CHAPTER 2
LITERATURE REVIEW

2.1 Physical and Chemical Properties of Lead

Lead (Pb) has an atomic number of 82, an atomic mass of 207.19, and is one of the elements comprising Group IVa of the Periodic Table. It is a bluish-gray soft metal with density of 11.34 g/cm^3. It melts at 327.43°C and boils at 1740°C.

Lead does not react with pure water without the presence of air. In the presence of atmospheric oxygen, however, acids including very weak acids like carbonic acid and even water may react with it. Plumbosolvency is reduced by small amounts of carbonate and silicate in the water thus, it decreases with water hardness. When heated in air, metallic Pb is oxidized to PbO and, upon further heating, to Pb_3O_4.

Lead is in the +2 oxidation state in most inorganic compounds. The Pb(II) salts, lead oxides and lead sulfide, do not readily dissolve in water, except for lead acetate, lead chlorate and, to some extent, lead chloride.

2.1.1 Organolead Compounds in Gasoline

The use of Pb as an antiknock additive as far back as the 1920s has created a contamination problem of global proportions. Tetramethyl lead (TML) and tetraethyl lead (TEL) are the most important organolead compounds owing to their use as antiknock agents in gasoline. At their boiling points -- 110°C and 200°C for TML and TEL, respectively -- or slightly below, these colorless liquids begin to decompose.

Until the 1970s, most gasolines contained TEL. Eventually, concerns about the risks posed by leaded gasoline mainly the increased release of Pb to the environment coupled with the grave health risk of such, especially on children, have

paved the way for the gradual reduction then total elimination of leaded fuels (Ashworth, 1991; Cunningham *et al.*, 1994).

2.2 Sources and Pathways of Lead Exposure

At any point during its mining, smelting, processing, use, recycling or disposal, the metal and its compounds enter the environment, with the atmosphere being the major initial recipient of emissions. Lead is released into the environment primarily through the air by the burning of gasoline and solid wastes. From the atmosphere, it is deposited onto soil, surface waters, and plants resulting to its inclusion into the food chain of animals and man. Lead in air is an important component of street dust. Harrison and Laxen (1981) stated that high concentrations up to 50,000 μg/g have been found at certain sites such as car parks and garage forecourts.

With regard to their significance to human health, the main sources of Pb in the environment come from its industrial and technological uses. Industrial processes such as the manufacture of paints, various metals, batteries, glass, clay, machinery and electronic capacitors are all sources of Pb (Ashworth, 1991; Oskarsson, 1989; Stubbs, 1973). The major dispersive and non-recoverable use of the metal, however, is in the manufacture and application of alkyllead fuel additives (WHO, 1977). For one thing, the combustion of leaded gasoline contributes to the major portion of Pb found in the atmosphere (Boeckx, 1986; Ewers & Schlipköter, 1991; Stoker & Seager, 1972; WHO, 1987).

Although vehicular Pb emissions have substantially declined in developed countries like the US since the 1970s (The Conservation Foundation, 1984), reductions in gasoline Pb content have been introduced fairly recently in Asian countries. Based on the World Health Organization's (WHO) 1992 report for the Philippines, for instance, Pb emissions from motor vehicles during 1988 in Metro Manila alone reached 598 metric tons (mt) and those in areas outside Metro Manila,

788 mt (Alto, 1995). Moreover, on that year, two out of eight monitoring sites along major thoroughfares in Metro Manila exceeded the WHO annual mean guideline range of 0.5 to 1.0 $\mu g/m^3$ by a factor of three. The introduction of low-lead gasoline in 1993, however, reduced Pb emissions from motor vehicles in Metro Manila and outside Metro Manila by 17% and 18% of the 1988 levels (Alto, 1995). From these figures, it can be presumed that air Pb levels likewise dropped significantly over the 1988-1993 period.

Similarly, in Thailand, 1993 monitoring data from 15 stations in Bangkok showed that air Pb levels ranged from 0.06 to 1.66 $\mu g/m^3$ during a 24-hour period (Boontherawara *et al.*, 1994; Pollution Control Department, 1993). Likewise, in 1994, it was found that the maximum air Pb in Bangkok within a 24-hour period was 2.93 $\mu g/m^3$, lower than the 10 $\mu g/m^3$ standard value for Pb in Thailand for the same period (Wangwongwatana, 1996). The drop in air Pb levels in Bangkok is linked to the reductions of Pb quantity in gasoline from 0.84 g/l to 0.45 g/l in 1984, and subsequent reductions to 0.15 g/l by 1992. Furthermore, premium unleaded gasoline was introduced to the market in 1991 (Boontherawara *et al.*, 1994).

The phase out of leaded gasoline can be a significant step in the reduction of a major source of exposure. Estimates in 1992 indicate that the market share of unleaded gasoline has reached about 72% worldwide (Wangwongwatana, 1996). However, recent estimates of the use of leaded gasoline in the Americas alone indicate that approximately 9,000 tons/year of Pb are still being added to gasoline (Finkelman, 1996). Approximately 75% of the Pb emitted in automobile exhaust is released as particulate lead (PbO_x particles) which can float in the air for a long time and can enter the human body through inhalation (Wangwongwatana, 1996). Additionally, the remaining percentage form vapor-phase organolead emissions which come from the production of unburned TML or TEL. Consequently, the highest concentrations of organolead compounds, particularly TML and TEL, are found in parking garages, gasoline stations, and busy streets in urban areas (Ewers & Schlipköter, 1991).

From a mass balance point of view, the transport and distribution of Pb from stationary or mobile sources is mainly through the air. Large amounts are also discharged into natural waters and onto soil, such as from wastewater discharge like sewage sludge (Harrison and Laxen, 1981). Lead tends to be localized near the points of such discharges.

Except in the immediate vicinity of mining, smelting, and manufacturing facilities, where concentrations are locally high, outdoor Pb levels increase from rural to urban locations. The concentration of Pb in air varies from 0.1-10 $\mu g/m^3$ in urban areas to 0.008-0.01 $\mu g/m^3$ in rural or remote areas. Moreover, areas with dense automobile traffic such as near urban freeways can reach 8.2-18 $\mu g/m^3$ (Harte *et al.*, 1991).

For the general population, exposure to the metal occurs from inhaled air, dust of various types, and food and water, with approximately 50/50 division between inhalation and ingestion routes (Lewis, 1993).

Lead in drinking water may come from the contamination of the water source or from the use of Pb materials in the water distribution system. Lead entry into drinking water by the latter is enhanced when the water supply is low in calcium and magnesium and has pH below 6.5.

Lead in the diet may come from crops absorbing it from the soil, dry fallout from the air onto leaves, absorption from cooking water, contamination during processing, solder from cans, and leaching from storage materials. The consumption of food and beverage prepared and/or stored in lead-glazed ceramics or pottery is a significant risk factor for elevated blood Pb levels among consumers.

Another important source of exposure is the use of lead-based paints which continue to pose a hazard particularly for children. Certain hobbies such as making

stained glass and molding tin soldiers are also associated with elevated Pb exposure (Oskarsson, 1989).

In addition, a fact that must not be neglected is that most acute and even fatal Pb poisoning cases in developing countries are associated with uncontrolled cottage industries, in particular battery recycling. On a global scale, 63% of all processed Pb is used in the manufacturing of batteries; as yet, no reliable and practical alternative technology is available (Finkelman, 1996).

2.3 Metabolism of Lead

2.3.1 Absorption, Distribution and Retention

There are different factors which affect the absorption of Pb from environmental sources. On the one hand, the amount of Pb available for entry into the human body must be considered. On the other hand, the chemical and physical forms of the metal are also important factors affecting its biological behavior in the body. Absorption is also influenced by host factors like age and physiological status. Adults absorb about 5-15% of ingested Pb and retain less than 5% while children absorb 50% and retain about 30% (Lewis, 1993).

Organic Pb compounds, like TML or TEL, are readily absorbed by the body through the skin or mucous membranes. Inorganic Pb, such as those compounds that are released from the combustion of leaded gasoline, is absorbed primarily through the gastrointestinal and respiratory tracts and is the main source of Pb for the body.

Little is known about Pb transport across the gastrointestinal mucosa. It has been speculated that Pb and calcium (Ca) may compete for a common transport mechanism, since there is a reciprocal relationship between the dietary content of Ca and Pb absorption (Goodman & Gilman, 1996). Nevertheless, the distribution of Pb in

the human body is relatively less vague. Experimental studies have shown that it is dispersed among several physiologically distinct compartments that differ in size and accessibility. Certain metabolic models have likewise been proposed by several authors to describe the absorption, distribution, deposition, accumulation and excretion of Pb both qualitatively and quantitatively in the different compartments and their interrelations (Todd *et al.*, 1996).

After absorption, inorganic Pb in the blood is distributed initially in the soft tissues, particularly in the tubular epithelium of the kidney and in the liver (Goodman & Gilman, 1996). This aspect is important since the contact between Pb and soft tissues such as kidneys and the nervous system is responsible for most of the metal's toxicity (Todd *et al.*, 1996).

In time, Pb is deposited mainly in the bones, and to some extent, in teeth and hair. Lead in the skeleton comprises the largest portion of the body burden. This compartment contains about 95% of all Pb in the human body in adults (Ewers & Schlipköter, 1991; Todd *et al.*, 1996; WHO, 1977) and approximately 70% in children (Boeckx, 1986; Todd *et al.*, 1996). Quantitative estimates of the biological half-life of Pb in bone vary. Data suggest that the half-life may depend on bone type (i.e., cortical or trabecular) or even bone-site. Generally, it is said that higher concentrations of Pb are found in the long bones although more Pb can be found in the flat bones after recent exposure (Goodman & Gilman, 1996). Likewise, it has been written that the concentration of Pb in bone increases throughout most of life (WHO, 1977). Thus, most estimates give a half-life of Pb in bone that is measured in years or even decades.

An unpleasant aspect of retained Pb in the bones is that it can be redistributed to other parts of the body long after its initial absorption. Indeed, Pb can be hypothetically remobilized from the skeleton under any circumstance that increases bone mineral turnover (Todd *et al.*, 1996). For instance, at times of Ca deficiency or greater Ca requirements, such as during pregnancy, Pb can be mobilized from bone and

enter the bloodstream (Harte *et al.*, 1991). Other potential examples include lactation, menopause, and hypermetabolic states including Paget's disease of bone and thyrotoxicosis (Todd *et al.*, 1996).

Lead mobilization during pregnancy is potentially very hazardous to the fetus since Pb passes across the placenta almost without hindrance (Barltrop, 1973; Davis & Svensgaard, 1987; Saxena *et al.*, 1994; Todd *et al.*, 1996; WHO, 1977). In addition, there exists a concern about the possible contribution to neuropsychological dysfunction for menopausal women as a result of mobilized Pb during bone demineralization (Todd *et al.*, 1996).

Unlike in the skeletal system, most soft tissues do not exhibit a significant age-related change in Pb concentration after the second decade of life (WHO, 1977). This is also observed with the concentration of Pb in whole blood and in blood serum. This makes the skeleton serve as a "sink" and thus reflects the long-term cumulative human exposure while the body fluids and soft tissues equilibrate reasonably fast and therefore reflect current and recent exposure. This, as well as the fact that it is conveniently accessible, make the blood the more frequently measured component. According to the Rabinowitz model, Pb in blood (PbB) makes up about 1% of the body burden, with a large portion of it found in the erythrocytes (Todd *et al.*, 1996, WHO, 1977).

The half-life of PbB usually is 2-4 weeks and a steady state is achieved in 5 months (Goodman & Gilman, 1996). Todd *et al.* (1996) specifically stated that PbB half-life is 36 ± 5 days.

2.3.2 Elimination

The biological half-time of Pb in the body is difficult to predict. The constantly decreasing availability of the major stores of Pb in osseous tissue makes it virtually impossible to describe the rate of loss from the body in simple terms. However, it is

clear that in man, the clearance of one-half of a body burden of the metal would require a number of years.

The elimination of Pb from the body is thought to be mainly through urinary excretion and by way of the gastrointestinal tract. Much of the ingested Pb passes through the body unabsorbed, and is eliminated in the feces. The greater portion of the Pb that is absorbed is caught by the liver and excreted, in part, in the bile. Urinary excretion is the more important route and past studies have shown that Pb concentration in urine is directly proportional to that in plasma. The WHO (1977) cited a study by Rabinowitz and colleagues (1973) on the relative contributions of the different routes of Pb excretion in man. This study gave losses in urine as 38 μg (76%), in gastrointestinal secretions as 8 μg (16%), and in hair, nails, sweat, etc. as 4 μg (8%), on a daily basis.

2.4 Toxicology of Lead

The hazardous effects on human health caused by Pb is a well-documented fact. The metal has been known to affect the human nervous system, the production of blood cells, kidneys, reproductive system, and behavior. At the typical levels to which individuals are normally exposed, the major organ systems affected are the blood and nervous systems (Lewis, 1993). Since its effects are cumulative, long-term (chronic) exposure to low concentrations is as hazardous as short-term (acute) exposure to high concentrations.

2.4.1 Hematological Effects

Although there are different toxic effects of the metal in soft tissues, the most sensitive system that is attacked is the hematopoietic system. The final effect is microcytic anemia, similar to that caused by iron deficiency (Boeckx, 1986).

Lead causes a decrease in red cell survival and a decrease in the rate of globin synthesis. It interferes with heme synthesis at several enzymatic steps. In the latter, Pb is known to inhibit δ-aminolevulinic acid dehydratase (ALAD). Delta-aminolevulinic acid synthetase (ALAS) is a rate-controlling enzyme in the heme biosynthetic pathway and is subject to negative feedback regulation. Thus, a decrease in heme synthesis due to ALAD suppression leads to an enhancement of ALAS activity, resulting to subsequent accumulation of δ-aminolevulinic acid (ALA) in blood and urine.

Ferrochelatase, the enzyme that catalyzes incorporation of iron into the porphyrin ring, is likewise inhibited by Pb. This causes a build-up of protoporphyrin IX in the erythrocyte. Measurement of erythrocytic protoporphyrin (EP) or zinc protoporphyrin has thus developed into a major diagnostic test in detecting Pb poisoning. In children, the threshold for EP in the erythrocyte is a Pb concentration of about 20-25 μg/dl (Boeckx, 1986).

2.4.2 CNS Effects, Neurobehavioral Deficits and Neuromuscular Effects

Both the central nervous system (CNS) and the peripheral nerves are also affected by increased Pb exposure. Heavy Pb exposure has been linked to encephalopathy in children and adults although it is much more commonly associated with childhood Pb poisoning. The main features are dullness, restlessness, irritability, headache, muscular tremor, hallucinations, and loss of memory and ability to concentrate. These signs and symptoms may progress to mental disturbance, convulsions, paralysis and coma.

At PbB concentrations of 80-100 μg/dl or higher, acute lead encephalopathy is observed in children (Goyer, 1993). Permanent neurological sequelae such as recurrent seizures, behavioral changes and mental retardation occur in children who have survived acute lead encephalopathy (Boeckx, 1986).

In recent years, concern has been focused on the impairments of CNS functions due to lower intensities of exposure that do not give rise to overt signs of Pb toxicity. Experimental evidence now suggests that blood Pb levels below 10 μg/dl can have the effect of diminishing the IQ of children (Lewis, 1993) and that effects on the CNS in infants and young children occur with PbB levels of 10-15 μg/dl (Goyer, 1993). Davis and Svensgaard (1987) linked impaired neurobehavioral development, among other things, with fetal exposures to Pb at blood levels as low as 10-15 μg/dl or possibly lower. More recently, Rice (1996) examined the congruence between epidemiological and experimental data with regard to the behavioral effects of Pb. This review stated that increased body burden of Pb results to decreased scores on measures of intelligence from early infancy through school age. Furthermore, neuropsychological deficits such as increased distractibility, short attention span, impulsivity, and inability to follow simple and complex sequences of directions are associated with increased Pb body burden.

In the peripheral nervous system, neuromuscular syndrome or lead palsy has been known to occur due to severe Pb poisoning. Such cases however, very rarely happen today. In recent times, attention has been given mainly to electrophysiologically detectable functional abnormalities that occur in the peripheral nerves without clinical neurological signs. Certain neurophysiological abnormalities recorded include slowing of the nervous motor conduction velocity, particularly that of the slower fibers, and electromyographic abnormalities like fibrillations and a reduced number of motor units in maximal contraction (Ewers & Schlipköter, 1991).

2.4.3 Renal Effects

Although the effects of Pb on the kidney are less dramatic than on the CNS and blood systems, nephropathy does occur. Two general types of effect have been described. The first one is a rather clear-cut renal tubular damage characterized by generalized aminoaciduria, hypophosphataemia with relative hyperphosphaturia, and

glucosuria. The condition is caused by decreased tubular reabsorption and, therefore, reflects proximal tubular damage. Such effects have been manifested in children with clinical Pb poisoning or undue high PbB levels, as well as in lead-exposed experimental animals (Ewers & Schlipköter, 1991; WHO, 1977).

Another form of renal effect is referred to as chronic lead nephropathy. It is characterized by slow development of contracted kidneys with arteriosclerotic changes, interstitial fibrosis, glomerular atrophy, and hyaline degeneration of the vessels. This syndrome is progressive and may end in renal failure.

2.4.4 Cardiovascular Effects

Several studies have shown the association of lead poisoning with cardiotoxic effects (electrocardiographic abnormalities) (WHO, 1977). Ewers and Schlipköter (1991) cited several studies that showed a positive relationship between PbB and blood pressure at moderately increased PbB levels. Such positive linkage has also been consistently reported in epidemiologic studies during the past 5 - 10 years (Schwartz, 1995). Thus, elevated blood pressure is identified as the critical effect of Pb in adults (Goyer, 1993).

Additionally, analyses of data from the British Regional Heart Study and US National Health and Nutrition Survey (NHANES II) -- two large-scale general population studies -- gave significant associations between PbB and elevated blood pressure in man. The above studies point toward moderately increased PbB levels of >30 μg/dl as being associated most clearly with blood pressure increases, although there exists the possibility that this effect can also occur at lower PbB levels. It must be noted however, that the lead-blood pressure effect is rather small compared with effects from age, body weight, and hereditary factors. As such, they are considered more significant determinants of blood pressure than Pb.

The biochemical and physiological mechanisms by which Pb affects the cardiovascular system to induce elevations in blood pressure have not yet been completely evaluated. Changes in vascular reactivity and sympathetic tone, both of which may be dependent on lead-related changes of the intracellular Ca ion concentration appear to cause increase of blood pressure (Ewers & Schlipköter, 1991; Schwartz, 1995).

2.4.5 Gastrointestinal Effects

The smooth muscle of the gut is affected by Pb resulting to intestinal symptoms that are an important early sign of exposure to the metal (Goodman & Gilman, 1996). Symptoms of the abdominal syndrome are vague and include anorexia, muscle discomfort, malaise, and headache. Constipation is also an early sign particularly in adults, but diarrhea occasionally occurs. Anorexia and constipation become more marked while intoxication progresses. Lead colic or severe abdominal pain caused by intestinal spasm is the most distressing feature of advanced abdominal syndrome.

2.4.6 Other Effects

In addition, other effects of severe intoxication of Pb on reproduction, such as sterility, abortion, stillbirths, neonatal mortality and morbidity have all been reported (Davis & Svensgaard, 1987; Ewers & Schlipköter, 1991; Goyer, 1993; WHO, 1977). Other reports have indicated associations with myocarditis and hepatic effects. Low levels of Pb also impair neurotransmission and immune system function (Lewis, 1993).

2.5 Measuring the Concentration of Lead in Blood

Human blood carries with it necessary components to all parts of the body and it can be obtained rather easily and frequently in routine procedures. The generally accepted measure of an individual's current exposure to Pb is the concentration of lead

in blood (PbB). As such, blood samples are usually analyzed for purposes of exposure assessment, surveillance and epidemiological studies in industrial hygiene as well as environmental health.

Namihira and colleagues (1993) investigated the Pb concentrations in blood and milk in lactating women living within a 200-m radius of three smelters in Mexico City to elucidate the potential hazard of Pb to infants and women in these areas. The mean PbB level was 45.88 μg/dl and the geometric mean of milk Pb level was 2.47 μg/dl. The Pb levels detected in women's blood in this study were considered high enough to pose a threat to the nursing infant. Furthermore, 63% of the population went over the 35 μg/dl limit recommended for working environments. The PbB levels and milk Pb levels presented a high correlation; therefore, the women in this study have high body burdens of Pb, and the nursing infant is at a high risk of Pb exposure via milk.

Likewise, a preliminary investigation was made in a hospital in Lucknow, India by Saxena and colleagues (1994). This was carried out to provide information on the possible range of Pb exposure in pregnant women and fetuses, to correlate high Pb levels with various socioenvironmental factors, and to examine any possible association between reproductive outcome and Pb levels in the population under study. Results from this study which showed incidence of higher Pb levels in maternal blood (17% and 38% with Pb >25 μg/dl in normal and abnormal deliveries, respectively) and cord blood (>10 μg/dl) reflect a need for regular monitoring and lowering of environmental Pb exposure.

Eidson and Tollestrup (1995) compared the pre- and post- cleanup PbB levels of residents living near an inactive lead/zinc smelter site in Socorro, New Mexico, where elevated soil Pb levels up to 24,800 mg/kg had been documented. They reported that PbB levels prior to the cleanup ranged from undetectable (<5 μg/dl) to 29 μg/dl; after the cleanup, they ranged from undetectable to 10 μg/dl.

Most studies involving PbB measurements, particularly in relation to exposure to leaded gasoline, have been carried out in industrialized countries. The first large population study to include the determination of PbB was carried out in the United States from 1976-1980. The NHANES II results showed PbB levels in the general population declining by 38% over the survey period -- the same period of air quality improvement (The Conservation Foundation, 1984). The decline parallels the reduced use of Pb in gasoline, which plummeted 68% between 1976 and 1981 as a result of federal regulations.

In 1984, the blood of long-distance runners in South Africa was determined to contain unacceptably high concentrations of Pb. Subsequently, a reduction in gasoline Pb content from 0.8 g/l to 0.4 g/l was made. In view of this reduction, a follow-up investigation of its effect on the PbB concentration of South African runners was undertaken in 1990 (Grobler *et al.*, 1992). The mean values of PbB concentrations were reported to have dropped from 52 to 13 μg/dl and from 20 to 8.5 μg/dl for the urban and rural trainers, respectively. The significant decrease in PbB levels was mainly attributed to the reduction in gasoline Pb levels. However, the results indicated that there still exists a certain degree of Pb exposure in athletes from non-remote areas.

Namihira *et al.* (1993) cited a WHO collaborative study in 16 capital cities including Mexico City conducted in 1980-1983. The Pb content in gasoline in Mexico is the highest (1 g/l) in the world. This was reflected in the results from the study which showed that the highest PbB levels were found in the population of Mexico City, where the median was 22 μg/dl.

In a more recent study by Wietlisbach and colleagues (1995), the trend and determinants of PbB levels in a Swiss region were analyzed over the 10-year period following the introduction of unleaded gasoline in 1985. Blood lead levels were measured in three representative samples of the adult population in 1984/1985, 1988/1989, and 1992/1993. The geometric mean PbB levels were 12.2. 8.7, and 6.8

μg/dl and 8.5, 6.0, and 5.2 μg/dl, in men and women, respectively. Their findings suggest that the changeover from leaded to unleaded gasoline has been the major cause of the PbB decline.

2.5.1 Electrochemical Methods for Lead Determination

Several approaches are available for the quantitation of Pb in environmental and biological samples such as atomic absorption spectroscopy (AAS), optical emission spectroscopy, x-ray fluorescence (XRF), isotope dilution mass spectrometry (IDMS), colorimetric and spectrophotometric analysis, and electrochemical methods. However, Pb in whole blood is normally determined using AAS, either with the Delves cup or the graphite furnace technique (Boeckx, 1986). Nevertheless, electrochemical methods (voltammetry) have also been employed for years to measure PbB concentrations (Ashley, 1994).

Earlier voltammetric methods involved the use of hot plate/acid digestion in the sample preparation then determination by anodic stripping voltammetry (ASV) or differential pulse anodic stripping voltammetry (DPASV) (Searle *et al.*, 1973; Duic *et al.*, 1973). More simple sample pretreatments have since been presented, however. Almestrand and co-workers (1987) cited the work of Morrell and Ghiridhar on Pb determination in whole blood by means of ASV using the ion exchange reagent Metexchange® instead of acid mineralization. A 1:20 sample dilution with this reagent allows the Pb in whole blood samples to be mobilized in electrochemically reducible species.

Additionally, Jagner *et al.* (1981) suggested potentiometric stripping as an alternative electroanalytical technique for Pb determination in whole blood. Unlike voltammetric techniques, potentiometric stripping analysis is not subject to interferences from other electroactive species (besides the Pb species of interest) that may be present in the matrix (Ashley, 1994; Wang, 1985). In this technique, which

was later used in a mode flow by Almestrand and co-workers (1987), the sample pretreatment involved only a 1:20 dilution in hydrochloric acid containing mercury ions. Potentiometric stripping technique was later exploited for the determination of cadmium and Pb in whole blood by Ostapczuk (1992) using 0.5 M HCl as supporting electrolyte to dilute the blood sample. More recently, Jagner *et al.* (1994) suggested another technique based on the principle of stripping potentiometry (Jagner *et al.*, 1981; Almestrand *et al.*, 1987) employing a computerized data acquisition system. This technique requires only microliter amounts of whole blood samples which are diluted in a two-step procedure. This was done first, with distilled water and then with a matrix modifier containing acid, ionic media, surface-active agent, and bismuth as an internal standard.