ANALYSIS OF LEAD IN COMMONLY USED EYELINERS AND ASSOCIATED BLOOD LEAD CHANGES.

By

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DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented in any previous application for a higher degree. All quotations are indicated and the sources of information are specifically acknowledged by means of references.

Eunice Yetunde Adesodun

Date:----------

DEDICATION

This work is dedicated to the memory of my late immediate sisters Toyin and Titi Adesodun.

ACKNOWLEDGEMENTS

I am very much grateful to my Supervisor Dr. (Mrs.) Neelam Jalil for supervising the project. I wish to thank Prof. K. Singh for his advice.

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E.Y. ADESODUN

ABSTRACT

An effort was made to study the chemical composition of commonly used eyeliners in Zaria and Kano, and to study the associated blood lead changes in patients attending Ahmadu Bello University Sick Bay and Ahmadu Bello university Teaching Hospital Zaria, for various medical ailments.

Physicochemical methods were used to analyse the Kuali samples. The colour (grey and black for white and black Kuali respectively), physical appearance (solid), and melting/decomposition point of the Kuali samples has been noted. The composition of the Kuali samples had. been ascertained on the basis of classical analysis, spot tests and atomic absorption spectrophotometry.

The results of the atomic absorption spectrophotometry revealed the presence of lead zinc, copper, antimony, tin, arsenic, manganese, cadmium, silver, iron and nickel.

Volumetric and gravimetric analyses were performed for the estimation of nonmetals. The nonmetals found were fluoride, chloride, bromide and sulphur.

The concentration of lead in each of the four samples is low compared with other metals in the samples. Sulphur has the highest concentration in all the nonmetals present.

Blood-lead concentrations were measured in thirty patients of whom twenty were using kuali and ten were nonusers of it. The mean blood lead concentration of the twenty people using the kuali is 90.05 ug100ml^{-1} compared with 29.70 uglOOml^{-1} in the ten people who are not using the kuali.

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ABBREVIATIONS

WHO	-	World Health Organisation
A.B.U.T.H.	-	Ahmadu Bello University Teaching Hospital.
A.B.U.S.B.	-	Ahmadu Bello University Sick Bay.
EDTA	-	Ethylene diamine tetraacetic acid.
T.C.A.	-	Trichloroacetic acid.
MIBK	-	Isobutylmethylketone.
APDC	-	Ammonium pyrolidinedithiocarbamate.
B.D.L.	-	Below detection Limit.
r.p.m.	-	Rate per minute

CHAPTER ONE

1.0 INTRODUCTION

1.1 POLLUTION: LEAD AS A POLLUTANT

Lead was not known to be a toxic substance in the past centuries and people used lead and its compounds freely. Series of researches have shown lead and its compounds to be very toxic and could be absorbed by the body. It is a toxic heavy metal which has been found to be one of the major pollutants in the environment (1 - 4).

Pollutant is a substance which adversely alters the environment by changing the growth rate of species, interferes with the food chain and interferes with health, comfort, amenity or property values of people (5).

1.2 SOURCES OF LEAD TOXICITY IN ADULTS

The major type of pollution caused by lead is from automobile exhausts.

Tetraethyl lead is added to gasoline as an antiknocking agent; phasing out of lead compounds with gasoline has resulted in alarmingly high levels of lead in the environment (6).

It has been reported that the atmospheric lead which occurs in particle sizes is readily retained in the

respiratory tract. Reports have also indicated that the soluble lead compounds of the proper particle size can rapidly be absorbed through the mucous membrane of the respiratory tract. People living near the roadside have higher blood-lead concentration than people living far away from the roadside (7).

The lead alkyl compounds in petrol may separate out in petrol-storage tanks over a period of years and concentrate to toxic levels in the sludge material that accumulates in the bottom of such tanks. Studies have shown that the people engaged in the cleaning of the tank were still found to have lead alkyl poisoning despite the provision of safety equipments for use (8).

Peterghem et. al., (9) carried out a study in which the blood lead concentration of technicians working with lead containing lubricants and of assemblers working in a cold mill department were measured and compared with values for men not occupationally exposed to lead. The results showed that these values were in general towards a higher side in people working with the oils.

1.3 SOURCES OF LEAD TOXICITY IN CHILDREN

A child born to a mother with high level of lead in the blood may have a significant level of blood-lead concentration as a result of transplacental lead transfer

leading to neonatal plumbism or even abortion. However, the common sources of childhood plumbism include household dust, chewing lead paints used on toys, cots, bedrails, furniture, indoor paints, licking decorators, paint brushes in addition to other sources like water supply, cooking utensils and food. Findings also confirmed that the occurence of excessive lead absorption in children is increased by household proximity to major urban highway or heavy traffic density (6). The inhalation of automobile exhaust is one of the important factors in the etiology of childhood lead poisoning in urban areas (10).

1.4 LEAD METABOLISM AND ITS TOXICITY

The usual dietary intake of lead in adults averages about 0.30 mg; out of this about 90% passes through the intestinal tract and is not absorbed. The usual respiratory intake of lead is estimated between 5 - 50 µg of lead per day. About 91% of the body lead is stored in the bone in a manner similar to calcium as lead carbonate.

Normal faeces may contain up to 0.28mg of lead per gm and normal urine up to 0.027 mg per litre per twenty four hour sample. Normal person may have concentrations of lead up to 0.03mg per 100ml of blood; lead is transported in plasma as insoluble lead phosphate (11).

Studies in adults revealed that as the sustained daily intake of lead rose above one mg per day, the levels of lead in the blood became higher; metabolic, functional and clinical responses followed. One of the best known adverse effects of lead is its inhibition of the activity of enzymes that are dependent on the presence of sulfhydryl (-SH) groups for their activity (10, 11). Lead affects the Acetyl co-enzyme A cycle interfering with the glycine metabolism. In particular, lead interferes with the formation of haemoglobin by impairing the utilization of iron in the bone marrow and shortens the life span of red blood cells thus resulting in anaemia.

Brain cells may be directly injured or their functions inhibited by lead. Since the Citric acid cycle is of paramount importance in the metabolism of brain the inhibition of the pyruvate and other oxidative system by lead will seriously interfere with the energy supply to the brain cells (11).

The earlier symptoms of lead poisoning are often unrecognised. Vague muscular pains, fatigue and muscular weakness precede the onset of classical symptoms of lead poisoning which include constipation, vomiting, abdominal colic, metallic taste and muscular paralysis by some weeks, and cerebral symptoms which include unconsciousness, optic atrophy, mental retardation in children and psychosis (12 - 15).

Lead exposure at doses below those producing symptoms appears to be associated with neuropsychologic deficit that may interfere with classroom performance of a child (16). David et. al., (17) had described that lead poisoning can produce a variety of effects in children; this may range from acute fatal toxicity or severe organic disability or relatively minor alteration in perception or cognition.

In adults a massive single dose of lead can result in death or severe brain damage and in many cases a chronic exposure to lead may result in a severe brain damage (17).

Hannier (18) described psychological changes from lead poisoning as slowness of performance, psychomotor disturbances, slight intelligence defects and personality changes.

In the past, clinical lead poisoning was widely believed not to happen when the lead concentration in the blood was between 20 and 80 $\mu g100ml^{-1}$ (18). Findings also showed that mild symptoms might be found in the presence of lead values between 60 and 80 $\mu g100ml^{-1}$ in the blood. As the level of lead in the blood rose above 80 $\mu g100ml^{-1}$, the risk of some symptoms increased sharply(11). In the absence of symptoms in children, blood-lead levels exceeding 80 $\mu g100ml^{-1}$ calls for immediate treatment and separation of the child from the source of lead.

The W.H.O has reported that occupational and environmental standards should, wherever possible, be based directly on mortality and morbidity rate (19). A value of 24.4 $\mu g100ml^{-1}$ is taken as normal for an occupationally exposed adult, whereas 55 $\mu g100ml^{-1}$ is abnormal (20).

An increased abdominal pain was found at a lead level greater than 110 $\mu g100ml^{-1}$ and fatigue at a value greater than 120 $\mu g100ml^{-1}$ (20). There is a general tendency for a higher average lead content in younger people and a lower lead content for those people who had passed middle age (21).

Subclinical lead poisoning results from level of lead not sufficient to produce symptoms of clinical poisoning, but enough to impair health and behaviour. Clinical lead poisoning was widely believed not to happen when the blood lead concentration is less than 80 $\mu g100ml^{-1}$, this may well be true, but it does not follow that lower levels are without effects, that is, lead poisoning could be subclinical. Haem synthesis may be impaired in the absence of symptoms at blood-lead level as low as 20 $\mu g100ml^{-1}$ (22).

Results of other findings showed that subclinical nerve damage could be detected in lead workers with no clinical neurological symptoms (23, 24).

1.5 AIMS AND OBJECTIVES OF THIS WORK

The relationship between pollution and lead intoxication has recently attracted considerable attention (18, 25). The review of literature showed a few instances of cosmetic plumbism (26, 27). There is hardly any report from African or Asian countries to this effect, where eyeliners are widely used traditionally either as a principle of hygiene, relief of eye strain, increase in visual acuity, or for cosmetic reasons.

In Nigeria, kuali is a naturally occuring mineral that is commonly used as a black eye-liner. It is usually applied to the conjunctival surfaces and to the eyelids by the teenage girls, young children, and women. The effect of long exposure to lead on childhood development and mainly on pregnant women, due to lead containing cosmetics is of particular concern for health reasons, when there is no legal restriction on the trade of lead containing cosmetics.

Therefore, a need was felt to analyse the commonly used naturally occuring "Kualis" - a mineral used as a black eyeliner, and to study the associated blood lead changes if any.

The following analytical studies have been carried out.

(1) Qualitative and quantitative analyses of some kualis.

(2) Analysis of the amount of lead in the blood of some people using the kualis.

(3) Analysis of the amount of lead in the blood of some people who are not using the kualis.

In order to achieve these objectives, kuali samples were purchased locally at Kano and Zaria markets.

Whole blood samples from twenty women who were kuali users and ten men who were kuali nonusers in the age group of 20 to 30 years, attending ABUSB and ABUTH Zaria for various medical ailments were collected and analysed; care was taken to include only those cases where the possibility of lead ingestion was ruled out. Blood samples were not collected from the following people:

1. Those who were mechanics, or painters by profession, since the concentration of lead in their blood could be raised slightly due to the nature of their work.

2. People living close to the main road.

The kuali samples were subjected to chemical analyses using atomic absorption spectroscopic, volumetric and gravimetric methods.

Blood samples were subjected to chemical analysis using atomic absorption spectroscopic method.

CHAPTER TWO

2.0 SAMPLING AND ANALYSIS

2.1 Sampling technique.

The technique used for sampling is very important in any analysis since this will affect the reliability of the result. The sample for analysis must be representative of the whole material to be analysed. A poor sampling system will not give a reliable result.

The basic aim of sampling includes the following:-

(i) to obtain a sample whose concentrations of determinants are identical to those in the sample of interest at the time of sampling.

(ii) to ensure that the concentration of the determinants in the samples will not change between sampling and analysis.

To achieve the aims above, a survey was conducted on sixty people mostly women who are using eyeliner, and questions were asked on the different types of eyeliner used before the blood samples were collected from some of them.

2.2 TIME INTERVAL BETWEEN SAMPLING AND ANALYSIS

The reliability of the analytical result also depends on the time interval between sampling and analysis. It is always better to carry out the analysis within certain time of sampling. It is also good to specify the maximum tolerable period. The time interval between the collection of blood samples and analysis was not more than seven days (35).

2.3 SAMPLE CONTAINERS

The reliability of the results also depends on the sample container used since this will affect the stability of the sample. A sample container that will introduce minimum contaminants or none to the sample should be used.

Sterile disposable syringe and needle have been found to be suitable for the collection of blood samples, and pyrex sample bottles are ideal for storing the blood samples.

In order not to contaminate the blood samples, the sample bottles should be of a minimum size, that is minimum air space should be left on top of the blood sample, for example, a 2.0ml or 2.5ml sample bottle should be used for storing 2ml of blood.

2.4 SAMPLE PRESERVATION.

A sample needs good preservation so that the composition of the sample does not change between the time of sampling and analysis. Some reactions can occur in the sample, which may affect the composition of the sample, if it is not preserved properly.

To prevent the blood from clotting, an anticoagulant was added (10% EDTA). The blood samples were kept in the freezer as soon as they were collected before the time of analysis.

2.5 THE EYELINERS STUDIED

Most of the people using the eyeliner have been using it from their youth. There are different types of eyeliners, these include the Kuali, eye brown pencil and some others. The one commonly used is kuali. There are different types of kuali, there is the 'white type', the 'black type' and the 'Arabian type'. Most of the people interviewed said they used the Kuali as cosmetics and for eye ailment.

2.5.1 DESCRIPTION OF SAMPLES

Name of Sample.

They are commonly called Kuali in Hausa language, Tiro in Yoruba language and Otanjele in Ibo language.

Purchase of sample.

Four Kuali samples were bought.

First Sample:

The first sample is the white type of kuali, bought in Sabon-gari market Zaria. It is opaque, metallic grey and brittle. It is normally sold in lumps each of about 9 - 10g. Five lumps were bought.

Second Sample.

The second sample is also the white type of kuali, bought in Sabon-gari Market Kano. It is opaque metallic grey and brittle. It is sold in lumps of about 5 - 6g each. Ten lumps were bought.

Third Sample.

The third sample is the black Kuali, it was bought in Sabon-gari market Kano. It is opaque, brittle and black in colour. It is sold in lumps of about 8 - 10g each. Six lumps were bought. The black Kuali is not available in Zaria market.

Fourth sample.

The fourth sample is the white type of Kuali called 'Arabian type' because it was packed in Saudi Arabia. It was bought in Sabon-gari market Zaria. It has been ground and it is normally sold in small bottles of about 3 ml volume. About 3g of the Kuali is in one bottle; six bottles were bought.

EXPERIMENTAL

2.5.2 PHYSICAL APPEARANCE OF SAMPLES.

The pysical appearance of the four samples were studied thoroughly and each of the samples was put in oven for two hours at 100°C, after which their appearances were studied.

2.6 PHYSICAL TESTS

2.6.1 SOLUBILITY

The solubility of the four samples were tested in the following:

(i) Cold and hot water,

(ii) Concentrated and dilute nitric, sulphuric, perchloric, hydrochloric, and acetic acids,

(iii) Aquaregia,

(iv) Some organic solvents, such as 95% ethanol, absolute ethanol, chloroform, liquid parafin.

2.6.2 CONDUCTANCE

The conductance of the samples could not be measured due to their insolubility in various nonpolar solvents.

2.6.3 MELTING/DECOMPOSITION POINTS

The melting/decomposition pointsof the four samples were measured.

2.7 CHEMICAL TESTS

2.7.1 FLAME TEST.

Some grains of each of the four samples were mixed with a drop of concentrated hydrochloric acid and introduced into the flame with a platinum wire which had been cleaned with concentrated hydrochloric acid (29, 38).

2.7.2 QUALITATIVE ANALYSIS

Qualitative analyses were performed on the Kuali samples, by using the classical analysis (29, 40) and spot test methods.

2.7.3 QUANTITATIVE ANALYSIS

Quantitative analyses were also performed on the Kuali samples by using atomic absorption spectroscopic, gravimetric and volumetric methods.

2.8 SPOT TESTS (28, 40)

2.8.1 PRINCIPLE OF SPOT TESTS

'Spot test analysis' is a term which refers to sensitive and selective test based on chemical reactions whereby the use of a drop of the test or reagent solution

is an essential step. The tests are microanalytical or semi-microanalytical in nature and are applicable for the investigation of both inorganic and organic compounds.

Spot tests are ordinarily run by using one of the following techniques.

1. By bringing together one drop each of the test solution and reagent on porous and non-porous supporting surfaces such as paper, glass, or porcelain.

2. By placing a drop of the test solution on a medium impregnated with appropriate reagents (filter paper, asbestor gelatine).

3. By placing a drop of reagent solution on a small quantity of the solid specimen.

4. By subjecting a drop of reagent or a strip of reagent paper to the action of liberated gases from a drop of the test solution or from a minute quantity of the solid specimen.

In spot test analysis, each of the cations and anions has its own principle of detection, since. they

can be detected with different reagents and under different experimental conditions, so the principle of each of the metals detected will be discussed briefly, before discussing the experimental procedure.

Tin.

The detection of tin is based on the fact that stannous chloride can react with phosphomolybdic acid and its salts, and reduce them to 'molybdenum blue' which is a colloidal dispersed mixture of lower molybdenum oxides.

Antimony.

The principle is based on the fact that antimony III salts have the property of reducing phosphomolybdic acid to 'molybdenum blue' when warmed, but unlike tin, they react with free phosphomolybdic acid or its soluble salts and not with insoluble phosphomolybdates.

The test is carried out in the solution obtained by warming the precipitate (residue I) with 1:1 HCl (Section 2.8.3). In this solution, the antimony is always present as the trichloride, and the tin as tetrachloride, so that the tin does not interfere with the test.

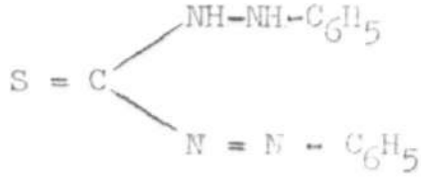
Silver.

It is based on the fact that on mixing manganese and silver solutions with alkalis, a black precipitate will be formed. The black precipitate consist of manganese dioxide and metallic silver.

The reaction takes place in very low silver ion concentration.

Lead.

The test for the detection of lead is carried out with dithizone (diphenylthiocarbazone). Dithizone I precipitatesthe red inner complex lead salt(II) from neutral solution.



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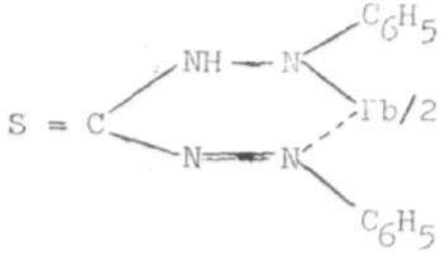


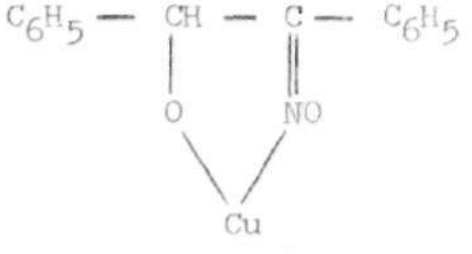
(II)

Copper.

The test was carried out with benzoinoxime.

Benzoinoxime(I) behaves towards copper ions as a dibasic acid in that it forms, in neutral or ammoniacal solutions, a green amorphous precipitate of copper salt with structure II.

(I)



(II)

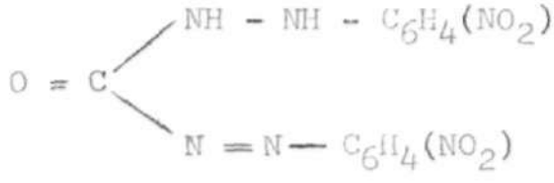
The benzoinoxime reaction was carried out after the residue has been taken up in a drop of dilute hydrochloric acid.

Cadmium.

The reagent used for the test was di-p-nitrophenylcarbazide. Cadmium hydroxide can be coloured

brown by di-p-nitrophenylcarbazide(I); on standing for some time, the colour changes to green-blue; this is because the carbazide is oxidized to carbazone(II). The colour change is accelerated by formaldehyde.

(I)



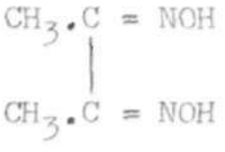
(II)

Iron.

The presence of iron can be detected with potassium thiocyanate. Iron(III) ions react with thiocyanat ions in acid solution to yield a red colour of ferri-thiocyanate ion.

Nickel.

The test for nickel was carried out with dimethylglyoxime(I). In the presence of large amount of nitrates and other oxidizing agents, small amount of nickel is not precipitated. A red precipitate of nickel dimethylglyoxime(II) is formed in an alkaline solution of nickel salt and dimethylglyoxime.



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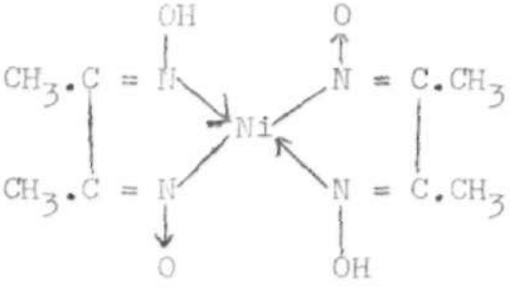


(II)

Zinc.

The test was carried out with dithizone. (diphenylthiocarbazone). Dithizone forms insoluble purple-red complex salts with zinc salts. The purple-red zinc salt, which can be formed in neutral, alkaline and acetic acid solutions, is soluble in carbon tetrachloride without change in colour.

Arsenic

The presence of arsenic was detected with the solution of stannous chloride. Compounds of arsenic(III) and arsenic(V) can be reduced to arsenic by solutions of stannous chloride which is strongly acidified with hydrochloric acid. The arsenic separates as a brownish black precipitate. The reduction was accelerated by heating. As a result of the fact that some arsenic compounds are volatile, the arsenic was converted into a heat resistant magnessium pyroarsenate.

Floride.

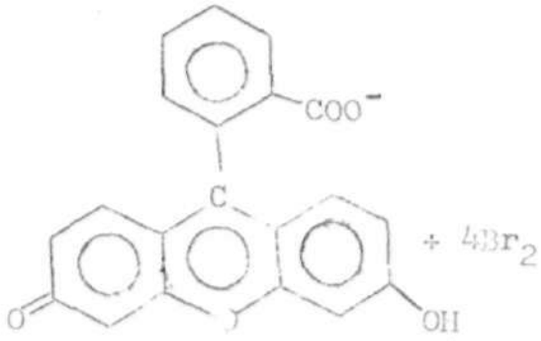
The test was carried out with zirconium alizarin solution. The addition of alizarin or alizarin sulfonate to dilute solutions of zirconium chloride containing hydrochloric acid results in a red-violet colour due to the formation of hydrosols of zironium compound with these dyestuffs. The dispersions turn yellow as soon as they are treated with excess fluoride. The zirconium combined with fluoride to form a complex $[ZrF_6]^{2-}$ anions.

Bromide

Bromide was detected by Fluorescein test.

Free bromine converts the yellow dye fluorescein(I) into red tetrabromofluorescein (eosin)(II). To carry

out this test for bromide, the bromide must be oxidized to free bromine. The liberation of bromine from bromides takes place on prolonged heating with an acetic acid - hydrogen peroxide mixture in the presence of fluorescein.



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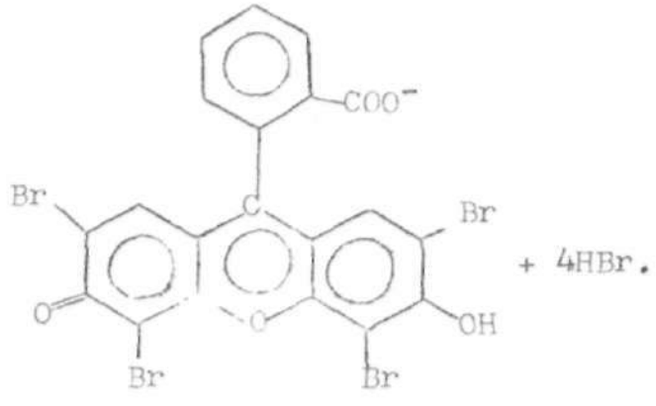

(II)

Chloride.

The test was carried out by the precipitation of silver chloride. Bromides and iodides were oxidized to bromine and iodine respectively, upon the addition of

hydrogen peroxide. If this oxidation is conducted in the presence of 8-hydroxyquinoline (oxine) this phenolic compound is brominated or iodized by the free halogen. The acid solution of the halogenated oxine does not react with silver nitrate and consequently only the unchanged chloride ions will be precipitated as silver chloride.

Sulphur. (sulphite)

Sulphites and free sulphorous acid even in concentrated solutions give only a pale-red colour with sodium nitroprusside. The colour is appreciably deepened by the addition of a saturated solution of zinc sulphate or nitrate and a few drops of a potassium ferrocyanide solution, then a red precipitate is formed.

Instrument and Apparatus.

1. Spot plates
2. Silica crucibles 2.5cm internal diameter,
3. Grinder. Herbert Alexander. England.

Reagents.

The reagents used were either of analytical grade or spot test grade.

1. Hydrochloric acid (S.G.1.18)
2. Nitric acid (S.G.1.42)
3. Sulphuric acid (S.G.1.84)
4. Acetic acid glacial
5. 5% phosphomolybdic acid
6. Ammonia concentrated
7. Potassium hydroxide 4M
8. Sodium hydroxide 10%
9. Manganese nitrate solution 0.05M
10. Potassium cyanide 10%
11. Potassium thiocyanate 1%
12. Stannous chloride
13. Magnessium chloride solution 10%
14. Hydrogen peroxide
15. Sodium nitroprusside solution 1%
16. Zinc nitrate
17. Potassium ferocyanide solution 0.5
18. Silver nitrate 1%
19. Dithizone solution 0.001%
20. Benzoinoxime 5%
21. Di-p-nitrophenylcarbazide 0.1%
22. Formaldehyde 40%
23. Dimethylglyoxime solution 1%
24. 8-hydroxyquinoline (oxine)
25. Fluorescein solution 1% - prepared by dissolving

1g of fluorescein dye in 100ml of 70% ethanol.

26. Zirconium-alizarin solution:- prepared by digesting Zirconium oxide with warm dilute HCl and filtered. The filterate was treated with a slight excess of an alcoholic solution of alizarin.

Preparation of sample solutions.

In order to carry out the spot test analysis on the four kuali samples. Feigl spot method of systematic analysis of mixtures had been followed.

Procedure.

The first, second and third samples were ground to a fine powder with a grinder.

10mg of the first sample was carefully weighed and put in a porcelain dish, 4ml of concentrated hydrochloric acid and 1ml of conc. nitric acid were added to the sample in the dish together with a few drops of bromine water.

The content was evaporated to dryness on a water bath.

The residue was taken up with a little 2M nitric acid, filtered and washed. The filtrate and residue were labelled solution I and residue I. respectively.

Residue I was tested for tin and antimony. A few drops of concentrated hydrochloric acid were run through the residue on the filter paper, and the test for antimony was made on the 2 drops of the filterate. The rest of the filterate and residue are treated with zinc chips and tested for tin.

A drop of solution I was tested for silver; the rest was heated with a few drops of sulphuric acid to white fumes, and the residue was taken up in about 2ml of water, filtered and washed. The precipitate and solution were labelled precipitate II, solution II. 4M potassium hydroxide was poured over the precipitate and the filterate was tested for lead.

Hydrogen sulphide gas was passed into solution II and the precipitate was filtered. The precipitate and the filterate were labelled as precipitate III, solution III respectively. Precipitate III was dissolved in hot 2M Nitric acid and the filterate got was used to test for copper and cadmium.

Solution III was oxidized with bromine water, the excess bromine was removed by concentrating the solution

to 2ml. The solution was divided into 2 portions. One portion was used to test for iron, nickel and manganese by suitable spot tests. 2M potassium hydroxide was added to the other portion and it was used to test for aluminium and zinc.

The above procedure was repeated for the second, third and fourth samples, after which the individual cations were detected in the four samples by various tests using the labelled solutions and precipitates above.

The four samples were digested in sodium carbonate solution as described in section 2.10, and the extracts were used for the detection of anions.

2.8.3 DETECTION OF CATIONS AND ANIONS

Detection of tin.

A quantitative filter paper was impregnated with 5% solution of phosphomolybdic acid and held over ammonia for a short time. A yellow spot of insoluble ammonium phosphomolybdate was got, and it was dried. The filter paper was kept in well stoppered bottles which was placed in the dark.

A drop of the test solution which has been warmed with 1:1 hydrochloric acid was placed on the filter paper. A deep blue colour was formed, which confirmed the presence of tin in the sample.

Detection of antimony.

A drop of the test solution was placed on filter paper which has been impregnated with 5% aqueous solution of phosphomolybdic acid, and held over steam. After about 2 minutes; a blue colour which confirmed the presence of antimony appeared.

Detection of silver.

A drop of 0.1M HCl was placed on the filter paper, followed by a drop of the test solution in the middle of moist fleck, and then a further drop of 0.1M HCl. The fleck was blackened on the addition of a drop of 0.05M manganese nitrate and a drop of 0.1M sodium hydroxide.

Detection of Lead.

A drop of the test solution was vigorously shaken in a small test tube with a drop of carbon tetrachloride solution of dithizone. The green reagent turned to a brick red colour.

Detection of Copper.

A drop of the weakly acidic test solution was treated on filter paper with a drop of a 5% alcohol solution of benzoinoxime, and held over ammonia. A green colour was observed. This indicated the presence of copper.

Detection of Cadmium.

A drop of the acidic test solution was mixed on a spot plate with a drop of 10% sodium hydroxide and one drop of 10% potassium cyanide; then, a drop of 0.1% alcoholic solution of di-p-nitrophenylcarbazide, and two drops of 40% formaldehyde were added with stirring. A blue-green precipitate was formed.

Detection of Iron.

A drop of the test solution was mixed on a spot plate with a drop of 1% potassium thiocyanate solution. A deep red colour which confirmed the presence of iron was got.

Detection of Nickel.

A drop of the test solution was mixed on a spot plate with 2 drops of saturated bromine water, and after 2 minutes, excess ammonia solution was added. This was followed by a drop of 1% alcoholic dimethylglyoxime solution. A reddish orange colour was got.

Detection of Zinc.

A drop of the test solution and a drop of a solution of dithizone in carbon tetrachloride were shaken together in a small stoppered test tubes, after about 2 minutes, a purple-red precipitate was got.

Detection of Arsenic.

A drop of the test solution was mixed in a small silica crucible, with 2 drops of ammonia, a drop of 10% hydrogen peroxide, and a drop of 10% magnessium chloride solution.

The mixture was evaporated slowly and then strongly ignited. The residue was mixed with 2 drops of a concentrated stannous chloride solution in 35% hydrochloric acid and warmed gently. A brownish-black precipitate confirmed the presence of arsenic.

Detection of fluoride

A strip of quantitative filter paper was impregnated with zirconium-alizarin solution. The dried paper was moistened with a drop of 50% acetic acid and then, a drop of the test solution was placed on the moist fleck. A yellow spot was got.

Detection of Chloride

A drop of the test solution was warmed in a microtest tube with a drop of oxine solution, a drop of hydrogen peroxide solution and microdrop of nitric acid for about 4 mins. A drop of 1% silver nitrate solution was added; after about 2 mins, a white turbidity was got.

Detection of Bromide

A drop of the test solution was mixed with a microdrop of 1% alcoholic fluorescein solution, and a microdrop of a 10:1 mixture of glacial acetic acid and 30% hydrogen peroxide; the content was evaporated to dryness in small silica crucible over the water-bath. A pink colour appeared which confirmed the presence of bromine from bromide.

Detection of Sulphite

A drop of 0.5M solution of potassium ferrocyanide was added to a drop of a cold saturated solution of zinc nitrate and then a drop of 1% solution of sodium nitroprusside was added. A white precipitate of zinc ferocyanide was got; after this, a drop of the test solution was introduced. A red precipitate was produced.

All the above tests were carried out for the first, second, third and fourth samples and the results got in each case are summarized in table 3.4.

2.9 ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF LEAD, COPPER, ZINC, ANTIMONY, IRON, ARSENIC, NICKEL, CADMIUM, TIN, SILVER AND MANGANESE. (30, 31, 32,39).

Principle of atomic absorption spectroscopy.

Atomic absorption spectroscopy is a technique which is widely used for metal analysis in biological fluids and tissues, in environmental samples such as air and water, and in occupational health and safety areas.

The method is based upon the absorption of radiation by free atoms in vapour state. The sample solution is aspirated into a flame and the sample element is converted to atomic vapour. The flame then contains atoms of that element. Some of the atoms are thermally excited by the flame, but most of them remain in the ground state. These ground state atoms can absorb radiation given off by the hollow cathode lamp which is used as the light source, containing the element to be determined. The wavelength of radiation absorbed by the atoms in the flame is the same as the wavelengths of radiation given off by the hollow cathode lamp.

The decrease in intensity is measured using a monochromator and detector systems. This decrease is related to the concentration of the element in solution.

The accuracy of the atomic absorption spectrometer can be increased by stability of the hollow cathode lamp which is the light source; this also depends on appropriate current, smooth nebulization and cleaniness of the burner.

Sensitivity of the apparatus is affected by various factors which include selection of burning gas, the state of the flame, efficiency of the nebulizer, working condition of the light source, optical systems and dispersion ability of the diffraction grating.

The type of fuel and oxidant to be used depends largely on the flame temperature required to atomise the sample. The most widely used flame for atomic absorption are the air-acetylene flame and the nitrous oxide acetylene flame with premix burners. The nitrous oxide acetylene flame with a high temperature is used for the elements that tend to form heat stable oxides in the air-acetylene flame. The air-acetylene flame absorbs a large fraction of the radiation at wavelengths below 200nm.

Instrument and Apparatus.

1. SP 1900 Unicam Atomic Absorption spectrometer. (air-acetylene flame was used) individual cathode lamp.
2. Elgastat deioniser.

Reagents.

All chemicals used were of analytical grade unless otherwise stated.

1. 0.1M EDTA: prepared by dissolving 37.224g of disodium dihydrogenethylene diaminetra-acetate (which was dried at 80°C for 2 hours) in water and making up to a litre.

2. Eriochrome Black T: prepared by dissolving 0.2g of the dyestuff in 15ml of triethanolamine with the addition of 5ml of absolute ethanol.

3. Xylenol orange: prepared by dissolving 0.5g of the compound in 100ml of water.

4. Murexide: prepared by shaking 0.5g of the powder with water and using the supernatant liquid for titration.

5. 0.025M potassium iodate: some potassium iodate was dried at 120°C for 1 hr and allowed to cool in a desiccator. 5.350g of the finely powdered potassium iodate was weighed and dissolved in a litre flask with deionise water. The solution was made up to the mark.

Preparation of standards for the individual metal ions.

Lead:

1000ppm stock solution of lead was prepared by dissolving 1.598g of lead nitrate in 25ml of nitric acid (1:1 v/v) and the volume was made up to a litre. The solution was standardized using 0.1M EDTA solution with xylenol orange indicator (32). An intermediate 10 ppm lead solution was prepared by diluting 1 ml of the stock to 100ml with water. From this 1 to 5 ppm lead standards were obtained. Calibration curve for lead is given in figure 2.1.

Copper.

1000 ppm stock solution of copper was prepared by dissolving 3.930g of copper sulphate pentahydrate in a small quantity of water and making up to one litre. The solution was standardized using 0.025M potassium iodate (32) 10 ppm intermediate copper standard was prepared by diluting 1 ml of the stock to 100 ml with water. Copper working standards in the range of 1 - 5 ppm were prepared from this. Figure 2.2 shows the calibration curve for copper.

Zinc.

A 1000 ppm stock solution of zinc was prepared by dissolving 4.398g of zinc sulphate heptahydrate in water and making up to a litre. The solution was standardized with 0.1MEDTA solution using Eriochrome Black T as indicator (32).

10 ppm intermediate standard solution of zinc was prepared by diluting 1 ml aliquot of the stock to 100ml. 1 - 5 ppm working standards were prepared by appropriate dilution of the intermediate with water. The calibration curve is shown in figure 2.3.

Antimony.

1000 ppm stock solution of antimony was prepared by dissolving 1g of antimony metal (granules) in 10ml of concentrated nitric acid. The solution was thoroughly mixed and diluted to one litre with deionised water. The solution was standardized with 0.025M potassium iodate (32) 100 ppm intermediate antimony standard was prepared by diluting 10ml of the stock solution to 100ml, antimony working standards in the range of 1 - 10 ppm were prepared from this. Figure 2.4 shows the calibration curve for antimony.

Iron.

1000 ppm stock solution of iron was prepared by dissolving 4.830g of Ferric chloride in deionised water. 1.5ml of conc. hydrochloric acid was added and the solution was diluted to one litre with deionised water. The solution was standardized using 0.1M EDTA and Eriochrome Black T as indicator (32). 100 ppm intermediate standard was prepared from the stock solution and iron working standards in the range of 1 - 15 ppm were prepared from this. The calibration curve is given in figure 2.5.

Arsenic.

A 1000 ppm stock solution of arsenic was prepared by dissolving 1.320g of arsenic trioxide As_2O_3 in 50ml of concentrated hydrochloric acid and diluting to one litre with deionised water. The solution was standardized with 0.025M potassium iodate (32). An intermediate solution of 10 ppm was prepared from the stock solution by diluting 1 ml of it to 100ml. Working standards in the range of 1 - 5 ppm were prepared from this. The calibration curve is given in figure 2.6.

Nickel.

1000 ppm stock solution of nickel was prepared by dissolving 4.953g of nickel nitrate hexahydrate in 200ml of deionised water. 1.5ml of concentrated nitric acid was added and made up to one litre with deionised water. The solution was standardized using 0.1M EDTA with murexide as indicator (32). 100 ppm intermediate standard was prepared by diluting 10ml of the stock solution to 100ml. Working standards in the range of 1 to 10 ppm were prepared from the intermediate standard. Calibration curve is given in figure 2.6.

Cadmium.

1000 ppm cadmium stock solution was prepared by dissolving 2.1071g of cadmium nitrate in water and making up to a litre. The solution was standardized with 0.1M EDTA with xylenol orange as indicator (32). 10 ppm cadmium intermediate standard was obtained from the stock by diluting 1ml of the stock to 100ml. 1 to 5 ppm cadmium working standards were obtained from the intermediate stock by appropriate dilution. The calibration curve is given in figure 2.7.

Tin.

A 1000 ppm stock solution of tin was prepared. 2.954g of stannous chloride pentahydrate was dissolved

in about 500ml of deionised water, and a few drops of concentrated hydrochloric acid was added. The solution was made to one litre with deionised water. The solution was standardized with 0.025M potassium iodate (32). An intermediate solution of 100 ppm was prepared from the stock solution. Working standards in the range of 1 - 10 ppm were prepared from this. The calibration curve is given in figure 2.8.

Silver.

1000 ppm stock solution of silver was prepared by dissolving 1.575g of anhydrous silver nitrate in deionised water, and 1.5ml of concentrated hydrochloric acid was added. The solution was diluted to one litre with deionised water. The solution was standardized using 0.1M EDTA and murexide indicator (32). 100 ppm intermediate standard was prepared, from which working standards in the range of 1 to 10 ppm were obtained. The calibration curve is given in figure 2.9.

Manganese.

A 1000 ppm solution of manganese was prepared by dissolving 4.069g of manganous sulphate tetrahydrate in water containing 1.5ml concentrated nitric acid and the volume was made up to one litre. The solution

was standardized using 0.1M EDTA with Eriochrome Black T indicator (32). An intermediate manganese solution of 10 ppm was prepared from the stock solution, by diluting 1ml of the stock solution to 100 ml. From this 1 to 5 ppm working standards were prepared. The calibration curve is given in figure 2.10.

2.9.1 PREPARATION OF SAMPLE SOLUTIONS.

Procedure.

The sample solutions were prepared by digesting the samples in perchloric acid.

1.0g of each of the samples was accurately weighed and poured inside a 250ml beaker. 40ml of concentrated perchloric acid was added to each in a beaker and the contents were put on a hot plate inside the fume cupboard. They were left for about 30 minutes on the hot plate after which they were left to cool. After cooling, each of the solutions was diluted to one litre with deionised water.

2.9.2 INSTRUMENTAL OPERATION AND SAMPLE ANALYSIS.

The appropriate wavelength was selected for each of the metals and appropriate hollow cathode lamps were inserted. All other instrumental conditions were adjusted. The instrumental conditions for the determination of each metal is given in table 2.1.

The standard solutions for the metals were first aspirated into the flame to obtain their absorbance; after this the sample solutions were aspirated into the flame; concentration of the metals in ppm in each of the sample solutions was read off from the calibration curves.

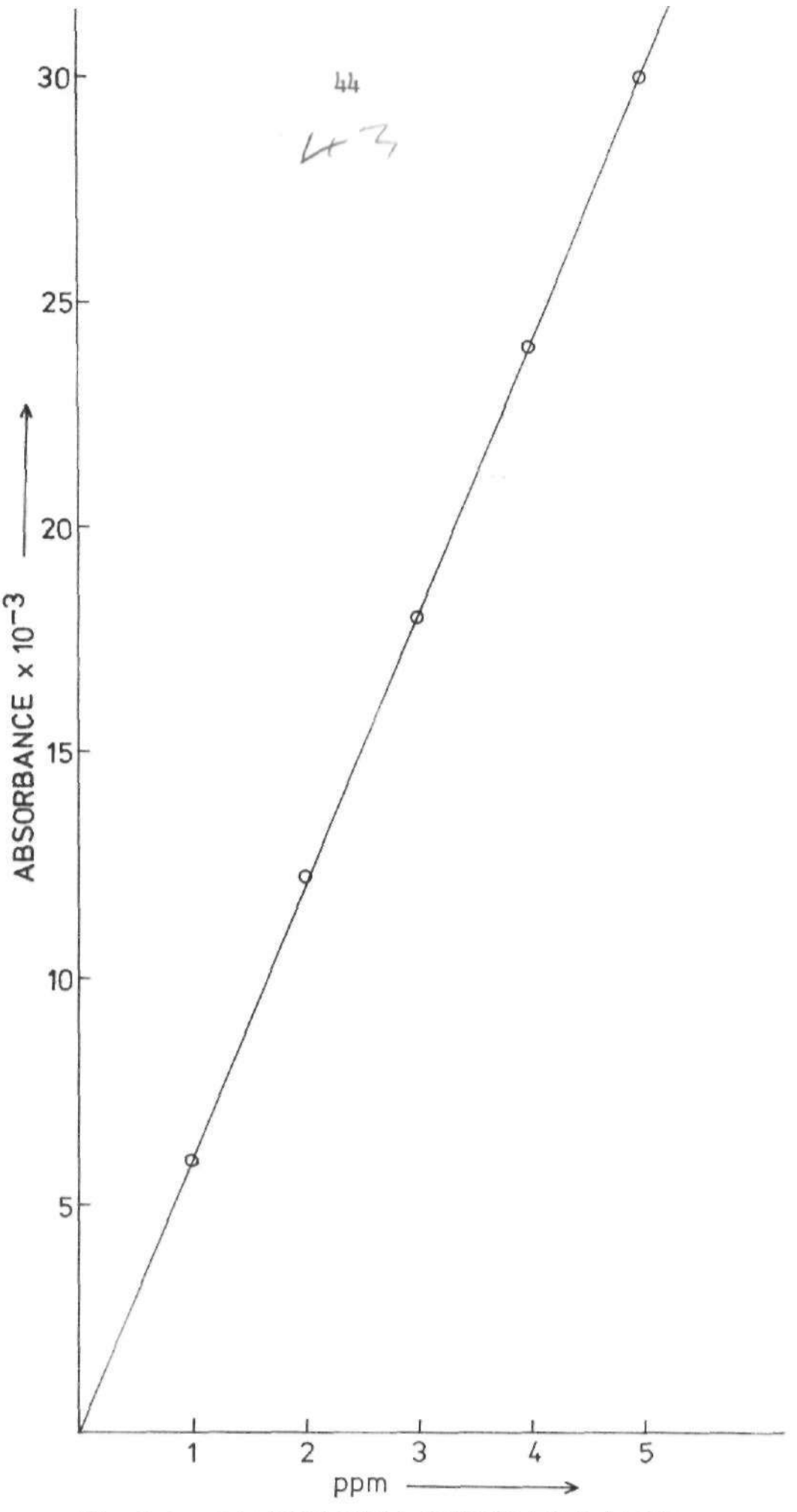

Fig. 2-1 CALIBRATION CURVE FOR LEAD.

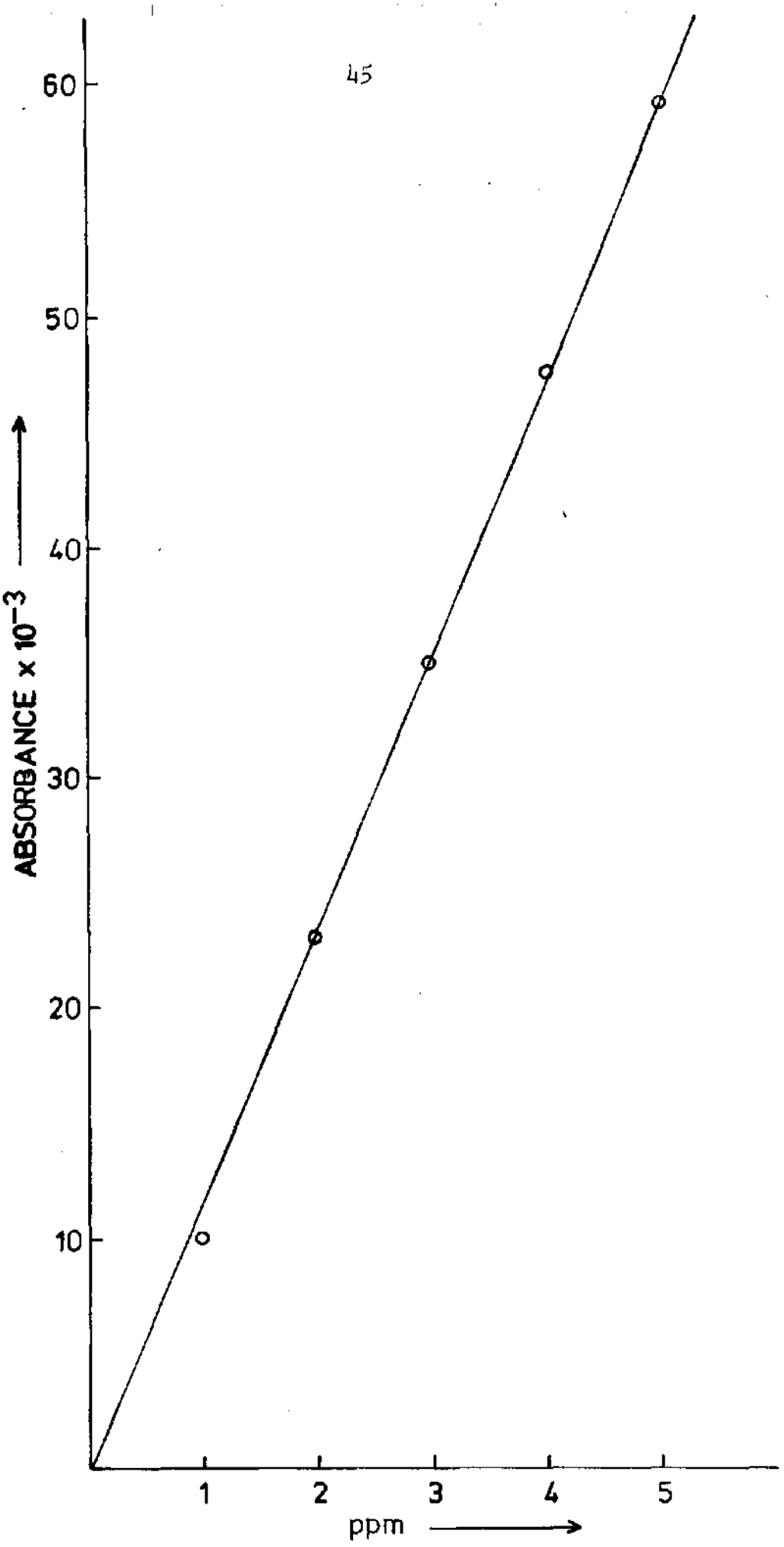

Fig. 2·2 CALIBRATION CURVE FOR COPPER.

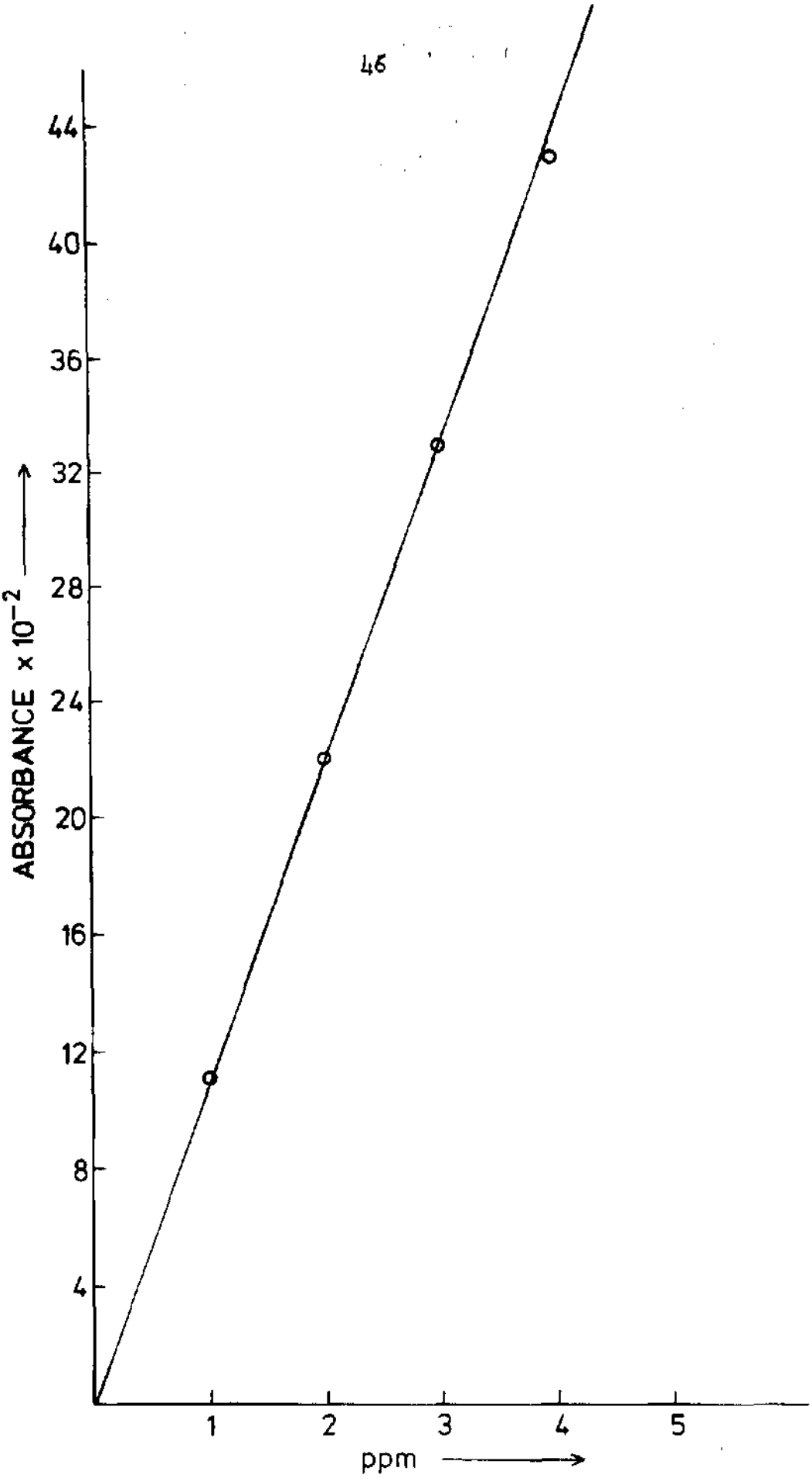

Fig. 2.3 CALIBRATION CURVE FOR ZINC.

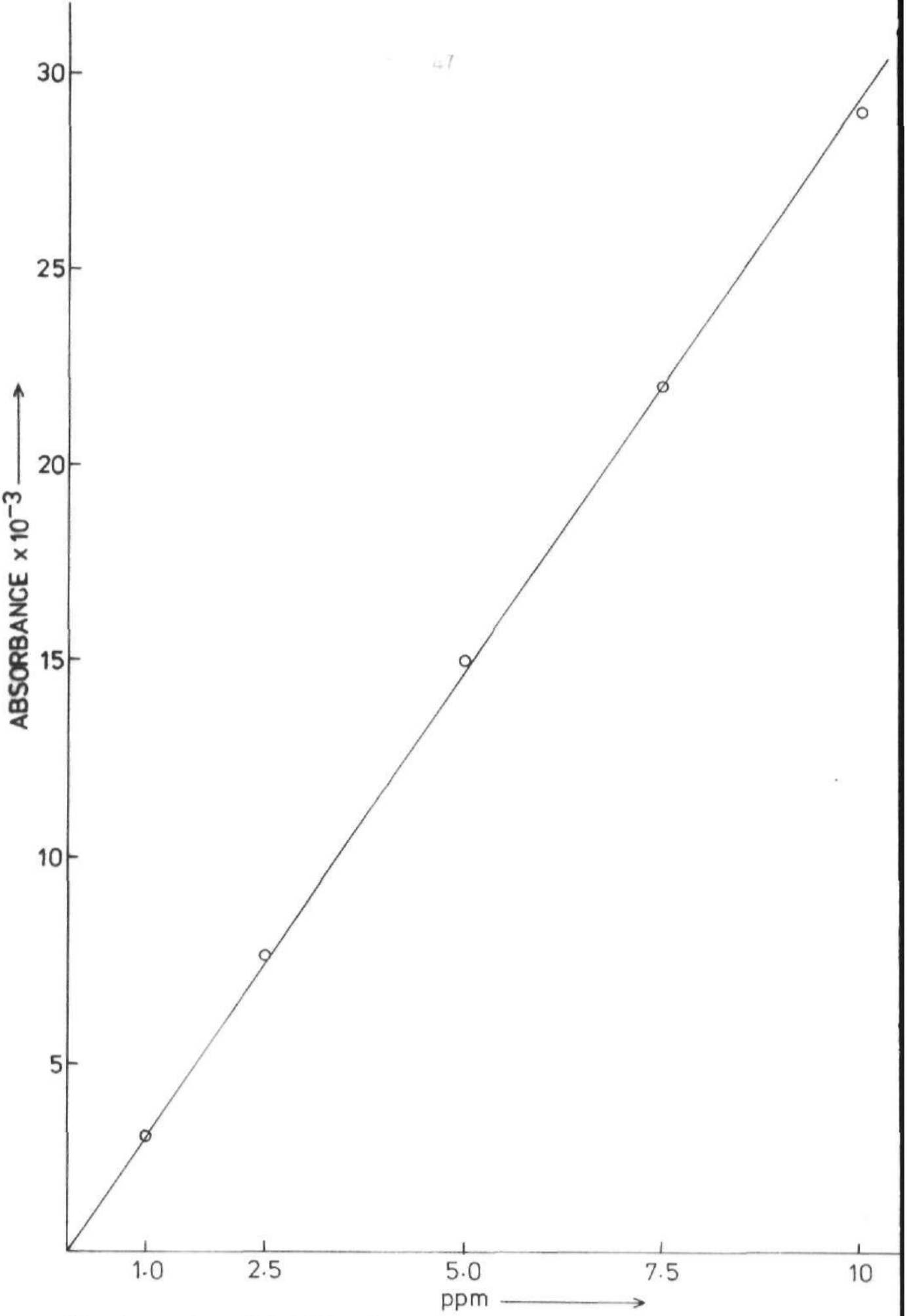

Fig. 2.4 CALIBRATION CURVE FOR ANTIMONY.

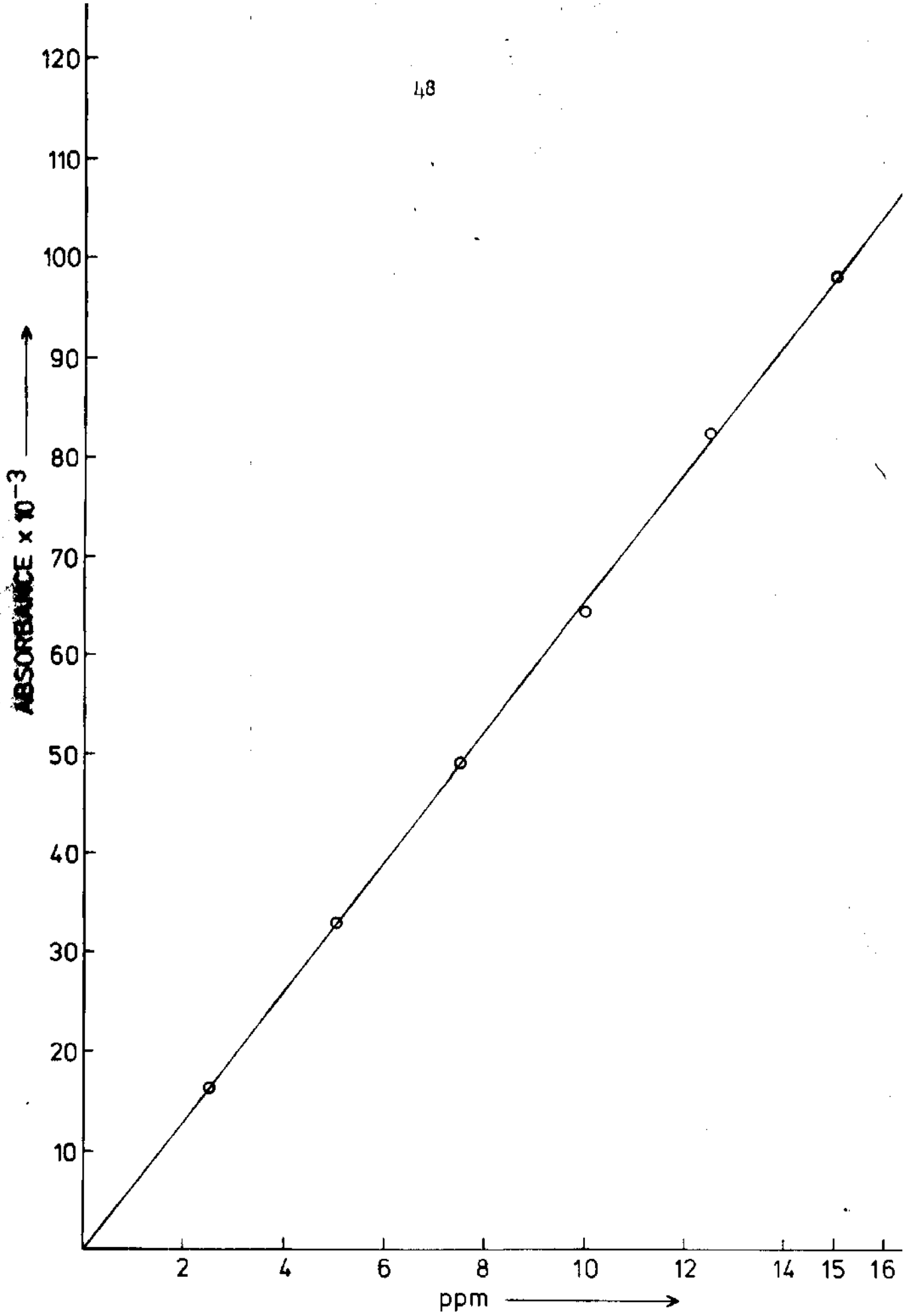

Fig. 2.5 CALIBRATION CURVE FOR IRON.

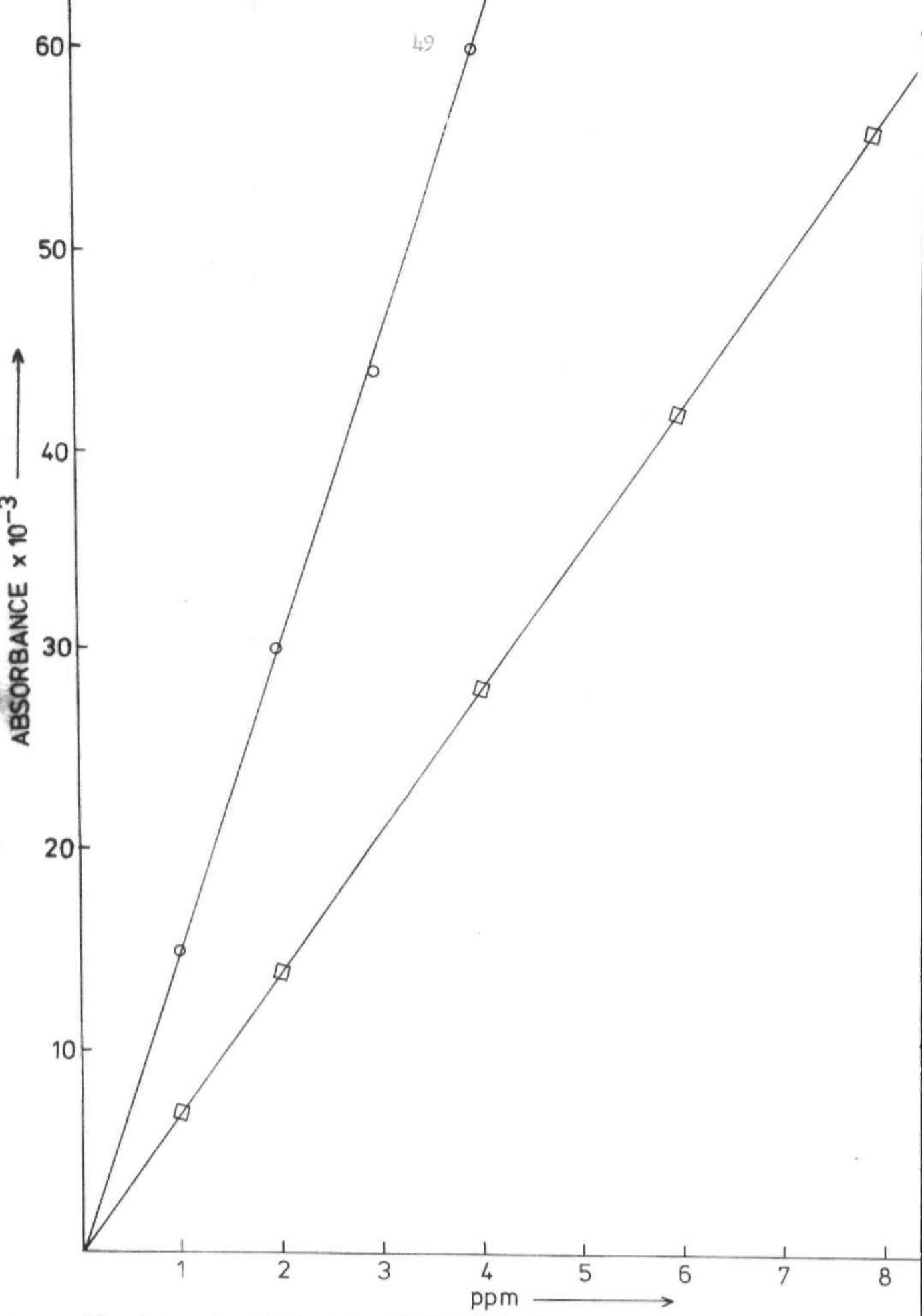

Fig. 2.6 CALIBRATION CURVES FOR ARSENIC AND NICKEL.
o ARSENIC, □ NICKEL

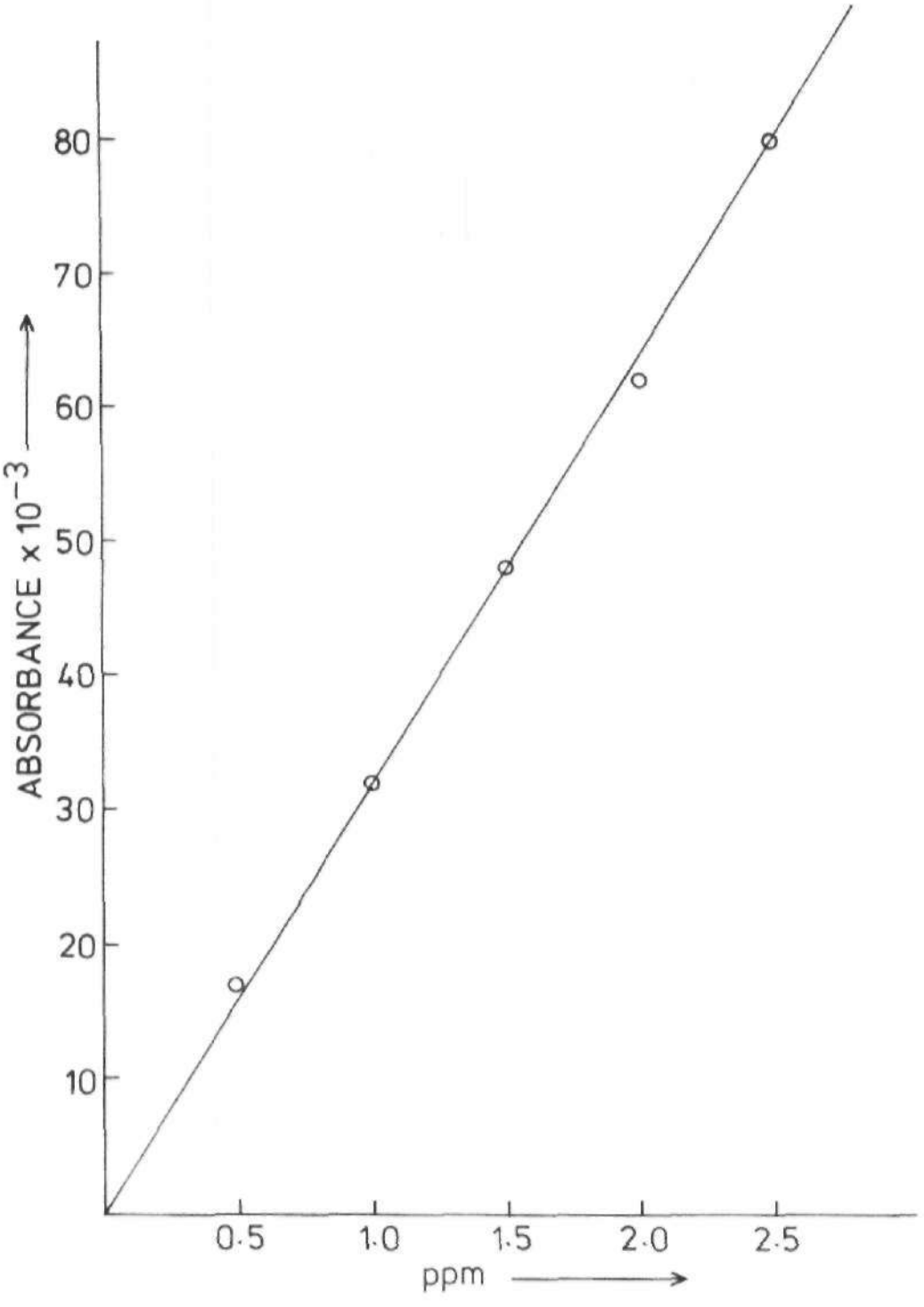

Fig. 2·7 CALIBRATION CURVE FOR CADMIUM

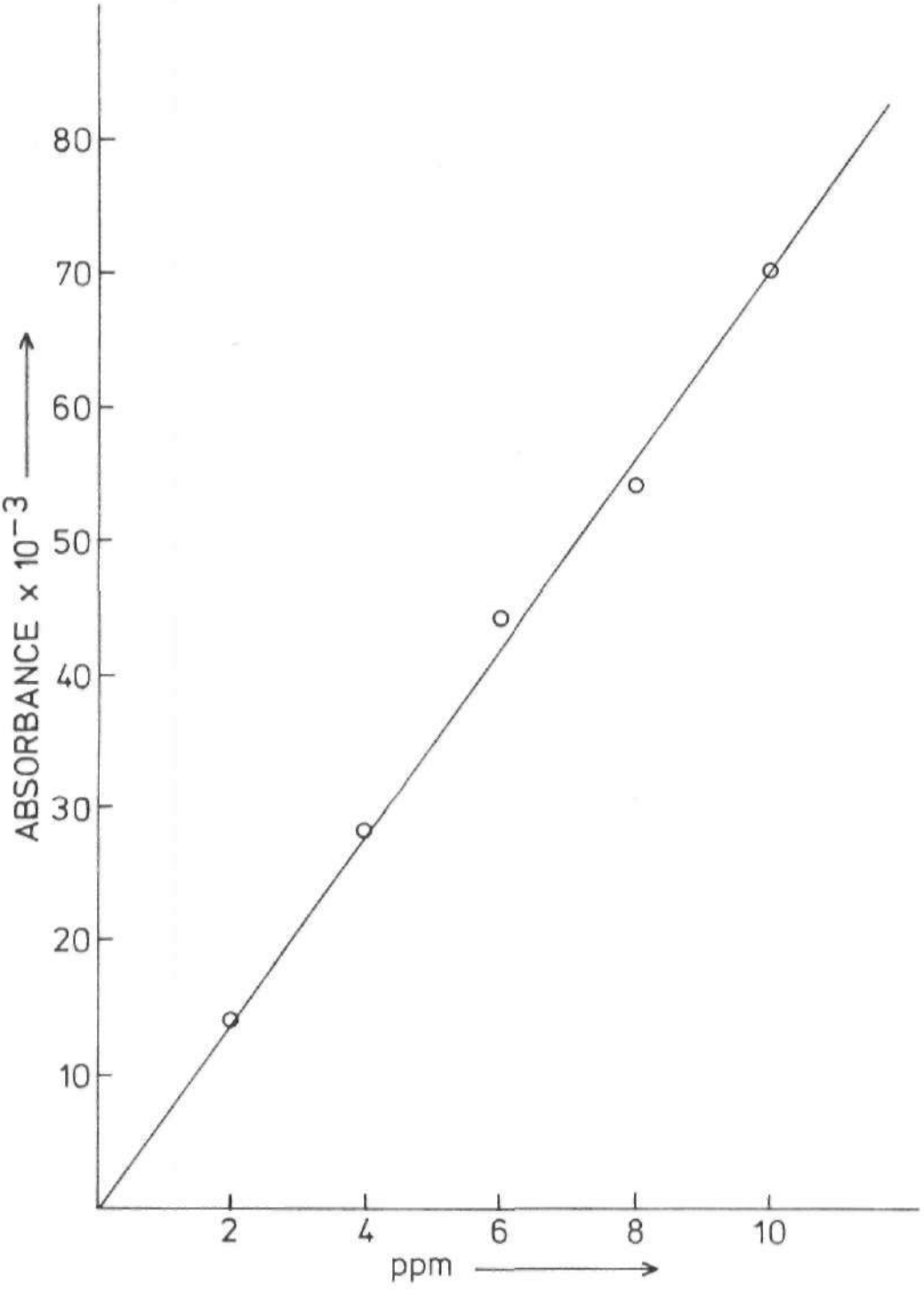

Fig. 2.8 CALIBRATION CURVE FOR TIN.

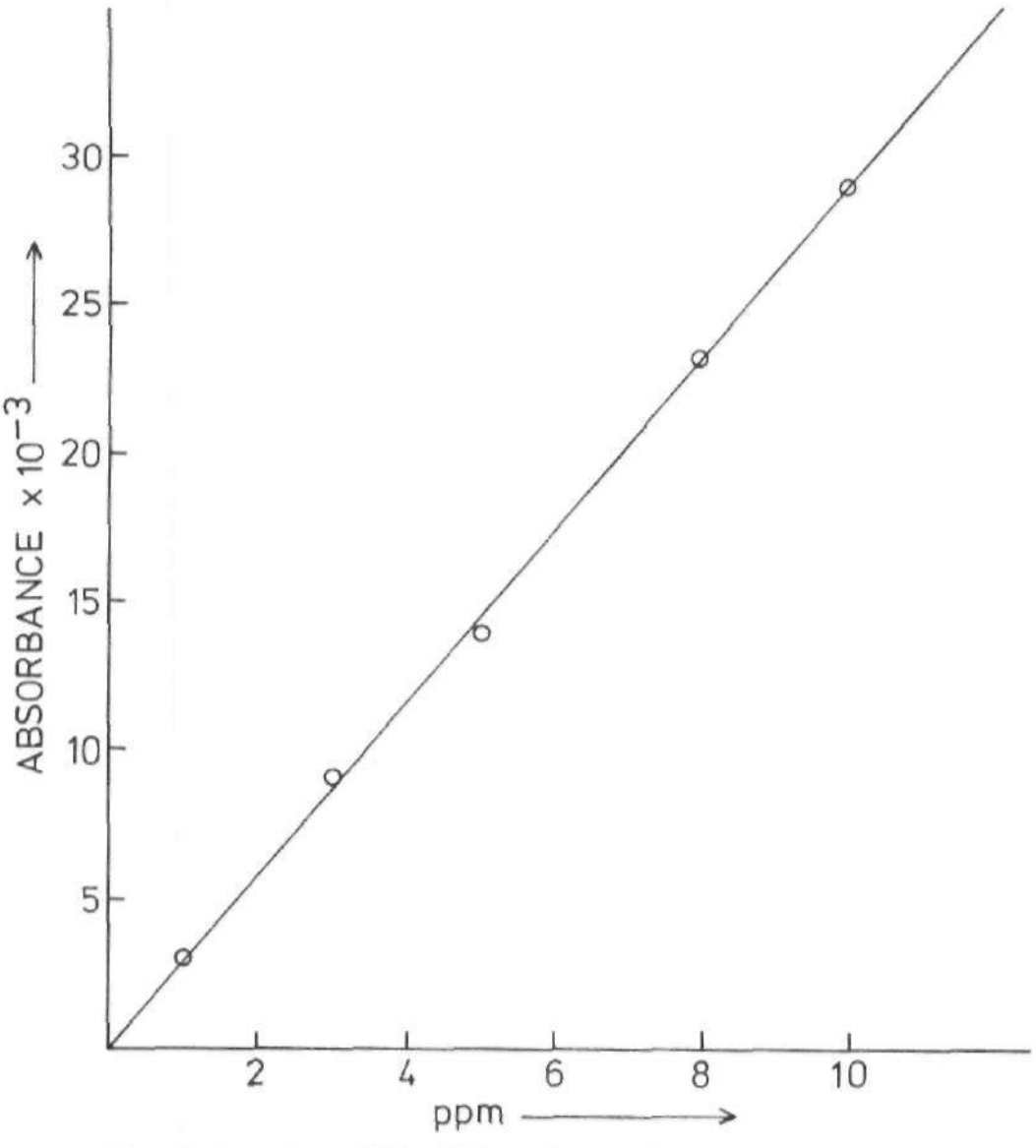

Fig. 2.9 CALIBRATION CURVE FOR SILVER

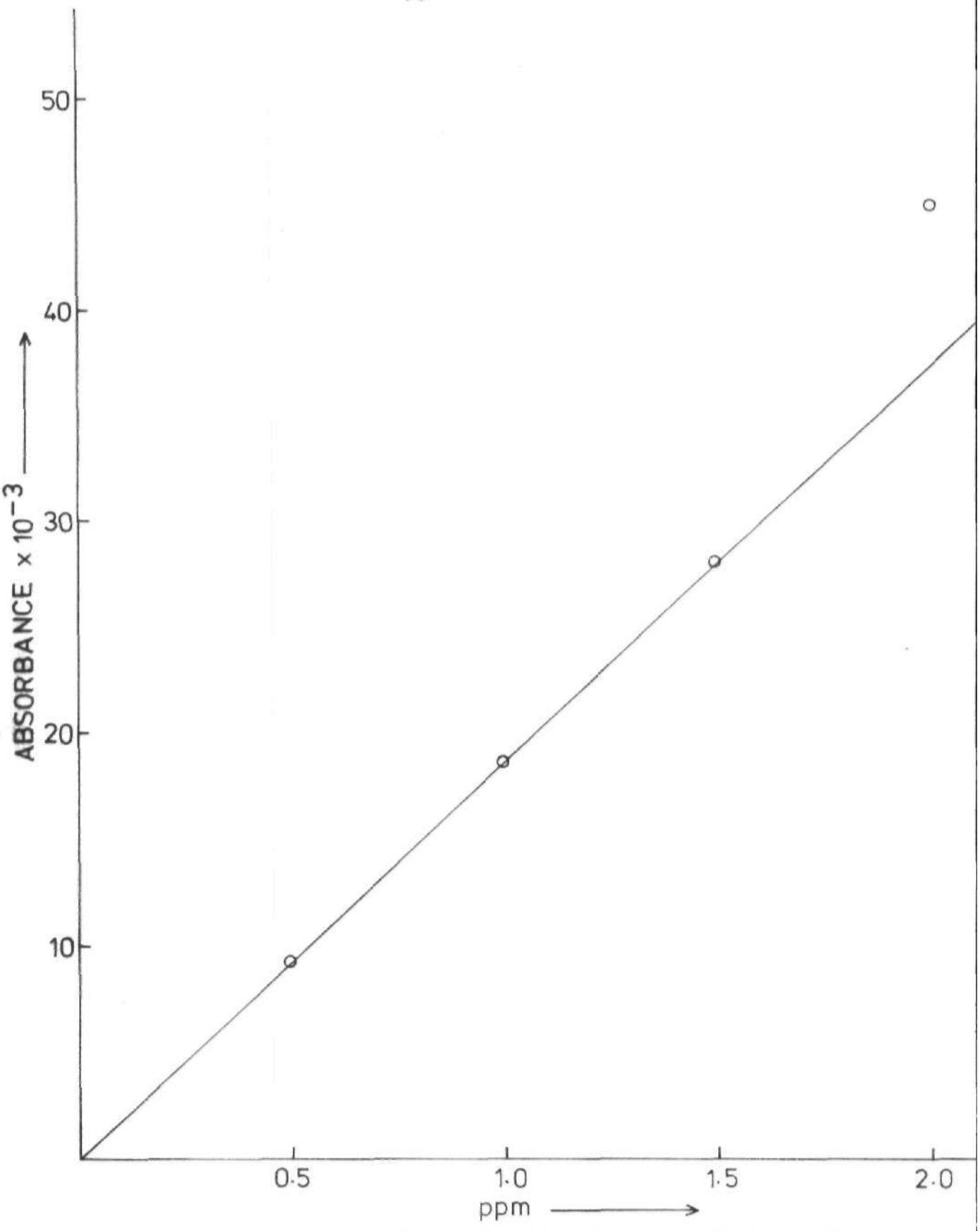

Fig. 2·10 CALIBRATION CURVE FOR MANGANESE.

2.10 DETERMINATION OF CHLORIDE, BROMIDE AND FLUORIDE BY VOLUMETRIC METHOD (32, 33).

The concentrations of bromides and chlorides in the four kuali samples were determined by volumetric method in which 0.1M silver nitrate solution was titrated against the solutions of the samples by using adsorption indicators, such as fluorescein and eosin.

Fluorescein indicator was used for the determination of chlorides, this indicator is a very weak acid. The optimum pH range is 7 to 10. Fluorescein cannot be used in solutions more dilute than 0.005M; and in solution more concentrated than 0.05N, if it is more concentrated, the precipitate coagulates too soon and interferes.

Eosin (tetrabromofluorescein) is used in the determination of bromides. It can be used down to a pH of 1 to 2; the colour change is sharpest in an acetic acid solution.

Eosin cannot be used for chloride titrations; this is because the eosinate ion can compete with the chloride ion before the equivalence point and thereby giving premature indication of the end point.

The disadvantage of adsorption indicators is that silver halides are sensitive to the action of light hence,

the titration has to be carried out with a minimum exposure of light.

The method of fluoride determination involves the titration of thorium nitrate against the fluoride solution. The thorium nitrate solution has to be standardised against a known fluoride solution. Since the reaction is pH dependent; it will give reasonable results at pH of 3.35 $\pm$ 0.10, and as such the sample solution has to be acidified with 1M perchloric acid to the said pH with buffer solution added. Indicator used was thymol blue.

Reagents and chemicals.

All reagents and chemicals used were of analytical grade unless otherwise stated.

1. Sodium carbonate anhydrous.
2. Fluorescein indicator: prepared by dissolving 0.2g of fluorescein powder in 100ml of 70% ethanol.
3. Silver nitrate 0.1M: prepared by dissolving 8.495g of anhydrous silver nitrate in 500ml of distilled water.

4. Eosin indicator: prepared by dissolving 0.1g of eosin in 100 ml of 70% ethanol.

5. Acetic acid 6M.

6. Buffer solution: prepared by dissolving 6.7g of glycine and 11g of sodium perchlorate in water; 11ml of 1M perchloric acid was added and the volume was made up to 100ml with water.

7. Thorium nitrate solution: prepared by dissolving 11.044g of thorium nitrate tetrahydrate in 0.001M nitric acid and the solution was made up to one litre with 0.001M nitric acid.

8. Thymol blue indicator: prepared by dissolving 0.2g of the thymol blue in 100ml of 70% ethanol.

Preparation of sample solutions.

The sample solutions were prepared by sodium carbonate fusion method. 1.0g of each of the samples was weighed and put inside a 150ml beaker separately. 3.0g of anhydrous sodium carbonate was added; each of the contents in the beakers was mixed thoroughly. 30ml of water was added to each and heated for about 20 minutes. The

resulting precipitate was filtered and the filterate was diluted to 100ml with distilled water.

Procedure.

Chloride determination.

25ml of the sodium carbonate extract of the first sample was pipetted into a 250ml conical flask. 5 drops of fluorescein indicator was added and it was titrated against 0.1M silver nitrate solution in a diffused light, with constant rotation of the flask. The titration was continued until the end point was reached, in which a pronounced pink colour was got. The titration was repeated with two other 25ml portions of the sample solution.

The above procedure was repeated for the second, third, and fourth samples. The amount of chloride in the samples were calculated from this relationship.

1ml 0.1M$AgNO_3$ ≡ 0.00355g of chloride.

Bromide determination.

25ml of the sodium carbonate extract of the first sample was pipetted into a 250 ml conical flask; a few ml of 6M acetic acid and five drops of eosin indicator

were added. The solution was titrated against 0.1M silver nitrate with constant stirring in a diffused light until the end point was reached. At the end point, a magneta colour precipitate was got. The titration was repeated with two other 25ml portions of the extract solution.

The above procedure was repeated for the second, third and fourth samples.

The amount of bromide in the samples were calculated from this relationship.

$$1ml\ 0.1M AgNO_3 \equiv 0.00799g \text{ of Bromide}$$

Fluoride determination.

5ml of the sodium carbonate extract of the first sample was pipetted into a 150 ml conical flask and diluted to 15ml with distilled water. The solution was acidified with 1M perchloric acid to pH of 3.35 ± 0.10. 2ml of buffer solution and 3 drops of thymol blue indicator solution were added to the mixture; the content was titrated with thorium nitrate to a deep red colour. The thorium nitrate was standardized against a known fluoride solution. The titration was repeated for three other 5ml portions of the extract.

The above procedure was repeated with the second, third and fourth samples.

The concentration of fluoride in the samples were calculated from the volume of thorium nitrate used since this was standardized against potassium fluoride solution.

2.11 DETERMINATION OF SULPHUR BY GRAVIMETRIC ANALYSIS (32, 34, 38).

This method of sulphur determination is used for the analysis of mineral sulphides. The sulphide in the mineral is oxidized to sulphuric acid and it is determined as barium sulphate. The oxidising agent used is a mixture of nitric acid and hydrochloric acid and a little bromine water.

The method has the advantage of not introducing any metallic ion but the iron in the sample has to be removed by treating the solution with an excess ammonia solution.

Precipitation of the barium sulphate is carried out in a weakly acidic solution in order to prevent the possible formation of the barium salts and furthermore, the obtainable precipitate may be easily filtered.

It is also of great importance to carry out the precipitation at boiling temperature, because the relative super saturation is less at higher temperatures.

Coprecipitation of the salt, if not taken care of, can also increase the amount of error in the analysis. Barium chloride and barium nitrate are readily coprecipitated; these can add to the weight of the barium sulphate, hence a high result will be got since the chloride is unchanged upon ignition, and the nitrate will yield barium oxide. The error due to chloride can be reduced by adding the hot dilute barium chloride solution slowly to the hot sulphate solution which is stirred constantly. The error due to nitrate can be avoided by evaporating the solution with a large excess of hydrochloric acid before precipitation.

Apparatus.

Platinum crucible 3.5cm internal diameter.

Reagents and chemicals.

All reagents and chemicals used were of analytical grade unless otherwise stated.

1. Nitric acid concentrated (S.G.1.42).
2. Hydrochloric acid concentrated (S.G.1.18)

3. Sodium carbonate anhydrous
4. Ammonia concentrated
5. Bromine water.
6. Barium chloride 5%.

Procedure.

1.0g of the first sample was weighed accurately and put in a beaker. 40ml of concentrated nitric acid and 10ml of concentrated hydrochloric acid were added along with little bromine water.

The solution was heated on a hot plate until the solution was reduced to only 5ml. After transfering the solution to a porcelain dish along with washings, a pinch of sodium carbonate was added and again evaporated to dryness and ignited to destroy the organic matter. The sodium carbonate is added to fix the sulphate as sodium sulphate which does not decompose at quite high temperatures. The ignited residue was cooled and dissolved in 20 - 30ml hydrochloric acid. The iron in the solution was removed by treating the solution with an excess of ammonia solution. The solution was filtered and the filterate was acidified with slight excess of concentrated hydrochloric acid.

TABLE 2.1

INSTRUMENTAL CONDITIONS OF THE ATOMIC ABSORPTION SPECTROPHOTOMETER.

	Lead	Copper	Zinc	Antimony	Iron	Arsenic	Nickel	Cadmium	Tin	Silver	Manganese
Principal resonance line (nm)	217.00	324.75	213.86	206.84	248.33	193.70	232.00	228.80	224.61	328.07	279.48
Slit width (nm)	0.15–0.20	0.15–0.20	0.10–0.15	0.15–0.20	0.10–0.15	0.15–0.20	0.10	0.15–0.20	0.10–0.15	0.15–0.20	0.15–0.20
Flame	A	A	A	A	A	A	A	A	A	A	A
Air flow rate (litre min^{-1})	5	5	5	5	5	5	5	5	5	5	5
Acetylene flow Rate (litre min^{-1})	0.80–1.00	0.80–1.00	0.80–1.10	0.90–1.10	0.80–1.00	1.10–1.40	0.80–1.10	0.80–1.00	1.10–1.40	0.80–1.10	0.80–1.00
Current (mA)	6	5	10	15	15	8	15	6	8	4	12
Best precision (mA)	5 - 6	4 - 5	8 - 10	12 - 15	12 - 15	8	12 - 15	5 - 6	7 - 8	4	10 - 12
Best sensitivity	3 - 4	2 - 4	4 - 7	10 - 12	7 - 11	6 - 7	7 - 11	3 - 4	4 - 6	2 - 3	6 - 9

A = Air acetylene

Taken from ref. 39.

The solution was heated to boiling and 50ml of boiling hot 5% barium chloride solution was added slowly with constant stirring. The solution was stirred vigorously and allowed to stand overnight. The precipitate was filtered off through an ashless filter paper no. 540 and washed with hot water until free from chloride.

The precipitate was taken in a platinum crucible, dried and ignited at 600°C. The residue was cooled in a dessicator and weighed as barium sulphate. The above procedure was repeated for the first sample twice, and the standard deviation calculated.

The procedure above was repeated for the second, third and fourth sample in triplicate, and their standard deviations calculated.

The weight of sulphur in each sample was got from this relationship.

$$\text{Wt. of sulphur} = \frac{0.1374 \times \text{wt of precipitate } (BaSO_4)}{\text{initial weight of sample}} g$$

2.12 ANALYSIS OF BLOOD SAMPLES (30, 31, 32, 35, 39).

Principle.

The blood samples were precipitated with trichloroacetic acid 5%, the lead in the blood samples were extracted into an organic solvent, and the organic layers were aspirated into the atomic absorption spectrometer.

An organic solvent can be used to selectively extract metals after complexing from an aqueous solution, and the solvent can then be aspirated directly into the flame. This has some advantages. The test element is separated from the sample thereby eliminating possible interferences. It is also obtained in a pure organic solvent; this results in a maximum atomisation efficiency.

Isobutylmethylketone is preferred for this purpose owing to its suitability as extractive agent and for use in premix burner systems. It is better to use an oxidizing flame (fuel lean) when an organic solvent is aspirated into the flame because the solvent must be burned (30, 31).

The blood samples were precipitated with 5% [illegible] trichloroacetic acid, the lead in the blood samples were extracted with ammonium pyrolidine dithiocarbamate 2% into isobutylmethyl ketone at a pH of 3.

Lead standards in the range of 0 to 2 ppm were prepared and similarly extracted into the organic layer as done for the blood samples. They were aspirated first into the flame before the blood samples were aspirated. During the aspiration of isobutylmethylketone (MIBK), the fuel was adjusted to give a very slightly yellow flame.

Instrument and Apparatus.

21G x 1½ Gillete sterile needle.

2ml sterile disposable syringe.

Auto bench centrifuge. Baird & Tatlock.

SP 1900 Unicam Atomic Absorption spectrometer.

Reagents.

All chemicals used were of analytical grade unless otherwise stated.

1. Trichloroacetic acid (TCA) 5%
2. Ammonium pyrrolidine dithiocarbamate (APDC) 2% - prepared by dissolving 2g of APDC in 100ml of deionised water and filtered. A fresh solution was prepared each day before use.
3. Ammonia concentrated
4. Isobutylmethylketone (MIBK) B.D.H: water saturated of it was prepared by mixing two volumes of MIBK and one volume of water in a separating funnel, and shaken for 20 minutes. This was allowed to stand for 10 minutes, after which the water phase was run off, and the organic phase was decanted.

Lead Standard.

A stock solution of 1000 ppm which is equivalent to 1000 $ugml^{-1}$ was prepared by dissolving 1.599g of lead nitrate in $25cm^3$ of nitric acid (1% v/v) and this was diluted to one litre with deionised water. Lead working standards in the range of 0 to 2 ppm were prepared by dilution of the stock solution. Each of the lead working standard solution was treated with 5ml of 5% TCA and the lead was extracted with 1.0ml of 2% APDC into 5ml of MIBK.

Analytical procedures.

Whole blood samples were collected in 2.5ml sample bottles each containing about 0.04ml of 10% disodium salt of EDTA and dried in the sample bottles before the collection of blood samples. As soon as the blood samples were collected, they were shaken thoroughly and kept in the freezer. Before the analysis was carried out, the blood samples were removed from the freezer and allowed to attain laboratory temperature.

Precipitation.

The blood samples were precipitated with 5% T.C.A. 2.0ml of the whole blood and 4ml of 5% T.C.A. solution were mixed in a centrifuge tube, allowed to stand for one hour with occassional stirring. After one hour,

the mixtures were spun for 10 minutes each at 3,000 r.p.m. The supernatant was decanted into a separating funnel. A further 4ml of 5% T.C.A. solution was added to each of the precipitate in the centrifuge tubes and stirred vigorously for a few minutes. The tubes were spun again for 10 minutes and the supernatant decanted into the separating funnel. The pH of the solution was adjusted to about 3.0 with concentrated ammonia (35).

Extraction.

1.0ml of 2% APDC solution was added to each of the solutions (supernatant), mixed and allowed to stand for 5 minutes. 5ml of M.I.B.K water saturated solution were added and the mixtures were shaken for 10 minutes with a shaking machine. The phases separated and the water phase was run off almost completely.

Instrumental conditions.

The intrumental conditions were the same as for lead in table 2.1.

Procedure.

The appropirate wavelength was selected and the lead hollow cathode lamp was set. All other instrumental conditions were adjusted. The standard solutions were first aspirated into the flame, after which the sample solutions were aspirated into the flame.

A calibration curve was prepared from the standard solutions intensity readings. The corresponding concentration of lead in ppm in each sample solution was read off from the calibration curve. The readings were converted into $\mu g 100ml^{-1}$. Calibration curve for lead is given in figure 2.11.

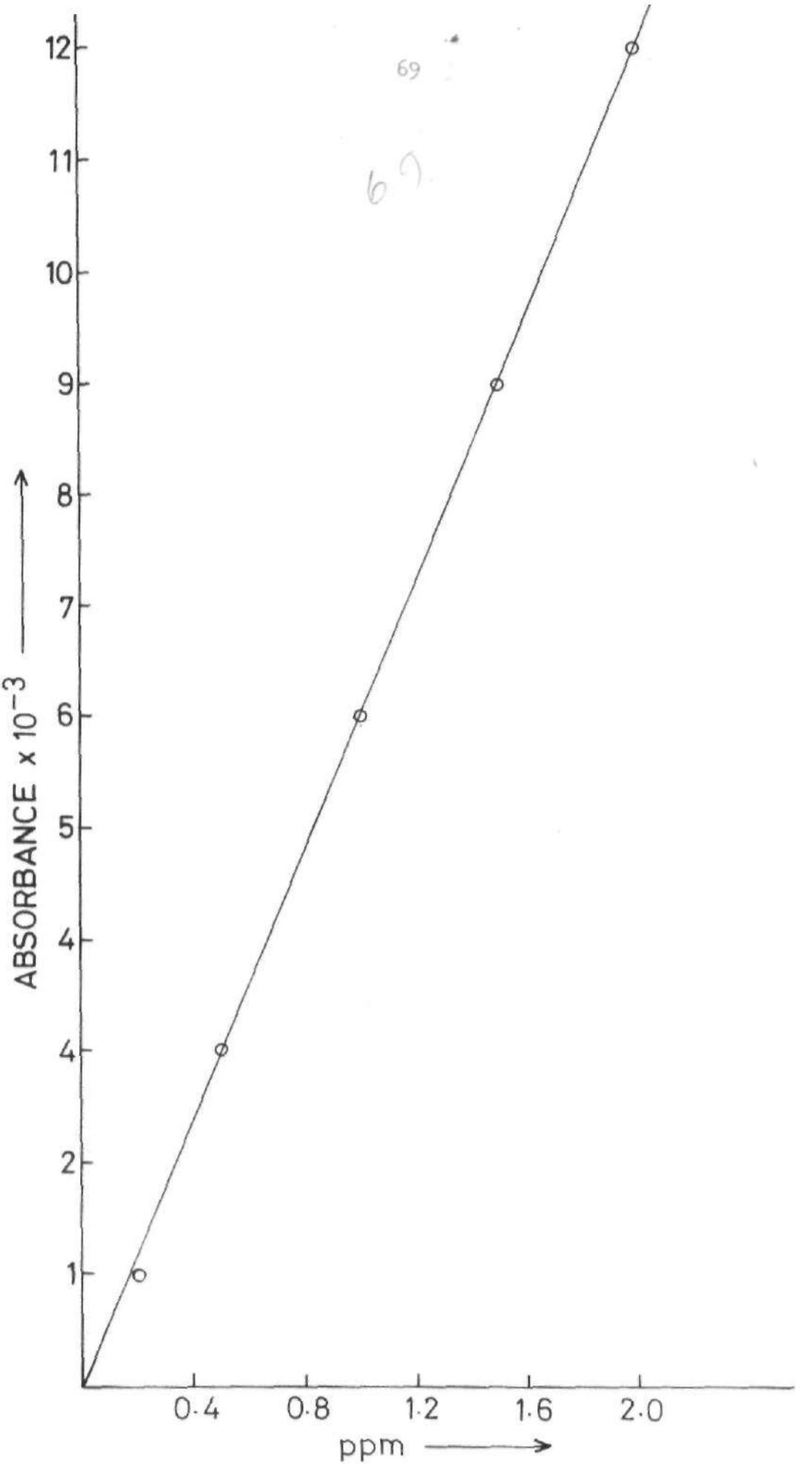

Fig. 2·11 CALIBRATION CURVE FOR LEAD (EXTRACTED INTO ORGANIC SOLVENT)

CHAPTER THREE

3.0 RESULTS AND DISCUSSION

3.1 RESULTS.

The results of the analysis of the Kuali and blood samples are shown below.

TABLE 3.1

PHYSICAL APPEARANCE OF SAMPLES

Sample	Physical Appearance	Physical Appearance after it has been placed in oven at 100°C for 2 hrs.
1.	Shape ranges from octahedral to cubic. It is brittle, opaque with metallic grey colour, greyish crystals were got after grinding.	There was no noticeable change in colour or shape.
2.	Shape ranges from cubic to octahedral. It is brittle, opaque with metallic grey colour greyish crystals were got after grinding.	There was no noticeable change in colour or shape.
3.	Cubic in shape, it is brittle, opaque and black in colour, black crystals were got after grinding.	There was no noticeable change in colour or shape.
4.	Greyish crystals	There was no noticeable change in colour or shape.

TABLE 3.2

SOLUBILITY OF SAMPLES IN WATER, CONCENTRATED AND DILUTE ACIDS, BASES AND SOME ORGANIC SOLVENTS.

Solubility in	Sample 1	Sample 2	Sample 3	Sample 4
Cold water	Not Soluble	Not Soluble	Not Soluble	Not Soluble
Hot water	" "	" "	" "	" "
Glacial CH_3COOH	" "	" "	" "	" "
Dil. and Conc. HNO_3	" "	" "	" "	" "
Dil. and Conc. H_2SO_4	" "	" "	" "	" "
Dil. HCl.	" "	" "	" "	" "
Conc. HCl	Partially soluble	Partially soluble	Partially soluble	Partially soluble
Aquaregia	Not soluble	Not soluble	Not soluble	Not soluble
Dil. $HClO_4$	Not soluble	Not soluble	Not soluble	Not soluble
Conc. $HClO_4$	Soluble	Soluble	Soluble	Soluble
Conc. and Dil. NH_4OH	Not soluble	Not soluble	Not soluble	Not soluble
Conc. and Dil. NaOH	Not soluble	Not soluble	Not soluble	Not soluble
95% C_2H_5OH	Not soluble	Not soluble	Not soluble	Not soluble
Chloroform	Not soluble	Not soluble	Not soluble	Not soluble
Liquid parafin	Not soluble	Not soluble	Not soluble	Not soluble

TABLE 2.3

DETERMINATION OF MELTING/DECOMPOSITION POINT AND FLAME TESTS OF THE SAMPLES.

Sample	Melting/Decomposition point	Flame Test
1.	Did not melt or decompose up till 400°C	Light blue, and light green colours were observed.
2.	Did not melt or decompose up till 400°C	Light blue and light green colours were observed.
3.	Did not melt or decompose up till 400°C.	Light blue, green, and bluish green colours were observed.
4.	Did not melt or decompose up till 400°C	Light blue and light green colours were observed.

TABLE 3.4

RESULTS OF SPOT TEST AND CLASSICAL ANALYSIS METHOD

Sample	Metals and nonmetals detected by spot test method.	Metals and nonmetals detected by classical Analysis.
1.	Antimony, silver, tin, iron, nickel, arsenic, lead, cadmium, sulphite, bromide, chloride and fluoride.	Lead, silver, arsenic antimony, tin, nickel, sulphite, bromide, fluoride and chloride.
2.	Silver, tin, nickel, antimony, arsenic, iron, lead, zinc, sulphite, bromide, chloride, and fluoride.	Lead, silver, arsenic, antimony, nickel, zinc, sulphite, bromide, fluoride and chloride.
3.	Lead, zinc, copper, silver, tin, antimony, iron, cadmium, nickel, arsenic, sulphite, bromide, chloride and fluoride.	Lead, silver, zinc, arsenic, tin, antimony, iron, nickel, sulphite, bromide, fluoride and chloride.
4.	Antimony, silver, tin iron, nickel, arsenic, lead, zinc, sulphite, bromide, chloride and fluoride.	Lead, zinc, silver, antimony, tin, iron, nickel, arsenic, sulphite, bromide, fluoride, and chloride.

TABLE 3.5

METALS DETECTED IN SAMPLES (IN PPM) BY ATOMIC ABSORPTION SPECTROMETRIC METHOD.

Metals	Sample 1	Sample 2	Sample 3	Sample 4
Manganese	*B.D.L.	*B.D.L	0.35 ± 0.01	B.D.L.
Iron	3.80 ± 0.60	2.80 ± 0.90	14.0 ± 2.20	4.00 ± 1.10
Nickel	4.80 ± 0.40	4.60 ± 0.80	4.00 ± 1.10	4.20 ± 0.65
Copper	B.D.L.	0.10 ± 0.001	1.25 ± 0.20	0.25 ± 0.04
Zinc	0.10 ± 0.02	0.50 ± 0.04	35.0 ± 1.00	1.00 ± 0.05
Arsenic	1.60 ± 0.20	1.80 ± 0.10	1.70 ± 0.40	1.80 ± 0.60
Silver	4.10 ± 1.90	3.40 ± 2.10	5.20 ± 1.90	4.50 ± 2.90
Cadmium	0.15 ± 0.02	0.10 ± 0.01	0.50 ± 0.03	0.10 ± 0.02
Tin	8.00 ± 2.10	11.3 ± 2.90	4.00 ± 1.20	6.80 ± 1.80
Antimony	3.40 ± 1.80	4.80 ± 2.50	4.00 ± 2.10	8.00 ± 3.60
Lead	1.65 ± 0.10	2.00 ± 0.50	3.00 ± 0.40	1.15 ± 0.60

*Below Detection Limit

TABLE 3.6

NONMETALS FOUND IN SAMPLES (IN MGG^{-1}) BY VOLUMETRIC AND GRAVIMETRIC METHODS.

Nonmetals	Sample 1	Sample 2	Sample 3	Sample 4
Fluoride	23.4 ± 4.60	24.9 ± 5.30	21.9 ± 5.50	23.2 ± 4.00
Sulphur	126 ± 21.0	125 ± 15.0	122 ± 19.0	102 ± 9.40
Chloride	5.60 ± 2.40	4.97 ± 2.90	9.50 ± 3.10	10.6 ± 4.20
Bromide	24.0 ± 2.00	43.2 ± 1.80	34.0 ± 2.10	48.0 ± 1.20

TABLE 3.7

BLOOD LEAD CONCENTRATION (IN $\mu g100ml^{-1}$) OF KUALI USERS AND KUALI NONUSERS BY A.A.S. METHOD.

KUALI USERS		KUALI NONUSERS	
No.	Conc. in $\mu g100ml^{-1}$	No.	Conc. in $\mu g100ml^{-1}$
1.	100.0	21	18.0
2.	142.0	22	34.0
3.	82.0	23	42.0
4.	118.0	24	25.0
5.	125.0	25	34.0
6.	75.0	26	18.0
7.	100.0	27	34.0
8.	75.0	28	25.0
9.	68.0	29	25.0
10.	68.0	30	42.0
11.	82.0		
12.	82.0		
13.	58.0		
14.	75.0		
15.	68.0		
16.	75.0		
17.	100.0		
18.	82.0		
19	92.0		
20.	134.0		

3.2 DISCUSSION OF RESULTS.

Physicochemical analyses were carried out on the Kuali samples and the results of the physical tests showed that all kuali samples possess some properties which are common to some metals and nonmetals; these include the shape, brittleness and opacity (table 3.1).

The four kuali samples were not chemically very reactive as reflected by their insolubility in some concentrated and dilute acids, bases and some common organic solvents. They were completely soluble in concentrated perchloric acid and partially soluble in concentrated hydrochloric acid with the evolution of hydrogen sulphide gas in both cases. This gave a mere possibility that sulphur is present in the kuali samples probably in the form of sulphide (table 3.2).

The kuali samples were very stable, since they did not melt or decompose up till 400^{o}C. The colours observed during the flame tests of the four samples gave an indication that some metals like antimony, zinc, arsenic and lead were probably present in the kuali samples (table 3.3). Much cannot be said about the conductivity of the kualis since these were not soluble in nonpolar solvents.

The metals and nonmetals detected in the kuali samples by spot test and classical analysis methods are shown in table 3.4. Almost all the metals and nonmetals in the kuali samples were detected by spot test; due to the limitation of the applicability of the classical methods on the elements present in small amounts, it was not possible to analyse them by this method.

Table 3.5 shows the result of the analysis carried out by A.A.S. method; included in the metals detected were lead, zinc, cadmium, copper, antimony, silver, tin, iron, nickel, arsenic and manganese. The concentrations of nickel, iron, tin, silver, and antimony in the first sample were found to be higher than the amount of other metals present in the sample. Tin has the highest concentration of 8.00 ppm. The concentrations of lead and arsenic in the first sample were 1.65 and 1.60 ppm respectively. The concentrations of zinc and cadmium in the first sample were very low, 0.1 and 0.15 ppm respectively whereas, the concentrations of copper and manganese in the sample were expected to be below detection limit of the instrument.

The second sample has large concentration of antimony, silver, tin and nickel, with tin having the highest concentration of 11.3 ppm in the sample. The concentration

of lead, iron, and arsenic in the sample were found to be 2.00, 2.80, and 1.80 ppm respectively. Zinc, cadmium and copper had low concentrations of 0.50, 0.10, and 0.10ppm respectively. The concentration of manganese was below detection limit.

The third sample was quite different in its composition, it had the largest concentration of zinc (35.0ppm) followed by iron (14.0ppm). This shows that zinc is the major metal constituent of the sample. The concentration of silver was about 5.20ppm. Antimony, tin and nickel appear to have the same concentration of 4.00ppm. Lead has a relatively low concentration of 3.00ppm. The concentrations of cadmium, copper, arsenic and manganese were very low.

The fourth sample has a large concentration of antimony, silver, tin, iron and nickel with antimony having the highest concentration. The concentrations of lead, zinc and arsenic were low and were found to be 1.15, 1.00 and 1.80ppm respectively. The concentrations of cadmium and copper were very low.

In general, all the four samples seemed to have a significant amount of antimony, silver, tin, iron, nickel with the exception of the third sample which has a very high concentration of zinc (35.0ppm).

The concentrations of cadmium, copper, zinc and arsenic were low in all the samples except in the third where the concentration of zinc was significantly high. The concentration of lead in all the four samples were low as compared to the concentrations of antimony, silver, tin, iron and nickel. Small amount of manganese was detected only in the third sample; whereas, the amount of manganese in the other three samples were below detection limit. Table 3.6 shows the result of the analysis of nonmetals in the four samples. The first sample has a large amount of sulphur which was 126 mgg^{-1}. The concentrations of fluoride and bromide were almost the same, 23.4 and 24.0mgg^{-1} respectively in the sample. The concentration of chloride was low, 5.6mgg^{-1}. In the second sample, the largest amount of nonmetal was sulphur 125mgg^{-1}, followed by bromide 43.2mgg^{-1}. The concentration of fluoride was 24.9mgg^{-1}; chloride has the lowest concentration of 4.97mgg^{-1} in the sample.

In the third sample, the largest amount of nonmetal was sulphur which was 122.0mgg^{-1}. The concentrations of bromide and fluoride were 54.0 and 21.9mmg^{-1} respectively. The concentration of chloride was low (9.50mgg^{-1})

In the fourth sample, sulphur has the largest concentration among the nonmetals present. The concentration

was slightly low (102mgg^{-1}) as compared with the concentration of sulphur in the other three samples. The concentration of bromide in the sample was a bit high (48 mgg^{-1}). This was followed by fluoride and chloride which were 23.2 and 10.6mgg^{-1} respectively.

The first sample had the highest amount of sulphur which was 126mgg^{-1}, whereas, the fourth sample had the lowest amount which was 102mgg^{-1} as compared with the amount of sulphur in the other samples. This leads to the belief that, there is much probability of the metals present in the form of sulphides, sulphates or sulphites. The fourth sample has the lowest amount of sulphur and the highest amount of bromide, as compared with the other three samples.

The concentration of fluoride in the four samples tend to be very close, 23.4, 24.9, 21.9 and 23.2mgg^{-1} for the first, second, third and fourth samples respectively. The concentrations of chloride in the first and second samples were nearly the same (5.60 and 4.96mgg^{-1}). Simillarly in the third and fourth samples the concentrations of chloride were very close 9.50 and 10.6mgg^{-1} respectively.

From the results of the analyses (table 3.5 and 3.6) it appears that the major constituents of the samples occur in the mineral probably as sulphide, sulphite or sulphate, but the action of concentrated hydrochloric and perchloric acids on the samples with the evolution of hydrogen sulphide gas, confirmed that some of the metals exist in the form of sulphide.

The results of spot test and classical analysis (table 3.4) showed that sulphite was detected in the analysis, instead of sulphide. This was due to the fact that the sample solutions were prepared with sodium carbonate and in the course of the preparation, the sulphide in the sample might have been oxidized to sulphite.

The results of the blood samples analysed were shown in table 3.7. The blood-lead concentration in kuali users ranges from 58 $\mu g100ml^{-1}$ to 142 $\mu g100ml^{-1}$ with a mean concentration of 90.05 $\mu g100ml^{-1}$, whereas, the blood lead concentration in kuali nonusers varied from 18.0 to 42.0 $\mu g100ml^{-1}$ with a mean value of 29.7 $\mu g100ml^{-1}$.

There was a significant change of lead levels in the blood of kuali users with a mean of 90.05 $\mu g100ml^{-1}$

as compared to the kuali nonusers, with a mean of 29.7 $\mu g100ml^{-1}$. Since the clinical lead poisoning manifest at 80 $\mu g100ml^{-1}$, it is expected that 12 people among the kuali users might have symptomatic lead poisoning.

Although, from the result obtained from A.A.S. analysis method, it would be seen that the concentrations of lead in the four samples were low, but this does not follow that the low concentrations cannot cause poisoning. Majority of the people interviewed said that they have been using the kuali right from their youth. Lead might be absorbed across the conjunctiva, from the drainage down the tear duct, or from rubbing the eyes and then licking the fingers. Thus an appreciable amount of lead may be absorbed through the skin also. Accordingly, this results in elevated blood-lead levels following the use of lead containing eye cosmetic (37).

Chilsom (11) had found that before the threshold concentration for poisoning is reached, some damages could be done to the body. It is possible that a content of lead in the body that is insufficient to cause obvious symptoms can give rise slowly to long lasting adverse effects, thus lead can cause a long term damage in the body.

It cannot be concluded for sure that all the amount of lead present in the blood of the people using the kualis came solely from the kualis, since there are some other minor sources by which lead can enter into their body but, at least, a large percentage of the lead in the blood came from the kualis.

3.3 SUGGESTION FOR FURTHER WORK.

In view of the present study on cosmetics plumbism, there is a need to assess the effects of raised lead level in the blood of the neonates or children born to mothers using lead containing cosmetics so as to establish if there is any transplacental transfer of lead in mothers using lead containing cosmetics.

3.4 SUMMARY AND CONCLUSION.

The study has been carried out to analyse some of the commonly used eyeliners, kualis and to measure the associated blood lead changes in the blood of the people using the kualis. The kuali samples were bought in Kano and in Zaria. The physical appearances of the Kualis were observed, and were found to have shapes ranging from cubic to octahedral. They are

brittle and opaque. The first second and fourth kuali samples are grey in colour. The third sample has a black colour.

Kuali samples were not soluble in concentrated and dilute bases and acids, except in concentrated hydrochloric acid and perchloric acid with the evolution of hydrogen sulphide gas. The Kuali samples were found to be stable since they did not melt or decompose up till 400^{o}C.

A qualitative test was conducted on the four Kuali samples by using spot test and classical methods of analysis. The four Kuali samples were analysed by atomic absorption spectrometer and were found to contain the following metals, lead, zinc, cadmium, copper, antimony, silver, tin, iron, nickel, arsenic and manganese. The nonmetals in the Kualis were analysed by volumetric and gravimetric methods, and were found to contain fluoride, sulphur, chloride, and bromide.

Whole blood samples, collected from some of the people using the Kualis and from those who were not using the Kualis were analysed. It was found that there was a significant change in the blood-lead levels in Kuali users as compared to the kuali nonusers.

A direct association between the use of Kuali and high blood-lead concentration was observed. The use of Kuallis is associated with high blood-lead concentrations. Kuali which is a naturally occuring mineral, is being purchased from the market and people grind it and use it freely. Since there is no legal restriction on the trade of lead containing eye cosmetics, there is need for wider publicity of lead poisoning due to lead containing eye cosmetics including Kuali.

The study suggests a preventive ban on lead containing eye cosmetics.

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