CHAPTER 1

INTRODUCTION

1.1 Lead

Lead has been an important metal in human societies over many thousands of years. Lead pipes for water supply was used from the period of the Roman Empire. However, the use of lead has increased dramatically since the early days of the industrial revolution. Some uses of lead are construction material for tank linings, piping and other equipment handling corrosive gas and liquids used in the manufacture of sulfuric acid, petroleum refining, some processes of halogenation, sulfonation, extraction, and condensation. It can also be used for x-rays and atomic radiation protection; in the manufacture of tetraethyllead as an anti-knocking agent in anti-explosion engines; pigments for paints; bearing metal and alloys; storage batteries; in ceramics, plastics and other electronic devices. Lead has also been used over the years as a glaze on pottery, in cosmetics and as sweetening wine [1].

While lead is an important factor and widely used in our daily life. On the other hand, lead is considered to be one of the most common toxic heavy metals in the environment. Mining, smelting and refining of lead, as well as the production and use of lead-based products give rise to release of lead into the environment: the air, surface water and deposit on the land. Through food, drinking water, air as well as a number of other sources, people are exposed to lead, and affected by this toxic heavy metal.

The Global Environmental Monitoring System (GEMS), in which UNEP, UNESCO, WMO and WHO cooperate, concentrates on major environmental features of climate and long-range pollution. Air pollution monitoring under GEMS is carried out in

60 cities in 50 countries. Due to the toxicity and most common use, lead is ascribed to be one of the heavy metals under this program, which includes the assessment, through biological monitoring, of air, water and food quality, and of the effects on human of exposure to organochlorine compounds and certain heavy metals [2].

1.1.1 Properties of lead

Lead is a one of the heavy metals in the IV A group of periodic table. The atomic number of lead is 82 and atomic mass is 207.2. The physical property of lead is flexible, malleable, easily melted, cast rolled and extruded. Its color ranges from bluish-white, silvery and grayish. Lead can slightly dissolve in water but fairly dissolve in nitric acid and hot sulfuric acid. The general properties can be shown in Table 1.1.

Table 1.1 Physical properties of lead [3].

Properties	Parameter
Melting point	327.4 °C
Boiling point	1740 °C
Density	11.34 g/cm^3 at 20 °C
Heat capacity	0.031 cal/g/°C
Resisitivity	20.65 $\mu\Omega$cm at 20 °C
Coefficient of expansion	29.0x10^{-6} at 0-100 °C
Vapor pressure	1.77 mm Hg at 1000°C
Viscosity of molten lead	3.2 centipoise at 327.4°C

Lead can be used in both metallic and lead compound form. Ryan [4] reported the production of lead in industries as follows: -

Storage battery   55-56 %

Metal product   16-18 %

Chemicals, gasoline anti-knock additive   16 %

Pigment   6-7 %

Others   3-4 %

1.1.2 Toxicity of lead [5]

The various sources of lead intake directly to the body can be divided in many ways as food, drink, inhalation. Lead in soils, sediment, dust also represents a potential highly important source of intake.

Lead absorbed into the human body enters the bloodstream initially, and rapidly attaches itself to red blood cells. There is a further rapid redistribution of the lead between blood, extracellular fluid and other storage sites, such that only about half of the fresh absorbed lead is found in the blood after only a few minutes. The storage site for lead is probably in soft tissue, as well as bone. There appears to be a steady accumulation of lead in bone throughout life that the amount entering long term storage increasing with uptake of lead. There is also evidence that not all of lead is immobile. Some will be continuously re-entering the bloodstream. The lead that remains in the bloodstream is lost steadily through excretion. And it is the lead circulation in the bloodstream, which is exerting adverse effects on the body. Hence, the concentration of lead in the bloodstream is an important parameter in the characterization of an individual's exposure to lead and in the relationship between exposure and occurrence of adverse consequences. The biological and adverse health effects of exposure to lead can be shown in Table 1.2.

The United State Environmental Protection Agency, U.S.EPA.[5] , in 1977 , also concluded that adverse effects to neurobehavior would be seen in some children at blood lead levels of 50 to 60 µg per 100 ml. Furthermore, intellectual deficits may occur in individuals with blood lead in the range of 40 to 80 µg per 100 ml during exposure.

Table 1.2 Symptoms of inorganic and organic lead poisoning.

Inorganic lead		Organic lead
Adults	Children	
Abdominal pain	Drowsiness	Disturbance in sleep pattern
Constipation	Irritability	Nausea
Vomiting	Vomiting	Anorexia
Physiological	Gastrointestinal	Vomiting
symptoms	symptoms	Vertigo and headache
Asthenia	Ataxia	Muscular weakness
Paraesthesiae	Stupor	

1.1.2.1 Metabolism of lead in man

The movement of lead from ambient air to the bloodstream is a two-part process: deposition of some fractions of inhaled air lead in the deeper part of the respiratory tract and absorption of the deposited fraction. The deposition rate is mainly determined by the particle size distribution of the inhaled particles and the ventilation rate. For adult humans, the deposition rate of particulate airborne lead occurring in rural and urban atmospheres is about 30-50%. All of the lead deposited in the lower respiratory trace appears to be absorbed so that the overall absorption rate is governed by the deposited rate. Respiratory uptake via respiratory tract in children, while not fully quantifiable, seems to be comparatively greater on a body weight basis [6,7].

Gastrointestinal absorption of lead mainly involves lead uptake from food, drinking water, and beverages as well as lead deposited in the upper respiratory tract and swallowed. It also includes uptake of non-food materials such as dust and paint chips, which may be unwittingly ingested by young children due to their mouthing activity and pica habits. The lead gastrointestinal lead absorption in human adult has been determined to in the order of 10-15%. The rate can be significantly increased under fasting conditions to 45% compared to lead ingested with food. For children, the fraction absorbed is much higher than in adults, ranging up to 50 % [8]. Absorption of inorganic lead compounds through the skin is much less significant than through the respiratory and gastrointestinal tract [9].

1.1.2.2 Biological and adverse health effects of exposure to lead

Lead has many adverse physiological and biochemical effects, which generally are of a deleterious. No evidence has been presented for an essential function of lead in the metabolism of human and animals. The adverse effects involved the central nervous system, kidney, cardiovascular system, and other organs and tissues, virtually every system in the body. Very severe lead exposure in human can cause coma, convulsions, and even death. It is particularly harmful to the developing brain and nervous system of fetuses and young children, and neural damage due to lead poisoning may be irreversible.

Over recent years, there has been mounting concern about more subtle chronic adverse effects to health at low level of exposure. Lower lead levels cause adverse effects on the central nervous system, kidney, and hematopoietic system. Blood lead levels as low as 10 μg/dl, which do not cause distinctive symptoms, are associated with decreased intelligence and impaired neurobehavioral development. Many other effects begin at these low blood levels, including decreased stature, decreased hearing ability to maintain a steady posture. Lead's impairment of synthesis of the active metabolite 1,25-$(OH)_2$ vitamin D is detectable at blood levels of 10-15μg/dl. Some studies have suggested harmful effects at lower levels. As yet, no threshold has been identified for the harmful effects of lead [10].

1.2 Voltammetry [11]

Voltammetry comprises microelectrolysis techniques in which the working electrode potential is forced by external instrumentation to follow a known potential-time function and the resultant current-potential and current-time curves are analyzed to obtain information about the solution composition. Depending on the shape of the potential-time perturbing signal and on the mode of the analyte transport, voltammetric techniques can be subdivided as linear potential sweep (dc) voltammetry, potential step methods, hydrodynamic methods, and stripping voltammetry.

Voltammetric techniques are distinct analytical tools for the determination of many organic and inorganic substances (primarily metal ions), which can be oxidized or reduced electrochemically (electroactive species) at trace level. The simultaneous determination of several analytes may also be possible with a voltammetric technique. Due to the analysis circumstances where the working electrode is a microelectrode, the sample solution volume is relatively large and the analysis time is short, the bulk concentration of the analyte is not changed significantly during the analysis. Thus, repeated measurements can be carried out in the same solutions. The selectivity of the techniques is moderate, but can be largely enhanced, e.g., by the combination of liquid chromatography with electrochemical detection.

The development of voltammetric techniques dates back to the pioneering work of Jaroslaw Heyrovsky (dc voltammetry with a dropping mercury electrode) in 1922 which was acknowledged by the Nobel Prize in 1959.
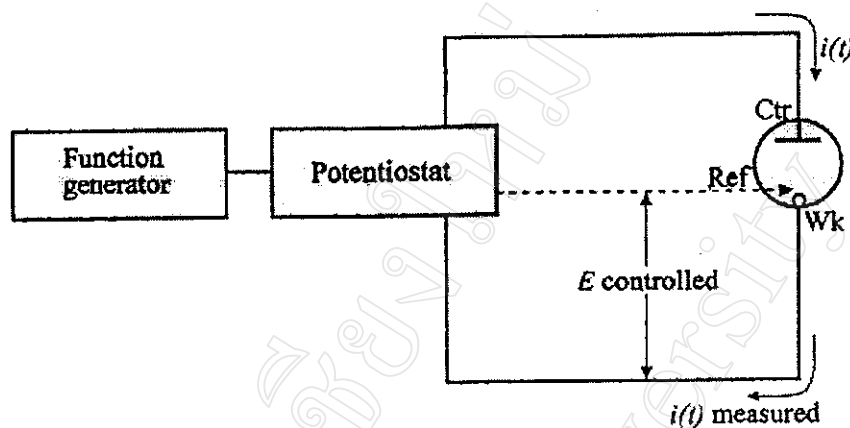
Fig.1.1 Schematic experimental arrangement for controlled potential experiments; Wk is the working electrode , Ctr is the counter electrode , Ref is the reference electrode [12].

The voltammetric cell incorporates the sample solution and a pair of electrodes of unequal size, as well as a reference electrode. The microelectrode is the working electrode, while the other current-conducting electrode is the auxiliary or counter electrode. A potentiostat control the voltage between the working electrode and the auxiliary electrode in order to maintain the potential difference between the working and the reference electrodes according to a preselected voltage-time program supplied by the function generator (or a microcomputer). The potential difference between the working and the reference electrodes is measured by a high input feedback loop (without current flow). The potentiostat is considered as active instrumentation, which has an external control on the working electrode potential experiment. However, for voltammetric analysis in aqueous solutions, a simpler two-electrode instrumentation (working and reference electrode incorporated) can also be used.

The working electrode is a ideally polarizable electrode, i.e., the electrode shows a large change in potential when an infinitesimally small current is passing through. The polarizability of the electrode is characterized by the horizontal region of the i-E curve of the electrode defining a potential window for analytical purposes, within which the

electrochemical oxidation/reduction measurements can be made. On the contrary, an ideally nonpolarizable electrode is an electrode of fixed potential, whose potential remains constant for the passage of a relatively small current, as is the case in microelectrolysis experiments. Nonpolarizable electrodes, such as electrodes of the second kind, or the large-surface mercury pool electrode, are used as reference electrodes in voltammetry. The auxiliary or counter-electrode is a current-conducting electrode (e.g., Pt wire). The voltammetric experiments are usually performed in quiescent solution in a presence of a large excess of inert salt, called supporting electrolyte.

1.2.1 Working electrodes

In voltammetry, different working electrodes can be used; among the most commonly employed ones are as follows:

## 1) The dropping mercury electrode (DME)

In classical polarography the dropping mercury microelectrode is used as a micro working electrode. The mercury drop is formed at the end of a glass capillary (length 10-20 cm, inside diameter 0.05 mm) as the capillary is connected by flexible tube to a reservoir about 50 cm in height. The mercury droplets are of highly reproducible diameter and of a lifetime ranging from 2 to 6s. The height of the Hg reservoir, i.e., the hydrostatic pressure of Hg control the drop time. The device is sometimes connected to a "knocker" to control the lifetime of drops. The advantages of the dropping mercury electrode are as follows:

- The constant renewal of the electrode surface eliminates the contamination of the electrode surface, which results in reproducible current-potential data.
- The charge transfer overvoltage of hydrogen ions present in the aqueous solvent is high on Hg, thus its reduction does not disturb the study of the

reduction processes of the electroactive species having more negative potential than the reversible potential of the proton discharge. In acidic solution, e.g., in 0.1 mol/l HCl solution at a potential more negative than −1.2 V , hydrogen gas evaluation is observed.

- Hg forms amalgams with many metals thus lowering their reduction potential.

The polarization range of the mercury electrode vs. SCE in aqueous solution in the absence of O_2 is between +0.3 and about −2.7 V. The cathodic potential limit is determined by the reduction of the cations of the so-called supporting electrolyte (a supporting electrolyte is added in order to ensure diffusion-controlled mass transport condition).The most negative polarization limit can be achieved in the presence of tetraalkylammonium cations, while the oxidation of the Hg :

$$2 \, Hg \rightleftharpoons Hg_2^{2+} + 2e^- \qquad (1)$$
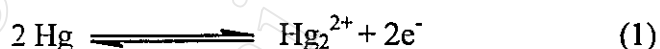
Sets a limit of polarization in the positive potential range. From this it can be concluded that reducible electroactive substances can be primarily analyzed with the Hg drop microelectrode.

2) The static mercury drop electrode (SMDE)

This electrode construction features the same elements of the dropping mercury electrode, e.g., Hg reservoir and glass capillary, but the reproducibility of drops size and the drop time are controlled more precisely. This is ensured by incorporating a solenoid-activated valve between the Hg reservoir and the capillary, and a mechanical knocker attached to the capillary. Thus, by appropriate setting of the opening and closing times of the valve, the time of dislodging of the drop can be set, and the device can be operated as a hanging mercury drop or in a dropping operation mode with controlled drop time.

The SMDE has all the characteristics of the dropping mercury electrode that make it especially advantageous for routine analytical work. An additional feature is due to the constant surface area of the drop, which will be discussed in connection with highly sensitive, current-sampled pulsed techniques.

3) Solid electrode [13,14]

For the determination of oxidizable components, solid electrodes are generally used. Among the different electrode (e.g., Pt, Au, C) used in electrolysis experiments, the different types of graphite electrodes have gained wide application in analytical voltammetry. This is attributed to features such as a large useful anodic potential range, a low electrical resistance, and an easily renewable electrode surface. Carbon paste [13] and glassy carbon electrodes [14] are used to monitor electrooxidation processes both in solution at rest, and in flowing electrolyte, e.g. in high-performance liquid chromatography. With the latter technique the analysis of electrochemically oxidizable substances, e.g., biogenic amines at the nanogram and picogram levels, can be accomplished in a low-volume voltammetric detection cell. The limit of useful anodic potential range is +1.5 V given by the evolution of O_2.

1.3 Adsorptive stripping voltammetry [15]

The demand for the detection of trace levels of substances of environmental or clinical significance is growing continuously. As a result, the technique of stripping analysis has been receiving considerable attention since it is the most sensitive electroanalytical technique. In stripping analysis the substance to be determined is preconcentrated into the working electrode prior to the actual electrochemical quantitation [15]. Most applications of stripping analysis have been concerned with heavy metal ions, which electrolytically deposit and form amalgam with mercury. In 1981-1983, it has seen that an increasing interest concerning stripping measurements of organic

and inorganic substances that cannot be accumulated by electrolysis. Alternative preconcentration schemes, based primarily on adsorptive accumulation, have been used for this purpose. The term adsorptive stripping voltammetry indicates an electroanalytical technique in which the analyte is preconcentrated first by adsorption onto the working electrode, followed by voltammetric measurement of the surface species. As a result, many important substances possessing surface-active properties are measurable at the nanomolar concentration level with detection limits comparable to those obtained in conventional stripping measurements of heavy metals.

1.3.1 Principles-operational parameters

Adsorption generally means the attachment of molecules or ions to the surface of electrodes. This phenomenon can affect the results of voltammetric studies and has been traditionally regarded as a problem. Recent work, however, has illustrated that certain advantages can be achieved via deliberate adsorption of species onto the electrode surface. For example, adsorption has been used as a means of surface modification to form a "chemically modified electrode". In adsorptive stripping voltammetry a spontaneous adsorption process is purposely utilized as a preconcentration step for trace measurements of important species that cannot be accumulated by electrolysis. The resulting voltammetric response of the adsorbed species is significantly larger than that of the solution-species alone. Hence, the detection limits are improved by several orders of magnitude as compared to the corresponding solution-phase voltammetric response. For example, detection limit near 2×10^{11} M were estimated for riboflavin and thiourea [16,17].

Anson [18] developed systematic generalization for the chemical basis of adsorption and established rules for predicting the adsorption of species on electrodes. Preliminary experiments concerning the nature of the adsorption and redox processes are usually performed using techniques such as chronocoulometry or cyclic and differential

pulse voltammetry. To achieve maximum sensitivity in the subsequent voltammetric response, optimum conditions for maximum adsorption should be utilized during the preconcentration step. The amount of analyte accumulated on the electrode surface is a composite of many variables such as the solvent, electrode material, potential, time, pH, ionic strength, mass-transport and temperature. Different analytes would respond differently to changes in these parameters, which could be useful analytically. In general, only strongly adsorbing species with relatively large ($>10^{-4}$cm) adsorption coefficients are suitable for adsorptive stripping measurements. In addition, the adsorption should proceed in a reproducible manner. Optimum conditions for maximum accumulation are usually found by examining the peak current enhancement (at a given accumulation time, over that without accumulation) using a $1x10^{-7}$M solution. Besides their effect on the peak current enhancement, some of the above parameters may influence other properties of the subsequent voltammetric measurement (e.g., reproducibility, peak shape). In various situations a compromise should be made to achieve the desired balance between sensitivity and reproducibility.

Batycka [19] and Wang [20] have evaluated the effects of accumulation potential and electrolyte composition on the adsorptive stripping response. The preconcentration should proceed at the potential of maximum adsorption. For neutral compounds, a potential close to the electrocapillary zero value would be most favorable (and thus open-circuit conditions may be used). The accumulation potential can be used to improve the selectivity and minimize interferences. For example, with a proper choice of the potential, interferences of urine constituents on the measurement of thiourea are eliminated [17]. In a similar way, other interferences, such as those associated with specific adsorption of halide ions or electrolytic deposition of metal ions, can be minimized. A unique property of differential pulse adsorptive stripping measurements is that highly dilute electrolyte solutions can be used. For certain analytes, the stripping current increases at lower electrolyte concentrations. In most cases, aqueous solutions are used; most neutral organic analytes are adsorbed from these solutions primarily because

of hydrophobic forces. A pH adjustment is essential mainly for acidic or basic analytes or when the stripping peak occurs at negative potentials. In addition, measurements of metal ions-based on the adsorption of their surface-active complexes-require optimization of the complexing ligand concentration. The choice of accumulation time requires special attention. Unlike anodic stripping voltammetry, were a linear increase of the peak current is observed with longer accumulation times, the adsorptive stripping signal levels off at prolonged periods. The saturation of the electrode surface will be discussed later. The length of the accumulation period depends on the expected analyte concentration. Typical accumulation times range from 1-5 min at the $1x10^{-7}M$ level to 10-20 min at $1x10^{-9}M$.

For most analytes the adsorption at the surface is fast and the overall rate of accumulation is governed by mass-transport. Thus, as in anodic stripping voltammetry, various forms of forced convection (stirring, rotation, flow, etc.) are employed during the preconcentration step. The convective transport is then stopped and, after a 15-sec rest period, the voltammetric measurement is performed in a quiescent solution.

Most adsorptive stripping procedures utilize the hanging mercury drop electrode for measuring reducible species, while carbon paste, wax-impregnated graphite, and platinum electrodes are used when oxidizable analytes are concerned. The hanging mercury drop electrode (especially with the static mercury drop design) offers the advantages of self-cleaning properties, reproducible surface area, and automatic control. With solid electrodes, a cleaning step may be required when the analyte is not desorbed during the voltammetric scan. Using carbon paste electrodes, the adsorptive accumulation of various compounds is accompanied by extraction into the electrode interior (pasting liquid). Because of their lower background current level, mercury electrodes offer lower detection limits (10^{-10}-10^{-11} M) as compared to those obtained at solid electrodes (10^{-8}-10^{-9} M). Relative standard deviations for replicate measurements at the mercury electrode range from 2 to 6% and from 5 to 12% at solid electrodes.

Depending upon the redox properties of the accumulated species, the species can be quantified by scanning the potential in the cathodic or anodic directions. A variety of voltammetric waveforms have been used for this purpose, including linear scan, differential pulse, square wave, staircase, ac, or subtractive modes. The differential pulse-stripping mode has been widely used because of its correction for the charging current and its commercial availability. When the redox reaction of the analyte is irreversible, the differential pulse mode offers little improvement in the response over linear scan. Under these conditions, linear scan is preferred due to its speed. Equations for the peak current and potential in differential pulse and linear scan measurements of surface-bound species have been given by Brown and Anson [21]. The measurement (stripping) step can be performed in a solution that is more favorable than the original sample [22]. For example, by transferring the electrode from the complex sample to a blank electrolyte solution, between the preconcentration and measurement steps, the selectivity is improved since interferences from solution-phase electroactive species are eliminated. Such operation can be easily accomplished using flow injection systems [23], which offer the additional advantages of speed, reproducibility, small sample volumes, and automation.

Independent of the stripping mode employed, saturation of the electrode surface results in deviations from linear calibration plots. At the low analyte levels (10^{-7}-10^{-10} M), for which the method is usually applied, linear adsorption isotherm is obeyed and the response is linear. Depending upon the operational conditions, deviations from linearity are observed at higher concentrations. The linear range differs from compound to compound based on its hydrophobicity, size, or orientation on the surface. Means to extend the linear range include the use of shorter preconcentration times, lower rates of forced-convection, and diluted samples. The use of calibration plots allows quantitation over the entire concentration range. The method of standard additions is suitable for quantitation with in the linear range; three additions are recommended to ensure that the response is within the linear portion.

1.3.2 Applications

Applications of adsorptive stripping voltammetry see Appendix B.

Table 1.3 Adsorptive stripping measurements of organic compounds.

Analyte	Working electrode	Electrolyte	Detection limit	Ref.
Heme	Hanging mercury drop	60% ethanol/H_2O	1×10^{-9} M	24
Chlorpromazine and other phenothiazines	Impregnated graphite, Carbon paste	Phosphate buffer	5×10^{-9} M	23,25
Adriamycin	Carbon paste	Acetate buffer	1×10^{-8} M	26
Butylated hydroxyanisole	Carbon paste	Phosphate buffer	2×10^{-8} M	27
Phenanthrenequinone	Carbon paste	0.1M $HClO_4$	1×10^{-9} M	28
Riboflavin	Static mercury drop	0.001M NaOH	2.5×10^{-11} M	16
Codeine, cocaine, and papaverine	Static mercury drop	NaOH	1×10^{-8} M	29
Dopamine	Platinum	Ethanol	5×10^{-8} M	30,31
Diazepam and nitrazepam	Static mercury drop	Acetate buffer	5×10^{-9} M	32
Cimetidine	Static mercury drop	0.1 M HCl	4×10^{-9} M	33
Nitro group-containing pesticides	Static mercury drop	Britton-Robinson buffer	5×10^{-10} M	34
Thiourea	Static mercury drop	0.1M $NaClO_4$	2×10^{-11} M	17
Trichlorobiphenyl	Static mercury drop	0.2M KF	4×10^{-8} M	35
Monensin	Static mercury drop	0.2M KF	1×10^{-7} M	36
Poly(ethylene glycols)	Static mercury drop	0.5 M Na_2SO_4	5×10^{-8} M	19
DNA	Static mercury drop	0.5 M McIlavine buffer	1×10^{-5} M	37

Table 1.4 Adsorptive stripping of metal ions via the adsorption of metal complexes.

Analyte	Ligand	Working electrode	Detection limit	Ref
Uranium	Pyrocatechol	Static mercury drop	2×10^{-9} M	38
Nickel	Dimethylglyoxime	Static mercury drop	4×10^{-10} M	39
	Bipyridine	Static mercury drop	2×10^{-8} M	40
Lanthanum, cerium, and praseodymium	Cresolphthalexon	Static mercury drop	2×10^{-10} M	41
Cobalt	Dimethlgloxime	Static mercury drop	1×10^{-10} M	42
Vanadium	Catechol	Static mercury drop	1×10^{-10} M	43
Copper	Catechol	Static mercury drop	1×10^{-11} M	44
Iron	Catechol,1-amine-2-naphtol-4-sulfonic acid	Static mercury drop	6×10^{-10} M	45

1.4 Pulse voltammetry

Since in this technique, i.e., normal pulse voltammetry (NPV), as well as differential pulse voltammetry (DPV), current sampling takes place at the end of the pulse, such as in pulse polarography where the sampling coincides with the end of the drop life, i.e., at the largest and most slowly growing surface, the phenomena in pulse voltammtry follow essentially the same theory and with corresponding sensitivity results; however, at stationary electrodes, even in pure supporting electrolyte, considerable residual currents, although markedly decreased at the end of the pulse, have been reported [46] , but could not be fully explained.

a) In normal pulse voltammetry and for the example of only ox of a redox couple, we preferably take E_{basic} (Fig.1.2) just before the cathodic decomposition potential, on application of the pulse an I_F (cathodic) starts at a high value, subsequently slowing down to the equilibrium value I_d (cf., Fig 1.3, with I_c only at an Hg electrode) which is sampled

at the pulse end and which agrees with a certain red/ox ratio at the electrode surface. However, after the pulse E has fallen back to E_{basic} all the red will be re-oxidized if the delay time between the pulses is sufficient; this means that even insoluble red on the electrode surface is cleaned off before the next pulse occurs, so that again the NPV yields excellent results. The reversibility of the electrode process can be checked from the E vs. $\log[(I_{c,d}-I)/I]$ by considering the slope value, $2.303RT/nF$ with irreversibility, such as for Au(III)/Au(0) at a graphite electrode, even a long delay time between the pulses cannot prevent a persistent depletion of Au (III) at the electrode; however, this could be overcome by stirring [47].

b) Using differential pulse voltammetry at stationary electrodes, excellent results can be obtained provided that oxidation and reduction are soluble, or with a mercury electrode if the resulting metal (if any) amalgamates, the voltammetric method can often be more rapid than the corresponding polarographic mode with its dependence on the drop time, provided that the delay time between pulses is not less than twice the pulse width (to avoid transient noise disturbances) and that the scan rate is not too fast (to limit dc distortion). When either ox or red is insoluble and so can precipitate on the electrode surface, NPV is to be preferred to DPV.
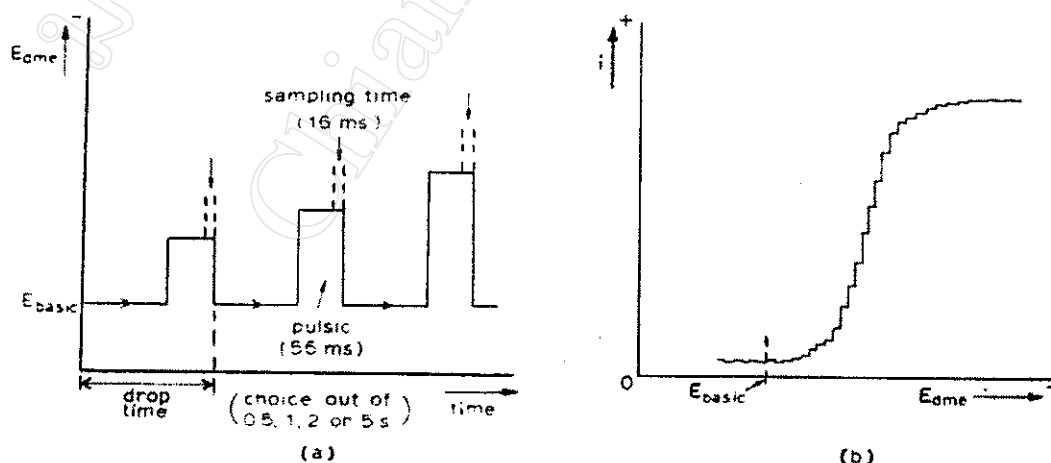

Fig 1.2 Normal pulse polarography, (a) sampling scheme, (b) current sampled.
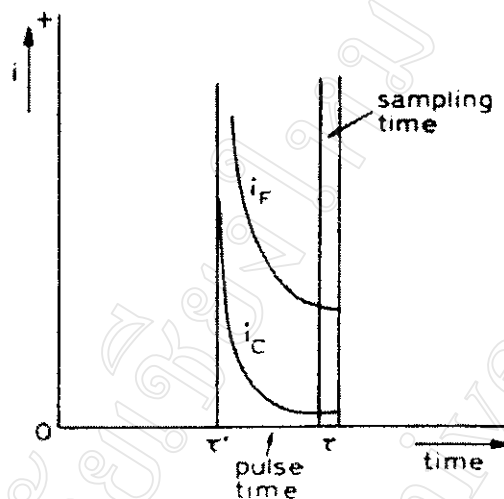
Fig 1.3 NPP, I_F and I_C vs. pulse-time course.

c) Differential double-pulse voltammetry [48] is another very interesting variant of pulse voltammetry at stationary electrodes, as it combines (see Fig.1.4) both the advantages of NPV (electrode cleaning at fallback of pulses) and those of DPV [an extreme, although not complete, elimination of residual current, especially when $\Delta i/\Delta E$ is based on the lower $\Delta E=(E_2-E_1)$ and $\Delta i/\Delta E$ on $\Delta E'=(E'_2-E'_1) = \Delta E$, respectively; moreover, for the stationary mercury electrode, owing to current sampling at the end of the pulse there is complete elimination of the charging current]. An increase in sensitivity can still be obtained by the determination of $\Delta^2 i/\Delta E^2$ on the basis of $(\Delta'i-\Delta i)$; computerization in the double-pulse technique is indicated, or course. It must be realized that the above differential valves in fact represent pseudo-derivatives (cf., under pulse polarography).
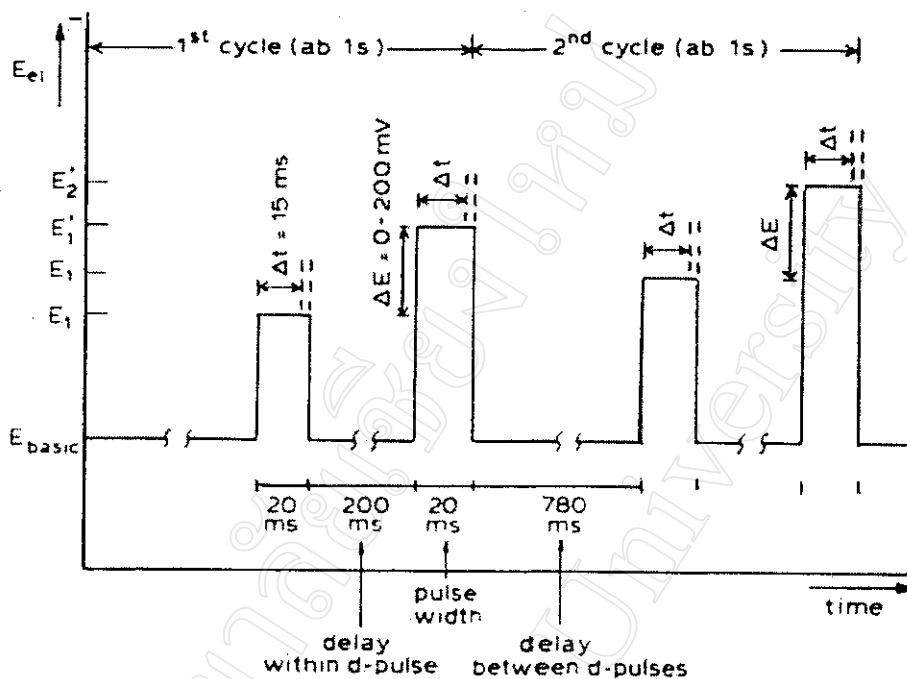
Fig.1.4 Sampling scheme of differential double-pulse voltammetry.

1.5 Chemically Modified Electrode

Although the idea of chemically modified electrode (or CMEs) was first demonstrated systematically only in the early 1970's this approach has assumed a dominant position in modern electrochemistry. These electrodes, which are made by incorporating specific chemical groupings or "microstructures" on other wise conventional electrode surfaces, are or interest because their responses have two completely separate components: the usual electrochemical component determination by the potential at which the electrode is maintained instrumentally and an additional chemical component determination by the reactivity of the attached group. Consequently, CMEs offer not only easily variable redox characteristics but also the possibility of adjustable physical and chemical properties (such as charge, polarity, chirality, permeability). Therefore, to a much greater extent than is possible with classical or

"unmodified" electrode, CMEs can be targeted for a specific application or investigation and rationally design to provide an optimal environment for that task.

Pioneering studies demonstrating the varied synthetic approaches by which modifiers can be incorporated and useful properties and applications that can result from modification have been the subject of several reviews [49,50]. In addition, hundreds of papers per year are published on CMEs. Of the disciplined directly affected by these developments, analytical chemistry is certainly one of the foremost, and virtually all the important analytical properties of electrodes-sensitivity, selectivity, reproducibility and even applicability-have been shown to be capable of enhancement by the judicious use of chemical modification. For example, CMEs capable of extremely selective binding and preconcentration of trace metals have been developed by utilizing well-known chelating agents such as EDTA and dimethyglyoxime as modifiers. Alternatively, effective rates of electron transfer have been improved for numerous compounds by attachment of electrocatalytic functional groups to the electrode surface. A few specific modifications-for example, the deposition of a polymer film such as Nafion-have been so successful that their use has become nearly routine. However, most modifications, although often well-conceived, offer advantages which have not yet been exploited in practical analysis.

The CMEs have been generally defined as a deliberate control of the molecular structure at the surface, aimed at tailoring the electrode to meet specific applications [50]. For the analytical chemistry this means the preparation of a sensor that makes the desired electrochemical reaction simpler and more rapid. Such a sensor is based on the incorporation of specific chemical groupings on the surface of a conventional bare electrode or in the bulk of a composite electrode like carbon paste. In this way, certain characteristic physicochemical and chemical properties of a chemical modifier in a solution are transferred onto the electrode. Moreover, for conditions of physical constraints, the modifier can promote new reactivities on the electrode surface. Thus, the

CMEs represent an electroanalytical device reflecting the response characteristics of two separate components:

i) the usual electrochemical component, controlled by the potential; and

ii) a specific chemical component given by the reactivity of a confined chemical modifier

In its role as a voltammetric sensor, the CMEs monitors the analyte concentration in solution without any significant effect on the level of its concentration in the bulk solution.

There are several concepts for the basic functions and applications of CMEs, and among these the following may be highlighted:

i) selective binding and preconcentration of analyte in the modifier layer;

ii) electrocatalysis of the redox reaction of an analyte with slow electron transfer at a bare electrode;

iii) incorporation of biomolecules, particularly enzymes for bioassay;

iv) permselectivity and membrane barrier effects for interfering electroactive and/or surface active species;

v) electrochemical detection of non-electroactive ionic analytes, and

vi) potentiometric response.

In many cases these concepts are combined such that the sensor attains sensitivity, selectivity, reproducibility and applicability that is considerably superior to those of a bare working electrode.

The introduction of CMEs with chemically inert surfaces as conventional working electrodes (usually represented as "ideally polarisable electrodes") has increased competitiveness. This has been such that electroanalytical chemistry has increased in scope from both the theoretical and practical standpoints.

As a result of the pioneering work of Lane and Hubbard [51], Miller [52], Murray [53] a wide range of CMEs responsive to analyte of both ionic and non-ionic types have been prepared. Also, several examples of practical analytical applications of CMEs have been reported. At the present time, CMEs have broad applications in voltammetry and as amperometric detectors in flowing streams [54,55].

1.6 Bioaccumulation

Research of chemically modified electrodes (CMEs) has experienced a quick development in the last years, especially in electroanalysis [56-58]. Since they allow preconcentration of the analyte prior to its electrochemical quantification, use of chemically modified electrode enormously improves selectivity and sensitivity in determination.

Among the methods used for the incorporation of modifiers to the electrodes are irreversible adsorption [59], covalent binding [60], coating with ion-exchange polymers [61], electrochemical polymerization [62] and direct incorporation of the modifier by mixing it with the carbon paste [63]. As for the mechanisms producing analyte accumulation on the electrode surface, they are very widely; among the most common mechanisms such as ion exchange [58], precipitation [63], complexation [64-66] and, recently, bioaccumulation [56,57,67,68] Success in their analytical applications depends mainly on the choice of modifier.

The ability of certain microorganism such as bacteria [69], yeasts [70], fungi [56,60,71], lichens [72], mosses [73,74] and water plants [75] for metal bioaccumulation has been known for quite a long time. High binding capacities have been observed under controlled conditions. Such binding or biosorption of metal ions to algae has been exploited since 1986 for effective removal and recovery of metal contaminants from water and in the selective recovery of valuable metals [76,77]. Biosorption of metal ions

to the alga cell wall is not dependent upon a living organism, and thus metal ion binding is often observed under conditions that would normally be detrimental to a living organism. Darnall et. al,[78] have reported that metal ions can be divided into three classes depending up on the pH dependence of the biosorption. The first class is comprised of metal ions that are tightly and rapidly bound at pH $\geq$ 5 and that can be stripped (or are not bond) at pH $\leq$ 2. Many metal ions fall into this class: Al(III), Cu(II), Pb(II), Cr(III), Cd(II), Co(II), Zn(II), Fe(III) and UO_2(VI). The second class in comprised of metallic anions that display the opposite behavior of class I metal ions, i.e., they are strongly bond at pH $\leq$ 2 and weakly bond or not bond at all at pH near 5. Ions in class II include $PtCl_4^{2-}$, $CrO_4^{2-}/Cr_2O_7^{2-}$, MoO_4^{2-} and SeO_4^{2-}. The third class of metal ions includes those metal ions for which there is no discernible pH dependence between pH 1 and 7, which includes Ag(I), Hg(I) and Au(III). These three ions are the most strongly bond of all metal ions tested. While the exact mechanism of binding of all metal ions is not clear at this time, it is clear that the biopolymers in the cell walls of algae are responsible for metal ion sorption. Nitrogen and sulfur have recently been shown to be ligating atoms for gold(III) and gold(I) [79]. Biosorption of metal ions in class I appears to occur via an ion-exchange process with metal cations competing with protons for negatively charged binding sites on the cell wall.

An NMR study on ^{113}Cd [80] (accumulated on a chlorella alga) revealed that metal binds to carboxylic groups belonging to the long polycarboxylic chains on the cellular membrane. Metabolism-dependent binding takes place only in living cells, able to accumulate metals through physico-chemical mechanisms: synthesis of metabolites capable of chelate formation with metal, diffusion through the cellular membrane or creation of a microenvironment around the cell allowing metal deposition or precipitation on the membrane. Deposition is usually a slow process, the metals that pass through the membrane setting in cellular organelles (vacuoles, chloroplasts, etc.) or staying in the citoplasm bond to proteins.

Wang et al.[56] have published a series of experiments carried out with alga-modified electrode in order to study incorporation of cationic and anionic complexes to the electrode

Gardea-Torresdey et. al.[57,67] performed voltammetric measurements with carbon paste electrode modified by algae, which allowed them to preconcentrate Cu(II) and Au(III)

Cornnor et. al.[68] described the development of carbon paste electrode modified by lichens for the detection of Pb(II) and Cu(II).

Wang et. al.[81] performed voltammetric measurements with carbon paste electrode modified by *Datura innoxia* , which allowed them to preconcentratrate gold.

1.7 *Pennisetum setosum*

Pennisetum setosum is a fast growing tufted annual or perennial grass weed, which has become a serious problem in southern Thailand. It occurs in young rubber, oil palm, coffee and orchards in Chumphon, Surat Thani and Nakon Si Thammarat provinces. It has also been identified as a roadside weed in the Eastern Thailand provinces of Chonburi, Rayong , Chanthaburi and Trat. *Pennisetum setosum* produces large numbers of seeds, which are carried, attached to clothing and animals and which are capable of germinating at all seasons. When grown in the greenhouse the plants show no obvious perennial characteristics except that if the plants are cut back before flowering they regrow vigorously [82].

The use of agriculture and biological materials for the removal and recovery of heavy metals from solution has been considerable investigated. The sorption capacity of these materials may be enhanced by chemical modifications [83].

Doungporn [84] modified *Pennisetum setosum* by crosslink-xanthate method [85] and use as anion exchanger. The result exhibits a high ability for ion exchanger.

Asawin [86] had used *Pennisetum setosum* modified by crosslink-xanthate method to remove metal ions especially Pb(II) from water in both batch and column processes.

1.8 Research Aims

The aims of this research can be summarized as follow:

1) To design and construct a sensor for the determination of lead(II).
2) To investigate the optimum conditions for the determination of lead(II) by bioaccumulation of lead(II) at the modified electrode.
3) To applied the proposed method for the determination of lead(II) in mineral water samples.