CHAPTER I

INTRODUCTION

Arsenic with an atomic number of 33 and an atomic weight of 74.9216 appears in group VA of the periodic table, to which nitrogen, phosphorus, antimony and bismuth also belong. It is represented by the symbol As (L. Arsenicum) and is classified as a nonmetal. According to the electronic structure with five orbital electrons in the outer shell, arsenic exhibits a valence of -3,0, +3 or +5 (1). The element is a steel-gray brittle crystalline material with poor heat and electrical conductivity (2). It can exist in other allotropic forms such as amorphous, vitreous arsenic and yellow arsenic. It does not melt under normal conditions but sublimes on heating.

1.1 Occurrence and applications of arsenic and its compounds

Arsenic has occasionally been found in the natural state, however, most arsenic is found in nature in a combined form. The native sulfides, realgar (As_2S_4) and orpiment (As_2S_3), have been known since ancient times. Most commonly, it occurs as arsenides and sulfarsenides of heavy metals such as silver, lead, copper, gold, cobalt and iron.

Arsenic is a rather ubiquitous element being widely disseminated throughout the earth's crust, in water, air, soil and living tissues. The ranges of these values are listed in Table 1. Arsenic occurs in the air in areas where coal is burned, particularly near smelters and refineries. Arsenic in soil results from agricultural use of arsenicals as pesticides, herbicides and insecticides. Arsenic in plant grown in contaminated soil was proved to be greater than in plant grown in soil not receiving manufactured arsenical applications (8). In addition, the amounts absorbed from soil by the aerial parts of plants were reported to be less than their roots (5).

In the body of man, arsenic ranked 12th (0.2 - 0.3 mg/ kg of the body weight) which was comparable to manganese, barium and iodine (7). A content of 14 - 21 mg in the standard man was resulted from a daily intake of 0.9 mg arsenic (6). It can be seen from Table 1 that foods of marine origin are much richer in arsenic than other. However, the arsenic contained in the organisms is apparently not toxic to animals or humans, and is readily excreted (5).

In a study of healthy adult human tissues by using neutron activation, the mean arsenic concentrations of most tissues were reported to lie between 0.04 and 0.09 μg/ g on the dry basis (6). The skin (0.12 μg/g), nails (0.36 μg/g) and hair (0.65 μg/g) were found to contain higher arsenic than other tissues, with no evidence of marked accumulation

Table 1 Concentration ranges of arsenic in nature and foods

Sample	Concentration range	Ref.
Earth's crust	2 - 5 µg/g	3
Sea water	3 µg/dm^3	4
Fresh water	< 10 - 1,100 µg/dm^3	5
Soil	1 - 40 µg/g	6
Algae (marine)	0.1 - 2.6 µg/g	5
Other seaweeds	0.7 - 12 µg/g	5
Food	in µg As/g of the fresh weight	
Cereals	0.18 ± 0.05	6
Fats	0.05 ± 0.01	6
Root vegetables	0.08 ± 0.01	6
Fruit and preserves	0.07 ± 0.01	6
Milk	0.05 ± 0.01	6
Meat (uncooked)	0.10 ± 0.05	6
Fish (fresh water)	2.0 ± 0.8	6
Fish (marine)	2 - 8	7
Oysters	3 - 10	7
Cow's milk	0.03 - 1.5 µg/cm^3	3

in any internal organ or tissue. The level of arsenic reported in human blood varied widely among individuals and among investigations with different methods analyzed. The quoted values ranged from 0.01 to 0.64 μg of As/cm^3 of the whole fresh blood (6,7).

Because of the brittle nature, arsenic has no use as a pure metal, it is often used in metallurgical applications as an alloying ingredient to increase hardness, corrosion resistance and toughness of metals. Arsenic and its compounds are widely used in industries, e.g. glass, paint, tanning and infrared lens industries (1). High purity arsenic element has an important use as a constituent in the production of semiconductor compounds such as gallium and indium arsenides.

Since many arsenical compounds are biologically active, once they had been used for the treatment of such parasitic diseases as trypanosomiasis, amebiasis and filariasis (5). Nowadays the advent of antibiotics rendered most of the medicinal products obsolete. The major applications are only in the area of pesticides, insecticides and herbicides. Some of the familiar pesticides are Paris green or copper acetoarsenite, lead and calcium arsenate and cacodylic acid. Sodium arsenite is used in cattle dips to control infection by ticks. Sodium arsenite in addition to disodium methyl-arsonate and cacodylic acid is used as herbicide . Arsenic acid is used as a cotton desiccant and defoliant prior to

harvesting. Other organic arsenicals such as arsanilic acid, 4 - nitrophenylarsonic acid and p - ureidobenzene arsonic acid are approved by the US Food and Drug Administration for use as growth promotants or therapeutic agents in poultry and swine feeds (5).

1.2 Effects of arsenicals on soil and plants

Arsenicals are readily adsorbed by soil colloids and are very immobile (8). Accumulation of arsenic in soil was found in the top 10 cm (9). Solubility reactions and soil types play a role in the sorption of arsenic by soil. Iron, aluminium and calcium are found to be important for arsenic fixation in soil (9). The contribution of calcium in the arsenic fixation is much smaller than that of iron and aluminium. Sorption of arsenate increased with the amount of active (extractable) iron or aluminium content of soil. Arsenic was reported to be more toxic on coarse-textured (i.e. sandy) than on fine-textured (i.e. high in clay) soil (5). This was explained in part by the marked increase in surface area as the clay content increases. Sorption of arsenic by soil was also time dependent (8).

Arsenic is not an essential plant nutrient but occasionally small yield increases have been observed at the low level of As, especially for tolerant crops such as potatoes, corn, rye and wheat. Root growth of lemon plants in solution culture was enhanced by 1 $\mu g/cm^3$ as arsenate or

arsenite but 5 $\mu g/cm^3$ of either form of arsenic was toxic and adversely affected both top and root growth (5). The growth of six vegetable crops were reduced by 50 % at levels of 6.2 to 48.3 μg of available arsenic per gram of soil as measured with the mixed acid procedure (5).

While the primary pathway of entry of arsenicals into plants is through leaves and stems, some may be absorbed by root. Absorption usually occurs within several hours after application. Arsenic is then translocated to other parts of plants such as rhizome, shoot and tuber (8).

1.3 Effects of arsenic on human beings

Toxicity of arsenic is influenced by the modes of entry which are oral administration, inhalation through the lung and absorption through the skin (10).

Elemental arsenic itself is considered to be nonpoisonous. It appears that after ingestion, most of arsenic which passes through the alimentary canal is in the unchanged form (10). Arsine gas is toxic in very low concentration in inhaled air (5). It causes hemolysis of the red blood cells, resulting in anemia, hemoglobinuria, renal damage and fatal (11). Derivatives of arsine are also skin and mucous membrane irritants, respiratory irritants and systemic poisons, for example, "lewisite" or chlorovinyl-dichloroarsine.

The symptoms of acute arsenic poisoning in man

following ingestion of trivalent arsenic are throat constriction, difficulty in swallowing and epigastric discomfort. Thereafter, violent abdominal pain accompanied by vomiting and diarrhea is experienced. In severe case, the victim may shock and have feeble pulse, and death commonly takes place within 24 hours (12). In the epidemic of arsenic poisoning in 1900, 70 deaths in Manchester and Liverpool resulted from consumption of beer containing more than 15 μg As/ cm^3 (3).

Chronic exposure to small toxic dose results in weakness, prostation and muscular aching with few gastrointestinal symptoms (12). Skin and mucosal changes usually develops together with a peripheral neuropathy and linear pigmentation in the fingernails. Headaches, drowsiness, confusion and convulsion are seen in both acute and chronic arsenic intoxication. The biochemical basis for these disturbances is probably an inhibition by trivalent arsenic of a wide range of enzymes. Enzymes containing active thiol groups are effectively inhibited through combination of arsenic with these groups (10).

The maximum long-term arsenic intake compatible with health and well being in man cannot be given with any precision because the variation in individual susceptibility is high and because the chemical form of the arsenic greatly affects its toxicity. Thus, the orchardist was found to ingest as much as 6.8 mg As /day without signs of intoxication, due presumably to the prior oxidation of the arsenic from the

trivalent to the much less toxic pentavalent form. In contrast, 30 mg As_2O_3 was found to be fatal (6).

An arsenic-thyroid antagonism in man was mentioned in reference 6 . A high incidence of goiter with deaf-mutism occurred in the Styrian Alps, the home of arsenic eaters, and in the Cordoba Province of Argentina where chronic arsenic poisoning was endemic (7). Arsenic level in the drinking water of this latter region was found as high as 1.4 mg/dm^3.

Arsenicals are placed in the group of suspected carcinogens (13). When arsenic trioxide is applied to skin, hyperkeratotic lesions appear on the skin with a delay of many years in some cases (5). These epidermiological evidences are precancerous but they can also be produced by sunlight, X-ray and thermal burns. Moreover, tumors and cancer were not produced in experimental animals by any arsenic compounds (13).

It was found that 2,3 - Dimercaptopropanol (dimercaprol) or British anti Lewisite (BAL) exerted a marked antagonistic effect to the toxic action of lewisite on animal tissue (12). It had also proved an efficient form of therapy in poisoning from organic and inorganic arsenicals (10). An alternatively effective antidote for arsenic poisoning due to accidental ingestion is freshly prepared ferric hydroxide (prepared from ferric chloride and milk of magnesia) which reacts with the arsenical forming a nontoxic compound (1).

1.4 Arsenic metabolism

The amount of arsenic absorbed and retained, and the routes of its excretion are influenced by the level and the chemical form in which it is ingested. The forms in which arsenic ordinarily occurs in foods, including the organically bound arsenic of shrimp, are well absorbed from the digestive tract, abdominal cavity and muscle tissues and rapidly eliminated mainly in the urine (5). Less than 10 % of the usual soluble forms of arsenic appear in the feces. Arsenic trioxide is also well absorbed but more of it is retained in the tissues (6).

Excretion of arsenates is faster than arsenites owing to the fact that homeostasis prevents arsenate accumulation by efficient urinary secretion while arsenite can bind to tissue proteins such as kelatin disulfides in hair, nails and skin, and is retained in the body for a prolonged period (10). Arsenites also accumulate in the leukocytes and do not cross the brain-blood barrier in man.

Urinary arsenic excretion rises with increasing arsenic intake so that total urinary arsenic excretion provides a useful index of exposure. Occupational studies indicated that workers who were chronically exposed to inorganic arsenic salts excreted an average of 70 μg As/dm^3 without symptoms of arsenic intoxication, and the level as high as 5 mg/dm^3 could be reached (5).

This study of arsenic analysis in some vegetables

is attributed to the fact previously mentioned that arsenic is widely distributed in nature and is toxic in some forms. The large amount of arsenicals imported (14) (as shown in Table 2) also implies that arsenicals are widely used in agriculture and industries. In addition, there is little information about arsenic level in environment or in biological materials in Thailand.

1.5 Literature survey of arsenic analysis in food and biological samples

Modern methods of arsenic analysis usually associate with the destruction of organic matter and the conversion of organoarsenicals into the inorganic form of the element. Both dry ashing and wet digestion procedures have been used. In dry ashing process, sample is ashed in muffle furnace. Many modifications were made by adding some chemicals as ashing aids, for example, magnesium nitrate (15,16), magnesium oxide-magnesium nitrate mixture (17,18), sodium bicarbonate and silver oxide (19). In wet digestion, such acid systems as concentrated nitric acid (20), nitric-sulfuric acid mixture (21 - 24), nitric-sulfuric-perchloric acid (25), and sulfuric acid (26), were used.

Spectrophotometry is the most commonly used method for determining low levels of arsenic in foods and biological samples, including colorimetry after generation and reaction of arsine with silver diethyldithiocarbamate (21,22,27,28)

Table 2 Amounts of arsenicals imported in kilograms (14)

Year	Arsenite	Arsenate	Arsenic trioxide, pentoxide and acid of arsenic	Organo arsenic compound
1971	10,227	16,330	124,158	5,086
1972	15,068	44	32,183	8,982
1973	(a)	(a)	95,000	8,656
1974	40,018	605	123,951	11,800
1975	(a)	6	12,022	12,006
1976	40,000	(a)	72,367	400
1977	15	(a)	74,895	39,078
1978	83 (b)	(a)	11,066 (c)	14,050(b)

(a) = no information

(b) = from January to April 1978

(c) = from January to May 1978

or with molybdate (19). More recently, procedures were developed in reducing arsenic to arsine, and arsine was determined by atomic absorption spectrophotometry (16, 23, 25, 29 - 34), flameless atomic absorption (15, 35, 36) or emission spectrophotometry (37). Though these instrumental methods of analysis are rapidly feasible and sensitive, there are some difficulties in quantitatively reducing arsenic to arsine and in collecting the gas before analysis (38). In addition, arsine is very toxic even in a small amount.

X-ray fluorescence method is limited by matrix effects and cost factors. It was applied to analysis of arsenic in marine samples (18), wine (39) and wet-digested samples from animal and plant tissues (20).

The determination of arsenic by neutron activation technique had been reported with satisfactory results. It took part in the investigation of food stuffs (40), vegetable (41), tobacco leaves (42) and plants (43-45). This technique is sensitive and requires no pretreatment of samples like other ones but nuclear reactor and sophisticated counting equipment are necessary.

The less frequently used techniques in trace analysis of arsenic in food are gas chromatography (46) and mass spectrophotometry (47).

Various electrochemical techniques have been investigated for the determination of arsenic at trace

levels (48 - 51). Electrochemical methods have great sensitivity in some cases approaching that of mass spectroscopy and neutron activation analysis. The instrumentation required is relatively simple and generally costs for less than that required for spectrochemical techniques (48). Another advantage of these techniques is their ability to distinguish between the different oxidation states of arsenic.

A comparative assessment was made of the reproducibility and sensitivity of the determination of arsenic in heat-resisting materials by using a vector polarography, an electronic polarography and oscilloscopic polarography (52). The last method is the most sensitive, but the necessity of oscilloscopic readout or of a sophisticated data acquisition system has prevented its widespread use. Anodic stripping voltammetry and differential pulse anodic stripping voltammetry of arsenic were studied by Forsberg et al (48). In spite of high sensitivity, this method is time consuming due to the preconcentration step by electrodeposition of ions prior to the stripping process and the complicated manipulation of electrodes. From information in the literature, it appeared that differential pulse polarographic technique could be used for arsenic determination in biological samples. Since commercial instruments are available, this method is reasonably alternative to other procedures.

Comparison of some analytical methods for the determination of arsenic is presented in Table 3 (53).

1.6 Differential pulse polarography (DPP)

The differential pulse polarographic technique was originally developed as an offshoot of square wave polarography in Britain in 1950 by G.C. Barker (54). The technique consists of superimposing a fixed height potential pulse at a regular interval on the slowly varying potential associated with dc polarography (see Figure 1). The pulse is so synchronized as to occur at a definite time in the life of each mercury drop. The current flow is sampled just before application of the pulse and again at the end of the pulse. The first current sampled is essentially equivalent to that obtained in the normal dc polarographic case.

Application of the pulse in the absence of a Faradaic reaction results in a current spike (Figure 2 b) due to the charging of the double layer. The charging spike decays to zero after a few milliseconds. If a pulse is applied in a potential region where a Faradaic reaction is occurring, the current-time behavior due to this Faradaic current is as shown in Figure 2 c. The current decays inversely as $t^{\frac{1}{2}}$. The sum of the currents (Figure 2 d) is observed experimentally. If a sampling of the current is taken at t_1 prior to pulse application and again at t_2 after the pulse has been applied, the capacity current

Table 3 Comparison of some analytical methods for arsenic (53).

Technique	Linear range	Limit of detection	Sample size	Recovery, %	Standard deviation	Substrate
Spectrophotometry: Silver diethyl-dithiocarbamate	1–20 μg	0.05 mg kg^{-1}	20 g	80–120		Total-diet samples
	0–20 μg	0.5 μg 0.02 mg kg^{-1}	10 g	98–109	12–14%	Chicken liver, kidney
	1–7 μg		1–4 g wet 0.5 g dry	91–98	6–8%	Marine fish, orchard leaves
Molybdenum blue	0–25 μg		5 g	91	±2% at 10 μg	Gelatin
	0–50 μg	10 μg	15 g	94	0.2%	Potatoes
	1–15 μg	0.5 μg	5 g	>90	5–16%	Sucrose, milk, blood
	1–4 500 μg		10 g	94–101		Animal tissue, food
	2–10 μg		100 g	87.6–109.3	0.037–0.225 mg kg^{-1} at 0.28–2.41 mg kg^{-1}	Red meat, poultry
Kinetic	Up to 25 μg	~1 μg per 25 ml	25 ml		2% at 10 μg	Lung tissue
Atomic-absorption spectrophotometry			0.3–0.6 g	80–103	1.0–1.4%	Shellfish, fish
	0.05–0.70 μg	10–20 ng g^{-1}	1 g	98 ± 5	1–2% at 0.1 μg	Fish, NBS materials
	0.1–1.0 μg ml^{-1}			100	2–6%	Standard solutions
		5 ng	50 ml			Water and sewage
Polarography	1–1 000 μg	1 μg		75 ± 4		Biological materials
	Up to 60 mg l^{-1}	0.22 μg l^{-1}			16% at 2 μg l^{-1}	Water, sludge, biological specimens
Anodic-stripping voltammetry	0.1–100 ng ml^{-1}	0.02 ng ml^{-1}		97	10–15%	Standard solutions
Neutron-activation analysis	4–20 μg kg^{-1}	1 μg kg^{-1}	1 g		5%	Kale, orchard leaves
		50 μg		90	6%	Biological material
Atomic-emission spectroscopy	3 ng–0.2 μg	1 ng ml^{-1}	1 ml	100	±5%	Water, sludge
		5 ng ml^{-1}	0.05–0.5g		±5%	Water, blood, plants
		0.1 μg ml^{-1}			3%	Standard solutions
X-ray fluorescence spectrometry		20–50 ng l^{-1}			±10%	Water
		50 ng ml^{-1}			±10%	Biological specimens

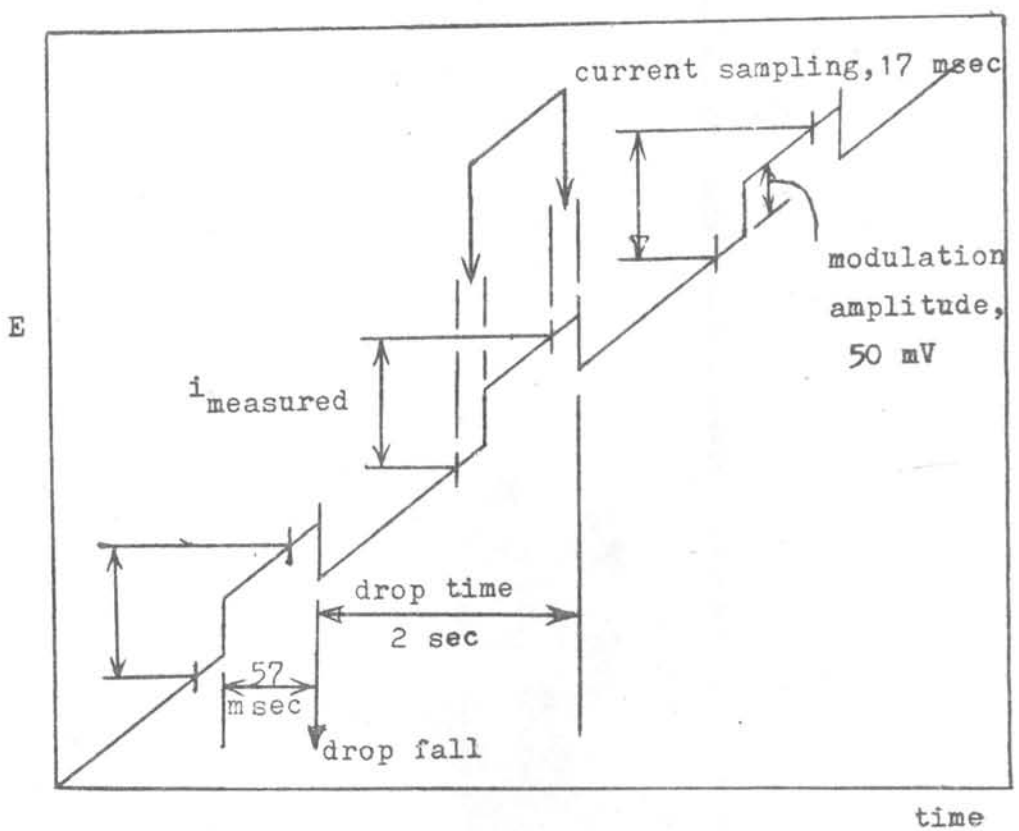

Figure 1. Potential excitation waveform used in differential pulse polarography.

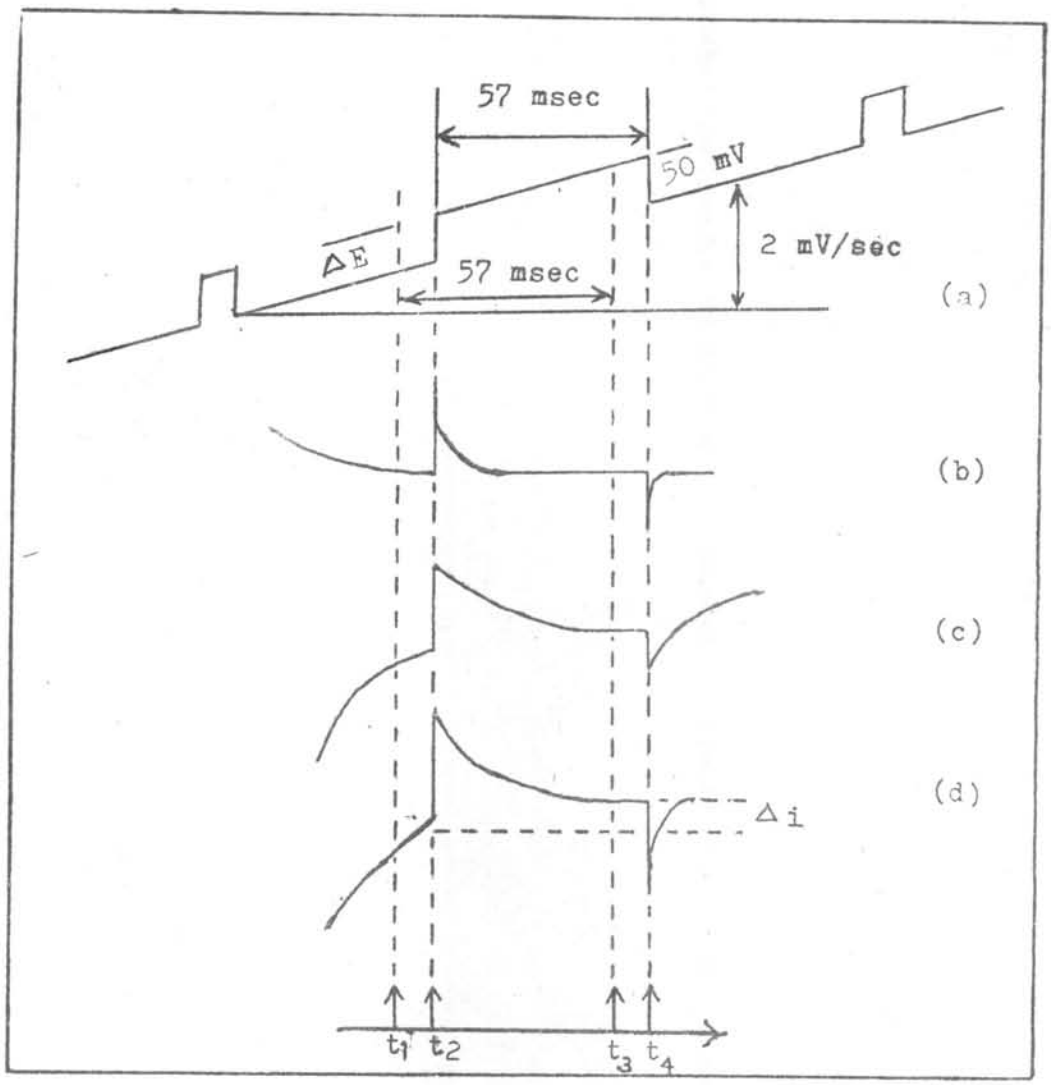

Figure 2. Potential-time behavior on application of voltage pulse (a) and resultant current-time behavior of (b) charging current only, (c) Faradaic current only, and (d) both charging and Faradaic current.

t_1, t_3 Time at which current is measured.

t_2 Time at which pulse is applied.

t_4 Time at which pulse is removed.

will be insignificant and the difference in currents between t_1 and t_3 will depend only on the Faradaic current. The effect of taking the current measurement in this manner is to increase the sensitivity and to eliminate the unwanted contribution of the charging current. The shape of the curve obtained is essentially a derivative of the normal polarographic curve(Figure 3). The clearly defined sharp peak allows precise measurement of the peak height and the exact location of the peak potential. The peak current can be simply measured from an extrapolated base line. The peak current is proportional to the concentration of the species studied, to all the other usual polarographic parameters, and to the amplitude of the pulse (54). The technique is applicable to both reversible and irreversible reactions.

Recently, Myers and Osteryoung (55) had reported the differential pulse polarography of arsenic in a number of supporting electrolytes, such as HCl, $HClO_4$, HNO_3, H_2SO_4. Its application was described by Holak for the analysis of arsenic in food (38).

In polarography it is desirable to perform quantitative measurement by a method of standard addition to compensate for matrix difference (38). The method is used when the peak height is proportional to the concentration of the substance being determined. The concentration of the unknown solution can be achieved by calculations (56). If V is the volume of the unknown

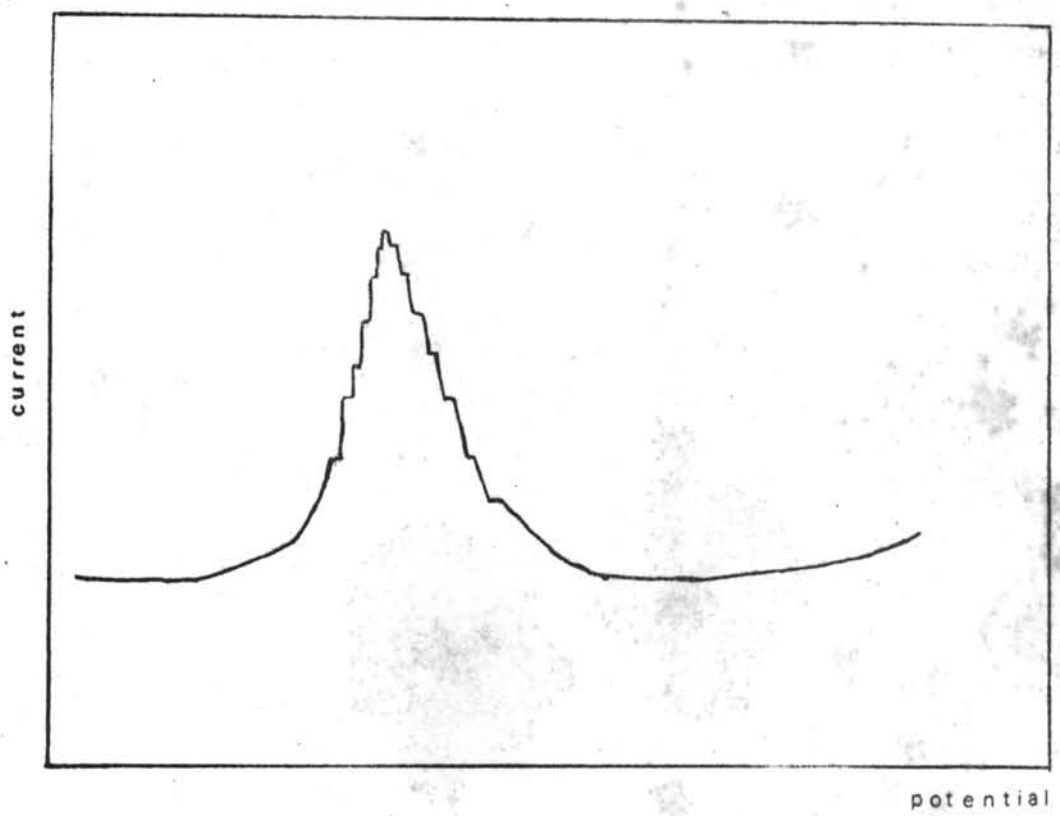

Figure 3. Typical differential pulse polarogram

solution in cm^3, C_x its concentration, i_1 its peak height at the peak potential; and if i_2 is the peak height resulted after the adding v cm^3 of a standard solution whose concentration is C_s, one has

$$i_1 = k C_x \qquad (1.1)$$

and

$$i_2 = \frac{k (V C_x + v C_s)}{V + v} \qquad (1.2)$$

Dividing eq. (1.2) by eq. (1.1) to eliminate the constant of proportionality k and rearranging the result yields

$$C_x = \frac{i_1 v C_s}{i_2 v + (i_2 - i_1) V} \qquad (1.3)$$

Differential pulse polarography associated with standard addition is a technique suitable for trace analysis of arsenic as previously described. The method was already used by Holak in the analysis of arsenic in foods (38).

Since vegetable has a part in food chain and arsenicals are widely used in agriculture and industries, the trace analysis of arsenic in vegetables by differential pulse polarography (DPP) was chosen for this study. In the present study, the DPP behavior of arsenite in various electrolytes were investigated and the interfering ions were eliminated by ion exchangers. The destruction of organic matter in vegetables was carried out by both dry ashing and wet oxidation comparatively.

Quantitative evaluation of arsenic in vegetables was obtained by means of standard addition method.