ANALYSIS OF TRACE METALS, TOTAL LIPIDS, AND CHOLESTEROL LEVELS IN BLOOD OF AJAOKUTA STEEL WORKERS

BY

MAKUN, HUSSAINI ANTHONY

B.Sc BIOCHEMISTRY A. B. U. ZARIA.

Thesis submitted to the Postgraduate School, Ahmadu Bello University, Zaria in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY

FACULTY OF SCIENCE

AHMADU BELLO UNIVERSITY

ZARIA

NIGERIA,

1991.

DECLARATION

I, hereby declare that the content of this thesis is purely a record of my own research work. It has not been accepted in any previous application for higher degree. All the sources of information are specifically acknowledged by means of reference.

H. A. Makun (Author)	Date
Prof. G. H. Ogbadu, Chairman, Supervisory Committee and Head of Department.	21/11/91 Date
Dr. J. A. Elegbede, Member, Supervisory Committee.	21/11/91 Date
Dean, Post-graduate School, A. B. U., Zaria.	11/3/92 Date

DEDICATION

This thesis is dedicated to the Ajaokuta Steel Workers and all other Nigerian workers that pay for the technological development of our country with their health.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to my supervisors, Prof. G.H. Ogbadu and Dr. J.A. Elegbede for the useful assistance and guidance which they offered throughout this work.

I am exceedingly grateful to my family for giving me the chance to study. Special gratitude is extended to Mama, Baba, Hassana, Hassan, Baba and Anti Meli who have given to me, their last coins during the period of this research. Bobso, who all regard as a member of our family cannot go unacknowledged for his interest and financial assistance at most stages of the work.

My love and thanks goes to Evelyn for the emotional care she always gave me. I am particularly impressed by my classmates who made the school conducive for learning. I am grateful to Stephen, Ricardo, Sparrow and Yakubu for the warm reception I received from them throughout the period of the course.

Finally, I thank God for allowing me to live and accomplish this research. I pray he gives others the strength to conduct meaningful researches that will help develop this country of ours.

ABSTRACT

Serum concentrations of total lipids, cholesterol, copper, Iron, magnesltim and zinc were determined In eighty-two AJaokuta Steel workers. Blood concentrations of antimony, arsenic, bismuth, cadmium, cobalt, lead, manganese and nickel were also determined in the steel workers. Similar determinations were made for twenty students of Ahmadu Bello University, Zaria, that served as the controls.

The mean serum total lipids in the controls (598.75 mg/l00ml) was not significantly (P/0.05) different from the value obtained for the steel workers (568.25 mg/100ml). Similarly, the serum cholesterol levels in controls (206.42 mg/100ml) did not differ significantly (P/0.05) from the levels in the steel workers (183.82 mg/l00ml).

There were no significant (P/0.05) differences between the serum copper concentrations In controls (1.26 ppm) and the levels in the steel workers (l.14. ppm). Also, there were no significant (P/0.05) differences observed between the serum magnesium content In controls (26.17 ppm) and the concentrations in steel workers (26.I4.3 ppm).

At 5% level of significance serum iron levels in controls (4. 28 ppm) did not differ significantly from iron levels in steel workers (3.62 ppm). However, serum zinc levels in stool workers (1.85 ppm) were significantly (P/O.05) higher than the levels in the controls (0.83 ppm).

Arsenic, antnmony, bismuth, cadmium, cobalt, lead, manganese and nickel were not detected in the blood of both the steel workers and their controls.

Except for the blood zinc concentrations which were significantly elevated in steel-workers as compared to levels in controls, oil other studied parameters were within normal range. The duration, of year of service in the industry did not have any significant (P/0.05) effect on the concentrations of the parameters studied. Similarly sex had no effect on the studied parameters except for serum total lipids which was. significantly (P/O.O5) higher in the female controls than in the male controls.

LIST OF FIGURES

FIGURE		PAGE
1	The pathway of haem synthesis with the stages inhibited by lead ...	32

LIST OF TABLES

TABLE		PAGE
1	Types of industrial wastes from iron and steel processing	4
2	Dose-response effect of lead absorption	36
3	Effect of sex on serum total lipids, cholesterol copper, iron, magnesium and zinc	52
4	Serum total lipids, cholesterol, copper, iron, magnesium and zinc concentration in controls and steel workers	53
5	Relationship between age, serum total lipids, cholesterol and trace metals levels	54

TABLE OF CONTENTS

TABLE		PAGE
Declaration	... ...	i
Dedication	... ...	ii
Acknowledgement	... ...	iii
Abstract	... ...	iv
List of Figures	... ...	vi
List of Tables	... ...	vii
CHAPTER ONE - INTRODUCTION		
1.1 General Introduction	... ...	1
CHAPTER TWO - LITERATURE REVIEW		
2.1 Total lipids	... ...	7
2.2 Cholesterol	... ...	9
2.3 Antimony	... ...	12
2.4 Arsenic	... ...	13
2.5 Bismuth	... ...	16
2.6 Cadmium	... ...	17
2.7 Cobalt	... ...	21
2.8 Copper	... ...	23
2.9 Iron	... ...	26
2.10 Lead	... ...	29
2.11 Magnesium	... ...	36
2.12 Manganese	... ...	38
2.13 Nickel	... ...	40
2.14 Zinc	... ...	42

TABLE OF CONTENTS CONT'D.

TABLE		PAGE
CHAPTER THREE - MATERIALS AND METHODS		
3.1	Chemicals	45
3.2	Blood samples	45
3.3	Determination of total lipids ...	46
3.4	Determination of Cholesterol ...	47
3.5	Determination of trace Metals ...	48
CHAPTER FOUR - RESULTS AND DISCUSSION		
4.1	Total lipids	51
4.2	Cholesterol	56
4.3	Trace Metals	58
Conclusions	... ...	63
References	... ...	65
Appendix	... ...	78

1.0 INTRODUCTION

Iron and steel manufacture is the means by which the ore is converted to metallic iron with pre-determined physical and chemical properties (Pounds, 1963). It produces the chief raw materials for a wide range of metal manufacturing industries, whose products include automobiles, ships, rail road equipment, household appliances, machinery for farming, construction materials and other industries.

Steel industry occupies a key position in modern industrial society and is now looked upon as a general indicator of a nation's business and technological activities (Pounds, 1963). For instance, goods derived from the finished products of iron and steel industry accounts for 75% of the annual income of the United States of America with over hundreds of billion of dollars capital income from its products (Warren, 1973). Other developed countries like Britain, U.S.S.R., France, Germany, and Sweden also depend on their iron and steel industry for technological and economic development. In view of its importance in a nation's development, the Nigerian government built an iron and steel industry of Ajaokuta, Kwara state in the early 80s.

Despite its vital role in the country's development, iron and steel manufacturing presents

one of the most diversified sets of occupational health problems, this observation was made at a conference of the American iron and steel institute, Washington D.C., in 1965. More than in most industries, the risks in iron and steel making are inherent in the process. The types of toxic industrial waste these processes that cause occupational hazard to workers are shown in table 1.

Trace metals are major components of industrial waste from iron and steel processing. Excess of these metals in the human body results in various diseases. For instance, Macdonald and Mclinnes, (1981) associated lead and cadmium with hypertension. Respiratory and gastro-intestinal disorders in steel workers have been associated with exposure to arsenic (Axelson et al., 1986), antimony (Schrumpf, 1972), cobalt (Mcdermott et al., 1966), copper (Aaseth and Norseth, 1986), magnesium (Browning, 1969) and zinc (Anseline, 1972). Industrial exposure to arsenic (Leonard and Lauwery, 1980), antimony (Browning 1969) and nickel (Fisher, 1956) results into skin lesion in steel workers.

Other diseases associated with metals are: neuropathy caused by excess of lead (Waldron, 1974; Glickman et al., 1984) or manganese (Rosenstock et al., 1971), anaemia due to lead poisoning (Kipling,

1985). and gonadal hypofunction which has been associated with cadmium poisoning (Dwivedi, 1983).

Apart from causing systemic disorder, divalent metals can compete with each other for ligands resulting in decrease in the blood and tissue levels of some essential elements (Prasad, 1978). This workers also demonstrated that there is decrease in blood copper, iron, manganese and zinc levels in cadmium exposed persons. Lead reduces the uptake of calcium, iron, copper, zinc and phosphorus (Behari, 1981). Divalent metals such as copper, cobalt, manganese and zinc can also bind to transferrin resulting in reduced absorption of iron (Prasad et al., 1974). Thus, it is possible that iron and steel making and its associated exposure to lead, cadmium and other divalent metals can result in deficiencies of some essential metals in steel workers.

High serum concentration of copper (Harman, 1968; Olatunbosun et al., 1986), manganese (Diozy, 1974) and zinc (Prasad et al., 1974; Osunkiyesi et al., 1986) have been associated with marked elevation of serum cholesterol. Elevated blood cholesterol has been associated with atherosclerosis and other cardiovascular diseases (Mattingly et al., 1956). It is therefore, possible that exposure to these metals

WASTES	SOURCES	COMPONENTS
Slags and Drosses	Smelting and Refining	Lead, Zinc, Copper, Manganese, Nickel, Chromium, Titanium and Aluminium.
Waste Solution	Electroplating Pickling and Etching	Cadmium, Nickel, Copper, Chromium, Cobalt, Gold, Silver Mercury and Molybdenum.
Metallurgical Dust	Smelting and Fabrication	Iron, Zinc, Lead, Copper, Arsenic, Antimony, Magnesium Bismuth, Cobalt, Nickel, Chromium.
Sludges and grinding Swarfs	Machining and Manufacturing	Chromium, Nickel, Cobalt, Iron, Manganese, Zircon, Tungsten and Diamond
Slimes and Tailings	Leaching and Ore treatment	Alumium, Iron, Copper, Phosphate, Uranium and precious Metals.
Industrial Gases	Pyrometallurgical Operation	Sulfur, Fluarine and Carbon Monoxide.

SOURCE:- Phoenix Quarterly fall, 1975, Institute of Scrap Iron and Steel.

in iron and steel industry might lead to atherosclerosis and other cardiovascular diseases.

Since cholesterol is a component of serum total lipids and it is elevated in excess presence of copper, zinc and manganese in the blood, it then implies that these metals can also affect blood total lipids levels in exposed individuals. In addition high serum copper content have been shown to result in hypolipidaemia (Harman, 1968; Osunkiyesi et al., 1986).

For the above mentioned reasons this project was undertaken to determine:

a. The blood levels of antimony, arsenic, bismuth, cadmium, cobalt, copper, iron, lead, magnesium, manganese, nickel and zinc in Ajaokuta steel workers and to compare them with levels in non-steel workers.

b. Serum total lipids and cholesterol in steel workers and to compare them with concentrations in non-steel workers.

c. The effect of length of service in steel industry on the levels of trace metals, total lipids and cholesterol in the blood of the workers.

From the results, we should be able to predict the occupational risk factors involved in iron and steel industry.

2.0 LITERATURE REVIEW

2.1 TOTAL LIPIDS:

The term total lipids have been defined as water insoluble biomolecules that can be extracted from cells and tissues by non-polar solvents e.g chloroform, ether and benzene, and it consistitute the generic name for all the classes of lipids in the human body which include fatty acids, triglycerides, steriods, phospholipids, shingolipids and cerebrosides (Stanley et al., 1975).

Various investigations have been carried out on the factors that affect the total lipids concentration within the human body. Sex, for instance affect plasma total lipids levels (Taylor, 1971). She reported a higher serum total lipids in Nigerian adult females as compared to their male counterparts. Lopez et al. (1967), however reported that females in developed countries have lower serum lipids levels than their male counterparts.

Hyperlipidaemia is highly hereditary in some families. Metabolic defects of genetic origin in nine sphingolipid storage diseases are known to cause accumulation of lipids in the blood and other tissues (Brady et al., 1971). Also endogenous hypertriglyceremia

is a dominate genetic disorder in which pre-B-lipoprotein (triglyceride carrier) and serum triglycerides are elevated (Stanley et al., 1975).

Experiments on young animals show that adrenaline, noradrenaline and growth hormones rise blood lipid levels while insulin promotes its storage and subsequent reduction in the blood. (Stanley et al., 1975). Therefore, adrenaline-increase during stress and insulin-lack in diabetic mellitus results in h hyperlipidaemia (Guyton, 1976).

The concentration of blood total lipid levels is very sensitive to the nutritional status of a person (Mann et al., 1975). They postulated that during starvation or in low energy intake, lipids stored in adipose tissues are mobilized into the circulatory system for energy production. This is believed to rise the level of lipids in the blood with a concomittant decrease in other tissues.

High fat diet which are more expensive than high carbohydrate diet in most countries, elevates serum total lipids levels as compared to the latter (Mann et al., 1975). This is believed to be one of the reasons why low income earners have lower serum lipid content as compared to the high income group. Using the same

reason, Mann et al. (1975) and Onitiri, (1977) explained why that lower serum lipid levels appears to be characteristic of many Nigerian communities as compared to their European counterparts.

Although most studies in Europeans and North Americans have demonstrated an age-related trend on the amount of serum lipids (Shaper and Jones, 1959; Antonis and Bersohn, 1960), Onitiri (1977) observed that serum lipid concentration did not alter with age in underprivileged Africans. He attributed this to differences in diet and income.

The interrelationship between metals and blood total lipid levels have been investigated. For instance, elevated serum copper has been known to cause hypolipidaemia (Osunkiyesi et al., 1986). This is because copper serves as a good catalyst for lipid peroxidation (Harman, 1968).

2.2 CHOLESTEROL:

Cholesterol is the parent 27-carbon atom steriod from which all other steriods in mammalian tissues are synthesized (Datta and Ottaway, 1979). It has been observed as the major constituent of the atheromatous plague and the pathological hall

mark of atherosclerosis (Mattingly et al., 1956). High serum cholesterol concentration have also been indicated as an aetiological factor in the incidence of myocardial infarction and hypertension (Taylor and Bamgboye, 1979).

Various factors have been highlighted to predispose individuals to high serum cholesterol levels. Guyton, (1975), for instance, has reported that saturated fat diet increase blood cholesterol content as much as 15 to 25 percent. This presumably results from increased fat deposition in the liver which then provides increased quantities of acetyl COA in the liver cells for production of cholesterol. In another independent epidemiological studies of sample population in Nigeria, Taylor and Bamgboye, (1979) demonstrated that a positive association exists between high fat diets and high serum cholesterol concentration. Castle, (1982) also noted that high level of income tend to increase blood cholesterol level in earners; this presumably results from the intake of the more expensive high fat diet.

Hypercholesterolaemia can be hereditary (Seftel et al., 1980). Thirty-four patients with homozygous familiar hypercholesterolaemia from twenty-seven families were shown to have high levels of cholesterol in their blood.

Plasma cholesterol tend to be higher in men than in women (Guyton, 1975; Datta and Ottaway, 1979). The female sex hormone, estrogen decreases blood cholesterol concentration in women (Guyton, 1979). He reported that administration of estrogen to men and chickens with cholesterol deposits in their coronaries has in some instances actually cleared the plagues. On the contrary, Taylor and Bamgboye, 1979 reported a higher cholesterol level in adult Nigerian females as compared to their male counterparts. This probably results from residual cholesterol deposit of pregnancies.

Blood cholesterol level tends to rise with age (Guyton, 1975; Stanley et al., 1975; Taylor and Bamgboye, 1979).

In diabetes mellitus, blood cholesterol is also rised (Stanley et al., 1975). This is believed to result from the general increase in lipid mobilization in this condition (Guyton, 1975). Serum cholesterol, also increases in renal retention diseases (Stanley, 1975).

High serum levels of copper (Harman 1968; Olatunbosun et al., 1976), Manganese (Diosy, 1974) and zinc (Prasad and Obserleas 1974) have been shown to elevate cholesterol levels in the blood. These

metals serve as catalysts in chlesterol synthesis (Harman, 1968).

2.3 ANTIMONY:

Metallic antimony is a lustrous silver-metal with a bluish tinge, and either a coarsely laminated or granular structure according to whether it is slowly or rapidly cooled (Beattie et al., 1972). Its alloys are used in metallurgical process, rubber, textile, paint, ceramic and glass industries. The oxides and sulphides forms of antimony are among the compounds used in chemical industries and have therapeutic purposes (Browning, 1969).

The commonest industrial exposure to antimony is during the minning, smelting and refining of antimony ore which is used in iron and steel industry for alloy production. Browning, (1969) have indicated that exposure to antimony alloys in this industry may result in to various forms of systemic, cardiac, respiratory and skin disorder.

Systemic toxic effects of antimony, for instance have been shown to result in to abdominal cramps, diarrhoea, vomitting, dizziness and nerve tenderness. Other symptoms include tingling, severe headache and

prostration (Beattie et al., 1972). Browning, (1969) also reported that upper respiratory irritation such as laryngitus, tracheities and pneumonitis ~~ex~~ extending from each hilus are common in antimony smelters.

Brieger et al. (1954) reporrted the presence of functional disorders of the heart and parenchymatous degeneration of the myocardium in workers of a plant that use antimony trisulphide. They also confirmed the mentioned cardiac disorders in animals exposed to 3mg per $1cm^3$ of antimony. in the atmosphere.

Eczema, dermatitis, irritation of the mucous membranes and severe skin lesions are common in workers of rubber and textile industries where antimony solution were used (Browning, 1969).

Brieger et al. (1954) found the mean blood antimony value of fifty antimony smelters to be 0.267 ppm. While the blood antimony level in healthy persons is between 0.0086 - 0.0091 ppm. (Browning, 1969).

2.4 ARSENIC:

Arsenic, in its stable form, is a steel gray, brittle and crystalline metalloid (Browning, 1969).

Although arsenic is not an essential element for human metabolism, it is constantly taken into the human body from contaminated food and water, and from metallurgical dust (Aaseth and Norseth, 1986).

Acute poisoning from inorganic arsenic has rarely occurred in industries but chronic occupational poisoning has been reported (Clay et al., 1977). These workers, however, reported only three fatal cases of arsine poisoning in Birmingham steel bronze factory. Acute poisoning from arsenic are frequent in drugs and drinking water (Browning 1969).

Parish et al. (1979) reported acute poisoning in two workers cleaning a clogged drain. The symptoms were nausea, vomitting, headache, bronze decolouration of skin, hemolysis and renal failure. The blood arsenic levels of the patients were 0.18 ppm and 0.20 ppm respectively. Parish et al. (1979) advised that blood arsenic concentration in arsenic exposed workers should be kept below 0.096 ppm.

The mortality experience of all pensioners from a copper smelter that were exposed to arsenic during their working life was studied by Pinto et al. (1978). They found that the overall mortality of the cohorts was 12.2% higher than for males living in the same area of the same ages and in the same period.

This excess mortality was due chiefly to respiratory cancer.

Axelson et al, (1978) demonstrated a high correlation between cardiovascular diseases mortality and an increasing trend of arsenic exposure. Other causes of death in arsenic exposed workers are lesions of the central nervous system, digestive and urinary diseases (Welch et al., 1982).

Chronic arsenic poisoning have been shown to increase the susceptibility of the skin to ultraviolent light and is associated with an increased incidence of rodent ulcers and other skin tumours (Renwich et al., 1981). There are indication that the mechanism for increase susceptible to UV light involves inhibition of the DNA-excision-repair enzymes (Leonard and Lauwerys, 1980). However, whether the enzymes involved is concerned with the excision of thymidine dimers or with their replacement or in the linking operations (Ligasestep) has not been clearly established. (Leonard and Lauwerys, 1980).

Wagner et al, (1974) and Parish et al, (1970) had independently determined the blood arsenic level in thirty non-arsenic exposed person and found it to be between 0.01 - 0.03 ppm.

2.5 BISMUTH:

Bismuth in the free state is a hard, pinkish-silver metal which usually occur as bismuth glance (Bi_2S_2), tetradymite ($Bi_2Te_2S_2$), bismuth Ochre (Bi_2O_3) and bismutite a natural basic carbonate (Browning, 1969). The purified form of the metal is used in preparation of a number of pharmaceutical products like bismuthyl tartrate and bismuth carboxylate. It is a constituent of steel alloys which is used to increase machinability in the metallurgical industry (Browning, 1969).

Orally ingested bismuth salts are used therapeutically to coat the intestinalmucosa in ulcers. However bismuth oxynitrate can be reduced to nitrite in the gastrointestinal tract by intestinal microflora and subsequent absorption of nitrite can cause methemoglobinemia in a child (Venugopal and Luckey, 1978). The same workers reported that a single intravenous injection of a bismuth salt has caused fatal bismuth intoxication in human infants. Bismuth salts for therapeutic purposes have been shown to cause nephrotoxicity and hepatitis in human subjects (Venugopal and Luckey, 1978).

Chronic bismuth intoxication produces alimentary symptoms, the mouth shows bismuth deposition in

epithelial cells, turning mucosa blue-grey (Arena, 1974). Other symptoms include, diarrhoea, vomitting, anorexia, digestive pains, skin lesions, peripherial neutitis, liver damage nephrotoxic action and blood dyscrasias.

Industrial poisoning from bismuth has not been recorded (Venugopal and Luckey, 1978), possibly because of the relative insolubility of its salts in water and tissue fluids prevents easy entry into the body.

The mean blood bismuth level in 1400 volunteers was found to be 0.0245 ppm with a range of 0.007 to 0.18 ppm (Rooney and Bowen, 1976).

2.6 CADMIUM:

Man absorbs cadmium together with food, air, water, cigarette smoke and ~~through~~ from the soil. Soluble cadmium is known to be more toxic than the particulate form in the dust (Houba and Remacle, 1982).

Nordberg, (1990) demonstrated that cadmium taken up from lungs and gastro-intestinal tract is transported via the blood to liver and kidney, in the form of metallothionein-bound cadmium. He also reported that cigarette smoking may increase tissue accumulation

since uptake of cadmium via the lungs is efficient (up to 50%) compared to uptake through gastro intestinal tract (about 5%).

Various industries in Sweden, USSR, West Germany, Japan, France and Belgium had cases of death due to cadmium poisoning in the 1930s and 1940s (Prasad, 1978). Subsequently it was realised that cadmium affect man adversely.

Cadmium may alter reproductive process particularly, the spermatozoa (Dwivedi, 1983). This worker reported that cadmium at dosages of 1mg/kg to 2mg/kg given orally inhibited choline acetyl transferase (CHAT) in all segments of spermatozoa obtained from rat. He also demonstrated that cadmium decreased spermatozoa motility when incubated with cadmium at a concentration of 5×10^{-4}M.

The most severe form of chronic cadmium poisoning is a painful bone disease known as Itai-Itai that occurred in Japan, in women above forty-five years (Berlin and Friberg, 1960). Exposure to cadmium was through the ingestion of rice and water containing about 500 ug/g and 600 ug/l of cadmium, respectively. Itai-itai disease is characterized by osteomalacia and received its name from the severe pain caused by

fractures of the softened bones. Nordberg, (1990) postulated that cadmium interferes with calcium absorption as well as promotes the loss of this mineral from the body and hence the softening of bones in Itai-Itai disease.

Cadmium can alter the metabolism and function of other elements, namely zinc, iron, manganese, copper and selenium (Fox, 1976). Prasad, (1978) subsequently demonstrated that the deficiencies of the mentioned elements results in marked increase in concentration of cadmium in the tissues and blood.

Hypertensive subjects have abnormally high concentration of renal cadmium (Lauwery et al, 1974). He also observed that elevated blood pressures in animals following chronic cadmium administration was common. The probable mechanism of cadmium-induced hypertension is its sodium retention ability which has been demonstrated in rats (Prasad, 1978). Lauwery et al, (1974) also reported that hypertension is responsible for 4% of death in sixty cadmium-exposed workers.

Cadmium-induced tubular proteinuria is due to a disease in reabsorption from the glomerular filter (Axelson et al., 1978). The low-molecular

weight proteinuria is regarded as the first sign of cadmium effect on renal function, subsequently renal dysfunction sets in and this is characterised by a decreased ability to concentrate urine and a reduction in Inulin. Axelson et al, (1968) and Lauwerys et al, (1974) reported renal lesions in rabbit after long term exposure to cadmium.

Acute poisoning due to cadmium, elicites gastro intestinal symptoms such as nausea, vomitting, diarrhoea and abdominal cramps (Beattie et al, 1972). These symptoms were observed in 13 pupils at a school in Stockholm, Sweden, that drank soft drinks containing 16 ppm of cadmium.

Kojima and Tamura, (1981) demonstrated that when cadmium was administered to mice within two days before elicitation for delay-type hypersensitivity (DTH), it resulted in suppression of the induction of primary delayed-type hypersentivity responses, memory T cell and suppressor T-cell activities.

The normal concentration of cadmium is very small, less than 0.01 ppm in blood of persons not exposed to high level of the metal (Fox, 1976). The Mean value obtained by Lauwery et al, (1974) was 0.007 ppm

in 22 persons. They also demonstrated that the mean cadmium level in industrial exposed workers is 0.092 ppm.

2.7 COBALT:

Cobalt is used in industry and in medicine. Industrially, it is used in various alloys to confer resistance to wear and corrision. It serves as a pigment or binder in the hard-metal industry; e.g steel and armament industry. Medically, cobalt forms four percent of vitamin B12 used for treating pernicious anaemia (Shinton and Singh, 1967). The radioactive isotope cobalt 60 is used in radiotherapy and gamma radiography.

The essentiality of cobalt to man and animals has been known for quite sometime now (Payne, 1977). Its deficiency is rare but excess intake produces gastro-intestinal upsets and abnormalities in skin, heart, blood or lungs (Payne, 1977).

Skin effects due to cobalt toxicity has been reported by Payne, (1977). It results in an allergic dermatitis producing the usual type of urticaria with erythematous papules. Minute traces are only necessary and the trades affected include building, printing, pottery, textile and steel manufacture.

Bonenfant et al, (1967) reported a severe epidemic of cardiomyopathy eruption with a fifty percent mortality rate in quebec. This was as a result of the presence of cobalt in high concentration in beer consumed by the natives. Similar outbreaks took place in Belgium and United States of America (McDermott et al, 1961). Adverse cardiac effect from cobalt have also been reported in hard-metal industries e.g. steel, engineering and armament manufactures (Payne, 1977).

Bech et al, (1962) reported ~~that~~ the occurrence of polycythaemia in some hard-metal workers. Payne, (1977) demonstrated that cobalts salts induce bone marrow hyperplasia with resultant polycythaemia. It is likely that cobalt inhibits the oxidative enzymes in the marrow and produces a tissue hypoxia thus stimulating erythropoiesis.

Experiments on rats and rabbits have produced both sarcomas and carcinomas as a result of the injection and chronic inhalation of cobalt particles (Morgan, 1983). However, the worker reported that evidences on the presence of pulmonary tumours in cobalt workers is not convincing.

Zolankiewics and Fashing, (1976) found the mean blood cobalt level in twenty normal persons to be 2.64×10^{-3} ppm. Meanwhile Andronikashvili and Mosulishvili, (1980) demonstrated that the safe limit of blood cobalt should not exceed 1 ppm in human subjects.

Bech and Barborik, (1977) found that the blood cobalt levels of hard metal workers with polythaemia ranged between 0.2 to 1.34 ppm.

2.8 COPPER:

Copper is an essential element and daily requirements according to W.H.O are 80 ug/kg body weight for infants and 30 ug/kg body weight for adults. Normal daily intakes are usually adequate between 1 and 3 mg (Aaseth and Norseth, 1986).

The most important function of copper is its presence as a component of a number of metallo-enzymes. These enzymes include cytochrome C oxidase, superoxide dimutase, ceruloplasmin and tyrosinase. Others include dopamine B-hydroxylase, lysyl oxidase and diamine oxidase. These enzymes play important role in iron metabolism and haemoglobin synthesis. The deficiency of copper, therefore results in anaemia, a fall in serum iron level and disorder of collagen and elastin metabolism (Shaw, 1980).

It is conventionally reported that more than ninety percent of total plasma copper is incorporated into ceruloplasmin while the remaining is loosely bound to albumin and amino acids (Solomons, 1979).

Diseases and other conditioning factors such as protein energy, malnutrition, malabsorption syndrome (Menke's disease), active ulcerative agents result in low serum copper levels (Solomons, 1979).

High serum copper on the other hand has been associated with high cholesterol levels in hypertensive patients (Olatunbosun et al, 1976, and Osunkiyesi et al., 1986). Harman, (1968) explained that copper serves as a good catalyst for the reaction of oxygen with lipids (Lipid peroxidation) and that lipid peroxidation tends to enhance atherogenesis. Using sixty male minipigs, the worker, demonstrated that lipid peroxidation contributes to atherogenesis.

Acute gastrointestinal disturbances with vomitting, epigastric burns and diarrhoea results from accidental ingestion of copper salts or foods containing copper in dose range of 25 to 75 mg (Asseth and Norseth, 1986).

In persons with Wilson's diseases, an inborn error of ceruloplasmin metabolism, serum copper

concentrations are extremely very low i.e 0.61 ± 0.21 ppm. Normal intake of copper gives rise to pathological accumulation in tissues such as brain, kidney and liver in these patients (Nordberg, 1990).

However, normal persons (without Wilson's disease) can tolerate considerable concentrations of copper (Aaseth and Norseth, 1986). The data of Scheinberg and Sternlieb, (1976) which supports this report showed that hepatic and serum concentration of copper miners despite years of exposure to an environment containing about one percent copper, are nomal.

Human copper toxicity has been reported inwomen using copper containing intrauterine contraceptive device (Scheinberg and Sternlieb, 1976); and some vineyard workers after years of spraying fungicidal copper sulphate (Prasad, 1978). Pulmonary copper deposition and fibrosis occurred in the lungs of the vineyard workers.

A considerable number of cases of childhood cirrhosis (Indian childhood cirrhosis, ICC) have been reported by Aaseth and Norseth, (1986). This disease has been observed in children of one to three years of age who displayed typical liver cirrhosis often with fatal outcome. High copper level have been found in the livers of these children and the use of brass

utensils for cooking is considered to be one important source of the excessive copper intake. Copper storage disease of the liver similar to ICC, has also been reported in Germany and Italy (Nordberg, 1990).

The mean value of serum copper for eighteen healthy men was found to be 1.065 ppm while that of ten women was 1.026 ppm (Parker et al., 1967). Dawson et al, (1968) showed the normal range of serum copper in one hundred clinically healthy persons to be 0.7 - 1.4 ppm. Subsequently, Osunkiyesi et al, (1986) gave a mean value of 1.04 ppm in twenty-nine normotensive persons.

2.9 IRON:

The industrial uses of iron and its compound are numerous and its ore forms the bulk of raw materials used in iron and steel industry.

It is an essential nutrient for mammals and it is involved in a number of physiological reactions which have been established by many workers (Lewis, 1971; Jacobs and Henry, 1962; Widdowson, 1974; Prasad, 1978). They demonstrated that iron deficiency is characterised by anemia, stunted growth, fatigue, lowered resistance to infection, anorexia and death.

Iron deficiency can lead to decreased rates of energy generation due to blocking of the synthesis of haemoglobin and other iron-containing enzymes of respiration. With low levels of iron, the heart must sustain a greater cardiac output to provide tissues with adequate oxygen (Saltman, 1983). This increased cardiac output, if sustained for long periods may encourage the development of systemic hypertension as indicated by Norman et al. (1975). Subsequently, Osunkiyesi et al, (1986) demonstrated significant lower serum iron levels in hypertensive patients as compared to normotensive controls (1.14 and 1.41 ppm respectively).

Acute iron poisoning causes a biphasic shock, initial but rapid increases in the respiration and pulse rate with congestion of blood vessels which leads to hypotension pallor and drowsiness in 6 - 8 hours (Venugopal and Luckey, 1978). This is followed by prostration, coma and finally death from peripheral cardiac failure in 36 hours. This is common in children that ingest 40 - 1,600 mg/kg body weight.

Chronic iron poisoning is characterised by haemorrhagic necrosis of gastrointestinal tract, hepatotoxicity and metabolic acidosis. Other symptoms are elevation of plasma levels of serotonin, histamine and a greatly prolonged blood-clotting time (Venugopal and Luckey, 1978).

However, Browning (1969, Arena, (1974) and Prasad, (1978) pointed out that there has been no case of either acute or chronic toxicity due to industrial exposure to iron. They indicated that most cases of iron toxicity are due to accidental ingestion of iron compounds or from its therapeutic uses.

Meanwhile, iron overload in mammals occur due to any of the following reasons:

a. Genetic abnormality: - Williams et al, (1974) found excessive iron in the liver biopsy and sera of twenty-eight out of forty-six relatives and postulated an intermediate from of inheritance. Mildly affected relatives representing the heterozygous state.

b. Excessive iron absorption as described by Williams et al, (1966) and Smith et al, (1969) can result in iron overload.

c. Tissue abnormality:- Increased release of iron from body stores as it occurs in necrotic hepatitis and chronic pancreatitis can elevate the blood iron concentration (Venugopal and Luckey, 1978).

d. Haematological disorder:- A variety of haematological diseases including haemolytic anaemia have been associated with increased blood iron (Lewis, 1971). Decreased survival time of red blood cells which

can be caused by stress has been demonstrated to increase blood iron concentration (Lewis, 1971; Venugopal and Luckey, 1978).

e. Self-induced overload:- Ingestion of iron tablets and oralcontraceptives for a prolonged period may develop into haemochromatosis (Shaw, 1980). Excessive iron deposits are derived from food and drinks prepared using iron-cooking utensils as is the case with the Bantu tribe (Venugopal and Luckey, 1978).

Adult males have a normal iron serum range of 0.6 - 1.5 ppm (Lewis, 1971 and Osunkiyesi *et al*, 1986) and values for females range from 0.5 - 1.3 ppm (Lewis, 1971). Serum iron levels decreases with age to levels of 0.4 - 0.8 ppm (Lewis, 1971).

2.10 LEAD:

Lead was one of the first metals with which man learned to work and it may well have been the first to poison him. It is used in making a variety of metal products including pipes, cable covering, foil, alloys and ammunition. The organic anti-knock agents for petrol are manufactured from lead (Grobler *et al*, 1984).

Lead poisoning is common in children living in urban areas and workers of hard-metal industries. It has been

shown that the mean lead level of motor mechanics was 0.276 ppm as compared to 0.034 ppm which is the mean value of lead in age-matched controls from the rural area (Grobler *et al*, 1984).

Glickman et al, (1984) measured saccadic eye movement of fifty-two lead exposed autobody shop workers and fifty-two matched controls with no history or occupational lead exposure. The result indicated that workers exposed to inorganic lead showed a decrease in saccade accuracy and an increase in overshoots as compared with the controls. Saccade maximum velocity was lower in lead exposed workers than in the reference group. They attributed this to the adverse effect lead has on the nerve conduction velocity.

Periheral neuropathy is one of the adverse effect of lead on the central nervous system. Waldron, (1978) and Glickman et al, (1984) reported that lead produces segmental demyelination or axonal degeneration in the peripheral nerves. It can also cause pre-synaptic block in neuro-transmission, an effect which can be overcome *in vitro* by adding excess calcium (Ca++) to the incubating media. Encephalopathy develops in adults as a result of massive absorption of lead (Waldron, 1978; Windér *et al*, 1983). They reported that the relationship between the onset of encephalopathy and blood lead

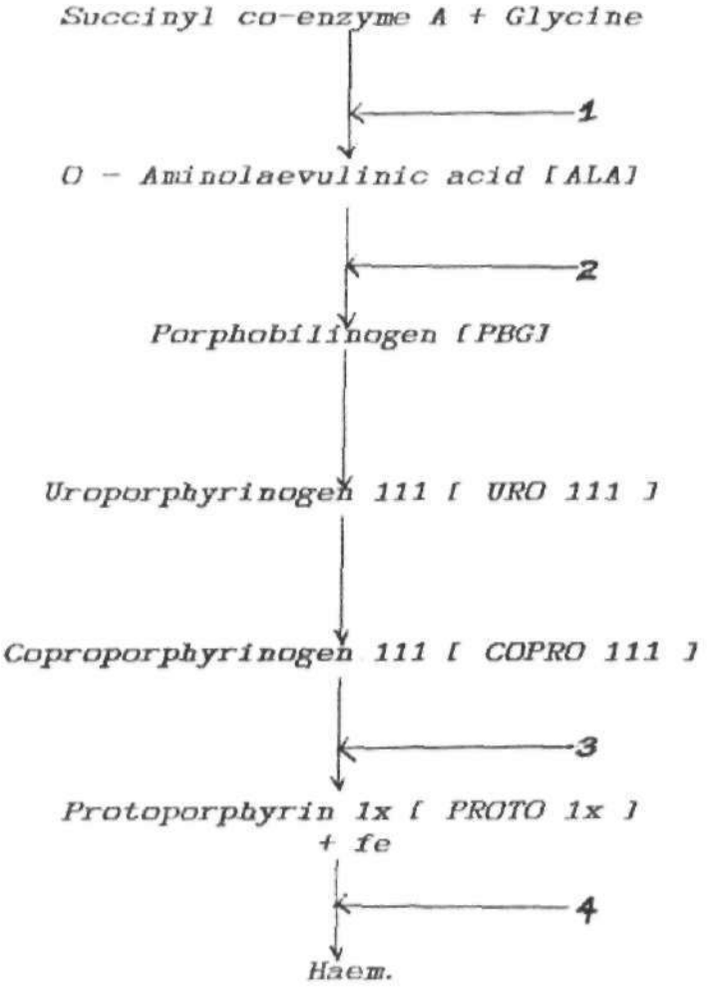
level is unlikely to be a simple one as the age of subject, acuteness of exposure and intercurrent illness are the factors accounting for individual variation. However, Winder et al, (1983) reported that other workers registered the occurrence of fatal encephalopathy in a child with a blood lead of 3.36 ppm and in two adults with brain levels of 5.8 ppm and 10.2 ppm respectively.

Recent animal studies indicated that in lead exposure, the brain of the off-spring may be particularly susceptible to trauma of parturition (Nordberg, 1990). However, only minor psychological changes have been reported to occur in workers in America whose blood lead were 0.7 ppm (Waldron, 1978).

The uptake of lead varies inversely with the amount of calcium, iron, phosphorus, copper and zinc in diet (Behari, 1981). He demonstrated that these mentioned elements are decreased in patients with lead poisoning.

Lead, like other heavy metals show a great affinity for sulphydryl group and is a potent enzyme inhibitor. Some enzymes involved in haem synthesis are inhibited by lead as illustrated in figure 1.

FIGURE 1 :- DIAGRAM SHOWING THE PATHWAY OF HAEM SYNTHESIS WITH THE STAGES INHIBITED BY LEAD (ARROWED AND NUMBER IN RED)



SOURCE:- Waldron. H.A. Health care of people at work. Workers exposed to Lead 1 . Inorganic head J. Soc. Occup. Med 28, 27 - 32. 1978.

The enzyme which seems most sensitive to lead is d-aminolaevulinic acid dehydratase (ALAD) that catalyses the formation of porphobilinogen (PBG) from 8-Aminolaevulinic acid (ALA), step 1 as shown in figure 1. Kipling, (1976) and a host of others have demonstrated the reduction in ALAD activity increase in protoporphyrin level in red blood cells and increase urinary excretion of ALA in lead workers. Urinary excretion of ALA in lead workers,and urinary excretion of coproporphyrin were also shown to be some of the major biochemical changes resulting from the effect of lead on haem synthesis.

Haemolytic studies conducted by McDermott et al, (1966) showed a haemoglobin content of less than 13 grams percent in seven out of nine employees of a small firm manufacturing car batteries who are exposed to lead. The blood lead range of the workers was 5.1 - 7.9 ppm with a urinary lead excretion of 5.35 ppm.

The above mentioned haemolytic studies and others demonstrate that anaemia develops in lead poisoning due to inhibition of haem synthesis and reduction of life span of the red blood cells.

Frank nephropathy among lead workers is rare now that industrial hygiene has improved but older literature

contains many reports of chronic lead exposure leading to kidney disorders. Waldron, (1978) and Nordberg, (1990) reported chronic lead exposure leading to interstitial nephritis with progressive impairment of renal function often accompanied by hypertension. The outcome was frequently fatal. These workers emphasized that this is no more common in lead worker.

Acute lead poisoning may produce the fanconi syndromes of aminoaciduria, more often seen in children (Kipling, 1976). He also reported that the production of gout in lead poisoning due to decrease tubular secretion of uric acid which results in impairment of renal function.

Biological monitoring of subjects exposed to lead is usually performed by measuring the concentration of lead in whole blood. It is believed that blood lead reflects the current exposure status of a person. However, it is generally agreed that blood contains lead in two forms, a non-diffusible form associated with the erythrocytes and a more biologically active form associated with the plasma.

Ong et al, (1990) showed that the mean blood lead level of forty-two lead stearate workers was 0.294 ppm and that for inorganic lead workers was 0.285 ppm.

There was a fifty percent reduction in activity of ALAD in red blood cells of both groups of workers.

Lilis et al, (1985) determined the blood lead levels of six hundred and eighty active copper smelter workers and they came up with a range of 0.4 - 0.5 ppm. The copper smelter workers showed mild central nervous system and musculoskeletal symptoms.

A concentration of 0.3 ppm blood lead is currently proposed as the acceptable upper limit in children while 0.4 ppm is the acceptable level for adults (Chisolm, 1956).

During the study of blood lead concentration in some people resident in Kaduna, Mamman, (1989) found the lead content in all the sample to be below detection limits (i.e 0.4 ppm).

A summary of dose-response effects of lead absorption is given in table 2.

Table 2: DOSE-RESPONSE EFFECTS OF LEAD ABSORPTION

BLOOD LEAD LEVEL PPM		EFFECTS
1.	0.2	Inhibition of ALAD.
2.	0.4	Elevation of Urinary ALA and coproporphyria.
3.	0.5	Peripheral neuropathy (Subclinical)
4.	0.6	Shortening of R.B.C life span
5	0.8	Anaemia
6.	1.3	Impairment of renal function
7.	1.5	Encephalopathy.

Adapted from Hernberg S. (1975) lead. In Zenz C. (ed) Occupational medicine. Principles and practical Application. Chicago year Book pp 715-769.

2.11 MAGNESIUM:

Magnesium is widely distributed in the earth crust as carbonate, silicate, sulphate and chloride. It serves as a constituents of alloys used in the steel industry.

The essentiality and function of magnesium to humans have been well established by many workers including Prasad, (1978) and Shaw, (1980). These workers reported that total depletion of magnesium could result

results in marked decrease in plasma, urinary and fecal magnesium and calcium. Decline in serum potassium level and metabolic alkalosis were observed in magnesium deficient persons (Prasad, 1978).

Oral ingestion of magnesium as therapeutic agent such as magnesium citrate and magnesium-containing antacids elevates serum magnesium concentration (Wacker, 1980).

Hypomagnesemia may occur due to the following conditions: absroption and excretion defects, gastrointestinal disorders, protein energy malnutrition and tetany (Wacker, 1980). Other conditions include liver cirrhosis, alcoholism, pancreatitis, kidney and endocrine disorder.

In a study of ninety-six magnesium exposed - industrial workers by Browning (1989), its main hazards are the elicitation of metal fume fever and burns with cutaneous injury resulting from penetration of the skin. The "metal fume fever" was analogous to that caused by zinc oxide while the burns differ in no way to second or third degree thermal burns.

Conjunctivitis and nasal catarrah were common in the ninety-six magnesium exposed workers. Elevation in

serum magnesium was observed in sixty percent of the workers, though they had no significant symptoms of ill health. In 10.3 percent the serum magnesium was twice the normal level (of 65 ppm). Seventy percent had a rise in their serum calcium concentration.

Osunkiyesi et al, (1986) determined the serum magnesium level in twenty-nine normotensive persons and found the mean value to be 23.11 ppm with a range of 19.20 - 28.54 ppm. Prasad, (1978) had a mean value of 32.0 ppm with a range of 20.09 to 36.45 ppm.

2.12 MANGANESE:

Manganese is used in iron and steel industry in the production of alloys, especially of steel (Ferro-manganese and silicospiegel) to deoxidize and recarburize the molten metal and also to give added strength and hardness (Browning, 1969).

Metalloenzymes namely pyruvate carboxylase and mitochondrial superoxide dimutase have manganese as components. However, it also activates a number of enzymes particularly the glycosyl transferases that are involved in polysaccharides and glycoproteins synthesis (Shaw, 1980).

Deficiency of manganese in human beings have been responsible for an abnormal depression of plasma

clotting proteins and of serum cholesterol levels (Doisy, 1974). Manganese has been reported to lower the blood sugar levels of some diabetic patients (Shaw, 1980).

Apart from the acute reaction of "metal fume fever" caused by the inhalation of high concentration of metals, manganese is also known to cause a chronic disease, "Manganism" occurring after prolonged exposure to this metal and its compounds (Rosenstock et al, 1971)

Cotzias et al. (1968) identified the symptoms of manganism to include hallucination, insomnia and mental confusion. He discovered that divalent and trivalent manganese induce degenerative changes in the nervous systems. Nephritis, liver cirrhosis, anorexia and muscular fatigue have been observed in patients of manganism (Rosenstock et al, 1971). Other symptoms reported by the same workers are, sexual impotence, monocytosis, leukoipenia and anaemia. The transitory psychiatric symptoms are followed by neurologic changes including disorders of the extra-pyrimidal system (Cotzias et al, 1968).

Patients with manganism had a serum manganese level of 0.075 ppm as against the normal level of 0.015 ppm (Rosenstock et al, 1971). Mean blood

manganese concentration in normal person was found to be 0.008 ppm (Cotzias et al, 1966)

2.13 NICKEL:

Nickel occurs mainly in deposits of sulphide ores and constitutes about 0.016 percent of the earth crust, (Browning, 1969). It has important roles in steel, batteries, ceramics and giass manufacturing. Other industrial uses of nickel are, in oils and alloy production, (Browining, 1969).

Nielsen, (1974) in his report has demonstrated that nickel plays important roles in metabolism and structure of membranes and protein (Synthesis of nickeloplasmin). Nickel stabilizes RNA and DNA against thermal denaturation (Kazimierz et al, 1982).

Exposure to industrial nickel dust causes nickel dermatitis, which is more commonly referred to as "nickel itch" (Fisher and Shapiro, 1956), Symeonides 1973). The dermatitis is ushered in by itching which lasts a week before the eruption appears. The primary eruption, erythematous, erythematopapular or follicular occurs and may be followed by superficial discrete ulcers or by eczema.

Acute nickel toxicity causes severe gastroenteritis following both oral and parenteral administration

(Venugopal and Luckey, 1978). They reported that nervous symptoms such as tremor, chorea-like movement and paralysis occur prior to death which results mostly from heart failure.

Chronic toxicity results in degenerative changes in heart muscles, brains, lungs, liver and kidney tissues (Symeonides, 1973). Venugopal and Luckey, (1978) also observed a high incidence in pulmonary diseases in nickel-exposed workers.

However, Symeonides, (1973) observed that metalic nickel toxicity is rare even in industrially exposed workers. He also pointed out that the main industrial hazard of nickel is that of exposure to nickel carbonyl poisoning, which is characterized by lesions of lungs, brain, heart and kidney (Kincaid et al, 1956; Symeonides, 1973).

Nelson, (1974) reported that the mean blood concentration of nickel in human subjects is 2.6 x 10-3 ppm with a range of 1.1 x 10-3 to 4.6 x 10-3 ppm. Blood nickel levels in twenty patients with acute nickel toxicity were within the range of 0.03 ppm to 0.12 ppm (Venugopal and Luckey, 1978).

2.14 ZINC:

The essentiality of zinc to plants, animals and man has been well established by many workers (Prasad, 1976; Venugopal and Luckey, 1978; Underwood, 1977). Many enzyme proteins and other macronutrients cannot function and some cannot even exist in the absence of minute amount of zinc.

The most important biological function of zinc appears to be related to its role in DNA and RNA synthesis and cell division (Prasad and Obserleas, 1974). Recent studies by these workers indicate that thymidine kinase, RNA polymerase and DNA polymerase are zinc dependent. Zinc also regulates the activity of RNase.

Owing to the widespread presence of this element in food and water supplies, zinc deficiency in man was once believed to be impossible. However, Prasad (1976) described zinc deficiency in eleven Iranian men and demonstrated that growth retardation and gonadol hypo-function were related to zinc deficiency. Other symptoms includes partial loss of taste sensation and hepatosplenomegaly (Prasad, 1974).

Inhalation of fresh fumes containing fine particles of zinc oxides gives a toxic reaction characterised by the acute onset of fever, shivering, dyspnoe, muscular

fatigue and nausea (Ross, 1974 and Ulvik, 1983). The disease which is known as zinc fever is a self-limited condition with complete recovery within 24 - 48 hours. The symptoms are easily misinterpreted as influenza, virus infection or pneumonia.

Ross, (1973) found that 40% of zinc fever was caused by welding galvanized steel on a zinc coated surface. However, fumes from many other welding metals including iron can be the cause of metal fume fever (Anseline, 1972).

The serum zinc level was raised for twelve days in eleven patients after the exposure to zinc fumes (Ross, 1974). The range was 4.04 to 5.64 ppm. Anseline, (1972) demonstrated an elevation of pulmonary isoenzyme of lactic dehydrogenase.

In contrast to Ross (1974) findings, Ulvik, (1983) found a subnormal values of serum zinc in a zinc fever patient. The range was between 0.3 to 0.4 ppm. Reasons for these subnormal values are not yet understood. However, Lekakis et al, (1980) reported that serum zinc levels are low in inflammatory conditions and tissue damage such as myocardial inarction.

Many investigators (Solomons, 1979; Prasad et al. 1974; and Prasad, 1976) have demonstrated a linear

relationship between albumin concentration and blood zinc level. Diseases like hepatic cirrhosis and kwashiorkor that depress blood albumin content have been shown to directly reduce blood zinc concentration (Solomons, 1979). Other conditions that lower blood zinc include, pregnancy (Prasad, 1974), inflammatory conditions (Solomons, 1979), and exogenous gonadal hormones such as contraceptive agents (Prasads, 1974).

Hyperzincemia may be genetically determined (Smith et al, 1970). They reported a high level of plasma zinc in five out of seven members of one family and in two of three second generation individuals. Increased level of plasma zinc was not accompanied by any adverse clinical features, the metabolic or absorptive defect accounting for this phenomonon remains unresolved.

Serum zinc values were determined in twenty-three healthy persons and the mean value was found to be 0.899 ppm (Parker et al, 1967). This value is in agreement with that of Widdowson et al, (1974) Osunkiyesi et al, (1986) found serum zinc range in twenty-nine person to be between 0.82 - 1.37 ppm.

3.0 MATERIALS AND METHODS

3.1 CHEMICALS

Tripalmitin, triolein and cholesterol were obtained from sigma chemical, Co. U.S.A. Sulphuric acid, phosphoric acid, chloroform, hydrochloric acid, ferric chloride (hydrated), lead nitrate, vanillin, cadmium nitrate and arsenic trioxide were obtained from May and Baker (M & B) Ltd, England and were of analytical grade. Manganous sulphate tetrahydrate, zinc sulphate heptahydrate, antimony granules, bismuth granules, purified cobalt copper metal, nickel metal and magnesium ribbon were purchased from British Drug House (BDH) chemical Ltd, London. Acetic acid, nitric acid and all other reagents used were of general purpose reagents grade, obtained from Hopkin and Williams Ltd, England.

3.2 BLOOD SAMPLE:

3.2.1 Collection of whole Blood:

Blood samples were obtained from eighty-two subjects who work in the factory production lines at Ajaokuta steel factory, Ajaokuta, Kwara state. Twenty students of Ahmadu Bello University, Zaria who have never worked in a factory were used as controls.

Venous blood samples were drawn from the fore arm of subjects into syringes containing either heparin

or ethylene diamine tetraacetic acid (10% EDTA) as anticoagulants.

Heparin was used as the anticoagulant in the whole blood samples collected from the steel workers while ethylene diamine tetraacetic acid (10% EDTA) served as the anticoagulant in the blood from the controls.

3.2.2 Preparation of serum samples:

Blood was allowed to clot for 1 to 2 hours at 37°C. The samples were left in a refrigerator overnight at 4°C and then centrifuged to separate the serum.

Both whole blood samples and sera were stored in pyrex sample bottles in the freezer at -20°C, until used for analysis.

3.3 DETERMINATION OF TOTAL LIPIDS:

Serum total lipids were determined using the phosphoric acid vanillin reaction method (Frings and Dunn, 1970). Aliquots of the serum sample (200 ul) were heated with 5.0 ml concentrated sulphuric acid at 100°C for 10 minutes. After allowing to cool to room temperature (25°C) 200 ul of the contents of each tube was transferred to a clean cuvette 112 x 75 mm) and 3.0 ml of phosphoric acid - vanillin reagent

was added. The cuvettes were allowed to stand in the dark for 60 minutes. Absorbances of specimens and standards were taken at 520 nm.

Standards of concentrations 200, 400, 600, 800 and 1,000 mg/100 ml were used to prepare a calibration curve (Appendix 1).

Total lipid concentration/100ml was determined as:

$$\text{Mg total lipid 100 ml serum} = \frac{AU}{As} \times Cs$$

Where:

Au = Absorbance of sample

As = Absorbance of standard

Cs = Concentration of Standard.

3.4 DETERMINATION OF TOTAL CHOLESTEROL:

Total serum cholesterol was determined by the colorimetric method of Zlatkis et al, (1953). 100 ul of serum samples were transferred into glass-stoppened centrifuge tubes and 4.9 ml of 10% ferric chloride acetic acid (1:2 v/v) was added to each tube. The contents of the tubes were mixed well and allowed to stand for 10 - 15 minute for the protein to flocculate. These were then centrifuged for 5 min. 1.5ml of concentrated sulphuric acid was added to 2.5ml of the

clear supernatant and the mixture was allowed to stand for 20 - 30 minutes. Absorbances were read at 560 nm.

Standards of concentrations, 100, 200, 300 and 400 mg/100ml were used to prepare a calibration curve (Appendix 1).

Total serum cholesterol was calculated as:

Mg total serum cholesterol/100ml serum

$$= \frac{Au}{As} \times Cs$$

Where:

Au = Absorbance of samples.

As = Absorbance of Standard.

Cs = Concentration of Standard.

3.5 DETERMINATION OF TRACE METALS:

Trace metals in both serum and whole blood were determined using an atomic absorption spectrophotometer (Pye Unicam sp 9). Magnesium, copper, iron and zinc were determined in serum while arsenic antimony bismuth, cobalt, cadmium, lead, manganese and nickel were determined in whole blood.

Samples were digested or diluted. The concentrations of the metals in ppm in each of the sample solutions

were read off from the calibration curves prepared for the metals (Appendix 1). The values obtained were multiplied by the appropriate dilution factors,

3.5.1 Preparation of sample solutions:

The serum samples for magnesium analysis were prepared by the method of Willis, (1974). Serum was diluted 1:100 with 0.1% (w/v) lanthanum chloride to suppress any interference from protein and phosphate. The diluted serum was aspirated directly into the atomic absorption spectrophotomoter (A.A.S).

Serum copper and zinc levels were measured by the method of Parker et al, (1967), Dawson et al. (1968). Serum samples were diluted 1:10 with deionized distilled water.

Serum iron was determined by the method of Bruce and Whiteside (1981). Samples were diluted in ratio 1:1 with 10% (w/v) trichloroacetic acid to precipitate the proteins. Determinations were carried out on the supernatant liquid after centrifuging.

Other trace metals, antimony, arsenic, biamith, cobalt, cadmium, manganaese, lead and nickel were determined in whole blood. The samples were digested using the "complete digestion technique" as described

by Shozo (1982). 9ml of the mixture of nitric acid, sulphuric acid and perchloric acid (3:1:2) was added to 1ml of the sample. This was then heated at 200 - 250^{o}C until the mixture was clear. The colourless solution was aspirated into the A.A.S for reading.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 TOTAL LIPIDS:

The effect of sex on serum total lipids concentration of controls and steel workers are presented in table 3. Contrary, to the reports of Lopez et al, 1967; Stanley et al, 1975; Datta and Ottaway, 1975, who indicated that adult males have higher serum total lipids levels than their female counterparts, female controls had significantly ($P<0.05$) higher serum total lipid content than male controls. This could be because of the increased lipid mobilization which is caused by female sex hormone shortly before their mentrual period (Taylor, 1971). It could also be attributed to better nutritional status of female controls relative to the male controls, since women of higher nutritional status have higher total lipid levels than men of lower income and nutritional status, (Onitiri, 1977). With the exception of the differences between blood lipid content of female controls and that of male controls, there was no significant ($P<0.05$) variation in serum total lipid levels of the female and male steel workers.

Serum total lipids concentrations (table 4) in the controls were slightly higher than that obtained

TABLE 3: EFFECT OF SEX ON SERUM TOTAL LIPIDS, CHOLESTEROL, COPPER, IRON, MAGNESIUM AND ZINC

	TOTAL LIPIDS (Mg/100ml)	CHOLESTEROL (Mg/100ml)	COPPER (ppm)	IRON (ppm)	MAGNESIUM (ppm)	ZINC (ppm)
Male Controls	573.45[a]	199.97	1.23	3.69	25.86	0.59[b]
	±81.39	±28.92	±0.33	±1.68	±3.51	±0.31
	(425-750)	(165-255)	(0.76-1.97)	(1.00-8.50)	(20.32)	(0.33-2.33)
	n=16	n=16	n=16	n=16	n=16	n=16
	a=25.1	a=25.1	a=25.1	a=25.1	a=25.1	a=25.1
Female Controls	7.00.00[a]	232.23	1.57	6.63	27.39	1.26
	±40.83	±38.99	±0.23	±1.49	±2.53	±0.74
	(650-750)	(173-255)	(1.76-1.82)	(5.00-8.50)	(23.8-29.7)	(0.66-2.33)
	n=4	n=4	n=4	n=4	n=4	n=4
	a=21	a=21	a=21	a=21	a=21	a=21
Male Steel	570.85	180.07	1.14	3.54	26.38	1.86[b]
Workers	±118.4	±43.98	±0.36	±1.98	±3.28	±1.03
	(350-925)	(91-287)	(0.61-2.12)	(1.00-8.50)	(19-33)	(0.52-3.50)
	n=80	n=80	n=80	n=80	n=80	n=80
	a=30	a=30	a=30	a=30	a=30	a=30
Female Steel	512.50	180.45	0.99	5.09	27.56	1.41
Workers	±17.80	±3.041	±0.32	±4.37	±1.15	±0.04
	(500-525)	(178-182)	(0.76-1.06)	(2.00-8.18)	(26.74-28.37)	(1.38-1.43)
	n=2	n=2	n=2	n=2	n=2	n=2
	a=23	a=23	a=23	a=23	a=23	a=23

NOTE:
Results are expressed as Mean ± Standard deviation.
Values in parenthesis are the ranges for each parameter.
n represents number of subject.
a represents the average age of subjects.
Statistical analysis: Figures with similar superscripts are significantly different at $P<0.05$.

TABLE 4: SERUM TOTAL LIPIDS, CHOLESTEROL, COPPER, IRON, MAGNESIUM AND ZINC CONCENTRATION IN CONTROLS AND STEEL WORKERS

	TOTAL LIPIDS (mg/100ml)	CHOLESTEROL (mg/100ml)	COPPER (ppm)	IRON (ppm)	MAGNESIUM (ppm)	ZINC (ppm)
Controls	598.75 ±90.49 (425-750) n=20 a=24.3	206.42 ±32.79 (165-255) n=20 a=24.3	1.23 ±0.33 (0.76-1.97) n=20 a=24.3	4.28 ±2.01 (1.00-8.50) n=20 a=24.3	26.17 ±3.34 (20-32) n=20 a=24.3	0.83[a] ±0.55 (0.33-2.33) n=20 a=24.3
Steel workers	568.25 ±105.74 (350-925 n=82 a=30	183.82 ±41.92 (91-287) n=82 a=30	1.14 ±0.39 (0.61-2.12) n=82 a=30	3.62 ±3.51 (1.00-8.18) n=82 a=30	26.43 ±3.26 (19-33) n=82 a=30	1.85[a] ±1.23 (0.52-5.48) n=82 a=30
Steel workers that have spent less five years in the industry	561.91 ±113.64 (400.925) n=21 a=26.5	172.96 ±89.85 (91-247) n=21 a=26.5	1.02 ±0.22 (0.76-1.36) n=21 a=26.5	3.56 ±2.02 (1.00-8.18) n=21 a=26.5	26.69 ±3.26 (19-32) n=21 a=26.5	1.74 ±1.24 (0.62-5.48) n=21 a=26.5
Steel workers that have spent five years and above in the industry	574.59 ±103.81 (350-300) n=61 a=33.5	194.67 ±43.93 (91-286) n=61 a=33.5	1.26 ±2.41 (0.61-2.12) n=61 a=33.5	3.69 ±1.96 (1.00-8.18) n=61 a=33.5	26.16 ±3.25 (19-33) n=61 a=33.5	1.94 ±1.90 (0.52-4.38) n=61 a=33.5

NOTE:
Results are expressed as Mean ± Standard deviation.
n represents number of subjects.
a represents average age of subjects.
Values in parenthesis are ranges for each parameter.
Statistical analysis: Figures with similar superscripts are significantly different at $P < 0.05$.

TABLE 5: RELATIONSHIP BETWEEN AGE, SERUM TOTAL LIPIDS CHOLESTEROL AND TRACE METAL LEVELS

Independent variable	Dependent Variable		Correlation Coefficient	Level of Significance
Age	Total lipids	1	-0.057	-
Age	Total lipids	2	-0.199	-
Age	Cholesterol	1	0.170	-
Age	Cholesterol	2	0.166	-
Copper	Total lipids	1	-0.364	P<0.10
Copper	Total lipids	2	-0.294	P<0.05
Magnesium	Total lipid	1	-0.299	-
Magnesium	Total lipids	2	-0.277	P<0.05
Iron	Total lipid	1	0.426	P<0.05
Iron	Total lipid	2	0.336	P<0.05
Zinc	Total lipid	1	-0.274	-
Zinc	Total lipids	2	-0.230	P<0.10
Copper	Cholesterol	1	0.285	-
Copper	Cholesterol	2	0.295	P<0.05
Iron	Cholesterol	1	0.123	-
Iron	Cholesterol	2	0.192	-
Magnesium	Cholesterol	1	0.258	-
Magnesium	Cholesterol	2	0.243	P<0.1
Zinc	Cholesterol	1	-0.163	-
Zinc	Cholesterol	2	-0.183	-
Copper	Iron	1	-0.265	-
Copper	Iron	2	-0.189	-
Copper	Magnesium	1	-0.237	-
Copper	Magnesium	2	-0.246	P<0.05
Copper	Zinc	1	-0.095	-
Copper	Zinc	2	-0.088	-
Iron	Magnesium	1	0.199	-
Iron	Magnesium	2	0.728	P<0.01
Iron	Zinc	1	-0.372	P<0.10
Iron	Zinc	2	-0.241	P<0.10
Magnesium	Zinc	1	-0.198	-
Magnesium	Zinc	2	-0.284	P<0.05

NOTE:

1 represents controls.
2 represents steel workers
- means no significance.

in the steel workers, which might be due to dietary variation (Mann et al., 1975 and Onitiri 1977). However, there were no significant ($P/0.05$) differences between the blood lipid content in controls and those in the steel workers.

The female controls had significantly ($P/0.05$) higher total lipid content than the female steelworkers. This could be because the former are of better nutrional nutritional status than the latter (Onitiri 1977). However there were no significant ($P/0.05$) differences between the total lipid concentration in the male controls and the levels in the male steel-workers.

The total lipid levels in the steel-workers that have spent less than five years in the industry did not differ significantly from the concentrations in workers that have spent more than five years in the industry, as such the duration of service in the steel industry did not affect the levels of total lipids in the steel-workers.

Table 5 and figure 2 indicate that there is a negative relationship between total lipid concentrations and blood levels of copper and zinc. This is consistent with previous reports (Prasad and Oberleas, 1974; Shaw 1980; Osunkiyesi et al., 1986), who indicated that high blood concentration of copper and zinc depresses

total lipid concentrations. Thus, the significantly ($P<0.05$) higher zinc levels in the steel workers as compared to controls could account for the lower blood lipid content of the former as compared to the levels in the controls.

The result (Table 5 and appendix 2) indicate that at 5% level of significance serum total lipids level raises with increase in blood iron. This could be because of the absolute demand for iron by enzymes involved in lipid synthesis)(Prasad 1978). Since copper and zinc depress serum total lipid concentration (Ozunkiyesi et al., 1986) and iron is a metabolic antagonist of these metals (Underwood 1977), iron can alleviate the suppression of blood total lipid by copper and zinc. This could be the reason why the total lipid content in steelworkers was normal despite the high serum zinc levels in these subjects.

Table 5 also indicates that there is an increase relationship between serum copper, magnesium and zinc levels and the total lipid concentrations. This is in agreement with the reports of Underwood 1977 and Prasad 1978. However the inverse relationship between these metals and total lipid content has been shown in this work to be insignificant. The results also show that there is an insignificant inverse relationship between age and total lipid levels in the studied subjects.

The iron and steel industry seems not to have any adverse effect on the blood total lipid of the steel workers, since the values obtained for these workers and their controls are within normal range. Normal serum lipid concentration in healthy persons falls within the range of 400 - 800 mg/100ml (Jacobs and Henry 1962; Frings and Dunn 1970).

CHOLESTEROL:

Serum cholesterol concentrations (Table 3) in both female controls and steel workers were slightly higher than those of their male counterparts. This observation is in line with the reports of Taylor and Reed et al. (1972), Bamgboye (1979) and Castle (1982) who indicated that adult females of better nutritional status have higher blood cholesterol than their male counterparts of lower nutritional status. Thus, the female subjects could be better off than their male counterparts in the sense of nutrition. This may also be the reason why the controls (Table 4) have a slightly higher blood cholesterol content than the steel workers.

There was no significant ($P>0.05$) variation in serum cholesterol levels of the male controls and those levels in the male steel workers. Similarly serum cholesterol concentration in the female controls did not

differ significantly ($P < 0.05$) from the concentration in the male steel workers. It was also observed that there were no significant ($P < 0.05$) differences in the cholesterol levels of the steel workers and those of their controls. No significant ($P < 0.05$) effect was observed in serum cholesterol content due to duration of service in the industry.

Table 5 and appendix 2 indicate that, there is an insignificant ($P < 0.05$) positive association between serum cholesterol content and the serum levels of copper, iron magnesium and zinc. This result also shows that serum cholesterol raises with age however this observation was insignificant ($P < 0.1$).

Blood cholesterol levels of both steel workers and their controls fall within normal range. The observed normal cholesterol levels for male and female population fall within the range of 162 - 306 mg/100ml and 157 - 327 mg/100ml respectively (Reed et al., 1972)

4.3 TRACE METALS:

There were no significant ($P < 0.01$) variations in the serum copper concentrations of all the studied groups. No significant ($P < 0.05$) differences were observed in the levels of copper in the subjects due to the effect of sex, working in the steel industry and duration of service in the factory.

Values obtained for serum copper levels in both steel workers and their controls are in conformity with the value in normal subjects. Parker et al, (1967) and Dawson et al, (1968) reported that the normal range of serum copper in clinically healthy person is 0.7 - 1.4 ppm.

Table 3 indicates that the female subjects had slightly higher blood iron concentration than their male counterparts. Since ingestion of iron tablets can elevate blood iron level (Shaw, 1980), it is possible that the female subjects take iron supplements. Moreso, in a discussion with the female controls, some of them agreed that they take iron tablets during and immediately after their menstrual period. However, the serum iron in both male and female subject did not differ significantly ($P < 0.01$). Similarly serum iron content in controls did not differ significantly ($P < 0.01$) from those in the two groups of steel workers.

The lack of variation in the iron levels of steel workers and controls is in agreement with the findings of Browning (1969); Arena (1974) and Prasad, (1978) who reported that acute and chronic iron toxicity have not been demonstrated in workers of iron and steel industry, and that most cases of iron toxicity are due to accidental ingestion of iron compounds or from its therapeutic uses.

Serum iron values obtained for controls and steel workers are slightly higher than normal values reported by Lewis (1971) and Osunkiyesi et al (1986). The elevated blood iron in the studied subjects could be as a result of ingestion of iron from tablets (Shaw 1980) or from food and drinks prepared in iron cooking utensils (Venugopal and Luckey 1978).

Apart from showing the level of blood iron in steel workers, this study (Table 5 and figure 3) demonstrate that there is an inverse relationship between blood iron and zinc. This agrees with the statement that iron is a metabolic antagonist of zinc (Underwood, 1977). Though not significant ($P/0.1$), magnesium concentration increases with rise in blood iron level.

There were no significant ($P/0.05$) differences in the serum magnesium levels in all the studied groups and the blood magnesium values obtained for both steel workers and their controls are within normal range. The observed normal blood magnesium levels for healthy persons fall within the range of 19.20 - 36.45 ppm (Prasad 1978). The effect of sex, working in the steel industry and duration of service in the industry did not have any significant ($P/0.05$) effect on the level of magnesium.

The serum zinc concentration in the steel workers (Table 4) were significantly ($P<0.05$) higher than those in the controls. Similarly the levels in the steel workers that have spent five years and above in the industry, were slightly higher than the levels in workers that have spent less than five years. It is noteworthy to mention that, while serum zinc concentrations in controls were normal, the levels in steel workers were higher than normal normal blood zinc concentration. The normal blood zinc range for clinically healthy persons is between 0.82 - 1.37 ppm (Parker et al. 1967; Widdowson et al. 1974; Osunkiyesi et al. 1986). Therefore, the elevated serum zinc in steel workers might possibly be due to exposure to zinc fumes in the industry. High serum zinc concentrations have been shown in welders-exposed to zinc oxide fumes during welding (Anseline, 1972; Ross 1974) and in steel workers exposed to metallurgical dust (Miller et al. 1973). However sex has no significant effect on the level of zinc in the studied subjects.

High serum zinc results in "metal fume fever" a self-limited condition with complete recovery in 12 - 48 hours (Ross 1974). The symptoms are fever, shivering, dysnoea, nausea and vomitting. This condition is protective, and tries to prevent injury of the respirary system (Prasad 1976).

Zinc interfers with the metabolism of iron, copper and calcium (Underwood 1977) and in zinc toxicity there are subnormal concentrations of these elements (Prasad 1976). With prolonged zinc toxicity, deficiencies of these elements ensues resulting into anaemia, "falling disease" (inefficient cross-links in elastin and collagen) and osteomalacia (softening of bones), (Prasad 1976).

Serum cipases are elevated in hyper-cholesteremia (Underwood 1977). Hyper-cholesteremia has been associated with atheroselerosis and other cardiovascular diseases (Mattingly et al 1956) as such zinc toxicity could be a predisposing factor in the incidence of cardiovascular diseases.

Blood concentrations of antimony, arsenic, bismuth, cadium, cobalt, lead, manganese and nickel were not detected in both controls and steel workers.

CONCLUSION

In view of the foregoing work we observed that:

1. Working in Ajaokuta Steel Industry did not have any significant ($P<0.05$) effect on the levels of serum total lipid and cholesterol in the workers.

2. The Steel Industry did not have any significant ($P<0.05$) effect on the levels of serum copper, iron and magnesium in the workers.

3. The steel workers had a significantly ($P<0.05$) higher serum zinc than the controls. The steel industry seems to have elevated the concentrations of serum zinc in the workers to above normal levels.

4. Except for the female controls that had significantly ($P<0.05$) higher serum total lipids than the male controls, sex did not have any significant ($P<0.05$) effect on the concentrations of serum, cholesterols, copper, iron, magnesium and zinc in both controls and steel workers.

5. The duration of service in the industry did not have any significant ($P<0.05$) effect on the concentrations of serum total lipids, cholesterol, copper, iron, magnesium and zinc in the steel workers.

6. Antimony, arsenic, bismuth, cadmium, cobalt, lead, manganese and nickel were not detected in the blood of both steel workers and their controls.

Except for the concentrations of zinc that were shown to be above normal in the steel workers, all other

studied parameters were within normal range, therefore the management of Ajaokuta Steel Industry should improve industrial hygiene with a view of eliminating zinc and other heavy metals from the metallurgical dust.

Symptoms associated with chronic toxicity of metals take long period before they manifest in exposed persons (Miller et al, 1973). As such, periodic monitoring of the levels of the studied metals in the steel workers will be useful in assessing the health of the workers and the occupational risk factors involved in iron and steel industry.

REFERENCES

1. Aaseth, J. and Norseth, T. (1986). Copper In Friberg L, Nordberg, G.F. and Vouk, V.B. (Eds). Handbook on the toxicology of Metals. 2. ed. Vol. 2. Elsevier, Amsterdam, the Netherlands. pp. 233-254.

2. Alderman, M.H. and Davis, T.K. (1976). Hypertension control at the work site. J. Occup. Med. 18, 793.

3. Andronikashvili E.L. and Mosulishvili L.M. (1980). Human leukemia and Trace elements, In Heimut sigel (eds), Metals, ions in Biology Systems.

4. Anseline, P. (1972). "Metal fume fever" The medical J. Australia 2, pp. 316-318.

5. Antonis, A. and Bersohn I. (1960). Serum total lipids in Africans. Lancet I pp. 998.

6. Arena, J.M. (1974). Poisoning: Toxicology, Symptoms and Treatment in: Venugopal B and Luckey T.D. (eds). Metals toxicity in Mammals. Phenum press. New York 2, 213-219.

7. Axelson, O.; Dahlgren E.; Jansson C.D and Rehnlund S.O. (1978). Arsenic exposure and mortality: A case referent study from a Sweden Copper smelter. Br. J. Ind. Med. 35: 8-15.

8. Axelson, O.; Dahlgren, E. and Poscator, M. (1968). Renal lesions in the Rabbit after long-term exposure to Cadmium. Arch of Environ. Health. 17: 24-29.

9. Beattie A.D.; Dagg, J.H.; Goldberg, A.; Wang, I. and Ronad, J. (1972). Lead poisoning in rural Scotland. Br. Med. J. 2: 488-491.

10. Bech, A. and Barborik E. (1977). Polycythaemia in hard metal workers In Payne. L.R. The hazards of Cobalt J. Soc. Occup. Med. 27: 20-25.

11. Behari, J.R. (1981). Effect of lead on zinc content of body organs, bone and serum of Rats. Chemosphere, 10(9): 1067-102.

12. Berlin M. and Friberg L. (1960). Bone Marrow activity and Erythrocyte destruction in chronic cadmium poisoning. Arch of Environ. Health 1: 478.

13. Bhave, S.A.; Pandit, A.N and Tanner, M.S. (1987). Comparison of feeding history of Children with Indian Childhood Cirrhosts and pained control. J. Pediatr. Gastroenterol. Nutr. 6: 562-567.

14. Bonenfant, J.L.; Miller, G. and Roy, P.E. (1967). Quebec. Beer-drinkers: Cardiomyopathy: Pathological Studies. Canadian Med. Assoc. J. 97: 910.

15. Brady, R.O.; Johnson, W.G. and Uhlendorf, B.W. (1971). Lipid storage diseases. Am. J. Med. 51: 423-431.

16. Brieger, H.; Semisch, C.W.; Stasney, J. and Paitnek, D.A. (1954). Industrial Antimony Poisoning. Industr. Med. Surg. 23: 521.

17. Browning E. (1969). Toxicity industrial Metals. Butterworths, London, pp. 23-33.

18. Bruce, A.M. and Whiteside P.J. (1981). Introduction to Atomic Absorption Spectrophotometer. Published by Pye Unicam Limited. pp. 46-47.

19. Castle, W.M. (1982). Cholesterol risk factors in black and white men in Zimbabwe and the effect of living standards. South African Med. J. 61(24): 926-929.

20. Chisolm J.J. (1986). Pediatric exposures to Lead, Arsenic, Cadmium and methryl Mercuty. In Nestle Nutrition. Trace elements in Nutrition of Children. pp. 31-36.

21. Clay, J.E.; Dale, I.; Cross, J.D. (1977). Arsenic Absroption Steel bronze workers. J. Soc. Occup. Med. 27: 102-104.

22. Cotzias, G.C.; Miller, S.T. and Edwards J. (1966). Neutron activation analysis. The stability of Manganese concentration in human blood and serum. J. Lab. Clin. Med. 67: 836-849.

23. Cotzias G.C.; Papavasilious, P.S.; Ginos, J.; Streck, A. and Diiby S. (1968). Metabolic Modifications of parkinson's diseases and of chronic Manganese poisoning. Annu. Rev. Med. 22: 305-326.

24. Datta, S.P. and Ottaway, J.H. (1979). Biochemistry Concise Medical textbooks, London. pg. 183-217.

25. Dawson, J.B.; Ellis, D.J.; and Newton, H. (1968). Serum Copper in normal person. Clin. Chem. Acta. 21: 23.

26. Doisy E.A. (1974). Effects of deficiency in Manganese upon plasma levels of clotting proteins and cholesterol in man: In Hoekstra W.G., Suttie J.W, Ganther, H.E. and Mertz W. (eds). Trace element metabolism in Animals. University Park Press, Baltimore, 2: 668-670.

27. Dwivedi, C. (1983). Cadmium-induced sterility: possible involvement in the cholinergic system. Arch, Environ, Contain Toxicol. 12(2): 151-156.

28. Fisher, A.A. and Shapiro A. (1956). Allergic eczemations contact dermatitis due to metallic nickel. J. of Am. Med. Assoc. 167: 717.

29. Fox M.R.S. (1976). Cadmium metabolism - A review of aspects pertinent to evaluating dietary cadmium intake by Man. In: Prasad A.S. (Ed) Trace Element in Human Health and Diseases. Academic Press, New York 2: 401-416.

30. Friedman H.S. (1962). Emotional stress and serum cholesterol level. Clin. Chim. Acta. 7: 100.

31. Frings, C.S. and Dunn, R.T. (1970). A colorimetric determination of lipids. Am. J. Clin. Path. 53: 89-91.

32. Gruyton, A.C. (1975). Lipid metabolism in textbook of medical physiology. 5th Edition. W.B. Saunders Company, U.S.A. pg. 916-927.

33. Glickman, L.; Valciukas, J.A.; Lilis, R. and Weisman, I. (1984). Occupational lead exposure. Effects on saccadic eye movement. Int. Arch. Occup. Environ. Health. 54(2): 115-126.

34. Grobler R.S.; Marsky L.S. and Rossouw R.J. (1984). Blood lead levels in people exposed to exhaust fumes. S. Afr. Med. J. 65(25): 984.

35. Harman D. (1968). Atherogenesis in mini pigs: Effect of Dietary fat unsaturation and of Copper. Circulation Vol. 38. Supplement VI: 8.

36. Hayashi, M.; Masatoshi, T. and Hiroshis, A. (1982). The distribution of environmental pollutants in pet animals: Copper and Manganese concentration in organs of house dogs. Dokkyo J. Med. Sci. 8(2): 65-68.

37. Houba, C. and Remacle J. (1982). Factors influencing toxicity of Cadmium. Particulate or soluble form and degree of complexation. Environ. Pollut. Ser. A. Eclo. Biol. 28(1): 35-44.

38. Jacobs S.L. and Henry R.J. (1962). Adult fasting normal ranges of total lipid in serum. Clin. Chim. Acta. 7: 270.

39. Kazimierz, J.Z. (1980). Antagonism by essential Divalent Metals and Amino acids of Nickel (11) - DNA binding in Vitrol. Toxicol & Appl. Pharmacol 82: 336-343.

40. Kipling, M.D (1976). Health hazards and powder metallury. (Iron, Zinc, Nickel, Chromium, Copper, Alminium, Lead and Cadmium). J. Soc. Occup. Med. 26: 81-84.

41. Kincald J.F.; Stanley, E.L.; Beckworth, C.H. and Sunderman F.W. (1956). Nickel poisoning III: Procedure for detection, prevention and treatment of Nickel Carbonye exposure. Am. J. Clin. Path 28: 107.

42. Kojima A and Tamura S. (1981). Effects of Cadmium on delayed-type Hypersensitivity in Mice. Japan J. Med. Sci. Biol. 34(5): 281-292.

43. Lauwery R.R.; Buchet, P.J.; Roels, H.A. and Brouwers, J. (1974). Epidemiological survey of workers exposed to Cadmium: Effects on kidney, lungs and several biological indices: Arch. Environ. Health 28: 148.

44. Lokakis J. and Kalofoutis A. (1980). Zinc concentrations in Myocardial infarction patients. Clin. Chem. 26: 1660.

45. Leonard, A. and Lauwerys R.R. (1980). Carcinogenicity, teratogenicity and nutagenicity of arsenic. Mutation Research 75: 49.

46. Lewis, S.M. (1971). Proposed recommendation for measurement of serum iron in human blood. In: Am. Clin. Path. 56: 543-545.

47. Lilis, R. Valciukas, J.A.; Malkin, J. and Weber, J.P. (1985). Effect of low level lead and arsenic exposure on copper smelter workers. Arch. Environ. Health. 40(1); 38-46.

48. Lopez A.; Krebl. A. and Hodge, R.E. (1967). Serum lipids and sex. Am. J. Clin. Nutr. 20: 808.

49. Macdonald E.E. and Mclinnes, G.T. (1981). Therapeutics of hypertension in work environment. J. Soc. Occup. Med. 31: 31-37.

50. Mamman C.A. (1989). Analysis of trace metals (Lead) in the blood of some hypertensive patients. Unpublished Diploma Thesis, Kaduna Polytechnic, Kaduna.

51. Mann, G.V.; Nicol, B.M. and Stare F.J. (1975). Serum lipids in Nigerians. Br. Med. J. 2: 1008.

52. Mattingly W.T.; Parmley F.L.; Smith, E.R.B. and Emmeth D.L., (1956). Lipid studies in health and Diseases. J. Am. Med. Assoc. 175(5): 536-541.

53. McDermott, P.H.; Delaney R.L; Egan, J.D. (1966). Myocardosis and Cardiac failure in Men. J. Am. Med. Assoc. 198: 253.

54. Miller, C.W.; Davis, M.W. and Goldman, A. (1973). Pneumoconiosis in Hard-Metal tool indusry Arch. Ind. Hygiene 8: 453.

55. Morgan L.G. (1983). A study into the health and mortality of men exposed to cobalt and oxides. J. Soc. Occup. Med. 33(4): 181-187.

56. Murth R.C.; Shyam, L.D.K.; Chandra, S.V. (1981). Effects of Manganese and Copper interaction on behaviour and biogenic amines in Rats fed a 100% casein diet. Chem-Biol. Interact 37(3): 209-308.

57. Norman, R.A.; Coleman, T.G.; Wiley, T.L.; Manning, R.D. and Guyton A.C. (1975). Separate roles of Sodium concentration and fluid volumes in salt-loading hypertension in dog. Am. J. Physiol. 229: 1068-1070.

58. Nelson, H.F. (1974). Essentiality and function of Nickel: In Hoekstra. W. (Eds). Trace element Metabolism in animals. University Park Press, Baltimore. pp. 381-392.

59. Nordberg, F.G. (1990). Human health effects of Metals in Drinking Water Relationship to cultural acidification. Int. J. Environ. Toxicol & Chem. 9: 887-894.

60. Norman, A.G. (1968). Implication of environmental stress. Arch. Environ. Health. 17(4): 571-578.

61. Olatunbosun, D.A.; Cole, T.O.; Boladeoku, J.O. and Adadevoh, B.K. (1976). Relationship of serum copper and zinc to human hypertension in Nigerians. W.H.O Bulletin. 53: 134-135.

62. Ing. C.N.; Chua L.H. and Teramoto K. (1990). Biological monitoring of workers exposed to Lead stearate. J. Appl. Toxicol. 10(1): 65-68.

63. Onitiri A.C. (1977). Serum lipids and lippoproteins in healthy Africans. Clin. Chim. Acta. 81: 57-61.

64. Osunkiyesi B.O.; Anjorin, F.I.; Jaiyesimi, A.E.; Idoko J.A. and Kazmi M.S. (1986). Trace metals in hypertensive patients in the guinea savannah of Northern Nigeria. Tropical cardiology. 12(48): 187-190.

65. Parker, M.; Humoller, F. and Mahler, D. (1967). Determination of Copper and Zinc in Biological materials. Clin. Chem. 13: 40.

66. Parish, G.G.; Glass R. and Kimbrough R. (1979). Acute Arsine poisoning in two workers cleaning a clogged Drain. Arch. Environ. Health. 34(4): 224-227.

67. Payne, L.R. (1977). The hazards of Cobalt. J. Sco. Occup. Med. 27: 20-25.

68. Pinto S.S.; Henderson, V. and Enterline P.E. (1978). Mortality Experience of Arsenic exposed workers. Arch. Environ. Health. 33(6): 325-331.

69. Pounds, J.G.N. (1963). The Geography of Iron and Steel. Hutchinson and Co. Publishers London, pages 55-73.

70. Prasad, A.S. (1976). Zinc deficiency in Man. Am. J. Dis. Child. 130: 359-361.

71. Prasad A.S. and Obserleas D. (1974). Thymidine Kinase activity and incorporation of thymidine into DNA, in zinc deficient tissue. J. Lab. Clin. Med. 83: 634-639.

72. Prasad, A.S. (1978). Trace elements and Iron in human metabolism. Academic Press, New York. CVol. 1 pp. 205-438.

73. Reed, A.H.; Cannon, D.C.; Winkelman J.W.; Bhasin, Y.P.; Henry, R.J. and Pileggi, V.J. (1972). Cholesterol levels in human serum. Clin. Chem. 18: 57-66.

74. Renwick, J.H.; Harrington, J.M.; Waldron H. and Dissanaike, D.S. (1981). Longterm effects of acute Arsenical poisoning. J. Soc. Occup. Med. 31: 144-147

75. Rooney, H. and Bowen, A. (1976). Human Blood Bismuth levels. In Hamalton E.L. (Ed). The chemical element and man. Measurements, Perspective, Applications. Academic Press New York. pp. 172-241.

76. Rosenstock, H.A.; Simon, D.G. and Meyer J.S. (1971). Chronic Manganism: Neurologic and Laboratory Studies during treatment with Levadopa J. Am. Med. Assoc. 217: 1354.

77. Ross, D.S. (1974). Welder's Metal fume fever. J. Soc. Occup. Med. 24: 125-129

78. Saltman P. (1983). Trace elements and blood pressure. Annals. Intern. Med. 98 (Part 2) 828.

79. Scheinberg, I.H. and Sternlieb, I. (1976). Copper toxicity and Wilson's disease. In: Prasad, A.S.; Trace elements in human Health and Disease. Vol. I. Academic Press, New York, pp. 415-438.

80. Seftel, H.C.; Baker, S.G.; Sandler, M.P.; Forman, M.B.; Joffer, I.B. Mendelsohn D.; Jenkins. T. and Mieny J.C. (1980). A host of hypercholesterolaemic homozygotes in South Africans. Br. Med. J. 281: 633-640.

81. Shaper, A.G. and Jones, K.W. (1959). Serum total lipids in Europeans and North Americans, Lancet II 534.

82. Shaw, C.L.J. (1980). Trace elements in the foetus and young infant: Copper, Mangnese, Selenium and Chromium. Am. J. Dis. Child. 134: 76-81.

83. Shinton, N.K. and Singh, A.K. (1967). Vitamin B12 Absorption by inhalation. Br. J. Haematology. 13: 75.

84. Shozo Nomoto, (1982). Sample preparations for analysis of trace metals in biological materials for atomic absorption spectrophoto metry and its detection limits. Clin. Chem. 3: 1097-1105.

85. Smith, I. and Sargent H. (1971). Iron overload. In Jacobs. A and Worwood M. (Eds). Iron in Biochemistry and Medicine. Academic.

86. Smith, J.C.; Zeller, J.A.; Brown E.D., and Ong. S.C. (1976). Elevated plasma Zinc. A heritable anomaly. In Prasa. A.S. (Ed). Trace elements and iron in human metabolism. London pp. 328-329.

87. Solomons, N.W. (1979). On the assessment of Zinc. and Copper nurtiture in Man. Am. J. Clin. Nutr. 856-871.

88. Stanley, D.; Passmore, R.; Brock, J.K. and Truswell, A.S. 1975. Human nutrition and Dietetics. 6th Edition. Great Britain page 73-89.

89. Symeonides. P.P. (1973). Allergic reaction after Nickel exposed: In Venugopal B and Luckey T.D. (Eds). Metal Toxicity in Mammals. Plenum Press New York, 1976. pp. 289-296.

90. Taylor, G.O.K. (1971). Studies of serum lipids in Nigerians. Trop. Geogr. Med. 23: 158.

91. Taylor, G.O. and Bamgboye A.E. (1979). Serum cholesterol and diseases in Nigeria. Am. J. Clin. Nutr. 32: 2540-2545.

92. Ulvik R.J. (1983). Subnormal serum zinc concentration in a patient with zinc fever. J. Soc. Occup. Med. 33(4): 187-189.

93. Underwood, J.E. (1977). Trace elements in human and animal nutrition. Academic press. New York. U.S.A. Pages, 16-37.

94. Venugopal B and Luckey, T.D. (1978). Metal Toxicity in Mammal 2 chemical Toxicity of Metals and Metalloid. Plenum Press New Yorm 2: pp. 213-219.

95. Wacker, E.C.W. (1980). Magnesium and Man. Harvard University Press, U.S.A. pp. 23-114.

96. Wagner, S.; Weswig P. and Ore, C. (1974). Arsenic in blood and Urine of forest workers. Arch. Environ. Health. 28: 77-79.

97. Waldron H.A. (1978). Health care of people at work: workers exposed to Lead, inorganic Lead. J. Soc. Occup. Med. 28: 27-32.

98. Warren, Kenneth, (1973). The American Steel Industry (1850-1970). A Geographical Interpretation. Clarendon Press, Oxford. pages 11-41.

99. Welch K.; Giggins, I. and Burchfiel C. (1982). Arsenic exposure, smoking and respiratory cancer in copper smelters workers. Arch. Environ. Health. 37(6): 325-335.

100. Widdowson, E.M.; Dauricey, J. and Shaw, J.C.L. (1974). Trace elements in foetal and early Postnatal development. Proc. Nutr. Soc. 33: 275-280.

101. Williams, McRoberts, (1973). Alteration in fractionated blood lead concentration in the development of inorganic Lead poisoning and

102. Willis, A.H. (1974). Iron Overload. In Jacobs A. and Worwood, M. (Eds). Iron in Biochemistry and Medicine. Academic Press, Inc. London. pp. 590-609.

103. Willis, A.H. (1974). The determination of metals in blood serum by atomic absorption spectrophotometry - 11 (Magnesium). Spectrochim. Acta, 6: 273.

104. Winder, C.; Carten L.L. and Lewis, P.D. (1983). The Morphological effects of Lead on the developing central nervous system. Neuropath and Appl. Neurobiol. 9: 87-108.

105. Wynder, E.L. (1980). Conference on the primary prevention of cancer: Assessment of risk factors and future directions. Preventive, Med. 9: 162-332.

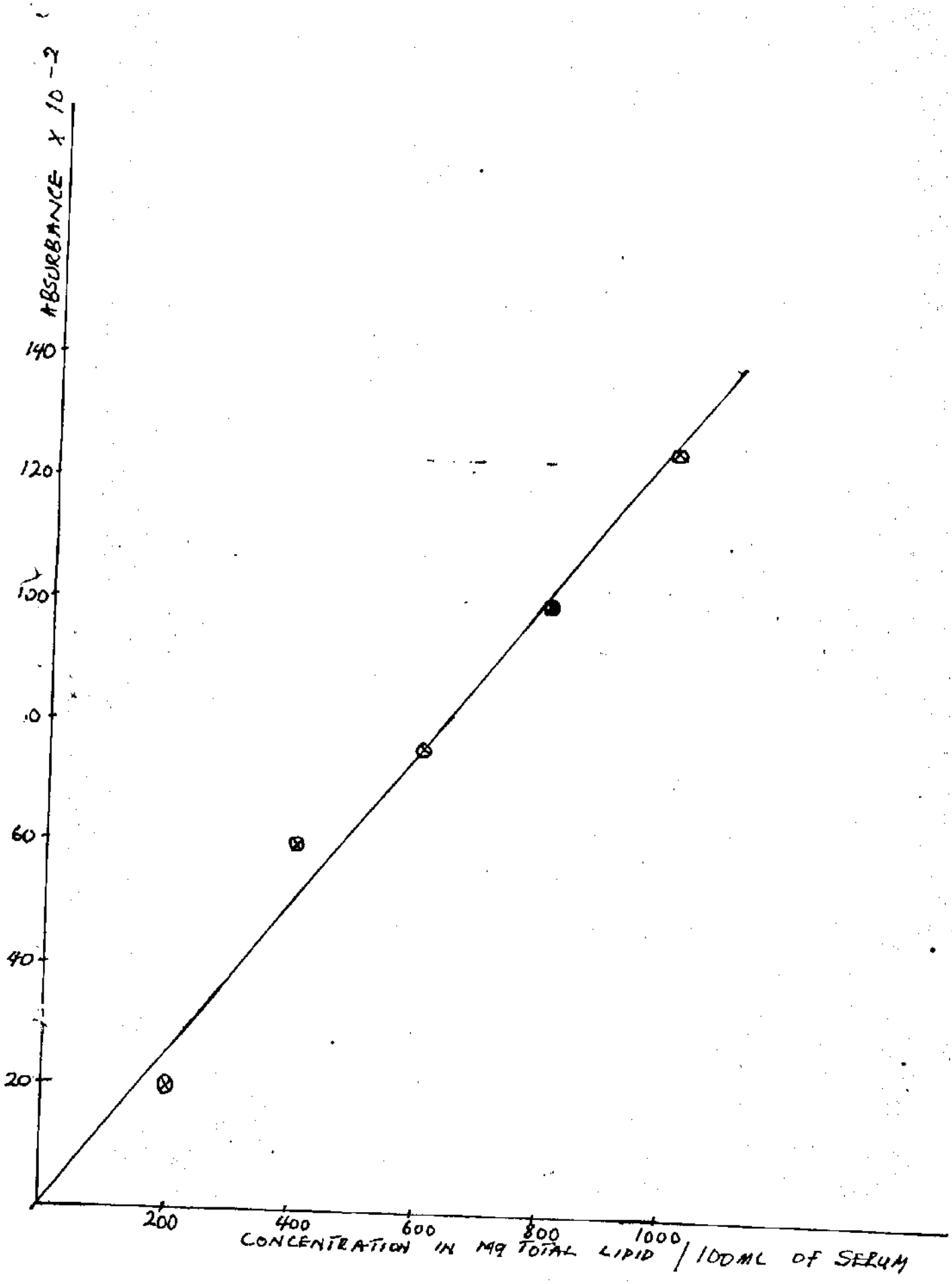
106. Zlatkis, H. and Boyle (1953). Determination of serum cholesterol. InL NORMAN T (Ed) Principles of Clinical Chemistry, Philandelphia, U.S.A. 1977. pages 605-620.

107. Zolankiewicz, D,D, and Fashing, J.L. (1976). Analysis of whole blood by Neutron Activation: A search for a Biochemical Indicator of neoplasia. Clin. Chem. 23(8): 1361-1365.

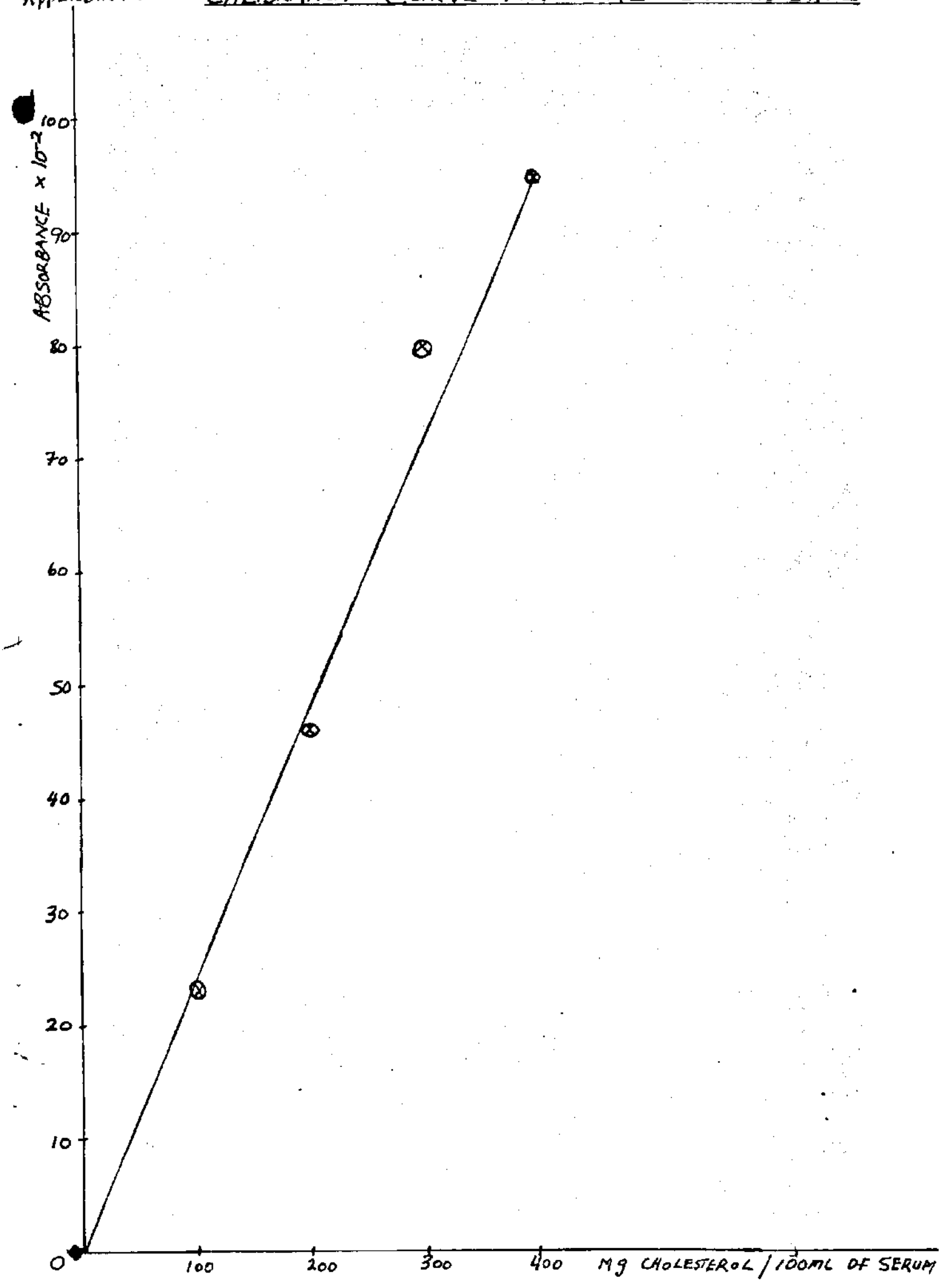
APPENDIX

APPENDIX 1: CALIBRATION CURVES FOR TOTAL LIPIDS, CHOLESTEROL AND TRACE METALS.

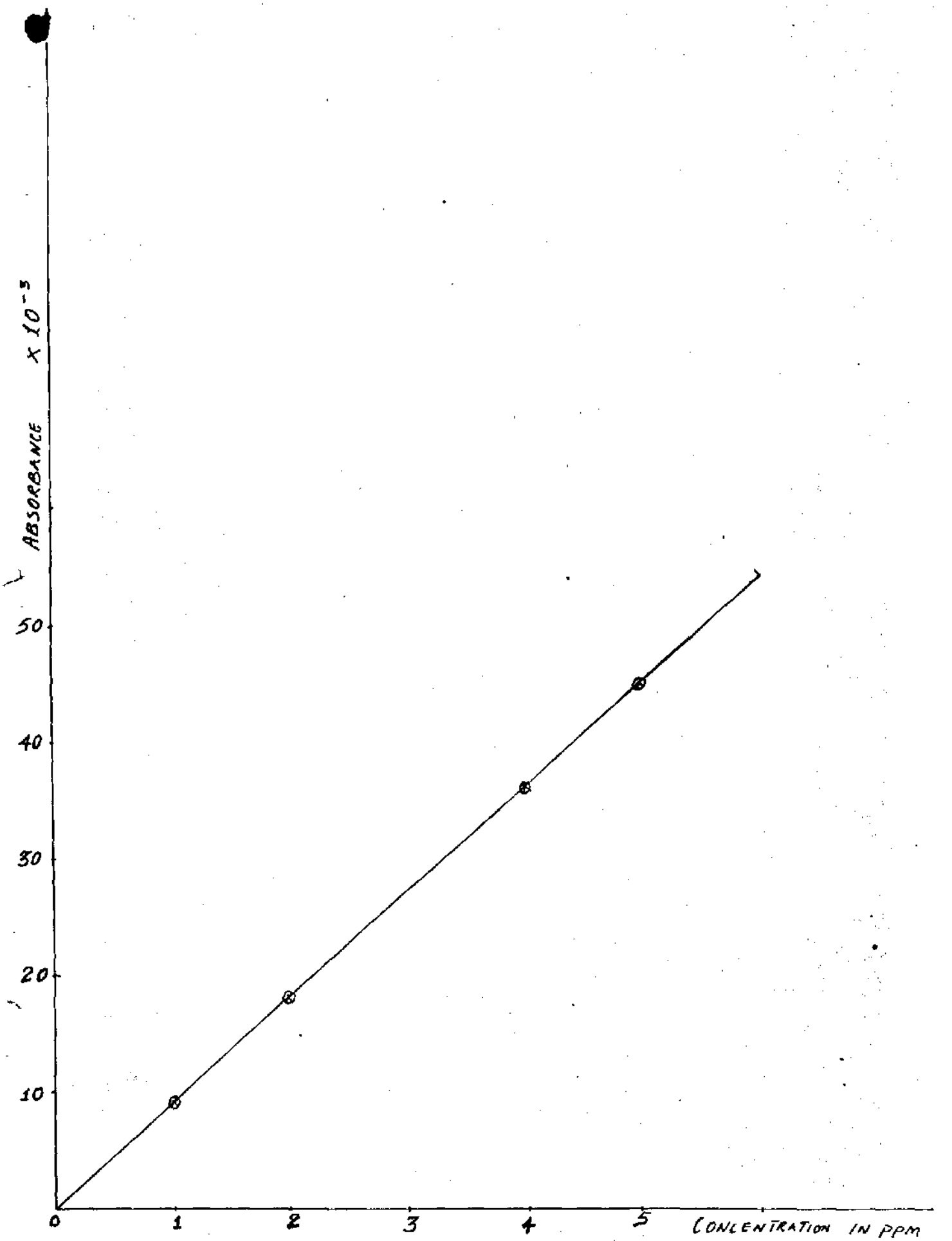
Appendix - 1.1 CALIBRATION CURVE FOR TOTAL LIPIDS



APPENDIX 1·2 CALIBRATION CURVE FOR TOTAL CHOLESTEROL

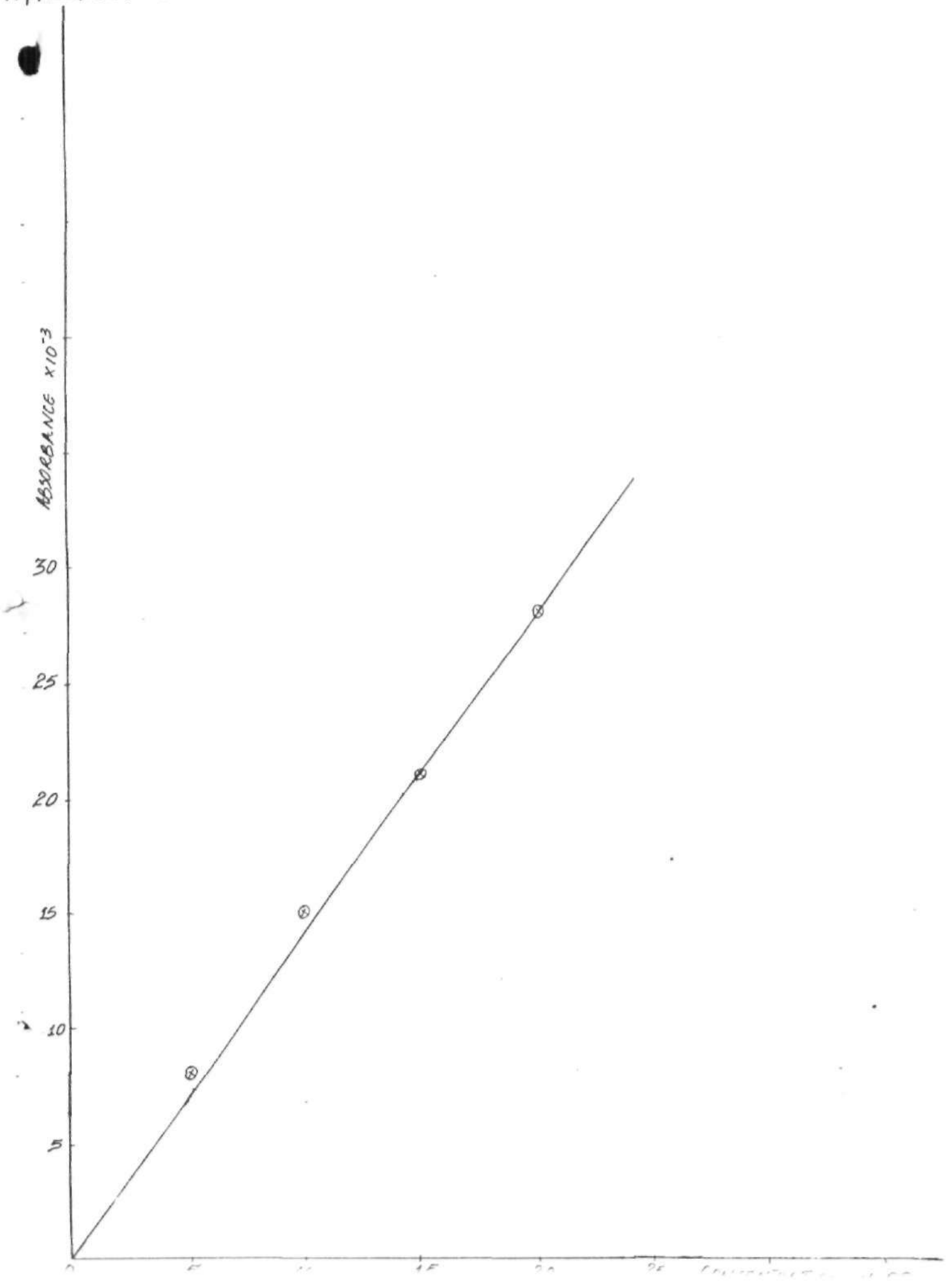


APPENDIX I·3 CALIBRATION CURVE FOR ANTIMONY

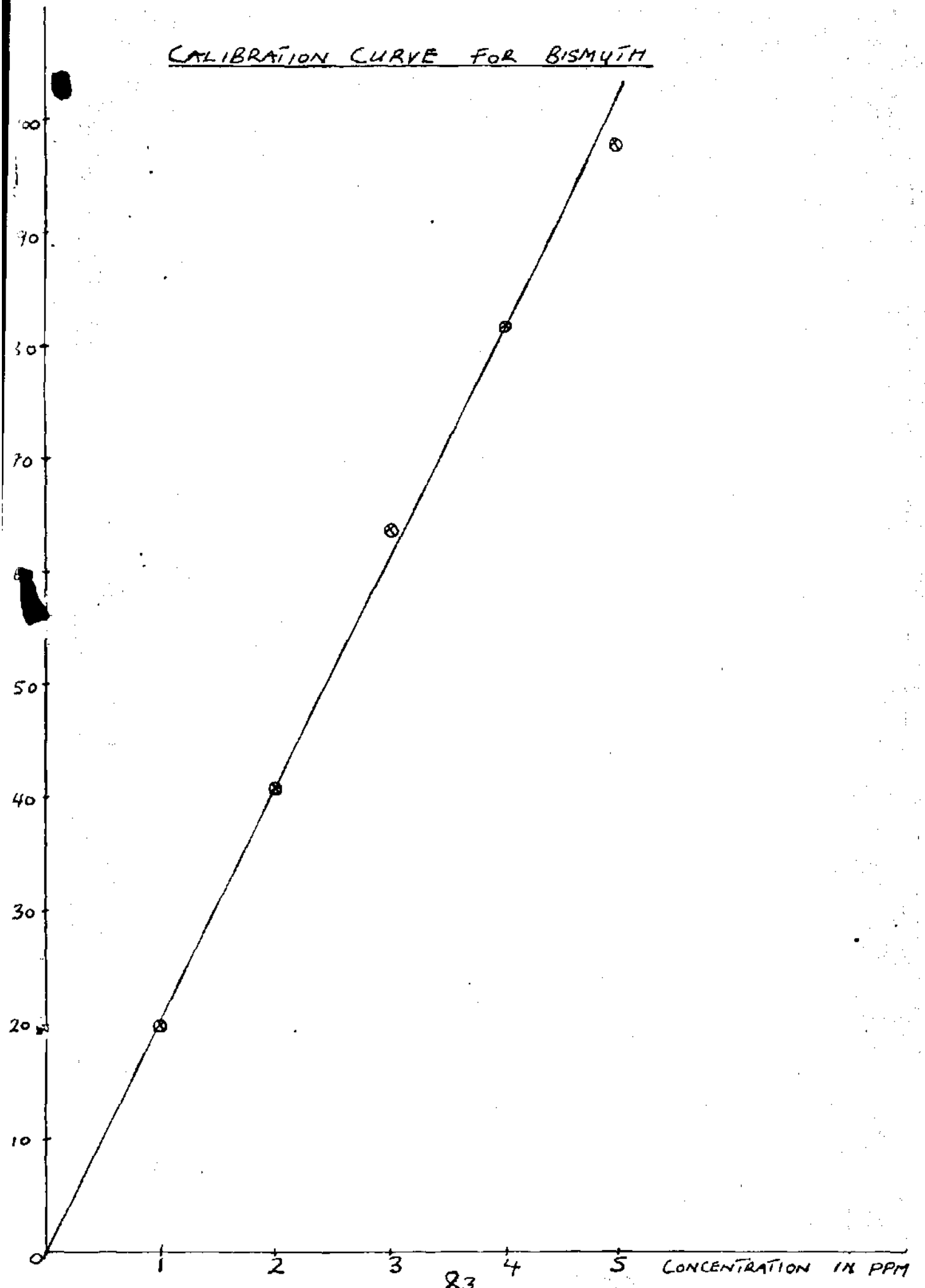


APPENDIX 1·4

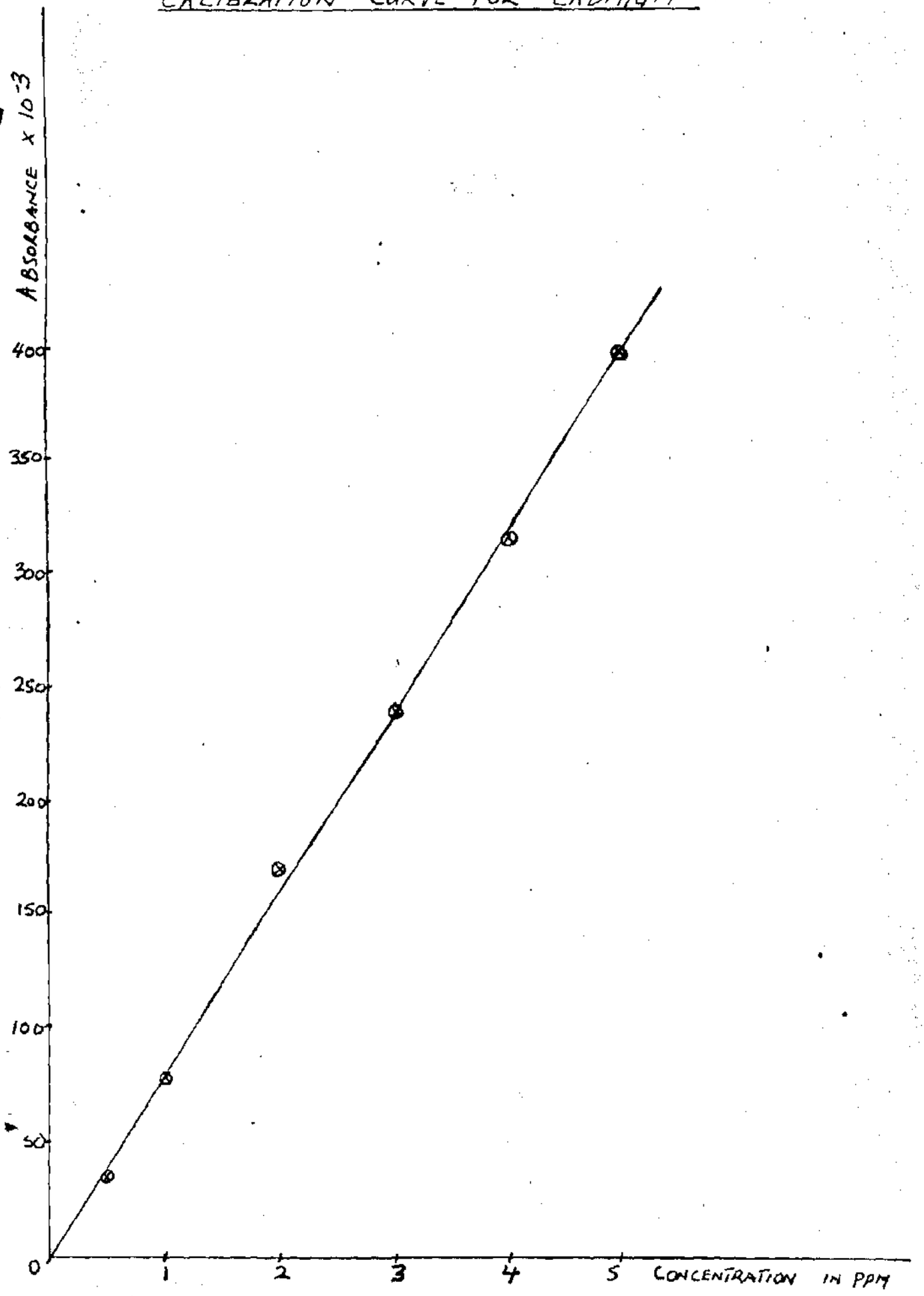
CALIBRATION CURVE FOR ARSENIC



CALIBRATION CURVE FOR BISMUTH

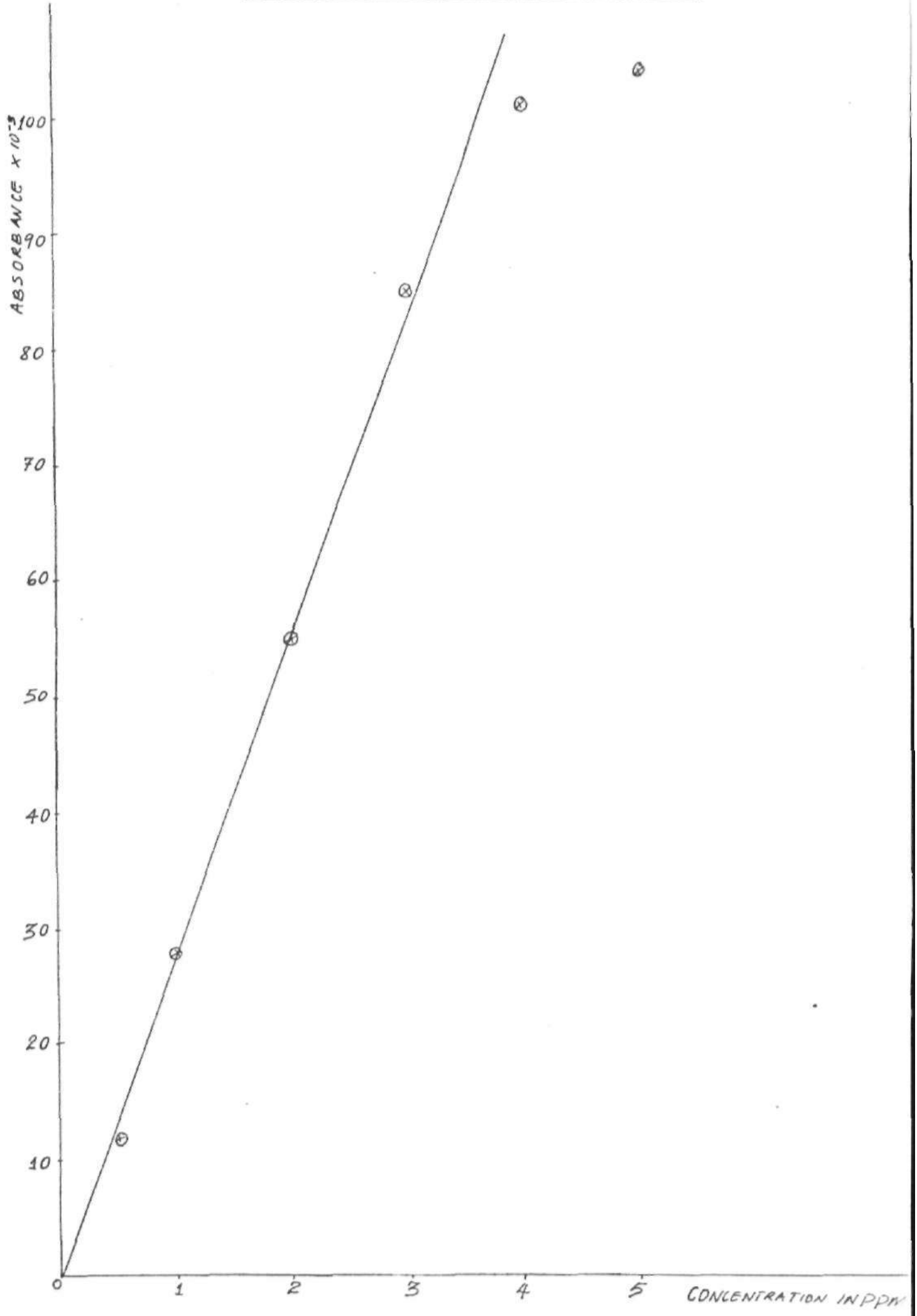


CALIBRATION CURVE FOR CADMIUM

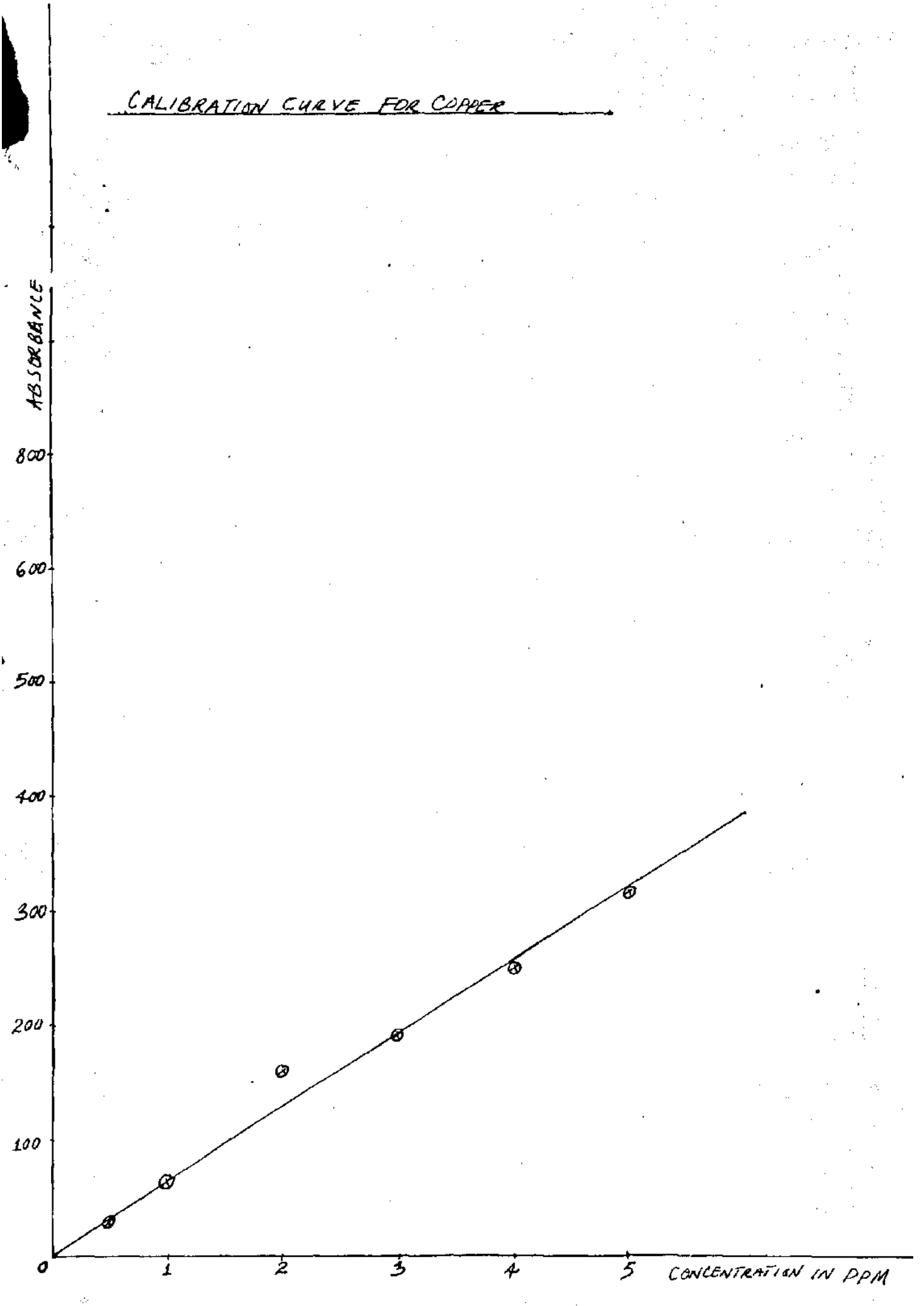


APPENDIX 1·7

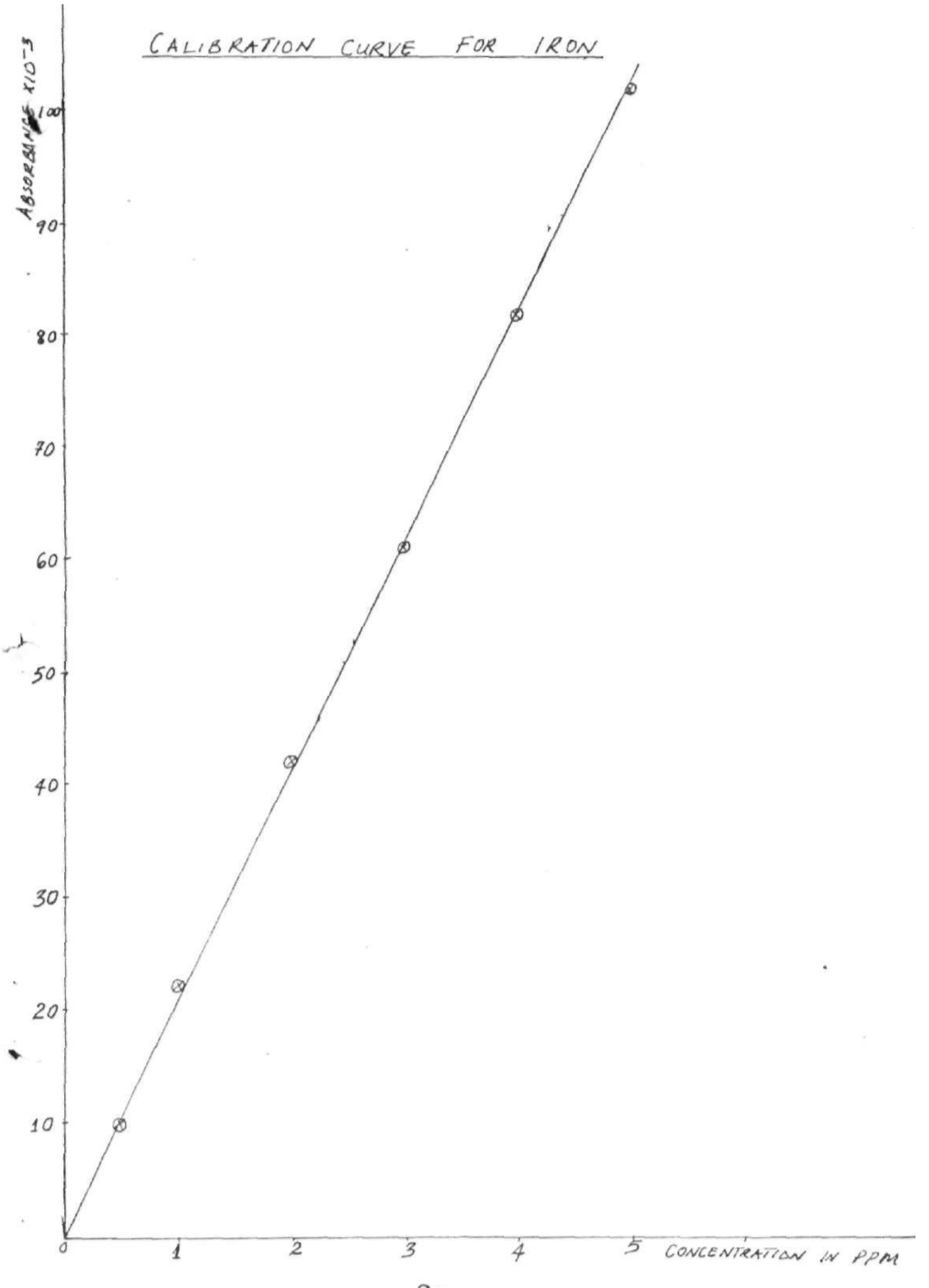
CALIBRATION CURVE FOR COBALT



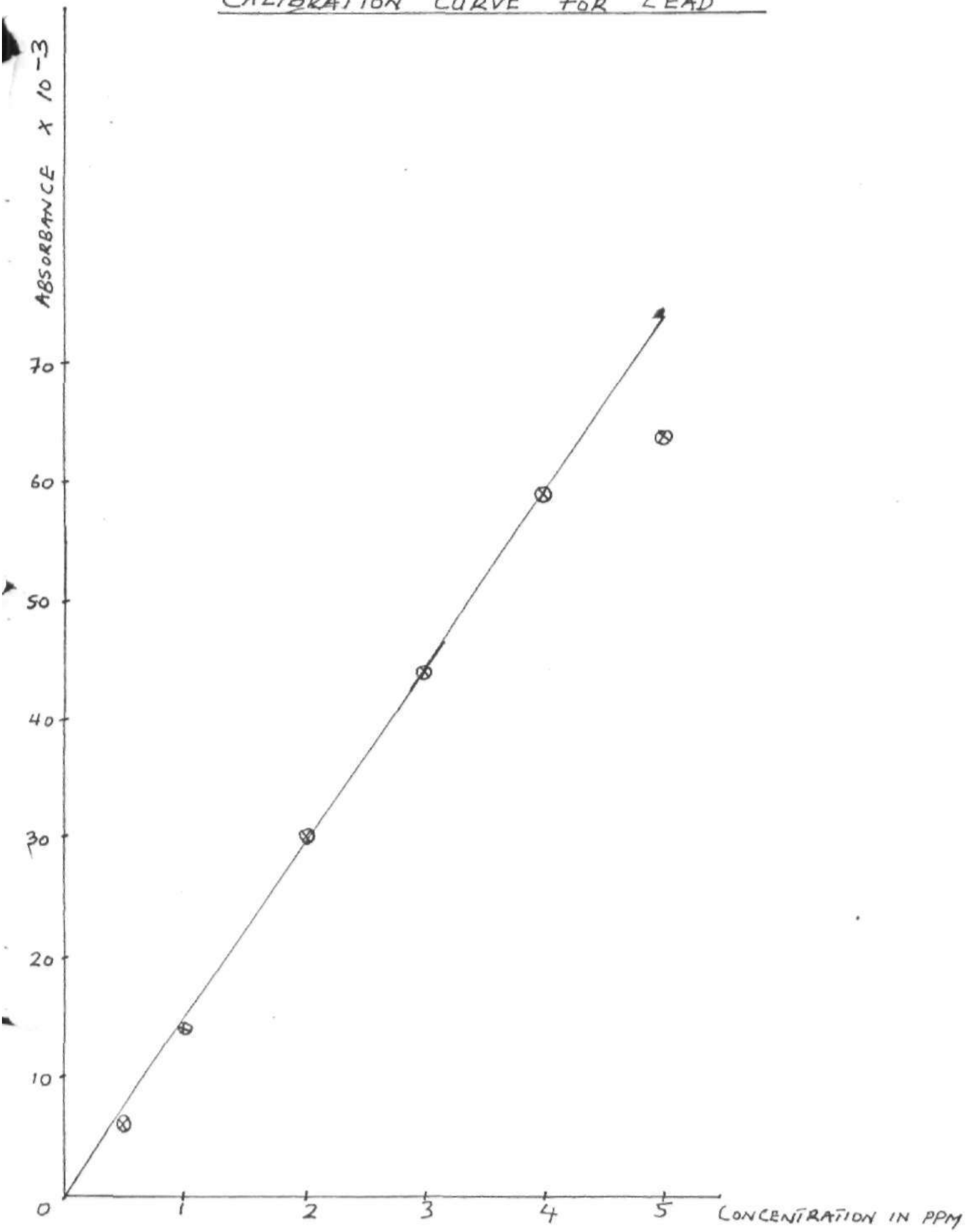
CALIBRATION CURVE FOR COPPER



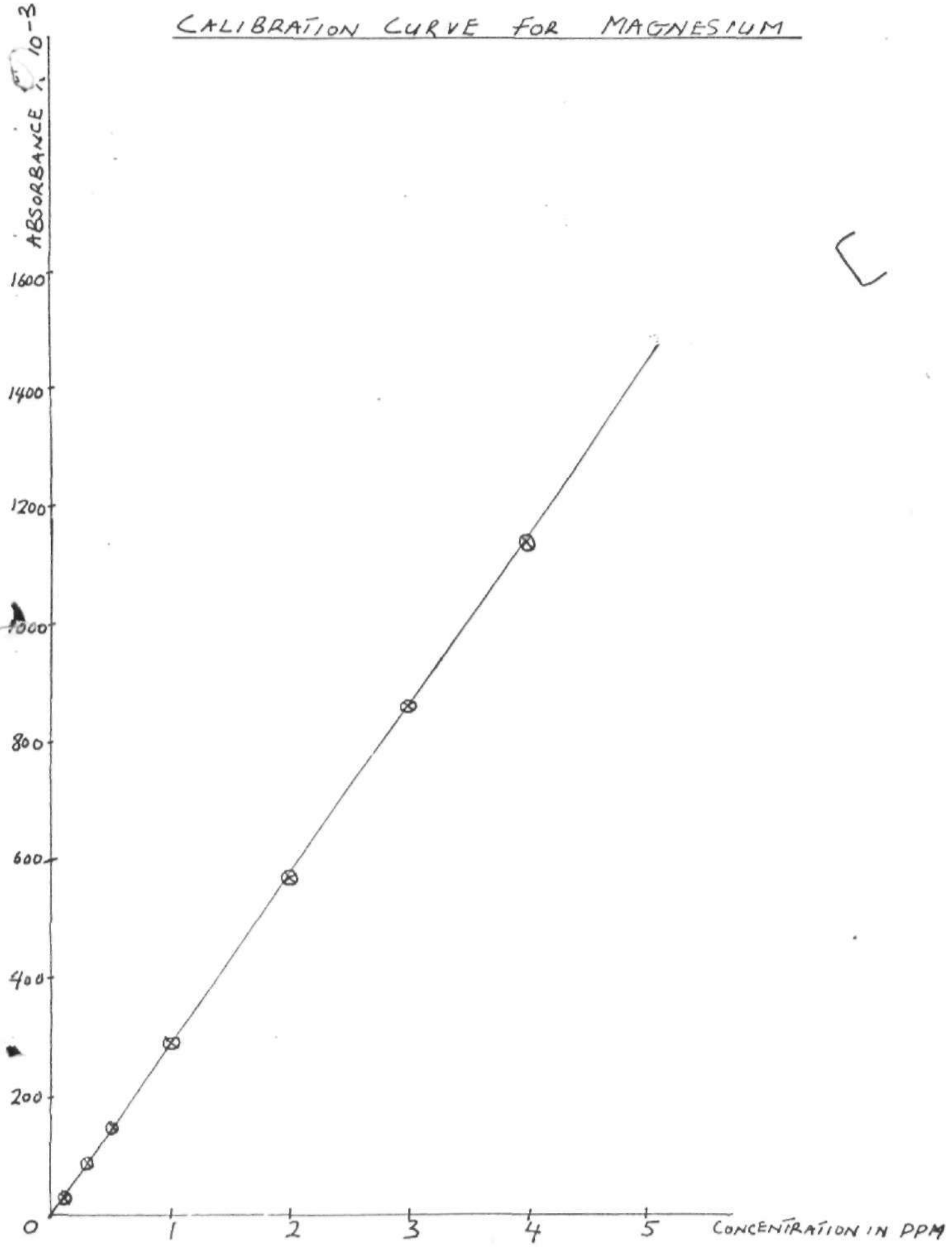
CALIBRATION CURVE FOR IRON



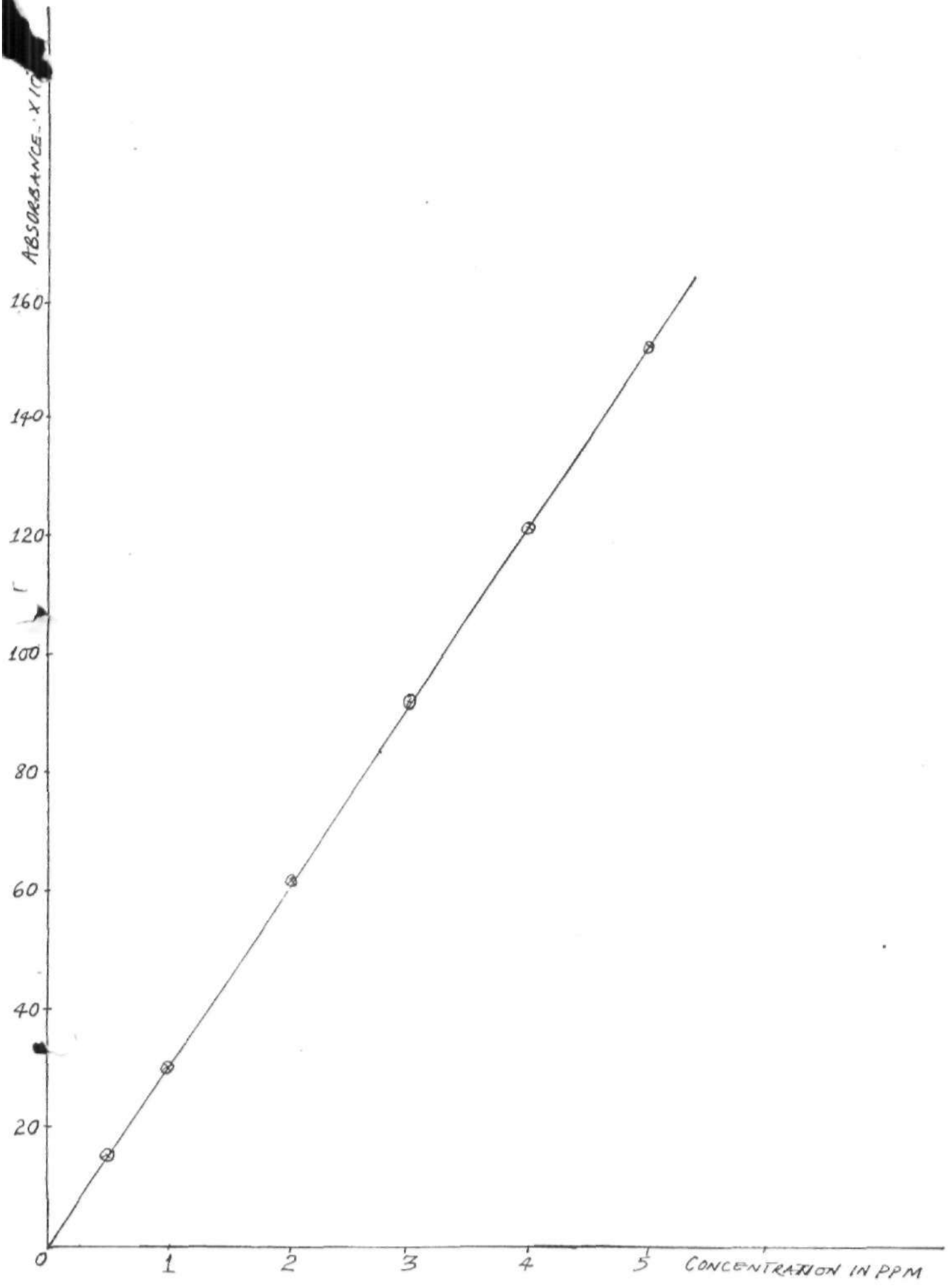
CALIBRATION CURVE FOR LEAD

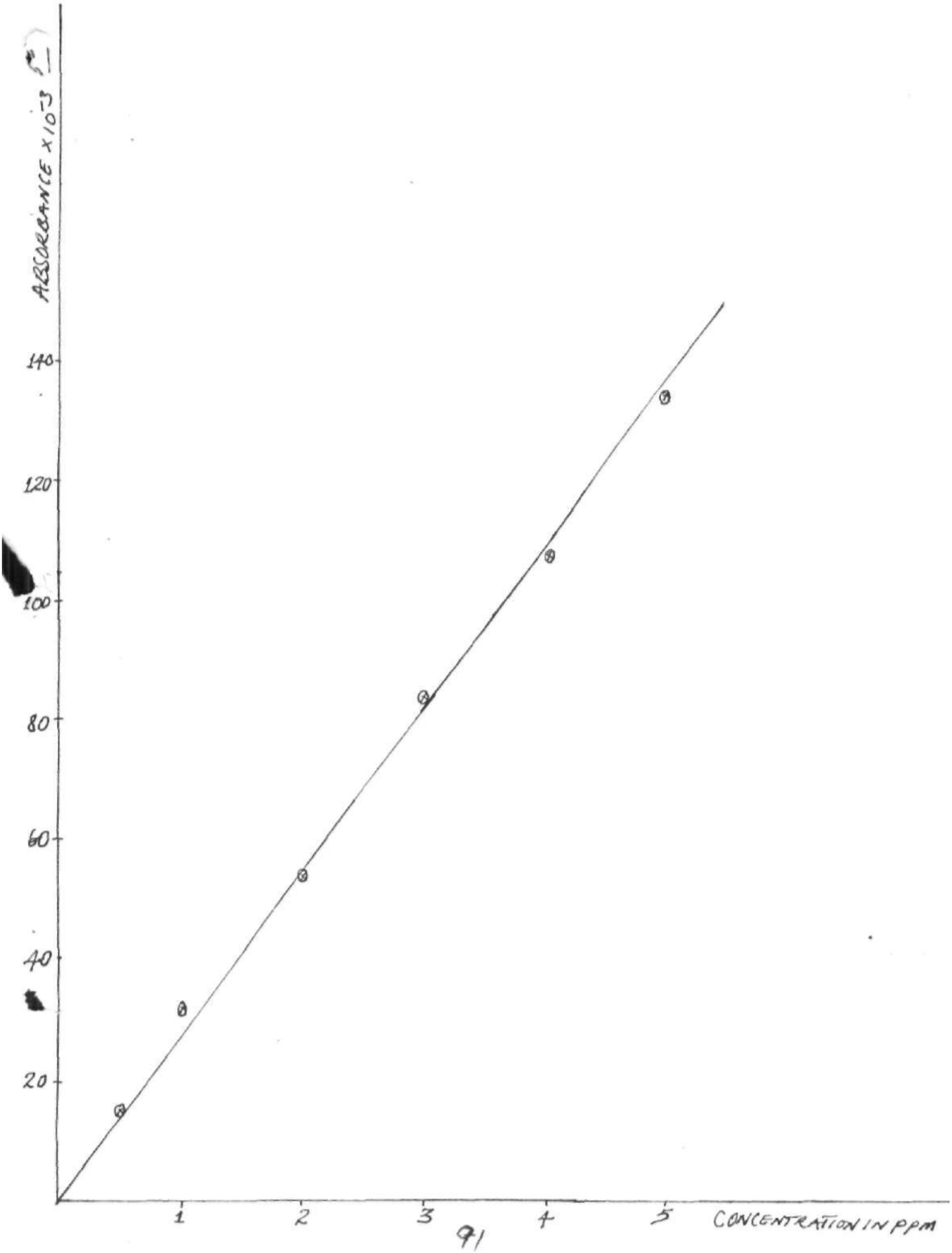


CALIBRATION CURVE FOR MAGNESIUM

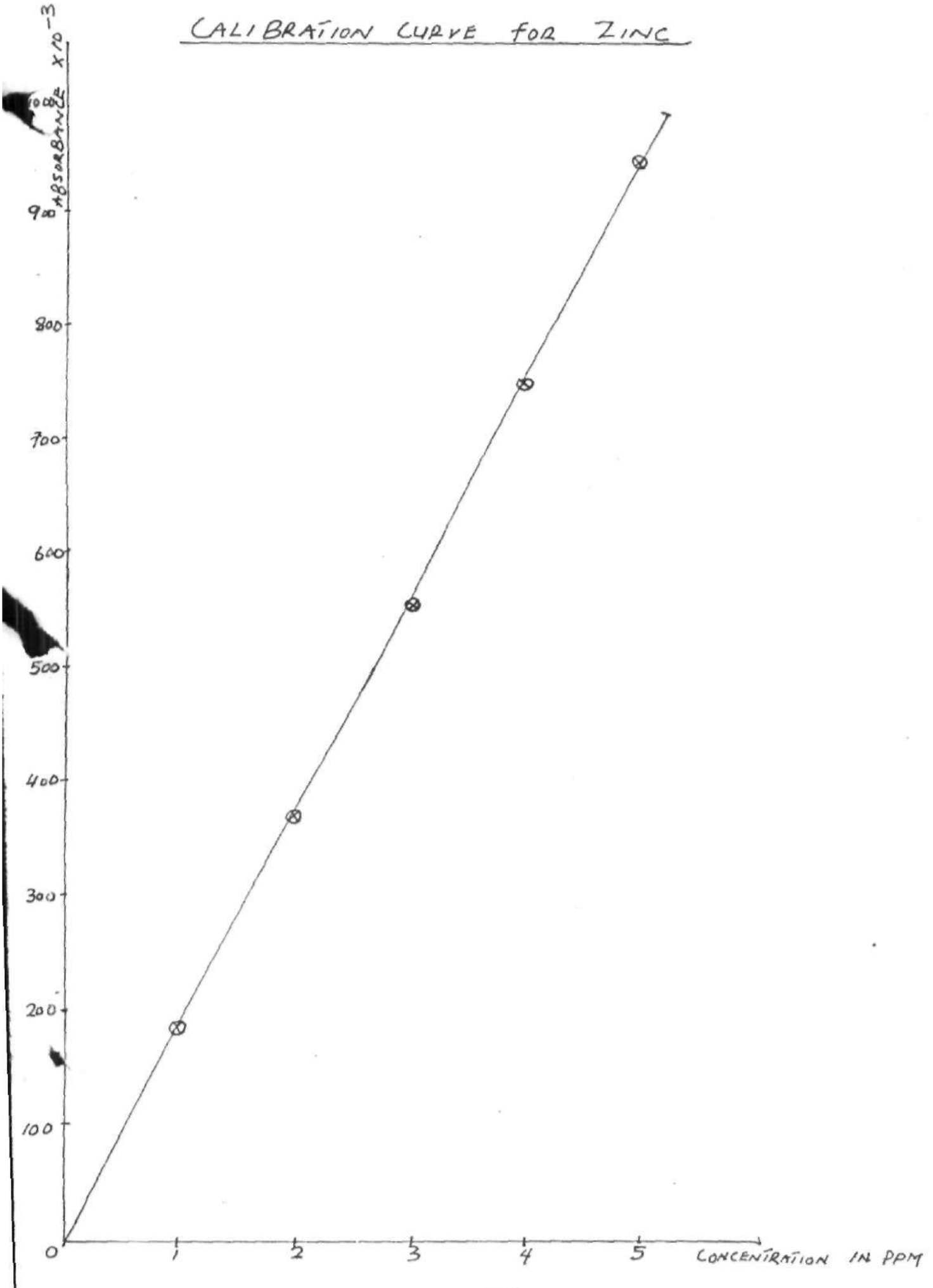


CALIBRATION CURVE FOR MANGANESE




ABSORBANCE X 10⁻³
140
120
100
80
60
40
20
1
2
3
4
5
CONCENTRATION IN ppm

CALIBRATION CURVE FOR ZINC

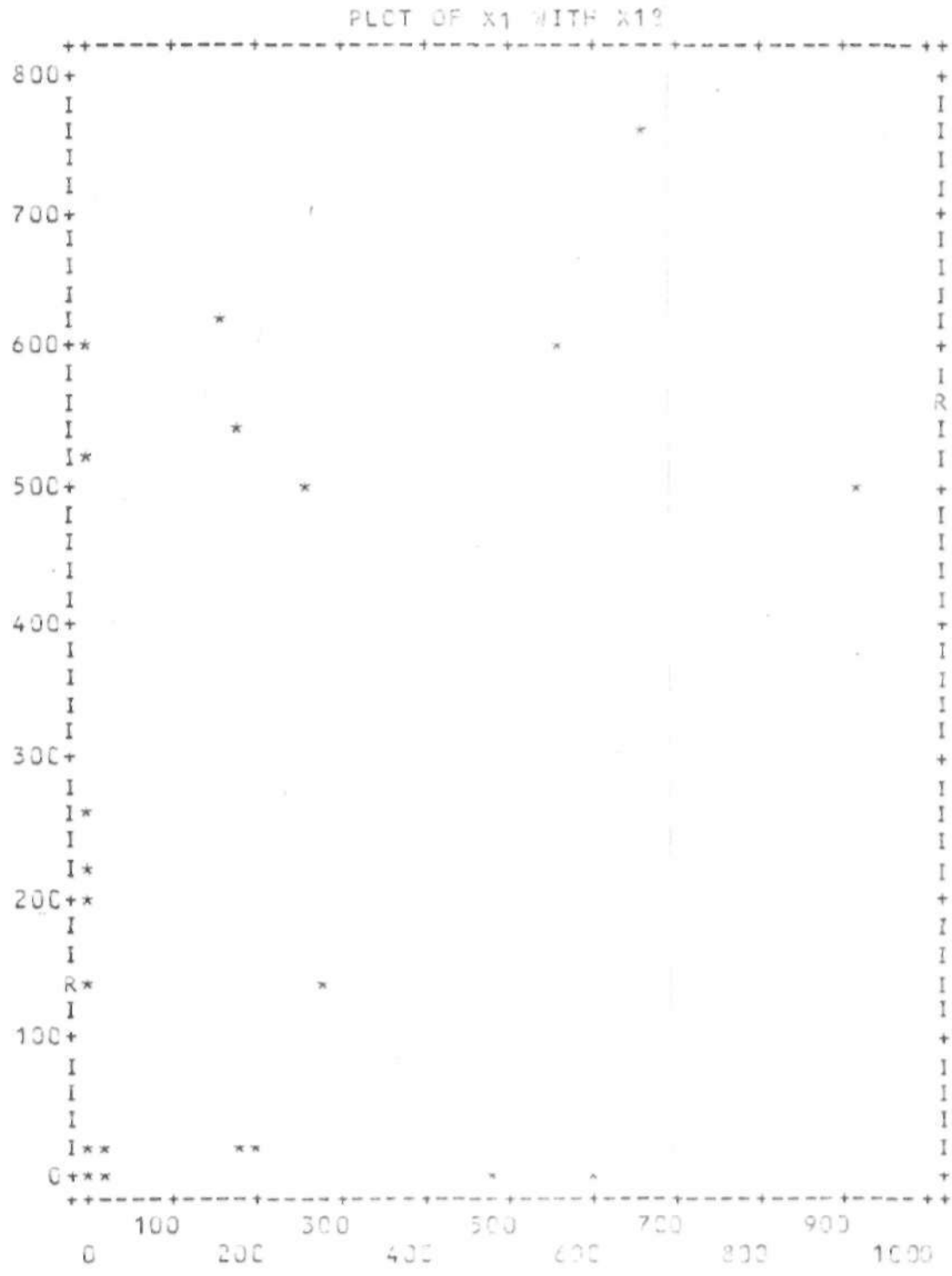


APPENDIX: 2

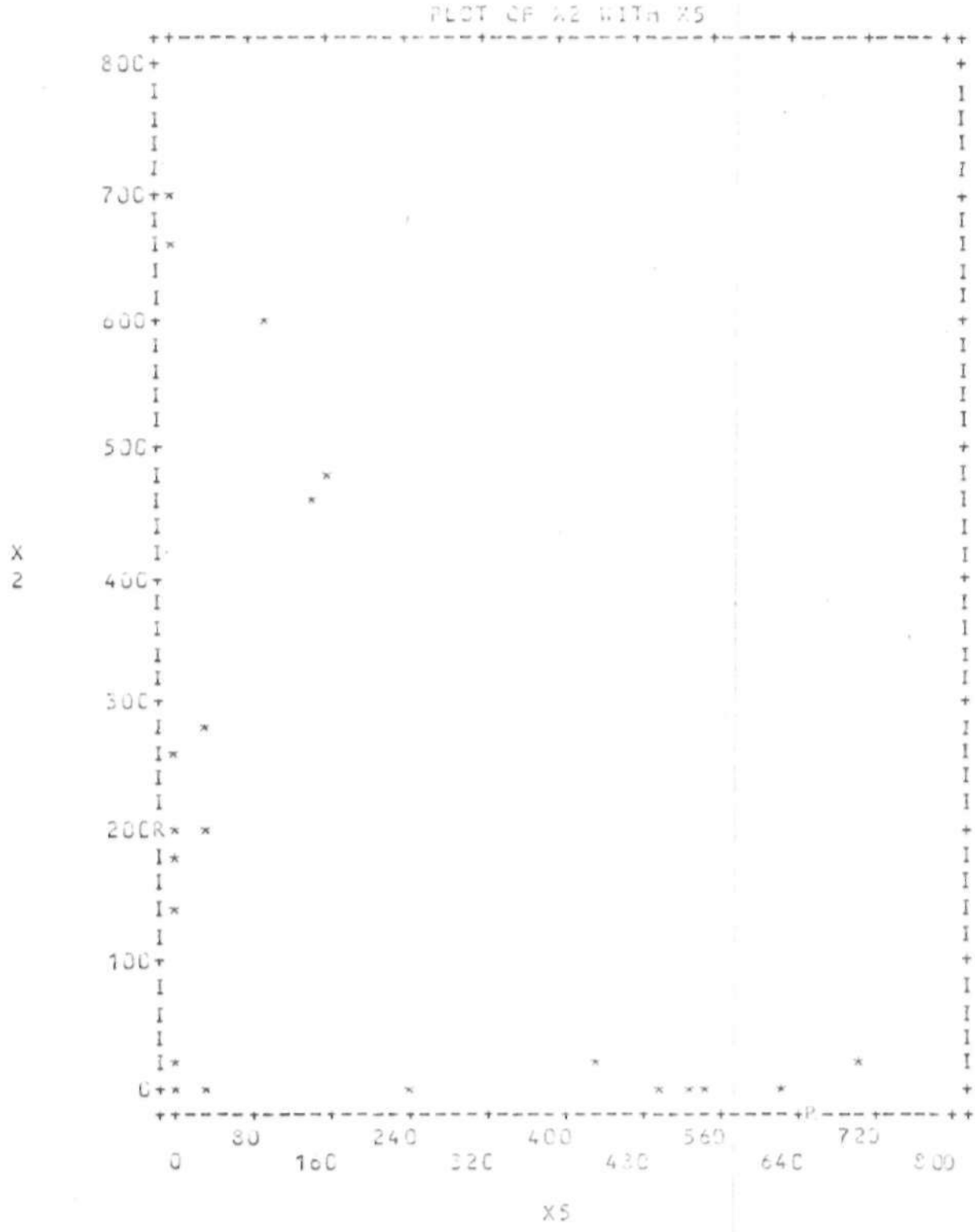
appendix - 2.1 Relationship between total lipid and Copper.

appendix - 2.2 Relationship between total lipids and Zinc

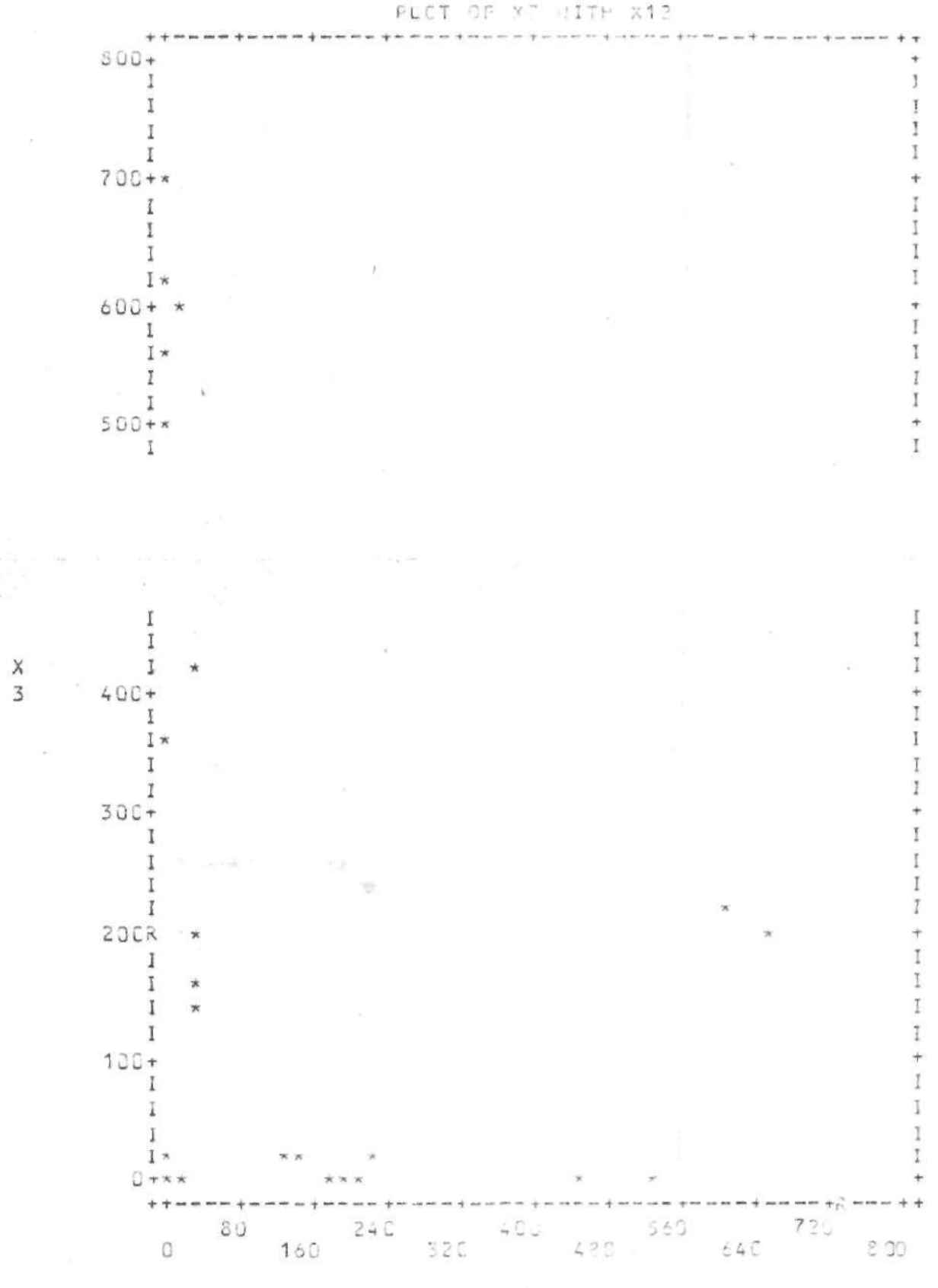
appendix - 2.3 Relationship between Iron and Copper.



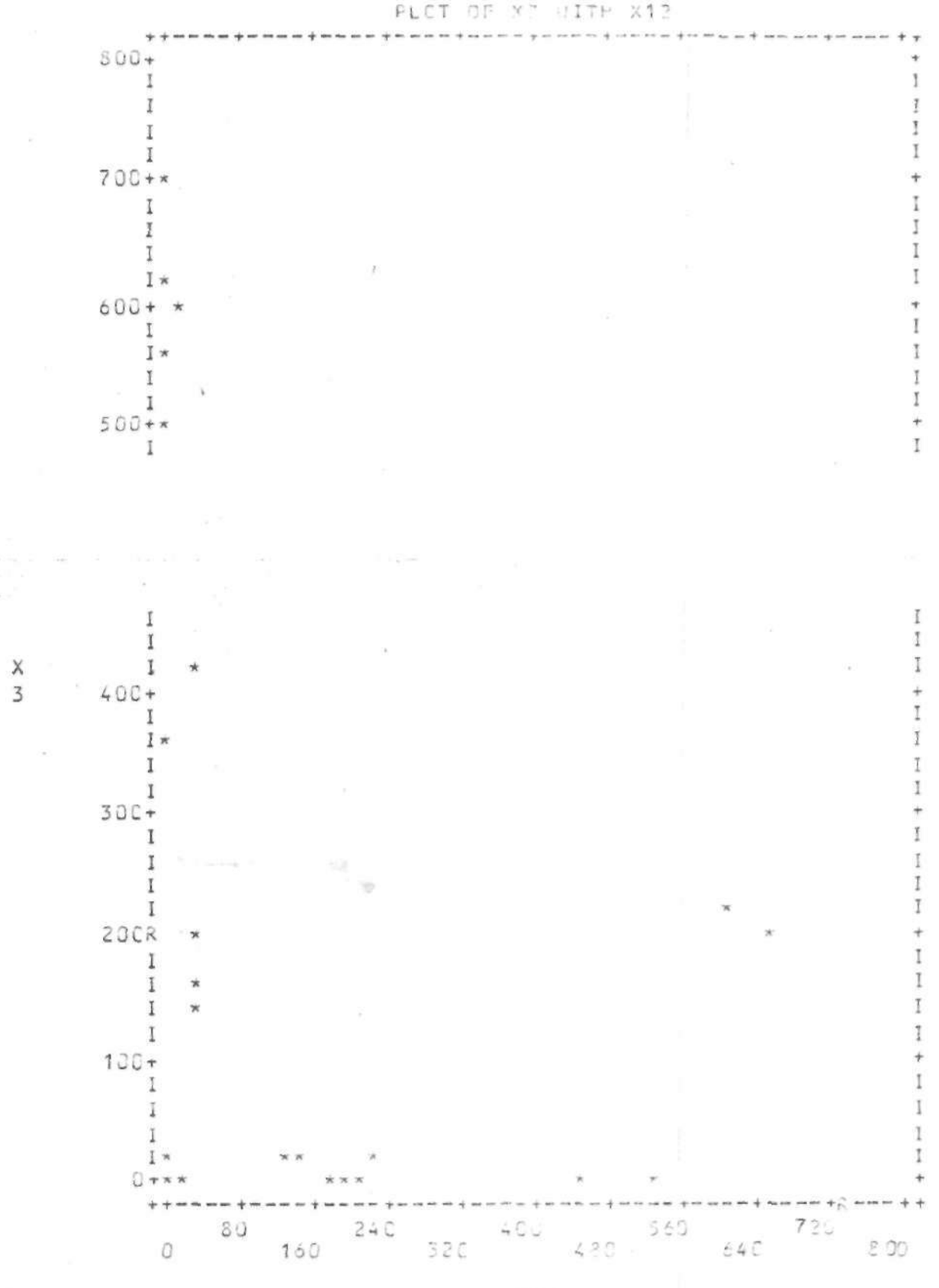
29 cases plotted. Regression statistics of X1 on X13:
Correlation .42595 R Squared .18144 S.E. of Est 232.939



29 cases plotted. Regression statistics of X2 on X5:
Correlation -.30728 R Squared .09442 S.E. of Est 211.7350
Intercept(S.E.) 198.28513(47.56728) Slope(S.E.) -.2994



29 cases plotted. Regression statistics of X3 on X12:
Correlation -.23042 R Squared .05309 S.E. of Est 226.4
Intercept(S.E.) 201.25590(50.33039) Slope(S.E.) b -.3



29 cases plotted. Regression statistics of X3 on X12:
Correlation -.23042 R Squared .05309 S.E. of Est 226.4
(Intercept(S.E.) 201.25690(50.30039) Slope(S.E.) b -.3