

SOME STUDIES IN NON-DISPERSIVE
ATOMIC FLUORESCENCE SPECTROSCOPY
FOR THE DETERMINATION OF ARSENIC
AND SELENIUM

by

JILLA AZAD, B.Sc., M.Sc., D.I.C.

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Chemistry Department,
Imperial College of Science and Technology,
London SW7 2AY.

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ABSTRACT

An outline of the analytical chemistry of arsenic and selenium is presented and the theory of atomic fluorescence spectroscopy is briefly explained.

A purpose built non-dispersive atomic fluorescence spectrometer is described with particular attention to the sources of radiation (EDLs).

Using the spectrometer a method for the determination of arsenic and selenium in aqueous solutions, soil digests and kale digests has been developed. The hydride generation technique is used for the introduction of the analyte into a cool argon-hydrogen entrained-air flame in which selenium and arsenic fluorescence is observed.

The interference effects of 15 concomitant elements have been studied and procedures for the elimination of the most serious effect, due to the presence of copper are recommended and applied to the analysis of real samples.

Procedures for the digestion of real samples are described and quantitative recoveries of the elements of interest are reported.

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TO MY PARENTS

WITH SINCERE THANKS

CONTENTSPage

TITLE PAGE	1
ABSTRACT	2
ACKNOWLEDGEMENTS	3
DEDICATION	4
CONTENTS	5

CHAPTER ONE

1. <u>ANALYTICAL CHEMISTRY OF ARSENIC AND SELENIUM</u>	10
1.1. HISTORY AND OCCURRANCE	10
1.2. PROPERTIES	11
1.2.1. Arsenic	11
1.2.2. Selenium	11
1.3. USES	12
1.3.1. Arsenic	12
1.3.2. Selenium	13
1.4. THE TOXICOLOGY OF ARSENIC, SELENIUM AND THEIR COMPOUNDS	14
1.4.1. Arsenic	14
1.4.2. Selenium	15
1.5. THE DETERMINATION OF ARSENIC AND SELENIUM	16
1.5.1. Arsenic	16
Gravimetric and Volumetric methods	16
Polarographic methods	17
Colorimetric methods	17
Gas-liquid Chromatography	18
X-ray Fluorescence	18
Emission Spectroscopy	18
Neutron Activation	19
Atomic Absorption Spectroscopy	19

	<u>Page</u>
1.5.2. Selenium	22
Gravimetric methods	22
Volumetric methods	22
Polarographic methods	23
Photometric methods	24
Emission Spectroscopy	26
X-ray Fluorescence	26
Neutron Activation	27
Atomic Absorption Spectroscopy	27
 <u>CHAPTER TWO</u>	
2. <u>ATOMIC FLUORESCENCE SPECTROSCOPY</u>	30
Interferences in Atomic Fluorescence Spectroscopy	37
Comparison of Atomic Fluorescence Spectroscopy with Atomic Absorption and Atomic Emission Spectroscopy	38
2.1. INSTRUMENTATION	41
2.1.1. The Spectral source	43
2.1.2. The Atom Cell	45
2.1.3. The Optics and Dispersive element	47
2.1.4. The detection and Signal Processing system	48
2.2. NON-DISPERSIVE ATOMIC FLORESCENCE SYSTEMS	49
2.3. THE NON-DISPERSIVE ATOMIC FLUORESCENCE SYSTEM EMPLOYED IN THIS STUDY	51
2.3.1. The Spectral source, Electrodeless Discharge Lamp	51
Introduction	51
Preparation of EDLs	54
Apparatus	54
Procedure	54
Operation of EDLs	59
Microwave Power and Coupling Devices	59

	The Spectral Characteristics of EDLs	65
	The Selenium Spectrum	65
	The Arsenic Spectrum	68
2.3.2.	The Atom Cell	71
2.3.3.	Detection and Signal Processing system	74

CHAPTER THREE

3.	<u>DETERMINATION OF SELENIUM BY NON-DISPERSIVE</u>	
	<u>ATOMIC FLUORESCENCE SPECTROSCOPY</u>	77
3.1.	INTRODUCTION	77
3.2.	EXPERIMENTAL	78
3.2.1.	Reagents	78
3.2.2.	Procedure	79
3.3.	OPTIMIZATION OF EXPERIMENTAL PARAMETERS	79
3.3.1.	Effect of Hydrochloric Acid and Sodium Borohydride Concentrations	83
3.3.2.	Optimization of Flame height and flame Composition	83
3.4.	CALIBRATION CURVE, LIMIT OF DETECTION AND PRECISION	87
3.5.	INTERFERENCE STUDIES	91
3.5.1.	Procedure	91
3.5.2.	The Lanthanum Nitrate Co-precipitation procedure	97
	Procedure	100
	Effect of pH on recovery	101
	Effect of time on recovery	101
3.5.3.	The Tellurium (IV) procedure	102
	Comparison of Lanthanum Hydroxide and Tellurium (IV) procedures	105

CHAPTER FOUR

4.	<u>THE DETERMINATION OF SELENIUM IN SOILS AND KALE</u>	109
4.1.	THE DETERMINATION OF SELENIUM IN SOIL	109
4.2.	THE DETERMINATION OF SELENIUM IN SOIL SAMPLES BY AFS	114
4.2.1.	The Digestion Procedure and Sample Preparation	114
4.2.2.	Procedures for Suppression of Interferences	115
4.2.3.	The effect of Potassium Bromide on the recovery of Selenium	116
4.3.	THE DETERMINATION OF SELENIUM IN KALE	120
4.3.1.	Experimental	126
4.3.2.	Procedure	127
4.3.3.	The effect of Temperature on Selenium recovery	130
4.3.4.	The effect of Potassium Bromide on Selenium recovery	130
4.3.5.	The effect of Digestion time on Selenium recovery	130

CHAPTER FIVE

5.	<u>THE DETERMINATION OF ARSENIC BY NON-DISPERSIVE ATOMIC FLUORESCENCE SPECTROSCOPY</u>	132
5.1.	INTRODUCTION	132
5.2.	EXPERIMENTAL	133
5.2.1.	Reagents	133
5.2.2.	Procedure	134
5.2.3.	Optimization of Experimental Parameters	134
	Effect of Hydrochloric Acid and Sodium-borohydride concentrations	134
	Optimization of flame height and flame composition	138
5.2.4.	Calibration curve, limit of Detection and Precision	138
5.2.5.	Interference studies	145
	Procedure	145

CHAPTER SIX

6.	<u>THE DETERMINATION OF ARSENIC IN SOILS AND KALE</u>	149
6.1.	THE DETERMINATION OF ARSENIC IN SOIL	149
6.1.1.	The effect of Potassium Iodide on the recovery of arsenic	153
6.2.	THE DETERMINATION OF ARSENIC IN KALE	156
6.2.1.	The effect of Temperature on Arsenic recovery	162
6.2.2.	The effect of Potassium Iodide on Arsenic recovery	162
6.2.3.	The effect of digestion time on Arsenic recovery	162

CHAPTER SEVEN

7.	<u>CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK</u>	164
7.1.	CONCLUSIONS	164
7.2.	SUGGESTIONS FOR FURTHER WORK	167
	Automation	167
	Optics	167
	Simultaneous multi-element analysis	168
	REFERENCES	169

CHAPTER ONE

1. ANALYTICAL CHEMISTRY OF ARSENIC AND SELENIUM

1.1. HISTORY AND OCCURRENCE

Arsenic has been encountered in nature by man since antiquity. In the First Century, Pliny stated that sandarach (arsenic trisulphide) is found in gold and silver mines and arsenicum (arsenic trioxide) is composed of the same matter as sandarach. Albertus Magnus is reputed in the Thirteenth Century to be the discoverer of metallic arsenic. It was not until 1649 that Schröder clearly reported the preparation of metallic arsenic by reducing arsenic trioxide with charcoal. By the Eighteenth Century the properties of metallic arsenic were sufficiently well known to classify it as a semimetal. Arnold de Villanova was the first to observe and describe the element which came to be known as selenium. It was not until 1817, however, that a reliable account of the isolation and identification of the new element was published by Berzelius (1). Berzelius and Gahn discovered the element during the burning of sulphur from falun pyrites. The new element was given the name of selenium, after the moon, because of its similarity to tellurium which 35 years before had been named after the earth.

The terrestrial abundance of arsenic is about 5 grams/ton although widely dispersed in nature. Some natural samples of arsenic have been found to vary in purity between 90% and 98%. The commonly associated impurities encountered in these samples are antimony, bismuth, iron, nickel and sulphur. Normally, arsenic is found combined as sulphides, arsenides, sulphoarsenides, arsenites and occasionally as the oxide and oxychloride. Arsenic is recovered as a by-product from the smelting of copper, lead, cobalt and gold ores. It may be obtained in the metallic form by the direct smelting of arsenopyrite at about 700°C in the absence of air. Commercially the metal is prepared by the reduction of arsenic trioxide with charcoal (2).

Selenium is an element which is widely distributed in small concentrations in the earth's crust, having an abundance around 7×10^{-5} weight per cent, which approximates to that of Cd and Sb (3). Minute amounts of selenium are often present in volcanic gases and magmas. Many rare minerals contain selenium as selenides (Cu_2Se , PbSe , HgSe). Selenium also occurs as an impurity in many sulphide ores, especially those with copper pyrites. The chief commercial source of selenium is the anode slime obtained from electrolytic refining of copper. It is similarly extracted from slimes obtained from lead chambers used in sulphuric acid manufacture.

1.2. PROPERTIES

1.2.1. Arsenic

Metallic arsenic is a steel-gray crystalline material which exhibits both low heat and electrical conductivity. In addition to the metallic form referred to as α arsenic, it can exist as a black, amorphous solid referred to as β arsenic, and also as a yellow allotrope. According to the periodic grouping arsenic is classified as a metalloid and belongs to group VI B . The principal valence states of arsenic are +5, +3, 0 and -3. In the elemental state it is stable in dry air and upon heating, the vapour sublimes and burns in air to form arsenic sesquioxide, As_4O_6 . It reacts with sulphur to form the compounds As_2S_3 , As_2S_2 and As_2S_5 . Hydrogen gas does not react directly with arsenic to form hydrides. Arsine, AsH_3 , can be formed chemically by the reaction of aluminium arsenide, AlAs , with HCl . Metallic arsenic is relatively inert to attack by water, alkalies and non-oxidizing acids. It will react with concentrated HNO_3 to form orthoarsenic acid, H_3AsO_4 . Thirteen isotopes of arsenic are known of which only one isotope, ^{75}As , is stable.

1.2.2. Selenium

As a member of group VI B of the periodic table, selenium displays a number of similarities to sulphur and tellurium in many of its properties.

The increasing metallic nature of the elements with increasing atomic weight is seen by the fact that oxygen and sulphur are electrically nonconducting, selenium and tellurium semiconductors and polonium a metal. The resemblance between selenium and sulphur is more pronounced in a number of respects than that between selenium and tellurium. Like sulphur, selenium has shown several allotropes e.g. trigonal (gray), α - monoclinic (red), β - monoclinic (red), vitreous (black), red amorphous and black amorphous.

The positive oxidation states of selenium are +4, +6 and only a few unstable compounds are in the +2 state. In selenides selenium assumes the oxidation state of -2. The availability of selenium 4d orbitals for bonding permits the formation of such compounds as SeCl_4 or SeBr_4 . The most stable compounds of selenium are those of the quadrivalent state. Selenium reacts directly with hydrogen, oxygen, halogens and sulphur, as well as with a large number of metals. Selenium dissolves in nitric and concentrated sulphuric acids, but does not dissolve in hydrochloric and dilute sulphuric acids. Seventeen isotopes of selenium are known (4), ranging in mass number from 70 to 87. Six are stable and the remainder radioactive.

1.3. USES

1.3.1. Arsenic

Because of its semimetallic properties arsenic is used in metallurgical applications as an additive metal. Addition of $\frac{1}{2}\%$ to 2% of arsenic to lead assists in the manufacture of lead shot to improve its sphericity. The addition of up to 3% arsenic to lead-base bearing alloys improves both their mechanical and elevated temperature properties. In minor additions arsenic will improve the corrosion resistance and raise the recrystallization temperature of copper. The largest quantity of arsenic is used in the form of chemical compounds. For example, lead arsenate is used to control fruit pests and sodium arsenite is used as a

weed killer. White arsenic trioxide, in addition to being a basic chemical for the preparation of other arsenic salts, is used as a decolourizing agent in glass manufacture. Arsenic sulphide is an ingredient in fireworks. It is also used as the compound As_2S_3 in the making of infrared lenses. High-purity arsenic exceeding 99.999% is used in the semiconductor industry. A series of low-melting point glasses containing high-purity arsenic have been developed for semiconductor and infrared applications (5).

1.3.2. Selenium

Selenium has a broad range of applications because of the variety of physical, chemical and electronic properties of the element. The principle applications are found in glass, pigments, steel, xerography and rectifiers. It was not until 1873 that its photoconductivity was noted, resulting in the development of the selenium photocell and thus the first practical application of the substance. Selenium pigments are used in paints, enamels, plastics, printing inks, glass, ceramics and rubber, e.g. cadmium sulphide selenide pigments are widely used and are preferred to the mercury red and the organic red pigments. Addition of selenium, in the form of iron selenide improves the machinability of stainless steels. There are also many minor uses of selenium: animal deficiency diseases such as white muscle disease in sheep have been counteracted successfully by the injection of small amounts of sodium selenite. Addition of sodium selenate in plating, especially in chrome plating, induces microcracking and leads to superior corrosion resistance. Many organic reactions including oxidation, hydrogenation, isomerization and polymerization are catalyzed by selenium and its compounds such as selenium dioxide. The catalytic activity of selenium has been reviewed by Kollonitsch and Kline (6).

1.4. THE TOXICOLOGY OF ARSENIC, SELENIUM AND THEIR COMPOUNDS

1.4.1. Arsenic

The toxicity of arsenic depends on its chemical state. Metallic arsenic and arsenious sulphides are almost inert while arsine, AsH_3 , is extremely toxic. The toxicity of other arsenical compounds can be classified between these two extremes. Man contains an estimated 20 mg of arsenic which is about as abundant as iodine in the human body.

From the toxicological view the arsenical compounds fall into three groups:

- 1) Inorganic arsenicals, for example "white" arsenic (As_2O_3) and the arsenate and arsenite salts.
- 2) Organic arsenicals in which arsenic is present in different oxidation states.
- 3) Gaseous arsenic (e.g. arsine).

The different oxidation states of arsenic have markedly different toxicological effects. Schroeder and Balassa (7) conclude that arsenate (As^{+5}) is non-toxic in normal amounts and is a normal constituent in food. By contrast As^{+3} is known to be highly inhibitory to enzyme systems which utilise thiol groups. The fatal dosage of As_2O_3 for man varies between 70 to 180 mg and the arsenic concentrations in blood, urine, nails and hair increases from 10 to 100 times the normal level in cases of acute poisoning. In industry arsenic is a rare cause of systemic poisoning but remains a primary toxicological hazard as a skin irritant. The major toxicity of arsine is due to haemolysis of the red blood cells, which only involves the mature cells; neither As_2O_3 nor As_2O_5 have this effect (8). Persons handling arsenic trioxide industrially wear special clothing and masks which are frequently changed. In processes emitting dusts and fumes, exhaust ventilation should be installed. The recommended maximum atmospheric concentration of arsenic in dusts, fumes or mists during an eight-hour daily exposure is 0.5 mg/m^3 . The maximum recommended exposure limit of arsine is 0.05 ppm of vapour in air per eight-hour period (9).

1.4.2. Selenium

The toxicology of selenium and its compounds is of considerable interest, particularly in view of the long established selenium poisoning of cattle foraging on seleniferous plants and the more recent extensive research confirming the nutritional essentiality of the element. The widely held belief that selenium is a toxic substance is due to the highly toxic nature of a number of plants growing in seleniferous soils which are able to accumulate as much as several thousand ppm of selenium. Although the toxic effects on humans of vegetation grown in seleniferous areas have been documented, no long-term systemic toxicity has been indicated (10). Indeed, seleniferous grains have come to be regarded as of higher nutritional value for animals than those containing no selenium. In most farming areas, the virgin soil contains so little available selenium that cultivated crops can not absorb more than traces. However the quantities absorbed vary from 0.1 to 30 ppm depending on the solubility in water of available selenium compounds. Chronic poisoning from selenium in animals occurs in the form of "blind staggers". Grain containing 10 ppm of selenium causes the typical "alkali disease" in pigs.

In industries where selenium compounds are used the atmospheric concentration should be below 1.0 mg/m^3 of air. The threshold limit given by the American Conference of Government Industrial Hygienists for hydrogen selenide is 0.2 mg/m^3 of air. Under ordinary conditions the threshold of toxicity to animals and probably also for man is placed at 3 to 4 ppm in the diet and more recently it has been stated that a concentration of 5 ppm in common foods or one tenth of this concentration in milk or water is potentially dangerous. The presence of methyl selenide in the breath, imparting a smell of garlic, is one of the early symptoms of selenium poisoning and this may occur with a daily ingestion of only a few milligrammes. It is claimed that the more refined and processed foods usually contain less selenium, that protein diminishes the toxicity

of selenium and that it is not so poisonous in high protein foods as in high carbohydrate foods (11). It is also claimed that some of the ingested selenium is stored as a protein compound in the tissues. In 1957 the role of selenium in nutrition became important when it was demonstrated that a diet to which selenium has been added at a level of 0.5 ppm prevented dietary liver necrosis in rats (12). There is also a relationship between selenium and vitamin E in reducing the incidence of retained placentae in dairy cows receiving a diet deficient in selenium. A number of papers have been published on the protective action of selenium against the toxic effects of cadmium and mercury (13) and it appears that there may be some nutritional adaptation of the organism to selenium (14). In an editorial article, "The Selenium Dilemma" (15), attempts have been made to balance the problem of dealing with selenium deficiency and cancer with the evidence that in certain circumstances the element and its compounds are carcinogenic. Hydrogen selenide is one of the most toxic and irritating selenium compounds. Although it is a gas with a very offensive smell it can cause olfactory fatigue. Thus at a concentration of 1 μg of SeH_2 per litre of air, the odour disappears quickly. Dudley and Miller (16) point out that at 5 μg per litre, SeH_2 is intolerable to man, causing eye irritation. The threshold limit value for hydrogen selenide is 0.05 ppm (by volume) of gaseous SeH_2 in air for 8 hours. (0.2 mg of Se as hydrogen selenide per cubic metre of air or 0.2 μg per litre).

1.5. THE DETERMINATION OF ARSENIC AND SELENIUM

1.5.1. Arsenic

Gravimetric and volumetric methods

The gravimetric determination of arsenic may be carried out by precipitation as arsenic trisulphide (As_2S_3), magnesium pyroarsenate ($\text{Mg}_2\text{As}_2\text{O}_7$) or as ammonium uranyl arsenate ($\text{NH}_4\text{UO}_2\text{AsO}_4 \cdot x\text{H}_2\text{O}$) (17). Several volumetric methods have been proposed for the determination of arsenic and are summarised in Table 1.

TABLE 1

VOLUMETRIC METHODS FOR DETERMINATION OF ARSENIC

<u>Method</u>	<u>Reference</u>
As silver arsenate (Ag_3AsO_4)	17
Oxidation by iodine	18
Oxidation by potassium iodate	18
Oxidation by potassium dichromate	19
Potentiometric titration using cobalt (III) acetate	20

Polarographic methods

Arsenic in the +5 state is not reduced at the dropping mercury electrode from any of the various supporting electrolytes, but arsenic in the +3 state does produce reduction waves under limited conditions (21). Bambach (22) developed a procedure for the determination of arsenic in biological material. The method is applicable to the determination of 1 μg to 1000 μg quantities of arsenic in various types of biological material using sample weights up to about 10 g. Kolthoff and Probst (23) discovered that +3 arsenic in strongly alkaline medium is oxidized to the +5 state at the dropping mercury electrode. In view of the difficulty of obtaining satisfactory reduction waves with arsenic compounds this anodic wave should be of considerable utility in practical analysis.

Colorimetric methods

Traces of arsenic may be detected colorimetrically because of the ease with which it can be separated from other elements. The "molybdenum blue" method is one of the best colorimetric methods for determination of arsenic. In this method the isolated arsenic is oxidised to As^{V} and added to ammonium molybdate to form a heteropoly molybdiarsenate which is then reduced by hydrazine sulphate to a strongly blue coloured complex (24),

the absorbance of which is measured at 8500 Å. The Gutzeit method (25) is a highly sensitive method which can detect as little as 0.1 µg of arsenic, but is not as precise and accurate as the molybdenum blue method. A method has been developed by George et al (26) to determine total arsenic in animal tissue. Dry ashing of the sample with hydrated magnesium nitrate is carried out. The ash is then moistened with water and finally made 3 M with respect to hydrochloric acid. To an aliquot zinc granules are added and the arsenic liberated is absorbed in a small volume of silver diethyldithiocarbamate. The absorbance is determined at 5400 Å. The method easily detects 0.1 ppm arsenic.

Gas-liquid Chromatography

The method described by Covello (27) involves the wet ashing of the sample and subsequent arsine generation which is mixed with helium as carrier gas. A stainless steel column at 50 to 90°C and a katharometer detector are used. The arsine peak is symmetrical and well separated from the hydrogen peak. A detection limit of 2 to 5 µg arsenic is obtainable.

X-ray Fluorescence

Using the method described by Reymont and Dubois (28), trace amounts of arsenic are coprecipitated with molybdenum as their sulphides and quantitatively determined by X-ray fluorescence. If the sample contains large amounts of lead, fluorescence line overlap is observed so precipitation of lead sulphide must be prevented. Linear calibration plots for both As^{3+} and As^{5+} from 1 to 350 µg arsenic may be obtained.

Emission spectroscopy

In this method developed by Lichte et al. (29) an arsine generator is directly connected to an excitation plasma. A 2450 MHz microwave generator is used with an Evanson quarter wave cavity to induce a plasma in argon at atmospheric pressure. Emission is measured at the 2349 Å arsenic line. The argon flow rate through the generator affects the arsine delivery rate to the plasma and hence the observed emission intensity.

The blank limited detection limit is estimated at 5×10^{-9} g. arsenic. Obviously reagents of higher purity would allow this to be reduced. Kirkbright et. al. (30) described an atomic emission method for arsenic determination using an inductively coupled high frequency plasma source. The detection limit obtained (0.11 ppm arsenic) compared favourably with flame atomic absorption spectroscopy. Recently Thompson et. al. (31) published a paper describing the determination of arsenic by employing hydride generation into an inductively coupled plasma source.

Neutron activation

Arsenic is particularly suitable for analytical investigations by neutron activation because there is only one stable isotope As^{75} which has a large cross-section for neutron capture, giving the radioactive isotope As^{76} . The half-life of As^{76} is 27 hours. The basic experimental procedure consists of irradiating the samples in an atomic pile and then digesting them with concentrated HNO_3 and H_2SO_4 . The digested sample together with 10 μg of As_2O_3 as a carrier are converted to arsine, which is collected in a mercury(II) chloride solution. The activity of As^{76} is counted using a Geiger counter. This method has been criticised by Kirshnan and Erickson (32). These authors have designed a method to overcome the errors of the above procedure by using a rapid ion-exchange separation. The lower limit of detection of their method is 10^{-10} g. Byne (33) has described a method for the analysis of hair samples for arsenic.

Atomic Absorption spectroscopy

The determination of arsenic by atomic absorption spectroscopy is not without difficulties. Two of the principal difficulties encountered are the relatively low intensity and stability shown by many arsenic hollow-cathode lamp sources and the high flame background and noise levels obtained at the resonance lines of this element below 2000 Å. At the 1937 Å resonance line using a premixed oxyacetylene flame, the detection

limit of arsenic is reported as 0.5 ppm (34). Kahn and Schallis (35) have demonstrated that the flame absorption of an air-acetylene flame is as much as 62% at 1937 Å whereas the absorption of the cooler argon-hydrogen-entrained air (Ar-H₂-EA) flame is only 15%, but the temperature of this cooler flame is still high enough to atomise the arsenic. Dagnall et al. (36) found that the flame absorbance for air-acetylene flames was very dependent on flame conditions and reaches a minimum for a flame on the verge of luminosity. Dagnall et al. also examined microwave excited electrodeless discharge lamps and also a number of different types of flames (37). Kirkbright et al. (38) described the application of a separate premixed air-acetylene flame. The use of a nitrous oxide-acetylene flame for determination of arsenic has also been described by Kirkbright and Ranson (39). The Marsh reaction of converting arsenic to arsine has been known for more than 130 years. This process of conversion is a part of the official methods of the Association of Official Analytical Chemists (40). Arsine was originally formed by the reaction of arsenic compounds with the hydrogen generated by the addition of zinc to hydrochloric acid solution (41). Pollock and West (42) used a magnesium-titanium(III)chloride reduction. Finally sodium borohydride was found to be a promising reducing agent (43) and has been used for the determination of arsenic using the hydride generation technique. Holak (44) has developed a method of collecting the arsine by freezing it in a U-tube, then introducing it into the flame by bringing the arsine to room temperature and sweeping it out of the tube using nitrogen. He obtained a detection limit of 0.04 µg arsenic. Dalton and Malanoski (45) showed that it is possible to eliminate the freezing step. Madsen (46) reacted the arsine with a dilute silver nitrate solution and then nebulized the resulting solution into an argon-hydrogen flame. Fernandez and Manning (41) collected the arsine, together with the excess hydrogen generated, for several minutes in a balloon reservoir, then

admitted the collected gases to the flame, by opening a valve, in a short burst of several seconds duration; the detection limit was estimated to be 0.02 μg arsenic. Khan (47) stresses the importance of using a deuterium background corrector to reduce the high absorption signal of hydrogen which occurs below 2100 Å. Maruta and Sudoh (48) determined arsenic using arsine generation employing different reductants. A "flameless" atomic absorption technique has been reported for arsenic determination by Chu et al. (49). In this method, arsenic is converted to its hydride, which is swept into an electrically heated absorption tube at 700°C, using argon. The background absorption is very low and an increase in sensitivity is obtained with a linear calibration curve up to 0.4 μg . Aslin (50) has also used a flameless atomic absorption technique to determine arsenic in geological material. Fleming (51) used a flame heated silica furnace to atomize arsine.

Direct determination of arsenic by atomic absorption spectroscopy has been applied successfully to several matrixes such as geological materials (50), combustible municipal solid waste (52), copper (53), (54), foods (55) and natural waters (56).

Boltz (57) and also Devoto (58) have developed an indirect method in which arsenic^V is converted into arsenomolybdic acid by the addition of ammonium molybdate. The acid is then extracted into methyl iso-butyl ketone (MIBK), and scrubbed with hydrochloric acid and water to remove excess molybdate. The heteropoly acid is extracted into alkaline aqueous medium and the Mo determined at 3133 Å (59). Yamamoto's (60) method is similar to Boltz's and Devoto's but the MIBK, which contains the arsenomolybdic acid, is aspirated directly into an air-acetylene or nitrous oxide-acetylene flame. The nitrous oxide-acetylene flame gave approximately a ten fold increase in sensitivity over the air acetylene flame. Sensitivity was 0.01 ppm of arsenic.

1.5.2. Selenium

Gravimetric methods

Selenium is commonly determined by precipitation as the element by reduction in a hydrochloric acid solution containing Se(IV) or Se(VI) with sulphur dioxide (61). As little as 2.0 ppm selenium in a 20 g. sample can be determined by this method. Different methods used for the gravimetric determination of selenium are summarised in Table 2.

TABLE 2
GRAVIMETRIC METHODS FOR DETERMINATION OF SELENIUM

<u>Method</u>	<u>Reference</u>
Mercury(II)nitrate precipitation	62
Reduction with Thiourea	62
Reduction with l-Amino-2-Thiourea	63
{ 4,5-dichloro-o-	
Phenylenediamine precipitation	64
Reduction with CuCl	65

Volumetric methods

Relatively few volumetric procedures for selenium have gained general acceptance, although electrochemical titration methods have been developed in recent years. One of the oldest volumetric methods is that of Norris and Fay (66) in which selenium(IV) is reduced by an excess of standard sodium thiosulphate solution. The excess sodium thiosulphate is back titrated with standard iodine solution. Different volumetric methods for selenium determination are summarized in Table 3.

TABLE 3
VOLUMETRIC METHODS FOR DETERMINATION OF SELENIUM

<u>Method</u>	<u>Reference</u>
Iodometry using addition of excess KI solution	67
Potassium permanganate oxidation	68
Complexing with KCN	69
Thermometric titration	70
Differential Potentiometric titration	71
Oxidation with Iodine Chloride in HCl	72

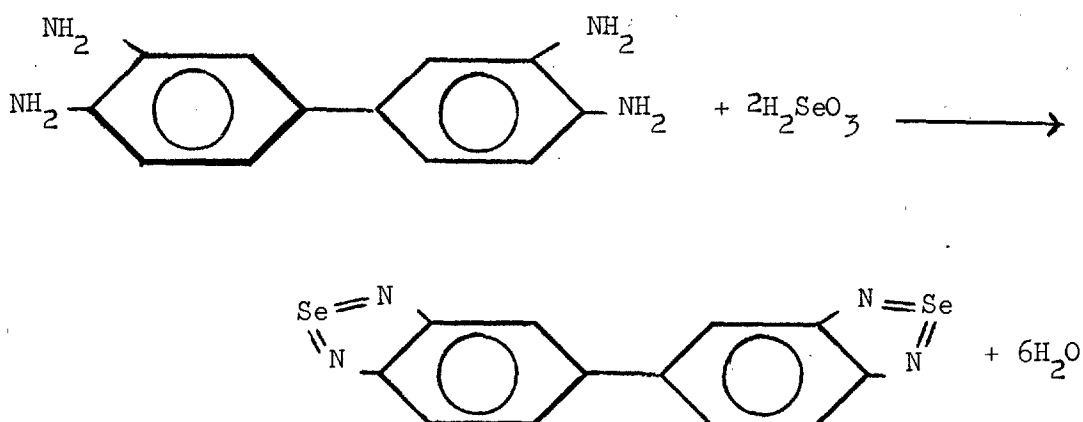
Polarographic methods

The polarographic behaviour of selenium in its various oxidation states has been studied systematically over a wide range of pH and supporting electrolytes (73). For analytical purposes, it appears that the best supporting electrolyte for Se(IV) reduction is 1 M NH_4Cl (pH, 8 to 9.5) containing 0.003% gelatin (73). A well-developed single wave is produced denoting reduction to Se^{2-} and the diffusion current is directly proportional to the Se(IV) concentration. A serious limitation in the application of polarography to the determination of selenium is the fact that the apparent height of the Se(IV) wave can be decreased if metal ions such as copper(II), which form insoluble selenides, are present in the solution. Following a prior separation of selenium by reduction to the element, Nangiot (74) found a sensitivity of 0.2 $\mu\text{g/ml}$ for Se(IV) in 0.7 M HBr. An interesting microdetermination of selenium has been developed which is based on the polarographic reduction of diphenylpiaszelenol formed by the reaction between Se(IV) and 3,3'-diaminobenzidine (75). In the presence of sulphite, the

selenosulphate ion in 0.6M NH_4OH - 0.6 M MNH_4Cl gives a square-wave polarogram (76). It is assumed that the mercury electrode catalyzes the decomposition of selenosulphate to sulphite with the latter being adsorbed on the electrode and reduced at the potential peak of the selenosulphate ion. Thus the reversible two electron reduction is that of selenium to selenide. In general polarography lacks the necessary specificity and sensitivity for the trace analysis of selenium, particularly in natural products.

Photometric methods

One of the most sensitive methods for the determination of selenium is based on the reaction of Se(IV) with 3,3'-diaminobenzidine at pH 2.5 (77), viz.



The intense yellow compound, diphenylpiaszelenol, which is formed can be extracted quantitatively by toluene at pH above 5. Beer's law is obeyed over the range of 5 to 25 μg of Se per 10 ml of toluene at the wavelengths 3400 and 4200 $\text{\AA}$. The use of E.D.T.A. permits the determination of selenium in the presence of iron, copper, molybdate, nickel, cobalt, tellurium, arsenic and up to 5 mg of vanadium (V). According to Cheng (77) the limit of sensitivity of the method is 50 ppb with a 1 cm absorption cell. Watkinson (78) utilized the strong fluorescence of the diphenylpiaszelenol at 5800 $\text{\AA}$ to determine as little as 0.02 μg of selenium. Interferences can be avoided by the extraction of selenium

with toluene-3,4-dithiol into a 50% mixture of ethylene chloride and carbon tetrachloride from strong hydrochloric acid solution. A bibliography ^{on} the use of 3,3'-diaminobenzidine as an analytical reagent especially for selenium has been compiled by Broad and Barnard (79). Other reagents such as 2,3-diaminonaphthalene and 4,5-diamino-6-thiopyrimidine which also form piasselenols with SeIV have been investigated. According to Lane (80), 3,3'-diaminobenzidine is less sensitive than 2,3-diaminonaphthalene and suffers from the disadvantage of a relatively high and variable blank. Coles (81) has chosen diaminonaphthalene as the most sensitive and suitable reagent for selenium determination with detection limit of 0.05 μg . The reagent 4,5-diamino-6-thiopyrimidine, can be used to determine Se(IV) directly in aqueous solution at pH 1.5 to 2.5 (82). It should be noted that reagents forming piasselenols are subject to air oxidation (82) and suitable precautions must be taken in their use.

Kirkbright and Ng (83) used thioglycollic acid to determine selenium in the presence of tellurium, provided that tellurium is oxidized to the hexavalent state. The yellow thioglycollate complex extracted into ethyl acetate obeyed Beer's law at 2600 $\text{\AA}$ in the range of 6 to 34 ppm. An older but still very useful photometric method for determining micro amounts of selenium is that in which a colloidal suspension of selenium is formed by a reducing agent such as SO_2 , tin (II) chloride or ascorbic acid. The use of methylcellulose permits a nephelometric determination of selenium in the range 0.7 - 4.5 μg Se per ml without interference from copper, iron or tellurium (84). Alternatively, the elemental selenium can be extracted into benzene or toluene and determined spectrophotometrically at 2760 $\text{\AA}$ with a sensitivity of 1 $\mu\text{g}/\text{ml}$ Se. Busev (85) has investigated a number of reagents containing sulphydryl groups such as 2-mercaptobenzimidazole and N-mercaptoacetyl-~~7~~ toluidine which react with selenium(IV). The resulting yellow compounds can be

extracted by a mixture of butyl alcohol and chloroform.

Emission Spectroscopy

As selenium has no sensitive spectral lines above 2200 Å the determination of selenium by optical emission spectroscopy is really beyond the range of the ordinary spectrographs and demands a vacuum spectrograph. However with the introduction of inductively coupled plasma which provides a uniquely effective excitation source for emission spectrometry, determination of very low levels of selenium has been possible.

Kirkbright et. al. (30) used an inductively coupled high-frequency plasma source to determine selenium down to 0.11 ppm. The calibration graph stabilised at 1960 Å was linear up to a concentration of 50 ppm of selenium in aqueous solution. The excellent detection limit of 0.8 ng/ml selenium has been reported by Thompson et. al. (31), using hydride generation technique together with an inductively coupled plasma as the source of excitation.

X-ray Fluorescence

If a suitable collection or separation method is employed, there is no difficulty in determining small amounts of selenium by X-ray fluorescence spectroscopy. Thus, selenium can be isolated using arsenic as the collector and hypophosphorous acid as the precipitating agent. The precipitate is collected on a micropore filter and analyzed for selenium by measuring the intensity of the selenium $K_{\alpha 1}$ peak. The detection limit for this method is 1 ppm (86). The intensities of the few X-ray emission lines of selenium are very low, so were not originally of much analytical value. With the advent of more accurate measuring devices, however, more accurate quantitative determinations can be performed. A novel approach which has been applied to the X-ray determination of selenium in semiconductor materials, utilizes the formation of a film of elemental selenium produced by the decomposition of the compound formed between $Se(IV)$ and 4,5-diamino-6-thiopyridine (87).

A linear relation was observed between concentration and X-ray intensity up to 800 μg of selenium.

Neutron Activation

A number of techniques have been developed for the determination of selenium by neutron activation particularly in biological material (88). There are only three radio-nuclides produced by thermal neutron irradiation which are useful for analytical purposes. These are ^{75}Se , ($t_{\frac{1}{2}} = 120$ days), ^{77}Se , ($t_{\frac{1}{2}} = 17.5$ Sec) and ^{81}Se , ($t_{\frac{1}{2}} = 18.6$ minutes). The long half life of ^{75}Se permits complete radiochemical separation of the element. However some workers have elected to permit the decay of shorter-lived nuclides, usually for a period of 5 to 7 days, to facilitate the direct measurement of ^{75}Se gamma radiation in the sample. Such procedure has the obvious disadvantage of serious delay in obtaining analytical results. The method has been applied to the estimation of selenium in steels (89). Traces of selenium in samples of Pt have also been determined by Morris et. al. (90). Using a radiochemical separation of ^{81}Se by distillation, Bowen and Cawse (88) were able to separate selenium from As, Br, Mn, Na and Zn and determine the element rapidly with a sensitivity of 0.005 μg .

Atomic Absorption Spectroscopy

The determination of selenium by flame spectroscopy presents some problems, for example, the selenium resonance lines lie in the far ultra-violet region of the spectrum below 2000 $\text{\AA}$ and this frequently leads to unfavourable signal to noise ratios resulting from atmospheric and background absorption of these selenium lines. Rann and Hambly (91) however, obtained a sensitivity of 1.0 $\mu\text{g/ml}$ selenium by atomic absorption spectroscopy using the 1961 $\text{\AA}$ resonance line and an air-acetylene flame. Kirkbright and Ranson (39) reported the use of a nitrous oxide-acetylene flame for the determination of selenium and a detection limit of 1.8 ppm. When a nitrogen shielded nitrous oxide-acetylene flame was used a detection limit of 2 ppm was obtained.

With the introduction of the hydrogen-argon entrained air flame (35) the problem of flame absorption was reduced considerably. The interference problems associated with Ar-H₂ flames were eliminated by developing a technique based on conversion of selenium to hydrogen selenide, SeH₂, and evolution of hydrogen selenide into the flame (41). A collection and storage device was used for the evolved SeH₂ prior to its introduction into the flame for atomic absorption determination (92). The reagents used to convert selenium to SeH₂ are the same reagents used for arsine generation. Fernandez (43) used sodium borohydride to generate SeH₂ and obtained a detection limit of 11 ng selenium.

Thompson and Thomerson (93) reported the use of a 17 cm long silica tube mounted in an air-acetylene flame in order to effect atomization of the generated hydrides. The advantages of this technique are that no collection vessel is required, that background flame absorption for selenium and arsenic determination is effectively eliminated and that the narrow silica tube gives a relatively large increase in sensitivity compared with direct sample injection into an argon-hydrogen flame. Smith (94) has published the first reported studies on the interferences encountered in the atomic absorption spectroscopic determination of Se and As. He used hydride generation technique and an argon-hydrogen flame. Pierce et al. (95) reported an automated technique for the detection of sub-microgram quantities of Se and As in surface waters. A detection limit of 0.01 µg/litre was claimed for selenium.

Non-flame atomic absorption spectroscopy has also been used to determine selenium. Baird et al. (96) determined trace amounts of selenium in wastewaters using a carbon rod as the atomizer. The carbon rod employed by them was the so-called "Mini-Massman" rod developed by Varian and a detection limit of 72 pg was claimed. Ihnat (97) used a carbon furnace for atomization and achieved a detection limit of 90 ng/ml. Kirkbright, Shan and Snook (98) used oxidizing agents such

as $K_2Cr_2O_7$, $KMnO_4$ and other reagents to enhance selenide formation in order to prevent the loss of selenium during drying and ashing process. An enhancement of the selenium signal was observed and the detection limit was reported to be 0.05 ppm using a sample size of 10 μ l. Goulden et. al. (99) have claimed an impressive detection limit of 0.1 ng/ml by dissociating hydrogen selenide in an electrically heated furnace in an atmosphere of hydrogen, oxygen and argon.

Another non-flame atomic absorption spectroscopic determination of selenium has been reported by Neve and Hanocq (100) employing the extraction of selenium with 4-chloro-1,2-diaminobenzene and a graphite-furnace atomiser. The detection limit reported was 10 ng/ml selenium and an excellent precision and reproducibility was achieved.

Direct atomic absorption spectroscopic determination of selenium has been applied to many real samples including, high purity copper (101), surface waters (95), foods (55), (102), glasses (103), blood (104) and plant material (104).

Indirect methods for the determination of selenium by atomic absorption spectroscopy are based on the selective formation of the complex $Pd (DAN Se)_2Cl_2$ which takes place on the reaction of selenite with 2,3 diaminonaphthalene (DAN) and palladium(II). The complex formation depends on the acidity conditions and the concentration of reagents (105). A sensitivity of 0.017 μ g/ml has been obtained for the complexed selenium when palladium is determined in the flame and few chemical interferences were observed.

CHAPTER TWO

2. ATOMIC FLUORESCENCE SPECTROSCOPY

Atomic fluorescence spectroscopy is based upon the absorption of radiation of a certain wavelength by an atomic vapour and subsequent radiational deactivation of the excited atoms. Both the absorption and the measured atomic emission processes occur at wavelengths which are characteristic of the atomic species present.

Atomic fluorescence was first observed in 1905, when Wood (106) succeeded in exciting fluorescence of the D lines of sodium vapour. Soon after this initial discovery, resonance radiation was observed for mercury, lithium, cadmium, zinc and many other elements, and has been summarised by Mitchell and Zemansky (107). The fluorescence of atoms in flames was first reported by Nichol and Howes (108) in 1923, and then by Badger and Mannkopff (109) who obtained weak fluorescence signals from barium, cadmium, calcium, mercury, sodium, silver and strontium when present at high concentrations in a flame irradiated by the resonance line of the appropriate metal. In 1962 Alkemade pointed out the possible analytical applications of this technique. The first analytical method was developed by Winefordner and his co-workers in a series of four papers published in 1964-1965 (110-113). A year later Dagnall, West and Young (114) utilized commercially available atomic absorption equipment for measuring atomic-fluorescence and Thompson (115) has since greatly extended this application. The basic experimental arrangement is shown in Figure 1.

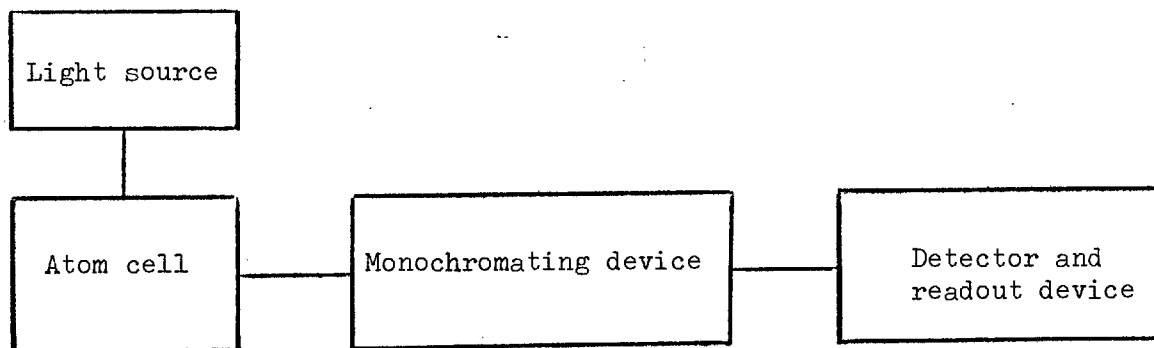


FIGURE 1 : BASIC COMPONENTS OF AN ATOMIC FLUORESCENCE SYSTEM

The source may be either a line source or a continuum and serves to excite atoms by absorption of radiation at the appropriate wavelength. The atoms are then deactivated partly by collisional quenching with flame gas molecules and partly by the emission of radiation of the same or of a longer wavelength. Thus fluorescence radiation can be observed at any angle to the incident exciting radiation. The wavelength of the emitted radiation is characteristic of the absorbing atoms and the intensity of emission is used as a measure of their concentrations. Instrumentation employed in this study is discussed in detail later. There are five basic types of atomic fluorescence:

1) Resonance fluorescence

Resonance fluorescence occurs when an atom emits a spectral line of the same wavelength as that used for excitation of the atom. Many of the atomic fluorescence measurements made by analytical chemists involve this type of fluorescence, since the transition probabilities for resonance transitions are usually much greater than those for other transitions.

2) Direct-line fluorescence

Direct line fluorescence occurs when an atom emits a spectral line of longer wavelength than the spectral line used for excitation. Both the exciting and the emitted lines originate from the same excited state.

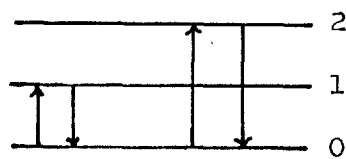
3) Stepwise-line fluorescence

Stepwise-line fluorescence occurs when the upper levels of the exciting line and of the emitted line are different. Radiatively excited atoms lose part of their energy, usually by collisional deactivation, before emitting fluorescence radiation of longer wavelength.

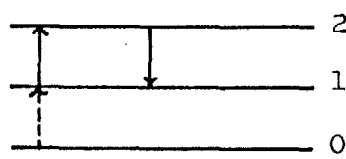
4) Sensitized fluorescence

Sensitized fluorescence occurs when an atom or molecule (donor) excited by an external source transfers its excitation energy to the sample atom (acceptor) by collision. The acceptor then undergoes radiative deactivation, resulting in atomic fluorescence. An example of this type is the fluorescence of thallium atoms at 3775 Å and 5350 Å which occurs when a mixture of thallium and a high concentration of mercury vapour is excited with the 253.6 nm mercury line. This type of fluorescence requires a higher concentration of donor species than can be obtained in flame cells, where the concentration of atoms is low and where atoms are primarily deactivated by collisional means. Therefore this phenomena is of academic interest only.

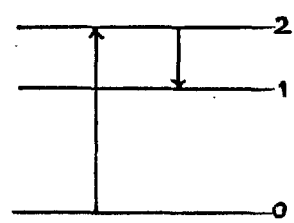
In addition to those mentioned above, multiphoton fluorescence was postulated. This mechanism assumes excitation by two (or more) identical photons. An interesting paper classifying in more detail the different types of atomic radiational transitions has been published by Omenetto and Winefordner (116). If the excitation energy is greater than the fluorescence energy then this type of fluorescence is termed stokes and if the fluorescence energy is greater than the excitation, then this type of fluorescence is termed anti-stokes. All possible types of atomic fluorescence processes are described in Figure 2.



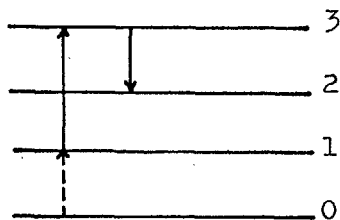
(a)



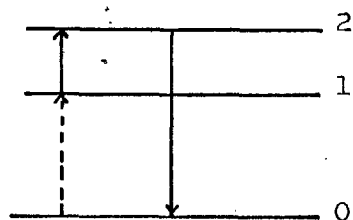
(b)



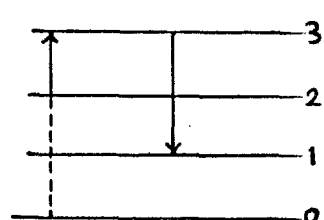
(c)



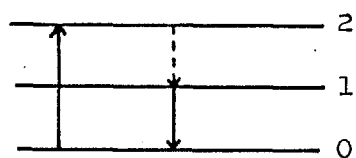
(d)



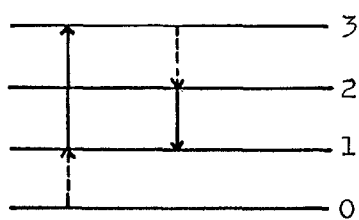
(e)



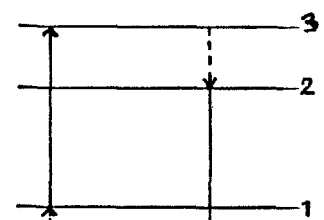
(f)



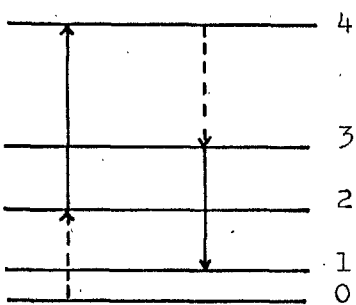
(g)



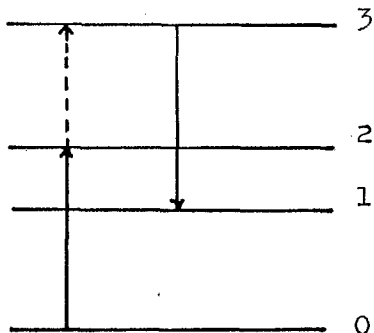
(h)



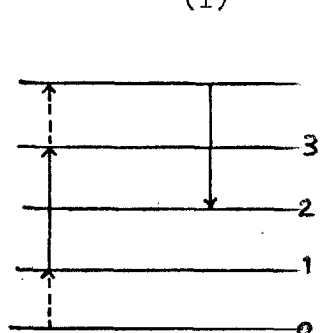
(i)



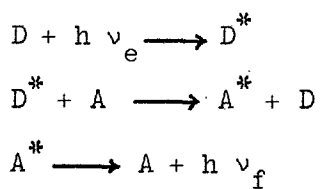
(j)



(k)

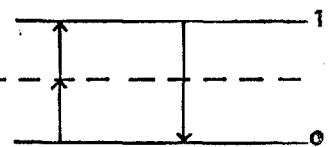


(l)



(m)

Hypothetical
level



(n)

FIGURE 2 : POSSIBLE TYPES OF ATOMIC FLUORESCENCE PROCESSES

- a = resonance
- b = excited state resonance
- c = stokes direct-line
- d = excited state stokes direct-line
- e = anti-stokes direct-line
- f = excited state anti-stokes direct-line
- g = stokes stepwise line
- h = excited state stokes stepwise line
- i = anti-stokes stepwise line
- j = excited state anti-stokes stepwise line
- k = thermally assisted stepwise line
- l = excited state k
- m = sensitized
- n = two photon excitation

These processes have been discussed in considerably greater detail by Kirkbright and Sargent (117).

In this section no attempt will be made to derive complex expressions for atomic fluorescence intensity, these calculations have been dealt with by Hooymayers (118), Zeegers and Winefordner (119) and Kirkbright and Sargent (117).

Under ideal conditions, viz.

- 1) the considered fluorescence transition is excited by absorption of energy of one wavelength only,
- 2) the entire fluorescence cell is within the solid angle viewed by the detector, and
- 3) none of the fluorescence emission is lost by reabsorption within the cell.

The integrated fluorescence intensity in a direction perpendicular to the exciting light beam is given by:

$$I_f = I_o w \frac{\Omega}{4\pi} A_T \phi$$

Where I_o is the radiant flux (expressed as energy per unit time per unit area of the fluorescence cell face on which it is incident) which excites the fluorescence under consideration, w is the width of the exciting beam of radiation, Ω is the solid angle over which the excited fluorescence is detected and measured, 4π is simply the total number of radians over which fluorescence is emitted from the cell, A_T is the total absorption factor for the spectral line at which the fluorescence is excited (and therefore depends on the absorption path length through the cell as well as atom concentration) and ϕ is the fluorescence yield (or power or efficiency), which is the fraction of the absorbed photons which are re-emitted as fluorescence radiation. The value of ϕ for a particular fluorescence transition will depend on the type of fluorescence and on the quenching of fluorescence which occurs in the cell. Quenching processes produce an overall reduction in the fluorescence intensity measured rather than a change in the shape of calibration graphs.

Under ideal conditions, mentioned earlier, it is found that for a continuum source:

$$F = aN \quad \text{at low concentrations}$$

$$\text{and } F = bN^{\frac{1}{2}} \quad \text{at high concentrations}$$

Where, F is the fluorescence intensity, N is the ground state population and, a and b are constants.

Moreover for a line source:

$$F = cN \quad \text{at low concentrations}$$

$$\text{and } F = d \quad \text{at high concentrations}$$

Where c and d are constants. These relationships are of little practical value, and it is necessary to extend the treatment to the non-ideal case,

when it is found that (117):

$$F = \frac{\Omega}{4\pi} l \phi \frac{8\pi}{(\lambda')^2 A N_0} \frac{g_0}{g_i} \int_0^\infty \alpha d\nu \cdot \int_0^\infty \beta d\nu'$$

Where Ω is the solid angle viewed by the detector,

l is the height of the atom cell,

ϕ is the fluorescence yield,

λ' is the wavelength of the fluorescence line,

A is the transition probability of the fluorescence line,

g_0 and g_i are the respective statistical weights of the lower and upper states,

N_0 is the population of the lower state,

$\alpha = I_\nu \exp. (-K_\nu \Delta L) [1 - \exp. (-K_\nu L)],$

and $\beta = \exp. (-K_{\nu'} \Delta w) [1 - \exp. (-K_{\nu'} w)].$

Where I_ν is the incident radiation flux at frequency ν ,

K_ν is the absorption coefficient at frequency ν ,

$K_{\nu'}$ is the absorption coefficient at frequency ν'

L is the length of the atom cell

and w is the width of the atom cell.

Evaluation of this equation enables theoretical analytical curves to be constructed (119). It may be seen that the intensity of the fluorescence emission depends upon:

- 1) the intensity of the exciting radiation from the spectral source,
- 2) the fraction of this radiation which is absorbed before it reaches that area of the atom cell viewed by the detector,
- 3) the fraction of this radiation absorbed by the atomic species under investigation,
- 4) the fraction of this absorbed radiation which is converted to fluorescence emission,
- 5) the fraction of this fluorescence emission which is absorbed before it is detected, and

- 6) the type of fluorescence process involved.

This last point, in particular, slightly complicates the situation encountered in non-dispersive work but, in general, the overall fluorescence intensity produced simultaneously by a number of lines may be considered as the summation of the individual intensities of each line.

Interferences in atomic fluorescence spectroscopy

Any process which modifies the size of the true fluorescence signal may be described as an interference. Such alterations to the analytical signal may result from any of a number of effects which are conveniently classified as spectral, physical or chemical interferences.

1) Spectral interferences

Such effects may arise either from the scattering of source radiation, by the overlap of the line profiles of two species present in the atom cell or by the detection of background emission. The scattering of incident radiation, typically by particulate material in the flame, is quite important in non-dispersive atomic fluorescence spectroscopy.

2) Physical interferences

Any alteration of the concentration of analyte atoms present in the atom cell which arises from a modification of the physical processes involved in nebulization and atomization may be termed a physical interference. Such types of interference may often be encountered when using flame cells.

3) Chemical interferences

This type of interference may be encountered when the concentration of the analyte atoms in the cell is altered as a result of reaction with concomitant materials present in the sample solutions. Such reactions may occur in either the solid or vapour phase but are usually quite easily overcome often by the addition of various agents to the sample solution or by the careful choice of flame stoichiometry.

While not exactly an interference, the process of fluorescence quenching may certainly reduce the intensity of the fluorescence emission as a result of collisional deactivation of excited analyte atoms by foreign atoms or molecules present in the atom cell. The quenching effect of molecules is generally greater than that of atoms and this is usually attributed to the large number of vibrational energy levels present within molecules which can facilitate the transfer of energy from the excited analyte atoms. Thus argon has found wide application as a flame diluent and separator.

Kirkbright and Sargent (117) have discussed the mechanism of quenching and also interference processes in detail.

Comparison of atomic fluorescence spectroscopy with atomic absorption and atomic emission spectroscopy

For the practising analyst, it is important to know how atomic fluorescence spectroscopy compares with the two other methods of atomic spectrometric analysis, and for which elements better results may be obtained with either atomic absorption spectroscopy (AAS) or flame emission spectroscopy (FES). The least well-established technique of these three is atomic fluorescence spectrometry (AFS). It exhibits certain advantages (and disadvantages) over atomic emission and atomic absorption spectrometry, being particularly suitable for non-dispersive measurements and simultaneous multi-element analysis. To a great extent atomic absorption spectrometry and atomic fluorescence spectrometry offer solutions to the same type of analytical problems. The merits of AFS in comparison with the other methods have been considered by different authors on the basis of theoretically calculated signal strengths and limit of detection (120). The paper by Winefordner and others (121) is the most extensive and covers all three methods. Assuming identical conditions, the ratio of signals from a dilute atomic vapour measured

by AFS and AAS is given by (121):

$$\frac{S_{AAS}}{S_{AFS}} = \frac{1}{Y\Omega/4\pi}$$

Where Y is the fluorescence quantum yield for the particular transition and $\frac{\Omega}{4\pi}$ is the fractional solid angle of excitation radiation collected by the entrance optics and falling on the atomic vapour. For the commonly employed monochromators $\frac{\Omega}{4\pi}$ is of the order of 0.01. When working with flames the fluorescence yield may be around 0.1 so that the signal measured by atomic absorption spectroscopy is approximately a thousand fold stronger than the fluorescence signal. Nevertheless, the signals observed in atomic fluorescence are invariably smaller than in atomic absorption. Winefordner says "If signals alone are considered, atomic absorption or atomic emission should be used for the measurement of analyte atoms in essentially all flames". The situation changes if noise is taken into account and the signal-to-noise ratio is considered. The total noise in emission and fluorescence flame spectroscopy may be expressed as:

$$N_{total} = \sqrt{N_{shot}^2 + N_{flame}^2}$$

Where N_{shot} is the shot-noise of the photodetector output as a result of the quantum nature of light and N_{flame} is the flame-flicker noise, which is the result of random fluctuations in the background intensity emitted by the flame. In comparison the total noise in atomic absorption spectroscopy is greater because the square of the light-source noise must be added in the above equation and simultaneously the shot-noise value is greater than for emission or fluorescence because a greater light flux is usually falling upon the photodetector surface. Therefore, considering signal-to-noise ratios for the three methods under identical conditions, i.e., instrumental system and flame (which in practice is rarely the case)

it can be concluded that limits of detection should be lower for atomic fluorescence spectroscopy than for atomic absorption spectroscopy at all wavelengths with low temperature flames and comparable with high temperature flames. These results are of limited practical validity and exceptions to this rule are rather more frequent than compliances with it because the quality of light sources for different elements proves to be of greater importance. The situation with heated graphite atomizers is similar. With most commercial apparatus the thermal background emission of the hot atomizer body can not usually be efficiently screened off unless a rather cold region of the atomizer is viewed and in such regions strong interferences usually occur.

Some of the advantages of atomic fluorescence spectroscopy are listed below:

- 1) For elements with their resonance lines in the ultra-violet region of the spectrum, the limits of detection obtainable by atomic fluorescence spectroscopy may be considerably better than those of flame-based atomic emission spectroscopy, which is comparatively insensitive in that area. An associated feature of this increased sensitivity is the greater linear concentration range given by atomic fluorescence compared to atomic absorption spectroscopy.
- 2) As the fluorescence process is dependent upon the absorption of radiation by ground state atoms, the technique is much less sensitive to temperature fluctuations within the atom cell than is atomic emission spectroscopy. Moreover, comparatively cool flames, which would produce virtually no emission intensity, may be used for the determination of many elements by atomic fluorescence spectroscopy.
- 3) Continuum or fairly broad-line light sources can be used with a less serious loss of sensitivity than for atomic absorption spectroscopy.

- 4) The attainable sensitivity is controlled by the intensity of the light source employed.
- 5) Atomic fluorescence spectroscopy is more promising in simultaneous multielement analysis than atomic absorption spectroscopy.

There are, however, a number of disadvantages associated with the technique including:

- 1) As in atomic absorption spectroscopy, a spectral source is required, usually a different one for each element.
- 2) Atomic fluorescence spectroscopy is not suited to the determination of those elements which have their resonance lines in the visible region of the spectrum. Such elements, being fairly easily excited produce intense emission signals which may overload the detection system and cause a marked increase in the noise level. Elements which form refractory oxides are also difficult to determine by atomic fluorescence spectroscopy as high-temperature flames, with their associated high background emission, are often required in order to produce adequate atomization.
- 3) The quenching effect of the gas species present in the atom cell reduces the fluorescence signal.

2.1. INSTRUMENTATION

The main aim of this project was to establish a precise, sensitive, rapid and simple direct method for the determination of arsenic and selenium in real samples particularly soil and biological samples. In considering which of the available methods is to be used to tackle these problems, two major requirements must be met. Firstly, it must have a high degree of selectivity and good sensitivity. Secondly the cost involved in its use must be considered. There are three contributions to the overall cost of an analysis. Apart from the initial outlay involved

in obtaining the appropriate equipment, it is necessary to consider running and operator costs. Atomic fluorescence spectroscopy exhibits certain distinct advantages over atomic emission and atomic absorption spectroscopy for the determination of selenium and arsenic as mentioned in section 2. Although the instrumentation used for atomic fluorescence measurements may be similar to that used in atomic absorption spectroscopy, the function of some of the basic components is not similar. In particular, the role of the monochromator is considerably less important in atomic fluorescence spectroscopy than in either atomic emission or atomic absorption spectroscopy. In atomic fluorescence spectroscopy the monochromator does not view the spectral source nor does it greatly affect the selectivity of the technique, which is chiefly governed by the purity of the source. In fact a monochromator limits the amount of radiation falling on the detector. This may be a very necessary function if the detector has a wide spectral response and the emission from the atom cell is high. It is evident though, that by minimizing such background emission and by using a detector with a limited spectral response, the monochromator may be dispensed with altogether. There are many advantages to be gained from using a non-dispersive system, such as:

- 1) The absence of a monochromator allows a much greater energy throughput in the system and a number of spectral lines may be viewed simultaneously for elements which exhibit complex fluorescence spectra. This increased sensitivity permits a greater linear concentration range than is observed using atomic absorption spectroscopy.
- 2) Atomic fluorescence spectroscopy is much less sensitive to temperature fluctuations within the atom cell than is atomic emission spectroscopy. This is particularly fortunate if a non-dispersive approach is to be used, since it is necessary to reduce the background emission from the atom cell as much as possible.

3) Given that an analysis of the trace elements in real samples involves the determination of several different elements, the value of rapid multi-element analysis becomes apparent. In non-dispersive atomic fluorescence spectroscopy making rapid, sequential multi-element analysis is easily achieved since, in changing from one element to another no adjustment of the detection system is necessary; only the substitution of the source of interest is necessary.

There are, however, a number of disadvantages associated with a non-dispersive atomic fluorescence system;

1) The requirements of a good source for atomic fluorescence spectroscopy are not easily met especially in non-dispersive work where the selectivity of the technique depends completely upon the spectral purity of the source. Even using a pure source, spectral overlap is considerably greater than in a dispersive system.

2) The principal difficulty encountered in non-dispersive atomic fluorescence spectroscopy is the problem of dealing with the scattering of radiation from the source by particulate material present in the flame. In this chapter, basic features of atomic fluorescence spectrometers are discussed after which the instrumentation used in this work is described in detail.

2.1.1. The Spectral source

Although many types of excitation sources may be used in atomic fluorescence spectroscopy, the most commonly used are the metal vapour discharge lamp (VDL), the hollow cathode lamp (HCL) and the microwave-excited electrodeless discharge lamp (EDL).

The VDL now finds much less application than in the past, since it suffers from the effects of line-broadening and self-absorption. Moreover, as the range of elements for which these sources may be used is quite small, they lack the versatility of the other types of source

presently available. HCLs are available for a wide range of elements but give insufficient intensity for most atomic fluorescence applications. However, intensity enhancement can be achieved either by operating the lamp for short periods of time at currents well above the manufacturer's recommended maximum or by using an additional discharge from a second cathode to excite the sputtered atomic vapour produced (122). The first high intensity lamps of this type have been used in the non-dispersive work of Larkins (123). The demountable HCL, has been described by Dinnin and Helz (124) and used for the atomic fluorescence determination of a number of elements (125). In general, the demountable HCL offers no great advantage over conventional lamps and is not widely used for routine analysis. The most popular type of narrow-line source, especially since the advent of suitable temperature-controlling devices, has been the EDL. The high frequency electrodeless discharge was discovered by Hittorf in 1884. Meggers et al. (126) in 1948-1950 described the preparation of an EDL of mercury. Radio frequency EDLs have been used as spectral sources for alkali metals (127) and for excitation of atomic resonance fluorescence of alkali metals (128).

Microwave excited EDLs have been used to obtain continuous sources in the vacuum U.V. using argon, hydrogen, krypton and xenon filler gases (129). The preparation of metal EDLs have been described in many publications (130-132). Winefordner (113) has used some commercially available EDLs for atomic fluorescence studies, with rather poor results mainly because of the EDL design. The real potential of the EDL as a source for atomic fluorescence and atomic absorption spectroscopy was realised by Dagnall, Thompson and West (130) and they described their preparation for several elements. Bartley (133) has compiled a comprehensive list of previously reported EDLs, which is of great value to those workers wishing to construct their own lamps.

The advent of the tunable organic dye laser has been of considerable importance to many atomic fluorescence workers since the narrow line width and high intensity of emission associated with this type of source enable excellent limits of detection to be obtained for many elements (134). The exceptionally high cost of laser-type sources has limited the extent to which they have found routine application in atomic fluorescence spectroscopy. Nevertheless, it seems certain that if a reasonably priced tunable laser source capable of operating in the ultra-violet region is produced, it would be used extensively for a variety of applications.

Continuum sources are widely used by many atomic fluorescence workers, since they enable the fluorescence of a wide range of elements to be excited using just one source. The most commonly available source of this type is high pressure xenon arc lamp. Scattering of radiation can be a serious problem in atomic fluorescence spectroscopy when using a continuum source.

2.1.2. The atom cell

Most analytical atomic fluorescence studies to date have been undertaken using a flame as the atom cell. This is due to the versatility of operation when using this type of atom cell for practical analysis. The optimum flame for atomic fluorescence spectroscopy should have the following properties;

- 1) high atomization efficiency for a wide range of elements,
- 2) low radiative background and noise near to the analyte fluorescence wavelength,
- 3) low partial pressure of quenching species,
- 4) long residence time of atoms in the optical path,
- 5) simplicity and safety of operation,
- 6) low cost.

Most of these properties are shown by cool flames (H_2 fuel).

Several types of flames, however, have been used in atomic fluorescence spectroscopy but the most commonly employed are those hydrogen flames which exhibit exceptionally low background emission. Hydrogen flames do not cause fluorescence quenching and also give good limits of detection for many elements. However their applicability is limited because of their low temperatures, which means that some elements can not be satisfactorily determined as a result of poor atomization or susceptibility to matrix interference effects. In contrast, the higher temperature of the air-acetylene flame helps to overcome matrix interference problems often encountered and it has proved to be a popular choice for many applications. In many cases even the air-acetylene flame temperature fails to give efficient atomization of the refractory oxides such as those of Al and Be (135) (136). This difficulty may be overcome by using the very hot, but considerably less stable, nitrous oxide-acetylene flames.

The major limitations of the nitrous oxide-acetylene flame however is the high level of background emission and the high concentration of fluorescence quenching species present. Winefordner and co-workers (137) have shown that by using a tunable laser as the spectral source and a nitrous oxide-acetylene flame very good sensitivity may be obtained for several rare-earth and refractory-oxide type elements.

Sample introduction into a flame atom cell is best effected in liquid form and a nebulization device which can present the sample solution to the flame in the form of a fine mist or spray is generally used. A variety of such devices are available but the most common are pneumatic nebulizers which are operated by a high velocity jet of the flame support gas, although ultrasonic nebulization is also used. For small sample volumes ($< 50 \mu l$) the performance of a flame as an atom cell may prove to be unsatisfactory, and the use of discrete atomisation

devices may be more favourable. These devices are normally resistively heated carbon furnaces, rods or cups or heated metal filaments. The advantages obtainable using these so called "non-flame atom cells" are greater sensitivity, as the whole sample volume (typically 5-10 μ l) may be evaporated and subsequently atomised into the path of the source radiation (cf. nebulisation: 2%-3% efficient) resulting in a transient signal. Also the composition of gases used to screen the heated filament or cup may be chosen to minimise quenching - for example oxygen can be eliminated completely by the use of an inert gas screen. The reader is referred to Kirkbright and Sargent (117) for a fuller explanation of these devices.

The cathode sputtering technique is a particularly useful method of atomization for the analysis of metals by atomic fluorescence spectroscopy (138). The major advantage of this method is that no chemical pre-treatment of the sample is necessary, although suitable solid standards are required. A useful method for the sample introduction of elements which form volatile hydrides is the hydride generation technique (139). Excellent limits of detection may be obtained for several elements, using this approach, which has already found many practical applications in atomic absorption spectrometry.

2.1.3. The optics and dispersive element

The role of the optical components in an atomic fluorescence system is to focus the radiation from the source onto the atom cell and to collect as much fluorescence radiation as possible.

Wavelength selection in dispersive atomic fluorescence systems is usually performed by prism or grating monochromators, although in some instances the use of simple interference filters is preferred. These filters have a higher transmission than that of a typical monochromator. Problems associated with the use of monochromators, such as wavelength

drift and poor light throughput can be overcome by the use of resonance monochromation described by Walsh and Russell (140).

Using this type of wavelength selection, fluorescence radiation from the atom cell is passed through an evacuated bulb which contains atoms of the analyte element. This stimulates characteristic resonance fluorescence, the intensity of which is proportional to the intensity of fluorescence radiation from the atom cell and can be detected and recorded in the conventional way. Owing to their limited life and instability of operation compared with ordinary dispersive devices, the practical applications of such monochromators are somewhat limited.

2.1.4. The detection and signal processing system

Although in theory various detectors could be used for detection of fluorescence signals, only photomultiplier tubes have become widely established. The most important characteristics of a photomultiplier are the noise equivalent power (NEP) and the dynamic range. NEP is defined as the radiant power giving the same response as the average amplitude of the dark-current noise, or, in terms of pulses, the power producing the number of pulses per unit of time equal to the number produced by the photomultiplier without illumination. The response characteristic of photomultiplier tubes depends primarily on the nature of the material used as the photocathode, which means that particular photomultipliers may be chosen for certain specific applications. This is remarkably important when a non-dispersive fluorescence system is used. All the completely non-dispersive systems reported to date have used solar-blind photomultiplier tubes. All the reported atomic fluorescence studies using a solar-blind photomultiplier tube have been carried out in flames with the exception of Gough (138), who used a cathode sputtering cell and Mounce (141) who employed a hot-wire "loop"

cell. Solar-blind photomultiplier tubes have been available for some years and their characteristics have been discussed by Dunkelman (142).

The measurement of electrical signals in atomic fluorescence spectroscopy invariably involves the monitoring of low electrical currents. The analytical signal must be distinguished not only from spurious signals originating from the optical components of the instrument (scatter, reflection, etc.) but also from photomultiplier noise, which may sometimes be comparable to or even higher than the useful analytical signal. The three methods most widely used are; direct-current measurement, alternating-current measurement with a lock-in amplifier and photon-counting techniques. Most flame atomic fluorescence spectroscopy measurements involve the use of an a.c. amplifier with a modulated spectral source but a fast-response unit is usually necessary to monitor the transient signals generated by many non-flame atom cells.

2.2. NON-DISPERSIVE ATOMIC FLUORESCENCE SYSTEMS

The schematic representation of a non-dispersive atomic fluorescence spectrometer is shown in Figure 3. Since no form of wavelength selection is employed a lot of unwanted background radiation, mainly from the atom cell may reach the detector and affect the performance of the system. The instrumentation used for non-dispersive atomic fluorescence spectroscopy is usually chosen with the intention of minimizing or avoiding the effects of background radiation. The most common method of achieving this is to use a detector of limited spectral response. As mentioned before, the solar-blind photomultiplier tube has proved to be extremely useful for most flame atomic fluorescence spectroscopy applications since it does not respond significantly to radiation of wavelengths greater than about 3000 Å. It means, for example, the intense hydroxyl band emission which is produced by many flame atom cells falls outside the range of the detection system and consequently the background

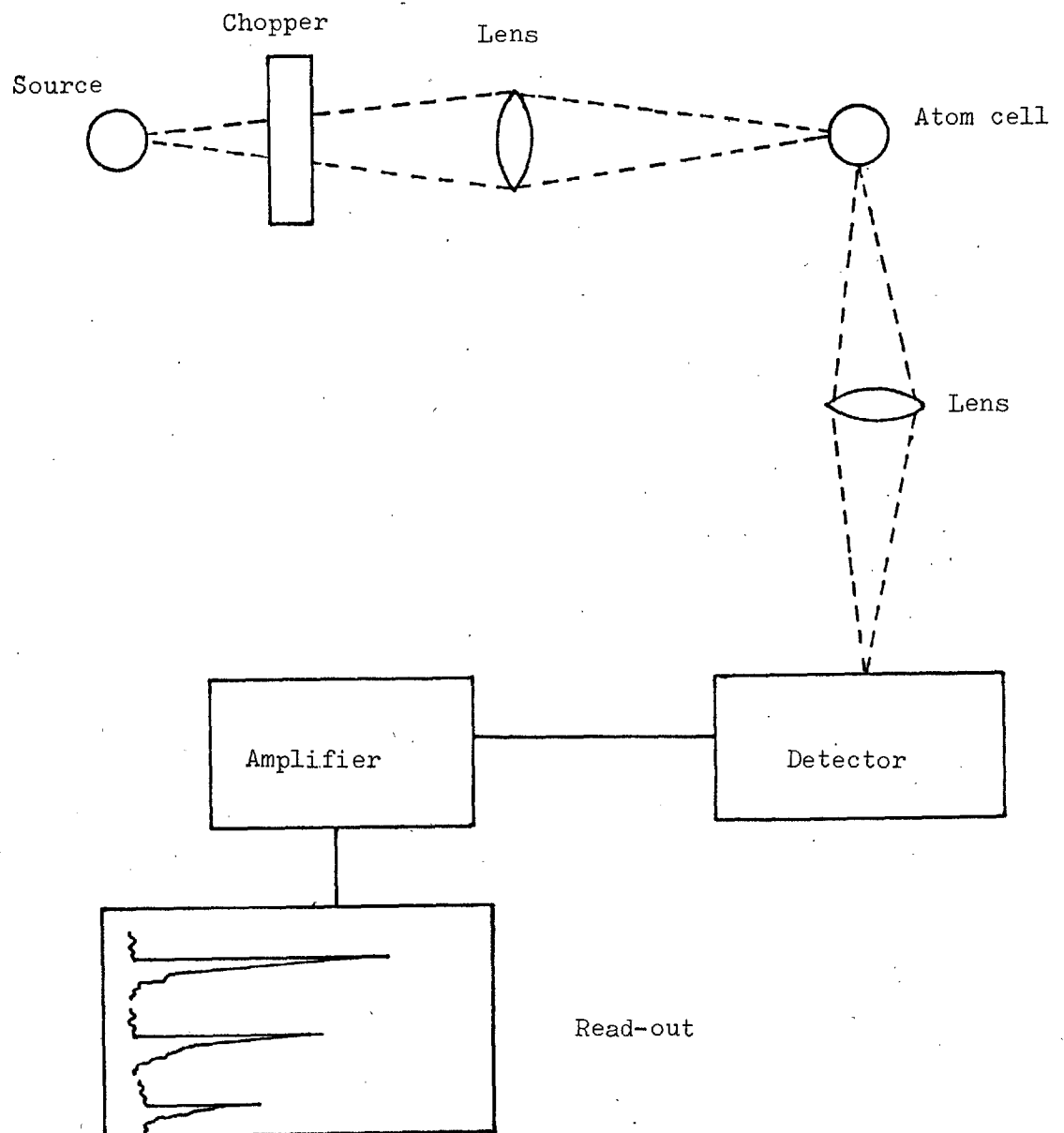


FIGURE 3 : SCHEMATIC DIAGRAM OF A NON-DISPERSIVE ATOMIC FLUORESCENCE
SYSTEM

radiation level is reduced. It is clear that by using such a detector it would be difficult to determine elements such as Cu, Ag or Cr whose resonance lines lie in the visible region of the spectrum. Flame separation can also be used to reduce the background emission from the atom cell. This means passing a stream of inert gas (usually nitrogen or argon) around the outside of flame such that the outer, secondary reaction zone is physically separated from the inner, primary reaction zone. An extended interconal zone is distinguished between these two zones and an overall decrease in the background emission level is observed. The effect of background emission can be further reduced by using a suitable electronic processing system. The most effective way to do so is to use a modulated spectral source together with a phase-sensitive detector. In this case only signals of the correct frequency and in phase with a reference waveform are accepted and the other components of the observed signal are rejected. A significant improvement in the signal to noise ratio results.

2.3. THE NON-DISPERSIVE ATOMIC FLUORESCENCE SYSTEM EMPLOYED IN THIS STUDY

Figure 4 shows a schematic representation of the instrument used in this study. Although the basic components resemble the components shown in Figure 3, two additional features are used to minimise the effect of unwanted background radiation. These are a solar-blind photomultiplier tube and a phase-sensitive a.c. detection system.

2.3.1. The spectral source, electrodeless discharge lamp

Introduction

Radio- and microwave-frequency electromagnetic fields are very efficient for generating and accelerating electrons and thereby maintaining a gaseous glow discharge without internal electrode contact with the plasma. This electrodeless system represents the ultimate in simplicity

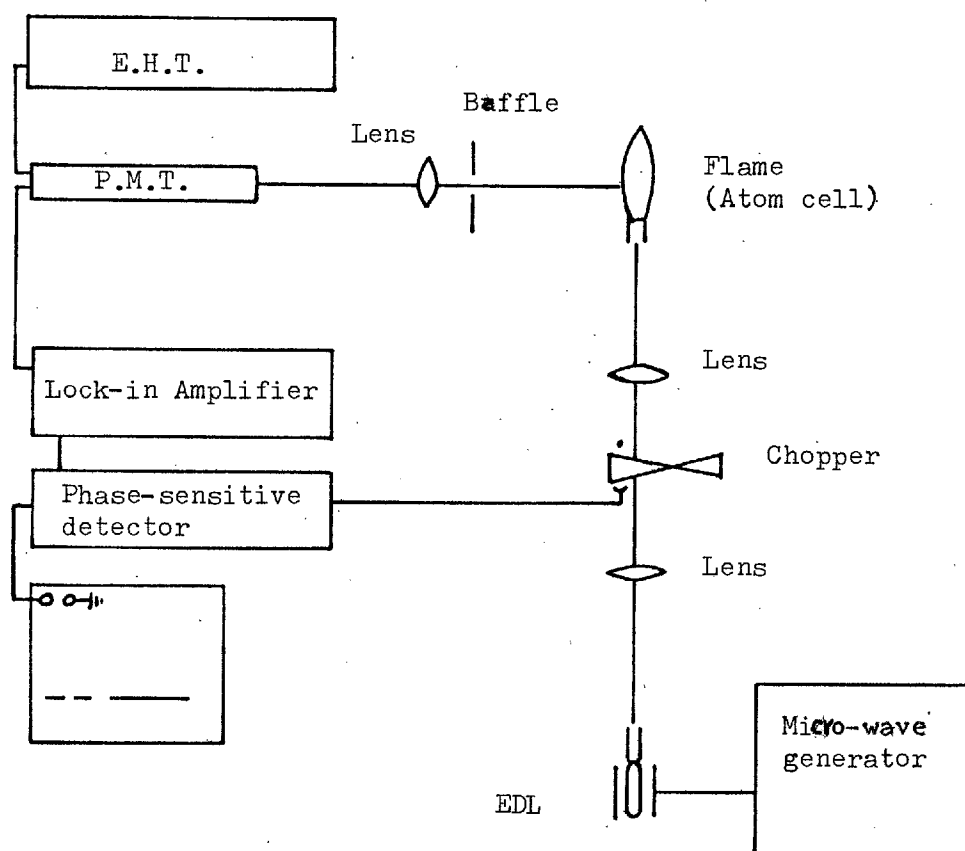


FIGURE 4 : SCHEMATIC DIAGRAM OF THE NON-DISPERSIVE ATOMIC
FLUORESCENCE SYSTEM EMPLOYED

among the other types of gaseous discharges and is not affected by some of the problems and limitations associated with systems requiring internal electrodes. The radio-frequency or microwave-frequency powered electrodeless discharge in sealed-off tubes containing an inert gas at low pressure and some volatile pure metal or metal salt (usually a halide), thus offers a means of obtaining a simple spectral line source with very good spectral characteristics when operated under proper conditions. Energy can be transferred effectively from the electric field to the gas for all frequencies up to about 10,000 MHz, (radio-frequency $2 \times 10^{5-9}$ Hz, microwave-frequency 10^{9-12} Hz). The microwave unit is somewhat simpler than either the R.F. or the d.c. unit to operate. Microwaves can be generated in the laboratory using commercially available instruments such as magnetrons. Thus with a magnetron placed at one end of a closed wave guide, electro-magnetic waves are formed and microwave energy is transmitted to the gases, through a quartz tube situated at the other end of the wave guide. Rose and Brown (143) have suggested the following mechanism in a steady state microwave discharge: "Electrons gain energy from the field and lose energy by elastic and inelastic collisions. Ionization of gas molecules provides a source of new electrons, and flow to the tube walls in the presence of density and space potential gradients provides the sink". The maximum electric field strength available in an empty wave guide is proportional to the square root of the input power. Thus as the power level is increased, the field strength will also increase until at some particular value it will be strong enough to initiate breakdown of the gas contained, in this instance, in a quartz bulb. To assist this process a source of external electrons can be introduced into the field by a sparking tesla coil applied to the quartz surface containing the (argon) gas; a discharge is then initiated.

Preparation of EDLs

Apparatus

The vacuum line used for the construction of EDLs and an EDL blank are shown in Figure 5. Mansfield (144) used a kinetic type system (10^{-6} torr) and an oil manometer instead of a mercury gauge which precludes any mercury vapour being sealed into the lamp,

Procedure

Quartz tubes, used as the blanks for preparation of EDLs, were first washed with concentrated HNO_3 and rinsed carefully with distilled water and acetone. The blanks were then left in an oven at about 100°C for 48 hours. They were removed from the oven, left to cool and were connected to the vacuum line. The system was pumped down to about 1 torr. Argon was introduced into the system and again repumped to about 1 torr. This was repeated two or three times. Using an oxy-propane hand torch the lamp bulb was heated at a temperature just below its softening point for about 10 minutes, during which argon was flushed and pumped out a few times to remove any released gases. The degree to which the inner quartz surface is degassed plays an important role in the useful lifetime of the lamp and its overall operation. The above procedure was found to be adequate for degassing the quartz but some authors have paid more attention to this step. For example Dagnall et. al. (145) heated the blanks up to 1000°C for a period of 12 hours. To avoid the decomposition of the quartz tube, it must be heated only once. The lamp blank was then allowed to cool with a high partial pressure of argon filling the vacuum line. Once the lamp was cool it was removed from the vacuum line and a small amount of the required element was introduced into the lamp blank using a micro-spatula. The lamp blank was then reconnected to the system and evacuated as before, flushed with argon and re-evacuated to a pressure of about 1 torr. Gentle heat, depending on

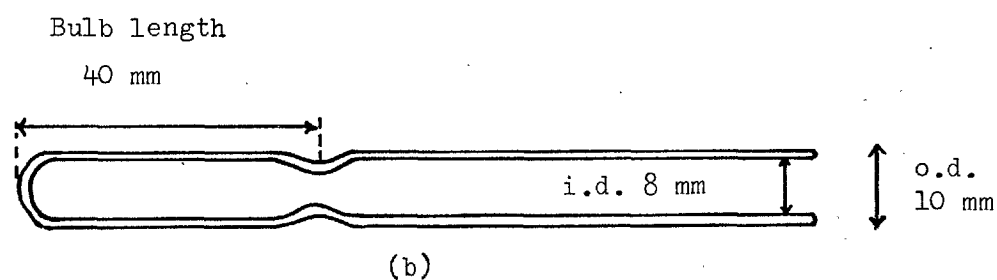
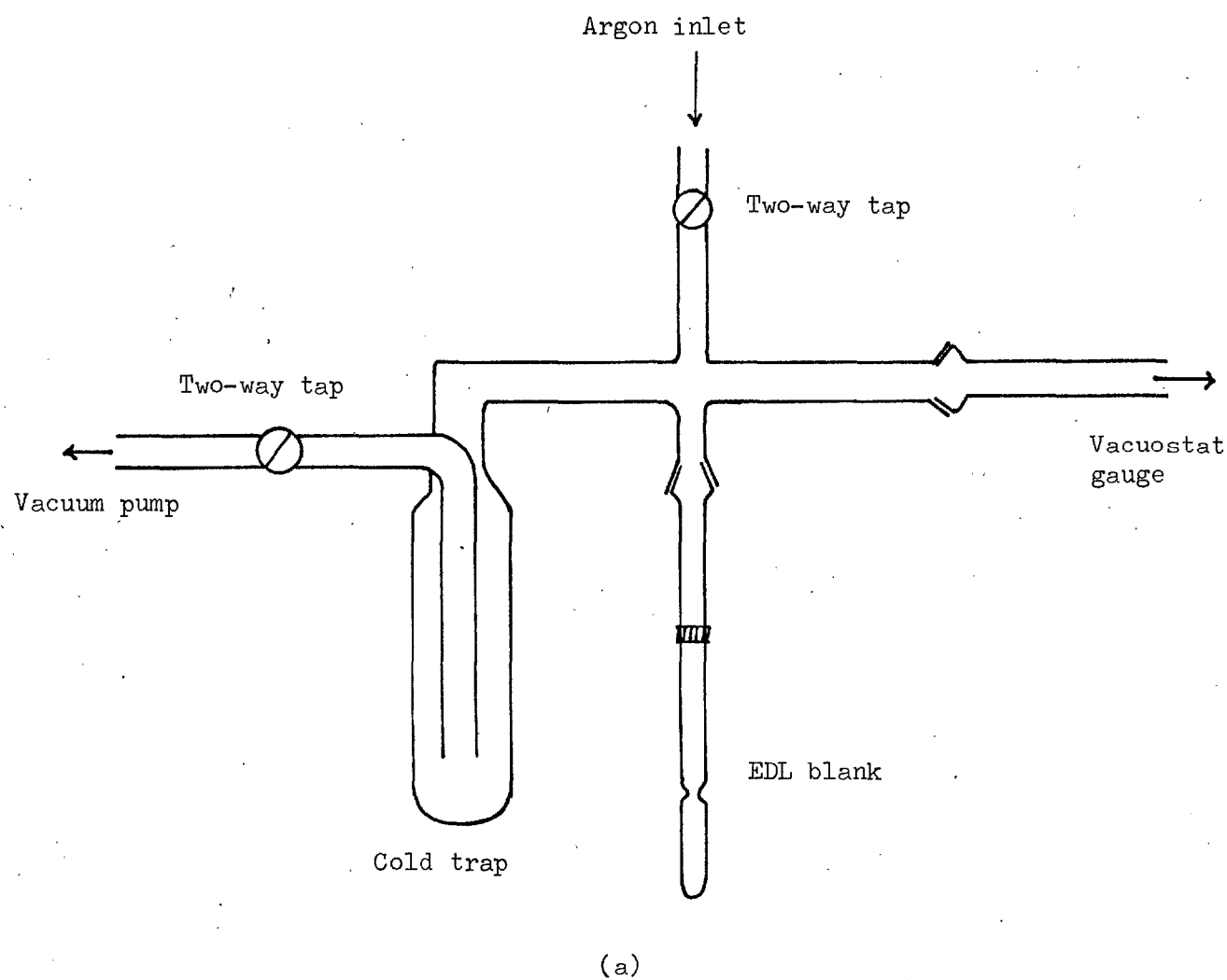


FIGURE 5 : a) THE VACUUM LINE USED TO CONSTRUCT THE ARSENIC AND
SELENIUM EDLs
 b) ELECTRODELESS DISCHARGE LAMP BLANK

the volatility of the material was applied to the base of the bulb to drive off any moisture. During this process, the whole system was flushed several times with argon and heating continued until the metal vaporised and condensed as a thin film around the cooler part of the bulb. The bottom of the lamp was submerged in cold water and it was possible to reduce the amount of introduced element by heating the thin deposited layer and passing a portion of it out of the bulb. Argon was introduced to the line and evacuated to a pressure of about 1 torr. Half of the lamp bulb was placed in cold water to keep the material cool at the base of the bulb whilst an oxy-propane torch was used to melt the quartz and rapidly seal the bulb at the prepared constricted region.

The intensity and stability of microwave excited EDLs are subjected to many variables such as lamp diameter and volume, nature and pressure of filler-gas, nature and mass of the material introduced into the lamp blank and microwave power applied to the lamp by means of certain coupling devices, most of which are interdependent and cause changes in metal vapour pressure. Insufficient information is available at present to evaluate correctly the effect of each of these variables and particularly their mutual interaction. It is therefore, not surprising that in reviewing the literature, contradictory statements can be found.

In this study the lamp blanks were made from transparent quartz tubing with 1 mm wall thickness. The blanks were made from 8 mm i.d. and 10 mm o.d. tubings as it is believed to give the optimum intensities, low noise and increased lifetime (130).

Mansfield et al. (144) have reported that there is a definite relation between the intensity of fluorescence and the length of the lamp bulb. Many authors have reported optimum lengths for the bulb for different EDLs and generally it is agreed that 2.5 - 3.5 cm is the best length for operation with a $1/4$ wave resonant cavity and

3.5 - 5.0 cm with a $\frac{3}{4}$ wave resonant cavity and A type antenna. In this study different bulb lengths for both elements were tried and 4.0 cm was found to be the most suitable bulb length.

Argon was chosen for preparation of EDLs used in this study because lamps filled with argon have shown indefinite lifetime, high intensity and excellent stability. Different argon pressures have been recommended by workers but the majority of published work from this department have reported 1 torr to 4 torr as the optimum argon pressure. After trying pressures between 1 to 5 torr, 1 torr was found to be the optimum pressure for the filler gas.

The type and the amount of material used in the construction of EDLs are two factors which play important roles in the performance of the lamps. There are a number of different types of EDLs using different materials: 1) element, 2) metal halide, 3) amalgam or metal halide with mercury and 4) other compounds.

The material placed in the lamp blank must have a vapour pressure of about 1 torr at $200 - 400^{\circ}\text{C}$ in order to be vaporised easily and to achieve maximum stability and intensity. It is this property that determines whether the metal itself or its more volatile compounds are employed. It is not possible to give a completely uniform scheme for the choice of material used for the preparation of EDLs. Table 4 indicates the range of elements and compounds available for use in the construction of EDLs.

It is difficult to give a general conclusion on the amount of material needed as it is rarely reported by workers.. Mansfield et al. (144) proved statistically that lamp intensity is independent of the amount introduced for a Cd EDL. In the first years, West, et al. used mg amounts and obtained excellent EDLs, but later they emphasised the necessity of a very small amount (146).

RANGE OF ELEMENTS AND COMPOUNDS AVAILABLE FOR USE IN THE EDL'S CONSTRUCTION

Al	$\text{Al} + \text{I}_2$	Pb	$\text{Pb} + \text{I}_2$
Sb	$\text{Sb} + \text{I}_2$	Mg	Mg
As	$\text{As} + \text{I}_2$	Hg	Hg
Ba	BaCl_2	Mo	$\text{Mo} + \text{Cl}_2$
Be	$\text{Be} + \text{I}_2$	Ni	NiCl_2
Bi	$\text{Bi} + \text{I}_2$	Nb	$\text{Nb} + \text{I}_2$
B	$\text{B} + \text{Cl}_2$	Pd	$\text{Pd} + \text{Cl}_2$
Br	Br_2	Pt	$\text{Pt} + \text{Cl}_2$
Cd	Cd	K	K
Cs	Cs	P	P
Ca	CaCl_2	Pr	PrCl_3
Cl	Cl_2	Rh	$\text{Rh} + \text{Cl}_2$
Cr	$\text{Cr} + \text{Cl}_2$ (CrCl_3)	Rb	Rb
Co	$\text{Co} + \text{I}_2$	Se	Se
Cu	$\text{Cu} + \text{I}_2$	Si	$\text{Si} + \text{I}_2$
Ga	$\text{Ga} + \text{I}_2$	Ag	AgCl
Ge	$\text{Ge} + \text{I}_2$	Na	Na
Au	$\text{Au} + \text{Cl}_2$	Sr	SrCl_2
Hf	$\text{Hf} + \text{Cl}_2$	S	S
In	$\text{In} + \text{I}_2$	Ta	$\text{Ta} + \text{I}_2$
I	I_2	Te	$\text{Te} + \text{I}_2$
Ir	$\text{Ir} + \text{Cl}_2$	Tl	$\text{Tl} + \text{I}_2$
Fe	$\text{Fe} + \text{I}_2$	Th	$\text{Th} + \text{I}_2$
La	$\text{La} + \text{Cl}_2$ (LaCl_3)	Sm	$\text{Sm} + \text{I}_2$
V	$\text{V} + \text{I}_2$	Ti	$\text{Ti} + \text{I}_2$
Zn	Zn	W	$\text{W} + \text{Cl}_2$
Zr	$\text{Zr} + \text{I}_2$	U	$\text{U} + \text{I}_2$

EDLs used in these studies were constructed using pure elemental selenium and arsenic powders as the introduced materials to the lamp blanks. Although arsenic iodide has been reported as the most suitable material for arsenic EDL construction, experiments indicated, however, that arsenic EDLs constructed using pure arsenic powder were more intense, showing the same degree of stability if operated under proper conditions. The amount of material introduced into the blanks ranged from 1 mg to an almost invisible amount. Experiments proved that lamps with the smaller amount of material show much more intensity and stability.

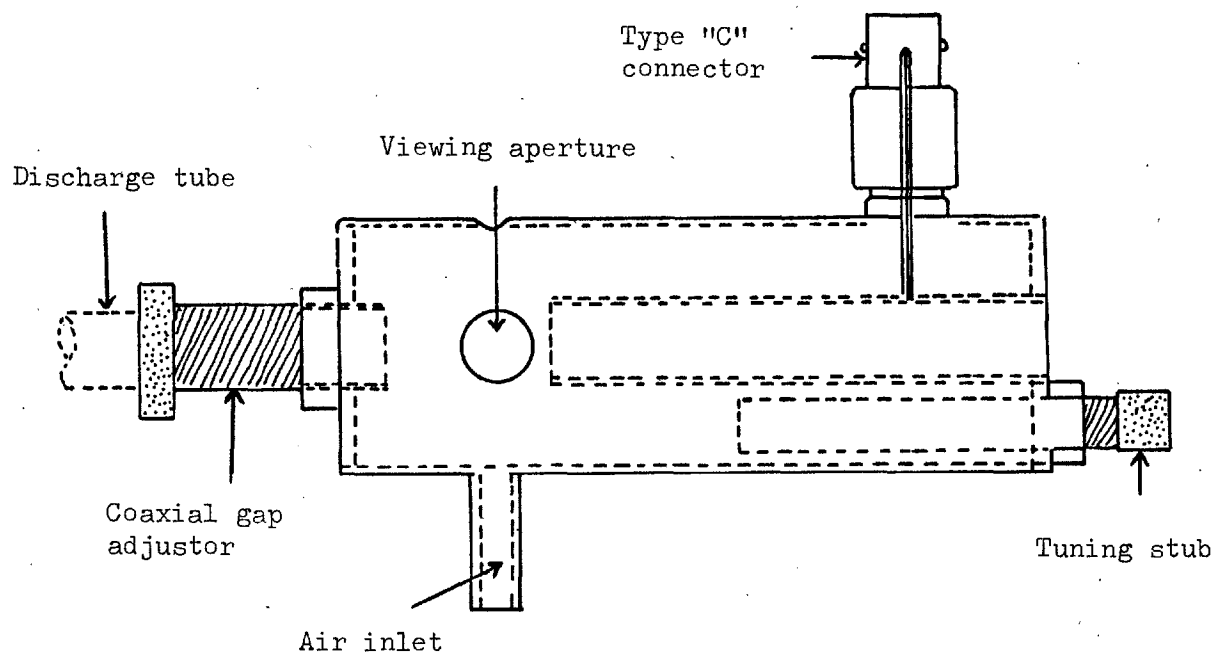
It has been my practical experience that the smallest amount possible should be used. A number of EDLs were constructed for each element and the best was selected for use in the determination of that element. The lamps were made following the procedure set out earlier in this chapter.

Operation of EDLs

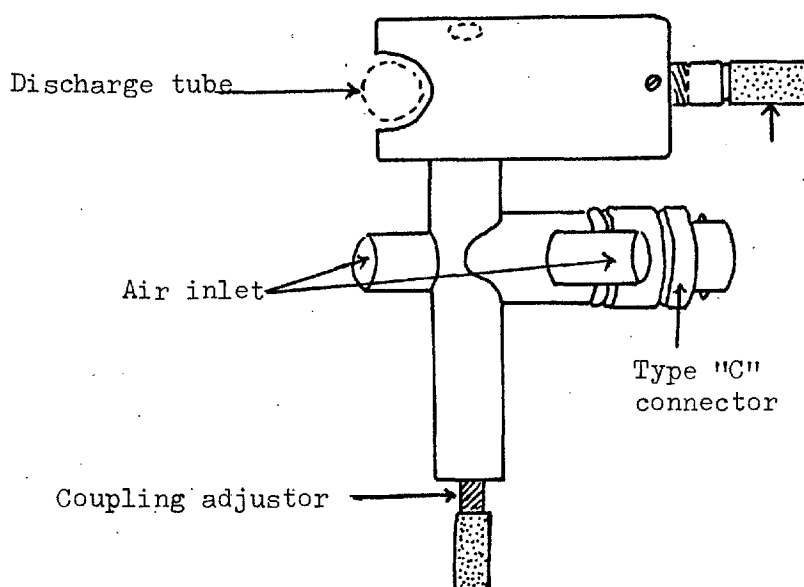
Microwave power and coupling devices

Most of the experimental work involving the use of EDLs for analytical atomic fluorescence studies has been carried out with commercially available microwave generators. Medical diathermy units operating at a frequency of 2450 MHz and at powers up to 150 W have been found to be useful as microwave generators. Modern microwave generators, such as the Microtron 200, (Electro-Medical Supplies Ltd., London) are designed for spectroscopic purposes. Suitable microwave generators are now available from a number of suppliers, together with the necessary coupling units; a reflected-power meter is either built-in or supplied as an accessory. A reflected-power meter gives a direct reading in watts of the power not being absorbed at the gas discharge cavity and its use greatly facilitates tuning for maximum efficiency and safety. Microwave energy from the generator is fed through the coaxial output cable and coupled to the

discharge lamp by means of either a resonant cavity or an antenna. The purpose of these devices is to transfer as much of the output power into the discharge lamp as possible. Sufficient energy must be absorbed to effect vaporization, excitation and maintenance of a suitable discharge. The efficiency with which the EDL operates depends to a large extent on the proper design and choice of these coupling devices. A resonant cavity transfers microwave energy to a discharge by matching its own resonance frequency to that of the microwave source. The more efficient the cavity the lower the reflected power. Various types of microwave resonant cavities have been developed (147), of these the $1/4$ -wave coaxial "Evanson" cavity and the $3/4$ -wave coaxial "Broida" cavity are the most popular. Various modifications of the two basic types exist which differ with respect to the location of tuning stubs, coupling adjustments and air cooling inlet. In most atomic fluorescence studies with EDL sources $1/4$ -wave and $3/4$ -wave cavities supplied by Electro-Medical Supplies Ltd., No 214L and 210L respectively have been employed. Figure 6 shows the schematic diagram of these two cavities. The $1/4$ -wave cavity is more efficient but its tuning is rather tedious and as the two tuning stubs are mutually dependent in operation, successive readjustment of each must be used to attain maximum efficiency. It is frequently thrown out of tune as the vapour and condensed phases shift within the lamp during operation. The $3/4$ -wave cavity is cylindrical in shape and can be air cooled. Because the lamp is almost totally immersed in the microwave field, the lamps prepared from relatively volatile or very volatile material are able to reach equilibrium conditions. In addition, the cylindrical cavity functions to some extent as a thermostat for the lamp by excluding extraneous draughts. During the course of these studies a $3/4$ -wave cavity was used exclusively, since the performances of both cavities were investigated and the $3/4$ -wave



a) A $3/4$ -wave Broida type cavity No. 210L



b) A $1/4$ -wave Evanson type cavity No. 214L

FIGURE 6 : THE $3/4$ -WAVE AND $1/4$ -WAVE CAVITIES

cavity was superior to $1/4$ -wave cavity for both arsenic and selenium EDLs.

In early atomic fluorescence studies, microwave excited EDLs were operated in the d.c. mode in conjunction with d.c. amplifiers. Measurement in the d.c. mode does not differentiate between atomic fluorescence and flame emission signals. To overcome this problem, signals can be modulated simply by using a chopper or electronic modulation. In AFS the light is chopped between the source and the flame, so that the steady thermal emission from the flame is not modulated and is rejected by the a.c. electronics. In general the lamp intensity is more sensitive to variation of power, position in cavity and cooling, when the lamp is modulated.

During the course of these studies the electronic modulation of lamps, using the modulation unit inside the microwave power generator, was attempted but it was not possible to achieve a stable output from the lamps. The resultant instability was observed for several selenium EDLs constructed at different pressures and containing different amounts of selenium. It was therefore finally decided to use a mechanical chopper to modulate the lamps.

Another important factor in the performance of EDLs is to provide a stable thermal environment while the lamp is operating. Precise temperature-control of EDLs can result in a considerable increase in emission intensity compared to the output at normal temperatures. Furthermore, the operation of an EDL at its optimum temperature reduces the dependence of its emission intensity on the applied microwave power so that an increase in the stability of output may result since the effect of fluctuations in applied power are minimised. The thermally stable environment offers the advantage of operating the lamp at its optimum emission intensity, often showing many orders of magnitude improvement. Large

microwave powers are no longer required to provide the thermal energy to vaporise the charge.

The cooling and heating of EDLs was employed to find out if any improvement in their performances was observed. When cooling was applied, the lamp discharge was extinguished after a few minutes. The rate of cooling was decreased to a minimum but the same sort of phenomena was observed for 6 different lamps. As it was not possible to maintain the discharge while cooling was employed, the idea was completely rejected. Heating was achieved by passing a heated air-stream around the lamp bulb. This involved the use of a resistively heated element over which is passed a supply of air. On heating, this air stream was introduced into the microwave cavity to provide a stable thermal environment in which the EDL could operate. Selenium EDLs were heated and their intensities were compared with unheated lamps intensities. Different temperatures were examined and all selenium EDLs showed almost negligible increase in their intensity. Figure 7 shows the effect of heating on the lamp intensities at different microwave powers.

All arsenic and selenium lamps showed very good stability and intensity at normal temperature when operated in a $3/4$ -wave cavity and under optimum conditions.

During the first hour of life the characteristics of an EDL can alter radically. It was found essential that all discharge tubes be "run-in" for the first two hours of the lamp's life at low powers; e.g. 30-40W; immediately after their construction. After this period a stable discharge and consequently stable output was achieved.

The life time of an EDL depends on many factors. In general, smaller diameter lamps exhibit shorter life-time due to the higher frequency of atom-to-wall collisions, particularly at high power levels. Filler gas pressure also governs lamp life-time. The higher the filler-gas

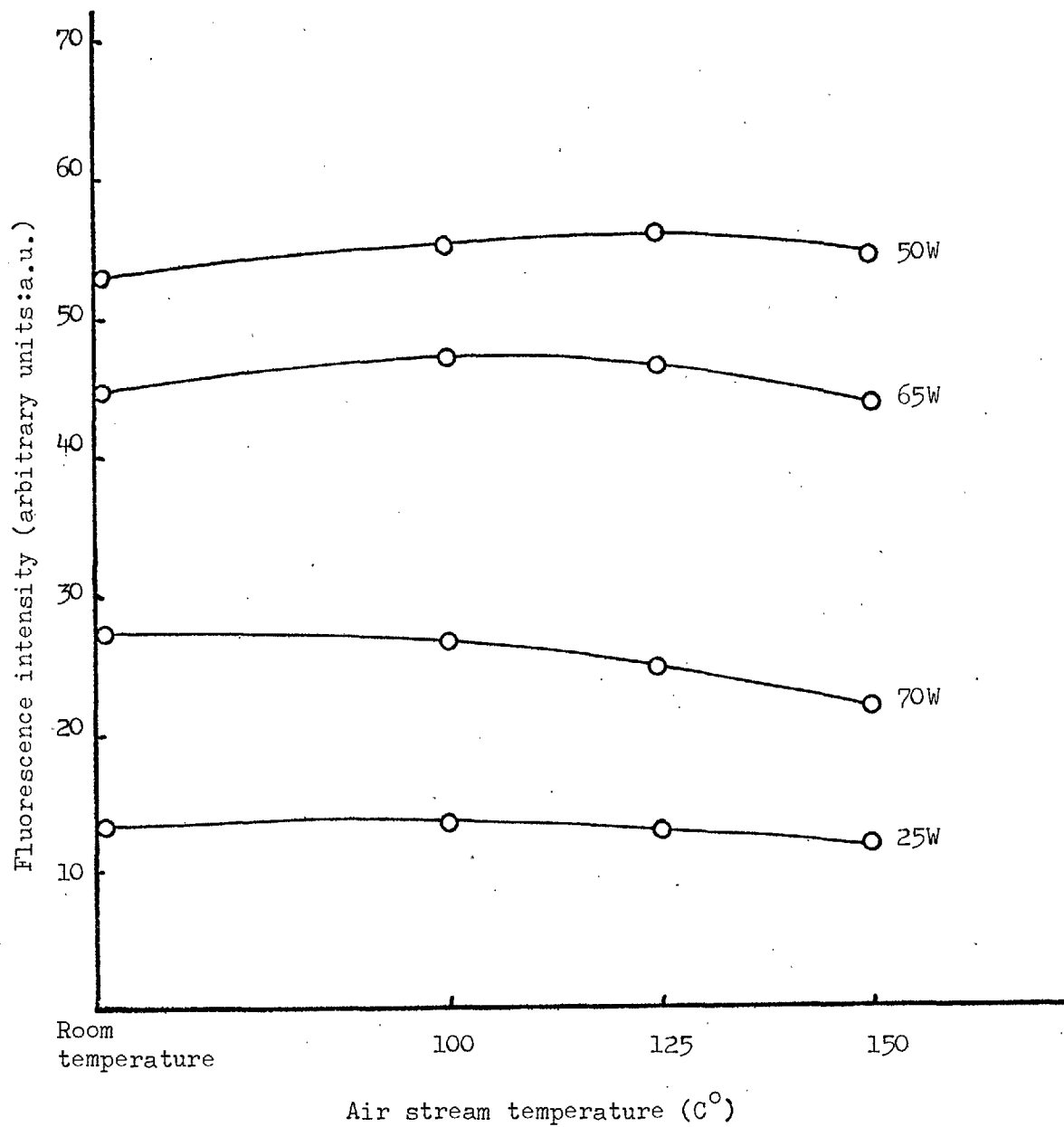


FIGURE 7 : THE EFFECT OF HEATING ON THE LAMP INTENSITIES AT
DIFFERENT MICROWAVE POWERS

pressure the longer will the lamp last. This phenomenon is related to the rate of "clean-up" of the filler-gas due to gettering action and diffusion into the material of the lamp walls. At very low pressures the clean-up process rapidly decreases the initial filler-gas pressure, resulting in very short lamp life-time. The clean-up process also occurs at higher pressures, but the total fraction of filler-gas effectively removed from the lamp atmosphere is considerably less, resulting in longer lamp lifetime.

Despite incomplete knowledge of the effect of all parameters discussed, EDLs exhibiting very good spectral characteristics and capabilities for exciting atomic fluorescence have been prepared. Good EDLs properly operated usually yield line intensities which are from 100 to 1000 times those of the corresponding conventional unshielded hollow cathode lamps, 10 - 100 times those of the shielded small bore hollow cathode lamps and up to 10 times those of boosted-output hollow-cathode lamp of the Sullivan-Walsh type.

The spectral characteristics of EDLs

Using a monochromator, it was possible to measure the line intensities from the sources. This does not give any indication of self-reversal at the line centre and is only a measure of relative intensities between lines and shows the lines present. The spectral range investigated was that of the response of the solar blind photomultiplier tube (1800 Å - 3200 Å).

The Selenium spectrum

The emission spectrum produced by a typical selenium EDL used throughout these studies and the stability of the lamp over a period of two hours, are shown in Figure 8.

This spectrum was obtained by focussing the radiation from the lamp onto the slit of a Techtron AA4 monochromator scanning at a rate of

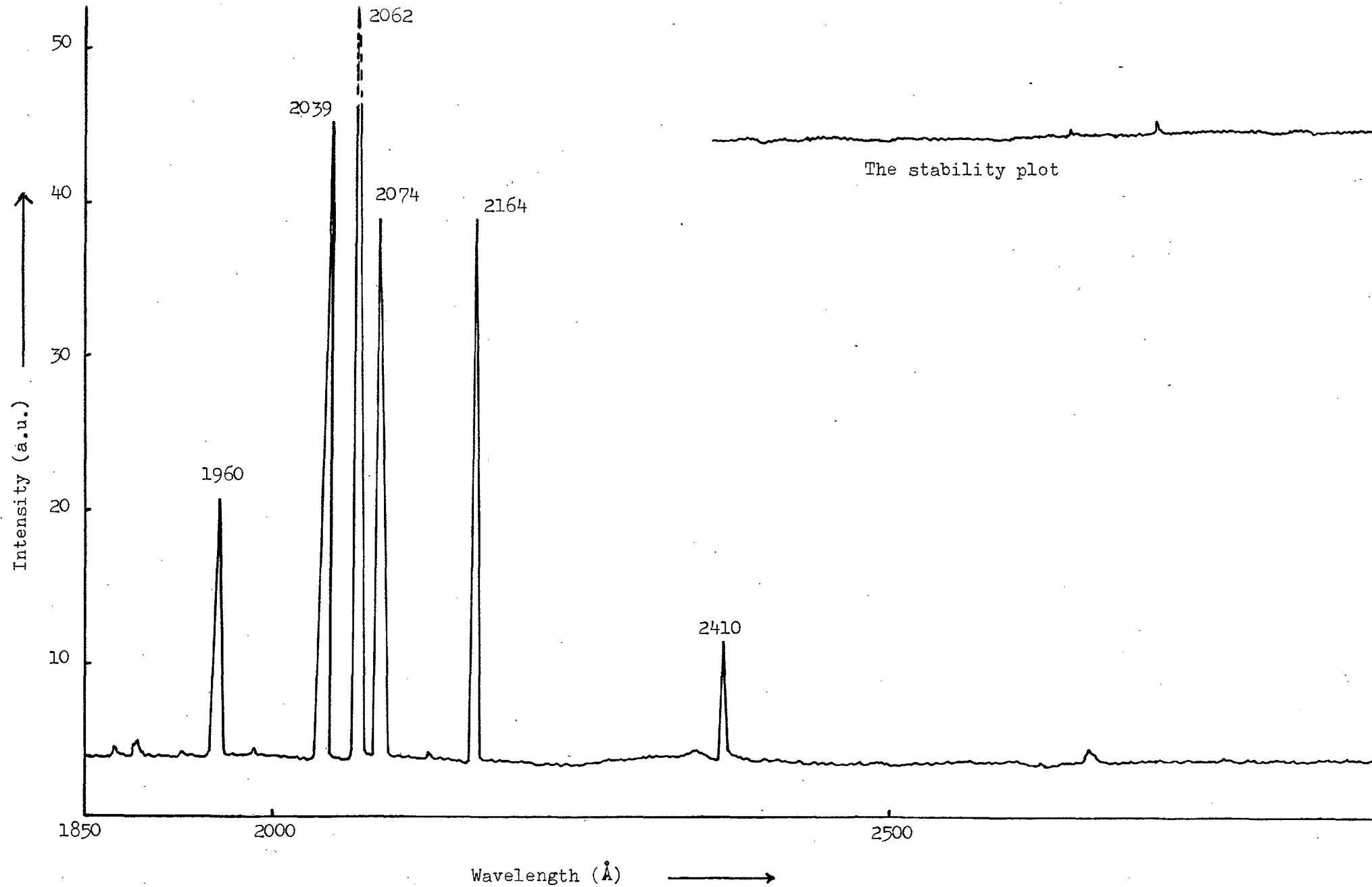


FIGURE 8 : THE SELENIUM LAMP EMISSION SPECTRUM AND ITS STABILITY PLOT

50 Å per min. and having a slit width of 50 μm . The photomultiplier (1P28) operating at 410 volts, was connected directly to a potentiometric chart recorder. All the expected selenium lines were observed and all of them were very sharp. Spectral characteristics of the observed selenium lines are listed in Table 5.

TABLE 5
THE SPECTRAL CHARACTERISTICS OF SELENIUM LINES

Wavelength (Å)	Energy Level (eV)	Transition
1960.9	0 - 6.323	${}^3\text{P}_2 - {}^3\text{S}_1^{\circ}$
2039.9	0.247 - 6.323	${}^3\text{P}_1 - {}^3\text{S}_1^{\circ}$
2062.8	0.314 - 6.323	${}^3\text{P}_0 - {}^3\text{S}_1^{\circ}$
2074.8	0 - 5.974	${}^3\text{P}_2 - {}^5\text{S}_2^{\circ}$
2164.0	0.247 - 5.974	${}^3\text{P}_1 - {}^5\text{S}_2^{\circ}$

The ground state of selenium ${}^3\text{P}_2$ is part of the triplet ${}^3\text{P}_2$, ${}^3\text{P}_1$ and ${}^3\text{P}_0$; see Figure 9.

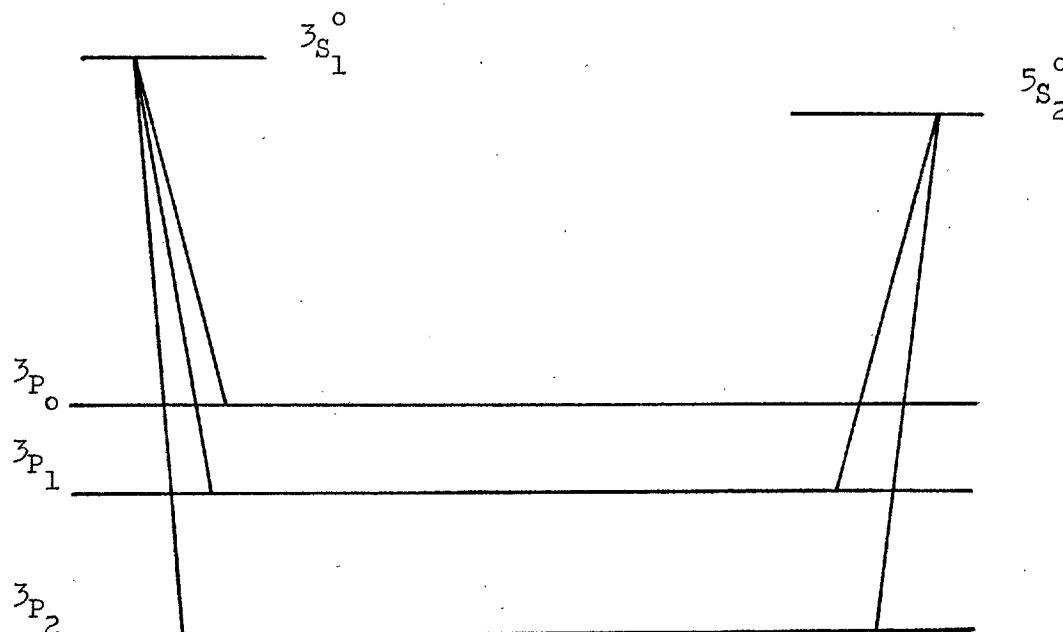


FIGURE 9 : THE SELENIUM GROUND STATE TRIPLET

The separation of these states is very small. There are thus three main contributions to the overall fluorescence intensity resulting from absorption of radiation to the $^3S_1^0$ excited state followed by deactivation to each of these three states. The 1960.9Å and 2039.9Å lines both absorb strongly whilst the 2062.8Å line absorbs only weakly. The 1960.9Å line emanating from the ground state absorbs about 5 times as strongly as the line emanating from the 3P_1 state which lies 0.25 eV above the ground state (148).

The relative intensities of the lines emitted by the spectral source give no true indication of the most intense fluorescence line, however, it is also necessary to consider the extent to which the absorption of each line by selenium atoms occurs. Experiments have indicated (148) that the major contribution to the measured fluorescence intensity results from resonance fluorescence at 1960.9Å and direct-line fluorescence at 2039.9 Å and 2062.8Å. It is possible that some atoms could be thermally excited to 3P_1 and 3P_0 states and that resonance fluorescence at 2039.9Å and 2062.8Å could occur but these processes are less probable than the direct line pattern described. Thompson (148) did not observe any atomic fluorescence signals for the 2075Å and 2164Å intercombination lines (3P_2 to $^5S_2^0$ and 3P_1 to $^5S_2^0$ transitions respectively). These two lines are quite intense but they are not of importance in contributing to the overall fluorescence signal.

The arsenic spectrum

The emission spectrum of the arsenic EDL at normal temperature is shown in Figure 10. This lamp was used throughout these studies. This spectrum was obtained by focussing the radiation from the lamp onto the entrance slit (50 µm) of a Rank Hilger monochromator equipped with an EMI 6256B photomultiplier operating at 900V and scanning at a rate of 50 Å per minute. Again signals were recorded at a potentiometric chart

recorder. The atomic fluorescence spectrum of arsenic is very complex. The main arsenic resonance lines are 1890Å, 1937Å and 1972Å. The spectral characteristics of these three lines are listed in Table 6.

TABLE 6
THE SPECTRAL CHARACTERISTICS OF ARSENIC RESONANCE LINES

Wavelength Å	Energy level eV	Transition
1890	0 - 6.557	$4s_{3/2}^o - 4p_{5/2}$
1937	0 - 6.398	$4s_{3/2}^o - 4p_{3/2}$
1972	0 - 6.285	$4s_{3/2}^o - 4p_{1/2}$

In addition to these three lines, 12 non-resonance fluorescence signals are observed. The 2381Å, 2456Å, 2437Å, 2492Å, 3032Å, and 3119Å lines are due to direct-line fluorescence, while the 2288Å, 2780Å, 2745Å, 2349Å and 2860Å lines are all examples of thermally assisted fluorescence processes (36). The relatively high fluorescence intensity of the 2349Å line is due to the low energy difference between the $4p_{5/2}$ and the $2p_{1/2}$ states (0.027 eV) and the favoured $2p_{1/2} - 2d_{3/2}^o$ spin-allowed transition. The fluorescence signals from the $2p_{3/2}$ state, which lies 0.21 eV above the $4p_{5/2}$ state are much weaker than those from the $2p_{1/2}$ state. These fluorescence signals (from the $2p_{3/2}$) are very weak in the cool diffusion flames, but are much stronger in the hotter air-acetylene flames, as would be expected.

Arsenic EDLs proved to be much more difficult to construct than selenium EDLs.

They were very sensitive to any small fluctuation in the applied microwave power and the discharge colour (light blue) tended to change

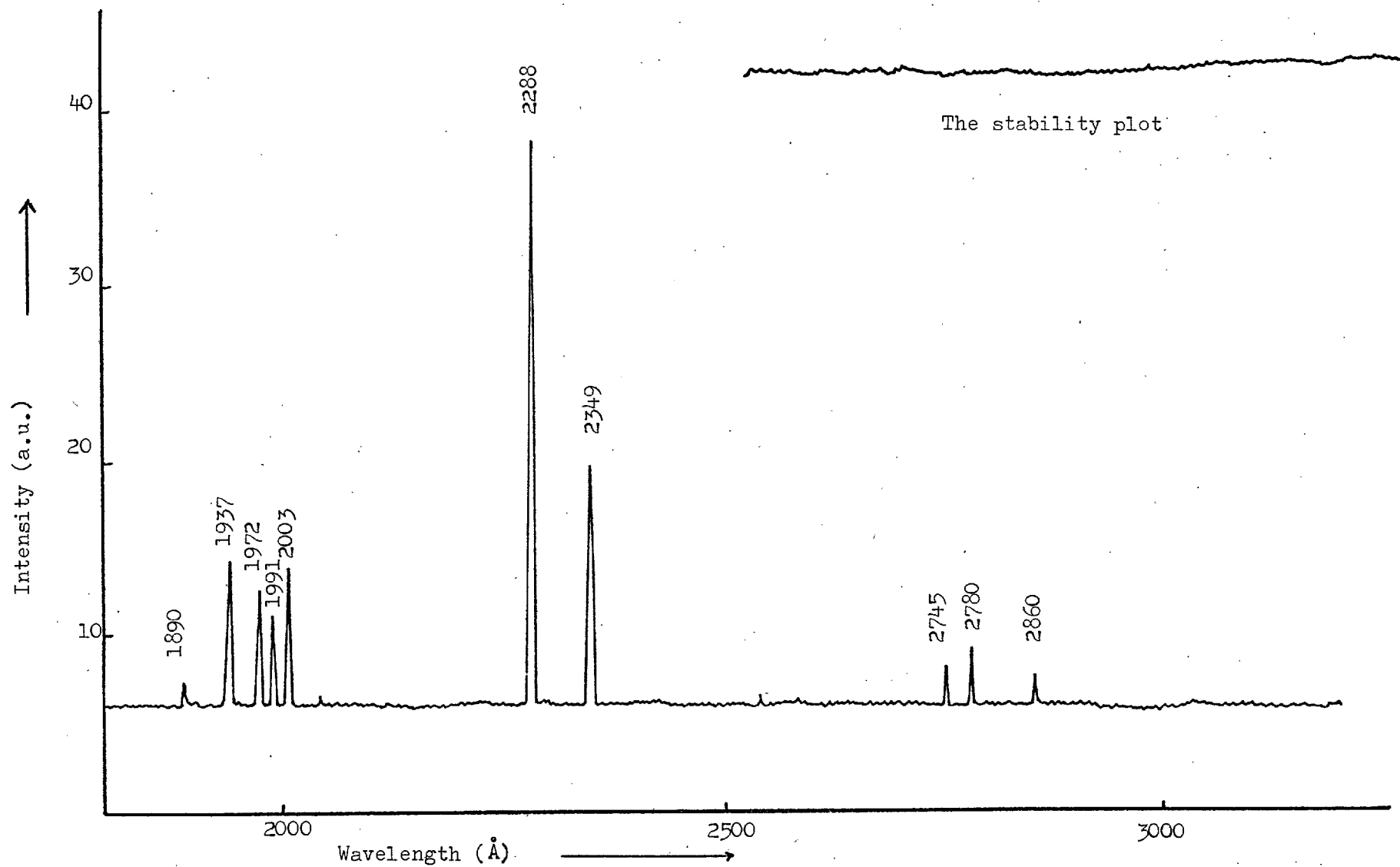


FIGURE 10 : THE ARSENIC LAMP EMISSION SPECTRUM AND ITS STABILITY PLOT

to a light green and then to a purple argon discharge colour. If the lamp was heated on a Bunsen flame, and lit, the normal blue colour appears again but the same phenomena occurred after about half an hour. This curious behaviour is difficult to explain but could be connected with the amorphous arsenic used, which slowly changes to a less volatile, more stable allotrope under low pressure conditions. The arsenic lamps were generally more unstable than selenium lamps as free arsenic tends to plate out on the walls of the quartz tube. The lamp used throughout these studies was chosen from a set of arsenic EDLs and showed very good stability over a period of 2 hours (Figure 10).

2.3.2. The atom cell

An argon-hydrogen entrained air flame was used throughout these studies. This flame was supported simply on a tube burner. Tubes with different internal diameters were examined and the most suitable one (7 mm i.d.) was used during the experimental work. The top of the burner was slightly narrower than the rest of the tube to give a sort of jet action to the gas mixture.

The flow rates of argon and hydrogen were monitored and controlled by passing each gas directly from the cylinder through a flowmeter tube. The burner could be moved 30 mm horizontally or vertically to facilitate optimisation of burner position with respect to the detector. The argon-hydrogen entrained air flame is a cool flame, a term applied generally to flames in which the oxidant necessary for combustion is fully supplied by diffusion or entrainment from the surrounding atmosphere. Argon is usually used as diluent and hydrogen as fuel. The argon-hydrogen entrained air flames are almost transparent, while hydrocarbon flames are luminous. The temperature of cool diffusion flames ranges from 280°C to 850°C depending on the exact region measured (149).

Despite this low temperature, cool flames are quite effective atomizers for several elements. The main advantages of cool flames using argon as diluent are: low background emission, high transmittance in the ultra-violet region below 2000 Å and lower concentration in the flame gases of molecular species with high quenching cross-sections, resulting in higher fluorescence yield.

Lower background emission and lower absorbance by flame gases leads to improved detection limits for arsenic and selenium. In the case of entrained air flames, because of the more energetic mixing with the atmosphere and a more vigorous combustion the temperature of the flame is not quite as low as that of diffusion flames. Smith, Stafford and Winefordner (150) reported a temperature of 2122 K, for the argon-hydrogen entrained air flame.

The flow rates of argon and hydrogen were optimized to establish the most suitable flame conditions for the experimental work.

Sample introduction was effected in this work by generating the hydride gases of the elements of interest inside a hydride generation cell and carrying them into the flame on a stream of argon. The hydride generation cell used throughout this work is shown in Figure 11.

Argon was bubbled through the sample solution and generated hydrides were thus swept into the flame. The bubbling system, however, caused poor reproducibility of the observed fluorescence signal and was consequently abandoned in favour of the system shown in Figure 11.

Cells with different volumes and shapes were examined and the most suitable volume was found to be around 50 ml. The side arm was used for the introduction of standard solutions into the generation cell, using a syringe pipette. The tap at the bottom of the cell facilitated the washing and cleaning of the cell.

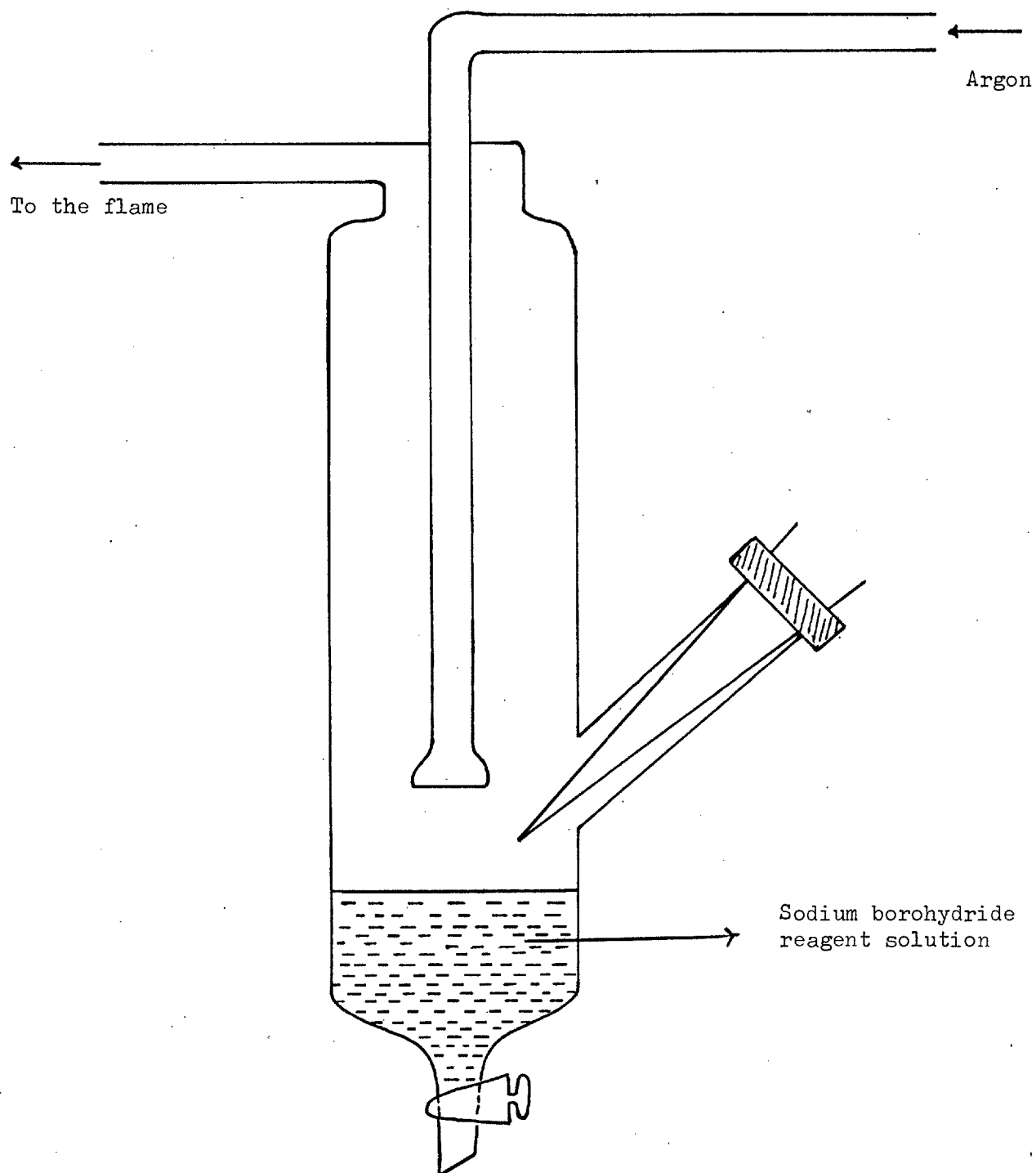


FIGURE 11 : THE HYDRIDE GENERATION CELL EMPLOYED

2.3.3. Detection and signal processing system

Although the instrument used in these studies is fairly simple the detection and signal processing system is more sophisticated. The modulation of the source radiation, using a mechanical chopper results in a modulated fluorescence signal of the same frequency (500 Hz) and phase. This signal plus some unwanted background radiation is detected by the PMT and converted into an equivalent electrical signal which is then amplified and fed into the phase sensitive detector. A reference signal from the chopper (Programmable Rofin Model 7500, 3-800 Hz, Rofin Ltd., Egham, Surrey) is fed to the phase sensitive detector where it is compared with the fluorescence signal. Any component of the fluorescence signal which is of the same frequency and phase as the reference signal is converted into a d.c. signal which is displayed on a readout device. An eleven stage, end window R431 solar-blind PMT, (Hamamatsu Co., Japan) was used through this work. The limit of its response to radiation is from 1800Å to 3200Å due to its Cs-Te coated cathode. Figure 12 shows the response characteristic of the PTM.

This type of PMT is particularly suitable for the determination of elements with their main resonance lines below or above 2000Å (eg Se, As,.....). A Brandenburg power supply Model 476R was used to power the PMT. It has a maximum out put of 2000 volts but the best signal/noise ratio was obtained at much lower voltages (600V). By increasing the supplied voltage, the actual magnitude of the fluorescence signal increased but the signal-to-noise ratio changed unfavourably. A Brookdeal lock-in, low noise amplifier (Brookdeal Electronics Type 540S) was used to amplify the signal from the PMT. This device is not frequency-tunable but the bandwidth may be limited by selecting the appropriate low and high pass filter frequencies (1000Hz - 100Hz).

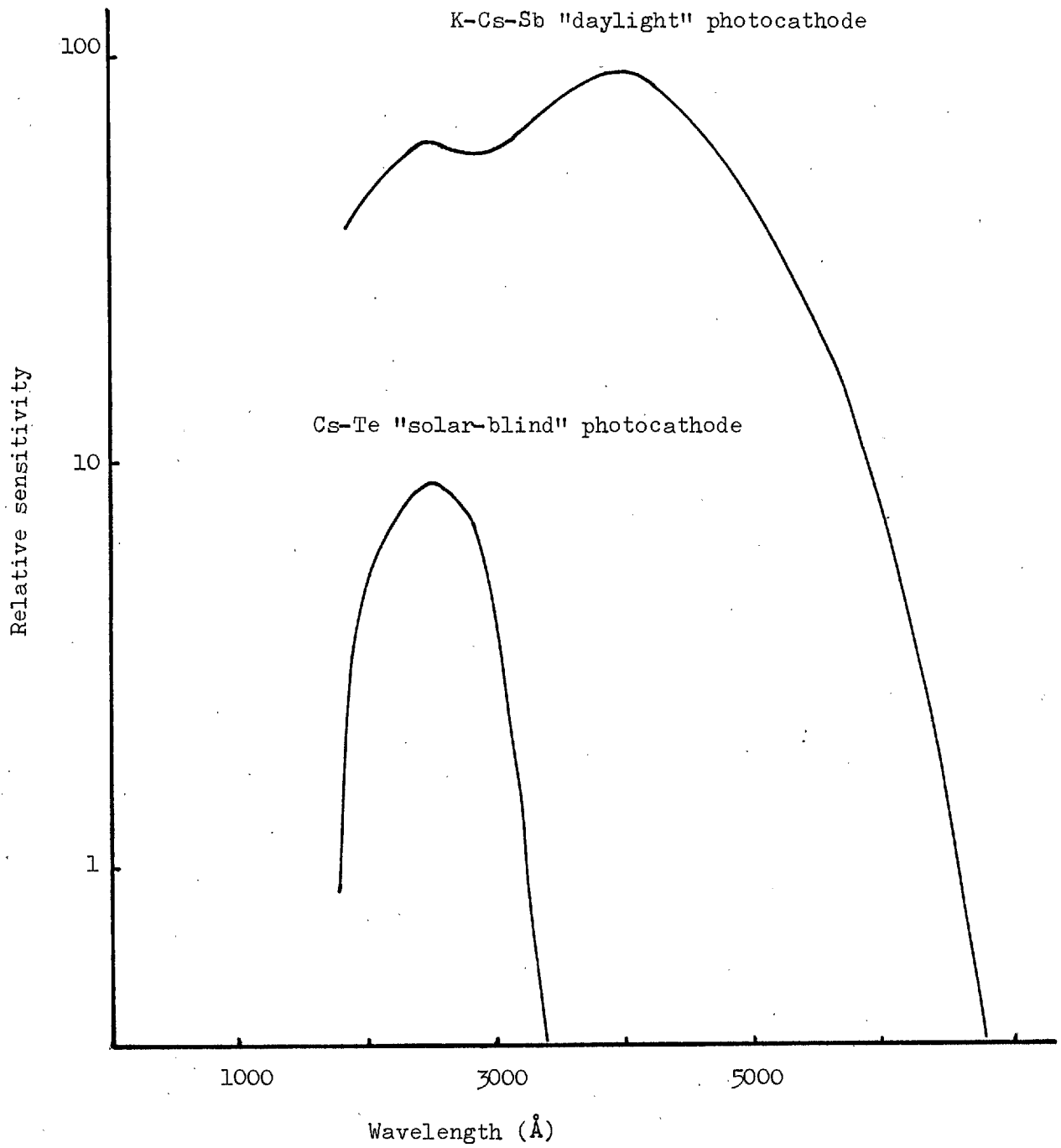


FIGURE 12 : THE RELATIVE SENSITIVITIES OF SOLAR BLIND AND DAYLIGHT PHOTOMULTIPLIERS

The maximum gain of +100 db is available but the optimum gain was found to be 40 db, considering the signal-to-noise ratio. A Brookdeal phase-sensitive detector (Brookdeal Electronics Type 411) was used to select that component of the signal from the amplifier, of the correct frequency and in phase with the reference waveform. The d.c. output from the phase-sensitive detector was displayed on a Servoscribe Model RE 511. 20 potentiometric recorder. The multi-range facility of this unit permits signal voltages of up to +20 volts to be measured. The instrument described above was used to determine selenium and arsenic at very low levels in both aqueous solutions and real samples. In the following chapters the experimental steps taken and the practicability of the system in real sample analysis are discussed.

CHAPTER THREE

3. DETERMINATION OF SELENIUM BY NON-DISPERSIVE ATOMIC FLUORESCENCE SPECTROSCOPY

3.1. INTRODUCTION

Dagnall et al (151) were the first authors to describe a method for the determination of selenium by atomic fluorescence spectroscopy. They used a selenium EDL as the excitation source together with an air-propane flame. It was found that there was no need to use a hot flame or a high-resolution monochromator. Linear calibration curves were obtained over the range 0.25 - 125 ppm of selenium with a slit-width of less than 2 mm and a detection limit of 0.15 ppm was reported. Cresser and West (152) have also determined selenium by atomic fluorescence spectroscopy, using an air-acetylene flame and automatic background correction. Detection limits of 1 and 0.2 ppm respectively for slit-widths of 0.1 and 1 mm were obtained.

Winefordner et al. (131) investigated the effect of an open quartz tube device and a quartz vacuum jacket device on the performances of EDLs used in flame atomic fluorescence spectroscopic determination of different elements. The selenium EDL was best operated at an "A" antenna with no vacuum jacket and a detection limit of 0.4 µg/ml selenium was obtainable. Thompson (115) found that very good detection limits could be obtained by using the hydride generation technique. He reported a detection limit of 0.00006 µg/ml for selenium, as twice the noise level on the base-line. In addition, Thompson and Wildy (153) demonstrated the advantages of using an electronically modulated spectral source in the determination of selenium. A detection limit of 0.1 µg/ml selenium and linearity over the range of 0.5 to 50 µg/ml selenium was reported. Browner and Manning (154) utilized a separated air-acetylene

flame and an H.C.L as the source of excitation. A detection limit of 45 $\mu\text{g/ml}$ was obtained and they concluded that the greater the reduction of flame emission provided by separation, the greater improvement in the detection limit. Multi-element EDLs have been used for the atomic fluorescence spectroscopic determination of selenium.

Cresser and West (155) used a dual-element EDL containing arsenic and selenium to obtain a detection limit of 0.8 $\mu\text{g/ml}$ for selenium and Marshall and West (156) used a multi-element source containing Bi, Se, Hg and Te, achieving a 10 $\mu\text{g/ml}$ detection limit for selenium. The first report of a non-dispersive atomic fluorescence system used for the determination of selenium was published by Larkins (123). He used a H.C.L₂ and a separated air-acetylene flame to obtain an aqueous detection limit of 6 $\mu\text{l/ml}$.

This chapter describes a sensitive, rapid and precise non-dispersive atomic fluorescence spectroscopic method for the determination of selenium in aqueous solutions. The interfering effects of different metal ions on selenium determination are studied and procedures for the suppression or elimination of copper interference effects are recommended.

3.2. EXPERIMENTAL

3.2.1. Reagents

Selenium(IV) stock solution was prepared by dissolving 100 mg of pure elemental selenium (Spec.pure grade - Johnson and Matthey Ltd.) in a minimum volume of concentrated nitric acid and diluting to 100 ml with 5 M hydrochloric acid (1000 ppm). Standard solutions were prepared by the appropriate dilution of this stock solution and were usually prepared freshly each day.

The sodium borohydride reagent was used as a freshly prepared 5% w/v solution in 1% sodium hydroxide solution. Tellurium dioxide solution was prepared by dissolving 4 g of Te O_2 in a minimum volume of

hot hydrochloric acid and diluting to 250 ml with distilled water.

Analytical reagent grade lanthanum nitrate, potassium bromide, hydrochloric acid, nitric acid and concentrated ammonia solution were used in all experiments.

3.2.2. Procedure

Using the instrument shown in Figure 4, Chapter 2, the following procedure was followed: with the flame ignited and argon passing through the hydride generation cell, sufficient time (ca. 20 seconds) was allowed for the replacement of any air in the apparatus. 2 ml of sodium borohydride reagent solution was then transferred to the generation cell via. the side-arm. 1 ml of acidified selenium standard solution was then pipetted into the sodium borohydride reagent using a syringe pipette whose tip was fitted with a rubber sleeve to ensure a gas-tight fit with the side-arm of the cell during sample introduction. The hydrogen selenide generated was then swept into the argon-hydrogen flame by the argon supply to the flame. The selenium atomic fluorescence signal was recorded at the chart recorder. The signal duration observed for a 5 $\mu\text{g/ml}$ selenium standard solution was ca. 8 seconds.

3.3. OPTIMIZATION OF EXPERIMENTAL PARAMETERS

Utilizing pure aqueous Se(IV) standard solution the experimental variables in the instrumental system employed were optimized to provide the best attainable sensitivity and precision. The operating power for the microwave-excited selenium EDL source, photomultiplier operating voltage, amplifier gain, hydride generation cell volume and sodium borohydride reagent and selenium standard solution volumes used in the hydride generation cell were each varied independently to establish optimum conditions for the determination of selenium. The optimum conditions established in this way are represented graphically in Figures 13-18.

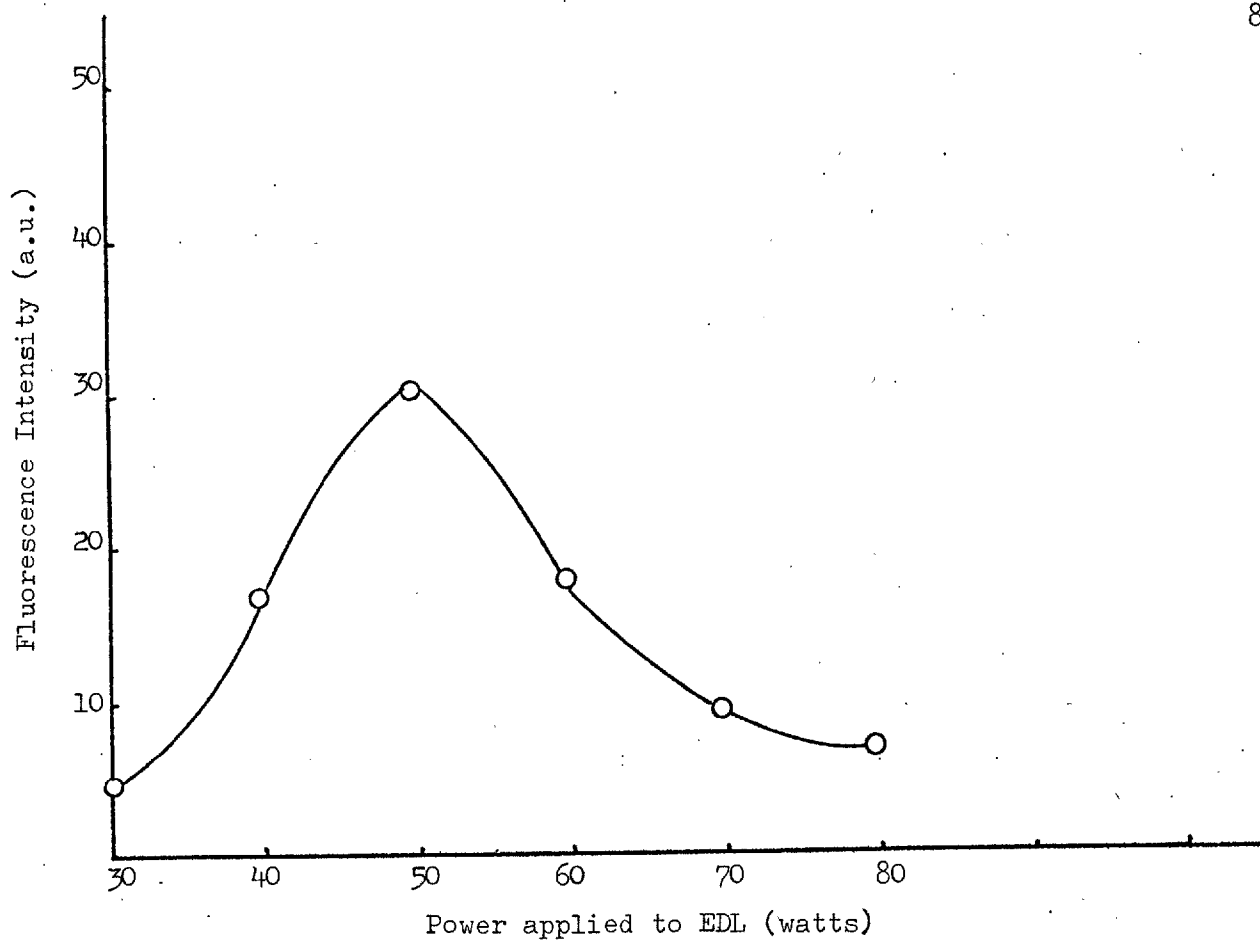


FIGURE 13 : THE EFFECT OF POWER APPLIED TO SELENIUM EDL ON FLUORESCENCE
INTENSITY

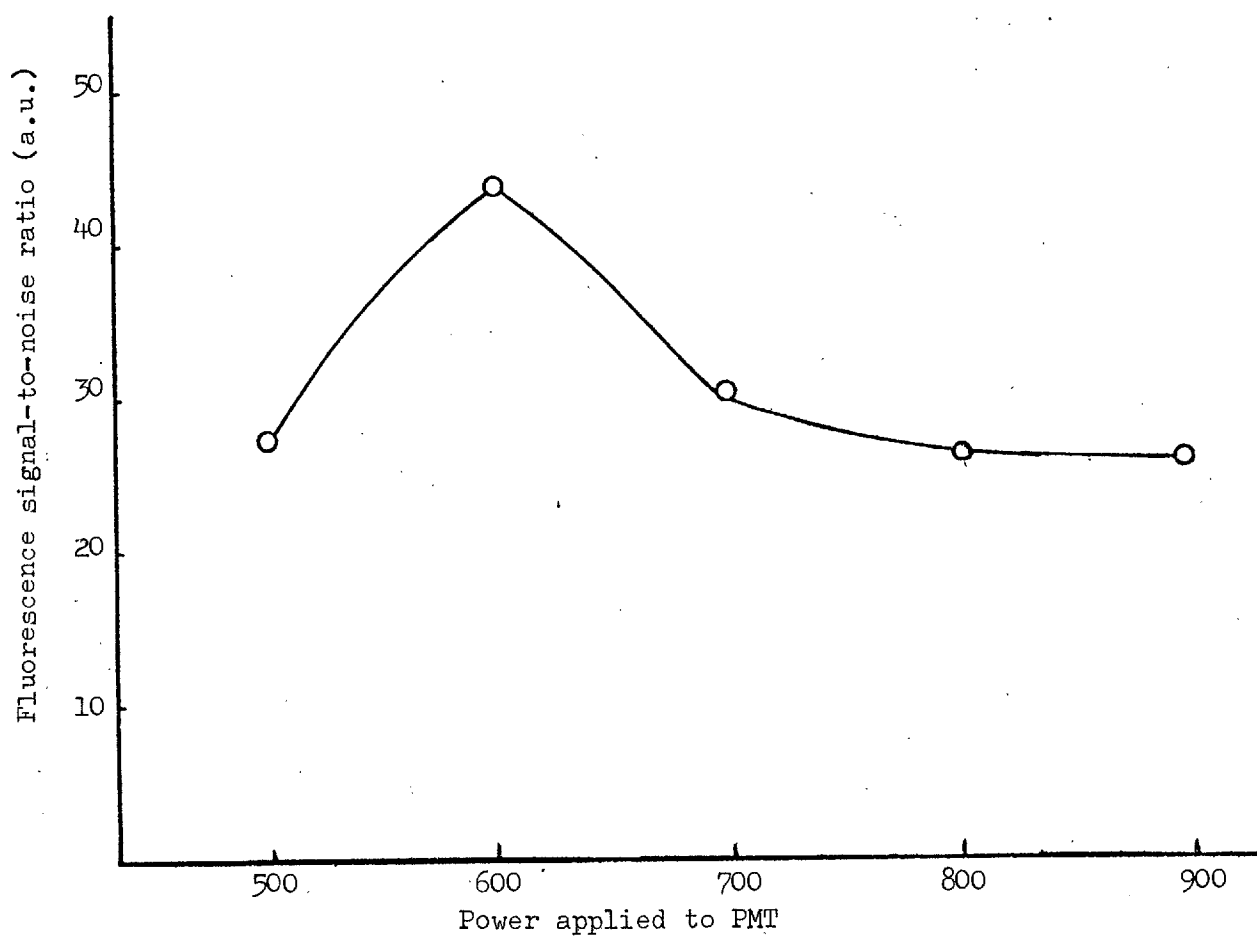


FIGURE 14 : THE EFFECT OF POWER APPLIED TO PMT ON FLUORESCENCE SIGNAL-
TO-NOISE RATIO

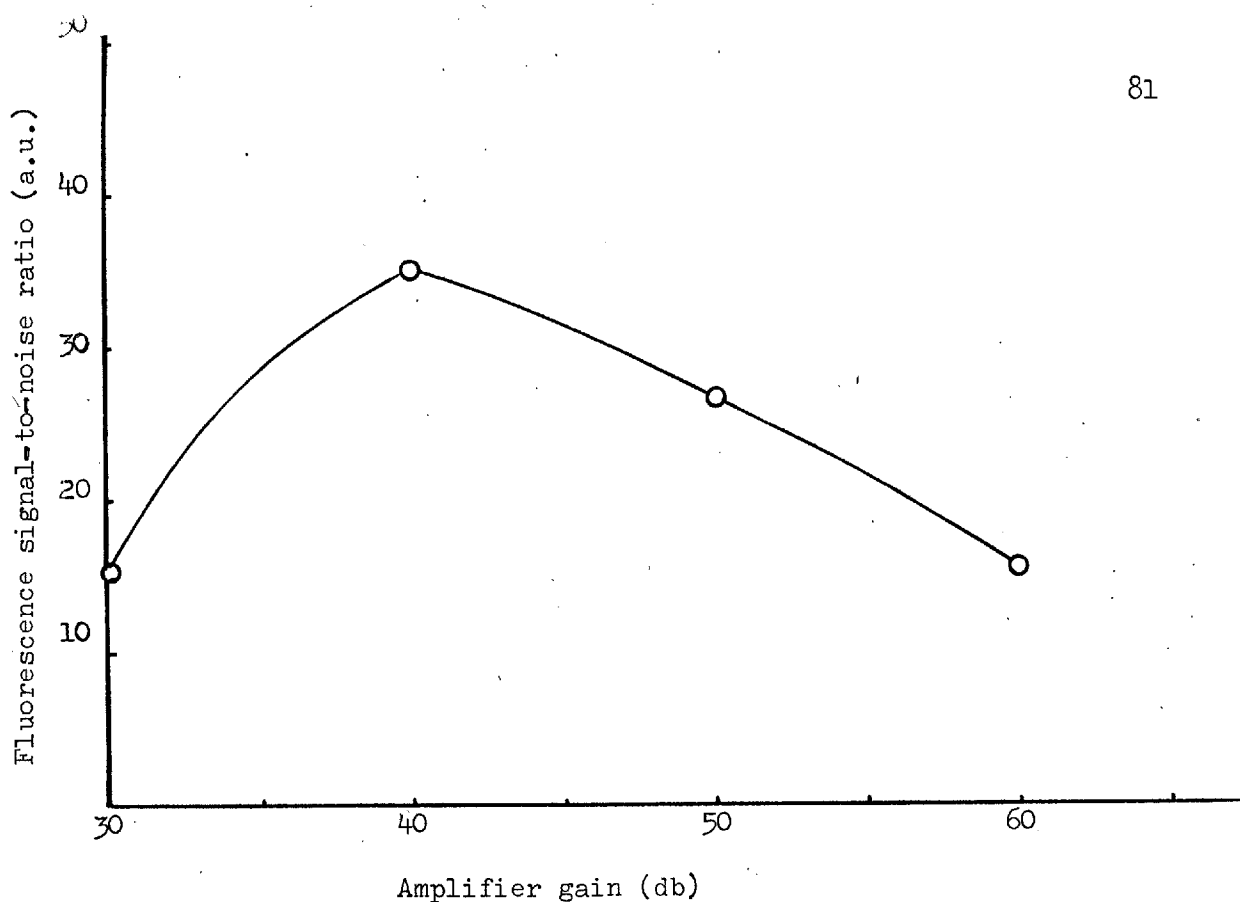


FIGURE 15 : THE EFFECT OF AMPLIFIER GAIN ON FLUORESCENCE SIGNAL-TO-NOISE RATIO

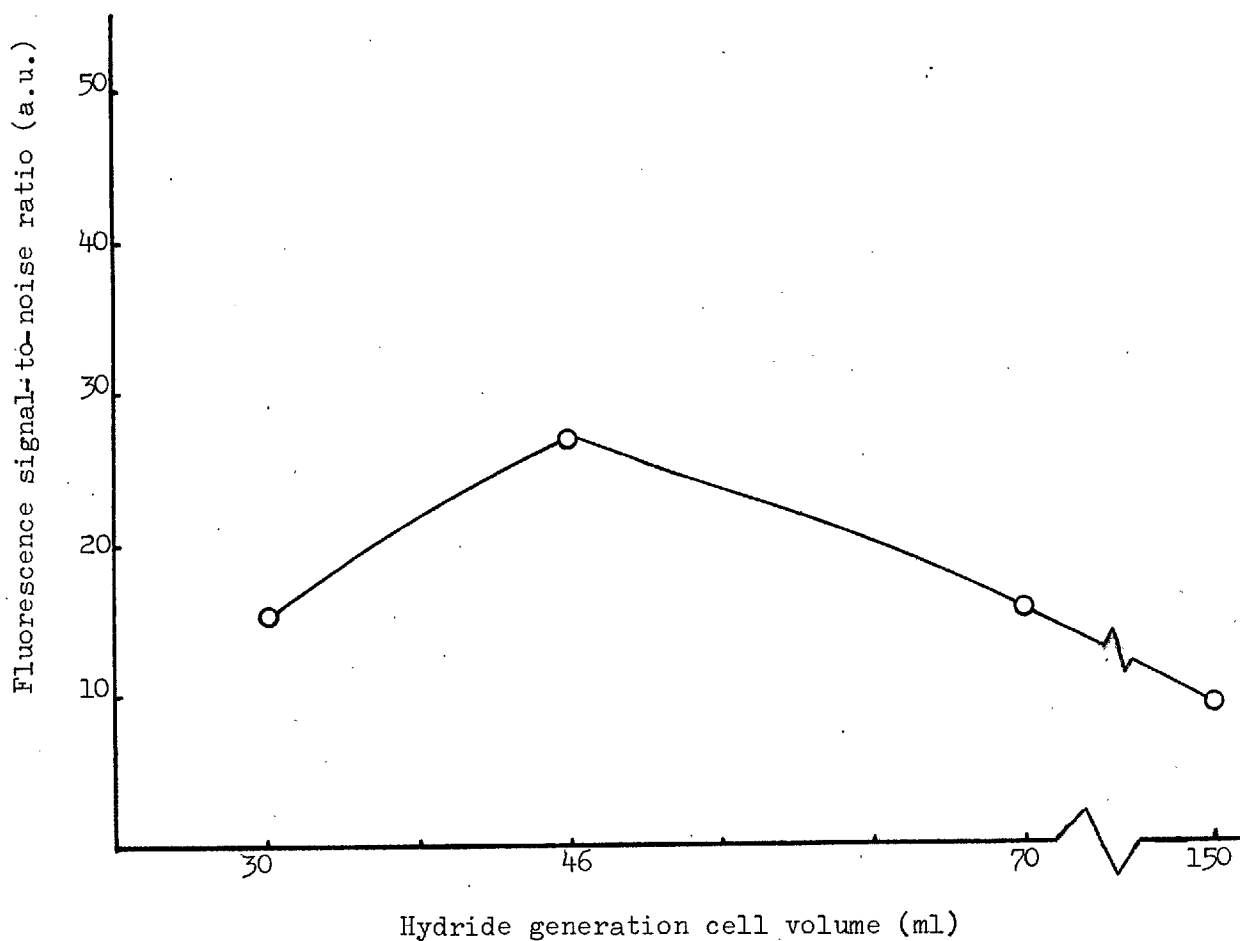


FIGURE 16 : THE EFFECT OF HYDRIDE GENERATION CELL VOLUME ON FLUORESCENCE SIGNAL-TO-NOISE RATIO

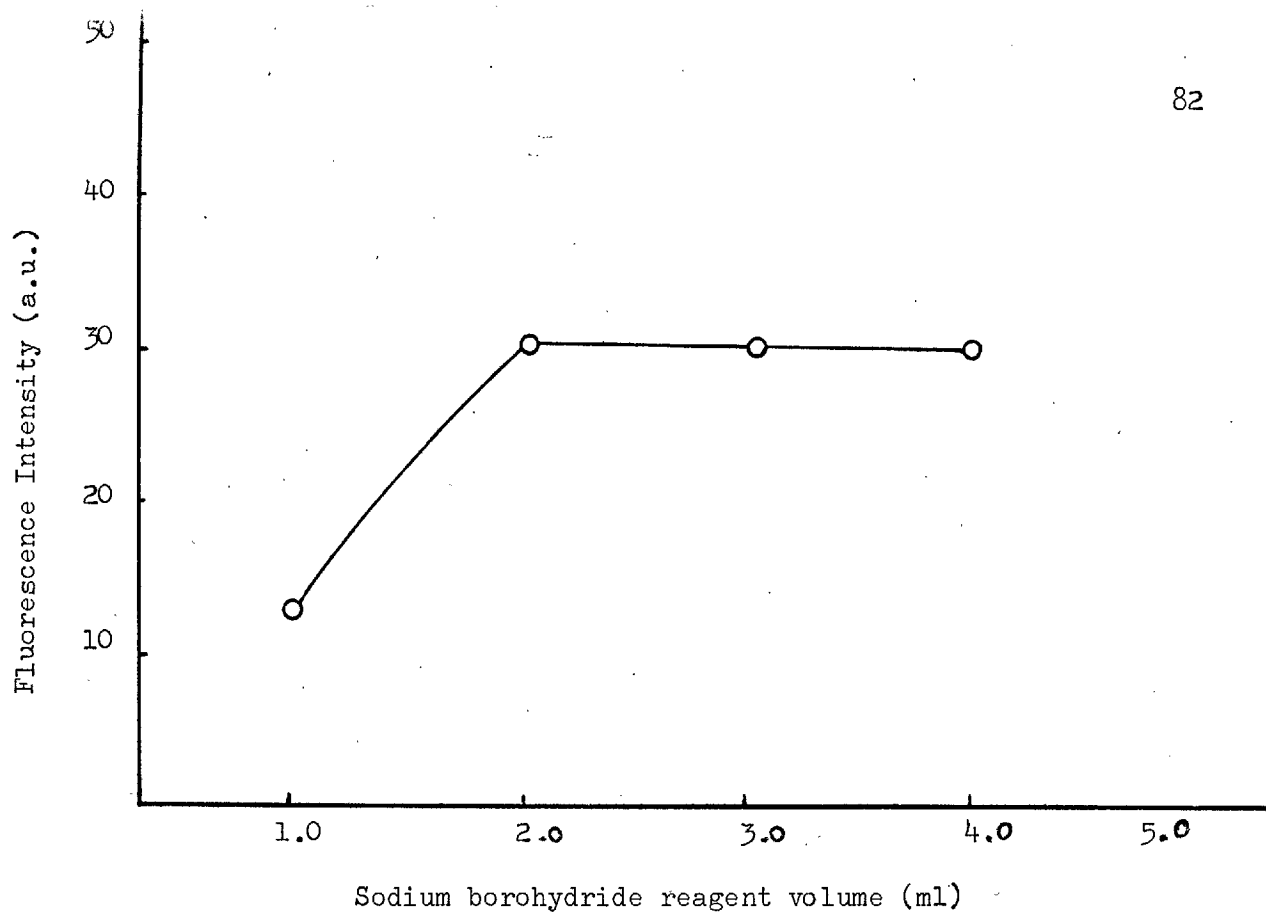


FIGURE 17 : THE EFFECT OF NaBH_4 REAGENT VOLUME ON FLUORESCENCE INTENSITY

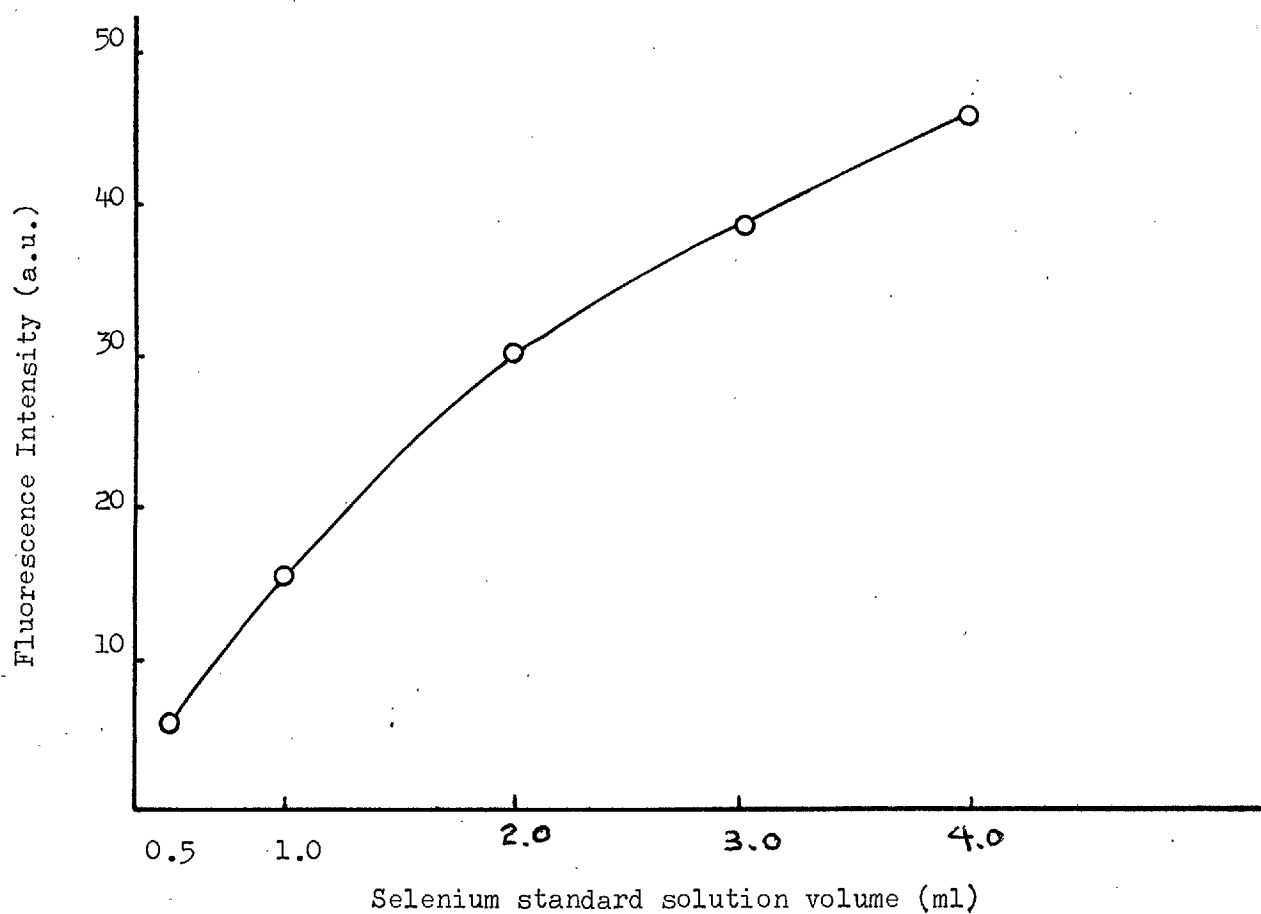


FIGURE 18 : THE EFFECT OF SELENIUM STANDARD SOLUTION VOLUME ON
FLUORESCENCE INTENSITY

3.3.1. Effect of hydrochloric acid and sodium borohydride concentrations

The effect of hydrochloric acid concentration of the standard solution, on the intensity of atomic fluorescence signal observed for 0.5 μg of selenium was investigated. The sodium borohydride concentration was maintained constant at 5% (w/v) for this experiment. Variation in the acid concentration present in the selenium standard solution has a pronounced effect on the efficiency of generation of hydrogen selenide only when the solution is less than 0.8 M with respect to hydrochloric acid. The atomic fluorescence signal remained more or less constant at higher acid concentrations. The results obtained are shown in figure 19. In all further work the hydrochloric acid concentration of the solutions to be analysed was maintained at 5 M. Using 1 ml volumes of selenium solution containing 0.5 μg selenium and which were 1 M with respect to hydrochloric acid, the effect of variation of sodium borohydride concentration on the atomic fluorescence signal was also investigated. Little variation in hydride generation efficiency was observed over the concentration range between 2% and 5% (w/v) of sodium borohydride. The results can be seen in Figure 20. A concentration of 5% (w/v) was chosen to be used in all further work.

3.3.2. Optimization of flame height and flame composition

An argon-hydrogen entrained air flame was used in this work. The argon and hydrogen gas flow-rates to the flame were optimized and with the optimum flow rates, burners with different internal diameters were examined and the results can be seen in Figure 21. By changing the viewing position of the flame the optimum viewing position was found to be about 25 mm above the top of the burner. The variation of the atomic fluorescence signal-to-noise ratio with various viewing position in the flame is shown in Figure 22.

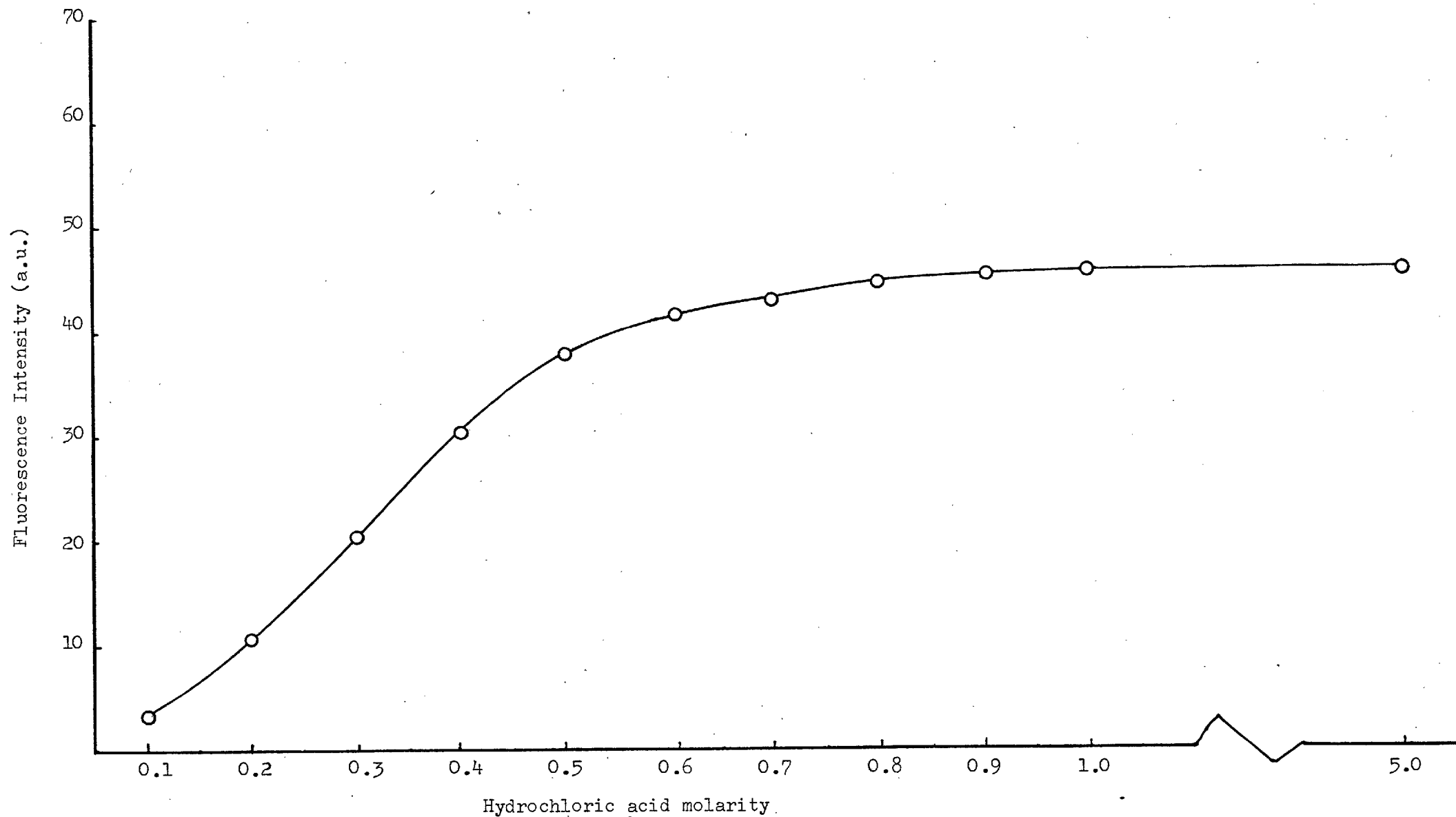


FIGURE 19 : THE EFFECT OF HCl CONCENTRATION ON THE DETERMINATION OF 0.5 $\mu\text{g/m}$ SELENIUM

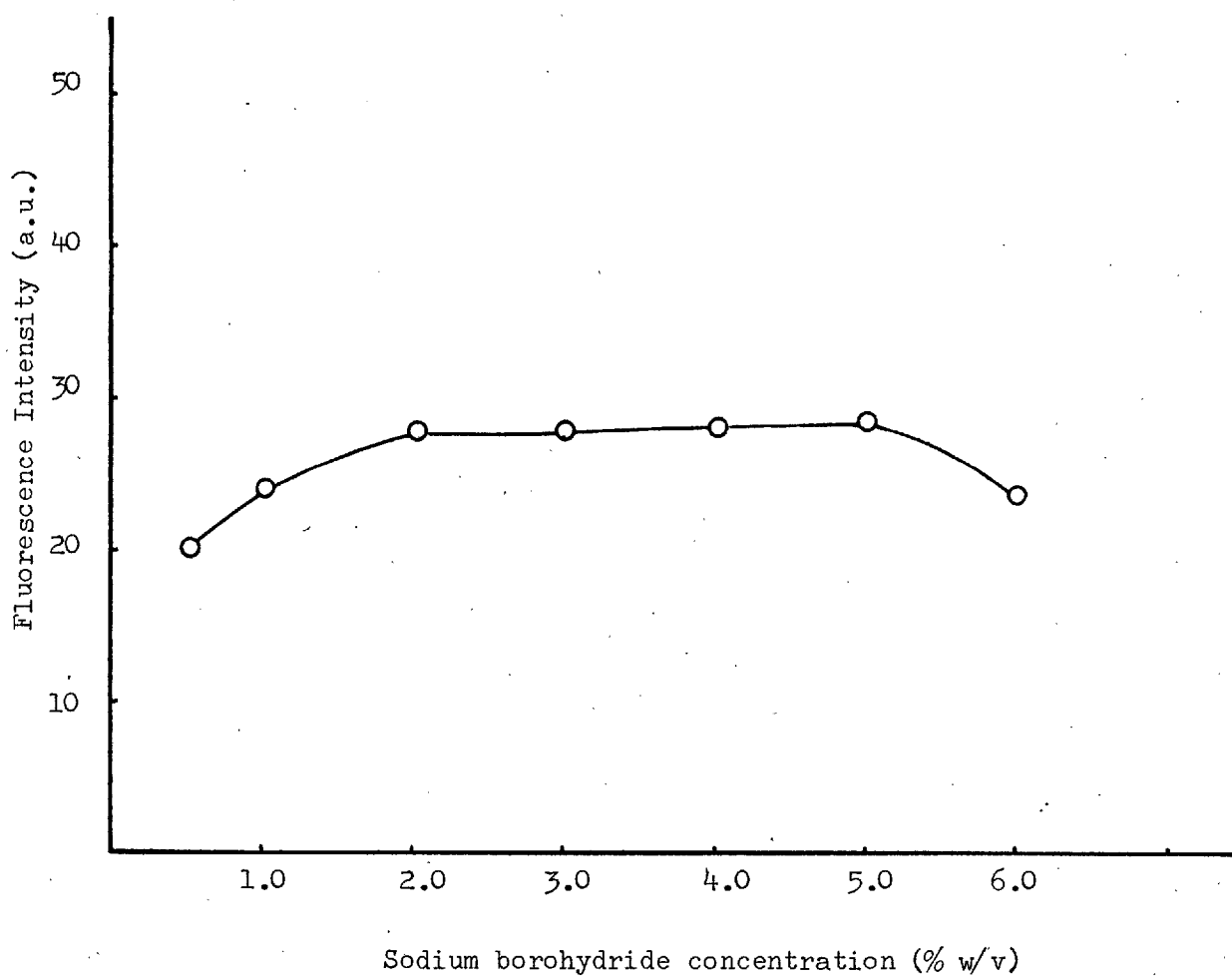


FIGURE 20 : THE EFFECT OF NaBH_4 CONCENTRATION ON THE DETERMINATION OF
0.5 $\mu\text{g/ml}$ SELENIUM

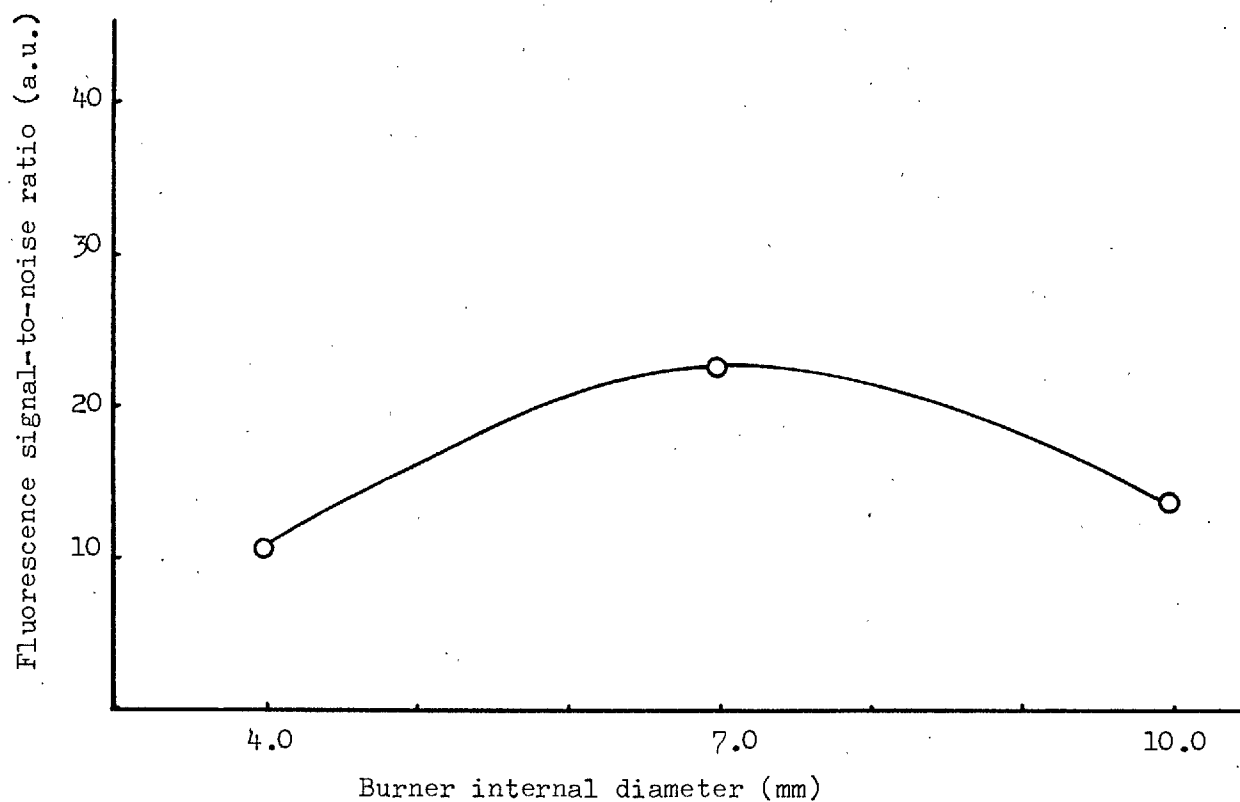


FIGURE 21 : THE EFFECT OF BURNER DIAMETER ON FLUORESCENCE INTENSITY

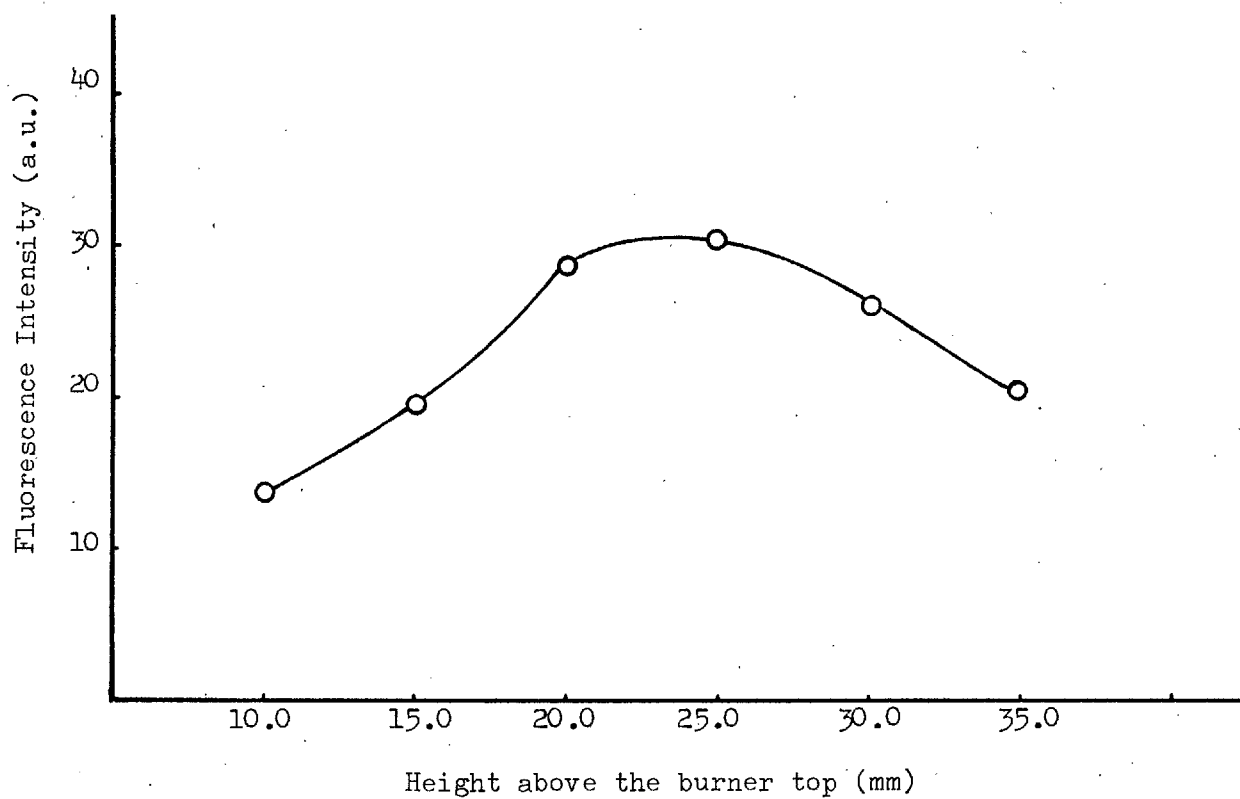


FIGURE 22 : THE EFFECT OF VIEWING POSITION ON FLUORESCENCE INTENSITY

3.4. CALIBRATION CURVE, LIMIT OF DETECTION AND PRECISION

The calibration curve for selenium, shown in Figure 23 was obtained by measuring the atomic fluorescence signals produced by a range of solutions of differing concentrations under optimum conditions. The calibration curve was found to be linear for selenium solutions containing between 10 and 500 ng/ml selenium in 1 ml sample volume i.e. for between 10 and 500 ng selenium. Signals obtained for aqueous solutions containing 0.1, 0.2, 0.3, 0.4 and 0.5 μg selenium are shown in Figure 24. The detection limit for selenium, defined as that mass of selenium required to produce a signal-to-noise ratio of 2 for the atomic fluorescence signal, was 10 ng selenium, under the conditions employed. A significant background blank signal was observed for selenium, equivalent to ca. 16 ng/ml selenium. It was probably due to the presence of selenium as an impurity in the sodium borohydride reagent employed. Two methods for removing this blank were investigated. Passing an inert gas such as helium through a 1% w/v sodium borohydride solution has been reported to remove selenium as its hydride (157).

To reduce the size of the blank signal by expelling the selenium hydride, argon was passed through the sodium borohydride reagent solution for 2 hours. The height of the blank signal did not change significantly.

Recrystallization of sodium borohydride **reagent** also did not have any effect on the height of the blank signal. The blank was then corrected for by subtraction in all quantitative analytical work undertaken. Ten repetitive determinations of selenium in a solution containing 100 ng/ml resulted in a relative standard deviation of 2.5%. The optimum operating conditions under which the calibration curve was produced may be summarised as follows:

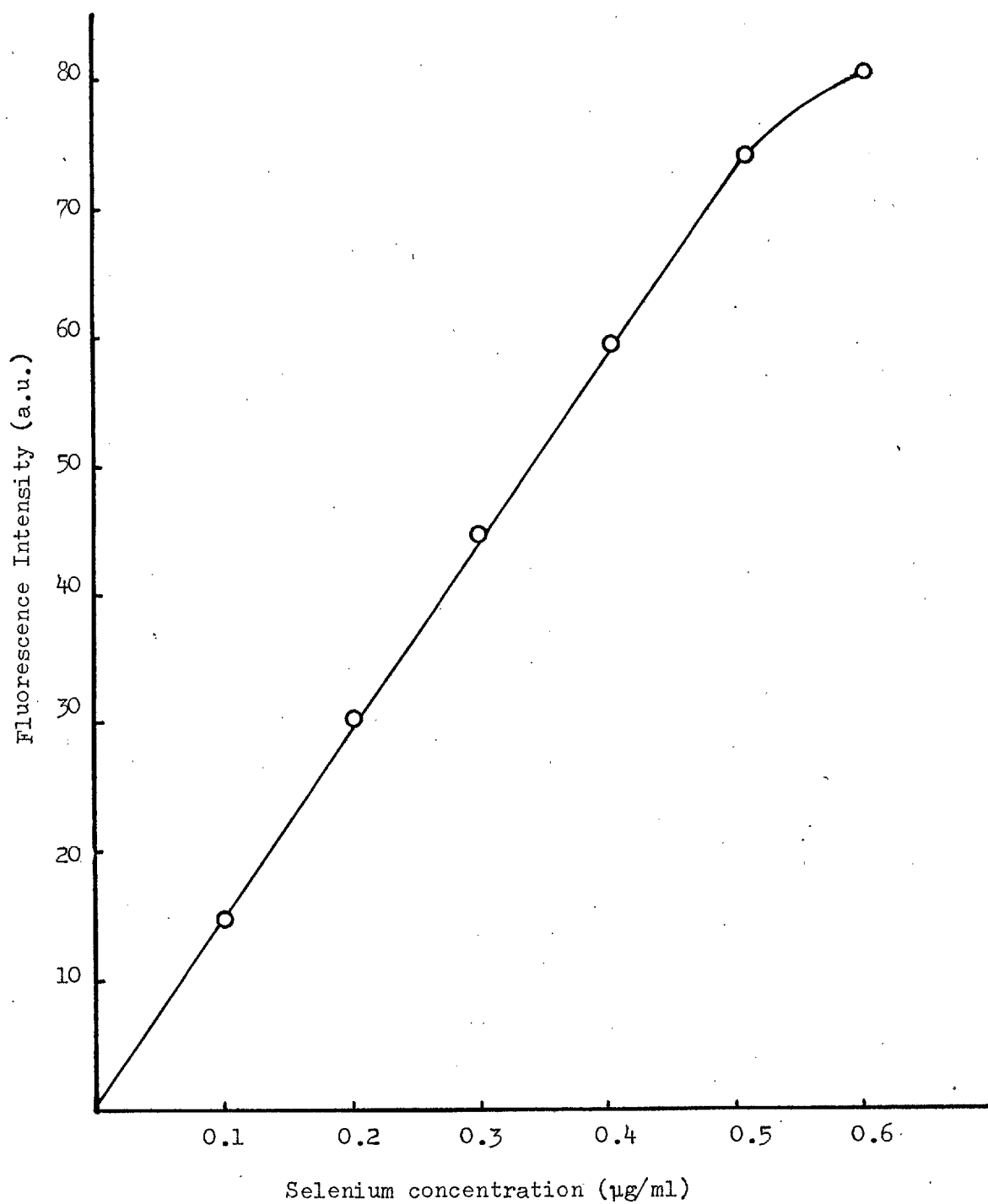


FIGURE 23 : AQUEOUS CALIBRATION CURVE FOR THE DETERMINATION OF
SELENIUM BY NON-DISPERSIVE AFS

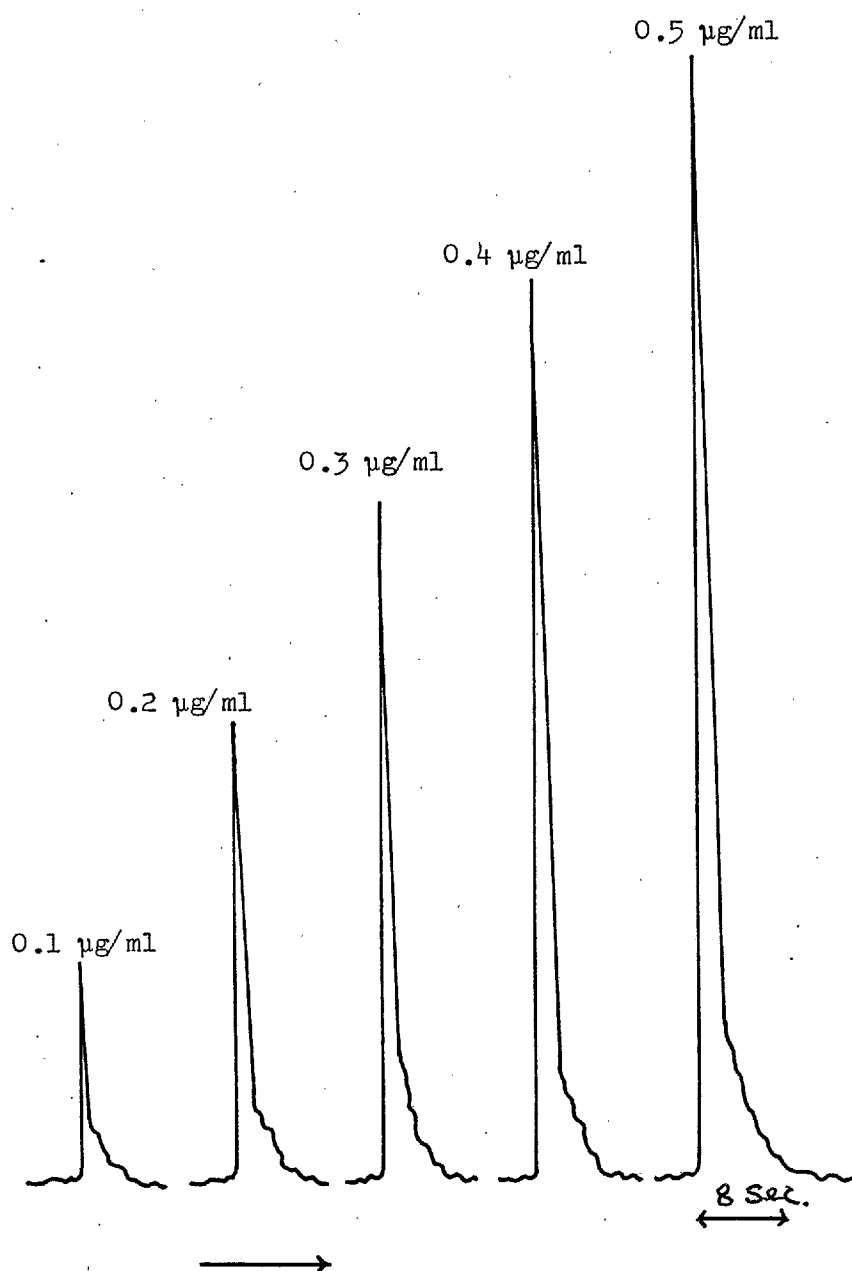


FIGURE 24: SIGNALS OBTAINED FOR SELENIUM STANDARD SOLUTIONS

Spectral source:

- 1) Applied microwave power - 50 W
- 2) Reflected power from cavity - 12 W
- 3) Modulation frequency - 500 Hz

Atom Cell:

- 1) Argon flow rate - 6.0 l/min.
- 2) Hydrogen flow rate - 3.3 l/min.
- 3) Viewing height above burner - 25 mm
- 4) Burner internal diameter - 7 mm

Photomultiplier:

- 1) Operating voltage - 600 v

Amplifier:

- 1) Input - normal protected
- 2) Frequency limit 100 Hz - 1000 Hz
- 3) Filter - internal
- 4) Meter damping - off
- 5) Gain - 40 db

Phase-sensitive detector:

- 1) Meter - 1 volt range (negative)
- 2) Zero - negative
- 3) Time constant - 1 sec. (internal)
- 4) Filter - on
- 5) Reference channel - A

Hydride generation cell volume - 46 ml.

Sodium borohydride reagent volume - 2 ml.

Selenium sample volume - 1 ml.

3.5. INTERFERENCE STUDIES

The determination of selenium by atomic absorption spectroscopy utilizing the hydride generation technique is well-known to be subject to interference from a number of heavy metal ions and in particular copper(II), which depresses the efficiency of the hydrogen selenide generation procedure using sodium borohydride as reducing agent (94). Similar interferences were expected in the atomic fluorescence spectrometric procedure developed here. A selenium standard solution containing 500 ng selenium was used to confirm the interference effect of copper(II) on the selenium determination. From the experiments carried out, it became clear that the presence of copper(II) as a concomitant element causes serious interference, as in the presence of 1000 $\mu\text{g/ml}$ copper, no atomic fluorescence signal was obtainable for selenium. The effect of copper concentration present in 0.5 $\mu\text{g/ml}$ selenium standard solution on the selenium atomic fluorescence signal is shown in Figure 25.

3.5.1. Procedure

The system was flushed with argon for 20 seconds, 1 ml of selenium standard solution (0.5 $\mu\text{g/ml}$) was placed inside the hydride generation cell followed by 1 ml of 1000 $\mu\text{g/ml}$ solution of the interfering element.

2 ml of sodium borohydride solution (5% w/v) was injected and the selenium atomic fluorescence signal recorded. A blank signal was recorded, following the same procedure but excluding the standard selenium solution. The work was carried out in the following order:

The atomic fluorescence signal for a standard selenium solution (with no interferent present) was recorded. This was followed by the analysis of two standard selenium solution with interfering element present, and then another standard selenium solution with no

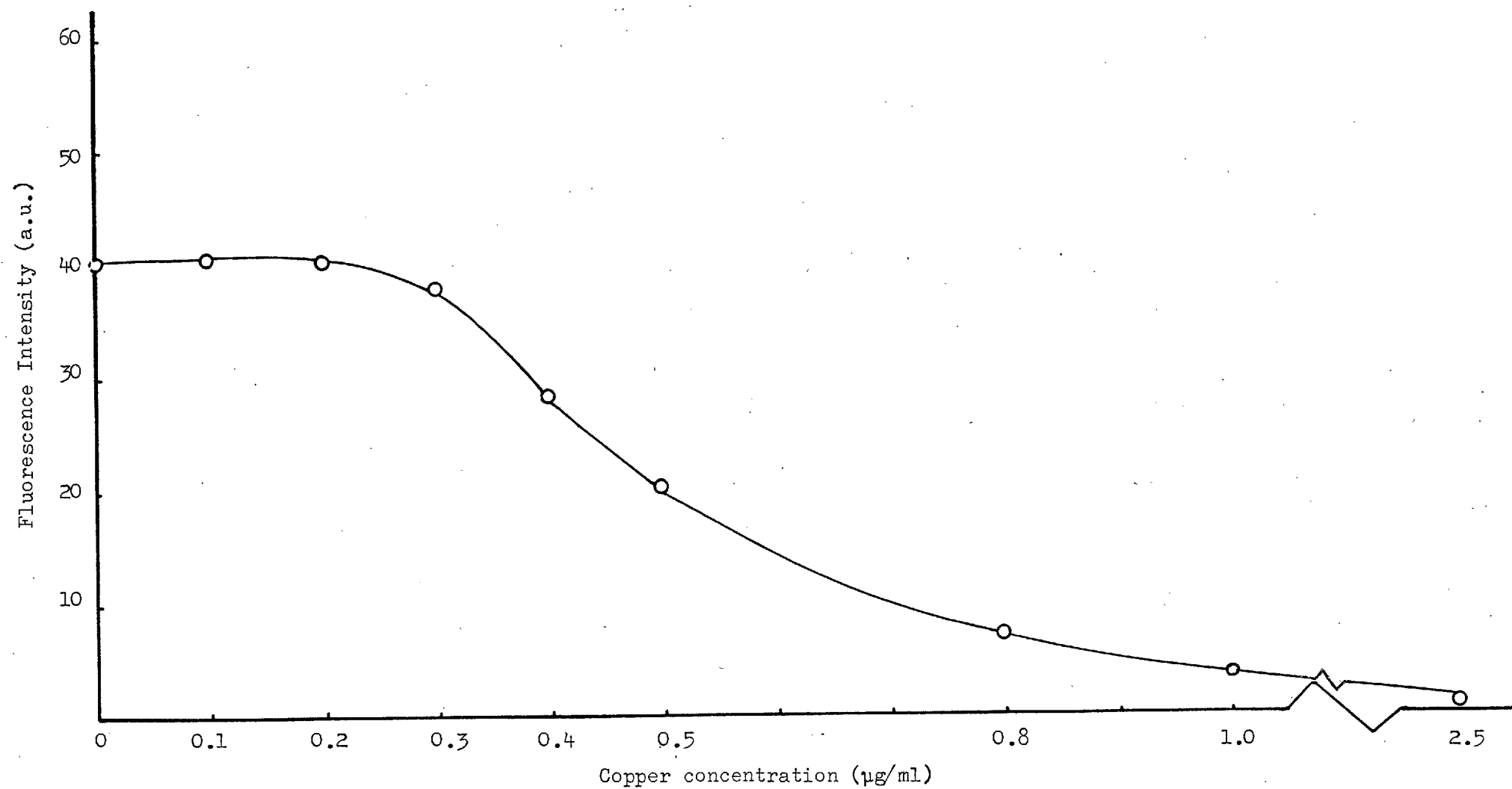
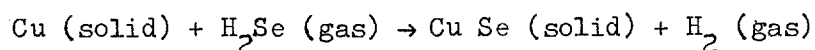


FIGURE 25 : THE EFFECT OF COPPER ON THE DETERMINATION OF 0.5 $\mu\text{g/ml}$ SELENIUM.

interferent present was analysed. In this way, a standard was always analysed immediately before and after those with interferent present. The interference effects of fifteen metal ions on selenium determination was investigated. These were the elements which do not form hydrides with sodium borohydride solution. Table 7 illustrates typical results of the depressive effects due to the presence of these metal ions on the analytical signals observed for selenium. Interferences are probably manifold, but they can be classified as follows:

a) Preferential reduction of the interferent

Preferential reduction of the interferent metal ion in solution to a different valency state or to the free metal is probably one of the most serious causes of interferences observed in selenium determination. The element of interest can be partly or completely co-precipitated or the volatile hydride formed with the addition of the reductant could be easily absorbed on the metal surface. Saleh (158) has proved that chemisorption of hydrogen selenide on metallic copper starts at -80°C and increases as the temperature increases. This is accompanied by evolution of hydrogen according to the following equation:



Thus this kind of interference can be caused by slowing down the rate of hydride generation or stopping it completely.

Many of the interfering elements form precipitates after sodium borohydride addition. As some of the sodium borohydride is used up in this way less is available for reduction of the element

TABLE 7

DEPRESSIVE EFFECT OF METAL IONS* ON THE ANALYTICAL SIGNALS OBSERVED
FOR 0.5 $\mu\text{g/ml}$ SELENIUM

<u>Metal ion</u> *	<u>% Depression of signal</u>
Na(I)	0
K(I)	2
Mg(II)	0
Mn(II)	0
Ca(II)	2
Hg(II)	0
Ba(II)	0
Al(III)	0
Fe(II)	36
Fe(III)	20
Pb(II)	40
Zn(II)	21
Co(II)	20
Cu(II)	99
Ag(I)	80
Ni(II)	65

* Interfering element concentration = 1000 $\mu\text{g/ml}$.

of interest to its hydride and a lower signal may be obtained.

b) Compound formation

The element of interest could react with the interfering element to form a stable, sometimes sparingly soluble, compound. Consequently sodium borohydride reagent has no reducing effect on the element of interest and hydride formation is completely prevented. Metal selenide formation is the most probable cause of interference of metals, particularly copper(II), on the determination of selenium.

c) Compound formation in the flame

Compound formation can occur in the cool argon-hydrogen flame. This may explain the mutual interference of virtually all the volatile hydrides on each other, observed by Smith (94). Thompson (159) found a complete suppression of selenium signal in the presence of excess copper, when using a high temperature air-acetylene flame to rule out the possibility of the interference, being caused by formation of compounds as observed in the comparatively cool flames.

Different methods to minimise or eliminate the interference of copper on the selenium determination were examined.

The use of E.D.T.A. as a masking agent was not possible because of the high acid concentration in selenium solution. Kirkbright et. al. (83) determined selenium and tellurium spectrophotometrically using thioglycollic acid (TGA) as a complexing agent. They showed that TGA reacts with Se(IV) to

form a soluble yellow complex over a wide range of hydrochloric acid concentrations. The yellow selenium - TGA complex could be extracted into oxygen-donor solvents and selenium measured photometrically. Ethyl acetate was chosen as the most convenient solvent for extraction. This method was investigated as a way of separating selenium from copper and some of the other interfering elements. The selenium - TGA complex was formed and extracted into ethyl acetate. As ethyl acetate is not miscible with water, sodium borohydride reagent had to be prepared in a suitable solvent. Dimethylformamide (DMF) was found to be suitable for this purpose, as it is miscible with ethyl acetate and sodium borohydride is soluble up to 15% w/v in this solvent. A 5% (w/v) sodium borohydride solution was prepared in DMF and was added to the ethyl acetate solution containing selenium, in the hydride generation cell. No atomic fluorescence signal of selenium was recorded. This was probably due to the presence of organic species in the flame and their consequent quenching effects on the selenium signal. The same results were obtained for solutions containing high selenium concentrations. No further investigation of this method was carried out.

Two other procedures were investigated and successfully applied to real samples, to avoid the copper interference effect on selenium determinations.

These procedures were:

- a) The lanthanum nitrate co-precipitation procedure
- b) The tellurium (IV) procedure to remove copper as a stable telluride.

3.5.2. The lanthanum nitrate co-precipitation procedure

Co-precipitation of trace elements with a collector is undoubtedly one of the most important methods of preconcentration and separation. In its most general sense co-precipitation refers to the precipitation of a compound simultaneously with one or more other compounds. A more restricted definition is the contamination of a precipitate by a substance which is normally soluble under the conditions of the precipitation. This definition is directly applicable to collectors. The ability to precipitate traces of ions at concentration levels where the solubility product of the trace ion is not exceeded is one of the most important properties of a collector. Some of the requirements for a collector are as follows:

- 1) It must be capable of collecting the required element as efficiently as possible.
- 2) A good collector is one that will gather the trace element quantitatively even when used in small amounts.
- 3) It must be more or less specific.
- 4) It must not, of course, be liable to cause interference in the determination of the trace element.
- 5) It must be precipitated in a form which can easily be removed from the solution.
- 6) The collector must contain an ion which forms a compound of low solubility with the trace element.

In order to co-precipitate trace elements, amorphous precipitates of high activity (hydroxides, sulphides, etc.) are most frequently used. Co-precipitation on such precipitants may be considered as a heterogeneous ion-exchange or chemical reaction which leads to formation

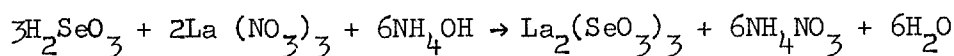
of a solid solution or of chemical compound of the microcomponent with the matrix compound. It is not always necessary to introduce a collector into the solution, some of the matrix may be used instead. This approach is particularly suitable in the case of hydroxides.

The distinction between the various types of co-precipitation is not sharp, but the following mechanisms may be distinguished:

solid solution formation, adsorption, chemical compound formation and occlusion. In any given example of co-precipitation it is not often possible to be certain which mechanisms are involved. The simplest type of collector is a slightly soluble solid which is added directly to the solution of the trace. It is preferable however, to form the slightly soluble solid in solution by adding a reagent, and then precipitating the collector by addition of a second reagent or by changing the pH of the solution. This is one of the most commonly used techniques, because advantage may be taken of the larger surface area and reactive properties of a newly forming crystal surface.

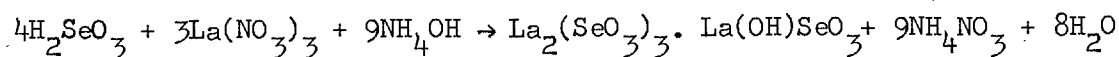
Lanthanum hydroxide has been used to separate gold, lead, bismuth, iron and aluminium from copper, zinc, cadmium, nickel and cobalt in high purity silver. Pitwell (160) used lanthanum hydroxide as a collector for aluminium at the 1 ppm level. In two papers the roles of iron(III)hydroxide as a collector of trace impurities in sea water (161) and of thorium hydroxide as a collector of molybdenum (162) were discussed. By analogy, the behaviour of lanthanum hydroxide as a collector of anionic species was studied. As selenium exists in ammoniacal solution as (SeO_3^{2-}) Reichel and Bleakly (53) studied the behaviour of lanthanum hydroxide as a collector, choosing (SeO_3^{2-}) or selenite ion as the model for the anionic species even though lanthanum hydroxide, by analogy to ferric hydroxide, would normally be expected to behave as a collector of cationic species. Lanthanum

hydroxide is a much stronger base than either ferric or aluminium hydroxide and is considerably more soluble in near neutral solutions. Moeller and Kremers (163) found that precipitation of lanthanum hydroxide began at pH 7.8, but Reichel and Bleakly (153) found that when a nitric acid solution containing lanthanum nitrate and selenious acid was neutralized with ammonium hydroxide, a colloidal white precipitate appeared at pH 2.9. According to my experiments this colloidal precipitate appeared at pH 3.0 which is in close agreement with Reichel and Bleakly. As neither lanthanum nor selenium precipitates at this pH, it must be assumed that the compound resulted from a reaction between lanthanum and selenium. Reichel and Bleakly performed some investigations to establish the stoichiometry of the reaction and found at pH 7.0 the stoichiometric ratio of selenium to lanthanum was 3:2 corresponding to the composition of normal lanthanum selenite, $\text{La}_2(\text{SeO}_3)_3 \cdot x \text{H}_2\text{O}$ contaminated by a small amount of basic lanthanum selenite: i.e.

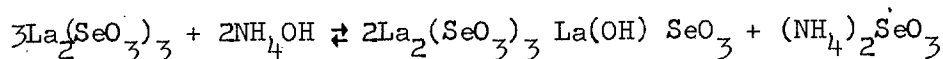


At pH 9-10 the selenium:lanthanum ratio was found to be 4:3 corresponding to the composition of basic lanthanum selenite, $\text{La}_2(\text{SeO}_3)_3$.

$\text{La}(\text{OH}) \cdot \text{SeO}_3 \cdot x \text{H}_2\text{O}$ contaminated by a small amount of normal lanthanum selenite, i.e.



They concluded that the transition from normal lanthanum selenite to basic lanthanum selenite may be written as



The basic lanthanum selenite formed is freely soluble in dilute acids but only sparingly soluble in water and 10% ammonium hydroxide solution.

It can be concluded that the action of lanthanum hydroxide as a precipitant and collector of selenium is characterized by the formation of sparingly soluble basic lanthanum selenite which is quantitatively retained by the precipitating lanthanum hydroxide.

The procedure reported by Kerbyson (54) in which the interference of copper on the determination of selenium by atomic absorption spectroscopy, via. generation of hydrogen selenide, was eliminated by removal of selenium from sample solution by co-precipitation from alkaline medium with lanthanum, was investigated.

Procedure

25 ml of 1 $\mu\text{g/ml}$ selenium solution is mixed with 25 ml of 1000 $\mu\text{g/ml}$ copper solution. 10 ml of 5% w/v lanthanum nitrate solution is added to the mixture. The solution is then diluted to about 100 ml using distilled water. 100 ml concentrated ammonium hydroxide solution is added slowly while stirring. The solution is left to stand for 1 minute and then filtered using a Whatman No. 50 filter paper. The precipitate and the beaker are washed twice with 5% ammonia solution followed with distilled water. The precipitate is dissolved into the original beaker using 30 ml hydrochloric acid (5M) and 40 ml concentrated ammonia solution is added to reprecipitate the lanthanum hydroxide. The precipitate is then left to stand for 1 minute and filtered and washed as before. The precipitate is finally dissolved in 10 ml of hot hydrochloric acid (5M) and diluted to 25 ml with 5M hydrochloric acid.

The mean recovery for 10 replicate analysis using this procedure was 99% with a relative standard deviation of 2.5%. Single precipitation was also investigated and the mean recovery was found to drop to 62.5%.

When a single precipitation procedure was applied, the precipitate had to be washed thoroughly several times with 10% ammonia solution and the loss of selenium at this stage was considerable. As the recovery of single precipitation was found not to be acceptable, double precipitation was applied for the rest of the work.

Effect of pH on recovery

Experiments were carried out to assess the effect of pH on selenium recovery. Solutions containing the same amount of selenium were adjusted with ammonium hydroxide to pH values of 7.0, 8.0, 9.0, 10.0 and 11.0. The precipitates were filtered and analysed for selenium. Complete precipitation of lanthanum hydroxide and 98% to 100.9% selenium recoveries occurred at pH 9.0 and above. When the pH of the solution was below 9.0, the colloidal precipitate tended to pass through the filter paper resulting in inconsistent and poor recoveries. The effect of pH on selenium recovery is shown in Figure 26. The amount of lanthanum nitrate necessary for the quantitative collection of selenium present in the sample solution was found not to be critical if added in an excess large enough for rapid and visual precipitation.

Effect of time on recovery

It was not possible to age the precipitate without loss of the trace element. Ageing implies digestion of a precipitate in contact with the solution from which it was brought down. Under these conditions recrystallization takes place and small crystals dissolve to be redeposited on larger crystals. Ageing of precipitates promotes crystal growth with liberation of occluded traces. Experiments were carried out to assess the effect of elapsed time between precipitation and filtration, on selenium recovery. The procedure was found to be most efficient when the lanthanum hydroxide

precipitate was filtered off as soon as possible after precipitation. The effect on the recovery of selenium, monitored via the selenium atomic fluorescence signal intensity, of elapsed time between precipitation and filtration is shown in Figure 27. When the precipitate was left in contact with the solution for some time, the ammoniacal copper solution tended to crystallize, resulting in lengthy and difficult filtration, high copper contamination and inconsistent recoveries. This method permits very high concentrations of copper (ca. 2000 µg/ml) to be tolerated in the determination of selenium.

3.5.3. The tellurium(IV) procedure

The second procedure investigated for suppression of the interference from copper, was simply the addition of tellurium(IV) to sample solutions immediately before the hydride generation procedure. This procedure has been described by Kirkbright and Taddia (164). Selenide ion (Se^{2-}) forms stable compounds with some metal ions, including copper(II). As was mentioned before, the most probable cause of copper interference in determination of selenium is the formation of copper selenide. If a more stable compound than the selenide can be formed, selenium is left free to react with the reducing agent and in fact copper is masked. The stability constants ($\log K_{\text{so}}$) of copper selenide and copper telluride are - 48.1 and -53.8, respectively. It can be seen that the stability constant of copper telluride is lower than copper selenide and consequently copper telluride is preferentially formed and thus copper interference is suppressed. Figure 28 shows the effect of variation in the tellurium(IV) concentration added to 0.5 µg/ml selenium standard solutions, on the signals recorded. A constant suppression of ca. 30% is attained at

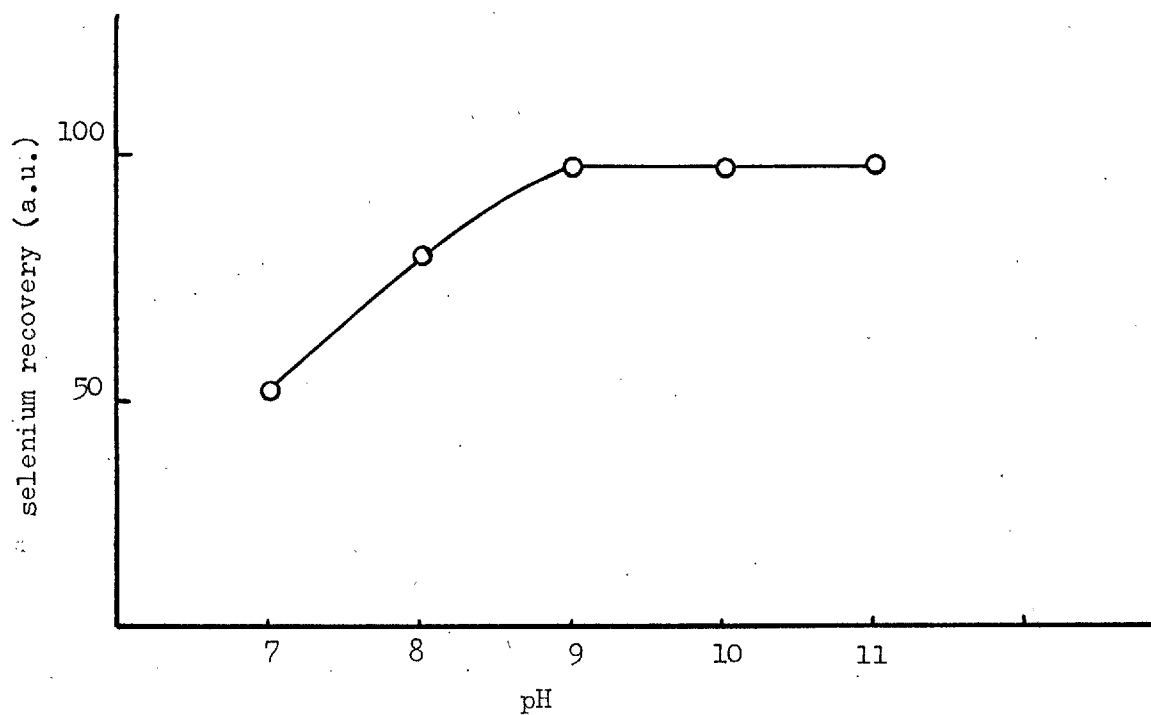


FIGURE 26 : THE EFFECT OF pH OF SAMPLE SOLUTION ON THE SELENIUM
RECOVERY

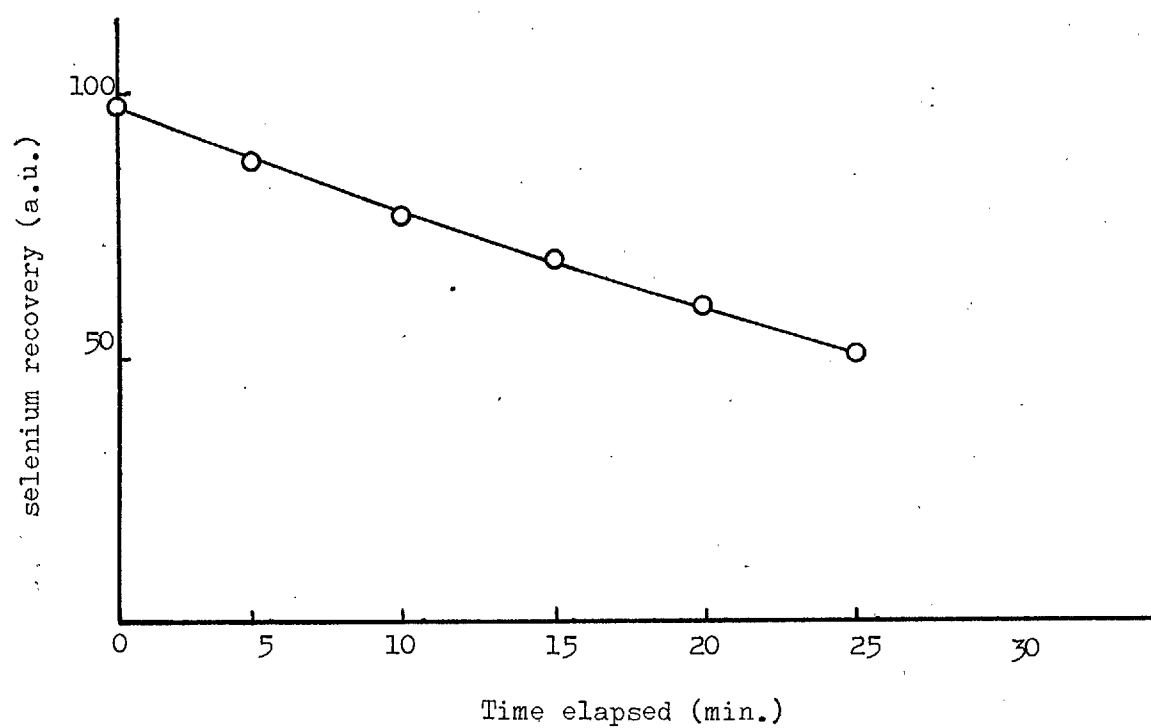


FIGURE 27 : THE EFFECT OF TIME ELAPSED BEFORE FILTRATION ON
THE SELENIUM RECOVERY

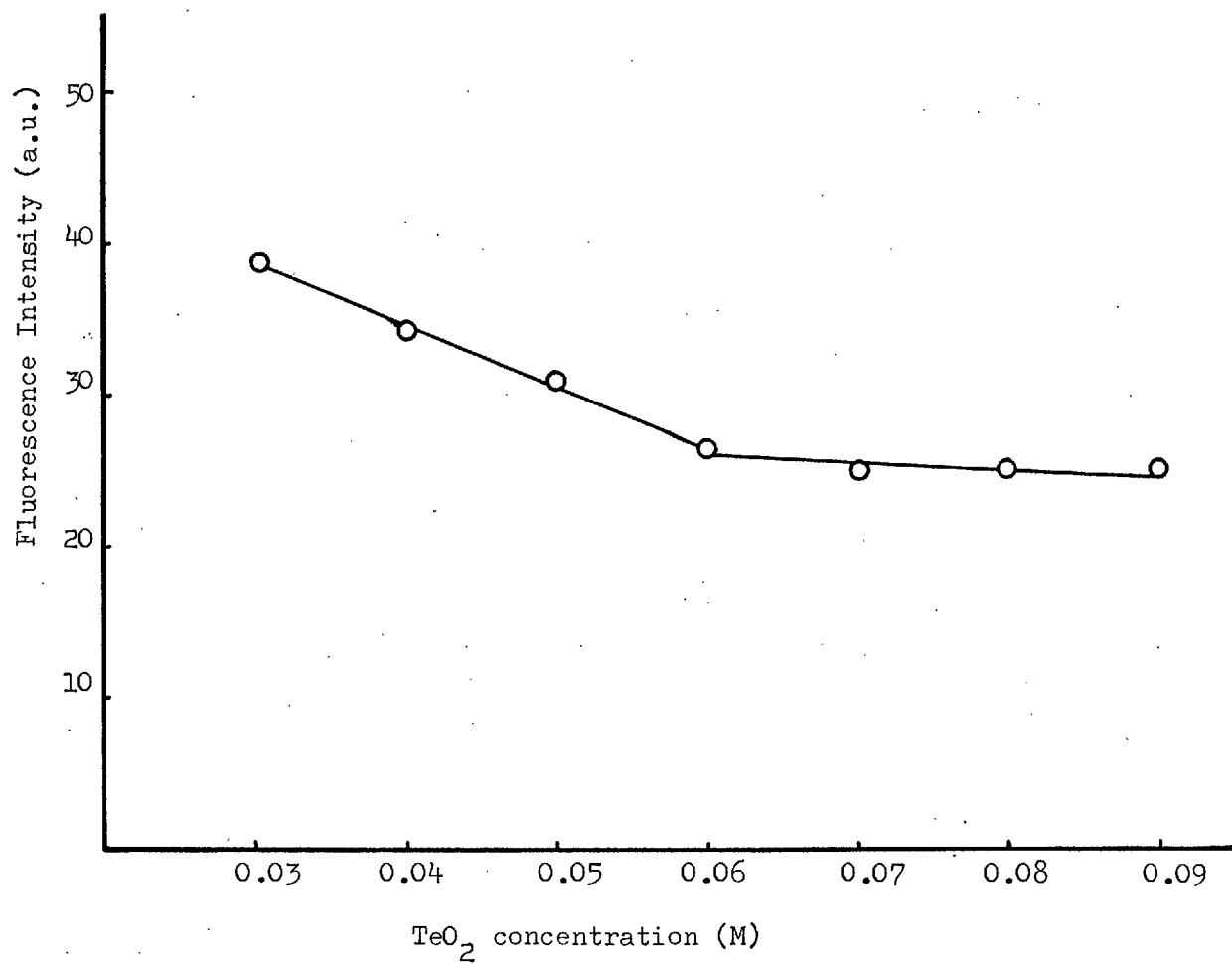


FIGURE 28 : THE EFFECT OF TeO_2 CONCENTRATION ON THE GENERATION OF SeH_2

tellurium(IV) concentrations between 0.06 and 0.08 M and no change in precision of the determination is observed. The presence of 0.06M tellurium(IV) enables relatively high concentrations of copper to be tolerated in the determination of selenium. Figure 29 shows the effect of increasing copper concentration on the atomic fluorescence signal observed from 0.5 $\mu\text{g/ml}$ selenium sample solutions containing 0.06 M tellurium(IV) solution. Addition of a concentration of tellurium(IV) to sample solutions, greater than 0.06M would permit extension of the tolerance of the procedure to higher concentrations of copper(II). Figure 30 shows a comparison of the analytical calibration graphs obtained for aqueous selenium solutions in the presence and absence of copper, utilizing tellurium(IV) to suppress copper interference. These data confirm the restoration of the selenium signal to ca. 70% of its value in the absence of copper, when tellurium(IV) is employed to suppress copper interference.

Comparison of lanthanum hydroxide and tellurium(IV) procedures

It is difficult to recommend which procedure is the most suitable for selenium determination as each has its own advantages. The lanthanum hydroxide co-precipitation procedure is a well established method. Excellent recoveries of selenium are obtainable if enough care is taken. However, to obtain good recoveries double precipitation must be employed. It is capable of eliminating the copper interference effect at copper concentrations as high as 2000 $\mu\text{g/ml}$. It not only eliminates the copper interference effect, but also eliminates the interference effect caused by silver, as silver forms a soluble complex in ammoniacal solutions and remains in the solution. However, this method is relatively time consuming compared to the tellurium(IV) method.

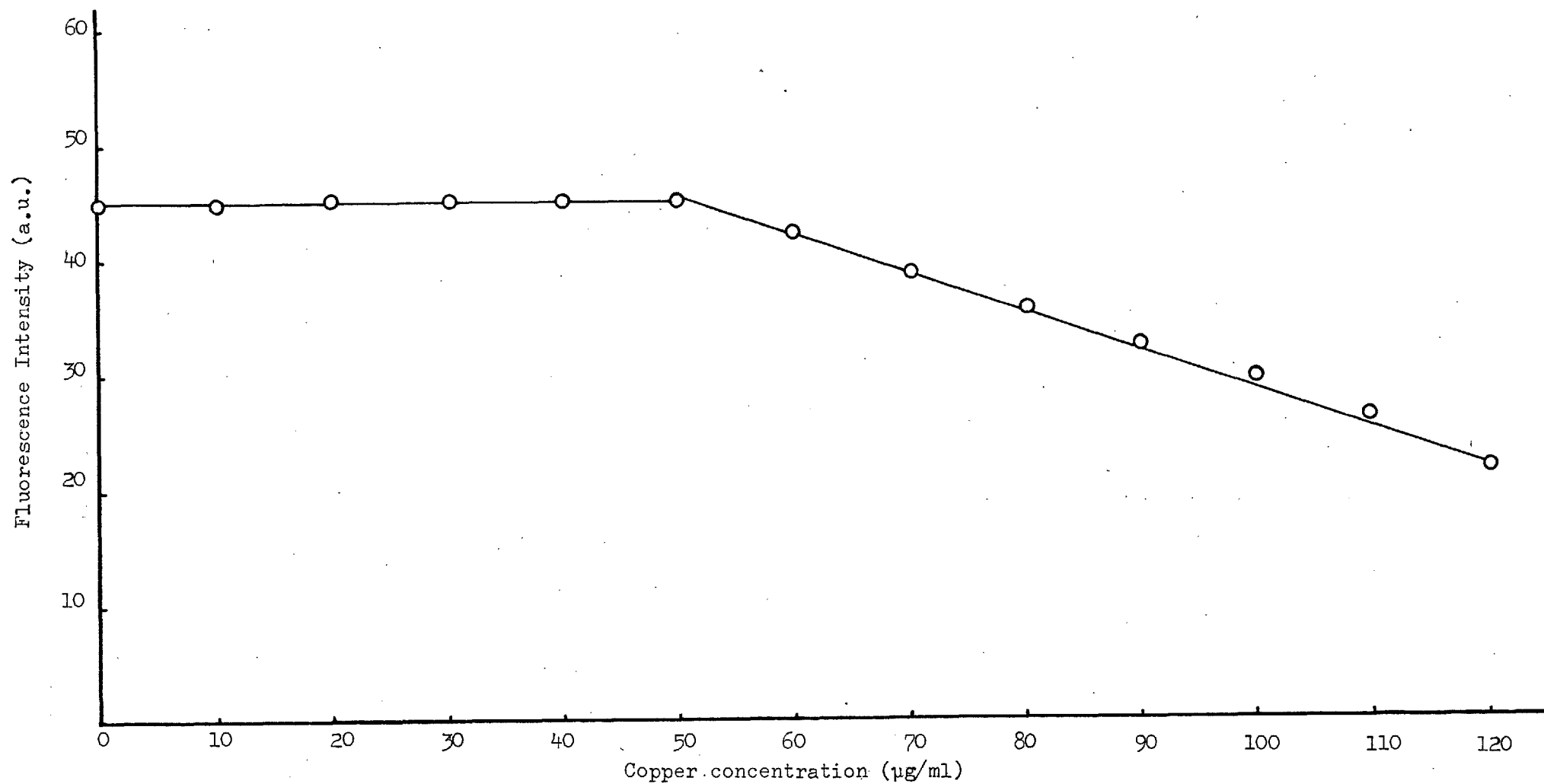


FIGURE 29 : THE EFFECT OF COPPER ON THE DETERMINATION OF 0.5 µg/ml SELENIUM IN THE PRESENCE OF 0.06M TeO_2

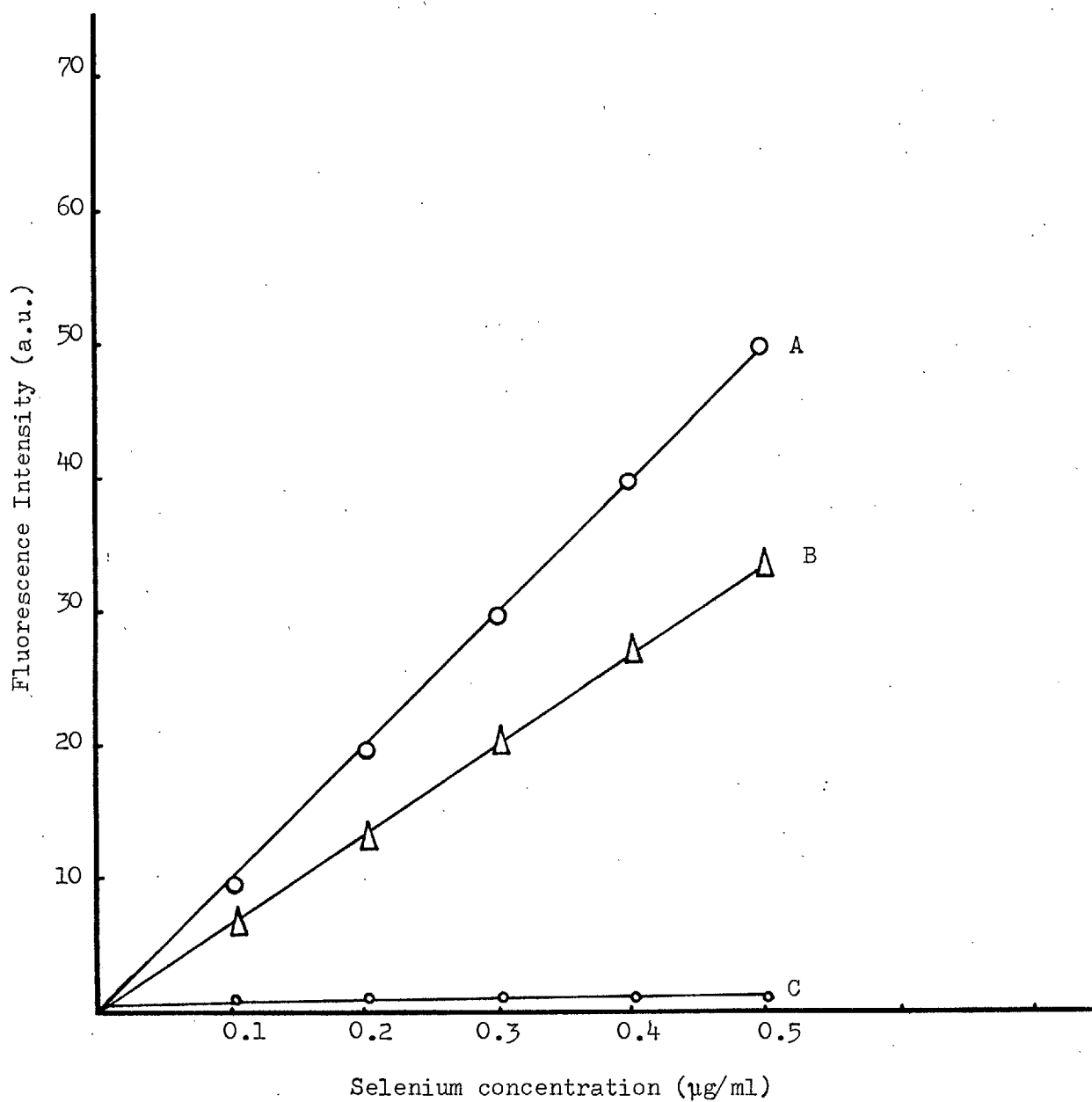


FIGURE 30 : COMPARISON OF ANALYTICAL CALIBRATION IN THE PRESENCE AND
ABSENCE OF 0.06M TeO_2

A : Aqueous selenium

B : Aqueous selenium + 0.06M TeO_2 + 20 µg/ml copper

C : Aqueous selenium + 20 µg/ml copper only

The tellurium(IV) procedure is simple, rapid and precise. By simply adding an appropriate amount of tellurium(IV) to the selenium sample solution the hydride generation procedure can be applied directly and immediately. The tellurium(IV) concentration used in the course of this study was enough to eliminate the interference effect of up to 50 $\mu\text{g/ml}$ copper. The concentration of copper in real samples of interest, (soil and food), should seldom exceed 50 $\mu\text{g/ml}$, so this cannot be considered as a serious disadvantage for this procedure. Although addition of more tellurium(IV) to the selenium sample solution might result in extension of this limit, there is the possibility of the excess tellurium being reduced to elemental tellurium after sodium borohydride addition. Selenium could then be co-precipitated or the efficiency of hydride generation may be partly suppressed. A constant suppression of 30% of the selenium analytical signal is attained when the tellurium(IV) procedure is employed. This means the detection limit is degraded by 30%. This can be tolerated, however, if the lowest possible detection limit is not very important, because of the simplicity and rapidity the procedure offers.

CHAPTER FOUR

4. THE DETERMINATION OF SELENIUM IN SOILS AND KALE

4.1. THE DETERMINATION OF SELENIUM IN SOIL

This section forms the very heart of this work, for only by the analysis of real and standard materials may a technique be fully evaluated. Unfortunately there were not any standard soil samples for selenium available and the results of the analysis were confirmed using an alternative method.

The amount of selenium in soil depends upon the quantity of selenium in the parent material, as modified by processes during or after soil formation that add or remove selenium.

The occurrence of selenium in soils is a topic of considerable interest particularly in view of its toxic effects on cattle through plants which can accumulate the element from seleniferous soils. There are also deficiency diseases prevalent in animals grazing in areas where soils are low in selenium. As indicated by Lakin and Davidson (1965) the selenium level in soil is related to the whole geochemical cycle of selenium. Factors of importance include the selenium content of host rocks, pH, and the nature of the drainage water. Selenium in soil may be derived from:

1. Rock formations.
2. Formations lying below the soil mantle.
3. Decomposition through the weathering of the host rock and subsequent transport of selenium by wind or surface waters.
4. Enrichment of the soil with selenium resulting from mining operations.

Sometimes metallic elements may be incorporated into soils through the use of pesticides, from sewage residues from industrial areas and by air pollution. Their accumulation by degrees, may ultimately lead to an aggravated toxic condition in the soil.

Table 8 lists the range of composition found in soils from the data given by Swaine (166), Vinogradov (167), Bear (168) and others.

The results of the analysis of several thousand soil samples in the U.S.A. have indicated a maximum concentration of selenium of less than 100 ppm, with the majority of seleniferous soils containing on average less than 2 ppm. The selenium content of most soils is estimated to be between 0.1 and 0.2 ppm. Selenium in soils may occur as selenites, selenates, elemental selenium and as selenium in association with minerals. It should be noted, however, that in seleniferous soils a significant amount of selenium in the form of soluble organic or inorganic compounds is present as a result of the decay of seleniferous plant materials. The high selenium content of soils in Ireland is particularly noteworthy, and the study of soil profiles there have shown a selenium content as high as 1200 ppm.

Many methods have been used for the determination of selenium in soils. Robinson et al. (169) described a method of isolating selenium from soils by distillation as its tetrabromide. The resulting solution was reduced with hydroxylamine and analysed colorimetrically. Klein (170) first treated the soil samples with nitric and sulphuric acids and then converted the selenium present to the tetrabromide with a mixture of hydrogen bromide and bromine. Distillation into hydrobromic acid gave a solution of selenious acid, which was estimated by a thiosulphate-iodine titration.

Plant-available selenium is estimated as water-soluble selenium, the soil samples being refluxed with water for 30 minutes and an aliquot of filtrate being concentrated by evaporation for analysis. Vogel (17) has described a method in which selenious acid is reacted with potassium permanganate for its estimation whilst Vendette (171)

TABLE 8

ELEMENTS IN SOIL

<u>Element</u>	<u>Mean ppm dry soil (range)</u>		<u>Element</u>	<u>Mean ppm dry soil (range)</u>	
Ag	0.1	(0.01-5)	Mg	5000	(600-6000)
Al	71000	(10000-300000)	Mn	850	(100-4000)
As	6	(0.1-40)	Mo	2	(0.2-5)
B	10	(2-100)	N	1000	(200-2500)
Ba	500	(100-3000)	Na	6300	(750-7500)
Be	6	(0.1-40)	Ni	40	(10-1000)
Br	5	(1-10)	O	490000	
C	20000		P	650	
Ca	13700	(7000-500000)	Pb	10	(2-200)
Cd	0.06	(0.01-0.7)	Ra	8×10^{-7}	($3-20 \times 10^{-7}$)
Ce	50		Rb	100	(20-600)
Cl	100		S	700	(30-900)
Co	8	(1-40)	Sb		(2-10?)
Cr	100	(5-3000)	Sc	7	(10-25)
Cs	6	(0.3-25)	Se	0.2	(0.01-2)
Cu	20	(2-100)	Si	330000	(250000-350000)
F	200	(30-300)	Sn	10	(2-200)
Fe	38000	(7000-550000)	Sr	300	(50-1000)
Ga	30	(0.4-300)	Th	5	(0.1-12)
Ge	1	(1-50)	Ti	5000	(1000-10000)
Hf	6		Tl	0.1	
Hg	0.03	(0.01-0.3)	U	1	(0.9-9)
I	5		V	100	(20-500)
K	14000	(400-30000)	Y	50	(25-250)
La	30	(1-5000)	Zn	50	(10-300)
Li	30	(7-200)	Zr	300	(60-2000)

determined selenium in soil using wet digestion of the soil samples and 2,3-diaminonaphthalene as a complexing agent. The resultant selenol was extracted into cyclohexane and measured fluorimetrically. An average coefficient of variation of 3.35 and recovery of 97.4% was obtained. Hemsted et al. (172) also used an acid digestion and 2,3-diaminonaphthalene as a complexing agent. They determined the selenium content of soil samples down to 0.04 ppm. This reagent together with the fluorimetric technique constitutes one of the most popular methods of selenium determination in soil samples.

Another method which is very frequently used in soil analysis for selenium is neutron activation. Kronborg and Steinnes (173) described the simultaneous determination of arsenic and selenium in soil samples using a neutron activation technique. Steinnes (174) later used this technique to determine selenium, arsenic and mercury simultaneously in soil samples. The high cost of the equipment and the availability of a neutron source is its limiting factor, otherwise it is one of the best and most precise techniques of soil analysis. Finally Severne and Brooks (175) have reported a rapid method for the determination of selenium in geological samples, (e.g. soils, rocks and sediments) which involves acid digestion of the sample with mineral acids, use of arsenic as a carrier for selenium and subsequent determination of selenium by conventional atomic absorption spectroscopy. A detection limit of 0.1 ppm was reported.

For the analysis of a soil for a particular element by chemical procedures, the element must be brought into solution. Two main methods of achieving this are a) fusion of the soil with subsequent digestion of the melt and b) digestion of the soil with strong acids. It is not possible to recommend one method as being better than the other as the suitability of either depends upon the element of interest. For

fusion of soils, sodium carbonate is most commonly used but potassium pyrosulphate, sodium hydroxide and sodium peroxide are used in certain cases.

The acids used for wet digestion of soils include perchloric, nitric, hydrochloric, hydrofluoric, phosphoric and sulphuric. The suitability of an acid or an acid mixture depends upon the element to be determined.

The choice of digestion reagent for a specific element is governed not only by the efficiency with which it converts that element into a soluble compound but also by conditions required for subsequent treatment. For many elements there are several methods of decomposition which would be applicable.

Different acids or acid mixtures which are usually used for soil digestion are: aqua regia, nitric acid, 2:1 nitric-perchloric acid mixture, hydrofluoric acid and sulphuric acid. All acid mixtures and acids mentioned above were examined to find out which gives the best selenium recovery. When aqua regia, or nitric acid, alone was used for the digestion of soil samples a suspension of carbonaceous material resulted which indicated a high organic carbon content of the soil samples. This fine suspension caused difficulty during the centrifugation and hydride generation processes. This is probably one of the reasons for the unpopularity of aqua regia as an acid mixture for soil sample digestion. When sulphuric acid, or hydrofluoric acid, was used for soil digestion, selenium recoveries were very poor because these acids were unable to destroy the organic constituents of the soil samples.

The mixture of nitric-perchloric acid did not introduce any problem, as complete destruction of organic matter and silicates was effected, passing selenium into the solution as its perchlorate.

Normal safety precautions were carefully exercised to avoid dangers associated with the use of perchloric acid.

Although dry ashing procedures have not been recommended for the determination of selenium in soil samples, an investigation was carried out to find out whether the loss of selenium is significant or can be tolerated. Sodium carbonate was used for the fusion of the soil samples and complete loss of selenium occurred, as expected.

The results of the experiments indicated that only the perchloric-nitric acid mixture digestion is capable of extracting selenium from the soil into the solution quantitatively. The sample is decomposed by heating with a mixture of perchloric and nitric acids, and the excess of nitric acid is evaporated. Evaporation to dryness must be avoided as selenium would be lost by volatilization. Nitric acid was found to be essential to attack firstly the readily oxidizable materials such as organic matter. The insoluble residue must be removed by filtration or centrifugation as it would interfere in the hydride generation process.

The value of laboratory data in soil studies depends on effective sampling. Every sample should represent a definite body or class of soil that is as homogeneous as possible. Soil samples must be dried either by air drying procedures or oven drying procedures. Oven drying has not been recommended by many workers, as some chemical changes in the soil composition are likely to occur. The air drying cabinet temperature should be kept around 25°C - 30°C while well ventilated.

4.2. THE DETERMINATION OF SELENIUM IN SOIL SAMPLES BY AFS

4.2.1. The digestion procedure and sample preparation

1 g soil samples were weighed into a series of test-tubes. 3.5 ml of concentrated nitric acid was added to each sample and the test tubes were covered and allowed to stand overnight. A few glass boiling

beads were added to each tube and then 1.5 ml of concentrated perchloric acid (72% w/v) was added to each. The tubes were then transferred to a cold aluminium digestion block whose temperature was raised steadily to 100°C over a period of 30 minutes. The block was maintained at this temperature for 30 minutes and then the temperature was raised to between 190 and 200°C and maintained at this temperature until digestion of the soil was complete. The final temperature of 200°C was not exceeded to avoid charring and the loss of selenium by volatilization. The test-tubes were then removed from the digestion block and allowed to cool. 2 ml of potassium bromide solution (2% w/v) was added to each and the test-tubes were allowed to stand in boiling water for 15 minutes to ensure complete reduction of selenium(VI) to selenium(IV). The solutions were then centrifuged and the residues rejected. The supernatant solution was taken for analysis.

Either the lanthanum nitrate/ammonia or tellurium(IV) addition procedure was applied to eliminate interference from copper. The solutions were then made 5M with respect to hydrochloric acid and analysed by the hydride generation technique using the non-dispersive atomic fluorescence spectrometer.

4.2.2. Procedures for suppression of interferences

a) Lanthanum nitrate co-precipitation procedure

0.5 ml of lanthanum nitrate solution (5% w/v) was added to each solution prepared for analysis by the digestion procedure described above. 2 ml of ammonia solution was then added and the solutions were mixed. After standing for one minute the solutions treated in this manner were centrifuged and the solution discarded. The precipitate was then dissolved in an appropriate amount of 5M hydrochloric acid.

b) Tellurium(IV) procedure

0.3 ml of 1.0 M tellurium dioxide solution was added to each solution prepared using the digestion procedure described above and then diluted to 5 ml with 5M hydrochloric acid.

4.2.3. The effect of potassium bromide on the recovery of selenium

The effect of potassium bromide on the recovery of selenium was investigated. Digestions were carried out following the procedure discussed above. A number of digested soils were treated with potassium bromide and analysed for selenium, while the identical soil digests were analysed without being subjected to potassium bromide pre-reduction. A decrease of 10-20% in selenium recoveries was observed for the soil samples analysed without pre-reduction by potassium bromide.

These experiments clearly indicated that a fraction of selenium exists as selenium(VI) in soil digest solutions as a strongly oxidizing mixture is used for their digestion. As the hydride generation procedure can only be applied to selenium in an oxidation state of four, it is therefore necessary to reduce any selenium(VI) present to selenium(IV) by the addition of potassium bromide solution to the digested samples. Selenium digestion recoveries were evaluated by the addition of known amounts of selenium to 1 g soil samples prior to their digestion. The recovery of the selenium was then determined by analysis after digestion. The results of these experiments are shown in Table 9. As can be seen from Table 9, the recoveries are very satisfactory and show a mean recovery of 100.5% with a relative standard deviation of 3.46%. The recovery figures are each an average of duplicate digestions. A mean recovery of 100.5% indicates the efficiency and reliability of the digestion procedure and the atomic fluorescence technique for the determination of selenium in soil samples. When acid or acid mixtures other than the nitric-perchloric acid mixture were used for soil digestion, very poor recoveries resulted. Table 10 compares the

SELENIUM RECOVERY FROM DIGESTED SOIL SAMPLESSample No. 4.

<u>Selenium concentration</u> <u>ppm</u>	<u>Selenium added</u> <u>ppm</u>	<u>Selenium found</u> <u>ppm</u>	<u>Recovery</u> <u>%</u>
0.7	0.1	0.83	104
0.7	0.2	0.87	97
0.7	0.3	0.94	94
0.7	0.4	1.18	107

TABLE 10COMPARISON OF SELENIUM RECOVERIES USING DIFFERENT ACIDS FOR DIGESTION

	<u>Selenium</u> <u>concentration</u> <u>ppm</u>	<u>Selenium</u> <u>added</u> <u>ppm</u>	<u>Selenium</u> <u>found</u> <u>ppm</u>	<u>Recovery</u> <u>%</u>
$\text{HClO}_4 + \text{HNO}_3$	0.7	0.4	1.18	107
H_2SO_4	0.7	0.4	0.50	45
HNO_3	0.7	0.4	0.47	42
HF	0.7	0.4	0.80	72
$\text{HNO}_3 + \text{HCl}$	0.7	0.4	0.60	54

recoveries of selenium obtained, using different acids and acid mixtures for soil digestion.

Nine soil samples were digested using the procedure described. Each sample was then analysed using both the lanthanum co-precipitation and the tellurium(IV) method of interference suppression. The results obtained for the selenium content of the soils analysed are shown in Table 11 and both methods of interference suppression are compared. As can be seen from Table 11 there is no serious quantitative difference between the results obtained by either method; the results obtained using the lanthanum nitrate co-precipitation procedure are, however, higher than those obtained using the tellurium(IV) procedure.

The lanthanum hydroxide procedure not only eliminates the suppressive effect of copper on the selenium determination but also eliminates the interfering effect of silver, as silver forms a soluble complex with ammonia solution. If we look at the stability constants of the selenides and tellurides of silver, ($\log K_{so}$ selenide = -63.7 and $\log K_{so}$ telluride = -71.7) it can be seen that the tellurium(IV) procedure should also eliminate the interfering effect of silver on the selenium determination. The reason that higher results are obtained when using the lanthanum hydroxide procedure is possibly due to the reduction of small quantities of excess tellurium to its elemental form after addition of sodium borohydride reagent solution. This elemental tellurium might be expected to co-precipitate or include selenium. Catalytic decomposition of generated hydrides is another possibility. The relative standard deviations observed for the two procedures indicated that the tellurium(IV) procedure produces more precise results than lanthanum hydroxide procedure, probably because as the procedure is simple, pre-treatment is minimised and is thus subjected to fewer experimental errors.

TABLE 11

COMPARISON OF RESULTS FOR THE SELENIUM CONTENT OF SOIL SAMPLES

<u>Soil Sample</u>	La(NO ₃) ₃ method (ppm)			Te(IV)method (ppm)			I.C.P.* (ppm)
	Mean	S.D.	R.S.D. %	Mean	S.D.	R.S.D.%	
1	0.37	0.017	4.5	0.35	0.009	2.6	0.38
2	0.36	0.016	4.4	0.35	0.012	3.4	0.33
3	0.24	0.010	4.1	0.23	0.015	6.5	0.23
4	0.70	0.014	2.0	0.68	0.015	2.2	0.69
5	18.70	0.640	3.4	18.6	0.49	2.6	19.2
6	111.00	2.930	2.6	110	2.45	2.2	-
7	0.29	0.014	4.8	0.28	0.012	4.2	0.28
8	0.31	0.020	6.4	0.30	0.015	5.0	-
9	0.30	0.023	7.6	0.29	0.018	6.2	-

* Inductively Coupled Plasma.

However, the relative standard deviations obtained for both procedures is below 10% and therefore quite satisfactory. In fact for seven of the nine determinations the relative standard deviations are below 5%.

The results obtained with both procedures also show extremely good agreement with those obtained by the hydride generation technique and optical emission spectrometry using an inductively-coupled argon plasma (ICAP) source.

The ICAP instrumental system employed for the comparison of results reported in this study has previously been described (31). Sample preparation procedures were identical to those described for the AFS determination of selenium and the lanthanum hydroxide procedure was employed to suppress interference from copper.

4.3. THE DETERMINATION OF SELENIUM IN KALE

The quality of food has been a matter for public concern over the last 100 years. In recent times, interest in metallic elements in food has broadened from the detection of gross criminal malpractices to investigations of chronic but possibly harmful effects on health of trace elements present as contaminants, together with the beneficial and nutritional requirements of those elements now known to be essential to human or animal life. It is not possible to draw a clear distinction between essential and toxic elements, especially in the case of selenium where there is a small margin between toxicity and deficiency. This makes it essential to find methods of selenium determination, at very low levels of selenium, in biological samples.

Selenium in plants mainly exist as complicated organic compounds in association with protein. In addition to organic compounds of selenium, many plants contain rather high proportions of soluble inorganic selenium.

The selenium content of plants varies over a wide range depending upon a number of factors. One of the most important of these is the kind of plant.

One type of plant has been referred to as indicator plants which grow on soils containing high levels of selenium. Much of the selenium in crop plants is associated with the plant protein and its distribution in the plant is related in general to the distribution of this protein, being higher in the seeds than in the straw. Selenium is an essential nutrient in several species but at higher levels in the diet it is extremely toxic. The nutritional requirement for selenium has been stated by Scott (15) to lie in the range 0.1 - 0.3 mg/Kg of diet whereas levels from 2 to 10 mg/Kg give rise to chronic toxicity symptoms. Hence there is a need for sensitive and accurate methods of analysis for selenium so that dietary intakes and possible hazards can be assessed with confidence.

Many methods have been used to determine selenium in biological materials and foods. Generally methods based on light absorption spectrometry (colorimetry) and fluorometry are the most widely accepted methods (176). Photometric methods based on the piazselenols particularly of 3,3'-diaminobenzidine and 2,3-diaminonaphthalene have gained wide acceptance in the analysis of selenium in both plants and animal materials. The fluorescence of the selenatiazole permits the determination of as little as 0.02 μ g of selenium in plant material (177). Using the oxygen flask combustion method Allawary and Cary (178) extended the fluorimetric 2,3-diaminonaphthalene procedure to the determination of selenium at very low levels (0.02 μ g), in mixed hay, mixed grains and beans. Nangriot (74) determined selenium in plants using a simple polarographic procedure.

As in selenium deficiency studies the critical selenium concentration appears to be in the range of 0.02 to 0.06 ppm, it is not surprising that

neutron activation methods have been developed. A detection limit of 0.005 μg selenium has been obtained using the nuclide $^{77}\text{Se}^{\text{m}}$, ($t_{1/2} = 17.5$ sec) (179). Also Heydorn and Damsgaard (180) used $^{81}\text{Se}^{\text{m}}$ and 1 g sample to determine selenium in biological materials. No pre-treatment of the samples was required.

Kotz and co-workers (181) used a teflon tube for the decomposition of biological material in nitric acid under pressure. The recovery of selenium in the ng range was $> 98\%$. This method is very useful when only limited amount of sample is available (≤ 500 mg). A microwave-induced plasma coupled to a tantalum-strip vaporization assembly, used for sample introduction, was used by Fricke (182) to determine selenium in food.

Atomic absorption spectroscopy has also been used for the determination of selenium in biological samples. Flame atomic absorption spectroscopy is not sensitive enough for this purpose. Shum (183) and co-workers used a flameless (graphite furnace) atomic absorption spectroscopic technique to determine selenium in food products. Recoveries of 99% were obtained together with a detection limit of 3 ng of selenium.

Vijan and Wood (184) determined selenium in vegetables using an automated non-flame atomic absorption spectroscopic system. They used a quartz-tube furnace as the atomizer and hydride generation technique to introduce selenium to the atomiser. A detection limit of 0.025 ppm was obtained. During recent years, however, procedures for liberating selenium as hydrogen selenide coupled with atomic absorption spectroscopy technique have gained widespread interest.

Relatively few biological materials have been analysed for a large number of elements by a large number of laboratories. One of the materials which has been intensively studied, however, is standard kale. Table 12 gives an indication of the kale composition.

TABLE 12
ELEMENTS IN KALE

<u>Element</u>	<u>Best mean value and range</u> <u>mg/Kg</u>		<u>Element</u>	<u>Best mean value and range</u> <u>mg/Kg</u>	
Ag		(0.03-0.5)	I		(0.063-0.27)
Al	38.2	(6.4-88.4)	In	< 0.3	
As	0.141	(0.11-0.22)	Ir		(0.0005-0.021)
Au	0.0022		K	24615	(20600-29300)
B	48.3	(39-56)	La	0.0865	(0.077-0.1)
Ba	4.55	(4-6.4)	Li		
Bi			Mg	1572	(1350-1700)
Br	26.1		Mn	14.73	(12.6-18)
Ca	40850		Mo	2.28	(1.5-3.1)
Cd	0.80	(0.38-1.06)	N	43089	
Ce		(0.14-0.46)	Na	2506	(1220-3250)
Cl	3415	(2180-4450)	Ni	< 0.2	
Co	0.0581	(0.041-0.081)	O	51500	
Cr	0.308	(0.18-0.42)	P	4489	(4020-4810)
Cs	0.0738		Pb	2.645	(1.6-3.8)
Cu	4.99	(3.6-6.5)	Pd	0.026?	
Dy	< 0.024		Ra		
Eu		(0.0066-0.012)	Rb	52.2	(49-57)
F	4.92	(4.2-6.2)	Re	< 0.3	
Fe	118.3	(88-157)	Ru	0.0045?	
Ga		(0.027-0.064)	S	15950	(13500-18000)
Ge			Sb	0.0689	(0.05-0.11)
Hf	< 0.07		Sc	0.00829	
Hg	0.167	(0.11-0.23)	Se	0.121?	(0.02-0.15)

TABLE 12 (cont.)

ELEMENTS IN KALE

<u>Element</u>	<u>Best mean value and range</u>		<u>Element</u>	<u>Best mean value and range</u>	
	<u>mg/Kg</u>			<u>mg/Kg</u>	
Si	237		Ti	(0.33-3.3)	
Sm		(0.022-0.16)	Tl	0.15	
Sn	0.26	(0.16-0.36)	U	0.011	(0.008-0.014)
Sr	98.9	(65-150)	V	0.36	(0.33-0.41)
Ta	< 0.1		W	0.0605	
Te			Zn	33.2	(30-38)
Th	0.0092		Zr	< 0.2	

To estimate the applicability of the non-dispersive atomic fluorescence system described in this study to this particularly important field, standard kale was analysed for selenium and the results obtained were compared to those specified for the kale.

Most analytical methods for the determination of metals and metalloids in biological materials require a preliminary destruction of organic matter in the sample. Despite the many variations on different methods used to destroy organic matter almost all fall into one of two main classes; dry ashing or wet oxidation. In the former, oxidation is accomplished by heating the sample to a relatively high temperature, usually between 400°C and 700°C , when atmospheric oxygen serves as an oxidizing agent. Chemical compounds may sometimes be added to aid the process. In wet digestions the temperature is much lower, liquid conditions are maintained throughout and the oxidation is carried out by oxidizing agents in solution. Gorsuch (185) investigated 250 reports on the destruction of organic material in foods. In 51% wet oxidation procedures were used and ashing was used in the remaining 49%. Wet oxidations were carried out using nitric/sulphuric acid mixtures, nitric/sulphuric/perchloric acid mixtures and nitric/perchloric acid mixtures.

The principal advantages of wet oxidation methods are that they are applicable to a wide variety of samples, are fairly rapid and are less prone to either volatilization or retention losses. Wet oxidations are normally carried out in Kjeldahl or conical flasks made from borosilicate glass or silica, employing relatively low temperatures and maintaining liquid conditions.

Most workers have recommended a nitric/perchloric acid mixture as the most suitable reagent for the determination of selenium in

biological material. The main problem is to destroy the organic matter without losing selenium. This can be achieved in the absence of fatty material, for example in plant material, although it is very necessary to maintain oxidizing conditions at all stages of the wet oxidation. This explains the efficiency of oxidizing mixtures containing perchloric acid. Controlled experiments have shown that some selenates are volatile above 200°C and therefore during the nitric/perchloric acid digestion the temperature has to be carefully controlled. The organic forms of selenium are also converted to volatile selenides during decomposition of the sample.

4.3.1. Experimental

A standard Bowen's kale (Department of Chemistry, University of Reading, UK) sample was analysed for its selenium content. According to Bowen (186) the selenium concentration in kale ranges from 0.02 to 0.15 mg/kg. Experiments have shown a bimodal distribution with two most probable values around 40 and 140 ng/g of selenium. Further work is necessary, however, to establish the correct value as it is probable that the loss of selenium through volatilization has given rise to the lower values.

The sample size taken for analysis is usually determined by two factors: (1) water content (wt %) and (2) fat, starch or sugar content. For products low in fat, starch or sugar and containing $> 50\%$ water, 5 to 10 g of sample is suitable (e.g. fruits, vegetables, fish and meat), whereas 3 to 5 g sample should be taken for products containing 10-50% water and 1 to 3 g for products containing $< 10\%$ water (e.g. flour, cereal and dried foods). For products high in fat or sugar (e.g. cheese, butter, oils, syrups and jams) sample size should be limited to 1 to 2 g.

4.3.2. Procedure

The kale powder (5% H_2O) was dried to constant weight at about $80^{\circ}C$. 5g portions of dried samples were weighed carefully into conical flasks and 3 glass beads were added to each followed by 30 ml of 5:1 nitric acid/perchloric acid mixture. The flasks were covered and allowed to stand for 24 hours. The flasks were then heated slowly on a hot plate until steady vigorous boiling was achieved. When the volume was reduced to about one-half the initial volume, the flasks were removed from the hot plate and 10 ml nitric acid was added to each flask. The flasks were then returned to the hot plate and heating was continued through the perchloric acid oxidation stage which is characterized by vigorous surface reaction and evolution of white fumes. Cautious addition of nitric acid in 1 ml aliquots was necessary to avoid charring and to maintain oxidizing conditions. The temperature was increased until the yellow green solution turned to a clear, colourless solution. Heating was continued for another 10 minutes and then the flasks were removed from the hot plate and left to cool. 2 ml of potassium bromide solution (2% w/v) was added to each flask and the flasks were allowed to stand in boiling water for 15 minutes to ensure complete reduction of selenium(VI) to selenium(IV). The solutions were then centrifuged and the residues rejected. The supernatant solution was taken for analysis.

The whole digestion process took about 4 hours to complete. One blank was digested for each three samples. The copper concentration in kale samples is much higher than the selenium concentration (see Table 12), consequently its interference in selenium determination is inevitable. Either the lanthanum nitrate/ammonia or tellurium (IV) procedure must be applied to eliminate the copper interference effect, if reliable selenium recoveries are to be achieved. Both these procedures

were applied to the kale digests and the results of the analyses performed by AFS are shown in Table 13.

No selenium fluorescence signals were observed when the digests were not treated with either lanthanum/ammonia or tellurium(IV).

As can be seen from Table 13 the results obtained by either the lanthanum hydroxide or tellurium(IV) procedure are in very good agreement with the result specified by Bowen. Again the lanthanum hydroxide procedure gives the higher result but in spite the simplicity of tellurium(IV) procedure, the relative standard deviation using this procedure is higher than when lanthanum hydroxide procedure is used.

In general digestion of kale samples was more complicated and needed much more care and attention than the soil sample digestions. Kale samples showed more sensitivity towards the final temperature of digestion than did the soil samples. Loss of elements through volatilization was more serious than in soil analysis if the temperature was not carefully controlled. As with the soil analyses, different acids and acid mixtures were used for digestion of kale samples to select the most effective digestion mixture. The only acid mixture which was able to extract the selenium content of the kale sample into the solution quantitatively was the perchloric-nitric acid mixture. With other acids no selenium recovery was obtained and even the recovery of known amounts of selenium added to the kale sample was not possible. Sample digestion recoveries using perchloric-nitric acid mixture for digestion were evaluated by the addition of known amounts of selenium to kale samples prior to their digestion. The recovery of selenium was then determined by analysing the samples after digestion. The results of these experiments are shown in Table 14. As can be seen from Table 14 the recoveries are very satisfactory showing a mean value of 99% and a relative standard deviation of 1.7%. These recoveries

TABLE 13COMPARISON OF RESULTS FOR THE SELENIUM CONTENT OF KALE SAMPLE

<u>La(NO₃)₃ method (ppm)</u>			<u>Te(IV) method (ppm)</u>			<u>Specified concentration</u> (<u>ppm</u>)
Mean	S.D.	RSD%	Mean	S.D.	RSD%	Best mean value 0.121
0.102	0.00809	7.93	0.100	0.0126	12	range 0.02 - 0.15

TABLE 14SELENIUM RECOVERY FROM DIGESTED KALE SAMPLES

<u>Se added to the</u> <u>kale sample</u> <u>(ppm)</u>	<u>Se found</u> <u>(ppm)</u>	<u>% Recovery</u>
0.1	0.200	99%
0.5	0.578	96%
1.0	1.080	98%
1.5	1.650	103%

are obtained if the final temperature does not exceed a certain level (190°C) and also if kale samples are digested long enough with the acid mixture. Digestion periods longer than three hours were sufficient and a four hour digestion period was chosen for all subsequent experiments, to ensure quantitative recovery of the element.

4.3.3. The effect of temperature on selenium recovery

The highest temperature at which the digestion could be carried out effectively without any loss of selenium through volatilization was established, using different final temperatures for digestion. As can be seen from Figure 31 the temperature can be raised to 190°C without any loss of selenium, above which a loss of ca. 30% is observed.

4.3.4. The effect of potassium bromide on selenium recovery

The addition of potassium bromide as a pre-reductant to convert selenium(VI) to selenium(IV), necessary for hydride generation, has already been mentioned. The effect on the generation efficiency was investigated by performing analysis in the presence and absence of potassium bromide. In the absence of this reagent a reduction in fluorescence intensity of 20% was observed, which implies a reduction in generation efficiency.

4.3.5. The effect of digestion time on selenium recovery

Kale samples were digested for different periods of time to establish the optimum digestion period to ensure quantitative recoveries of selenium. Samples which were digested for less than three hours did not give quantitative recoveries. The recoveries were satisfactory for digestion periods longer than three hours and a four hour period was chosen and applied. The effect of digestion time on selenium recovery is shown in Figure 32.

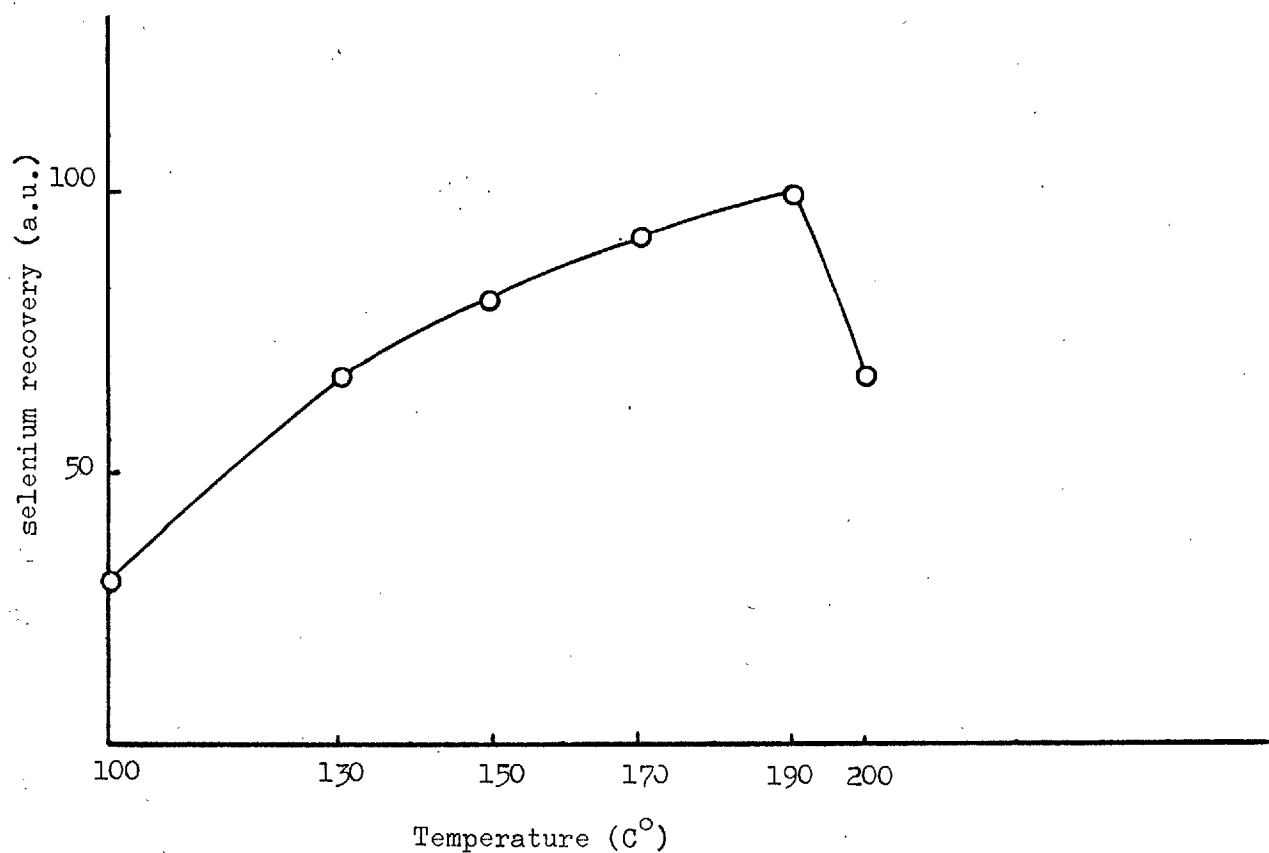


FIGURE 31 : THE EFFECT OF DIGESTION TEMPERATURE ON THE SELENIUM RECOVERY

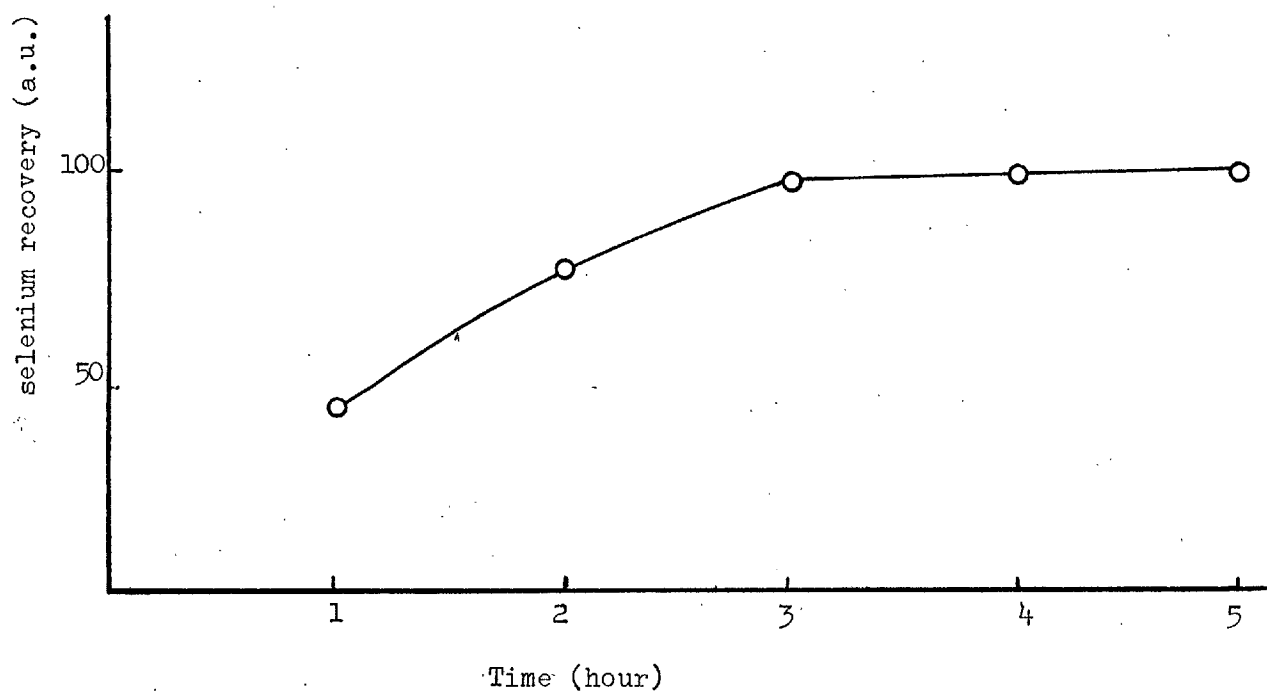


FIGURE 32 : THE EFFECT OF DIGESTION TIME ON THE SELENIUM RECOVERY

CHAPTER FIVE

5. THE DETERMINATION OF ARSENIC BY NON-DISPERSIVE ATOMIC FLUORESCENCE SPECTROSCOPY

5.1. INTRODUCTION

In the dispersive atomic fluorescence spectroscopic determination of arsenic, the 1890 Å and 1937 Å arsenic resonance lines are usually used, but Fulton and co-workers (187) reported a detection limit of 25 µg/ml at 2349 Å using an arsenic-antimony dual-element EDL. They found that the stability of the dual-element lamps did not depend upon the power applied and that dual-element EDLs can be as stable as single element lamps. Cresser and West (155) have also used a dual-element E.D.L. containing arsenic and selenium. They obtained a detection limit of 25 µg/ml for arsenic at 1937 Å.

Dagnall et al. (36) determined arsenic using an arsenic E.D.L. as the source and different flames as the atom reservoir. They investigated the effect of flame temperature on the arsenic signal by using normal air-acetylene, air-propane and air-hydrogen flames and also diffusion flames of nitrogen-hydrogen and argon-hydrogen. A detection limit of 0.2 µg/ml of arsenic was obtained using a nitrogen-hydrogen flame. Thompson and Wildy (153) utilized an electronically modulated arsenic EDL and an air-acetylene flame to determine arsenic. A detection limit of 0.2 µg/ml arsenic was obtained with a linear range of 0.5 to 100 µg/ml arsenic. One of the lowest limits of detection obtained to date was reported by Thompson (115) who reported a detection limit of 0.0001 µg/ml using an electronically modulated arsenic E.D.L. and the hydride generation technique for sample introduction.

The first non-dispersive atomic fluorescence determination of arsenic was reported by Larkins (123). He used an H.C.L. as the source and a separated air-acetylene flame. A detection limit of

6 $\mu\text{g/ml}$ arsenic was obtained. Later Vickers and co-workers (188) used an arsenic E.D.L. and an argon-hydrogen flame to obtain a detection limit of 0.3 $\mu\text{g/ml}$ arsenic.

In the work of Tsujii and Kuga (189) the arsine generation technique was applied followed by fluorescence measurements in a hydrogen-argon-entrained air flame. A detection limit of 2 ng was obtained for a 50 ml sample. They made some improvements in their technique four years later (190) and indicated that in the previous work some limitations existed which could be overcome by using a hydride generation technique, similar to that reported by Thompson and Thomerson (93), coupled with a small argon-hydrogen-entrained air flame. They claimed the extremely good detection limit of 0.05 ng arsenic.

Nakahara and co-workers (191) utilized the arsine generation technique and a newly designed burner to determine arsenic at the nanogram level. They tried both dispersive and non-dispersive atomic fluorescence systems and obtained detection limits of 15.2 ng and 2.3 ng respectively.

Finally Nakahara and co-workers (192) determined arsenic down to 2 ng for a 20 ml sample, using sodium borohydride and 0.5 ng for a 20 ml sample using Zn - SnCl_2 -KI by a non-dispersive atomic fluorescence system.

In this chapter a rapid and sensitive method for the determination of arsenic in aqueous solutions is described. The non-dispersive atomic fluorescence spectroscopic system, described before, is used and the interfering effects of some metal ions on the arsenic determination are studied.

5.2. EXPERIMENTAL

5.2.1. Reagents

Arsenic(III) stock solution was prepared by dissolving 0.132 g of arsenic oxide (As_2O_3) in a few mls. of 5M hydrochloric acid solution

while mild heating was applied. When the powder was dissolved completely the solution was diluted to 100 ml using 5M hydrochloric acid solution. Standard solutions were prepared by the appropriate dilution of this stock solution and were prepared freshly each day.

The sodium borohydride reagent was used as a freshly prepared 5% w/v in 1% sodium hydroxide solution. Analytical reagent grade potassium iodide and hydrochloric acid were used throughout the work.

5.2.2. Procedure

The experimental procedure outlined in section 3.2.2., Chapter 3, was adopted in these studies.

5.2.3. Optimization of experimental parameters

Using pure aqueous arsenic (III) standard solutions the experimental variables were optimized to provide the best possible sensitivity and precision. The operating power for the arsenic E.D.L., photomultiplier operating voltage, amplifier gain, hydride generation cell volume, sodium borohydride reagent and arsenic standard solution volumes used in the arsine generation cell were each varied and the optimum conditions were established. The optimum conditions established experimentally are represented schematically in Figures 33 - 38.

Effect of hydrochloric acid and sodium borohydride concentrations

The effect of the acidity of standard arsenic solutions with respect to hydrochloric acid, on the intensity of atomic fluorescence signal observed for 0.5 μg of arsenic was investigated. The concentration of sodium borohydride reagent was kept constant at 5% w/v for this experiment. The effect of varying the acidity of standard solutions, on the analytical signal was less pronounced for arsenic than it was for selenium.

The variation of acid concentration present in the arsenic standard solution had an effect on the efficiency of arsine generation only when the standard solution was less than 1 M with respect to

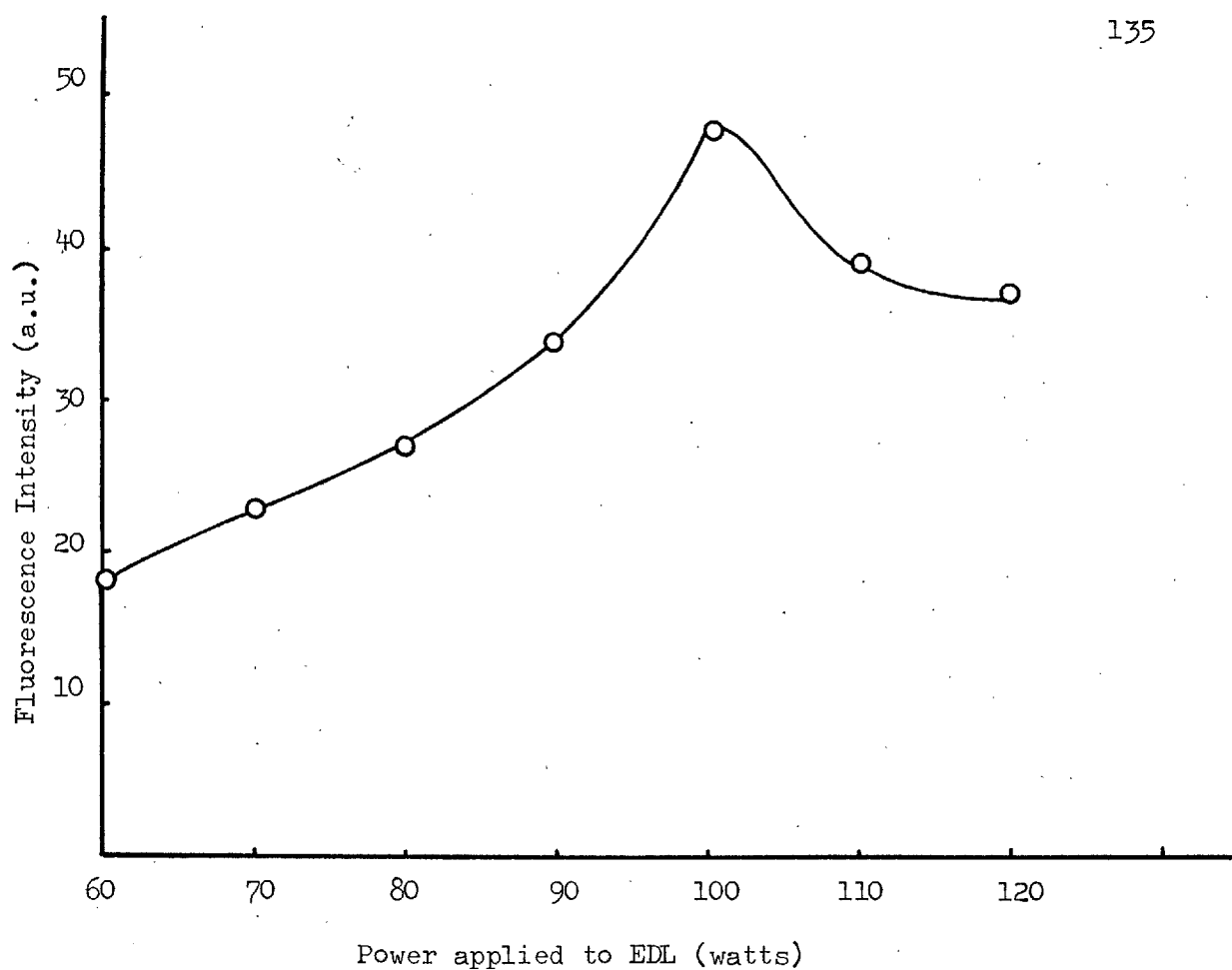


FIGURE 33 : THE EFFECT OF POWER APPLIED TO THE ARSENIC EDL
ON FLUORESCENCE INTENSITY

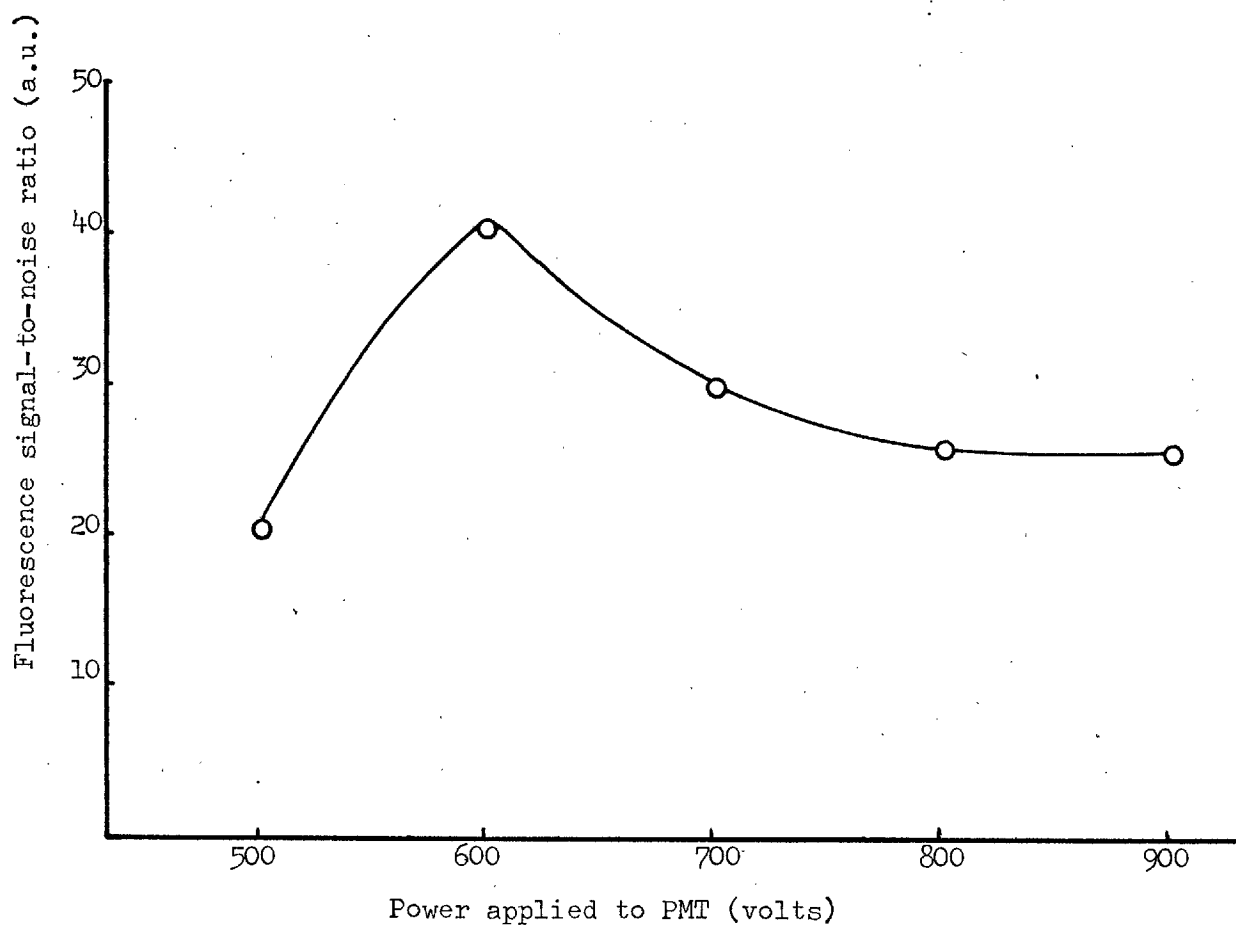


FIGURE 34 : THE EFFECT OF POWER APPLIED TO PMT ON FLUORESCENCE
SIGNAL-TO-NOISE RATIO

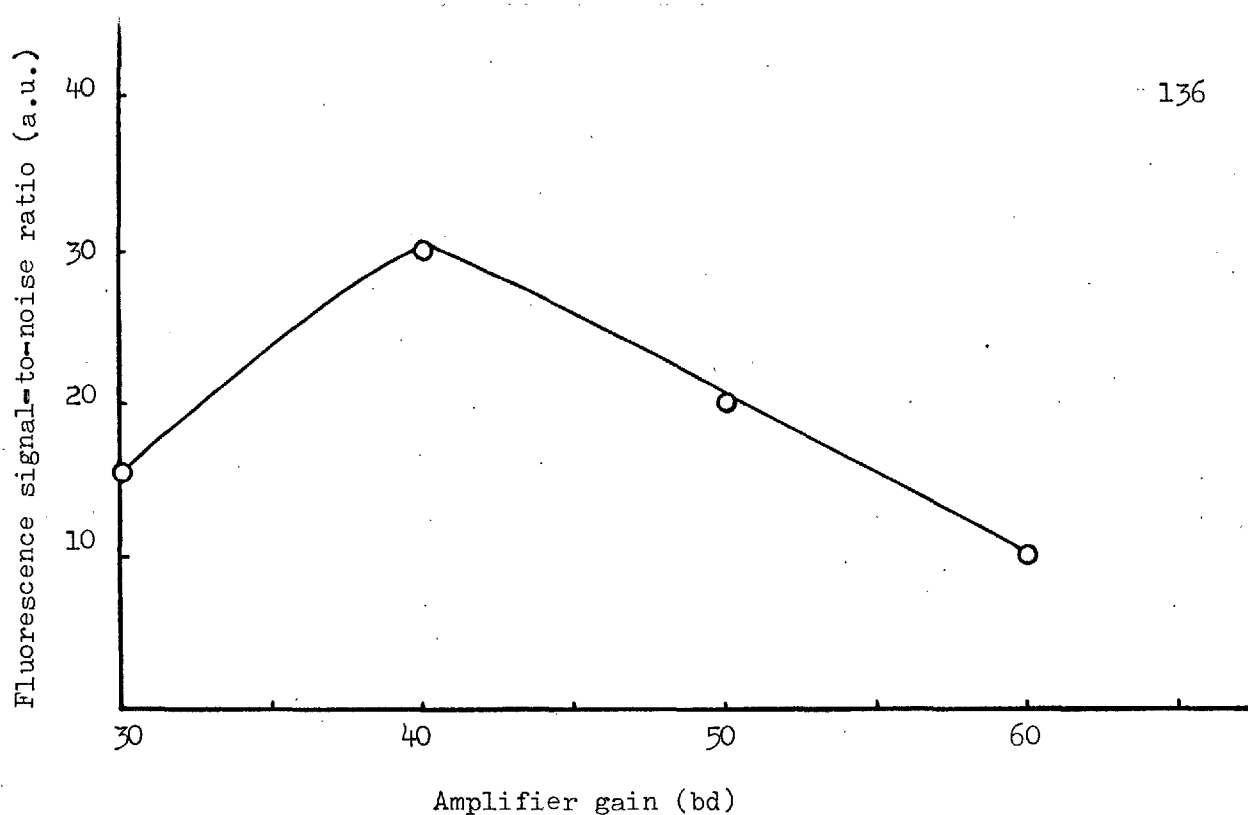


FIGURE 35 : THE EFFECT OF AMPLIFIER GAIN ON FLUORESCENCE SIGNAL-
TO-NOISE RATIO

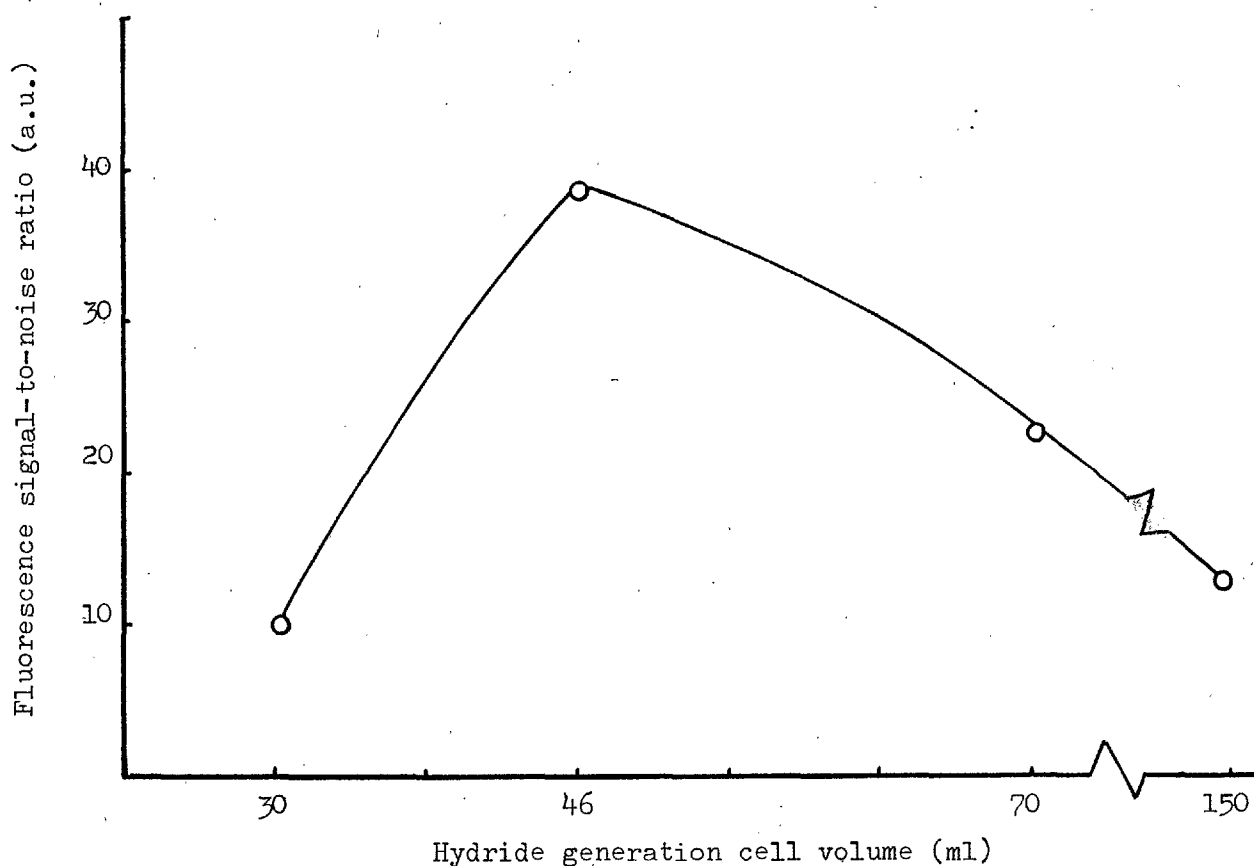


FIGURE 36 : THE EFFECT OF HYDRIDE GENERATION CELL VOLUME ON FLUORESCENCE
SIGNAL-TO-NOISE RATIO

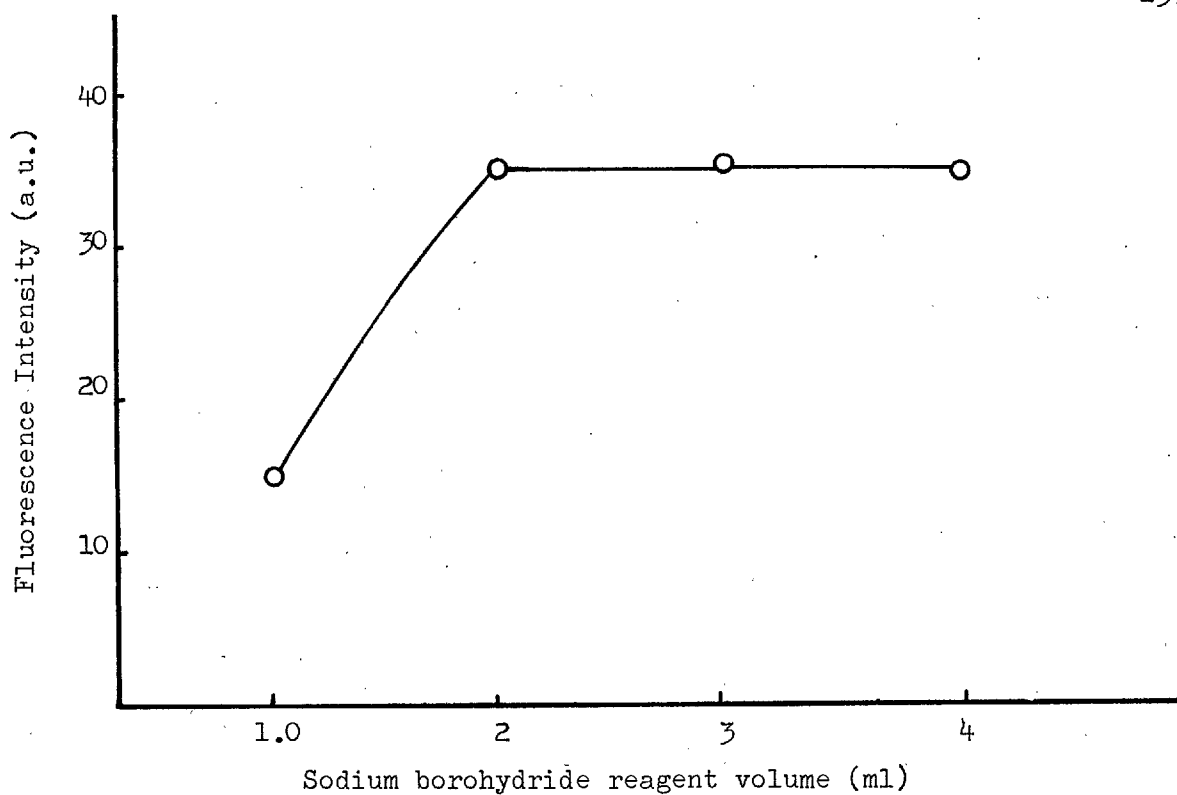


FIGURE 37 : THE EFFECT OF NaBH_4 REAGENT VOLUME ON FLUORESCENCE INTENSITY

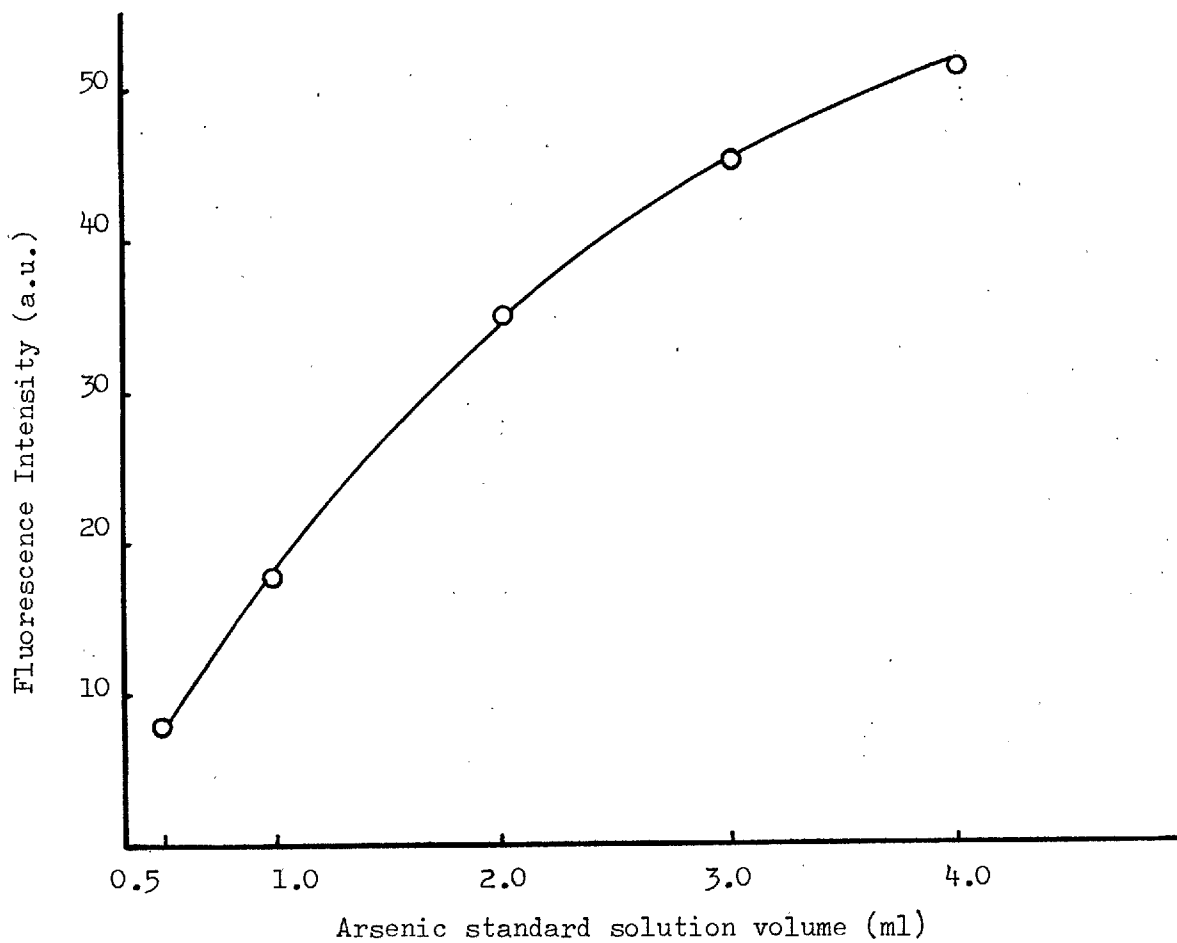


FIGURE 38 : THE EFFECT OF ARSENIC STANDARD SOLUTION VOLUME
ON FLUORESCENCE INTENSITY

hydrochloric acid. The atomic fluorescence signal remained constant at higher acid concentrations. The results of this experiment are shown in Figure 39. A constant hydrochloric acid concentration of 5M was chosen throughout the rest of the experimental work.

The effect of variation of sodium borohydride solution concentration on the arsenic atomic fluorescence signal was investigated using 1 ml volumes of 0.5 µg/ml arsenic standard solutions. Very little variation in arsine generation efficiency was observed over the concentration range of 1% to 5% w/v of sodium borohydride. A concentration of 5% w/v was chosen and used throughout the further work. The results of this experiment are shown in Figure 40.

Optimization of flame height and flame composition

An argon-hydrogen entrained air flame was used for arsenic determination. The flow rates of both argon and hydrogen gases were optimized to give the best signal to noise ratio. Burners with different internal diameters were tried while optimum flow rates to the flame were used. These results are shown in Figure 41. The viewing position in the flame was optimized and 25 mm above the burner was chosen as the best viewing position. Figure 42 shows the result of this experiment.

5.2.4. Calibration curve, limit of detection and precision

The calibration curve for arsenic shown in Figure 43 was obtained by measuring the atomic fluorescence signals produced by a range of solutions of differing concentrations. These solutions were prepared by appropriate dilution of the 1000 ppm arsenic stock solution. The calibration curve was linear for arsenic solutions containing between 30 and 600 ng/ml arsenic in a 1 ml sample volume (i.e. between 30 and 600 ng arsenic). Figure 44 shows signals obtained for arsenic solutions containing 100 to 600 ng/ml arsenic. The smallest observable amount of

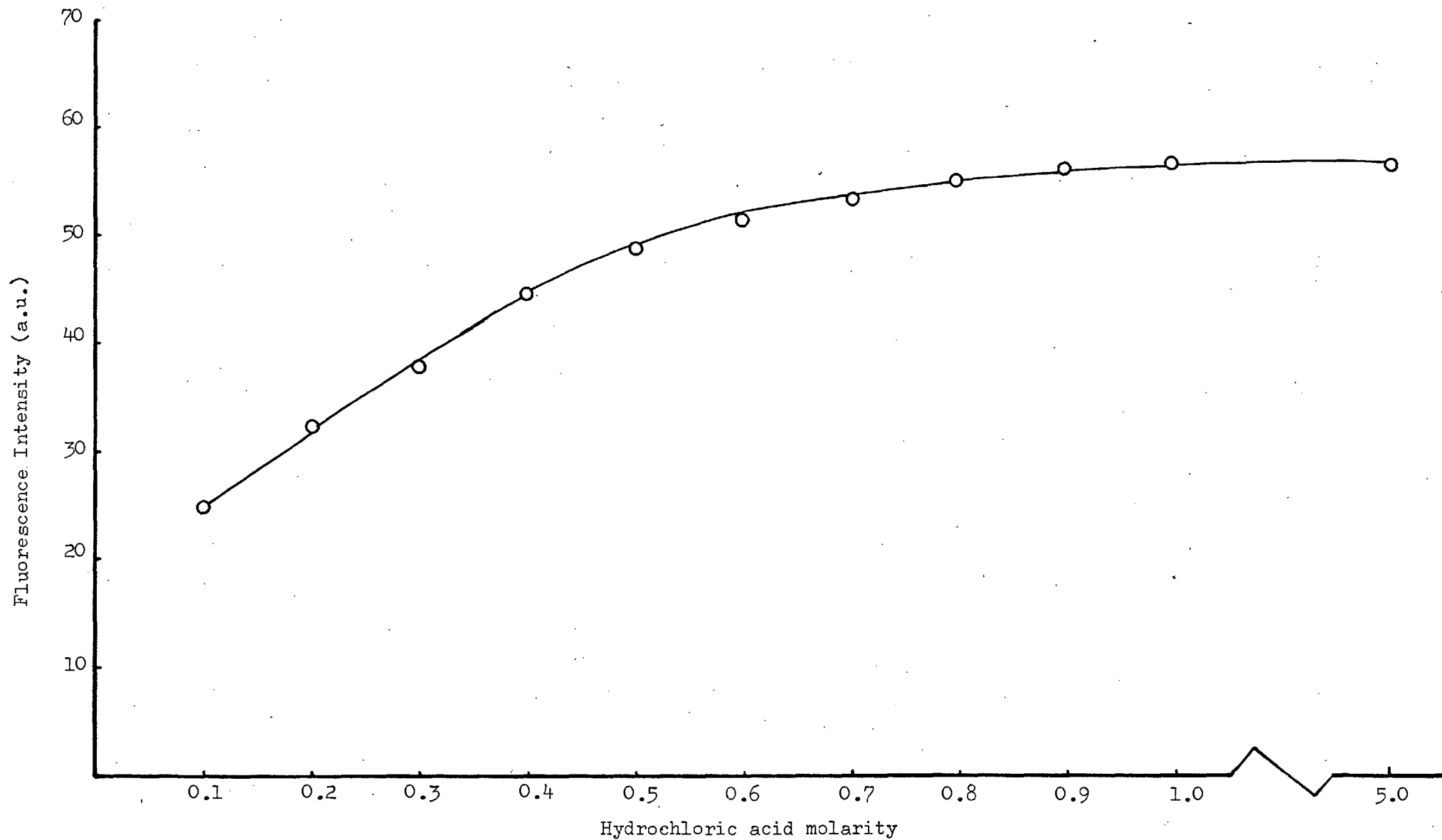


FIGURE 39 : THE EFFECT OF HCl CONCENTRATION ON THE DETERMINATION OF 0.5 $\mu\text{g/ml}$ ARSENIC

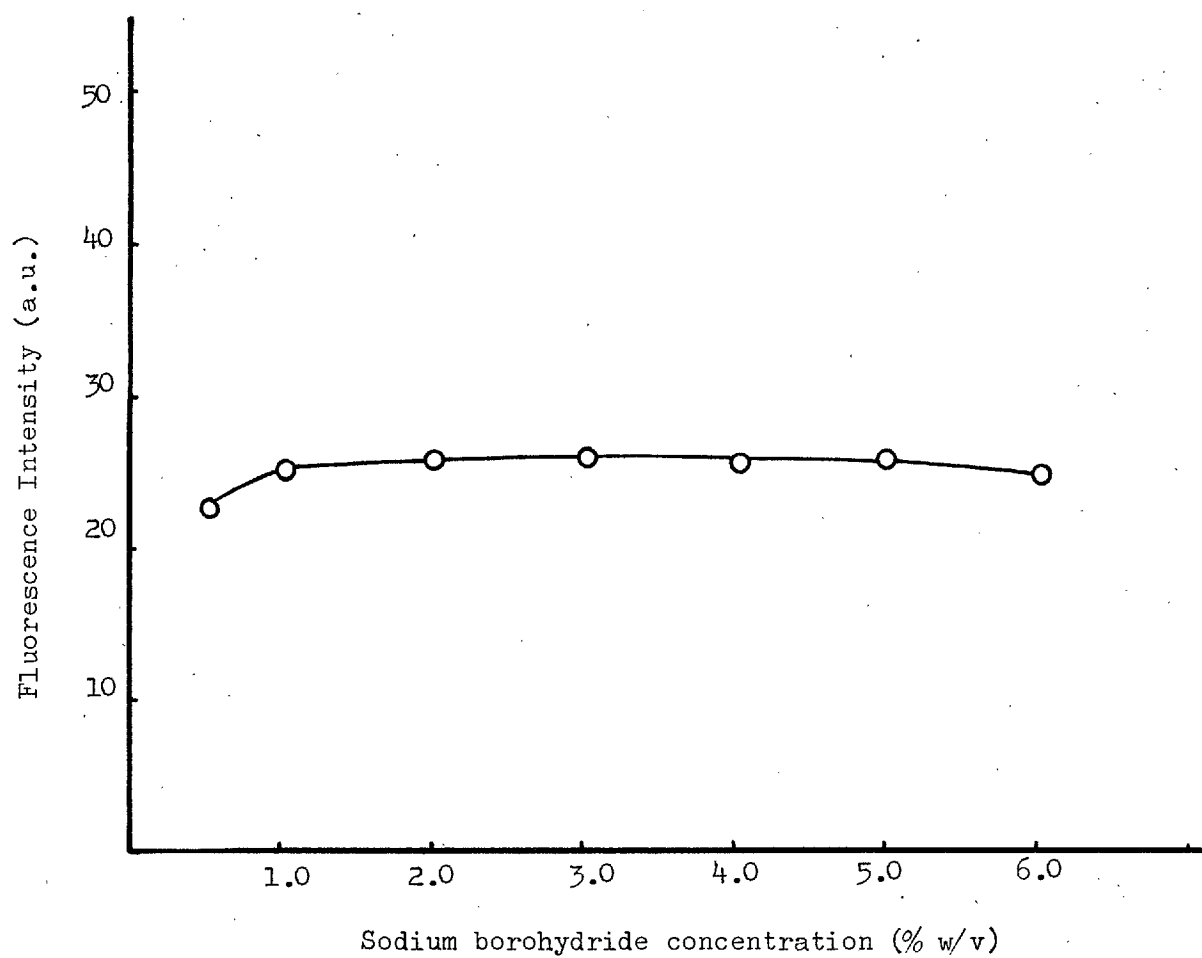


FIGURE 40 : THE EFFECT OF NaBH_4 CONCENTRATION ON THE DETERMINATION
OF 0.5 $\mu\text{g/ml}$ ARSENIC

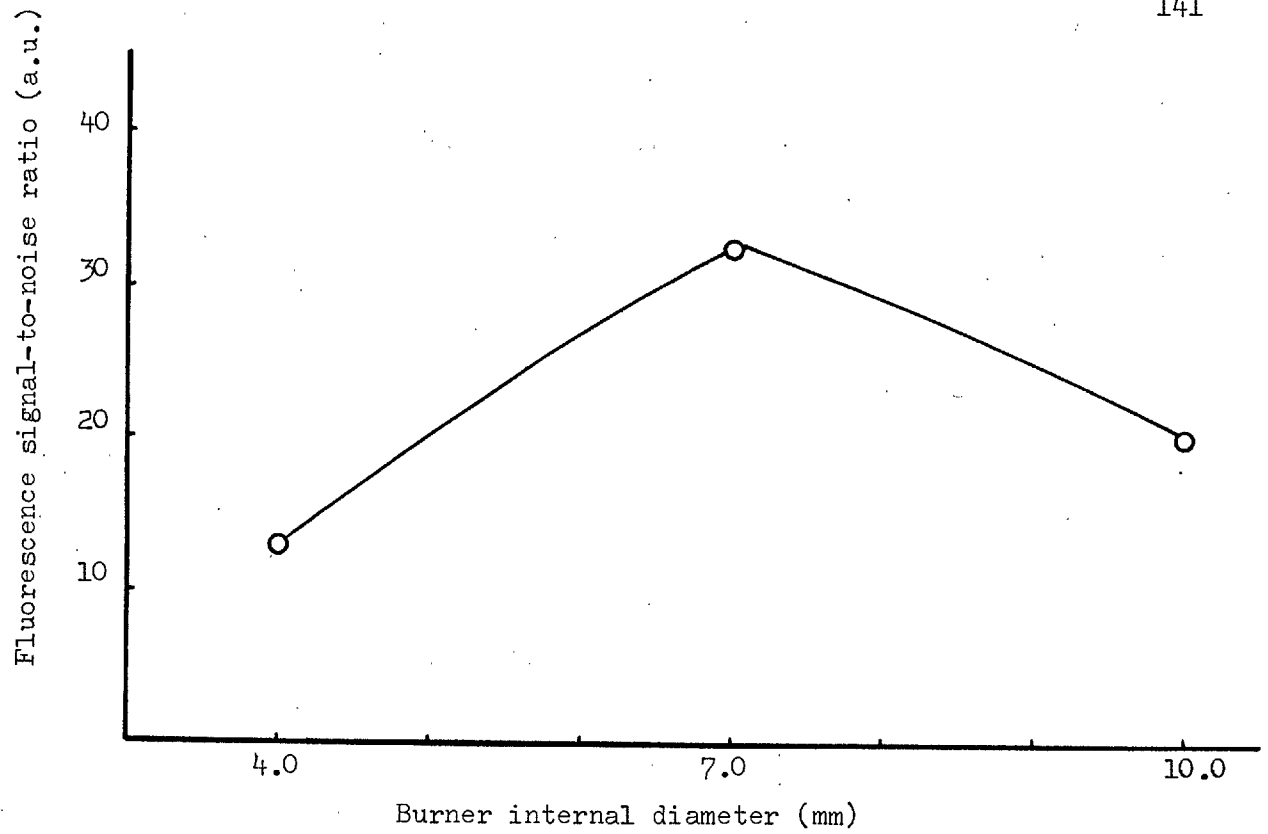


FIGURE 41 : THE EFFECT OF BURNER DIAMETER ON FLUORESCENCE SIGNAL-TO-NOISE RATIO

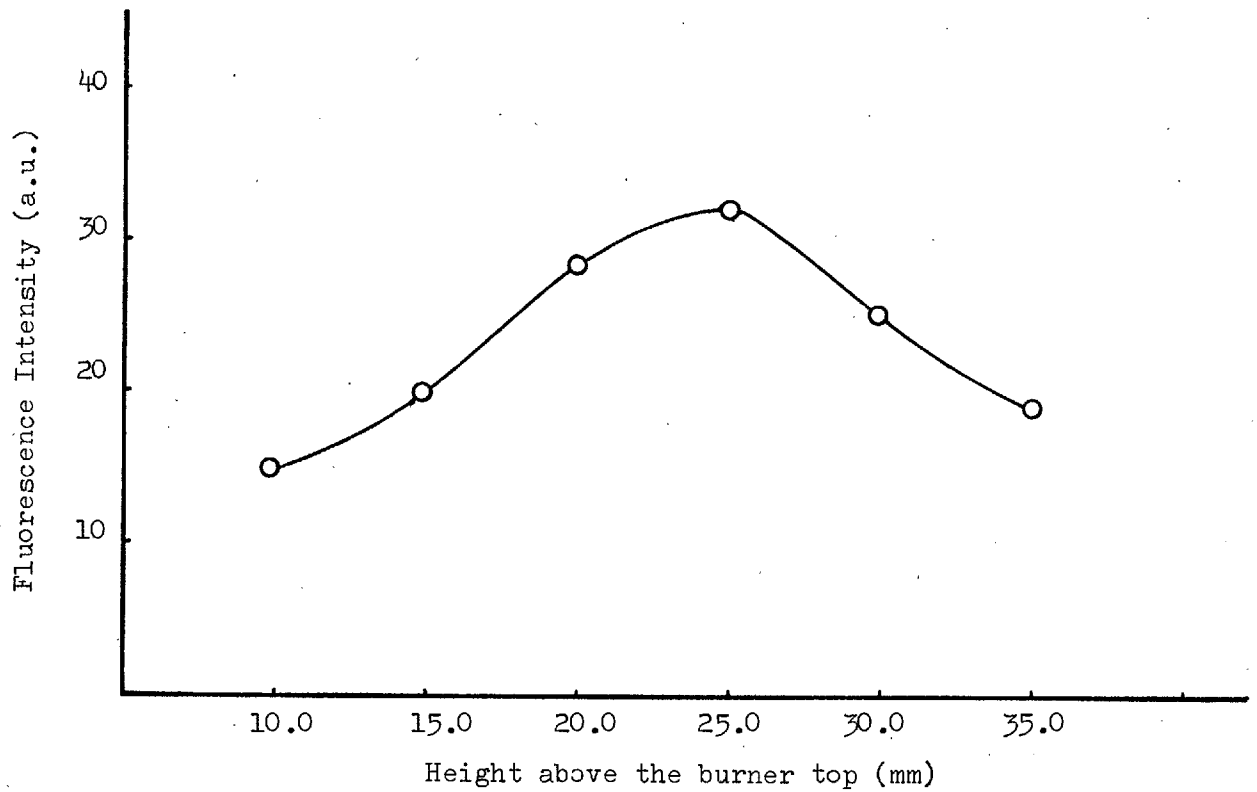


FIGURE 42 : THE EFFECT OF VIEWING POSITION ON FLUORESCENCE INTENSITY

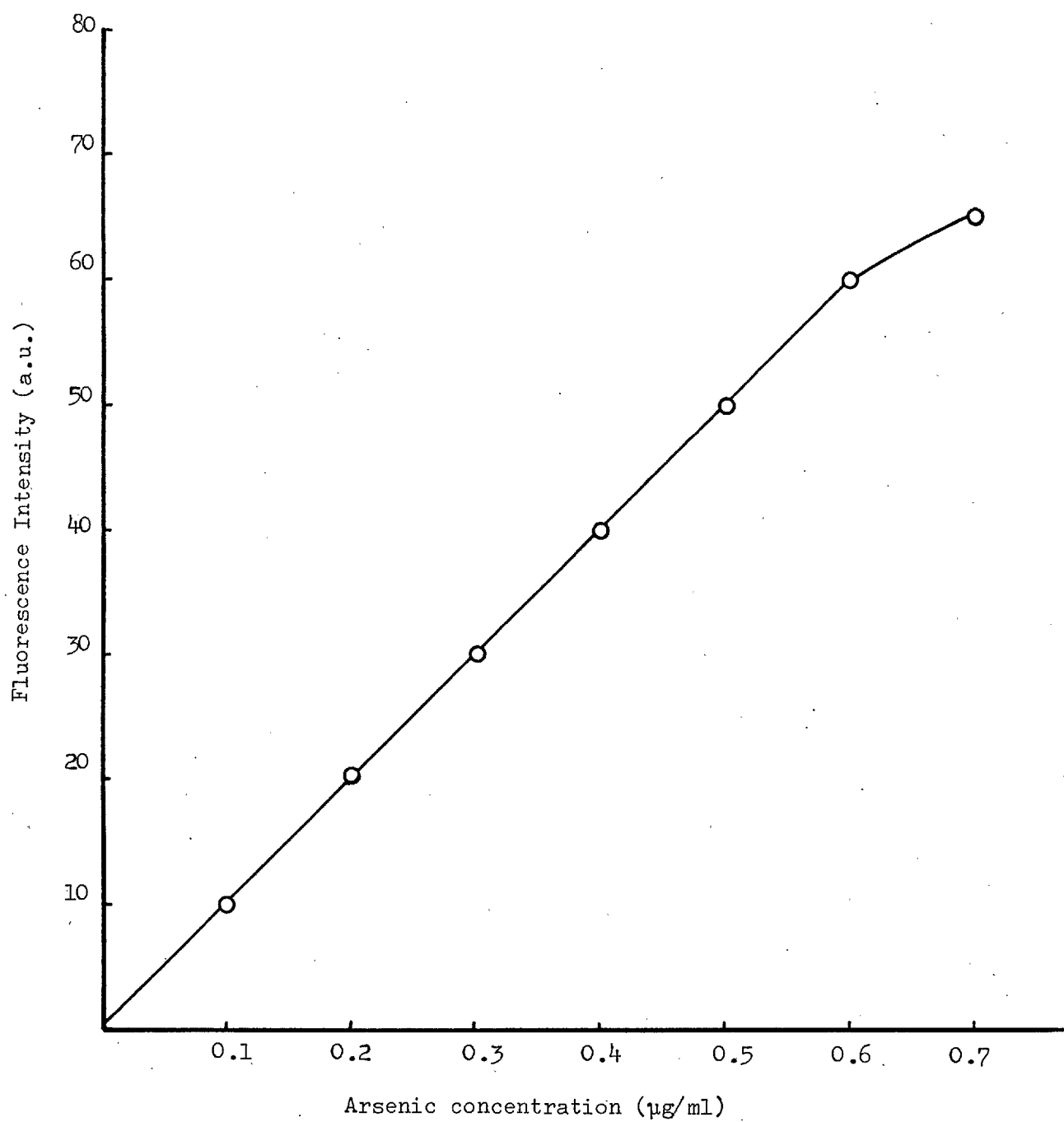


FIGURE 43 : AQUEOUS CALIBRATION CURVE FOR THE DETERMINATION OF ARSENIC
BY NON-DISPERSIVE AFS

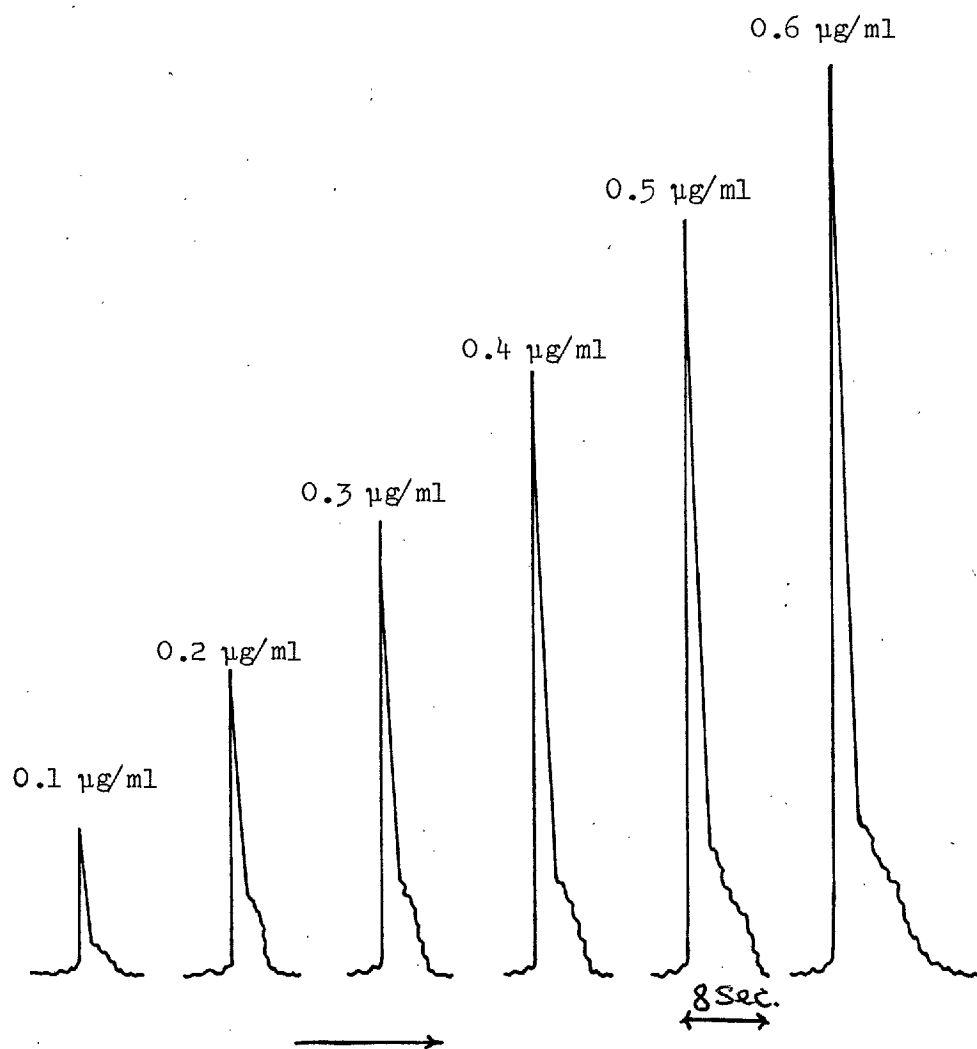


FIGURE 44 : SIGNALS OBTAINED FOR ARSENIC STANDARD SOLUTIONS

arsenic was 30 ng which is higher than the theoretical detection limit (the mass of arsenic required to give a fluorescence signal twice the height of the peak to peak noise value) calculated for optimal conditions.

The blank signal was quite significant and was equivalent to ca. 15 ng/ml arsenic. Many workers have noted the presence of a high blank signal while measuring arsenic. This blank has been traced to the reagents used to make up the standard solutions or those used to generate arsine.

Recrystallization of sodium borohydride and passage of argon through the sodium borohydride solution to expel the arsine, did not decrease the size of the blank signal considerably. Argon was passed through the hydrochloric acid, used to make up the standard arsenic solutions for 2 hours but the reduction of the blank signal was not significant. The blank was therefore corrected for by subtraction in all further quantitative analytical work. A relative standard deviation of 3.56% resulted when ten repetitive determinations of arsenic solutions of 500 ng/ml concentration were performed. The optimum operating conditions at which the arsenic calibration curve was constructed are summarised below:

Spectral source

- 1) Applied microwave power - 100 w
- 2) Reflected power from cavity - 25 w
- 3) Modulation frequency - 500 Hz

Atom cell

- 1) Argon flow rate - 8 l/min
- 2) Hydrogen flow rate - 3.5 l/min
- 3) Viewing height above burner - 25 mm
- 4) Burner internal diameter - 7 mm

Photomultiplier

- 1) Operating voltage - 600 V

Amplifier

- 1) Input - normal protected
- 2) Frequency limits - 100 Hz - 1000 Hz
- 3) Filter - internal
- 4) Meter damping - off
- 5) Gain - 40 db

Phase sensitive detector

- 1) Meter - 1 volt range (negative)
- 2) Zero - negative
- 3) Time constant - 1 sec. (internal)
- 4) Filter - on
- 5) Reference channel - A

Hydride generation cell volume - 46 ml

Sodium borohydride reagent volume - 2 ml

Arsenic sample solution volume - 1 ml.

5.2.5. Interference studies

The determination of arsenic by atomic absorption spectroscopy and atomic fluorescence spectroscopy is well-known to be subjected to interference from a number of metal ions. An arsenic standard solution containing 0.5 µg/ml arsenic was used to study the interfering effects of different metal ions on the arsenic determination.

Procedure

The procedure employed for this study has been described in section 3.5.1. Chapter 3.

Table 15 illustrates typical results of an investigation into the depressive effect of metal ions on the analytical signals observed for arsenic.

TABLE 15

DEPRESSIVE EFFECT OF METAL IONS* ON THE ANALYTICAL SIGNALS
OBSERVED FOR 0.5 $\mu\text{g/ml}$ ARSENIC

<u>Metal ion</u> *	<u>% depression of signal</u>
Na(I)	0
K(I)	2
Mg(II)	2
Mn(II)	0
Ca(II)	0
Ba(II)	0
Hg(II)	0
Al(III)	3
Fe(II)	6
Fe(III)	2
Pb(II)	2
Zn(II)	3
Co(II)	9
Cu(II)	7
Ag(I)	9
Ni(II)	90

* Concentration of metal ion = 1000 $\mu\text{g/ml}$ (or ppm).

As can be seen from Table 15 nickel is the only element which interferes seriously. The suppression caused by other elements investigated is below 10% and may often be neglected. The effect of nickel concentration present in a 500 ng/ml arsenic standard solution, on the arsenic atomic fluorescence signal can be seen in Figure 45 which indicates that the interfering effect of nickel becomes significant only when its concentration is more than 10 times the arsenic concentration present in solution.

The interfering effect of nickel on the arsenic determination can be explained by the fact that when nickel is added to an arsenic solution, there is a change in the volatility of arsenic due to the formation of stable nickel arsenide. Consequently the efficiency of sodium borohydride as a reducing agent and its ability to generate arsine is reduced. Ediger (193) has reported the effect of nickel on arsenic volatility. According to him arsenic in an acid mixture alone is stable to about 600°C when losses begin during the charring process but when nickel is added arsenic is stable at charring temperatures up to 1400°C due to the formation of stable nickel arsenide.

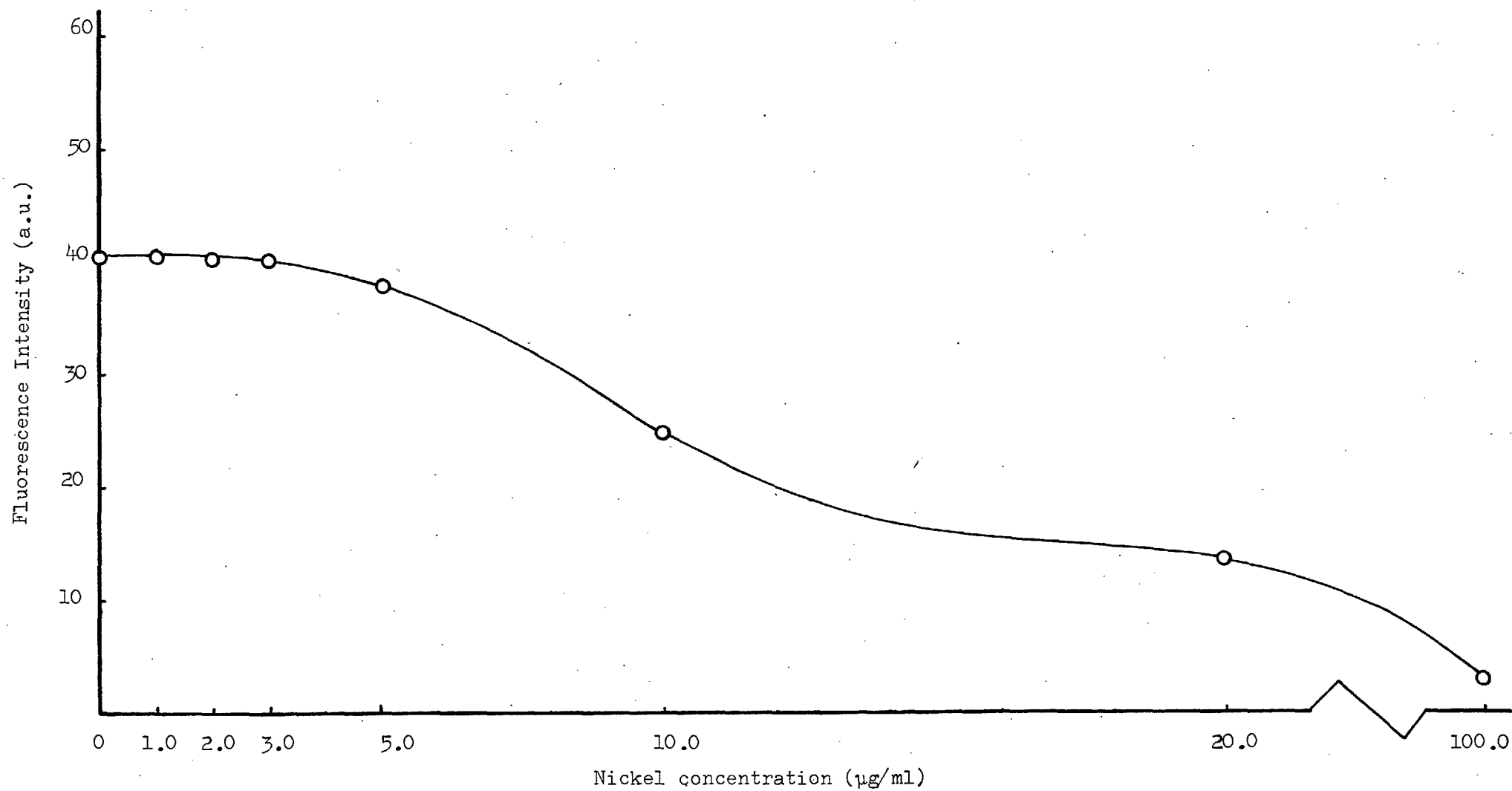


FIGURE 45 : THE EFFECT OF NICKEL ON THE DETERMINATION OF 0.5 µg/ml ARSENIC

CHAPTER SIX

6. THE DETERMINATION OF ARSENIC IN SOILS AND KALE

6.1. THE DETERMINATION OF ARSENIC IN SOIL

Arsenic is present in small amounts in nearly all soils and is widely distributed in plants, some of which can accumulate the element in their roots. One of the most widespread arsenic minerals is arsenopyrite, AsFeS . Arsenic is sometimes added to soils in the form of arsenical sprays used to combat fungi and other plant diseases. If the soil is sandy the applied arsenic can be very toxic to plants but toxicity is less likely with clay soils, particularly if they contain much iron. There are different methods of countering arsenic toxicity in soil, the best amongst them is to add lime or hydrated iron (III) oxide.

Accurate and rapid determination of arsenic is very important for analytical chemists dealing with environmental pollution problems. The classical methods such as the Marsh method or Gutzeit method are time consuming and require considerable skill.

Different methods, however, have been applied for the determination of arsenic in soils. Traces of arsenic were generally determined by either the Gutzeit method (194), which was adopted by the Association of Official Agricultural Chemists, or the molybdenum method (195). Almond (194) made some modifications to the Gutzeit method to make it more suitable for the determination of arsenic in field soils. He used lead acetate to react with hydrogen sulphide to separate it from gaseous arsine.

^{et al.}
Woolson^{et al.} (195) used the colorimetric method, based on an arsenomolybdenum blue colour development, on an arsenic (III) distillate.

Many instrumental methods have been used to determine arsenic in soil samples. Shigeru et al. (196) used the neutron activation

technique for determination of arsenic in many soil samples. About 0.1g of fine powder of the soil was irradiated and fused with $\text{NaOH} + \text{Na}_2\text{O}_2$ in a nickel crucible. Finally the activity due to ^{76}As was measured, obtaining a detection limit of 0.005 ppm arsenic. Kronborg and Steinnes (1973) determined arsenic and selenium simultaneously in soil samples using the same technique. More than 300 soil samples were analysed using 200 mg sample size. The reproducibility of the method was estimated to be about 5% for both elements.

Gastinger (197) determined arsenic spectrophotometrically using silver diethyldithiocarbamate reagent. Arsine was generated and passed through the reagent solution in pyridine and the absorbance of the solution was measured at 5330 Å. A calibration curve from 2-20 µg of arsenic was presented.

A rapid determination of trace elements including arsenic, in organic-rich soils by automatic X-ray fluorescence spectrometry was performed by Leake and Peachey (198). The measurements were carried out without any prior removal of the organic matter and the accuracy obtained was adequate for geochemical prospecting. Sakamoto *et. al.* (199) used a microwave plasma detector in the gas chromatographic analysis of arsenic in soil samples. Gas chromatography was used to separate arsine from large amounts of hydrogen which were evolved from an acid solution containing arsenic compounds, after treatment with zinc metal. The arsenic 2288 Å line intensity was monitored. A limit of detection of 0.2 ppm and a relative standard deviation of 3% for 10 ppm arsenic were obtained. Atomic absorption spectroscopy has also been used for the determination of arsenic in soil samples. Hamme *et. al.* (200) used a conventional atomic absorption instrument to determine arsenic in soil samples. Up to 93.4% of the arsenic was recovered from soils to which known amounts of organic arsenic were added. Comparison of standards made from inorganic arsenic dissolved in dilute nitric acid, with

standards of organic arsenic solution showed excellent correlation in the range of 0.5 - 40 ppm showing that the atomic absorption technique did not differentiate between organic and inorganic arsenic. Melton et. al. (201) described a procedure for arsenic determination in soils, based on the direct introduction of arsine into the flame of an atomic absorption spectrometer. Determination of arsenic at ppm levels was possible, with recoveries of added arsenic ranging from 93.0% to 106%. Yasushi et. al. (202) also used hydride generation and atomic absorption techniques together with a wet digestion of soil samples. Arsine was generated and swept to an argon-hydrogen-air flame. Absorption at the 1937 Å arsenic line was measured and a sensitivity of 0.02 µg was obtained.

A flame-less atomic absorption spectroscopic technique was used by Aslin (50) to determine arsenic in geological materials e.g. soil and rocks. Arsenic was separated from the sample, after acid attack, via hydride generation using sodium borohydride as a reductant. The generated hydride was passed into a heated quartz cell where atomic absorption measurements were made. The detection limit based on 500 mg of digested sample was 160 ng/g arsenic.

For the determination of arsenic in soil, whatever the nature of the soil sample, it is usually essential to convert arsenic into a soluble inorganic form. It must also be remembered that arsenic is a volatile metalloid and great care must therefore be exercised in all operations involving heating to avoid losses of the element by volatilization. Different procedures have been applied to bring the arsenic content of soil samples into solution. Fusion, normally with sodium or potassium hydroxide in nickel crucibles, or acid digestion usually using a mixture of nitric and perchloric acids are the most frequently used methods. Most workers have recommended an

acid digestion procedure, using a perchloric-nitric acid mixture. The soil sample is first digested with nitric acid alone and then with a mixture of nitric and perchloric acids. Pre-treatment with nitric acid is essential in order to reduce the danger of explosion which could occur with hot perchloric acid in contact with organic matter.

Different acids and acid mixtures were investigated to establish which was the most effective digestion solution for the determination of arsenic in soil samples. When aqua regia or nitric acid alone was used the problem of residual carbonaceous particles was encountered. Sulphuric acid and hydrofluoric acid were not able to destroy the organic matter and release arsenic. A mixture of nitric-perchloric acid (2:1) was successfully employed and the efficiency of this acid mixture for digestion of soil samples resulted in quantitative recoveries of arsenic.

Knowledge of normal concentrations of arsenic in soils is not extensive, however, it has been determined in soils from various geographical regions and its concentration in most of these samples was found to be within the range 1 - 10 ppm.

As was indicated in Table 8 the amount of arsenic in soils varies from about 0.1 ppm to 40 ppm, having a mean value of 6 ppm.

In this study nine soil samples which were previously analysed for their selenium content, were analysed for arsenic.

The procedure followed for digestion of these soil samples was the same as that described for selenium determination in soils, and can be found in section 4.2.1. Chapter 4.

The nickel concentration in the soil samples was found to be below 15 ppm and therefore not high enough to interfere in the determination. No attempt was made to eliminate its presence.

6.1.1. The effect of potassium iodide on the recovery of arsenic

The effect of potassium iodide on the recovery of arsenic was investigated. As the hydride generation technique is only applicable to arsenic (III), it is necessary to make sure that all arsenic present in the solution after digestion is in this oxidation state. Potassium iodide solution has been used as a pre-reductant in many arsenic determinations in real samples. 0.5 ml of potassium iodide solution (10% w/v) were added to each soil digest solution and the solutions were analysed for their arsenic content.

The same soil digest solutions were analysed for arsenic without being treated with potassium iodide. A reduction of 10-15% in arsenic recoveries was observed.

It is therefore necessary to add potassium iodide to the digest solutions at least two minutes before the addition of sodium borohydride reagent, if quantitative recoveries of arsenic are to be obtained.

Sample digestion recoveries were evaluated by the addition of known amounts of arsenic to 1 g soil samples prior to their digestion. The recovery of arsenic was then determined. Table 16 indicates the results obtained by these experiments.

As can be seen from Table 16 arsenic recoveries are satisfactory, showing a mean value of 98.0% and a relative standard deviation of 2.0%. When acids or acid mixtures other than nitric-perchloric acid mixture were used, poor arsenic recoveries were obtained. The recoveries of arsenic, using different acids and acid mixtures for digestion of the soil samples are compared in Table 17. Each figure in these tables is an average of a duplicate analysis.

Nine soil samples were digested in nitric-perchloric acid mixture and analysed to determine their arsenic content using the procedure described in section 4.2.1. The results obtained are shown in Table 18.

TABLE 16ARSENIC RECOVERY FROM DIGESTED SOIL SAMPLES

Sample No. 1.

<u>Arsenic Concentration</u> <u>ppm</u>	<u>Arsenic added</u> <u>ppm</u>	<u>Arsenic found</u> <u>ppm</u>	<u>Recovery</u> <u>%</u>
2.12	0.5	2.63	97
2.12	0.8	2.97	102
2.12	1.0	3.12	100
2.12	1.5	3.40	94

TABLE 17COMPARISON OF ARSENIC RECOVERIES USING DIFFERENT ACIDS
FOR DIGESTION

	<u>Arsenic</u> <u>concentration</u> <u>ppm</u>	<u>Arsenic</u> <u>added</u> <u>ppm</u>	<u>Arsenic</u> <u>found</u> <u>ppm</u>	<u>Recovery</u> <u>%</u>
Aqua regia	2.28	1.0	2.70	82
Nitric acid	2.28	1.0	2.30	70
Sulphuric acid	2.28	1.0	2.65	80
Hydrofluoric acid	2.28	1.0	2.90	88
Nitric-perchloric acid	2.28	1.0	3.23	98

TABLE 18ARSENIC CONTENT OF SOIL SAMPLES

<u>Soil Sample</u>	<u>Mean value ppm</u>	<u>S.D.</u>	<u>R.S.D. %</u>	<u>I.C.P.* ppm</u>
1	2.12	0.177	8.35	1.80
2	2.43	0.210	8.64	2.50
3	2.28	0.161	7.06	2.28
4	4.50	0.344	7.64	4.46
5	17.02	0.837	4.91	-
6	50.43	4.171	8.27	-
7	8.08	0.789	9.76	7.00
8	21.42	1.619	7.56	-
9	33.55	2.930	8.73	-

* Inductively Coupled Plasma.

The very high levels of arsenic observed in some of these soil samples are probably due to the soils being contaminated by pesticides containing arsenic. The relative standard deviations are higher than those obtained for the selenium determinations. Arsenic is more volatile than selenium and consequently its loss during the digestion period can be more serious. As can be seen in Table 18 the results show good agreement with those obtained using an optical emission spectrometer with an inductively-coupled plasma as the emission source.

6.2. THE DETERMINATION OF ARSENIC IN KALE

Arsenic is widely distributed throughout the biosphere although it has no known biological function. Arsenic occurs naturally at relatively high concentrations in sea foods probably as a result of contamination from industrial effluents and in some herbs and plants because of its use in insecticides and fungicides. Arsenic is known to be toxic at very low levels of intake and there are no known deficiency symptoms. Arsenic is present in biological materials to a greater or lesser extent as a contaminant as a result of increasing industrialization and associated pollution of the biosphere. Contamination may arise from a number of different sources. Thus crops will contain various amounts of contaminants according to the nature of the soil, fertilizer and insecticide treatment and proximity to industrial activity.

Experiments have shown an arsenic concentration of 0.110 ppm to 0.220 ppm (mg/Kg) in kale samples. The mean value of 0.140 ppm has been observed in most laboratories and with few exceptions, analyses of arsenic in kale agree well with one another. Different methods have been used for determination of arsenic in biological samples including kale of which neutron activation and atomic absorption

spectroscopy are the most popular. Thus Haller et al. (203) used a neutron activation technique together with a large volume, high resolution Ge(Li) detector to determine 15 elements including selenium and arsenic in plant tissues. The samples were freeze-dried, subjected to neutron activation and analysed. The method did not require any chemical or ashing manipulation and consequently many inherent errors associated with chemical determinations were eliminated. Routine determination of arsenic in kale by a neutron activation technique has been reported by Kroon and Das (204). Samples were irradiated and after purification with an ion exchange resin the ^{76}As was isolated by precipitation as the metal and the chemical yield determined by weighing. Standard kale powder was analysed for arsenic, giving a value of 0.146 ppm. Heydorn et al. (180) determined arsenic and selenium simultaneously in kale samples using neutron activation analysis. No pre-treatment of samples before activation was required. Irradiated samples were decomposed and chemically separated by precipitation. The arsenic content of the kale sample was found to be 0.110 ppm while 0.140 ppm was the recommended value. Multielement analysis of 36 elements in kale samples by neutron activation technique has been reported by Nadkarni and Morrison (205). The amount of arsenic was found to be 0.150 ppm. Finally Steinnes (206) used solvent extraction to separate the interfering elements and determined 11 elements, including arsenic, in Bowen's kale sample. He found 0.130 ppm arsenic while the specified value was 0.141 ppm.

After neutron activation techniques, atomic absorption spectroscopy is the most popular technique for the determination of arsenic in biological samples. Hosogaki et al. (207) decomposed samples with $\text{H}_2\text{SO}_4 - \text{HNO}_3$ mixture and then generated the arsine which was absorbed by

an aqueous solution. This solution was aspirated into an air-hydrogen flame and arsenic absorption was measured.

They determined arsenic down to 0.2 ppm and a linear calibration curve between 0.2 to 1 ppm was obtained. Kamada et al. (208) have also used arsine generation and atomic absorption using an argon-hydrogen flame to determine arsenic in biological samples. The sensitivity for 1% absorption was 0.7 ppb arsenic and the calibration curve was linear up to 50 ppb arsenic. Eichelberger et al. (209) used a non-flame atomic absorption spectroscopic method to determine arsenic in biological samples. The method has been found to be suitable for rapid routine determinations of arsenic especially in toxicological research. Total arsenic in biological materials has been determined by Ishizaki (210) using a flameless atomic absorption spectroscopic method. Arsenic in ashed biological sample was separated by extraction into chloroform from 1N HCl. As (V) was then reduced to As(III) with KI, and quantitatively back-extracted with $\text{Mg}(\text{NO}_3)_2$ solution. Calibration curves were linear from 0.2 to 6 ng of arsenic. Arsenic recoveries higher than 95% and relative standard deviations of 11% were obtained.

Ihnat et al. (211) analysed food samples including Bowen's kale for arsenic, using acid digestion, hydride evolution and atomic absorption spectroscopy. An argon-hydrogen-entrained air flame was used to decompose the generated arsine and a method detection limit of 25 ng arsenic was obtained. The recoveries of inorganic arsenic added ranged from 70% to 125%.

Arsenic is one of the most important toxic elements encountered in nature. Arsenicals are used as pesticides and fungicides and thus introduce a very high risk of contamination of plants and vegetables by arsenic. Therefore it is very important to find an accurate, rapid and precise method for the determination of this

element in biological materials. The non-dispersive atomic fluorescence system used in these studies was used to determine arsenic in standard kale and the results obtained were compared to those specified for the kale. As was mentioned before it is necessary to destroy the organic matter and convert the arsenic content of the kale into a soluble inorganic form, before any determination can take place. Dry ashing procedures have not been recommended for determination of arsenic in biological materials by many authors, acid digestion being preferred in most cases. Different acids and acid mixtures were investigated to establish the most effective digestion mixture. When acids or acid mixtures other than nitric-perchloric acid mixture were used, no arsenic recovery was observed. Changing the ratio of acids in the acid mixtures, increasing the temperature used for digestion and prolonging the digestion period all failed to improve the recovery of arsenic and no measurable arsenic was extracted into the solution. Even the recovery of known amounts of arsenic added to the kale samples was not possible. Nitric-perchloric acid mixture (5:1) was the only acid mixture strong enough to destroy the organic matter completely and effectively pass the arsenic into solution. It was found essential to maintain the oxidising conditions at all stages of the wet oxidation and at the same time control the final temperature of the digestion.

The procedure used for digestion of standard kale has already been described in Chapter 4 section 4.3.2. The digestion process took about 4 hours to complete and one blank was digested for each three samples.

As indicated in Table 15, nickel is the only element which interferes seriously in determination of arsenic by non-dispersive atomic fluorescence spectroscopy. According to Table 12 the nickel concentration in kale is lower than 0.2 ppm and consequently no interfering effect was expected or observed.

Table 19 indicates the result obtained using non-dispersive AFS for the arsenic content of kale which is compared to the value specified by Bowen. It can be seen from Table 19 that the result obtained using the non-dispersive atomic fluorescence system is in very good agreement with the specified value and the relative standard deviation is acceptable. In fact for a volatile element like arsenic in a complex matrix such as kale, the standard deviation of 5.78% is good and with many techniques, difficult to achieve. Sample digestion recoveries obtained when the nitric-perchloric acid mixture was used for digestion, were assessed by the addition of known amounts of arsenic to the kale samples prior to their digestion. The recovery of arsenic was then determined by analysing the digested samples. The results of these experiments can be seen in Table 20.

Table 20 shows the recoveries to be quite satisfactory with a mean value of 95.25% and a relative standard deviation of 1.84%. Each figure is the average of duplicate analysis. These recoveries were obtained under specific conditions. Any pronounced variation in the final temperature used for digestion of kale samples, does introduce variations in the obtained recoveries. The final temperature must not only be kept constant but also it should not exceed a certain level (170°C). The digestion time also has a pronounced effect on recoveries. Digestion periods shorter than 3 hours resulted in poor recoveries.

As mentioned before, the kale analysis was generally more complicated than the soil analysis and determination of arsenic in kale itself was more difficult than determination of selenium in kale. Arsenic was easily lost if the final temperature was not kept under constant observation and the effect of digestion time on recoveries was more pronounced than observed for selenium determinations. Under optimum conditions, however, quantitative recoveries of arsenic were obtainable.

TABLE 19COMPARISON OF RESULTS FOR THE ARSENIC CONTENT OF KALE SAMPLE

<u>Arsenic concentration found</u> (ppm)		<u>Specified concentration</u> (ppm)
Mean *	0.155	Best mean value 0.141
S.D.	0.00897	
R.S.D.	5.78%	Range 0.11 - 0.22

* Nine analysis

TABLE 20ARSENIC RECOVERY FROM DIGESTED KALE SAMPLE

<u>Arsenic added to</u> <u>the kale sample</u> (ppm)	<u>Arsenic found</u> (ppm)	<u>% Recovery</u>
0.1	0.200	93%
0.5	0.578	98%
1.0	1.080	98%
1.5	1.650	92%

6.2.1. The effect of temperature on arsenic recovery

Different final temperatures for digestion of kale samples were investigated to establish the highest temperature, at which digestion could be performed without any loss of arsenic through volatilization. Figure 46 shows the results of these experiments. As can be seen the final temperature of 170°C can be used without any loss of arsenic. When temperatures higher than 170°C were used a reduction of ca. 40% in the arsenic recovery was observed.

6.2.2. The effect of potassium iodide on arsenic recovery

The effect of potassium iodide as a pre-reductant to convert As(V) to As(III) in digested sample solutions has been mentioned before.. Analyses were performed in the presence and absence of potassium iodide and a reduction in fluorescence intensity of arsenic of about 30% was observed in the absence of potassium iodide. This suggests that addition of potassium iodide is necessary if quantitative recoveries of arsenic are expected. Therefore potassium iodide was added to the digested samples 2 minutes before hydride generation and subsequent fluorescence measurements were carried out. The 2 minute period was found to be sufficient, although longer periods have been reported by some authors.

6.2.3. The effect of digestion time on arsenic recovery

The effect of digestion time on arsenic recovery was investigated by digesting the kale samples for different periods of time and comparing the arsenic recoveries obtained. Quantitative recoveries of arsenic were obtained for digestion periods longer than 3 hours and a 4 hour period was adopted for all experimental work undertaken. The effect of digestion time on arsenic recovery is shown in Figure 47.

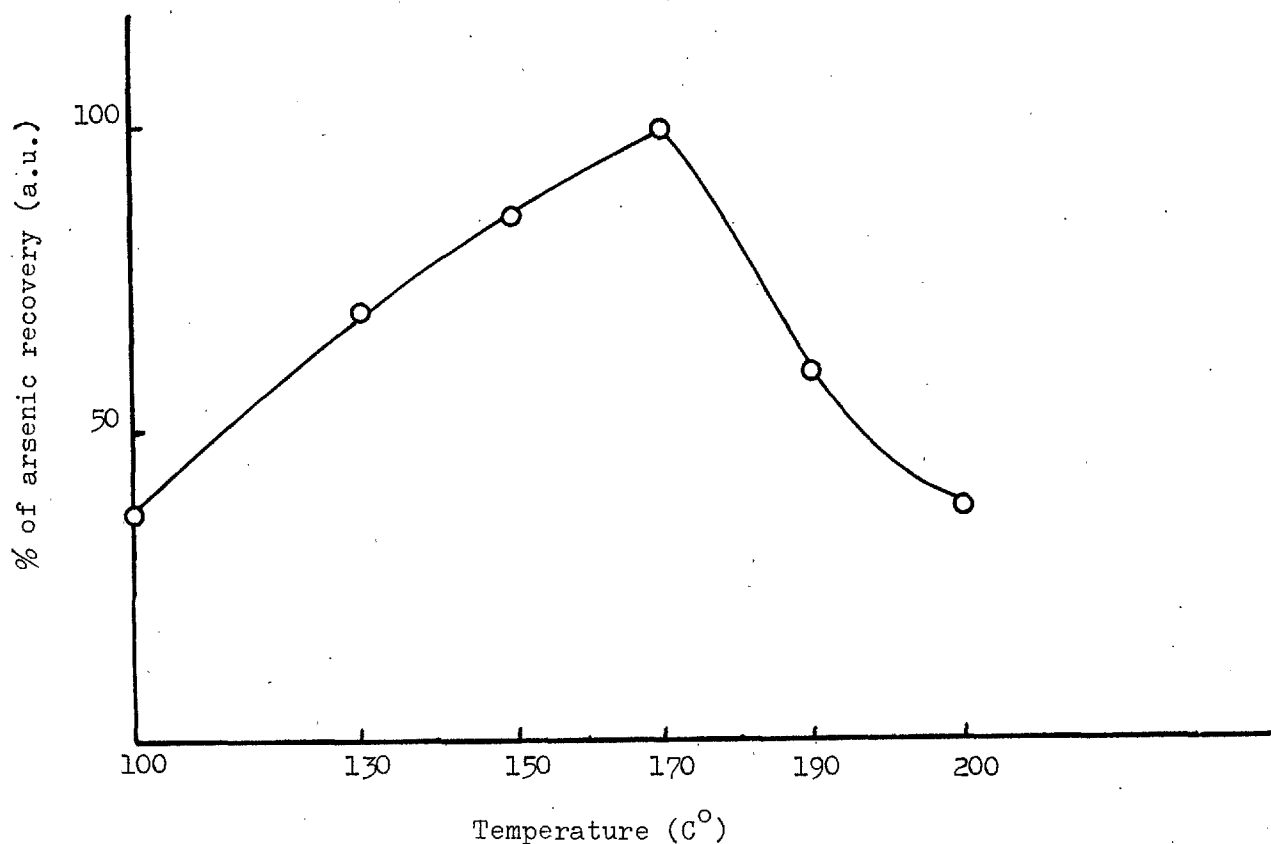


FIGURE 46 : THE EFFECT OF DIGESTION TEMPERATURE ON THE ARSENIC RECOVERY

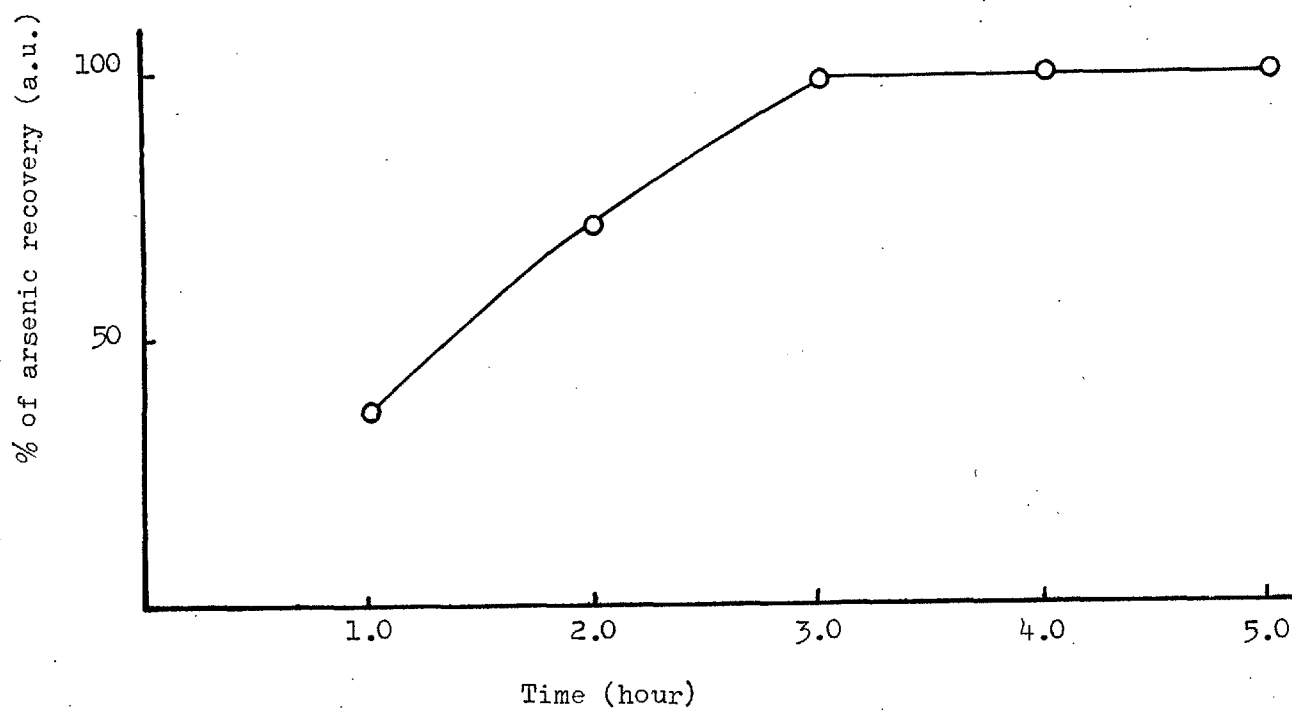


FIGURE 47 : THE EFFECT OF DIGESTION TIME ON THE ARSENIC RECOVERY

7. CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

7.1. CONCLUSIONS

During the past few years, there has been an increasing realization of the importance of trace element chemistry in biological systems. This awareness has been stimulated by the rising concern in industrial nations of the impact of man on his environment and its biological effect on him. Of primary significance is the role played by trace elements and whether or not they are beneficial to the biochemistry of man. Recently it has been recognized that a number of trace elements are required nutrients. The essential trace elements for plant and animal life to date include, Co, Cr, Cu, B, F, Fe, I, Mn, Mo, Se and Zn with Ni, Sn and V possibly essential. To this list of important elements must be added those of toxicological concern, i.e. Li, Be, Ba, Ni, Ag, Cd, Hg, As, Sb, Pb and Br.

Arsenic and selenium from these two lists were chosen as the elements of interest in this research work.

As the principal resonance lines of arsenic and selenium are all in the far ultraviolet region of the spectrum, below 2000 Å, determination of these elements by flame spectrometry presents some problems. For example, unfavourable signal to noise ratios result due to atmospheric and background absorption of these lines. Experiments have shown that AFS is superior to both AAS and AES for determination of arsenic and selenium.

As mentioned earlier the role of the monochromator in AFS is less important than in either AAS or AES and AFS can be employed without any means of monochromation. The advantages and disadvantages of a non-dispersive atomic fluorescence system have been discussed

earlier in this thesis. In comparison with the other flame spectroscopic techniques, non-dispersive AFS enables similar or better limits of detection to be obtained particularly for those elements whose main resonance lines lie in the ultraviolet region of the spectrum. In addition, the linear dynamic ranges of the growth curves usually extend over at least three orders of magnitude, enabling the analysis of a number of samples of widely differing concentrations to be made much more rapidly and conveniently than by AAS, for example. Since non-dispersive AFS features no form of wavelength selection, the selectivity of the technique depends wholly on the spectral purity of the excitation source. Fortunately, very satisfactory sources are available commercially or can be made in the laboratory. The EDL sources used during the course of this work were constructed in the laboratory and their intense and stable output provided reliable selectivity of the technique. There can be no question that the limits of detection quoted in this work could be substantially improved by using better or more efficient instrumental units. Atomic fluorescence instrumentation usually consists of different parts, few of which have been specifically designed for the application in which they find themselves and this frequently limits the performance of the instrument.

In comparison with an atomic absorption or atomic emission spectrometer of similar capability, the cost of a non-dispersive atomic fluorescence instrument would be considerably lower. Excellent EDLs can be prepared in the laboratory at virtually no cost. In terms of speed and simplicity of operation, it is difficult to imagine a more suitable technique for real sample analysis. Beyond the dissolution of samples, a series of elements may be determined in any one sample solution simply by inserting the appropriate excitation sources into the instrument.

Of all the features to be considered in assessing a quantitative analytical technique, precision and accuracy are usually the most important. Speed of operation, simplicity of instrumentation, multi-element capabilities etc. are all of little consequence if the technique is unable to provide accurate and precise data. In order to assess the performance of the technique a standard kale sample was analysed. As standard soil samples for selenium and arsenic were not available, known amounts of elements were added to the soil samples and then samples were analysed for their selenium and arsenic contents.

In each determination nine separate weighings of each sample were taken to enable the overall precision of the technique to be assessed. In general, the relative standard deviation for each determination was less than 10% and frequently less than 5%. Thus non-dispersive AFS seems to be capable of making sufficiently accurate and precise determinations of these two elements of interest in the field of biological material analysis.

The general conclusion can be summarised as follows: It has been demonstrated that arsenic and selenium can be determined at trace levels in soil and kale samples using the technique of non-dispersive atomic fluorescence spectrometry. The technique is both sensitive and precise. Although the hydride generation procedure for determination of selenium is normally subject to severe interference from copper, this effect has been eliminated by employing chemical pretreatment of samples, using lanthanum hydroxide as a co-precipitant or the addition of tellurium (IV) to form the stable copper telluride. Both methods have been applied successfully to the determination of selenium in soils and kale digests. Neither of these two methods can be preferred to the other unless all governing

factors of a particular analysis are taken into account.

The digestion procedures used to dissolve the soils and kale samples have proved to be effective, resulting in quantitative recoveries of elements. These recoveries are obtained under the conditions specified in the appropriate chapter.

7.2. SUGGESTIONS FOR FURTHER WORK

There are three areas in which the technique might usefully be improved. No attempt has been made to discuss how these developments can be established. These areas are:

Automation

Most analytical procedures can benefit by becoming, at least, partly automated. As an instrument intended for use on a routine basis, a non-dispersive atomic fluorescence spectrometer employing an automatic sample changer and an automatic readout device would require the minimum of attention during operation. When the hydride generation technique is employed, the use of a pumping system to automatically introduce the reagent to the cell and wash the generation cell after each determination, would greatly improve the speed and precision of the analyses. This feature would not add greatly to the overall cost of the instrument and would serve to underline the suitability of the technique for routine analysis.

Optics

In this work the source of radiation was focussed as a 1:1 image on the flame using two 7.5 cm focal length fused silica convex lenses. A similar lens was used to focuss the flame as an inverted 1:1 image on the PMT. It must be stressed that this optical arrangement was not considered as an ideal arrangement. The whole optical system could be improved by using more suitable components such as a concave mirror to increase the amount of radiation passing through the atom cell. This would improve the sensitivity of the technique.

Simultaneous multi-element analysis

The ease with which AFS, in general, lends itself to simultaneous multi-element analysis makes this aspect worthy of attention. Still the additional expense involved in developing such a system might outweigh any advantages gained.

REFERENCES

1. J.J. Berzelius, Annales de Chimie et du Physique, Paris, Series 2, Tome 7, 199, (1817).
2. C.H. Jones, Chem. Met. Eng., 23, 957, (1920).
3. M.J. Fleischer, J. Chem. Ed., 31, 446, (1954).
4. K.W. Bagnall, "The Chemistry of Se, Te and Po", Elsevier, ~~AMSTERDAM~~ ~~LONDON~~ ~~NEW YORK~~ (1966).
5. S. Flaschen, D. Pearson and W. Northover, J. Am. Cer. Soc., 43, 274, (1960).
6. V. Kollonitsch and C.H. Kline, Ind. Eng. Chem., 55, (12), 18, (1963).
7. H. Schroeder and J. Balassa, J. Chron. Dis., 19, 85, (1966).
8. S.S. Pinto, S. Petronella, D. Johns and M.F. Arnold, Arch., Ind. Hyg. and Occ. Med., 1, 437, (1950).
9. "Hygienic Guide Series", Detroit, Michigan, American Industrial Hygiene Assoc., (1964), (1965).
10. J.R. Glover, Ann. Occ. Hyg., 10, 3, (1967).
11. V.C. Morris and A. Levander, J. Nutrit., 100, 1383, (1970).
12. K. Schwartz and C.M. Foltz, J. Amer. Chem. Soc., 79, 3292, (1957).
13. J.H. Koesman, W.H.M. Peeters, C.H.M. Keudstall, P.S. Tjiol and J.J.M. de Goeiz, Nature, 245, 385, (1973).
14. W.G. Jaffe and M.C. Mondragon, J. Nutrit., 97, 431, (1969).
15. M.L. Scott, J. Nutrit., 103 (6), 803, (1973).
16. H.C. Dudley and J.W. Miller, J. Ind. Hyg. Toxicol., 23, 470, (1941).
17. Vogel, "Textbook of Quantitative Inorganic Analysis", 4 Ed., Longman, London, (1978).
18. J. Grant, "Volumetric Analysis", 13 Ed., Butterworths Scientific Publications, (1955). ~~LONDON~~
19. M.K. Gandikota, B. Sarojini and G. Gopalarao, Indian J. Chem., 12 (2), 203, (1974).
20. M. Hanif, M.A. Ghughtai and M.A. Chaudhri, Pakistan J. Sci. In. Res., 15 (1-2), 37, (1972).
21. J.J. Lingane, Ind. Eng. Chem. Anal. Ed., 15, 583, (1943).

22. K. Bambach, Ind. Eng. Chem. Anal. Ed., 14, 265, (1942).
23. I.M. Kolthoff and R.L. Probst, Anal. Chem., 21, 753, (1949).
24. H.J. Morris and H.O. Calvery, Ind. Eng. Chem. Anal. Ed.,
9, 447, (1937).
25. E.B. Sandell, "Colorimetric Determination of Traces of Metals",
3rd Ed., 289, (1959).
26. G.M. George, L.J. Frahn and J.P. McDonnell, J. Ass. Off. Anal.
Chem., 56, 793, (1973).
27. M. Covello, G. Giampa and E. Ciamillo, Farmaco. Ed. Prat.,
22, 218, (1967).
28. T.M. Raymont and R.J. Dubois, Anal. Chim. Acta , 56, 1, (1971).
29. F.E. Lichte and R.K. Skogerboe, Anal. Chem., 44, 1480, (1972).
30. G.F. Kirkbright, A.F. Ward and T.S. West, Anal. Chim. Acta ,
64, 353, (1973).
31. M. Thompson, B. Pahlavanpour, S.J. Walton and G.F. Kirkbright,
Analyst, 103, 568, (1978).
32. S.S. Kirshnan and N.E. Erickson, J. Forens. Scie., 11, 1, (1966).
33. A.R. Byne, Anal. Chim. Acta , 59, 91, (1972).
34. V.A. Fassell and D.W. Golightly, Anal. Chem., 39, 466, (1967).
35. H.L. Kahn and J.E. Schallis, Ato. Abso. Newslett., 7, 5, (1968).
36. R.M. Dagnall, K.C. Thompson and T.S. West, Talanta, 15, 677, (1968).
37. R.M. Dagnall, K.C. Thompson and T.S. West, Ato. Abso. Newslett.,
6, 117, (1967).
38. G.F. Kirkbright, M. Sargent and T.S. West, Ato. Abso. Newslett.,
8, 34, (1969).
39. G.F. Kirkbright and L. Ranson, Anal. Chem., 43 (10), 1238, (1971).
40. "Official Methods of Analysis", 11th Ed., A.O.A.C., Washington D.C.
41. F.J. Fernandez and D.C. Manning, Ato. Abso. Newslett., 10,
86, (1971).
42. E.N. Pollock and S.J. West, Ato. Abso. Newslett., 11, 104, (1972).
43. F.J. Fernandez, Ato. Abso. Newslett., 12 (4), 93, (1973).
44. W.H. Holak, Anal. Chem., 41, 1712, (1969).
45. E.F. Dalton and A.J. Malanoski, Ato. Abso. Newslett., 10, 92, (1971).

46. R.E. Madsen, Jr., *Ato. Abso. Newslett.*, 10, 57, (1971).
47. H.L. Khan, *Ato. Abso. Newslett.*, 7, 40, (1968).
48. T. Maruta and G. Sudoh, *Anal. Chim. Acta*, 77, 37, (1975).
49. R.C. Chu, G.P. Barron and P.A.W. Baumgarner, *Anal. Chem.*, 44, 1476, (1972).
50. G.E.M. Aslin, *J. Geochem. Explor.*, 6, 321, (1976).
51. D.E. Fleming and G.A. Taylor, *Analyst*, 103, 101, (1978).
52. B.W. Haynes, *Ato. Abso. Newslett.*, 17, 49, (1978).
53. W. Reichel and B.G. Bleakley, *Anal. Chem.*, 46(1), 59, (1974).
54. M. Bedard and J.D. Kerbyson, *Canadian J. of Spect.*, 21 (3), 64, (1976).
55. J.F. Fiorino, J.W. Jones and S.G. Capar, *Anal. Chem.*, 48 (1), 120, (1976).
56. S. Nakashima, *Analyst*, 103, 1031, (1978).
57. D.F. Boltz and R.S. Danchik, *Anal. Lett.*, 1, 901, (1968).
58. G. Devoto, *Boll. Soc. Ital. Biol. Sper.*, 44, 425, (1968).
59. G.F. Kirkbright, A.M. Smith and T.S. West, *Analyst*, 92, 411, (1967).
60. Y. Yamamoto, T. Kumamarn, Y. Hayashi, M. Kanke and A. Matsui, *Talanta*, 19, 1633, (1972).
61. H. Goto and Y. Kakita, *Japan Analyst*, 3, 299, (1954).
62. G.S. Deshmukh and K.M. Sankaranarayanan, *Chem. Abst.*, 48, 63 20C, (1954).
63. R.A. Nadkarni and B.C. Haldar, *J. Indian Chem. Soc.*, 41 (12), 813, (1964).
64. C.A. Starace, ^{IST-}*Chem* ~~Analyst~~, 55 (3), 74, (1966).
65. G.J. Misra and J.P. Tandon, *Indian J. Chem.*, 5 (11), 560, (1967).
66. J.F. Norris and H. Fay, *Am. Chem. J.*, 20, 278, (1898).
67. J.S. McNulty, E.J. Center and R.M. MacIntosh, *Anal. Chem.*, 23, 123, (1951).
68. S. Barabas and W.C. Cooper, *Anal. Chem.*, 28 (1), 129, (1956).
69. L. Erdey, O. Gimesi and G. Rady, *Talanta*, 11(3), 461, (1964).
70. M.J. Dastoor and B.C. Haldar, *Indian J. Chem.*, 5, 335, (1967).
71. S. Barabas and P.W. Bennett, *Anal. Chem.*, 35 (2), 135, (1963).

72. A. Kishioka, J. Chem. Soc. Japan, Pure Chem. Sect., 85 (10), 647, (1964).
73. J.J. Lingane and L.W. Niedrach, J. Am. Chem. Soc., 70, 4115, (1948).
74. P.J. Nangniot, Electroanal. Chem., 12, 187, (1966).
75. C. Le Peintre, Compt. Rend., 252, 1968, (1961).
76. B. Ya. Kaplan and I.A. Sorokovskaya, Zavod. Lab., 30, 783, (1964).
77. K.L. Cheng, Anal. Chem., 28, 1738, (1956).
78. J.H. Watkinson, Anal. Chem., 32, 981, (1960).
79. W.C. Broad and A.J. Barnard, Jr., Chemist-Analyst, 50, 124, (1961).
80. J.C. Lane, Irish J. Agr. Res., 5, 177, (1966).
81. L.E. Coles, J.A.P.A., 12, 68, (1974).
82. F.L. Chan, Talanta, 11, 1019, (1964).
83. G.F. Kirkbright and W.K. Ng, Anal. Chim. Acta , 35, 116, (1966).
84. W.C. Cooper, "Standard Methods of Chemical Analysis", 6th Ed., Van Nostrand, 1, 925, (1962).
LONDON
85. A.I. Busev, Talanta, 11, 485, (1964).
86. J.D. Kerbyson and B.J. Givens, Noranda Research Centre, Unpublished work.
87. F.L. Chan, "Advances in X-ray Analysis", New York, Plenum Press, 7, 542, (1964).
88. H.J. Bowen and P.A. Cawes, Analyst, 88, 721, (1963).
89. F.J. Conrad and B.T. Kenna, Anal. Chem., 39, 1001, (1967).
90. D.F.C. Morris and R.A. Killick, Talanta, 10 (3), 279, (1963).
91. C.S. Rann and A.W. Hambly, Anal. Chim. Acta , 32, 346, (1965).
92. Y.Y. Yamamoto, Y. Kumamaru, Y. Hayashi and M. Kanke, Anal. Lett., 5, 717, (1972).
93. K.C. Thompson and D.R. Thomerson, Analyst, 99, 595, (1974).
94. A.E. Smith, Analyst, 100, 300, (1975).
95. F.D. Pierce, T.C. Lamoreaux, H.R. Brown and R.S. Fraser, Applied Spectroscopy, 30 (1), 38, (1976).
96. R.B. Baird, S. Pourian and S.M. Gabrielian, Anal. Chem., 44 (11), 1887, (1972).

97. M. Ithnat, *Anal. Chim. Acta*, 82, 293, (1976).
98. G.F. Kirkbright, H.C. Shan and R.D. Snook, in press.
99. P.D. Goulden and P. Brooksbank, *Anal. Chem.*, 46, 1431, (1974).
100. J. Neve and M. Hanocq, *Anal. Chim. Acta*, 93, 85, (1977).
101. J.D. Mullen, *Talanta*, 23, 846, (1976).
102. M. Ithnat and H.J. Miller, *J. of AOAC*, 60 (6), 1414, (1977).
103. R. Hermann, *At. Abso. Newslett.*, 16 (2), 44, (1977).
104. O.E. Clinton, *Analyst*, 102, 187, (1977).
105. H.K.Y. Lau and P.F. Lott, *Talanta*, 18, 303, (1971).
106. R.W. Wood, *Phil. Mag.*, 10, 513, (1905).
107. A.C.G. Mitchell and M.W. Zemansky, "Resonance Radiation and Excited Atoms", University Press, Cambridge, (1961).
108. E.L. Nichols and H.L. Howes, *Phys. Rev.*, 22, 425, (1923).
109. R. Mannkopff, *Verhandl. D. Deutsch Phys. Ges.*, 14, 16, (1933).
110. J.D. Winefordner and T.J. Vickers, *Anal. Chem.*, 36, 161, (1964).
111. J.D. Winefordner and R.A. Staab, *Anal. Chem.*, 36, 165, (1964).
112. J.D. Winefordner and R.A. Staab, *Anal. Chem.*, 36, 1367, (1964).
113. J.D. Winefordner, J.M. Mansfield and C. Veillon, *Anal. Chem.*, 37, 1049, (1965).
114. R.M. Dagnall, T.S. West and P. Young, *Talanta*, 13, 803, (1966).
115. K.C. Thompson, *Analyst*, 100, 307, (1975).
116. N. Omenetto and J.D. Winefordner, *Applied Spectroscopy*, 26, 555, (1972).
117. G.F. Kirkbright and M. Sargent, "Atomic Absorption and Fluorescence Spectroscopy", Academic Press, (1974).
118. H.P. Hooymayers, *Spectrochim. Acta*, 22B, 567, (1968).
119. P.J. Zeegers and J.D. Winefordner, *Spectrochim. Acta*, 26B, 161, (1971).
120. C.T.J. Alkemade, "Atomic Absorption Spectroscopy", Ed. by R.M. Dagnall and G.F. Kirkbright, Butterworths, London (1970).
121. J.D. Winefordner, V. Svoloda and L.J. Cline, *CRC Critical Reviews in Analytical Chemistry*, 1, 233, (1970).

122. J.V. Sullivan and A. Walsh, *Spectrochim. Acta* , 21, 721, (1965).
123. P.L. Larkins, *Spectrochim. Acta* , 26B, 477, (1971).
124. J.I. Dinnin and A.W. Helz, *Anal. Chem.*, 39, 1489, (1967).
125. J.I. Dinnin, *Anal. Chem.*, 39, 1491, (1967).
126. W.F. Meggers and K.G. Kessler, *J. Opt. Soc. Am.*, 40, 737, (1950).
127. W.E. Bell, A.L. Bloom and J. Lynch, *Rev. Sci. Inst.*, 32, 688, (1965).
128. D.H. Burling, V.Czajkowski and L. Krause, *J. Opt. Soc. Am.*, 53, 1269, (1965).
129. P.G. Wilkinson and E.T. Byram, *Applied Optics*, 4, 581, (1965).
130. R.M. Dagnall, K.C. Thompson and T.S. West, *Talanta*, 14, 551, (1967).
131. K.E. Zacha, M.P. Bratzel, Jr., J.D. Winefordner and J.M. Mansfield, Jr., *Anal. Chem.*, 40 (11), 1733, (1968).
132. T.C. Rains, W. Snelleman and O. Menis, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 4, (1968).
133. D.F. Bartley, Ph. D. Thesis, London University, (1975).
134. L. M. Fraser and J.D. Winefordner, *Anal. Chem.*, 44, 1444, (1972).
135. R.M. Dagnall, G.F. Kirkbright, T.S. West and R. Wood, *Anal. Chem.*, 42, 1029, (1970).
136. R.M. Dagnall, G.F. Kirkbright, T.S. West and R. Wood, *Anal. Chim. Acta* , 47, 407, (1969).
137. N.Omenetto, N.N. Hatch, L.M. Fraser and J.D. Winefordner, *Anal. Chem.*, 45, 195, (1973).
138. D.S. Gough, P. Hannaford and A. Walsh, *Spectrochim. Acta*, 28B, 197, (1973).
139. D.C. Manning, *At. Abso. Newslett.*, 10 (6), 123, (1971).
140. B.J. Russell and A. Walsh, *Spectrochim. Acta* , 15, 883, (1959).
141. R.P. Mounce, R.M. Dagnall, B.L. Sharp and T.S. West, *Proc. Soc. Anal. Chem.*, 10, 271, (1973).
142. L. Dunkelman, W.G. Fowler and J. Hennes, *Applied Optics*, 1, 695, (1962).
143. D.J. Rose and S.C. Brown, *Phys. Rev.*, 98, 310, (1955).
144. J.M. Mansfield, M. P. Bratzel, H.O. Norgordon, D.O. Knapp, K.E. Zacha and J.D. Winefordner, *Spectrochim. Acta* , 23B, 389, (1968).

145. M.D. Silverster and W.J. McCartly, *Spectrochim. Acta* , 25B, 229, (1970).
146. K.M. Aldous, D. Alger, R.M. Dagnall and T.S. West, *Lab. Prac.*, 19, 587, (1966).
147. F.C. Fehsenfeld, K.M. Evanson and H.P. Broida, *Rev. Sci. Instr.*, 36, 294, (1965).
148. K.C. Thompson, Ph. D. Thesis, University of London, (1968).
149. R.M. Dagnall, K.C. Thompson and T.S. West, *Analyst*, 92, 506, (1967).
150. R. Smith, C.M. Stafford and J.D. Winefordner, *Anal. Chem.*, 41, 946, (1969).
151. R.M. Dagnall, K.C. Thompson and T.S. West, *Talanta*, 14 (1), 557, (1967).
152. M.S. Cresser and T.S. West, *Spect. Lett.*, 2 (1), 9, (1969).
153. K.C. Thompson and P.C. Wildy, *Analyst*, 95, 776, (1970).
154. R.F. Browner and D.C. Manning, *Anal. Chem*, 44, 843, (1972).
155. M.S. Cresser and T.S. West, *Anal. Chim. Acta* , 51, 530, (1970).
156. G.B. Marshall and T.S. West, *Anal. Chim. Acta* , 51, 179, (1970).
157. E.A. Sullivan, "Sodium Borohydride: Handling, Uses, Properties, Analytical Procedures", Booklet No. 01915, Vetron Corporation, Benerley, Mass., U.S.A.
158. J.M. Saleh, *J. Chem. Soc.*, 68, 1520, (1972).
159. K.C. Thompson, Personal Communication, Through Reference No. 94.
160. L.R. Pitwell, *Applied Spectroscopy*, 12, 54, (1958).
161. Y.S. Kim and H. Zeitlin, *Anal. Chim. Acta* , 46, 1, (1969).
162. Y.S. Kim and H. Zeitlin, *Anal. Chim. Acta* , 51, 516, (1970).
163. T. Moeller and H.E. Kremers, *J. Phys. Chem.*, 48, 395, (1944).
164. G.F. Kirkbright and M. Taddia, *Ato. Abso. Newslett.*, In press.
165. H.W. Lakin and D.F. Davidson, *Symposium-Selenium in Biomedicine*, O.H. Muth, Ed., Avi Publishing Co., Inc., Westport, Conn., 27, (1967).
166. D.J. Swaine, *Commonw. Bur. Soil Sci. Tech. Comm.* 48 Commonwealth Agricultural Bureau, (1955).
167. A.P. Vinogradov, "Geochemistry of Rare and Dispersed Elements in Soils", Chapman and Hall, London (1959).
168. F.E. Bear, "Chemistry of the Soil", 2nd Ed., Chapman and Hall, London, (1964).

169. W.O. Robinson, *Ind. Eng. Chem. A.E.*, 6, 274, (1934).
170. A.K. Klein, *J. Ass. Off Agric. Chem.*, 24, 363, (1941).
171. M. Levesque and E.D. Vendette, *Can. J. Soil Sci.*, 51 (1) 85, (1971).
172. W.R. T. Hemsted, M. Sina and S. Cekicer, *Analyst*, 97, 383, (1972).
173. O.J. Kronborg and E. Steinnes, *Analyst*, 100, 835, (1975).
174. E. Steinnes, *Acta. Agric. Scand.*, 27 (2), 110, (1977).
175. B.C. Severne and R.R. Brooks, *Talanta*, 19 (2), 1467, (1972).
176. *Official Methods of Analysis*, 12th Ed., AOAC, Washington D.C. (1975).
177. L.M. Cummins, J.L. Martin and D.D. Maag, *Anal. Chem.*, 37, 430, (1965).
178. W.H. Allaway and E.E. Cary, *Anal. Chem.*, 36, 1359, (1964).
179. R.C. Dickson and R.H. Tomlinson, *Int. J. Appl. Radiat. Isotopes*, 18, 153, (1967).
180. K. Heydorn and E. Damsgaard, *Talanta*, 20 (1), 1, (1973).
181. L. Kotz, G. Kaiser, P. Tschoepel and G. Toelg, *Fresenius' Z. Anal. Chem.*, 260 (3), 207, (1972).
182. F.L. Fricke, *Talanta*, 23, 317, (1976). of
183. G. T. C. Shum, H.C. Freeman and J.F. Uthe, *J. AOAC*, 60 (5), 1010, (1977).
184. P.N. Vijan and G.R. Wood, *Talanta*, 23, 89, (1976).
185. T.T. Gorsuch, *Analyst*, 84, 135, (1959).
186. H.J. Bowen, *J. Radioanalytical Chemistry*, 19, 215, (1974).
187. A. Fulton, K.C. Thompson and T.S. West, *Anal. Chim. Acta*, 51, 373, (1970).
188. T.J. Vickers, P.J. Slevin, V.I. Muscat and L.T. Farias, *Anal. Chem.*, 44, 930, (1972).
189. K. Tsujii and K. Kuga, *Anal. Chim. Acta*, 72, 85, (1974).
190. K. Tsujii and K. Kuga, *Anal. Chim. Acta*, 97, 51, (1978).
191. T. Nakahara, T. Tanaka and S. Musha, *Bull. Chem. Soc. Jpn.*, 51, 2046, (1978).
192. T. Nakahara, S. Kobayashi and S. Musha, *Anal. Chim. Acta*, 104, 173, (1979).

193. R.D. Ediger, Ato. Abso. Newslett., 14 (5), 127, (1975).
194. H.Y. Almond, Anal. Chem., 25 (11), 1766, (1953).
195. E.A. Woolson, J.H. Axley and P.C. Kearney, Soil Sci., 111 (3), 158, (1971).
196. O. Shigeru, Y. Michihiko, Radioisotop^Es, 19 (12), 565, (1970).
197. E.G. Gastinger, Mikrochim. Acta , 4, 526, (1972).
198. R.C. Leake and D. Peachey, Ins. Mining Met. Trans., Sect. B, 82, 25, (1973).
199. T. Sakamoto, H. Kawaguchi and A. Mizuike, Bunseki Kagaku, 25 (2), 81, (1976).
200. N.A. Hamme, A.L. Young and J.H. Hunter, U.S. Nat. Tech. Information Serv. Ad Rep. No. 728628, 21, (1970).
201. J.R. Melton, W.L. Hoover, J.L. Ayers and A. Peggy, Soil Sci. Soc. Amer. Proc., 37 (4), 558, (1973).
202. N. Yasushi, H. Nagai, D. Kubota and S. Himeno, Bunseki Kagaku, 22 (12), 1543, (1973).
203. W.A. Haller, L.A. Rancitelli and J.A. Cooper, J. Agr. Food Chem., 16 (6), 1036, (1968).
204. J.J. Kroon and H.A. Das, RCN, Rep. RCN-124, 8, (1970).
205. R.A. Nadkarni and G.H. Morrison, Anal. Chem., 45 (2), 1957, (1973).
206. E. Steinnes , Anal. Chim. Acta , 78, 307, (1975).
207. R. Hosogaki, T. Terashima and H. Shimura, Eisei Kagaku, 15 (6), 368, (1969).
208. T. Kamada, H. Okuda, T. Kumamaru and Y. Yamamoto, Eisei Kagaku, 19 (6), 314, (1973).
209. P. Eichelberger, M. Kuehnert and U. Pfueller, Arch. Exp. Veterinaarmed, 27 (4), 675, (1973).
210. M. Ishizaki, Bunseki Kagaku, 26 (10), 667, (1977).
211. M. Ihnat and H.J. Miller, J. of ~~the~~ AOAC, 60 (4), 813, (1977).