was found to be 0.908.

Key words: Pb (II)-ascorbic acid, De Ford-Hume, Square-wave voltammetry (SWV), cyclic voltammetry (CV), stability constant.

1. INTRODUCTION

1.1. Heavy metals

Minerals containing heavy metallic elements occur widely in rocks and soils. When they do occur, cations of the heavy metal are liberated and find their ways in to surface water. Mercury, cadmium, arsenic, lead, copper and zinc are heavy metals that have been extensively mined and whose environmental levels have been strongly influenced by man. All are rather toxic to living organisms and may be regarded as pollutants [1].Trace quantities of the salts of some heavy elements are essential to human existence. Yet a number of them are also particularly hazardous and toxic to the human body such that ingestion of minute quantities is responsible for brain damage and death [2].

The potential toxicity, reactivity, transport, bioavailability and bioaccumulation of various heavy metals are controlled to a very large extent by their physico-chemical forms. Hence, speciation studies i.e. the identification and quantification of different physico-chemical forms of a metal are important in understanding the role trace metals play in natural environmental systems or environmental health. The data from speciation studies has a wide application in pollution control, material transportation, metal availability and toxicity in water, wastewater treatment and also serves as a basis of setting water quality standards.

1.1.1. Historical background of Lead

Lead was one of the first metals known to man. Probably the oldest lead artifact is a figure made about 3000 BC. All civilizations, beginning with the ancient Egyptians, Assyrians, and Babylonians, have used lead for many ornamental and structural purposes. Many magnificent buildings erected in the 15th and 16th centuries still stand under their original lead roofs.

Lead was first used to produce water pipes in Roman times, and until the 1950s was still used extensively in the United Kingdom (UK). Since then lead pipes have been largely replaced by copper ones, which are easier to fabricate and to bend; and, more recently, in cold-water

systems by plastics ones, plastic being cheaper than copper. In hard-water areas, in which the pH of the water does not drop below 7, lead pipes are quite stable. A layer of lead, calcium and magnesium carbonates is rapidly built up and acts as a protective coating against dissolution of the lead. In soft-water areas, in which the pH of the water may be below 5, the lead is relatively soluble: water that has been in contact with the lead for a period of time may contain over 1 mg Pb dm^{-3}. The European Community (EC) limit for lead in drinking water is 50 μg dm^{-3}, but the World Healthy Organization (WHO) is now recommending that the limit should be reduced to 10 μg dm^{-3} [3].

Lead is especially prone to accumulation in surface horizons of soil because its low water solubility within an environmentally relevant pH range results in very low mobility. Soil water contains only about 0.05-0.13% of the total soil lead concentration. Lead speciation is rather simple and Pb^{2+} is the dominant soluble form. It forms a number of highly insoluble precipitates including $Pb(OH)_2$, $Pb_3(PO_4)_2$, and $PbCO_3$ as well as to the formation of lead-organic complex. Plant uptake factors for lead are low (0.01-0.1) due to very low water solubility. Due to past uses of lead in industrial processes and consumer products (e.g., paint, gasoline), urban soils often contain high lead concentrations, up to 1840 mg/kg. Neurological problems, especially in children, are the principal concern for chronic lead exposure. Past uses of lead solder in food and beverage cans lead to significant human exposures. Consumption of lead contaminated soil itself, rather than crop contamination, is a more likely exposure hazard [3, 4].

Lead occurs naturally in the earth's crust, in ores such as galena, lead (II) sulfide (PbS). However, human activity has resulted in atmospheric lead, mainly as $PbSO_4$ and $PbCO_3$. Lead is generally resistant to corrosion, but will dissolve in low pH acid water. Beside such weak solutions, a significant fraction may be present in an undissolved form, colloidal particles, or larger particles of lead (II) carbonate, lead (II) oxide, and lead (II) hydroxide. Lead may be

leached out, or washed out in suspension from pipes or from soil after heavy rains or flooding especially in acid conditions. Hence, lead contaminated waters may be found near foundries producing metal alloys containing lead, such as brass and bronze; and also near petroleum refineries, where leaded gasoline is produced. Tetraethyl lead (TEL), an organic alkyl compound is used to increase the gasoline grade, measured in Octane number and thus prevents knocking in petrol engines [5].

1.1.2. Physical properties of Lead

Lead is a heavy, soft, bluish-grey metallic solid. It has no special taste or smell, but when it burned imparts a metallic, sweetish taste. It is both ductile and malleable. Ductile means capable of being drawn into thin wires. Malleable means capable of being hammered into thin sheets. It has a shiny surface when first cut, but it slowly tarnishes (rusts) and becomes dull. Lead is easily worked. Working a metal means bending, cutting, shaping, pulling, and otherwise changing the shape of the metal. The melting point of lead is 327.4°C (621.3°F), and its boiling point is 1,740°C (3,164 °F). Its density is 11.34 grams per cubic centimeter. Lead does not conduct an electric current, sound, or vibrations very well [6].

1.1.3. Chemical properties of Lead

Lead will not dissolve in pure water; however, it may react with impurities in water to produce soluble lead compounds. Lead is soluble in nitric acid and slightly soluble in sulfuric acid. Lead is a moderately active metal. It reacts more rapidly with hot acids. It does not react with oxygen in the air readily and does not burn [6].

1.1.4. Complex formation of Lead

Complex compounds are compounds in which the central atom or ions (generally metals) are attached to more atoms or groups of atoms they would be expected from the charge on the central atom or ion. The atom surrounding the central atom or ion is called ligand. Some ligands contain more than one atom that can be attached to the central atom or ion and these tends to form strong complexes called chelates.

In lead-contaminated soils, biota and vegetation influence the transformations of lead together with environmental characteristics such as soil pH, organic matter content, texture, redox-potential and presence of other elements. R. Turpeinen (2002) found that lead complexed with dissolved organic matter may migrate from the surface soil layer to mineral soil, thus raising the concern of lead contaminating the groundwater [3, 7].

There are four general classes of lead interactions: specific adsorption, co-precipitation, cation exchange, and organic-complexation. Specific adsorption involves partly covalent bonds of the lead metal with lattice ions on soil particle surfaces. Co-precipitation involves formation of water insoluble precipitates from lead ions (cations) and anions such as carbonate, sulfide, or phosphate. Cation exchange is non-specific interaction of lead with negative surface charges on soils minerals, such as clay. Finally, soil organic matter (e.g., humus) adsorbs lead by forming chelate complexes, with carboxyl groups playing a predominant role. Given the chemical basis for each of the four general classes of lead absorption, it is clear that soil type is a fundamental determinant of lead metal transport and fate [4].

1.1.5. Toxic effect of Lead

The toxicity of lead is a consequence of the ability of Pb^{2+} to interfere with several enzymes. Because lead causes a large variety of toxic effects, including gastrointestinal, muscular, reproductive, neurological and behavioral and genetic malfunctions, the fate of lead in the environment is of great concern [7]. It has also a number of well-defined toxic effects upon human being including the production of anemia, disturbances of hemoglobin synthesis, hypertension, and damage to the nervous systems and kidneys [1, 4].

The biochemical and molecular mechanisms of lead toxicity are poorly understood, but emerging data suggest that some of the effects of lead may be due to its interference with calcium in the activation of protein kinase C (PKC) and /or through production of reactive oxygen species (ROS). Also, it was reported that lead increased the level of lipid peroxidation [8, 9].

1.1.6. Uses of Lead

Metallic lead is used in the production of batteries, products used to shield X-rays and various metal products (pipes, solder, flashing, ammunition/bullets, fishing weights, electronics and alloys with other metals). A survey of lead compounds shows some of their uses are as follows. Tetraethyl lead is used to make other leaded compounds (tetra-alkyl leads) used in leaded fuels (petrol). Its compounds are used in the manufacture of plastics, rubbers, metals, matches, ammunition, fireworks and explosives. They are also used in pigments, dyes, paints and coatings, rodenticides, insecticides, pottery glazes, brake shoes, flame retardants for plastics, lead lighting, catalysts for industrial production and epoxy curing agents [7].

1.2. Vitamin C (L - Ascorbic Acid)

Vitamin C (also called L-Ascorbic acid) is a six-carbon lactone which is synthesized from glucose by many animals. It occurs as a white or slightly yellow crystal or powder with a slight acidic taste [10]. It can be easily destroyed under the exposure to oxygen, metals, light and heat. It is freely soluble in water; sparingly soluble in alcohol; insoluble in chloroform, ether, and benzene. Albert Szent-Gyorgyi was awarded the 1937 Nobel Prize in Medicine for the discovery of vitamin C [11].

Vitamin C is a carbohydrate-like substance involved in the metabolic functions including synthesis of collagen, maintenance of the structural strength of the blood vessels, metabolism of certain amino acids, and the synthesis or release of hormones in the adrenal glands. It is also used as in the basis of connective tissue, which is found in skin, ligaments, cartilage, vertebral discs, joint linings, capillary walls, and the bones and teeth. Collagen, and thus vitamin C, is needed to give support and shape to the body, to help wounds heal, and to maintain healthy blood vessels. Specifically, ascorbic acid works as a coenzyme to convert proline and lysine to hydroxyproline and hydroxylysine, both important to the collagen structure [12, 13].

Vitamin C also aids the metabolism of tyrosine, folic acid, and tryptophan. Tryptophan is converted in the presence of ascorbic acid to 5-hydroxytryptophan, which forms serotonin, an important brain chemical. Vitamin C also helps folic acid convert to its active form, tetrahydrofolic acid, and tyrosine needs ascorbic acid to form the neurotransmitter substances dopamine and epinephrine. Vitamin C stimulates adrenal function and the release of norepinephrine and epinephrine (adrenaline), our stress hormones; however, prolonged stress depletes vitamin C in the adrenals and decreases the blood levels. Ascorbic acid also helps thyroid hormone production, and it aids in cholesterol metabolism, increasing its elimination and thereby assisting in lowering blood cholesterol [14].

Vitamin C is considered to be one of the most prevalent antioxidative components of fruit and vegetables, and it could exert chemo preventive effects without apparent toxicity at doses higher than the current recommended dietary allowance of 60 mg/d. It has also been used as a dietary supplement intended to prevent oxidative stress–mediated chronic diseases such as cancer, cardiovascular disease, hypertension, and neurodegenerative disorder [15].

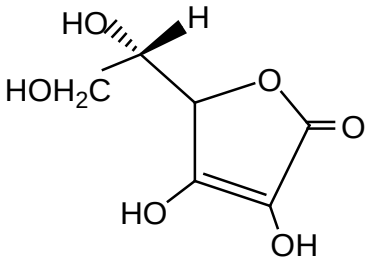
Vitamin C also indirectly protects the fat-soluble vitamins A and E as well as some of the B vitamins, such as riboflavin, thiamine, folic acid, and pantothenic acid, from oxidation. Ascorbic acid acts as a detoxifier and may reduce the side effects of drugs such as cortisone, aspirin, and insulin; it may also reduce the toxicity of the heavy metals lead, mercury, and arsenic [9, 16]. It also detoxifies carbon monoxide, sulfur dioxide, and carcinogens, so it is the only immediate protection we have against the bad effects of air pollution and smoking. It has also been shown that ascorbic acid increases the therapeutic effect of different drugs and medicines by making them more effective. Thus, less of a drug is required if it is taken in combination with large amounts of ascorbic acid. Diabetics could reduce their insulin requirements if this were practiced.

Vitamin C is the most commonly consumed nutrient supplement and is available in tablets, both fast-acting and time-released, in chewable tablets, in powders and effervescent, and in liquid form. It is available as L-ascorbic acid and various mineral ascorbate salts, such as sodium or calcium ascorbate.

Scurvy is an avitaminosis resulting from lack of vitamin C, since without this vitamin, the synthesized collagen is too unstable to perform its function. Scurvy leads to the formation of liver spots on the skin, spongy gums, and bleeding from all mucous membranes. An important note is that many medical problems have been found to be associated with low blood levels of vitamin C. These problems include various infections, colds, depression, high blood pressure, arthritis, vascular fragility, allergies, ulcers, and cholesterol gallstones. Most of these symptoms and problems can be easily avoided with minimal supplementation of vitamin C or a diet well supplied with fresh fruits and vegetables. Since the average diet has much less vitamin C than that of our ancestors, it is important for us to be aware of our ascorbic acid intake [11, 17].

The best-known sources of vitamin C are the citrus fruits—oranges, lemons, limes, tangerines, and grapefruits. The fruits with the highest natural concentrations are citrus fruits, rose hips, and acerola cherries, followed by papayas, cantaloupes, and strawberries. Good vegetable sources include red and green peppers, broccoli, Brussels sprouts, tomatoes, asparagus, parsley, dark leafy greens, cabbage, and sauerkraut. There is not much available in the whole grains, seeds, and beans; however, when these are sprouted, their vitamin C content shoots up. Animal foods contain almost no vitamin C; though fish, if eaten raw, has enough to prevent deficiency symptoms [11].

The Recommended Dietary Allowance (RDA) for adults is considered to be 60 mg. We need only about 10–20 mg to prevent scurvy, and there is more than that in one portion of most fruits or vegetables. Infants need 35mg; about 50mg between ages one and fourteen and 60 mg afterward are the suggested minimums. During pregnancy, 80 mg are required; 100 mg are needed during lactation. Realistically, between 100–150 mg daily is a minimum dosage for most people [15].



L-ascorbic acid (vitamin C)

Chemical formula $C_6H_8O_6$

Molecular weight 176.12

2. ELECTROANALYTICAL TECHNIQUES

Voltammetry is a versatile technique for research purposes. It allows the search into several aspects of electrochemical reactions, namely those reactions in which electrons are exchanged between reagents and products. For such reactions it is possible to investigate the dependence of current on the potential when an electrode is dipped into the reaction environment. Generally those laws are very complicated, just like the redox reactions and the environment in which they take place [18].

Voltammetry is an electroanalytical technique based on the measurement of current flowing through an electrode dipped in a solution containing electro-active compounds, while a potential is imposed upon it [19, 20]. Voltammetry is typically performed using a three electrode potentiostat, which accurately controls the applied potential. The redox reaction takes places at the working electrode, because the working electrode is where the reaction or transfer of interest is taking place. The reduction or oxidation of a substance at the surface of a working electrode, at the appropriate applied potential, results in the mass transport of new materials to the electrode surface and the generation of current. This electrode could be composed of several materials. Usually, it has a very little surface in order to assume quickly and accurately the potential imposed by the electrical circuit. The electrode can be solid (gold, platinum or glassy carbon) or formed by a drop of mercury hanging from a tip of a capillary. If the electrode is formed by a drop of mercury rhythmically dropping from a capillary, the analytical technique is called Polarography [18].The second electrode is a reference electrode, which maintains a constant potential throughout the experiments, and the third electrode is the counter electrode, which complete the electrical circuit. The counter electrode also known as the auxiliary electrode, is often much larger than the working electrode to minimized current density at the electrode surface [20].

The common characteristics of all voltammetric techniques is that they involve the application of a potential (E) to an electrode and the monitoring of the resulting current (i) flowing through

electrochemical cell [19].The potential, or independent variable is applied to the working electrode as a function of time (t) in order to evoke a response in the form of current (i) or the dependent variable, which is unique to the analyte of interest. The pattern in which the potential changes with time is denoted as the waveform.

Analytical, physical, inorganic and biological chemists widely use voltammetric techniques for a variety of purposes, including fundamental studies of oxidation and reduction processes in various media, adsorption processes on surfaces, electron transfer and reaction mechanisms, kinetics of electron transfer processes, and transport, speciation and thermodynamic properties of solvated species. Voltammetric methods are also applied to the determination of compounds of pharmaceutical interest and when coupled with high performance liquid chromatography (HPLC), they are effective tools for the analysis of complex mixtures.

The analytical advantages of the various voltammetric techniques include excellent sensitivity with a very large useful linear concentration range for both inorganic and organic species (10^{-12} to 10^{-1}), a large number of useful solvents and electrolytes, a wide range of temperatures, rapid analysis times (seconds), simultaneous determination of several analytes, the ability to determine kinetics and mechanistic parameters, a well developed theory and thus the ability to reasonably estimate the values of unknown parameters, and the ease with which different potential wave-forms can be generated and small current measured.

The use of the voltammetric techniques is the basis of the comprehension of the laws concerning several electrochemical phenomena and has a great importance in several technological fields, like: research of corrosion- proof materials (corrosion is a consequence of a series of electrochemical reactions), research of new electrodic processes for chemical industries (in fact, for example, million of tons of aluminum, chlorine, soda are produced by means of electrochemical reactions) and production of new type of batteries that can store rapidly great quantities of energy. It is also used as the quantitative analysis of trace of metals (or, anyway, of those reducible or oxidizable chemicals) at µg/L levels or less [18].

2.1. Cyclic Voltammetry (CV)

Cyclic voltammetry is the most effective and versatile electroanalytical technique for the study of electroactive species. Its versatility combined with ease of measurement has resulted in extensive use of cyclic voltammetry in the filed of electrochemistry, inorganic chemistry, organic chemistry and biochemistry. It is often the first experiments performed in an electrochemical study of an inorganic and an organic compound, a biological material, or an electrode surface. The effectiveness of cyclic voltammetry results from its capability for rapid observing redox behavior over a wide potential range [21, 22]. It is the most widely used techniques of all voltammetric methods by both electrochemists and non electrochemists alike. It allows the analyst to mechanistically study redox systems, especially the assignment and characterization of redox couples [20].

Cyclic voltammetry consists of cycling the potential of an electrode, which is immersed in an unstirred solution, and measuring the resulting current. The potential of the working electrode is controlled verses a reference electrode such as a saturated calomel electrode (SCE) ($Hg/Hg_2Cl_2/Cl^-$) or $Ag/AgCl/Cl^-$. The controlling potential that is applied across these two electrodes can be considered an excitation signal. The excitation signal for cyclic voltammetry is a linear potential scan with a triangular wave-form as shown in Fig.1 (a). This triangular potential excitation signals sweeps the potential of the electrode between two values, sometimes called the switching potentials [21, 22].

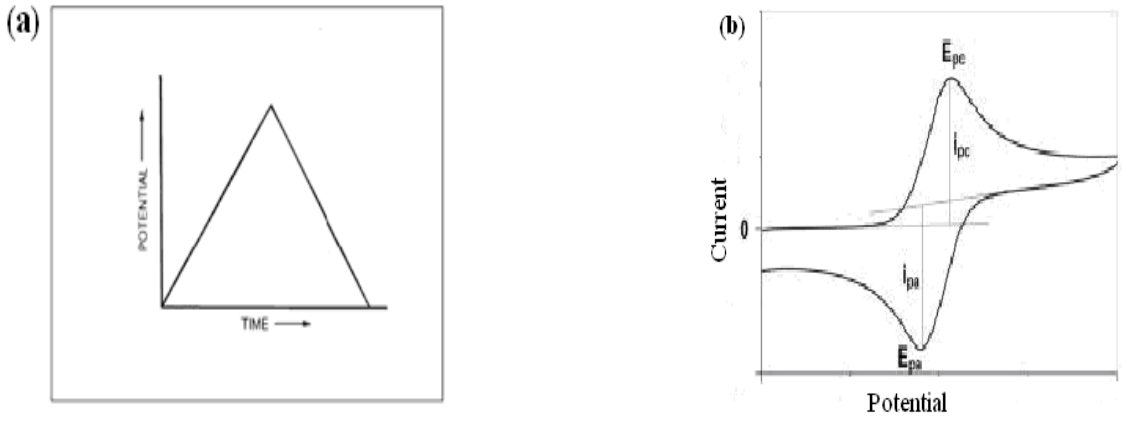


Fig.1**. (a)** Excitation waveform of cyclic voltammetry and (**b)** response obtained for the reversible cyclic voltammetry.

A cyclic voltammogram is obtained by measuring the current at the working electrode during the potential scan. The current can be considered as the response signal to the potential excitation signal. The voltammogram is a display of current (vertical axis) verses potential (horizontal axis). Because the potential varies linearly with time, the horizontal axis can also be thought of as a time axis [19-24].

Cyclic voltammetry has become an important and widely used electroanalytical technique in many area of chemistry. It is rarely use for quantitative determinations, but it is widely used for the study of redox processes, for understanding reaction intermediate, and for obtaining stability of reaction products [19, 23, 24].

The important parameters of a cyclic voltammogram are the magnitude of the anodic peak current (i_{pa}), cathodic peak current (i_{pc}), anodic peak potential (E_{pa}), and cathodic potential peak (E_{pc}). A redox couple in which both species rapidly exchange electrons with the working electrode is termed as electrochemical reversible couple. Such couple can be identified from a cyclic voltammogram by measurement of the potential difference between the two peaks potential.

The formal reduction potential, $E^{o'}$, for a reversible couple is centered between E_{pa} and E_{pc} [21, 23, 24]:

$$E^{0'} = \frac{E_{pa} + E_{pc}}{2} \quad \text{----------} \quad (1)$$

The number (n) of electrons transferred in the electrode reaction for a reversible couple can be determined from the separation between peak potentials:

$$\Delta E_p = E_{pa} - E_{pc} \approx \frac{2.303RT}{nF} \quad \text{----------} \quad (2)$$

where n is the number of electrons transferred and E_{pa} and E_{pc} are the anodic and cathodic peak potential, respectively, in volts. Thus for a reversible redox reaction at 25 ^{0}C with n electrons ΔE_p should be 0.0592 /nV or about 60mV for one electron. In practical this value is difficult to attain because of such factors as cell resistance.

$$E_p - E_{p/2} = \frac{2.20RT}{nF} = \frac{56.5}{n} \text{ mV} \quad \text{……..} \quad (3)$$

where E_p, the peak potential and $E_{p/2}$ is the potential corresponding to $i_{p/2.}$

The peak current for a reversible system is described by the Randles-Sevcik equation at 25 ^{0}C:

$$i_p = 2.69 \text{ x} 10^5 \ n^{3/2} A \ D^{1/2} C \ v^{1/2} \quad \text{----------} \quad (4)$$

where i_p is the peak current in amperes, n is the number of electrons transferred, A is the electrode area in cm^2, D is the diffusion coefficient in cm^2/s, C is the concentration in mol/cm^3 and v is the scan rate in V/s.

The peak potential at 25 ^{0}C is defined (for reduction) by:

$$E_p = E^{0'}_{O,R} - \frac{0.029}{n} \quad \text{----------} \quad (5)$$

where E_p and $E^{0'}_{O,R}$ are expressed in volts, $D_O = D_R$, and $E^{0'}_{O,R}$ is the formal electrode potential corrected to the reference electrode being used.

According to equation (4), i_p is proportional to $v^{1/2}$ and a plot of i_p verses $v^{1/2}$ should be a straight line for a reversible system. The relationship to concentration is particularly important in analytical applications and in studies of electrode mechanisms. Although the peak current increases with scan rate, the potential at which the peak occurs in invariant with scan rate as indicated by equation (5).

The value of i_{pa} and i_{pc} should be close for a simple reversible (fast) couple, that is:

$$\frac{i_{pa}}{i_{pc}} \approx 1 \quad \text{----------} \quad (6)$$

However, the ratio of peak currents can be significantly influenced by chemical reactions coupled to the electrode processes [21, 23, 24]

In the case of an irreversible system, the equation for peak current, i_p, is:

$$i_p = (2.99 \times 10^5)\, n(\alpha n_a)^{1/2}\, A\, D^{1/2}\, C\, v^{1/2} \quad \text{----------} \quad (7)$$

where α is the transfer coefficient and n_a is the number of electrons in the rate determining step of the electrode process.

The peak potential, E_p, is:

$$E_p = E^{0'} + \frac{RT}{\alpha n_a F}\left[-0.78 + \frac{\ln k_1}{D_O^{1/2}} - \frac{1}{2}\frac{\ln(\alpha n_a F v)}{RT}\right] \text{----------} \quad (8)$$

$$E_p = k - \frac{2.303RT}{2\alpha n_a F}\log v \text{----------(9)}$$

$$\text{where } k = E^{0'} + \frac{RT}{\alpha n_a F}\left[-0.78 + \frac{\ln k_1}{D_O^{1/2}}\right]$$

where k_1 is related to the heterogeneous rate constant of the electron transfer reaction.

The difference in peak potential and half peak potential is given by

$$E_p - \frac{E_p}{2} = \frac{48mV}{\alpha n_a} \text{----------(10)}$$

At 25^{o}C E_p shifts by $30/\alpha n_a$mV for each decade change in ν,

$$E_p = \frac{30}{\alpha n_a}\log v + \text{constant} \text{----------(11)}$$

Electrochemical irreversibility is caused by slow electron exchange of the redox species with the working electrode. It is characterized by a separation of peak potential that is greater than 0.059/n V, that is about 70mV and that is dependent on the scan rate. In this case equation (1) is not applicable. It is important to recognize that electrochemical irreversibility also influences the peak current ratio. The more irreversible a couple becomes, the smaller will be the peak current, i_p, on the reverse scan. This is often due to both the smaller rate constant and the fact that significant product has diffused away from the surface by the time the reverse peak potential, E_p, is reached. Because of these complications, it can be dangerous to obtain quantitative information from the peak current, i_p, ratio for extremely irreversible couples [23].

2.2. Square-Wave Voltammetry (SWV)

The excitation signal in SWV consists of a symmetrical square-wave pulse of amplitude E_{sw} superimposed on a staircase waveform of step height ΔE, where the forward pulse of the square wave coincides with the staircase step. The net current, i_{net}, is obtained by taking the difference between the forward and reverse currents ($i_{for} - i_{rev}$) and is centered on the redox potential. The peak height is directly proportional to the concentration of the electroactive species and direct detection limits as low as 10^{-8} M is possible [18, 24].

Square-wave voltammetry has several advantages. Among these are its excellent sensitivity and the rejection of background currents. Another is the speed (for example, its ability to scan the voltage range over one drop during polarography with the DME). This speed, coupled with computer control and signal averaging, allows for experiments to be performed repetitively and it increases the signal to- noise ratio.

The net peak current for the irreversible system is given by [18].

$$I_p = \text{constant } \alpha\ n^2 \Delta E\, E_{SW} (fD)^{1/2}\, C \quad \text{----------(12)}$$

where ΔE is the step potential, f is square wave frequency, E_{sw} is the square wave amplitude, α is the transfer coefficient, n is the over all electron transfer, C is the bulk concentration, and D is the diffusion coefficient of the electro active species.

Applications of square-wave voltammetry include the study of electrode kinetics with regard to preceding, following, or catalytic homogeneous chemical reactions, determination of some species at trace levels, and its use with electrochemical detection in HPLC [20].

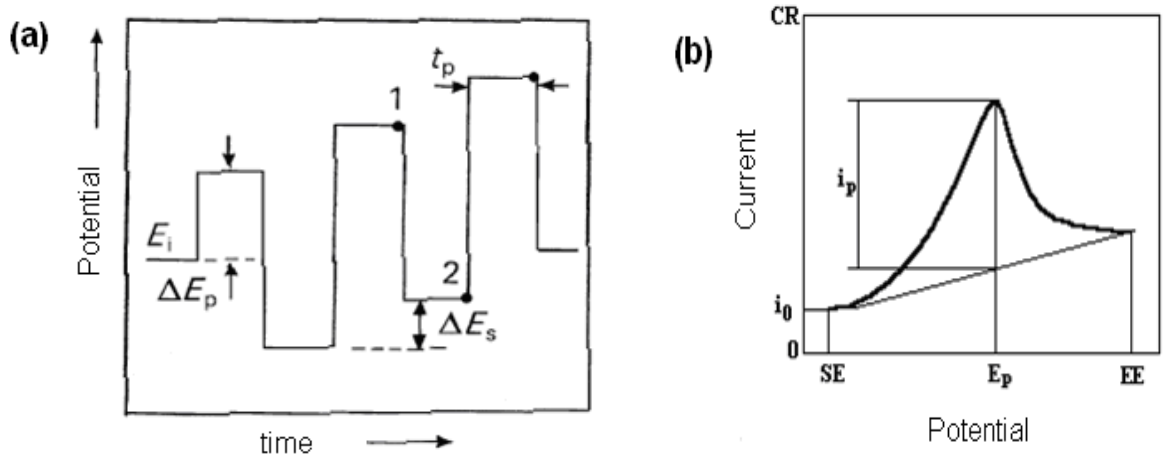


Fig.2. **(a**) Excitation waveform of square wave voltammetry and (**b)** Response obtained by square wave voltammetry.

3. LITERATURE REVIEW OF VOLTAMMETRIC DETERMINATION OF STABILITY CONSTANTS OF LEAD COMPLEXES

Stability constants of metal complexes are extremely important. In theoretical chemistry, relationships must often be found between such quantities and certain properties of the metal ion or the ligand. Stability constants are widely used in analytical chemistry in devising new methods or estimating interfering effects. They must also be considered in such areas as the kinetics of reaction in solution involving metal complexes; the biological effects of metal ions etc [25]. Stability constants numbers are expressed as the logarithm of the real stability value (number).

Stability constants of metals complexes have been determined by many different methods such as spectroscopy [26, 27, 28] (i.e. atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), inductively coupled plasma-mass spectrometry (ICP-MS) [29], X-ray fluorescence (XRF), thermal lens spectrometry (TLS) [30]), potentiometry [26, 27] and voltammetry techniques [27, 28]. However, spectroscopy techniques require expensive instrumentation and are additionally time-consuming. They cannot be used for routine in-field monitoring of a large number of samples [31]. So, voltammetric techniques and methods are very often used for studying the metal-ion complexation and speciation with different ligands in natural aquatic environment, life sciences, pharmaceutical and all other branches of the chemical industry. They have attractive feature, including sensitivity, easy operation procedures, and portability [32].

The convectional voltammetric techniques for studying the metal-ion complexation usually involve the use of mercury-based electrodes, e.g., hanging mercury drop electrode or mercury film-coated electrodes, including substrates such as glassy carbon or most recently iridium. Mercury is considered to be the electrode of choice for a metal stripping analysis, but disposal problems and high cost are major drawbacks. Most importantly, however, mercury toxicity has been a concern and has motivated the effort to develop mercury free electrodes [33].

Lead is a poisonous chemical which is widely distributed in nature and occurs in the form of inorganic or organic compounds. The presence of lead in food and water above a certain level presents a serious threat to public health, such as cancerization and hypogenesis. It is also affects the brain and the nervous system, together with other organs and several metabolic pathways. Therefore, it is extremely important to monitor trace or ultra trace lead in the environment [29, 31]. The most usual techniques employed in the routine analysis of this metal and its stability constant of the complexes had been studied by potentiometric, voltammetric, and spectroscopic methods [27].

The formation of the complex compounds of lead (II) with different ligands and their stability constant of the complexes had been reported by voltammetric methods, which is shown in table1.

Table 1. The stability constant of some complex compounds of lead (II) using different techniques and electrodes, which is listed below.

No	Ligands	formula	Stability constant(logβ)	Voltammetric techniques	electrode	reference
1	DOM from water sample	PbL	4.2 ± 0.02	DPASV	HMDE	[34]
2	Picolinic acid	PbL, PbL_2, PbL_3, $PbL_2(OH)$	4.49±0.02,7.58±0.03,9.59±0.02 , 11.46 ± 0.01	DPP	DME	[35]
3	Chloro-complex	PbL, PbL_2, PbL_3, PbL_4	14.5±0.4,74.3±2.5, 129.2±3.2, 11.2±1.0)	DPP	DME	[36]
4	Calcein-blue (CB)	PbL_2	16.48 ± 0.37	CSV	HMDE	[37]
5	DEDC	PbL	15.6±0.03	DCP	DME	[28]
6	Glutamic acid	PbL,PbL_2	4.91±0.1, 7.93±0.04	ASV-DC	DME	[38]
7	TAPSO	PbL,PbL_2,$PbL_2(OH)$,$PbL_2(OH)_2$	3.7±0.1,6.6±0.1, 12.9±0.1, 17.8±0.1	DCP	DME	[39]
8	L-ascorbic acid	PbL,PbL_2	9.3 ± 0.2 ,18.0 ± 0.1	SWV	SMDE	[16]

3.1. Calculations of Stability Constants

Stability constants are well known tools for solution chemists, biochemists and chemist in general to help determine the properties of metal-ligand reactions in water and biological systems [40]. Generally, ionized metals in water consist of the metallic ion attached to a few water molecules and carry the positive electric charge of the metal ion.

3.1.1. Stability Constant Determinations from Reversible Reductions

The method used for measuring stability constants of simple reversible mononuclear complexes using the De Ford and Hume method. The De Ford and Hume method for measuring simple complex formation is based on solving the following series of F-functions [41].

$$F_0(L) = \text{antilog}\left(\frac{0.4343nF}{RT}\Delta E_p + \log_{10}\left[\frac{(i_p)_M}{(i_p)_C}\right]\right) \quad \text{..... (13)}$$

where n, F, R and T have their same meanings as in the Nernstian equation, i.e. n is the transfer of electron(s) in redox reaction, F is faraday constant in C, R is universal gas constant J/K- mol and T is temperature in K. ΔE_p is the change in reduction peak potentials, $(i_p)_M$ and $(i_p)_C$ are the corresponding diffusion or peak currents of the metal and complexed metal respectively.

By sequential differentiation of eqn. (13), consecutive F-functions are obtained as:

$$F_1(L) = \frac{F_0(L) - \beta_0}{[L]} \quad \text{..... (14)}$$

$$F_2(L) = \frac{F_1(L) - \beta_1}{[L]} \quad \text{..... (15)}$$

$$\vdots$$

$$F_N(L) = \frac{F_{N-1}(L) - \beta_{N-1}}{[L]} = \beta_N \quad \text{..... (16)}$$

where [L] is the ligand concentration and $\beta_0, \beta_1 \ldots \beta_{n-1}$ are the consecutive stability constants. β_0 is unity by definition.

A measure of peak potential in polarographic method shift serves to determine both the co-ordination number and the stability constant of the complex. It can fairly simply be shown that the shift, to a first approximation, by ignoring activity coefficients, may be expressed in the following relationship

$$\Delta E_p = (E_p)_M - (E_P)_C = \frac{RT}{0.4343nF} \log_{10}\beta_{MLp} + p\frac{RT}{0.4343nF} \log_{10}[L] \quad \text{---------- (17)}$$

in which $(E_p)_M$ and $(E_p)_C$, are the peak potentials of the simple and complexed species, respectively, p, is the co-ordination number of the complex, β_{MLp} is stability constant, and [L] is the ligand concentration.

Peak potentials of complexed metal ions shift with changing activity of the complexing ligand in accordance with:

$$\frac{\Delta E_p}{\Delta \log_{10}[L]} = - p\frac{2.303RT}{nF} \quad \text{----------(18)}$$

Hence the number of ligands, p, bound in the complex is found from a plot of $\log_{10}[C]$, against ΔE_p , which, in the present case, should be linear [40].

From consecutive plots of F-functions against the varying concentrations, consecutive stability constants can be obtained as intercepts of the plots. Some interesting characteristics of these plots are that the last complex gives a plot which is linear and parallel to the concentration axis while a straight line with a positive gradient indicates the immediately proceeding complex. The rest of the plots are curvatures. These characteristics help in establishing the number of complex ions formed [42].

The relation between the change in half-wave potential and the free-ligand concentration [L] for a reversible metal - complex is

$$\frac{0.4343nF}{RT}\Delta E_p + \log_{10}\left[\frac{(i_p)_M}{(i_p)_C}\right] = \log_{10}\beta_{MLp} + \Delta \log_{10}[L] \quad \text{..... (19)}$$

3.1.2. Stability Constant Determinations from Irreversible Waves

The De Ford and Hume method [41] can be used to determine the stability constants of the irreversible complexes according to the equation:

$$\frac{0.4343\alpha nF}{RT}\Delta E_p + \log_{10}\left[\frac{(i_p)_M}{(i_p)_C}\right] = \log_{10}\beta_{MLp} + p\log_{10}[L] \quad \text{..... (20)}$$

where β_{MLp} is the stability constant of the MLp complex (where M, metal; L, ligand), [L] is the concentration of ligand, ΔE_p is the distance of the peak potentials of the free metal ion and the complex in polarographic method, p is the ligand/metal value, and $(i_p)_M$ and $(i_p)_C$ are the peak currents of the free metal ion and the complex, respectively.

For irreversible systems, shift peak potential is given by:

$$\Delta E_p = (E_p)_C - (E_p)_M = \frac{2.303RT}{\alpha nF} \log_{10} \beta_{MLp} - p\frac{2.303RT}{\alpha nF} \log_{10}[L] \quad \text{----------(21)}$$

Peak potentials of complexed metal ions in polarographic method shift with changing activity of the complexing ligand in irreversible system in accordance with:

$$\frac{\Delta E_p}{\Delta \log_{10}[C]} = -p \frac{2.303RT}{\alpha nF} \quad \text{----------(22)}$$

where, α is the transfer coefficient, all other terms are mentioned above.

4. EXPERIMENTAL PART

4.1. Reagents and Chemicals

The reagents and chemicals used were lead nitrate (Nice, Wagtech International LTD., UK, purity 99%), L-ascorbic acid (Aldrich-chemie D-7924, Steinem, purity 99%), sodium perchlorate (BDH chemicals LTD. Poole, England, purity 97%), HCl (Riedel-deHaen AG), NaOH (BDH Poole, England) and alumina (for polishing the working electrode). Deionized water was used through out the work.

4.2. Apparatus, Electrolytic Cells and Electrodes

The voltammetric measurements were performed with BAS 100A, electrochemical analyzer [Bioanalytical systems (BAS), USA], which was connected to PC (Acer computer). The voltammetric cell was connected with a gas inlet and a three electrode system consisting of a bare platinum electrode (surface area, 0.23cm^2) as working electrode, a Ag/AgCl/(saturated KCl) electrode as reference electrode and a platinum wire as a counter electrode (an auxiliary electrode) was used for all measurements. A magnetic stirrer with a hot plate [model 04803-02] from Cole Palmer Instrument Company was also used for stirring in pH adjustments. The pH of the supporting electrolyte solution was measured with a Hanna instrument digital pH 301 meter with a combination glass electrode.

4.3. Procedures

All voltammetric experiments were carried out in the presence of sodium perchlorate solution, which maintain the ionic strength. The procedure for preparing lead nitrate and ascorbic acid samples were as following: 0.001656g lead nitrate and 0.17612g L-ascorbic acid were carefully measured then dissolved in sodium perchlorate solution, transferred to a 50 mL flask and diluted to scale with sodium perchlorate solution. The pH was adjusted to 5. Then cyclic voltammogram were recorded in the potential range from -600mV to -200mV with a scan rate of 100 mV/s. Square wave voltammetric measurements were taken from -600 to 100 mV using

the Osteryoung square wave voltammetric technique. The net current responses of the different voltammetric measurements were recorded. The parameters for square wave voltammetric measurements were: a potential step of 5 mV, square wave amplitude of 50 mV and frequency of 25 Hz. Dissolved oxygen which interferes with voltammetric analysis was removed by purging the solutions with nitrogen gas for about 10 min. A magnetic stirrer with a hot plate [model 04803-02] from Cole Palmer Instrument Company was also used for stirring in pH adjustments. Then the pH of the solutions was adjusted by adding drops of concentrated HCl and NaOH. All measurements were performed at room temperature.

5. RESULTS AND DISCUSSION

In this paper the stability constant of lead complex with L-ascorbic acid at a bare platinum electrode and in sodium perchlorate supporting electrolyte has been studied using cyclic volammetry and square wave voltammetry. The optimum pH observed to study of the complex formation of lead- ascrobate complex and the stability constant of this complex was pH 5.

5.1. Cyclic Voltammetric (CV) Investigation

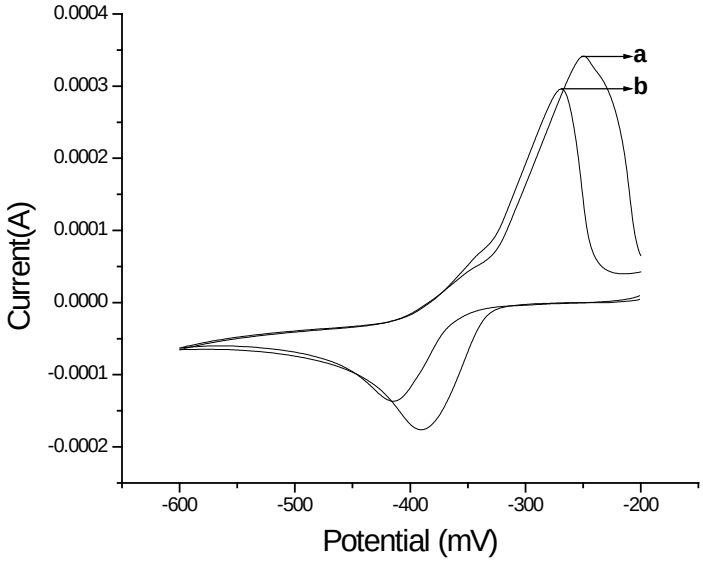


Fig.3. Cyclic voltammogram of $1x10^{-4}$ M Pb (II) in 0.1 M $NaClO_4$ supporting electrolyte (pH 5.0) at scan rate of 100mV/S. **a**) free Pb (II) ion and **b**) in the presence of $2x10^{-2}$M L-ascorbic acid.

Fig.3 shows cyclic voltammogram $1x10^{-4}$ M Pb (II) in the presence of $2x10^{-2}$M L-ascorbic acid in 0.1 M $NaClO_4$ at platinum electrode at pH 5. As a result of the interaction of Pb (II) with L-ascorbic acid, a negative shift of the anodic peak potential was observed on the voltammograms. This peak is believed to be a complex formation between lead and L-ascorbic

acid i.e. is lead-ascorbate complex. This peak appears 19 mV more negative than the peak potential for the free lead (II) ion. The peak potential of free lead (II) ion is -250 mV and its complexed lead (II) ion is -269 mV.

5.2. Square Wave Voltammetric (SWV) Investigation

Experiments have been carried out using the square wave voltammetric technique. Fig.4 shows the square wave voltammogram of $1x10^{-4}$ M Pb (II) in the presence of $2x10^{-2}$M L-ascorbic acid in 0.1 M $NaClO_4$ at pH 5.0. The addition of $2x10^{-2}$M L-ascorbic acid to a $1x10^{-4}$ M Pb (II) solution results shifts of peak potential and a gradual decrease in the peak current of the complexed Pb (II) ion. This is probability due to a complex formation between lead (II) ion and ascorbic acid. The peak for the free lead (II) ion is more positive than in the complexed lead (II) ion. In other wards, the peak potential of the complexed lead (II) ion is more negative than the free lead(II) ion, when the ligand (L-ascorbic acid) is added. It shifts to negative peak potential of 15 mV, which is in close agreement with the literature [16]. But the peak current of the free lead (II) ion is increases, which is shown in Fig.4. The peak potential of free lead (II) ion is -340 mV and its complexed lead (II) ion is -355mV.

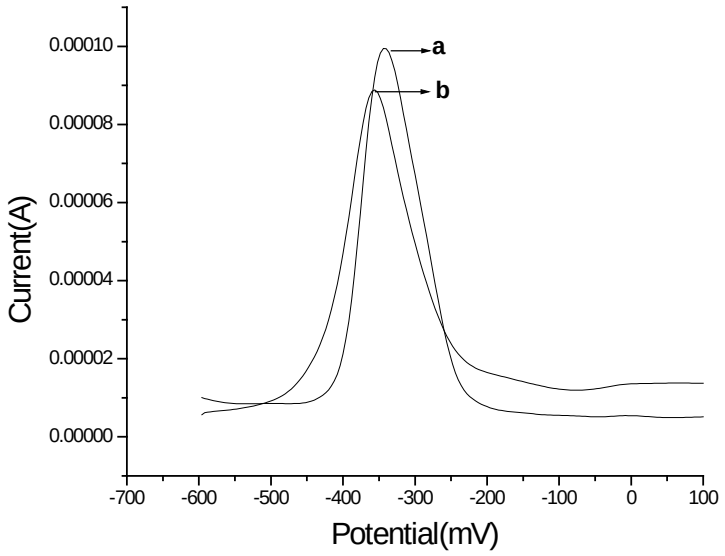


Fig.4. Square Wave Voltammogram of $1x10^{-4}$ M Pb (II) in 0.1 M $NaClO_4$ (pH 5.0). **a**) free Pb (II) ion and **b**) in the presence of $2x10^{-2}$M L-ascorbic acid.

5.3. Effect of pH

Fig. 6 shows a set of square-wave voltammogram (SWV) of the electrolyte containing $1x10^{-4}$M Pb (II) in 0.1M $NaClO_4$ in the absence of ligand (L-ascorbic acid) while solution pH was varied from 4 to 9. The voltammetric peak of Pb (II) shifted to lower (more negative) potentials with increasing the pH value. The change of the peak potential (ΔEp) of the optimized pH (i.e. pH 5) and pH 9 is about -103.35 mV. This is a consequence of the formation of labile, lead hydroxide complexes, which is in close agreement with the literature [16]. From the dependence of the shift of the peak potential (ΔE_p) on the increasing concentration of hydroxide ions or pH (Fig. 8), it can be seen that at pH = 5 there are no hydroxide complexes present in the measured system and also the peak height increase at this pH, which is shown in Fig.7. Hence, this pH value was chosen for further investigations.

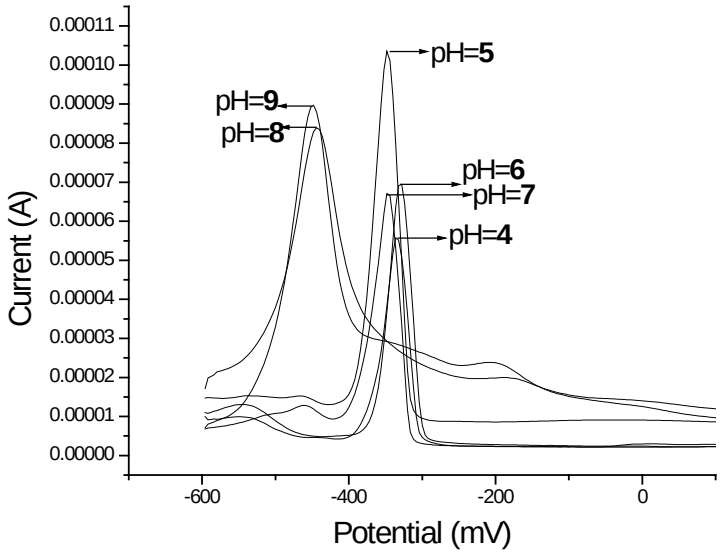


Fig.5. Square Wave Voltammogram of $1x10^{-4}$M Pb (II) in 0.1M $NaClO_4$ with the indicated pH values .

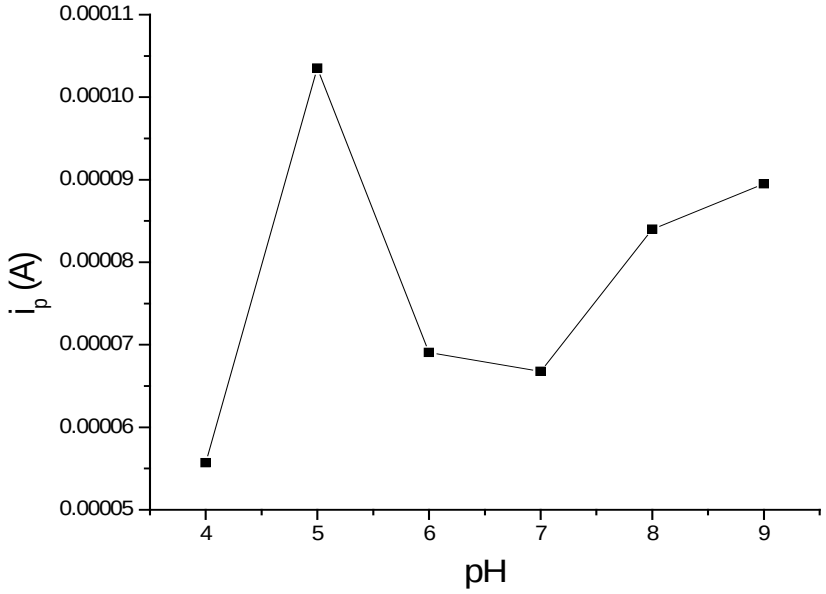


Fig.6. Plot of Square Wave Voltammogram of peak current as a function of $1x10^{-4}$M Pb (II) in 0.1 M $NaClO_4$.

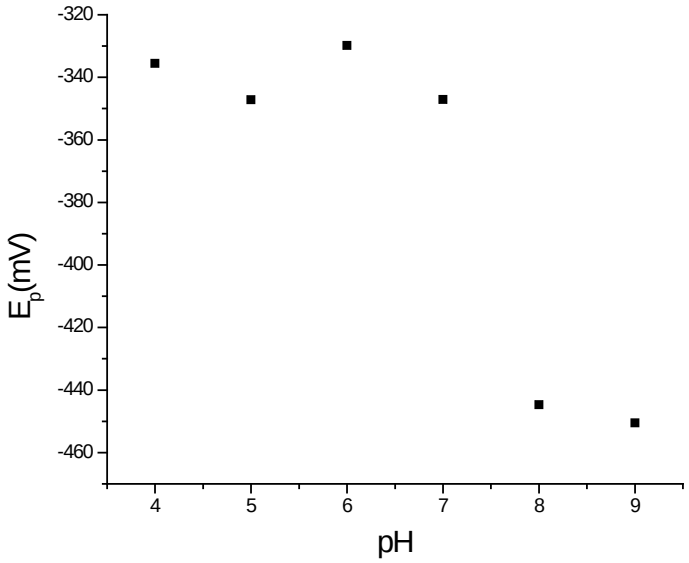


Fig.7. Plot of Square Wave Voltammogram of peak potential as a function of pH of $1x10^{-4}$M Pb (II) in 0.1 M $NaClO_4$.

5.4. Effect of Scan Rate

The cyclic voltammogram of $1x10^{-4}$ Pb (II) in the presence of $2x10^{-2}$ M L-ascorbic acid solution was run at different scan rates as shown in Fig. 8. As the scan rate changes from 25 to 150 mV /S; there is a shift in the cathodic peak potentials and cathodic peak currents of lead-ascrobate complex. The electrode reaction and the complex formation of lead-ascorbate complex at surface of the electrode are linearly dependent on the square root of the scan rates. The dependence of peak current on the square root of scan rates is shown in Fig.9, which is linear with a slope-$8.6767x10^{-5}$ [23].

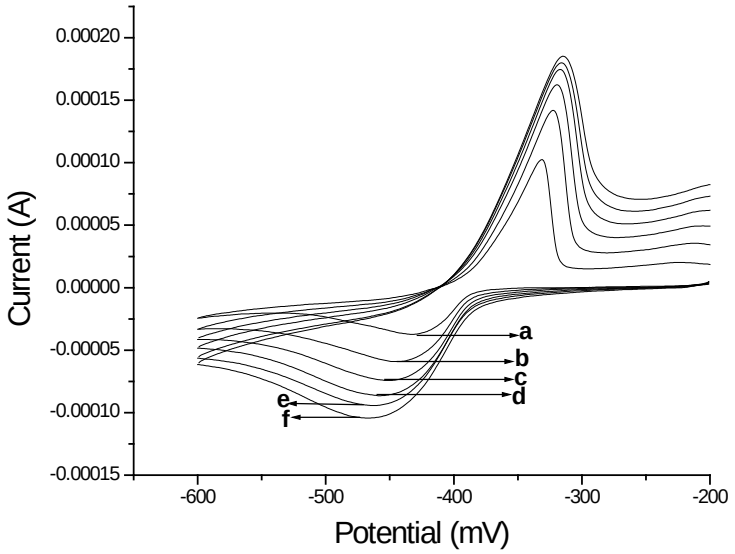


Fig.8. Cyclic Voltammogram of lead-ascrobate complex of $1x10^{-4}$ M Pb (II) in the presence of $2x10^{-2}$ M L-ascorbic acid in 0.1 M $NaClO_4$ of (pH 5) with different scan rates. a) 25, b) 50, c) 75, d) 100, e) 125 and f) 150 mV/S.

The dependence of peak current (i_p)and peak potential (E_p) at scan rate (see Fig. 8).

Table 2. The influence of scan rate on peak current and peak potential for the formation of lead-ascrobate complex, with cyclic voltammogram of $1x10^{-4}$M Pb (II) in the presence of $2x10^{-2}$M L-ascorbic acid in 0.1 M $NaClO_4$ as supporting electrolyte (pH 5).

V (mV/S)	25	50	75	100	125	150
$v^{1/2}$ (mV/S)	5.000	7.071	8.660	10.000	11.180	12.247
log v (mV/S)	1.3979	1.6989	1.8750	2.0000	2.0969	2.1760
i_{pc}(A) (10^{-5})	-3.6347	-5.8247	-7.4426	-8.6510	-9.3678	-10.474
E_{pc} (mV)	-433	-447	-454	-459	-464	-468

From table (2) it is easy to conclude that, as the logarithm of the scan rate increases the peak potential (E_{pa}) lead-ascrobate complex also increases, which is shown in Fig.10, which also in agreement to eqn.11 and the value of αn_a, which was calculated from the slope of cathodic peak potential (E_{pc}) verses the logarithm of scan rate is 0.72. It also shows that there is cathodic peak current and anodic peak current of the complexed lead (II) ion, which is shown in Fig.8 and the ratio of the cathodic peak current and the anodic peak is greater than one (from eqn.6). Also, there is a great separation of anodic and cathodic peak potential, that means, from eqn.2 ΔE_p at 25mV/S scan rate 102 mV and changing the scan rate at 150mV/S becomes 152 mV, hence, the change of peak potential (ΔE_p) is greater than 60mV, therefore, the electrochemical reaction of the lead-ascrobate complex is irreversible [21, 23, 24].

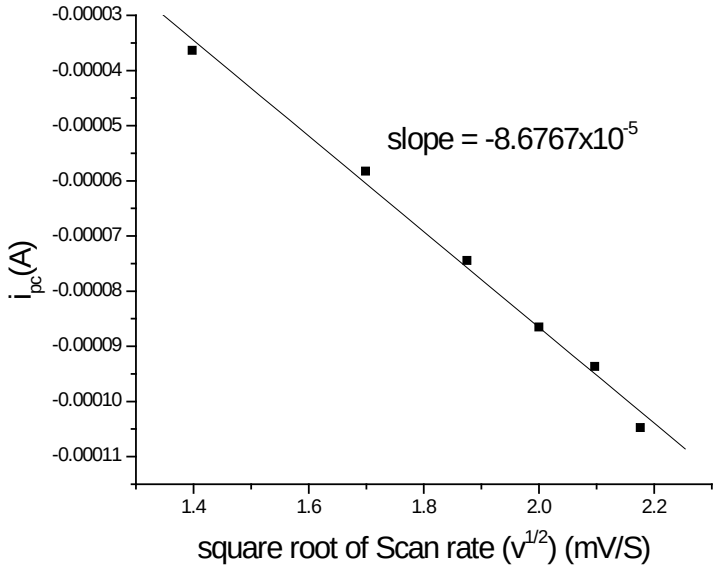


Fig.9. Plot of cyclic voltammogram of cathodic peak current as a function of square root of Scan rate ($v^{1/2}$) in the formation of lead – ascorbate complex of $1x10^{-4}$M Pb (II) in the presence of $2x10^{-2}$M L-ascorbic acid in 0.1 M $NaClO_4$ as supporting electrolyte (pH 5). (R= 0.9967).

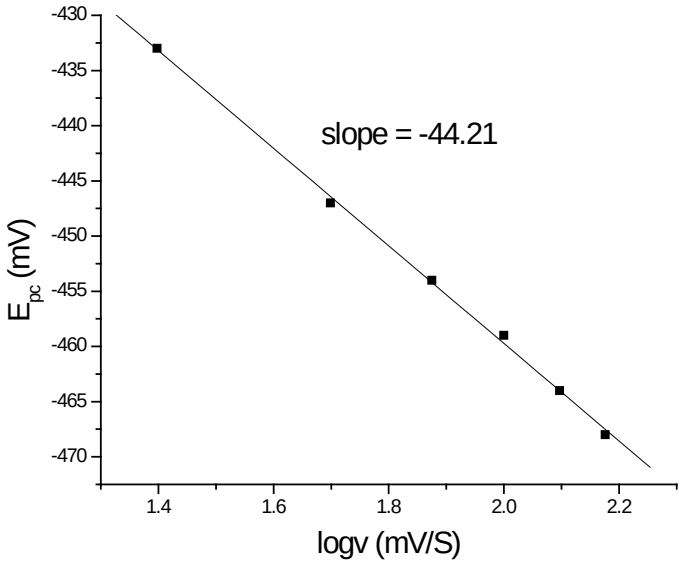


Fig.10. Plot of cyclic voltammogram of cathodic peak potential as a function of log of scan rate (logv), in the formation of lead-ascorbate complex of $1x10^{-4}$M Pb (II) in the presence of $2x10^{-2}$M L-ascorbic acid in 0.1 M $NaClO_4$ as supporting electrolyte (pH 5). (R= 0.99928)

5.5. Effect of Concentration

The effect of concentration was studied using cyclic voltammetry and square wave voltammetry of 0M, $2x10^{-2}$M, $4x10^{-2}$M, $6x10^{-2}$M, $8x10^{-1}$M and $1x10^{-1}$M L-ascorbic acid in $1x10^{-4}$M Pb(II) in 0.1 M $NaClO_4$ at pH 5.0. L-ascorbic acid is an electroactive compound that forms a complex with lead (II) ion. When the concentration of L-ascorbic acid was raised the peak current decreases successively in both of the techniques and the peak potential of the lead-ascorbate complexes shift toward more negative. This indicates there is the formation of lead-L-ascorbic acid complex i.e. a quite stable complex of lead (II) ion is formed. These systems investigated exhibited a labile character, having a single, irreversible cyclic voltammogram peak that shifted towards more negative potential values with an increase in the ligand concentration, and some changes in the peak height. At very low ligand (L-ascorbic acid) concentrations, the free metal species, Pb^{2+}, would dominate.

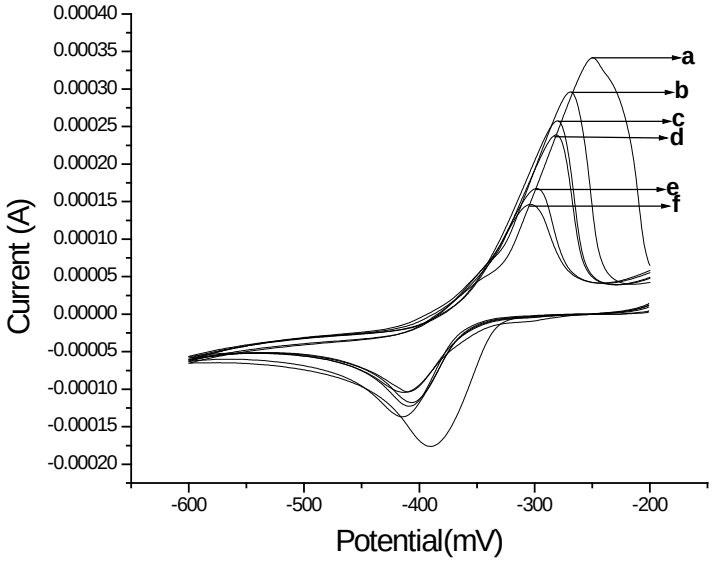


Fig.11.Cyclic voltammogram response of current as a function of potential of lead-ascrobate complexes at different concentrations of L-ascorbic acid in $1x10^{-4}$M Pb (II) in 0.1 M $NaClO_4$ (pH 5.0) with a scan rate of 100mV/S a) 0M, b) $2x10^{-2}$M, c) $4x10^{-2}$M, d) $6x10^{-2}$M, e) $8x10^{-2}$M and f) $1x10^{-1}$M.

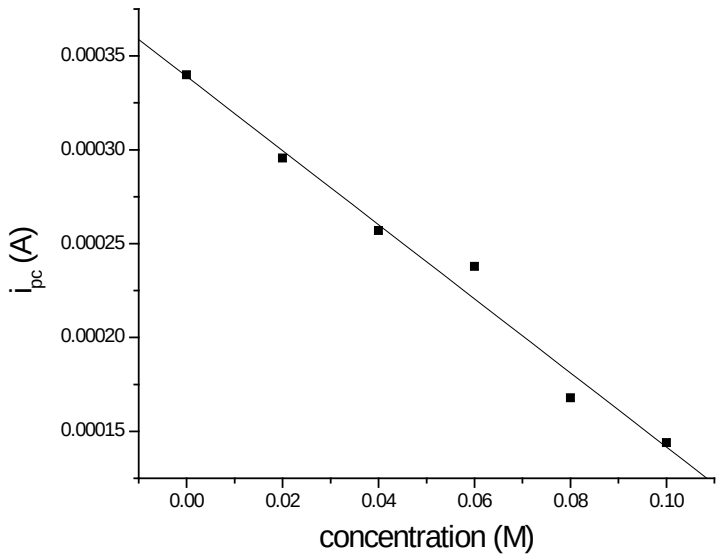


Fig.12. Plot of Cyclic voltammogram of anodic peak current as a function of concentration of L-ascorbic acid in $1x10^{-4}$M Pb (II) in 0.1 M $NaClO_4$ at pH 5.0 with a scan rate of 100mV/S (R = 0.99086)

As can be seen from the cyclic voltammogram and square wave voltammogram of current peaks as a function of concentration of L-ascorbic acid, the peak current decrease with increasing the concentration of L-ascorbic acid, which is shown in (Fig.12) (R =0.99086) and (Fig.14) (R = 0.99176), respectively. This is indicative of complex formation between the lead (II) ion and L-ascorbic acid. At high ligand (L-ascorbic acid) concentration, the lead peak current decreases.

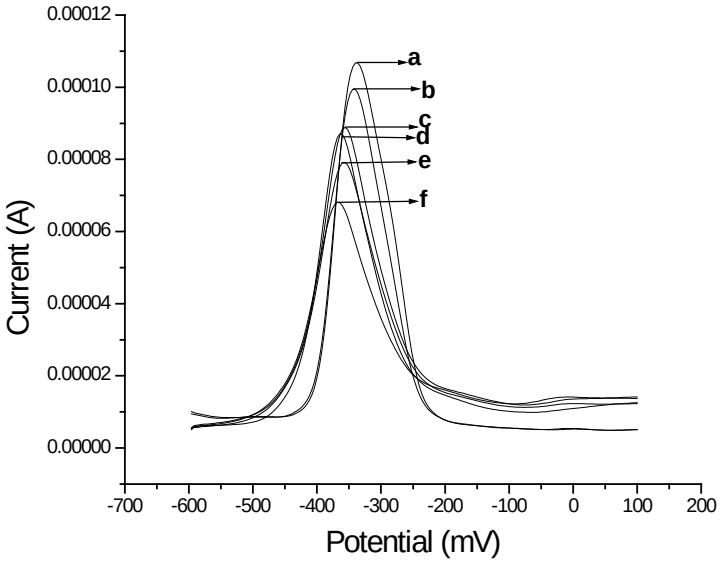


Fig.13. Square Wave Voltammogram of response of current as a function of potential of lead-ascrobate complex at different concentrations of L-ascorbic acid in $1x10^{-4}$M Pb(II) in 0.1 M $NaClO_4$ at pH 5.0 a) 0M, b) $2x10^{-2}$M, c) $4x10^{-2}$M, d) $6x10^{-2}$M, e) $8x10^{-2}$M and f) $1x10^{-1}$M.

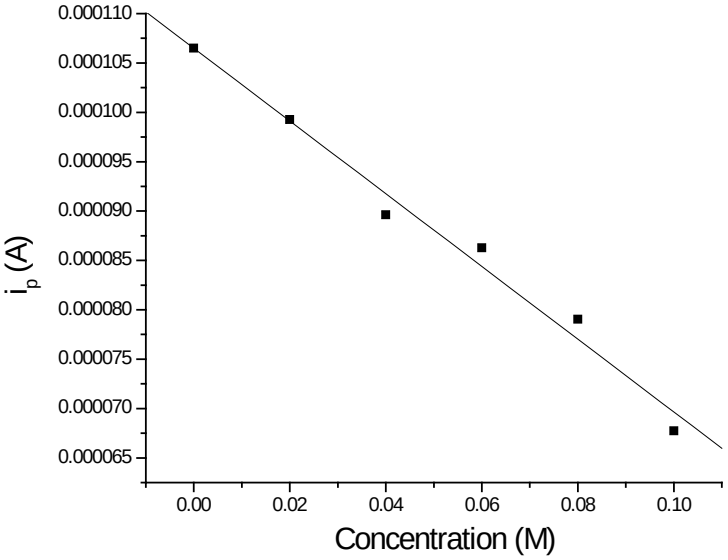


Fig.14.Plot of Square Wave Voltammogram of peak current as a function of concentration of L-ascorbic acid in $1x10^{-4}$M Pb (II) in 0.1 M $NaClO_4$ at pH 5.0.(R = 0.99176)

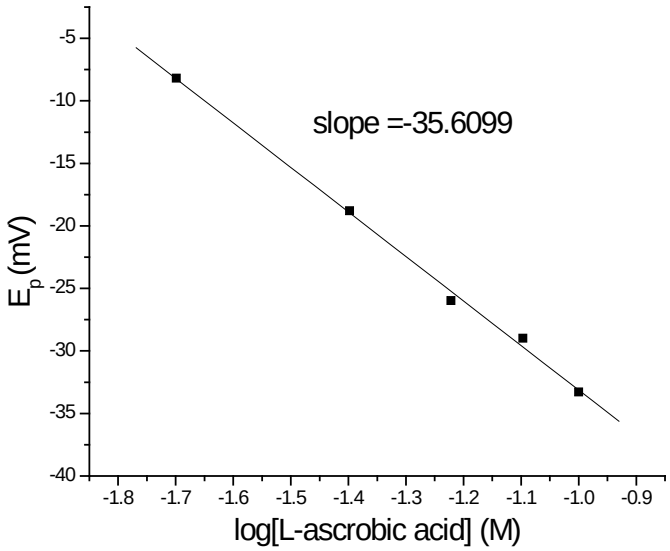


Fig.15. Plot of Square Wave Voltammogram of change of peak potential of free and complexed lead (II) ion as a function of the log concentration L-ascorbic acid in $1x10^{-4}$M Pb (II) at pH 5.(R= 0.99857).

Fig.15 shows square wave voltammogram data of the shift of the peak potential (ΔE_p) verses increasing logarithm concentration of ligand (L-ascorbic acid).The modified De-Ford and Hume method is used to determine the stoichiometries and stability constants of irreversible lead (II)-ascrobate complexes. That means, eqn. 21 was used to calculate the stability constant lead (II)-ascrobate complexes.

$$\Delta E_p = (E_p)_C - (E_p)_M = \frac{2.303RT}{\alpha n_a F} \log_{10} \beta_{MLp} - p\frac{2.303RT}{\alpha n_a F} \log_{10}[L] \quad \text{----------(21)}$$

The temperature was 23^{o}C, αn_a was calculated from eqn.11, which is about 0.72 and p was calculated from a plot of the change of peak potential (ΔE_p) against the logarithm of the L-ascorbic acid concentration, which is linear (Fig.15) (R = 0.99857) and the slope is $-3.56099x10^{-2}$, which is about 0.43 (eqn.22), which indicates the formation of one complexe.

The change of the peak potential (ΔE_p) of the lead-ascrobate complex and the free lead in $2x10^{-2}$M L-ascorbic acid was 15mV. Then by applying the modified De-Ford and Hume equation, (eqn.21), the stability constant log β 1:1of PbL was 0.908.

The stability constant of lead- ascrobate (logβ 1:1of PbL) complex on platinum electrode, which is irreversible peak, was 0.908 where as on dropping mercury electrode in reversible peak was 9.3 ± 0.2 [16]. This difference is due to the nature of electrode.

6. CONCLUSION

The complexation reaction occurring between Pb (II) ions and L-ascorbic acid were performed using cyclic voltammetry (CV) and square wave voltammetry (SWV), which allow the identification of the complex formed as well as the determination of their stability constant. In addition, the results show that the De-Ford and Hume method is applicable for irreversible complexes. It was found also that the electrode reaction of lead(II) (1×10^{-4} M) is irreversible in 0.1 M $NaClO_4$ and that lead (II) ascorbate complexes are electrochemically labile to the L-ascorbic acid concentration of $2x10^{-2}$ M, which were the conditions in the solution used for the determination of the stability constants by square-wave voltammetry.

At pH = 5 there is no evidence of lead hydroxides. The chosen pH for establishing the stability constants was 5. The result of the stability constant of lead-ascrobate complex ($\log\beta_{PbL}$) in 1:1 of irreversible peak from square wave voltammogram data was 0.908.

7. REFERENCES

1. C. Walker, Environmental Pollution by Chemicals, Hutchinson Educational LTD., London, Melbourne, **1971**, 45-48.
2. E. Meyer, Chemistry of Hazardous Materials, Prentice-Hall, Inc., London, **1977**, 205-206.
3. F. W. Fifield and P. J. Haines, Environmental Analytical Chemistry, Blackie Academic and Professional, London, 1st edn., **1997**, 348-349.
4. L. R. Curtis and B. W. Smith, Heavy Metal in Fertilizers, Oregon State University Corvallis, Oregon, **2002**, 8-12.
5. D. T. Win, M. M. Than and S. Tun**,** A U J.T., 6 (**2003**) 187-192.
6. L. M. Lamp, Physical and Chemical Properties of Metals, Blackie Academic and Professional, London, 2000, 34-36.
7. R. Turpeinen, Interactions between Metals, Microbes and Plants Bioremediation of Arsenic and Lead Contaminated Soils, Neopoli, Lahti, **2002**, 19.
8. M. G. Shalana,, M. S. Mostafa, M. M. Hassouna, S. E. H. El-Nabic and A. El-Refaied, Toxicol., 206 (**2005**) 1–15.
9. R. C. Patra, D. Swarup and S. K. Dwivedi, Toxicol., 162 (**2001**) 81–88.
10. A. A. Izuagie and F.O. Izuagie, Res. J. Agric. & Biol. Sci., 3 (**2007**) 367-369.
11. O. R. Fonorow, the Vitamin C Foundation; the Nature of Vitamin C, 6 (**2006)** 4-8.

* A U J.T. = Assumption University Journal of Thailand.

12. S. Englard and S. Seifter, the Biochemical Functions of Ascorbic Acid, Annual Review of Nutrition, **1986,** 365–406.
13. A. A. Etim and M. H. Etukudo, Pakistan Journal of Nutrition, 5 (**2006**) 490-491.
14. M. Elson, the Complete Guide to Diet and Nutritional Medicine, Staying Healthy with Nutrition, Published by Celestial Arts, **1984**.
15. K.W. Lee, H. J. Lee, Y. J. Surh and C. Y. Lee, Am. J. Clin. Nutr., 78 (**2003**) 1074–1078.
16. G. Branica, M. Metiko-Hukovi and D. Omanovi, Croat. Chem. Acta, 79 (**2006**) 77-83.
17. J. L. Svirbelyl and A. Szent-Gyorgyi, The Biochemical Journal, 27 (**1933**) 279-285.
18. P. Protti, Introduction to Modern Voltammetric and Polarographic Analysis Techniques, Amel Electrochemistry, 4th edn., New York, **2001**.
19. S. P. Kounaves, Hand books of Instrumental Techniques for Analytical Chemistry, John Wiley and Sons, Inc., chapter 37, New York, 711.
20. W. R. Lacourse, Pulsed Electrochemical Detection in HPLC, John Wiley and Sons, Inc., New York, **1997**, 47-48.
21. D. T. Sawyer, W. R. Heimeman and J. M. Beebe, Chemistry Experiments for Instrumental Methods, John Wiley and Sons, Inc., New York, **1984**, 80-85.
22. L. L. Merritt, J. A. Dean, F. A. Settle and Willard, Instrumental Methods of Analysis, Litton educational publishing, Inc., 6th edn., New York, **1981**, 712-713.

23. P. T. Kissinger and W. R. Heinemann, Laboratory Techniques in Electroanalytical Chemistry, Marcel Dekker, Inc., New York, 2nd edn, **1996**.

24. A. J. Bard and L. R. Faulkner, Electrochemical Methods: Fundamentals and Applications, John Wiley and Sons, Inc., 2nd edn, New York, **2001**, 227-228.

25. M. T. Beck, Pure & App. Chem., 49 (**1977**) 127-135.

26. M. S. Diaz-Cruz, J. M. Diaz-Cruz, J. Mendieta, R. Tauler and M. Esteban, Anal. Biochem., 279 (**2000**) 189–201.

27. M. Casado, S. Daunert and M. Valiente, Electroanalysis, 13 (**2001**) 54-60.

28. A. L. B. Marques and G. O. Chierice, J. Braz. Chem. Soc., 9 (**1998**) 531-538.

29. F. N. Carrijo, L. C. Brasil and N. M. Coelho, J. Braz. Chem. Soc., 16 (**2005**) 1.

30. M. A. Proskurnin, V.V. Chernysh, S. V. Pakhomova, M.Y. Kononets, A. P. Smirnova and D. A. Nedosekin, Anal. Sci., 17 (**2001**) 1169-1172.

31. G. Yanping, W. Wanzhi, G. Xiaohua, Z. Jinxiang and Y. Jian, Intern. J. Environ. Anal. Chem., 87 (**2007**) 521–533.

32. B. S. Grabaric, Z. Grabaric, J. M. Doaaz-Cruz, M. Esteban and E. Casassas, Anal. Chim. Acta, 363 (**1998**) 2610-278.

33. A. Manivannan, R. Kawasaki, D. A. Tryk and A. Fujishima, Electrochim. Acta, 49 (**2004**) 3313-3318.

34. M. C. E. Rollemberg, M. L. S. Goncalves, A. M. A. Mota and F. B. Jimenez, Anal. Chim. Acta, 384 (**1999**) 17-26.

35. I. Cukrowski and S. A. Loader, Electroanalysis, 10 (**1998**) 877-885.

36. I. Pietas, D. Omanovic and M. Branica, Anal. Chim. Acta, 401 (**1999**) 163–172.

37. E. Fischer and M. G. Van den Berg, Anal. Chim. Acta, 432 (**2001**) 11–20.

38. M. M. C. Dos Santos, M. L. S. Gonpdves and S. Cupelo, Electroanalysis, 8 (**1996**) 178-182.

39. C. M. M. Machado, I. Cukrowski and H. M. V. M. Soares, Electroanalysis, 17 (**2005**) 1291-1301.

40. D. R. Crow and J. V. Westwood, The Study of Complexed Metal Ions by Polarographic Methods, Academic Press, New York, **1964**, 57.

41. D. D. DeFord and D. N. Hume, J. Am. Chem. Soc., 73 (**1951**) 5321.

42. E. Chekmeneva, J. M. Diaz-Cruz, C. Arino and M. Esteban, Anal. Biochem., 348 (**2006**) 252–258.