COLORIMETRIC DETERMINATION OF SOME TRANSITION METALS IN SELECTED INI-ANT MILK FORMULAE.

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DECLARATION

I hereby declare that this thesis contains the report of my research work and has not been presented in any previous application for a higher degree. All information from other sources have been acknowledged by means of references.

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CERTIFICATION

This thesis entitled ''Colorimetric Determination of Some Transition Metals in Selected Infant Milk Formulae'' by CHIME, Charles Chukwuma, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, Nigeria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late Father, Emmanuel; my mother, Benice; my sisters, Felicia, Charity and Tina; and my brothers, Jonathan, and Samuel.

ABSTRACT

Infant formulas were digested with nitric acid and sulphuric acid (5:1); furnace ashed at 500 ± 10°C; and treated with 10% solution of trichloroacetic acid (TCAA). The resulting concentrates were analyzed colorimetrieally for iron, using 1,10-phenanthroline, and 2,2'-Bipyridyl as ligands; 2,9-dimethyl-1,10-phenanthroline for copper; Dithiozone in carbontetrachloride for zinc; Potassium periodate for manganese; Nitroso-R-salt for Cobalt; and Dithiozone for Lead. Atomic Absorption Speetrophotometric (AAS) analyses of the metal ions in the three sample preparations were also done. Most coexisting metal ions found to interfere were conveniently masked prior to the determination. Recoveries of added ion for each of the metal ions were investigated colorimetrically using the three preparations. On statistical comparison of the results obtained from the colorimetric methods with the standard (AAS) method, the accuracy and acceptability of the colorimetric methods were assessed.

Six organic reagents namely: Benzidine, 1,2,3-trihydroxylbenzene (Pyrogallol), Rodizonic acid, Flourescien, Picric acid, and Mercaptoethanol were tested for their colored complex formation with iron, copper, zinc and manganese. Pyrogallol found to form stable complex that could enable the colorimetric determination of iron, was exploited in estimating iron in the sample preparations. The results obtained were acceptable and relate well with the AAS method.

Other essential parameters in infant formula were determined and compared statistically with the values displayed by the manufacturers. It was found that only the results for copper, zinc and manganese compared very well with the manufacturers values. The results for the other parameters varied from sample to sample.

TABLE OF CONTENTS

Page

DECLARATION ..... (i)

CERTIFICATION ..... (ii)

DEDICATION ..... (iii)

ACKNOWLEDGMENTS ..... (iv)

ABSTRACT ..... (v)

TABLE OF CONTENTS ..... (vi)

LIST OF TABLES ..... (x)

LIST OF FIGURES ..... (xi)

LIST OF APPENDICES ..... (xii)

ABBREVIATIONS AND SYMBOLS ..... (xiii)

CHAPTER 1

1.0 Introduction 1

1.1 Aims of the Research 2

CHAPTER 2

2.0 REVIEW OF LITERATURE 3

2.1 Colorimetric Reaction 3

2.2 Trace Elements Components of Milk 5

2.3 Analysis of Trace Elements in Milk 6

2.4 Major Components of Milk 9

2.5 Analysis of the Major Components of Milk 9

CHAPTER 3

EXPERIMENTAL 12

3.1 Sampling 12

3.2 Reagents 12

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Page

3.3 Methods 13
3.3.1 Sample Pretreatment 13
3.3.2 Colorimetric Determination of Trace Element Component 13
3.3.2.1 Determination of Iron Colorimetrically Using
1,10-phen. and 2,2'-Bipy 13
3.3.2.2 Colorimetric Determination of Copper Using
2,9-Dimethyl-1,10-phen. 14
3.3.2.3 Colorimetric Determination of Zinc Using Dithizone. 15
3.3.2.4 Colorimetric Determination of Manganese
Using Potassium Periodate 16
3.3.2.5 Colorimetric Determination of Cobalt Using Nitroso-R-Salt 16
3.3.2.6 Colorimetric Determination of Lead Using Dithizone 17
3.3.2.7 Recovery of Added Ion 17
3.3.3 AAS Determination of the Metals 18
3.3.3.1 Determination of Iron 18
3.3.3.2 Determination of Copper 18
3.3.3.3 Determination of Zinc 19
3.3.3.4 Determination of Manganese 19
3.3.3.5 Determination of Calcium and Mangnesium 19
3.3.4 Flame Photometric Determination of Sodium and Potassium 20
3.3.5 Colorimetric Determinatin of Phosphorous 21
3.3.6 Determination of Chloride 21
3.3.7 Determination of Moisture Content 22
3.3.8 Determination of Ash Content 23
3.3.9 Determination of Titrable Acidity 23
3.3.10 Determination of Fat Content 24
3.3.11 Determination of Protein Content 24
3.3.12 Determination of Lactose Content 25

Page

3.3.13 Development of New Colorimetric Reagents for the Determination of the Trace Metals 25
3.3.14 Colorimetric Determination of Iron Using Pyrogallol 26

CHAPTER 4

4.0 RESULTS AND DISCUSSIONS 27
4.1 Sample Preparations 27
4.2 Iron Determination 27
4.2.1 Statistical Comparison of Results from Iron Determination 27
4.3 Copper Determination/Statistical Comparison of Results 32
4.4 Zinc Determination/Statistical Comparison of Results 36
4.5 Manganese, Cobalt and Lead Determination 37
4.6 New Colorimetric Reagent for the Determination of the Metals 41
4.7 Determination of Major Components and Statistical Comparison of Results 47

CHAPTER 5

5.0 CONCLUSION AND SUGGESTIONS 56

REFERENCES 58

APPENDICES 65

LIST OF TABLES

Table Page

4.1 Elemental Determination Using the Wet Digestion and Colorimetry.....29

4.2 Elemental Determination Using Furnace Digestion and Colorimetry.....30

4.3 Elemental Determination Using the (TCAA) Filtrate and Colorimetry.....31

4.4 Added Ions Recovered Using the Wet Digestion and Colorimetry.....33

4.5 Added Ions Recovered Using the Furnace Digestion and Colorimetry.....34

4.6 Added Ions Recovered Using the (TCAA) Filtrate and Colorimetry.....35

4.7 Elemental Determination Using the Wet Digestion and (AAS).....38

4.8 Elemental Determination Using Furnace Digestion and (AAS).....39

4.9 Elemental Determination Using (TCAA) Filtrate and (AAS).....40

4.10 Iron Determination Using the Digests and (TCAA) Filtrate and Pyrogallol as Colorimetric Reagent.....44

4.11 Added Iron Recovered Using the Digests and (TCAA) Filtrate and Pyrogallol as Colorimetric Reagent.....45

4.12 Major Elements Determination Using the Wet Digestion and (AAS).....46

4.13 Major Elements Determination Using Furnace Digestion and (AAS)/FAES.....53

4.14 Major Elements Determination Using the (TCAA) Filtrate and (AAS)/FAES.....54

4.15 Summary of Results for Other Parameters in the Formulas.....55

LIST OF FIGURES

Figure Page

4.1 Graph of Absorbance Against Time for Iron-Rodizonic acid Complex and Iron-Mercaptoethanol Complex....42
4.2 Graph of Absorbance Against Time for Iron-1,2,3-trihydroxylbenzene (Pyrogallol) Complex....43
4.3 Calibration Graph For Iron Determination Using Pyrogallol....49
4.4 Rigbom Plot for Iron Determination Using Pyrogallol....50
4.5 Graph of Absorbance Against Wavelength for Iron-Pyrogallol Complex....51
4.6 Graph of Absorbance Against Wavelength for the Interference of Copper in the Determintion of Iron Using Pyrogallol and Its Masking With Thiourea....52

LIST OF APPENDICES

Appendix — Page

A: Calibration, Absorption and Rigbom Curves for the Colorimetric Determination of Iron.....65

B: Calibration, Absorption and Rigbom Curves for the Colorimetric Determination of Copper.....71

C: Calibration, Absorption and Rigbom Curves for the Colorimetric Determination of Zinc.....74

D: Absorption, Rigbom and Calibration Curves for the Colorimetric Determination of Manganese.....77

E: Absorption, Rigbom and Calibration Curves for the Colorimetric Determination of Cobalt.....80

F: Absorption, Rigbom and Calibration Curves for the Colorimetric Determination of Lead.....83

G: Calibration Curve for the Colorimetric Determinatin of Phosphorous...86

H: Calibration Curves for the (AAS/FAES) Determination of the Elements.....87

I: Statistical Comparison of Results.....95

ABBREVIATIONS AND SYMBOLS

AAS	Atomic Absorption Spectrophotometry or Spectrophotometer
FAES	Flame Atomic Emmision Spectrophotometry
cm^3	Cubic Centimeter
PPM	Parts per million
mg	Milligramme
µg	Microgramme
g	Gramme
%	Per cent
pH	Hydrogen Ion Index
1,10-Phen	1,10-Phenanthroline
2,2'-Bipy	2,2'-Bipyridyl
TCAA	Trichloroacetic acid
SD	Standard Deviation
%RSD	Percent Relative Standard Deviation
nm	Nanometer
wt	Weight
M	Molar
L	Litres
λ_{max}	Wavelength of Maximum Absorption
etc.	et-ce-tera
e.g	Example
ppb	Parts per billion

CHAPTER 1

1.0 INTRODUCTION

The Colorimetric determination of inorganic substances with organic ligands are based on reactions which yield products which (in solution) absorb radiation within the visible range of the electronic spectra (Colin, 1977). Consequently, the tendency of transition metal elements to react with organic ligands producing complex species that absorb radiation within this range has been exploited for their colorimetric determination.

Transition metals possess qualities such as the presence of *d*-electron orbitals which participate in colour forming reactions with organic reagents having suitable functional groups. This work has put into consideration the potentials of some metal -ligand coloured complex formation in estimating transition metal ions in infant milk formula.

Even though many techniques have been used in the past to determine these metal ions, colorimetry is still considered as sensitive for, and capable of offering comparable results. Various organic ligands have found applications in the colorimetry of metals. However, what dictates the use of a particular ligand is the existence within it of a group which is capable of interacting with the metal ion so as to produce a complex that is coloured, stable, and representative of the concentration of the metal ion in the solution (Holzbecher *et.al.*, 1976).

It has become imperative to determine trace metal ions in infant milk with respect to a couple of reasons. The levels of copper and iron in milks can be controlled to achieve good keeping qualities. Also it was necessary to investigate the levels of zinc, manganese, and other trace elements to be able to monitor their uptake by babies. Nevertheless, routine monitoring of iron in infant milk would reduce the scourge of iron deficiency anemia in babies.

Few techniques of milk powder analysis have the inherent simplicity of colorimetry. Doubtless, the atomic absorption spectrophotometric (A.A.S) method is good, but it is not readily available for place to place monitoring of metal ions in infant milks. This

underscores the need for the development of available, portable and affordable sensitive colorimetric method, the motivation for this work.

1.1 Aims of the Research.

The first goal of this work is to determine metals such as Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Cobalt (Co), and Lead (Pb) in wet digest, dry digest and trichloroacetic acid filtrate of infant milk formula, using standard colorimetric methods and atomic absorption spectrophotometric (AAS) method. Moreover, the improvement of the colorimetric methods based on the minimization of interferences would be carried out.Furthermore, a comparison of the colorimetric methods with the AAS method for each of the preparation procedures would be undertaken with view to finding out which of the methods and preparation techniques offer better results.

To develop new colorimetric methods for the determination of Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn) in infant milk formula, six organic reagents namely:- Benzidine, Pyrogallol, Rodizonic acid, Flouresien, Picric acid and Mercapto-ethanol would be investigated and a comparison of the results obtained from the developed method with AAS method would be made for each of the preparation procedure. The objective is to ascertaining the degree of reliability of the developed method.

It is also the aim of this work to assess the levels of Sodium, Potassium, Calcium, Magnesium, Phosphorous Chloride, Protein, Fat, Lactose, Ash and Moisture in the infant formula, using standard methods. The results obtained with these standard methods would be compared with the manufacturers specification, to checking the level of agreement, of the two methods.

Nevertheless, the high cost of sophisticated equipment such as AAS for the determination of metals in infant milk formula, has elicited the search for cheaper and equally sensitive colorimetric methods, a paramount aim of this work.

CHAPTER 2

2.0 REVIEW OF LITERATURE

2.1 Colorimetric Reaction

The reactions of inorganic substances with the reagents which yield coloured products suited for colorimetric, spectrophotometric or turbidimetric determinations are called colorimetric reactions. The products are normally metal salts or complexes. In some instances a new organic substance is synthesized. (Babko, 1968).

For chelation to occur, the ligand must contain at least two donor atoms capable of bonding to the same metal atom. Elements that act as donors are the more electronegative elements on the right hand side of the periodic table. They are primarily found in group (V) (nitrogen, phosphorous, arsenic and antimony) and group (VI) (oxygen, sulphur, selenium and tellurium) (Collin, 1977).

The formation of a stable metal complex requires the presence of a basic or acidic functional group in the molecule of the reagent in those positions which allow the formation of a five or six -membered Chelate ring.

Colour results from the absorption of radiation in some part of the visible region of the electromagnetic spectrum. Absorption usually extends into the near infra-red or UV regions and is associated with excitation of electrons within the complex from lower to higher energy levels. Electronic transition giving rise to absorption spectra can be classified (into various types) depending on the chromophoric group present.

The *d*-electron chromophore is characterized by a metal ion with an unfilled *d*-level and combined with particular donor atoms. This chromophoric group is found in complex compounds of transition metals and can be denoted as MX_n, where M is the central metal ion and X the donor atom. Another type of transition are those involving a *d*-element and the π -electron system of the ligand molecule. The transition could be from the ligand to

metal or from the metal to the ligand. These (transitions) are called charge-transfer transitions and they result in intense absorption bands.

The metal ligand transition (M → L) which is characterized by the transfer of a *d*-electron from the central field of the metal ion suggests that the metal must be in the lower oxidation state. Also there must be a possibility of accepting this electron in an unfilled orbital of the ligand. Such ligands are found among organic compounds with a double bond or a chain of conjugated double bonds attached to the donor atom (McWhinnie and Miller, 1967), for example, the complexes of iron (II) with ligands such as 8-hydroxyquinoline, 2-picolinic acid, 2,2-bipyridyl, 1,10-phen, etc. For all these complexes the absorption bands are found in the visible region and are assigned to the transfer of a t_{2g} electron to a free anti-bonding π - orbital of the ligand.

The ligand metal (L →M) transition which involves transfer of an electron from the ligand to the metal, is found with complexes where the d-shell metal can be reduced easily to lower oxidation state by accepting one electron. At the same time, the ligand molecule must have a π - electron system to which an acidic group is attached. This group interacts with the metal ion and provides non-bonding electrons free for transfer to the metal ion. Such groups are -OH, -SH, =NH. This type of complex can be exemplified by the complexes of iron (III) with 8-hydroxyquinoline, Tiron and Sulphosalicylic acid (Holm and Everett, 1966).

Organic reagent used in colorimetric reactions should be sufficiently stable and resistant to aerial oxidation or to photometric decomposition. It must also possess a π -electron chromophoric groups in its molecule for colour to be produced. In the absorption spectrum of a product there must be a characteristic intensive absorption band at a sufficient distance from that of the reagent or of substance being determined. λ_{max} (product) - λ_{max} (reagent) $\geq$ 100nm. The reagent for which λ_{max} (product) $\equiv$ λ_{max} reagent are less suited for spectrophotometric determinations (Collin, 1977).

The complex forming properties of organic reagents can be influenced by introducing suitable substituent into their molecules. The stability of the complex is usually increased by higher basicity of the ligand. This can be achieved for example when electron donor

substituents (e.g. alkyl groups) are present in the position *meta* or *para* to the active grouping (Holzbecher, 1976).

When a bulky substituent is close to the functional grouping the reactivity of the reagent may be decreased or lost owing to steric-effect. The reaction of the metal ion with the (particular) functional groups is thus prevented. By this, it is possible to increase the selectivity of the reagent. (Dunn, 1959).

The replacement of O by S as donor atom often leads to high selectivity and sometimes even to higher intensity of colour (Dwyer and Meller, 1964). Though some reagents form sufficiently suitable complexes, they do not possess properties suitable for spectrophotometry. A more suitable derivative can be obtained by introducing chromophoric groups into the molecules or increasing the number of aromatic or heterocyclic rings (Borg and Becker, 1940) in this way greater delocalization of electrons can be achieved which brings about a bathochromic shift in the spectra of the metal complex.

2.2 Trace Elements Components of Milk

The trace elements in milk come from the food eaten by the animal and ultimately from the soil in which the food were grown. The elements are chromium, cobalt, copper, iodine, manganese, zinc and iron (Byron and Arnold 1965). Although foodstuffs are the primary source of trace elements in milk, a certain quantity could be obtained from the water supply, from insecticides and spray residues, from glass containers or from the metal cans and processing equipment of the dairy.

The trace element cobalt in milk forms the centre of vitamin B_{12} molecule. Hence it is important in animal nutrition. It was first reported by Bertrand and Macheboveuf, (1925). Values for normal milk are usually less than 1μg/l (Archibald, 1951; Ellis and Thompson, 1945). Much attention has been given to the copper content of milk because of its catalytic effect on the development of oxidized flavour in milk and its products. It is essential to the formation of haemoglobin. Daily requirement for copper is about 2mg for adults and 0.05mg per Kg body weight for infants (Archibald, 1958, Davis, 1931).

The presence of iron in milk was reported by Bunge, (1889). It is always present in milk usually in the range 100-900 ug/l. It is important as constituent of heamoglobin and as component of respiratory enzymes. 0.15mg/kg of body weight is recommended in humans (Archbald, 1958, Robert and Sturt, 1959). Manganese in milk is essential as integral part of the liver enzyme arginase. Between 20 - 30 µg/l has been reported in cow milk.

Of all the trace elements found in milk, zinc is consistently present in the largest amount. Average value for normal milk would be 350 µg/l. It is however needed in small amounts by humans (Baron and Arnold, 1965). Molybdenium, nickel and iodine are also trace elements found in milk. Nickel is not present in cow milk but can be found in milk from its storage and processing.

2.3 **Analyses of Trace Elements in Milk**

Many works have been reported on the analysis of trace elements in milk and milk products. Smith (1967) reported two rapid methods for the determination of trace Cu in milk. The methods involve addition of known amount of Cu to samples to be determined, and the modification of Diling (1963) method; and comparison with the dry ashing procedure. The need for pH adjustment was eliminated by the use of zinc-dibenzyldithiocarbonate. The methods were found to agree with the dry-ashing procedure and are reproducible.

Manganese was determined at ppb level in dairy products by activation analysis (Das *et.al.*, 1967). This was done by oxidation of ^{56}Mn to the heptavalent state followed by extraction of tetraphenylarsonium permanganate into chloroform. Down to 25 µg/l of Mn was reported. Manganese in milk at the submicrogram amount was determined by ashing the dried sample in a muffle furnace at 500°C and complexing with Na-diethyldithiocarbamate. (Hearly, 1966). The complex was extracted into chloroform at pH 5.2. Results obtained were reproducible and compared well with AAS methods.

A precision method for the determination of zinc in food, especially milk and milk products has been reported (Higgins and Pickering, 1971). Techniques involving colorimetry, polarography and AAS, yielded similar values for the samples. However, AAS had smaller average relative standard deviation. In another development, a method involving dry ashing of milk powder at 400°C and dissolution of the ash with acetic acid followed by quantitative transfer into seperatory funnel, buffering to pH 5 - 6 and extracting with 0.04% dithizone in CCl_4, (Traskov and Gotsulyazk, 1967) has been used in determining traces of zinc. Sensitivity was 1.3 x 10-6g/g milk.

Copper and zinc are readily determined in milk by AAS. Two methods are presented, one involves simple dilution and aspiration into the burner of the instrument, the other involve trichloroacetic acid precipitation prior to aspiration. (Mary *et. al.*, 1967). Satisfactory results in precision and recovery of added metals were obtained with both methods (Alfred and William, 1967).

Iron was determined in milk ash (Galtseva and Lisoya, 1966) using the yellow complex produced when iron complexes with sulfosalicylic acid. Calibration curve was constructed using $FeSO_4(NH)_2SO_4.6H_2O$. (Reproducibility was satisfactory). Also, some methods for the determination of iron in dried milk were compared by Dedicova, Libuse and Dolezalek (1975). Colorimetric methods using *O*-phen, or α, α'-dipyridyl after deproteination of the sample solution were most suitable. A modified trichloroacetic acid method could be harnessed for the deproteination (Hanne and Knud, 1966).

The 1:1 orange-yellow Cu(I) -*bis* (6-methyl-2-pyridyl) glyoxal dihydrazone complex was used in the spectrophotometric determination of Copper in milk (Varcarcel and Pino 1973). The $\lambda_{max.}$ was 440nm and extraction of the complex was accomplished with $PhNO_2$. Relative error was below 0.13%. Cu has also been determined in milk products by complexation with diethyldithiocarbamic acid, extraction of the complex with amyl acetate and carrying out photometry of the yellow complex, (Anon, 1978). Spectrophotometric determination of copper can also be achieved at 500nm with 6-phenyl-2, 3-dihydro-1,2,4-triazine-3-thione.

Trace elements encountered in milk did not interfere in the method (Karapetia, 1982). Recoveries of added Cu was 99.82% (Zhu and Liu, 1993, Nakoro and Huyeshi 1993, Malik *et al.*, 1993; Murky 1994; Zhang 1994).

For the determination of Cu, Fe and Mn in milk, flameless AAS was used. (Johnson, 1976). The only sample pretreatment needed was a simple acid precipitation. Suitable acids for this were H_2SO_4 for Cu and Fe, and HCl for Mn. Mn and Zn in milk were determined by digestion of sample with a conventional microwave oven acid digestion system employing PTFE reactors. There was good agreement with certified milk values of the element when the method was used (Dela 1995, Sirois, 1962).

Iron was determined spectrophotometrically as the orange-red complex formed by Fe(II) with 1,10-phen at pH 4-6 (Chem Soc. 1978). Any Fe(III) present was reduced by ascorbic acid. Average relative standard deviations for collaborative studies by seven laboratory were 2.2 - 7.0% (Anon, 1981, Yi Liu Ying, 1980). Pyridine-2-carbaldehyde-2-hydroxy-benzoylhydrazon was also used as a selective reagent for the extraction and spectrophotometric determination of Fe(II) (Gallego *et. al.*, 1979).

A method involving ion exchange separation and AAS determination of µg quantities of Cu ,Fe and Zn in infant milk formula powder was reported (Aziz, 1994). The method involves digestion with HNO_3 and elution of the resulting solution through a column of Amberlite 400 (chloride form) and determination of the adsorbed Cu, Fe and Zn with AAS. Recoveries were 99.6 - 100.5% (Mittal, 1993).

In the method of Davydova *et al*, (1978) for the colorimetric determination of Lead (Pb) in milk products, a weighed sample was ashed and dissolved in 1:1HCl. After treatment with K_2SO_4 followed by centrifugation and pH adjustment to 9, dithizone in $CHCl_3$ was used to extract the Pb. Colorimetric determination was performed at 520nm after pooling the extracts. In another development, Pb was also estimated in milk by FAES using a base digest (Authur, 1977). Cadmium, lead and copper were determined by

inverse differential pulse polarography (Fariwar *et al.*, 1979). Milk powder analyzed contained Cd, Pb and Cu in the ppb level.

2.4 Major Components of Milk

The major components of milk include water, fat, protein, sugar salts of Ca, Na, P, Mg, K and chlorides. (Webb and Johnson, 1965). The salts influence the heat stability of milk, stability towards alcohol coagulation and age-thickening of sweetened condensed milk. Milk fat is derived in part from triglycerides of the blood and in part from synthetic activities of the mammary gland (Kurtz 1965). Milk contains supplements for other proteinaceous food that may be deficient in one or several amino acids. The only sugar in milk is a disaccharide that is composed of glucose and galactose. Water is present in the largest amount in milk.

2.5 Analysis of the Major Constituents of Milk

Calcium and magnesium content of milk were determined complexometrically as reported by Josef and Libuse, (1966) and Sarude, (1966). In their work, HCl treated milk serum was liberated from interferring phosphate by ion exchange Ca and magnesium concentrations were then determined McCormick (1973) by titration with EDTA in the presence of Eriochrome blue S.E. indicator. Milk samples analyzed by AAS showed standard deviations for various elements Ca 1.6%, Mg 1.6%, K 1.9% and Na 2.4%. Recoveries of added Ca, Mg, K and Na in milk were quantitative and reproducible comparison of results obtained with photometry, direct titration with EDTA and oxalate - permanganate titration, was also made. (Murthy, and Rhea 1967).

The formal titration results used in the calculation of the protein content in milk were used for the determination of Ca as well. (Gajdusek 1975). The Ca content was calculated from the difference between the acidity of milk before and after addition of ammonium oxalate. A new method for K and Ca determination in milk based on direct determination by flame photometry was reported by Bende, *et. al.*, (1973) who obtained comparable results with those given by other methods.

In milk assay, the common extraction techniques for routine analysis namely dry ashing and wet digestion were time consuming and hazardous. A method involving the use of trichloroacetic acid was evaluated for the AAS determination of Ca, Na and K. by Zucchetti *et. al.*, (1993). Recoveries (ranging from 93 - 99.5%) indicate good accuracy and precision. The effect of interference during the AAS determination of these ions including phosphorous was reported by (Lopez and Lozano 1992). The work revealed that the methods were free from interference. Phosphorous was rapidly and directly determined in milk powder solutions by optical emission spectrophotometry with and inductively Ar plasma source (Guna *et. al.*, 1977). The limit of determination was 0.1% $\mu g/cm^3$. (Kawaguchi and Hiroshi, 1978).

Nitrogen and phosphorous in milk have been determined by using a complex catalyst for mineralization. The method was 30-folds as rapid as by Kjeldahl method and is comparable to Kjeldahl in accuracy (Burkus 1971). Phosphorous has also been determined spectrophotometrically in dry ash of milk. (Bedessen *et al*, 1969). The phosphorous is determined colorimetrically after reaction with bisulfite - hydroquinone and ammonium molybdate with recoveries of about 95 - 105%.

Fat, protein and lactose content of milk were determined using Fourier transform IR spectroscopy (Linge and Hope, 1993). The fat protein and lactose content are predicted of which the former two are compared with Reose - Gottlieb and Kjeldabl reference values respectively. (Habbs, and Lawrence 1972; Beitz and Philips 1977). Similarly, the protein content of milk was analyzed colorimetrically. (Cioccia and Anna, 1995). The method measures nitrogen colorimetrically in Kjeldahl digests. It has the advantage of eliminating the distillation and titration steps of the Kjeldahl method. The results agreed with those of the micro Kjeldahl digestion. (Florence and Eric 1977; Tronov, 1978).

Colorimetric methods for the determination of lactose was reported (De Carvalho and Itamar 1978, Teles *et al*, 1978). In the method, lactose reduced picric acid under alkaline medium and the picramate produced was determined colorimetrically at 470nm in a solution of sodium carbonate and NaOH. A simple quick and accurate method based on the determination of excess silver after precipitation of Cl^- with a standardized

$AgNO_3$ solution acidified with HNO_3, by flame photometric determination of chloride in milk was reported by Gutshe and Bafi (1968). Also, Kacskovics *et al* (1968) compared potentiometric methods with Mohr or Volhard methods of determining chlorides. Results showed that the potentiometric method was more accurate (Roy, 1972).

A dye amide black 10B was used in determining the protein content of milk (Galina, 1966; Jain and Tandon 1965). Very high correlation is shown between the dye absorption value and Kjeldahl nitrogen value. (Konrad, 1966). Also, free fat in powdered milk was determined by four methods (Dabrowski, 1977). The method included extraction with petroleum ether, ether and hexane, and extracting free fat by centrifugation. The use of extraction with pet ether was recommended because of its precision (Molnar and Poole 1967).

CHAPTER 3

3.0 EXPERIMENTAL

3.1 Sampling

Infant milk formulas in 450g tins were purchased from the Samaru market. Five formulas from different manufacturers were procured. The samples so obtained were opened, homogenized and stored in the refrigerator until when needed. The samples batch number (BN). expiry dates (BB), and manufacturers are as follows:

S/No	Sample	BN	BB	Manufacturers
1	Nestle NAN	CSBAV 0024A	08/98	Nestle Nederland b.v for Nestle Nigeria
2	Cowbell	65130	11/98	Cowbell A.G Hofstrasse France
3	Bebelac No. 1	00711	06/98	Nutricia N.V Zoetermeer Halland
4	Wyeth SMA	5M241C	11/98	Wyeth Nutritionals Ireland Askeaton Co. Limerick Ireland for Wyeth Nig. Limited
5	1-er	P 13	03/98	L'enpal par Celia France.

3.2 Reagents

All reagents used were of analytical reagent grade. Standard solutions were prepared using distilled water. Stock solutions of digest/trichloroacetic acid (TCAA) filtrates and standards were stored in the refrigerator until needed.

3.3 Methods

3.3.1 Sample Pretreatment

Wet Digestion: About 5.0g of the dried milk sample was weighed into a 100cm^3 Kjeldahl's flask. Then 10cm^3 of conc. HNO_3 (1.41, 70% w/v) was added and the mixture was warmed gently on a heating mantle. 2cm^3 of conc. H_2SO_4 (1.84, 98% w/v) was added and the mixture was heated on a heating mantle for two hours. It was heated for two hours with the addition of drops of conc. HNO_3. The heating was continued until white fumes was observed. The digest was cooled and filtered into a 100cm^3 volumetric flask, it was neutralized with NH_4OH (1:3) solution, and was treated with 1cm^3 of conc. HCl and made up to the mark with water.

Dry Ashing: About 5.0g of the milk sample was weighed into porcelain crucible and was transferred into the oven and carbonized for 1 hour. It was then transferred into a carbolite furnace and was heated at 550°C for 4 hours. The ash resulting from the treatment was taken up in 1cm^3 of conc. HCl, transferred into a 100cm^3 volumetric flask and was made up to the mark with water.

Trichloroacetic acid (TCAA) Treatment: About 5.0g of the milk sample was reconstituted, transferred into 50cm^3 volumetric flask and made up to the mark with warm water. The mixture was transferred into a 250cm^3 conical flask, on a hot plate maintained at 100°C and was heated to boiling. 10% TCAA solution was added with stirring. The mixture was boiled for a further 20 mins cooled and filtered into a 100cm^3 volumetric flask. The filtrate was then made up to the mark with water.

3.3.2 Colorimetric Determination of Trace Element Components.

3.3.2.1 Determination of Iron (Fe) Using 1,10-Phen. and 2,2-Bipy, as Ligands.

About 0.0702g of ferrous ammonium sulphate was weighed and dissolved in 50cm^3 of water and transferred into a litre volumetric flask. Then 10cm^3 of conc. HCl was added

and the solution was made up to the mark with water. This solution corresponds to 10ppm solution of Fe(III). Aliquots of the solution covering the range 0.1 - 6.0 ppm of Fe(III), were pipetted into a series of 50cm^3 volumetric flask containing 2cm^3, 3cm^3 and 5cm^3 aliquots of the digests/filtrate. Another 50cm^3 flask was set aside for blank.

For colour development using 1,10-phenathroline 1.0cm^3 of 10% solution of hydroxylammoniumchloride, 2.5cm^3 of 0.1% solution of 1.10-Phen and 2.0cm3 of 10% sodium acetate solution were pipetted into each of the flasks including blank. In the determination using 2,2'-Bipy, standards for calibration, prepared from 50 ppm stock solution covered the range 0.1 - 16ppm Fe(III). Colour development was done by adding 1.0cm^3 of 10% hydroxylammoniumchloride, 2.5cm^3 of 0.5% 2,2'-Bipy solution and 2.0cm^3 of 10% sodium acetate solution to the set of flasks. The set of solutions in each case were left to stand for 30 minutes, made up to the mark with water, and determined colourimetrically on a Corning 253 colourimeter (Chem. Soc., 1978).

The absorbance readings obtained were used in constructing the absorption, calibration and Rigbom curves. Concentrations of iron in the samples were then computed from the calibration curves.

Calculation

$$\text{Fe mg/100g sample} = \frac{\text{I(ppm) x solution volume (cm}^3\text{) x 10}}{\text{(Volume of Aliquot taken) x (weight of sample)g}}$$

where I(ppm) = ppm Fe from the calibration curve

3.3.2.2. Colourimetric Determination of Copper (Cu) using 2,9-Dimethyl-1,10-phen.hydrochloride as Ligand.

0.1964g copper sulphate ($CuSO_4.5H_2O$). was dissolved in water. and was transferred into 1 litre flask in which 5cm^3 conc HCl has been added and was made up to the mark with water. This stock solution of copper corresponds to 50ppm in Cu(II). 0.5 cm^3 - 5.0cm^3 aliquots of the stock covering the range 1.0 - 10ppm Cu(II) were pipetted into a

set of 25cm^3 volumetric flasks. Aliquots of the digest/filtrate were also taken and transferred to another set of 25cm^3 volumetric flasks, into which 1.0cm3 aliquot of the stock solution corresponding to 2.0ppm Cu(II) have been added. Another 25cm^3 flask was set aside for blank. Colour development was according to the method of Snell and Leslie (1971). A Corning 253 colourimeter was used for the determination. Calibration, absorption and Rigbom curves were constructed from the results and the concentration of copper in the samples was computed from the calibration curve.

Calculation

$$\text{Cu mg/100g sample} = \frac{\text{C(ppm) x solution volume (cm}^3\text{) x 10}}{\text{(volume of aliquot taken) x (weight of sample)}}$$

where, C(ppm) = ppm Cu from graph - 2.0ppm added Cu

3.3.2.3 Colorimetric Determination of Zinc (Zn) Using Dithizone in CCl_4 as Ligand

20ppm stock solution of Zn(II) was prepared by 0.08794g of zinc sulphate ($ZnSO_4.7H_2O$), dissolving in some quantity of water transferring into 1 litre volumetric flask in which 5cm^3 of HCl was added and making up to the mark with water. 0.05cm^3 - 1.00cm^3 aliquots of the stock solution covering the range 0.1 - 2.0mg Zn were pipetted into a set of 20cm^3 separatory funnels. Aliquots of the digests/TCAA filtrate were also pipetted into another set of 20cm^3 separatory funnels. Solvent extraction and subsequent colourimetric determination were according to the method of Charlot (1964).

A Corning 253 colorimeter was used in running both the standards and samples, and from the results calibration, Absorption, and Rigbom curves were constructed. Concentration of Zinc in the sample was computed from the calibration curve.

Calculation

$$\text{Zn mg/100g sample} = \frac{\text{Z(ppm) x solution volume x 10}}{\text{(volume of aliquot) x (weight of the sample)}}$$

where Z(ppm) = ppm Zn from the calibration curve.

3.3.2.4 Colorimetric Determination of Manganese Mn Using Potassium Periodate as Ligand.

Standard 10ppm solution of Mn was prepared by dissolving 0.041g Manganese sulphate in water, transferring into 1litre volumetric flask and making up to the mark with water. Standards ranging from 0.1 - 6.0 (ppm) in Mn were transferred into a series of $50cm^3$ volumetric flasks. Also portions of the digest/TCAA filtrate were transferred into $50cm^3$ volumetric flasks in which 2.0ppm Mn has been added. Another $50cm^3$ volumetric flask was set aside for blank. Colour was developed according to the method of (Charlot, 1964). Colurimetric determination was done using Corning 253 colorimeter, and from the results of the standards, calibration, absorption and Rigbom curves were constructed. The concentration of Mn in the digests/filtrate was determined from the calibration curve.

Calculation

$$\text{Mn mg/100g sample} = \frac{\text{M(ppm)} \times \text{solution volume } (cm^3) \times 10}{(\text{volume of aliquot } (cm^3)) \times (\text{weight of sample})}$$

where M(ppm) = ppm Mn from graph - 2.0ppm added Mn.

3.3.2.5 Colorimetric Determination of Cobalt (Co) Using Nitroso-R-Salt as Ligand.

10ppm standard solution of Co was prepared by dissolving 0.04g of cobaltous chloride, in a $100cm^3$ of water, the resulting solution was transferred into a litre volumetric flask and was made up to the mark with water. Standard solutions containing 0.2 - 1.6ppm Co were prepared by pipetting 0.2 to $1.6cm^3$ of the stock solution into $10cm^3$ volumetric flasks. Aliquots of the digest/TCAA filtrate were transferred to another sets of $10cm^3$ volumetric flasks. Another $10cm^3$ volumetric flask was set aside for blank. Colur was developed according to AOAC, (1965) The colour intensity of each sample was read on a Corning 253 colorimeter, and from the results obtained, calibration, absorption and

Rigbom curves were made. From the calibration curve the concentration of Co in the samples were computed.

Calculation

$$\text{Co mg/100g sample} = \frac{\text{N(ppm) x solution volume x 10}}{\text{Volume of Aliquot x weight of sample}}$$

Assuming N(ppm) = ppm Co from the calibration curve

3.3.2.6 Colorimetric Determination of Lead (Pb) Using Dithizone in CCl_4 as Ligand.

A 10ppm standard solution of Pb(II) was prepared by dissolving 0.01598g of lead nitrate $Pb(NO_3)_2$, in water in a litre volumetric flask. From the stock solution, aliquots covering the range 0.1 - 2.0ppm. Pb(II) were pipetted into a series of $20cm^3$ separatory funnels. Also, aliquots of the digest/TCAA filtrate were treated in a similar manner. The method of Charlot, (1964) was used in complexation and extraction of the Pb ion. Calibration, Rigbom and absorption curves were constructed from the results obtained by running the solution on a Corning 253 colorimeter. Pb ion in the sample was determined from the calibration curve.

Calculation

$$\text{mg of Pb/100g sample} = \frac{\text{P(ppm) x solution volume x 10}}{\text{volume of Aliquot taken x weight of sample}}$$

where P(ppm) = ppm Pb from the calibration curve

3.3.2.7 Recovery of Added Ion

0.1mg of each of the ion was added to 5g portion of the sample. Then wet digestion, dry ashing and TCAA treatments were repeated as in (Section 3.3.1). Colour development and determination for each of the digests/filtrate follows the same procedure as in (Section 3.3.2.1 - 6). The experiment was repeated using 0.2 and 0.3mg of each ion.

Calculation

$$\% \text{ Recovery} = \frac{\text{ion recovered (mg)}}{\text{ion added (mg)}} \times \frac{100}{1}$$

where; ion recovered = Ion found (when there was addition - Ion found (when there was no addition)

3.3.3 AAS Determination of the Metals

3.3.3.1 Determination of Iron

About 7.0025g iron(II) ammonium sulphate was dissolved in water in a litre volumetric flask giving 1000mg iron solution. $10cm^3$ of this solution was diluted to $100cm^3$ to obtain 100mg/l iron from which 0 - 15mg/l was prepared through serial dilution. Aliquots of the digests/TCAA filtrate were used without further dilution. The entire solutions were then aspirated at wavelength of 248.3nm. A calibration curve was prepared. A Pye-Unicam Model SP1900 AAS was used for the determination. Calculation follows the pattern in (Section 3.3.2.1).

3.3.3.2 Determination of Copper (Cu)

A stock solution of Copper was prepared by dissolving 0.03938g copper(II) sulphate in water in a litre flask to give 10ppm copper solution. A 0 - 6ppm working standards were prepared from this 10ppm solution by pipetting 0 - $15cm^3$ aliquots, into a set of $25cm^3$ flasks and diluting to the mark. Aliquots of the digest/TCAA filtrate were transferred into $25cm^3$ volumetric flasks in which $5.0cm^3$ (2.0mg) of the 10ppm solution have been added, and made up to the mark with distilled water. Each of the standards, sample and blank were aspirated at wavelength of 324.7nm using a Pye-Unicam Model SP1900 AAS. A calibration curve was prepared. Calculation was made as in (Section 3.3.2.2)

3.3.3.3 **Determination of Zinc**

A 20mg/l stock solution was prepared by dissolving 0.08794g of zinc sulphate ($ZnSO_4.7H_2O$) in water in a $1000cm^3$ flask. Standards ranging from 0 - 1.5 ppm of Zn were prepared by diluting appropriate portions of the stock solution to $25cm^3$ in separate volumetric flasks. $10cm^3$ aliquots of the digests/TCAA filtrate were each diluted to $25cm^3$ with water. The solutions were aspirated at λ = 213.8nm.

3.3.3.4 **Determination of Manganese**

A 10 ppm Manganese stock solution was prepared by dissolving about 0.0406g manganese sulphate ($MnSO_4.4H_2O$) in water containing $1cm^3$ of conc. H_2SO_4 in a litre volumetric flask. Then, 0 - 6ppm working standards were prepared. $15cm^3$ aliquot of the digest and $5.0cm^3$ standard. 10ppm Mn solution were transferred into $25cm^3$ flask and diluted to the mark. The solutions were aspirated at 279.5nm .

3.3.3.5 **Determination of Calcium and Magnesium**

A stock solution corresponding to 20ppm was prepared from $CaCO_3$, and 0 - 10ppm standards were made from the stock by pipetting $0.0cm^3$ - $25cm^3$ into a set of $50cm^3$ volumetric flasks and making them up to the mark with water.

Similarly, a stock solution corresponding to 10ppm Mg was prepared from $MgSO_4$, and 0.0 - 1.5ppm standards were made from the stock by pipetting $0.0cm^3$ - $7.5cm^3$ into a set of $50cm^3$ volumetric flasks and making them up to the mark with water.

Aliquots of the digests/TCAA filtrate were taken and diluted to $25cm^3$ with distilled water. The standard and the samples were aspirated at 422.7nm for Ca and at 285.2nm for Mg using a Pye-Unicam Model SP1900 AAS.

Calculations

$$\text{mg of Ca/100g sample} = \frac{\text{Ca (ppm) x Solution volume x 10}}{\text{Volume of Aliquot x weight of sample}}$$

$$\text{mg of Mg/100g sample} = \frac{\text{Mg(ppm) x solution volume x 10}}{\text{Volume of Aliquot x weight of sample}}$$

where:

Ca(ppm) = (ppm) Ca from the graph

Mg(ppm) = (ppm) Mg from the graph

3.3.4 Flame Photometric Determination of Sodium and Potassium

1.5002g of sodium chloride and 2g of potassium chloride were oven dried at 120°C for 1 hr. On cooling for 30 min., 0.2540g of NaCl and 0.190g of KCl was each dissolved separately in a litre volumetric flask and made up to the mark with water, to obtain a 100ppm Na and K stock solutions respectively. From the Na stock, working standards from 0 - 15ppm were prepared, and from the K stock, working standards corresponding to 2 - 30ppm K were prepared. Aliquots of the digest/TCAA filtrate were taken and diluted to 25cm^3 with water. The solutions were determined in the (Corning EEL) photometer after the instrument was set to 0% and 100% emissions with the highest and the lowest standards respectively for each ion. Calibration curves were prepared from the readings of the standards.

Calculation

$$\text{mg of Na/100g sample} = \frac{\text{Na(ppm) x solution volume x 10}}{\text{Volume Aliquot x weight of sample}}$$

$$\text{mg of K/100g sample} = \frac{\text{K(ppm) x solution volume x 10}}{\text{Volume of Aliquot x weight of sample}}$$

where: Na(ppm) = Sodium concentration from the curve

K(ppm) = Potassium concentration from the curve.

3.3.5 Colorimetric Determination of Phosphorous

A stock solution of 20ppm phosphorous was prepared by dissolving 0.0768g $NaHPO_4$ in water and the volume made up to 1 litre with water. Aliquots of the stock were pipetted into $50cm^3$ volumetric flasks. These solutions when made up to the $50cm^3$ mark with distilled water contain 0.5 - 6.0ppm P. Another $50cm^3$ volumetric flask was set aside for blank. Portions of the digests/TCAA filtrate were also transferred into $50cm^3$ volumetric flasks. Then $5cm^3$ of 6N HNO_3, $5cm^3$ of ammonium vanadate solution, and $5cm^3$ 5% ammonium molybdate solution were added to each of the flasks including blank. After dilution to the marks, colorimetry was carried out using (Corning 253) colorimeter at λ_{max} = 460nm. Calibration curve was constructed and used in determining the concentration of P in each sample.

Calculation

$$\text{mg of P/100g sample} = \frac{\text{P(ppm) x Solution volume x 10}}{\text{Volume of Aliquot x weight of sample}}$$

where:

P(ppm) = concentration of Phosphorous from the curve.

3.3.6 Determination of Chloride

Standardization of $AgNO_3$ Solution: About 2.92g of dried NaCl was dissolved in water in a litre volumetric flask. The solution was about 0.05M. About 0.85g of $AgNO_3$ crystals was dissolved in a $100cm^3$ volumetric flask and was diluted to the mark with water. This solution was slightly more than 0.05M $AgNO_3$. The $AgNO_3$ solution was put in the burette and was titrated against $25cm^3$ of the standard 0.05M NaCl solution in

which 1cm^3 of 5% potassium chromate has been added, in a small conical flask. At the end of the titration, 25cm^3 of 0.05M NaCl require 24.8cm^3 of the $AgNO_3$ solution.

Calculation

Equation: $NaCl + AgNO_3 \longrightarrow NaNO_3 + AgCl$

1MNaCl ≡ 58.5gNaCl in 1000cm^3 water ≡ 35.5g Cl^-

0.05MNaCl ≡ 2.925g NaCl in 1000cm^3 water ≡ 1.775Cl^-

Hence. 1cm^{3}NaCl ≡ $\frac{1.775}{1000}$ = 0.001775 Cl^-

But 25cm^3 NaCl ≡ 24.8cm^3 $AgNO_3$

1cm^3 NaCl ≡ 0.992cm^3 $AgNO_3$

Hence 0.992cm^3 ≡ $AgNO_3$ ≡ 0.00177gCl^-

Determination of Cl^- in the Samples.

1.0g of the sample was weighed and reconstituted in a 50cm^3 conical flask using 20cm^3 of warm water. Then, 10 drops of 10% potassium chromate solution (prepared by weighing accurately 10g of K_2CrO_4, transferring into 100cm^3 volumetric flask, dissolving with about 30cm^3 of alcohol and making up to the mark with alcohol) was added. The mixture was then titrated against the standard $AgNO_3$ solution until a red-wine end point was reached. The average volume of $AgNO_3$ used for the titration was used in calculating the Cl^- content of the samples.

Calculation

mg of Cl^-/100g sample = Titre value x 178.427

3.3.7 Determination of Moisture Content of the Samples

Clean porcelain crucibles were dried to constant weight in an air oven at 105°C for 1 hr. They were then cooled in a desiccator and weighed. About 1g of the sample was

weighed into the crucible. The crucible with the content was reweighed to know the weight of the sample. It was kept in the oven at 80°C for six hours, and was cooled in desiccator. The oven drying and weighing were repeated at intervals of 1hr until constant weight was obtained. The loss in weight during drying represent the moisture content.

Calculation

$$\% \text{ moisture} = \frac{\text{weight loss(g)}}{\text{weight of sample}} \times \frac{100}{1}$$

3.3.8 Determination of Ash Content of the Samples

A clean porcelain crucible was ignited in the furnace at 550°C for 10 min. and was transferred to a desiccator to cool after which it was weighed. About 1.0g of oven dried powdered sample was weighed into the crucible and the crucible with the content weighed again. It was placed in an oven set at 150°C until smoking ceased. It was then placed in the furnace and the temperature was gradually increased until 550°C. The temperature was maintained for 8 hours. The sample was removed from the furnace, cooled in a desiccator and then weighed. The weight of the residue in the crucible corresponds to the ash content of the sample.

Calculation

$$\% \text{ Ash} = \frac{\text{weight of Ash}}{\text{weight of sample}} \times \frac{100}{1}$$

3.3.9 Determination of Titrable Acidity of the Samples

About 2.5g of the sample was weighed and reconstituted in a $50cm^3$ volumetric flask with warm water. $10cm^3$ of the resulting mixture was pipetted into a $50cm^3$ conical flask. Then 5-drops of 1% phenolphthalein indicator was added and the mixture was

titrated against standard 0.1M NaOH solution until a faint pink colour at the end point was observed.

Calculation

Equation: $NaOH + CH_3CH(OH)COOH \longrightarrow CH_3CHOHCOO\,Na + H_2O$

40g NaOH neutralizes 90g of acid

1 litre of 1MNaOH nuetralizes 90g acid

Hence 1 litre of 0.1M NaOH neutralizes 10g of acid

$1cm^3$ of 0.1M NaOH neutralizes 0.01g of acid.

$$\% \text{ Acid} = \frac{\text{Titre value} \times 0.01}{\text{Weight of sample}} \times \frac{100}{1}$$

3.3.10 **Determination of the Fat Content of the Samples.**

About 1.0g of the sample was weighed and placed in $30cm^3$ Kjeldahl flask. Then, $10cm^3$ of conc. HNO_3 and $2cm^3$ of conc. H_2SO_4 were added and the mixture heated on a heating mantle for 1 hr. The mixture was cooled and poured into separatory funnel and then $15cm^3$ of petroleum ether was added, and the mixture was shaken for 1 min. The ether layer was transferred into a weighed evaporating dish and the ether was separated from the fat by evaporation at room temperature for 24 hours. The dish and the fat was then weighed and the difference in weight gave the crude fat content of the sample.

Calculation

$$\% \text{ fat} = \frac{\text{weight of fat}}{\text{wight of sample}} \times \frac{100}{1}$$

3.3.11 **Determination of Protein Content By Kjeldahl's Method**

About 2.5g of the milk powder was placed in a $100cm^3$ Kjeldahl's flask and $25cm^3$ of conc H_2SO_4 was added. Then a heating mantle was used to apply heat gently in a fume

cupboard until fumes were observed. Then about 5.0g of K_2SO_4 and about 0.3g of $CuSO_4$ crystals were added. Heating was continued until the content of the flask was clear. Then heating was prolonged for a further 1 hr. The content of the flask was cooled and transferred into Kjeldalh's apparatus where the ammonia in it was steam distilled into $50cm^3$ of 0.1N H_2SO_4 in which 8-drops of methyl-red indicator has been added. After the distillation, excess of acid was titrated with 0.1N NaOH to yellow end point.

Calculation

Equation: $H_2SO_4 + 2NH_3 \longrightarrow (NH_4)_2SO_4$

98g of H_2SO_4 neutralizes34g of ammonia containing 28g of nitrogen.

But, 1 litre of 0.1N H_2SO_4 contains 4.9g of pure H_2SO_4.

Hence, 98g of H_2SO_4 will be contained in 20 litres.

i.e 20 litres of H_2SO_4 = 28g of nitrogen

$$\% \text{ Nitrogen} = \frac{(\text{volume of } H_2SO_4 \text{ used} - \text{Titre value}) \times 28}{20000ml \times \text{weight of sample}} \quad \frac{100}{1}$$

% Protein = % Nitrogen x 6.38 (AOAC conversion factor)

3.3.12 Determination of Lactose

This was determined by difference

% lactose = 100 - (% Protein + % Ash + % Fat).

3.3.13 Development of New Colorimetric Reagent for the Determination of the Trace Metals in Infant Milk.

Six organic compounds namely: Benzidine, 1,2,3-trihydroxylbenzene (pyrogallol), rhodizonic acid, flourescien, picric acid, and mercapto ethanol were proposed. 1% solutions of each organic compound was made and they were set aside for the tests. Standard solutions corresponding to 10ppm of the metal ions were prepared. Each of the metal ions was tested in turn with each of the proposed reagents, in the following manner. Aliquots of the stock solution of the metal was transferred into a test tube.

Then about 5-drops of the reagent was added, observations and pH were recorded. The content of the tube was discharged and a new aliquot of the metal ion was introduced. The pH was varied using a series of buffer solutions until the pH range of 1 -12 was exhausted.

In some instances where reduction of the metal ion was needed, hydroxylammonium chloride solution was added. The pH of the test solutions were monitored with the aid of high quality pH-hydrion paper with (1 - 12) range. Reagents that produced intense colour with the metal ion was used in determining the ion in solution and in the milk samples colorimetrically.

3.3.14 Colorimetric Determination of Fe(III) with 1,2,3-trihydroxylbenzene (pyrogallol)

100ppm solution of Fe(III) was prepared by dissolving 0.3510g of ferrous ammonium sulphate in water into which $3cm^3$ of conc. HCl has been added in a $500cm^3$ volumetric flask. Aliquots of this stock solutions corresponding to 0.5 - 50ppm in Fe(III) were pipetted into a series of $50cm^3$ volumetric flasks. Another $50cm^3$ flask was set aside for blank. Aliquots of the digest/TCAA filtrate were transferred also to $50cm^3$ volumetric flasks.

Then, $2cm^3$ of 1% reagent solution followed by $5cm^3$ of 10% sodium acetate solution (pH 5 -6) were added to each of the flasks. Colour was allowed to develop for 30 mins. The absorbances of the solution were taken on a Corning 253 Colorimeter. The λ_{max} of the complex was determined by taking the absorbances of 5 - 10ppm solutions between the wavelength range of 440 - 680nm. The results obtained were used in constructing the absorption, calibration and Rigbom curves. Concentration of Fe in the digests/TCAA filtrate was read from the calibration curve. Calculation follow the pattern in (Section 3.3.2.1). Recoveries of added iron follow the method in (Section 3.3.2.7).

CHAPTER 4

4.0 RESULTS AND DISCUSSIONS

4.1 Sample Preparation

To ensure quantitative and reproducible results, the sample preparation procedures were carefully carried out. Wet digestion of samples was stopped when the solutions became clear signifying the total destruction of organic matter. After filtration, the filter paper was washed several times with water to ensure good recovery of ions that might be trapped in it. The temperature of the carbolite furnace used for the dry ashing was increased slowly to ensure uniformity in the destruction of organic matter and to guide against the loss of some volatile inorganic components. (Jackson 1938). During the precipitation of fat and protein using TCAA, the temperature was maintained at 100 ± 5°C to achieve optimum result. Resultant precipitate was washed several times to enable quantitative recovery of the ions (Coni, 1994).

4.2 Iron (Fe) Determination

To be able to determine Fe colorimetrically in the preparations, the effect of other ions in milk was investigated. It was found that Copper (Cu) presented serious interferences. However, it was possible to mask 50ppm of Cu with 10% thiourea, when determining 1.0ppm of Fe by the 1,10-phen and 2,2'-Bipy methods. The absorption curves for the two methods gave λ_{max} at 510nm for Fe (-1,10-phen)$_3^{2+}$ and λ_{max} at 520nm for Fe(-2,2'-Bipy)$_3^{2+}$ Appendix A, (III) and (IV). From the calibration and Rigbom curves Appendix A (I), (II), (V) and (VI)., the detection limits were found to be 0.35 - 3.7ppm for Fe(-1,10-phen)$_3^{2+}$ and 0.63 - 5.25ppm for Fe(-2,2'-Bipy)$_3^{2+}$.

4.2.1 Statistical Comparison of Results For Fe Determination.

Tables 4.1, 4.2 and 4.3 show the results obtained using colorimetric techniques for the three sample preparations. Tables 4.7, 4.8 and 4.9 show the results by AAS method for

the three preparations. A statistical comparison of the two set of results, using the student-*t* test for pair data experiment, at 95% confidence limit, was done. Appendix I (I). On comparison of the *t*s calculated from the difference between the two sets of results with t = (2.77 at f = 4 degrees of freedom). It was evident that the 2,2'-Bipy method using the wet digest gave the least value for t, which implies better comparison with the AAS method. This result was followed by 2,2'-Bipy method using TCAA filtrate; 1,10-phen method using TCAA filtrate and 1,10-phen method using wet digest, in that order. It was not possible to deduce from the student-*t* test obtained from the 1,10-phen. and 2,2'-Bipy dry digests' whether they can give statistically equivalent results with AAS, as their values were very close to $t_{0.05}$.

However the percent relative standard deviation, a measure of the level of experimental error (Tables 4.1, 4.2 and 4.3) for 1,10-phen method was least on the average, using the TCAA filtrate, followed by wet digest and then dry digest. When 2,2'-Bipy method was used, the trend changed from the wet digest to the furnace (dry) digest. The lower values obtained with the dry digest could be due to the loss of some ions on ignition. 1,10-phen can complex other substances in the digest, thus reducing its concentration available for complexation with iron. The TCAA filtrate contains some surfactants, which could flocculate ions and offer better determinations (Coni, 1994).

Table 4.1: Elemental Determination Using The Wet Digestion and Colorimetry. (Mean Concentrations, mg/100g).

S/No	Formula		Fe		Cu	Zn	Mn	Co	Pb
			1,10-Phen.	2,2`-Bipy					
1	NAN	Mean	6.25	6.55	0.36	4.58			
		SD	0.04	0.07	0.02	0.06	ND	ND	ND
		%RSD	0.64	1.07	5.55	1.31			
2	COWBELL	Mean	5.41	5.29	0.35	2.63			
		SD	0.06	0.03	0.02	0.08	ND	ND	ND
		%RSD	1.11	0.57	5.71	4.91			
3	BEBELAC	Mean	4.09	5.09	0.47	5.53			
		SD	0.06	0.06	0.02	0.04	ND	ND	ND
		%RSD	1.46	1.81	4.25	0.72			
4	SMA	Mean	6.15	5.92	0.64	3.63			
		SD	0.10	0.11	0.01	0.02	ND	ND	ND
		%RSD	1.63	1.86	1.56	3.17			
5	I-ER	Mean	6.44	6.24	0.96	3.40			
		SD	0.04	0.05	0.03	0.04	ND	ND	ND
		%RSD	0.62	0.80	3.13	2.86			

SD = Standard Deviation

RSD = Relative Standard Deviation

ND = Not Detected

Table 4.2: Elemental Determination Using Furnace Digestion and Colorimetry. (mg/100g Powder)

S/No	Formula		Fe		Cu	Zn	Mn	Co	Pb
			1,10-Phen	2,2`-Bipy					
1	NAN	Mean	5.47	5.07	0.82	3.14			
		SD	0.05	0.05	0.01	0.04	ND	ND	ND
		% RSD	0.91	0.99	1.22	1.27			
2	COWBELL	Mean	2.60	2.19	0.41	2.04			
		SD	0.15	0.06	0.01	0.05	ND	ND	ND
		% RSD	5.77	2.74	2.44	2.45			
3	BEBELAC	Mean	3.72	3.47	0.40	3.80			
		SD	0.02	0.05	0.01	0.04	ND	ND	ND
		% RSD	0.54	1.44	2.50	1.05			
4	SMA	Mean	6.29	6.04	0.16	22			
		SD	0.05	0.03	0.02	0.02	ND	ND	ND
		% RSD	0.81	0.50	12.50	0.62			
5		Mean	6.71	6.49	0.13	3.11			
	1-ER	SD	0.03	0.05	0.02	0.08	ND	ND	ND
		% RSD	0.45	0.77	15.38	0.57			

SD = Standard Deviation

RSD = Relative SD

ND = Not Detected

Table 4.3 Elemental Determination Using (TCAA) Filtrate and Colorimetry.

(Mg/100g powder)

S/No	Formula		Fe		Cu	Zn	Mn	Co	Pb	
			1.10-Phen	2.2'-Bipy						
1	NAN	Mean	5.78	5.80	0.43	3.49				
		SD	0.02	0.03	0.02	0.02	ND	ND	ND	
		%RSD	0.35	0.52	4.65	0.57				
2.	COWBELL	Mean	5.17	5.07	0.31	2.85				
		SD	0.12	0.05	0.01	0.03	ND	ND	ND	
		%RSD	2.32	1.00	3.23	1.05				
3.	BEBELAC	Mean	3.72	3.01	0.30	4.26				
		SD	0.02	0.01	0.01	0.03	ND	ND	ND	
		%RSD	0.54	0.33	3.33	0.71				
4.	SMA	Mean	8.11	8.62	0.42	3.60				
		SD	0.04	0.02	0.02	0.05	ND	ND	ND	
		%RSD	0.50	0.23	4.76	1.39				
5.	1-ER	Mean	6.38	6.18	0.45	3.91				
		SD	0.02	0.13	0.03	0.01	ND	ND	ND	
		%RSD	0.31	2.10	6.67	0.26				

SD = Standard Deviation

RSD = Relative SD

ND = Not Detected.

To test for the accuracy and reproducibility of the methods, recovery tests were carried out (Table 4.4, 4.5 and 4.6). More Fe ions were recovered in the dry digest followed by the TCAA filtrate and wet digest, for both methods. Percent recoveries of the methods ranged from 86% to 99.02%, testifying to the reliability of the methods, and their suitability for colorimetric determinations.

4.3 Copper (Cu): Determination/Statistical Comparison of Results

The 2,9.dimethyl-1,10-phen, method was adopted for the determination of Cu as it is sensitive and selective for Cu ions (Snell *et. al.*, 1971). The hydrochloride derivative of the reagent was used to eliminate solvent extraction and enabling aqueous determination, with the slight interference offered by Fe masked with EDTA. Other ions did not pose any interference to the determination. The λ_{max} for the determination was found to be 450nm and at this λ_{max}, the detection limits were found to be 0.41 to 4.10mg/l [Appendix B (I) (II) (III)]. The results obtained by the colorimetric method using the three preparations are as shown in Tables 4.1, 4.2 and 4.3. Also, Tables 4.7, 4.8 and 4.9 show the results obtained by the AAS method using the three preparations.

A student *t* test on the differences obtained for both methods, and the preparation procedures was made at 95% confidence limit. The calculated *t* for the furnace digest was least relative to $t_{0.05} = \pm 2.77$ at (f= 4 degrees of freedom), followed by the t for the wet digest and that for the TCAA filtrate. Although the furnace procedure has the lowest *t*, showing better comparison with AAS method, it is obvious that the methods generally gave statistically equivalent results with the AAS method. Appendix I (II).

Table 4.4: Added Ions Recovered Using the Wet Digestion and Colorimetry (Mean % Recoveries)

S/No	Formula		Fe		Cu	Zn	Mn	Co	Pb
			1,10-Phen	2,2'-Bipy					
1	NAN	Mean	90.33	93.33	84.33	85.00	96.67	89.43	81.10
		SD	3.68	4.03	6.13	4.08	1.70	0.80	1.50
		% RSD	4.07	4.32	7.27	4.80	1.76	0.89	1.92
2	COWBEL	Mean	89.44	90.33	89.33	82.77	85.37	85.93	92.77
	L	SD	3.42	0.42	3.29	2.08	0.90	0.33	2.08
		% RSD	3.82	0.52	3.52	2.51	1.05	0.38	2.24
3	BEBELAC	Mean	90.61	90.20	83.67	81.10	88.33	89.43	85.00
		SD	2.39	3.02	2.62	1.56	1.25	1.02	4.08
		% RSD	2.64	3.35	3.13	1.92	1.42	1.14	4.80
4	SMA	Mean	93.55	86.00	90.00	85.00	95.62	82.87	88.87
		SD	2.34	5.35	0.00	4.08	4.44	3.60	1.60
		% RSD	2.50	6.22	0.00	4.80	4.28	4.34	1.80
5	1-ER	Mean	93.89	95.55	77.23	83.87	99.27	88.83	83.33
		SD	3.47	0.42	12.21	2.81	1.02	1.03	9.43
		% RSD	3,70	0.44	15.81	3.35	1.03	1.16	11.30

Table 4.5: Added Ions Recovered Using the Furnace Digestion and Colorimetry (Mean % Recoveries)

S/No	Formula		Fe		Cu	Zn	Mn	Co	Pb
			1,10-Phen	2,2'-Bipy					
1	NAN	Mean	96.43	95.00	70.53	83.87	97.37	89.73	89.43
		SD	4.58	7.07	10.06	2.81	1.69	1.43	3.41
		% RSD	4.75	7.44	15.12	3.35	1.74	1.59	3.81
2	COWBELL	Mean	96.44	88.33	89.33	88.33	93.73	91.67	81.10
		SD	6.12	6.24	6.65	6.24	3.88	2.36	1.56
		% RSD	6.35	7.06	7.44	7.06	4.14	2.57	1.92
3	BEBELAC	Mean	99.05	99.39	85.00	83.87	86.67	87.27	85.00
		SD	4.61	6.85	4.08	2.81	0.47	4.46	1.63
		% RSD	4.65	6.82	4.08	3.35	0.54	5.11	1.92
4	SMA	Mean	97.05	87.83	90.00	88.86	99.70	89.10	86.03
		SD	5.59	4.94	0.00	1.60	0.67	4.41	1.10
		% RSD	5.76	5.62	0.00	1.80	0.67	4.95	1.28
5	I-ER	Mean	97.78	92.94	78.87	87.20	95.67	82.67	93.33
		SD	3.92	6.33	6.82	2.09	0.47	2.87	2.86
		% RSD	4.01	6.81	8.65	2.39	0.49	3.47	3.06

Table 4.6: Added Ions Recovered Using the (TCAA) Filtrate and Colorimetry (Mean % Recoveries)

S/No	Formula		Fe		Cu	Zn	Mn	Co	Pb
			1,10 Phen	2,2'-Bipy					
1	NAN	Mean	97.28	90.55	81.10	91.00	99.33	93.43	91.27
		SD	1.77	4.78	1.56	1.41	0.47	1.56	1.58
		% RSD	1.82	5.28	1.92	1.55	0.47	1.67	1.73
2	COWBELL	Mean	92.44	91.78	92.77	96.10	89.43	95.50	91.60
		SD	2.53	4.01	2.08	2.84	0.80	0.71	2.01
		% RSD	2.74	4.37	2.24	2.96	0.89	0.74	2.19
3	BEBELAC	Mean	94.40	94.28	85.00	87.20	87.53	88.60	91.67
		SD	2.45	2.34	4.08	6.14	1.05	0.29	2.87
		% RSD	2.60	2.48	4.80	7.04	1.20	0.33	3.13
4	SMA	Mean	91.10	93.00	88.33	83.86	98.17	97.53	91.57
		SD	1.56	3.56	2.36	2.81	2.72	6.12	2.19
		% RSD	1.71	3.83	2.67	3.35	2.61	6.27	2.39
5	I-ER	Mean	90.33	94.78	86.67	91.67	98.60	96.77	89.17
		SD	9.32	4.25	4.72	2.36	1.51	2.47	0.62
		% RSD	8.10	4.48	5.45	2.57	1.53	2.55	0.70

Appendix I (III). Even though, at 95% confidence limit, the (TCAA) method gave the best result, relative to AAS, the other two methods still compare well with (ASS) results.

4.5 Manganese, Cobalt and Lead Determination

For the manganese determination, the potassium pexiodate method was used because of the relative availability of the reagent and the reduced interferences of other metals with the method. Tables 4.4, 4.5 and 4.6 show recoveries of added manganese ranging from 87.53 - 98.60% for (TCAA) method, 85.37 - 99.27% for the wet digest and 86.67 - 99.70 for the furnace digest. This might mean that the method is suited for the determination of manganese in solutions. However, when the manganese content of the three preparations Tables 4.1, 4.2 and 4.3 were determined, unreliable results were obtained. Attempts at increasing the quantity of the sample digested and pre-concentration of the digest could not show the presence of manganese. Even though manganese was detected using the (AAS) method. Tables 4.7, 4.8 and 4.9, the percent RSD are so high that the results could hardly be relied upon.

It is evident from Tables 4.1, 4.2 and 4.3 that Cobalt and Lead might not be present in the three preparations. However, Tables 4.4, 4.5 and 4.6 testify to the reliability of the methods adopted for their determination as the percent recoveries recorded conform to the acceptable standards. The (AAS) results Tables 4.7, 4.8 and 4.9 also proved negative thereby substantiating the colorimetric results.

Tables 4.7 - Elemental determination using the Wet digestion and AAS (mg/100g powder)

S/No	Formula		Fe	Cu	Zn	Mn	Co	Pb
1	NAN	Mean	6.40	0.36	2.88			
		SD	0.05	0.01	0.28	ND	ND	ND
		%RSD	0.78	2.77	9.72			
2	COWBELL	Mean	6.00	0.42	0.42	0.16		
		SD	0.12	0.06	0.60	0.01	ND	ND
		%RSD	2.00	14.28	18.92	5.06		
3	BEBELLAC	Mean	4.44	0.35	3.63	0.07		
		SD	0.06	0.02	0.66	0.02	ND	ND
		%RSD	1.35	5.72	18.18	2.86		
4	SMA	Mean	8.84	0.35	4.07	0.14		
		SD	0.11	0.02	0.05	0.01	ND	ND
		%RSD	1.24	5.72	1.23	6.29		
5	I-ER	Mean	7.20	0.34	3.50	0.11		
		SD	0.01	0.03	0.01	0.04	ND	ND
		%RSD	0.14	8.82	0.29	33.6 5		

Table 4.9 Elemental determination using (TCAA) filterate and (AAS) (mg/100g powder)

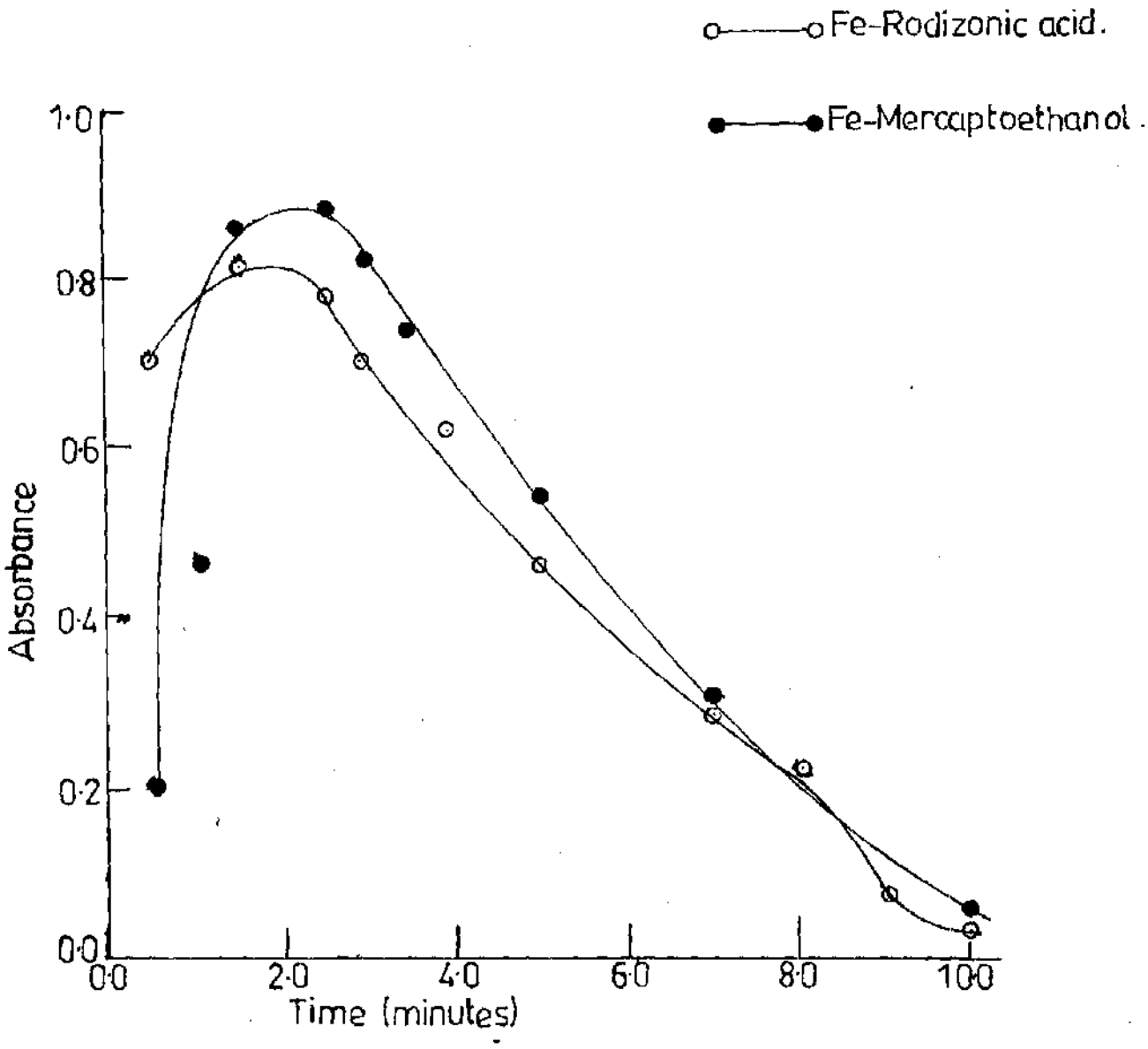
S/No	Formula		Fe	Cu	Zn	Mn	Co	Pb
1	NAN	Mean	5.80	0.32	3.68	0.03		
		SD	0.01	0.01	0.05	0.02	ND	ND
		%RSD	0.17	3.13	1.36	50.00		
2	COWBELL	Mean	5.38	0.31	2.75	0.05		
		SD	0.03	0.01	0.04	0.001	ND	ND
		%RSD	0.56	3.23	1.46	2.04		
3	BEBELAC	Mean	5.68	0.29	4.15	0.05		
		SD	0.07	0.01	0.14	0.0005	ND	ND
		%RSD	1.23	3.45	3.37	10.64		
4	SMA	Mean	9.76	0.38	3.97	0.13		
		SD	0.01	0.01	0.04	0.01	ND	ND
		%RSD	0.10	2.63	1.01	9.45		
5	1-ER	Mean	6.27	0.29	3.78	0.04		
		SD	0.04	0.01	0.01	0.003	ND	ND
		%RSD	0.64	3.45	0.26	8.33		

ND ⇒ Not detected.

4.6: New Reagents for Iron, Copper, Zinc and Manganese Determination

Based on their functional groups, a selection of organic reagents were employed for the determination of the metal ions in the samples. The trials were based on the pH of the solutions as it is vital in coloured complex formation. (Charlot, 1964). For all the reagents tested no coloured complex was noticed for copper, zinc and manganese for they form colourless complexes or do not complex at all with these reagents. Only 1,2,3-trihydroxylbenzene (pyrogallol), Rodizonic acid, and mercaptoethanol gave coloured complex with λ_{max} at 560nm between pH 5 - 8; λ_{max} at 430nm at pH 3 - 4; and λ_{max} at 520nm at pH 10 - 12 respectively with Iron versus distilled water blank. However, the complexes of iron with Rodizonic acid and mercaptoethanol were unstable to enable quantitative determination, as evidenced in figure 4.1, where the colours developed and disappeared within a short time

In the case of iron with pyrogallol, colour development was within five minutes and was stable for about forty four minutes after which turbidity was noticed (Fig. 4.2).



Fig·4·1:Graph of absorbances against time for Fe-Rodizonic acid complex (λ_{max} = 430nm), and Fe-Mercaptoethanol complex (λ_{max} = 520nm).

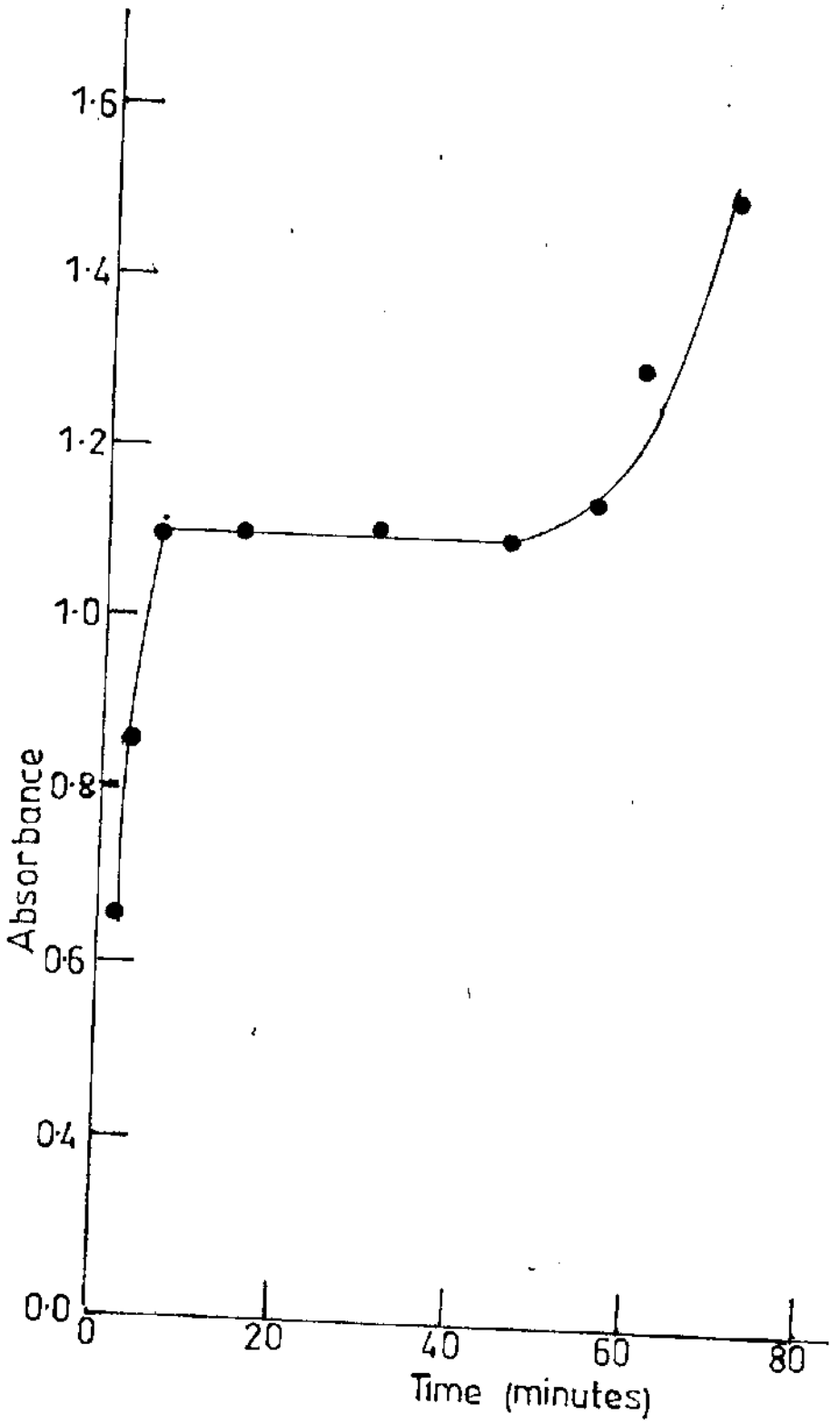


Fig 4.2: Graph of absorbance against time for Fe–pyrogallol complex (λ_{max} = 560 n.m)

Table 4.10 Iron determination using the digestion /TCAA Filtrate and pyrogallol as Colorimetric reagent (mg/100g powder).

S/No.	Formula		Wet Digestion	Furnace Digestion	TCAA Filtrate
1	NAN	Mean	6.28	5.62	5.02
		SD	0.03	0.02	0.01
		%RSD	0.40	0.36	0.19
2	COWBELL	Mean	4.67	4.85	4.20
		SD	0.47	0.05	0.20
		%RSD	10.10	0.36	4.76
3	BEBELAC	Mean	6.18	5.40	5.62
		SD	0.08	0.71	0.02
		%RSD	1.29	13.15	0.36
4	SMA	Mean	7.17	6.69	7.47
		SD	0.12	0.41	0.13
		%RSD	1.67	6.13	1.74
5	I-ER	Mean	6.91	5.55	4.95
		SD	0.11	0.05	0.05
		%RSD	1.59	0.90	1.01

Table 4.11 Added Fe recovered Using the digest/TCAA Filtrate and Pyrogallol as Colorimetric reagent (Mean % Recovery)

S/No	Formula		Wet digestion	Furnace Digestion	TCAA filtrate
1	NAN	Mean	88.43	83.95	82.80.
		SD	1.23	4.51	0.28
		%RSD	1.39	5.37	0.34
2	COWBELL	Mean	79.00	76.00	78.93
		SD	0.82	0.65	2.41
		%RSD	1.04	0.86	3.53
3	BEBELAC	Mean	80.02	80.50	85.96
		SD	4.37	3.84	0.53
		%RSD	5.46	4.77	0.62
4	SMA	Mean	86.83	81.80	88.46
		SD	3.41	2.78	1.23
		%RSD	5.46	3.40	1.39
5	I-ER	Mean	82.15	81.92	90.43
		SD	2.50	2.80	1.55
		%RSD	3.04	3.42	1.72

Table 4.12 Major Elements determination using the wet digestion and (AAS) (mg/100g powder).

S/No	Formula		Ca	Mg	Na	K	P
1	NAN	Mean	406.50	37.20	128.67	530.39	206.35
		SD	6.50	0.16	2.62	0.03	2.59
		%RSD	1.60	0.43	2.04	0.01	1.26
2	COWBELL	Mean	441.00	42.03	134.93	451.00	213.60
		SD	1.42	0.05	0.09	8.28	3.96
		%RSD	0.32	0.11	0.07	1.84	1.85
3	BEBELAC	Mean	383.33	36.17	118.50	570.83	245.00
		SD	2.49	0.13	1.08	0.85	0.82
		%RSD	0.65	0.36	0.91	0.15	0.34
4	SMA	Mean	351.80	42.42	137.67	477.50	241.67
		SD	0.36	0.10	0.24	2.04	4.64
		%RSD	0.10	0.23	0.17	0.43	1.92
5	I-ER	Mean	453.67	58.81	273.37	685.00	221.77
		SD	4.49	0.06	1.24	2.45	1.36
		%RSD	0.98	0.10	0.45	0.36	0.61

The λ_{max} and detection limits of the complex were found to be 560nm and 1.00 - 19.95ppm respectively Fig. 4.3, 4.4 and 4.5. The effect of diverse ions commonly found in milk was investigated and it was only copper that interfered seriously. 4.00ppm of Cu ions interfered during the determination of 10ppm of Fe but this interference could be masked with 10% solution of thiourea (Fig. 4.6).

Table 4.11 shows the added iron recovered as determined by the method in the three sample preparations. It is obvious from the table that the (TCAA) filtrate with recoveries ranging from 79 - 90% offers the best sample preparation method for the determination. Wet digests with 79 - 88.43% follows closely and the furnace digest with recoveries of 79 - 83.95%. The low percent recoveries could be due to incomplete complexation and to some aerial oxidation of the iron. However, the low values of percent RSD testify to minimum levels of experimental error with the method.

A *t*-test at 95% confidence limit performed on the difference obtained from the results of the colorimetry (Table 4.10) with AAS results showed *t*-calculated for the wet method to be ±0.56 and the furnace method to be ±1.27. However, with the TCAA method a $t = \pm 3.11$ which is higher than $t_{0.05} = 2.77$ was obtained showing that it doesn't give statistically equivalent result with the AAS method. [Appendix I (IV)].

4.7 Determination of Major Components of the Sample/Statistical Comparison of Results

An assessment of the other components of the samples namely: Na, K, Ca, Mg and P, and other parameters such as moisture, ash, acidity, protein, fat, lactose and chloride content of the samples was made. This was with the view to assess the dietary qualities of the formulae. Tables 4.12, 4.13 and 4.14 show the results obtained for the macro elements using the three sample preparations, and Table 4.15 shows the results for the other components of the

samples. In other to relate the values obtained for each of these determinations with the manufacturer's quoted values, it was necessary to perform a student-*t*-comparison test.

Appendix I (V) shows the various *t*-calculated obtained from the differences between the experimental and manufacturers' mean values for each of the parameters at 95% confidence limit and at f = 2 degrees of freedom, $t_{0.05} = \pm 4.304$, from a closer look at the table the *t*s for some of the parameters for the individual milk samples exceeded $t_{0.05}$. This tendency connotes that those results might not belong to the same statistical population with the manufacturers' result. This could be linked to the method of analysis used by the manufacturers. The results obtained for Cu, Zn, and Mn conform to the results quoted by the manufacturers, as evident by their low *t*s. The results from other parameters vary from sample to sample. The disagreeable results obtained from comparison of the moisture content can be attributed to handling during the analysis, as the milk samples attract moisture rapidly on exposure.

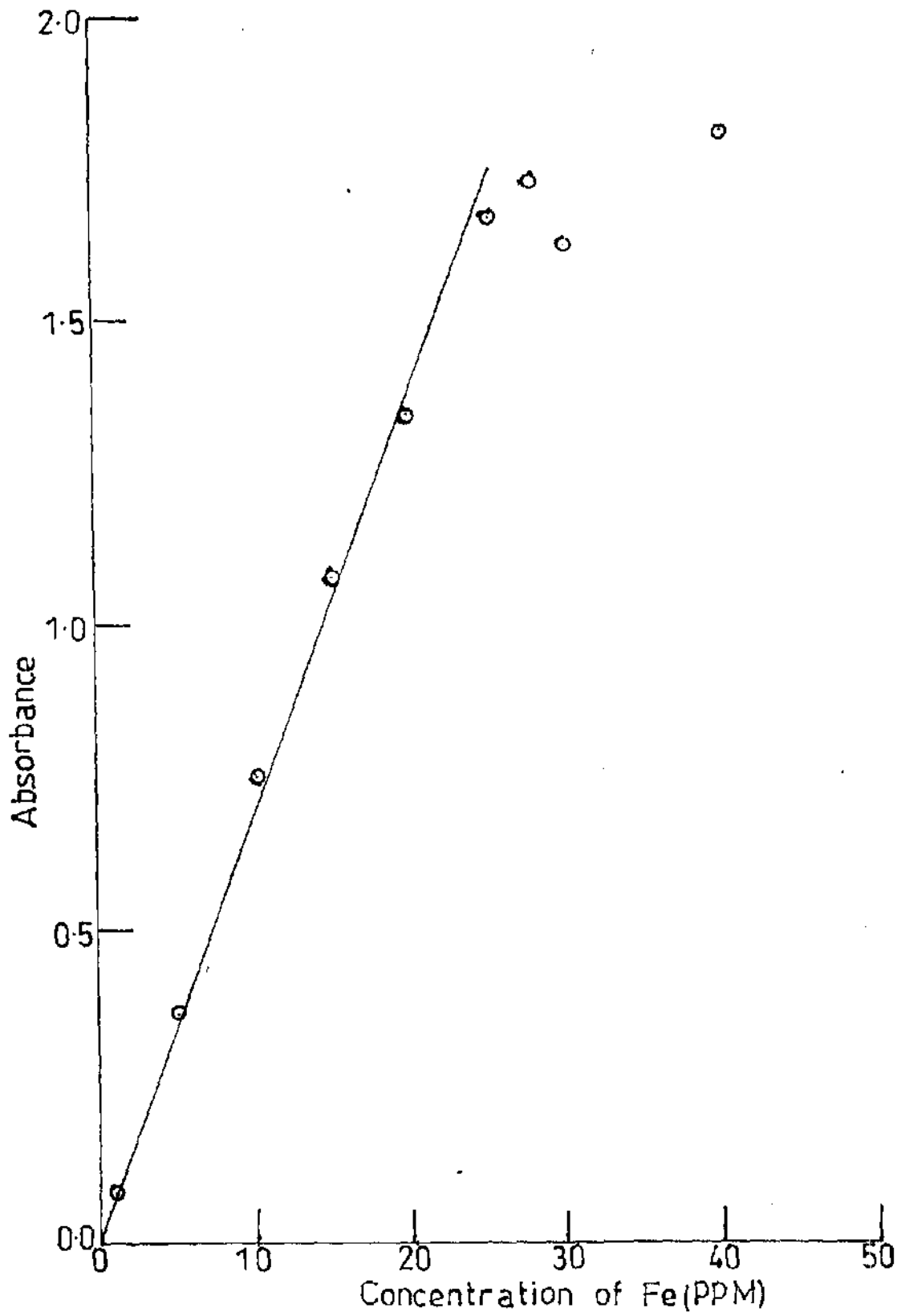


Fig.4·3: Calibration graph for Fe determination using pyrogallol at (λ_{max} = 560nm).

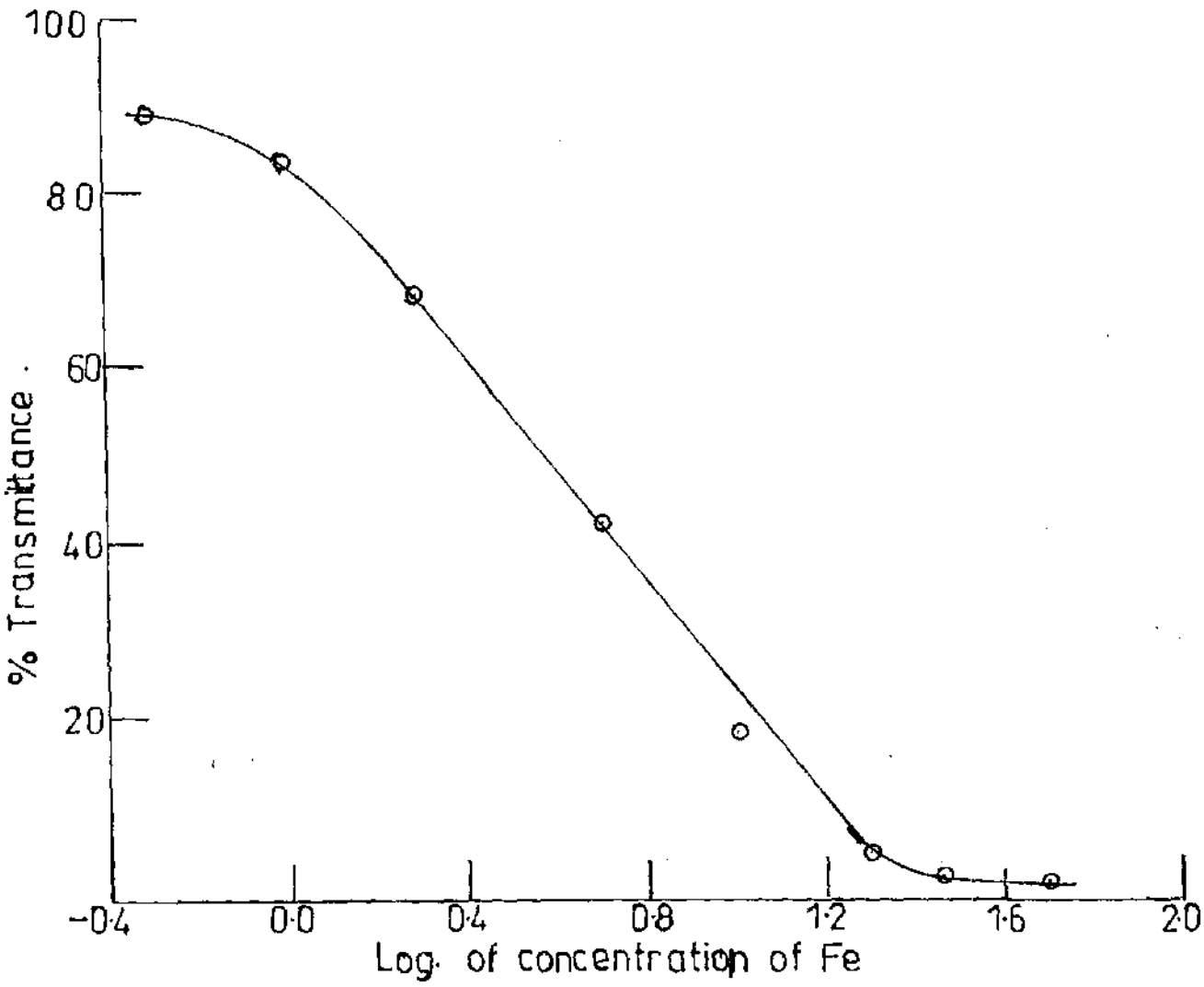


Fig.4.4: Rigbom plot for Fe determination using pyrogallol.

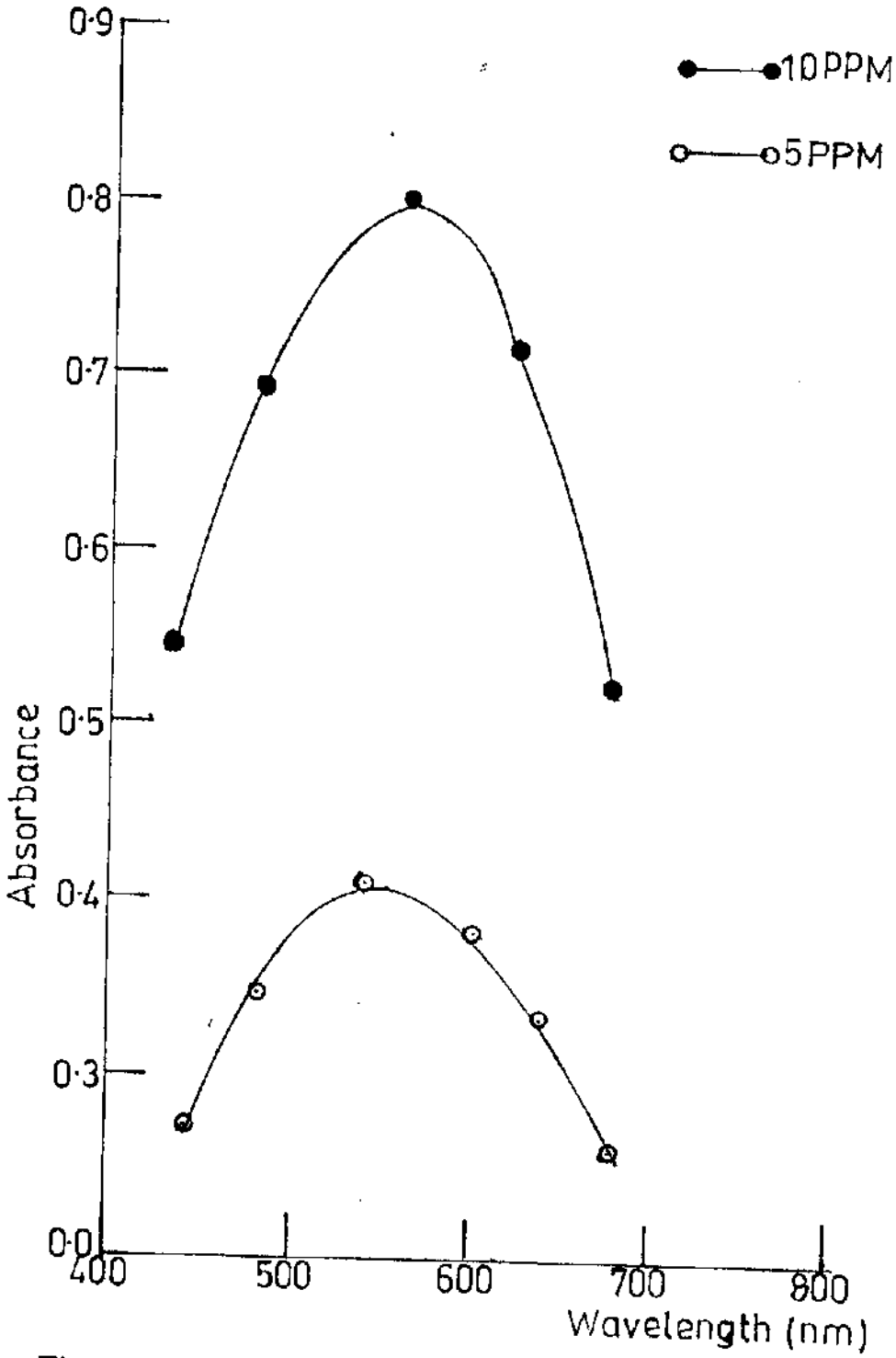


Fig.4·5: Absorption spectra for Fe-pyrogallol complex.

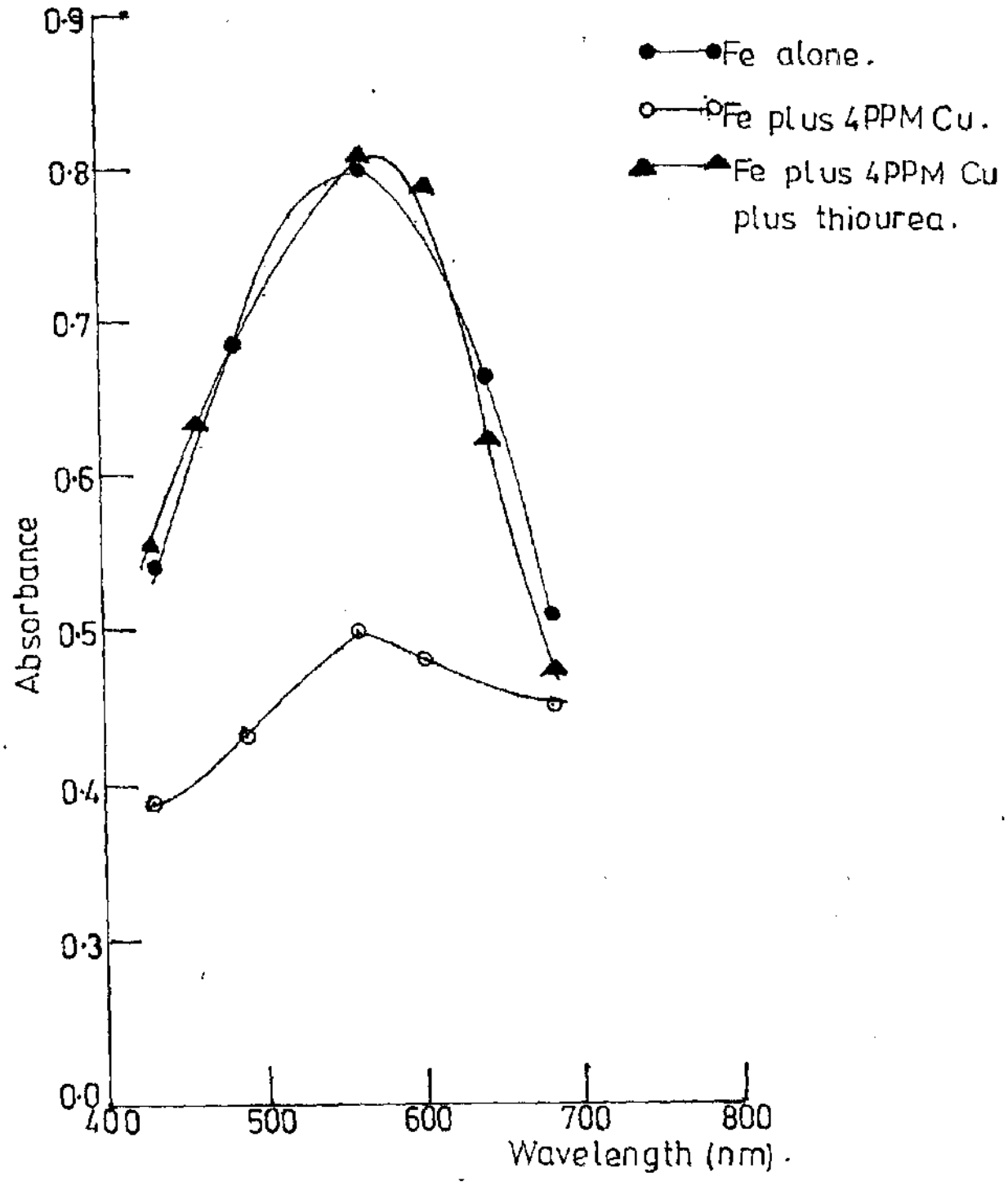


Fig.4.6: Absorption spectra for the interference of Cu in the determination of Fe with pyrogallol, and it's removal with thiourea.

Table 4.13 Major Elements Determination using Furnace Digestion AAS/FES (mg/100g)

S/No	Formula		Ca	Mg	Na	K	P
1	NAN	Mean	420.00	35.22	122.93	527.18	194.13
		SD	4.08	0.14	0.34	8.64	7.77
		%RSD	0.97	0.40	0.28	1.63	4.00
2	COWBELL	Mean	453.67	39.76	133.80	436.41	205.94
		SD	0.47	0.93	0.16	2.20	0.30
		%RSD	0.10	2.34	0.12	0.51	0.15
3	BEBELAC	Mean	343.67	33.38	118.58	568.06	242.07
		SD	1.89	0.13	0.11	0.51	1.11
		%RSD	0.55	0.39	0.09	0.09	0.46
4	SMA	Mean	333.67	40.04	135.86	475.23	235.00
		SD	0.34	0.04	0.37	0.29	1.41
		%RSD	0.10	0.10	0.27	0.06	0.60
5	I-ER	Mean	425.47	53.69	270.57	685.41	217.16
		SD	0.97	0.24	0.31	7.18	0.60
		%RSD	0.23	0.45	0.12	1.05	0.28

Table 4.15 Summary of the Results in (g/100g) of Other Parameters in the Formulae

S/No	Formula		Moisture	Ash	Acidity	Protein	Fat	Lactose	Chloride (mg/100g)
1	NAN	Mean	5.54	3.10	0.62	12.72	24.00	60.34	0.48
		SD	0.01	0.09	0.01	0.07	0.24	0.23	0.004
		%RSD	0.18	2.90	1.29	0.55	1.01	0.38	0.83
2	COWBELL	Mean	6.07	2.98	0.50	1030	28.94	57.79	0.37
		SD	0.01	0.03	0.004	0.03	0.20	0.21	0.01
		%RSD	0.10	1.04	0.80	0.29	0.69	0.36	2.16
3	BEBELAC	Mean	2.98	3.01	0.56	12.09	29.27	55.64	0.43
		SD	0.02	0.05	0.01	0.02	0.07	0.03	0.01
		%RSD	0.67	1.66	0.89	0.17	0.24	0.05	1.16
4	SMA	Mean	3.51	3.15	0.55	12.26	26.94	57.65	0.32
		SD	0.03	0.07	0.01	0.03	0.03	0.09	0.05
		%RSD	0.86	2.22	0.91	0.24	0.11	0.16	15.92
5	I-ER	Mean	2.51	3.05	0.63	12.87	41.44	42.64	0.45
		SD	0.02	0.01	0.02	0.01	0.50	0.50	0.06
		%RSD	0.80	0.46	3.17	0.06	1.21	1.17	13.33

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

Colorimetric method is ideal for rapid and selective determination of the metals in infant milk formula. This is evidenced in the comparability of the results obtained using the methods with atomic absorption spectrophotometric (AAS) technique. The interference of coexisting metal ions were satisfactorily reduced to achieve equivalent results with the AAS method.

The reagents - 2,2`-bipyridyl and 1,10-phenanthroline, used in the determination of iron give equivalent results with the AAS method. However, sample preparation involving wet *digestion prior to colorimetric determination using 2,2`-Bipyridyl offers the best* result. In copper analysis furnace digestion prior to colorimetric determination gave the best result while in zinc, the trichloroacetic acid method was better. However, further improvement in the trichloroacetic acid method would be necessary with regards to its simplicity and rapidity. Manganese, cobalt and lead were not detected in colorimetric *method. It is suggested that an attempt be made at modifying the colorimetric methods* for very trace determination of these metals.

Among the six reagents investigated in the search for new colorimetric reagents only pyrogallol gave positive result with iron (III) ion. The sensitivity of the method was found to be low as depicted by its detection limit. Although this is not a limitation to the determination of iron in infant milk, the method could detect iron at a very low concentration. The results obtained with this new method gave equivalent results with AAS method. However, the wet digestion preparation method prior to colorimetric determination, offers the best result using the method. Attention should be focused on improving the sensitivity of the method and also adapting it for the determination of iron(II).

The agreement of the experimental results of analysis of the other parameters in the infant milk formula, with the manufacturers specifications has been limited. Only the results for copper, zinc, and manganese compared well.

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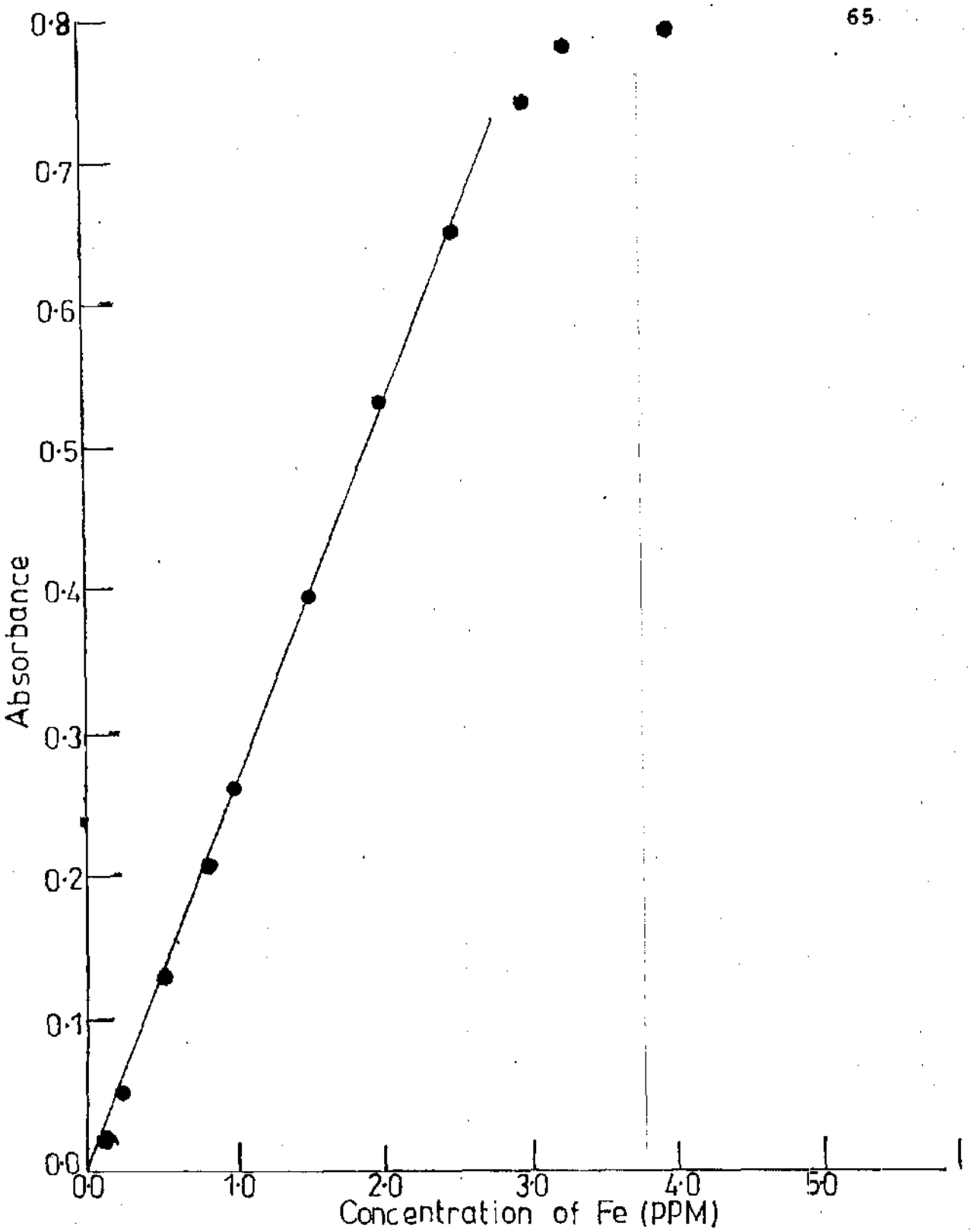
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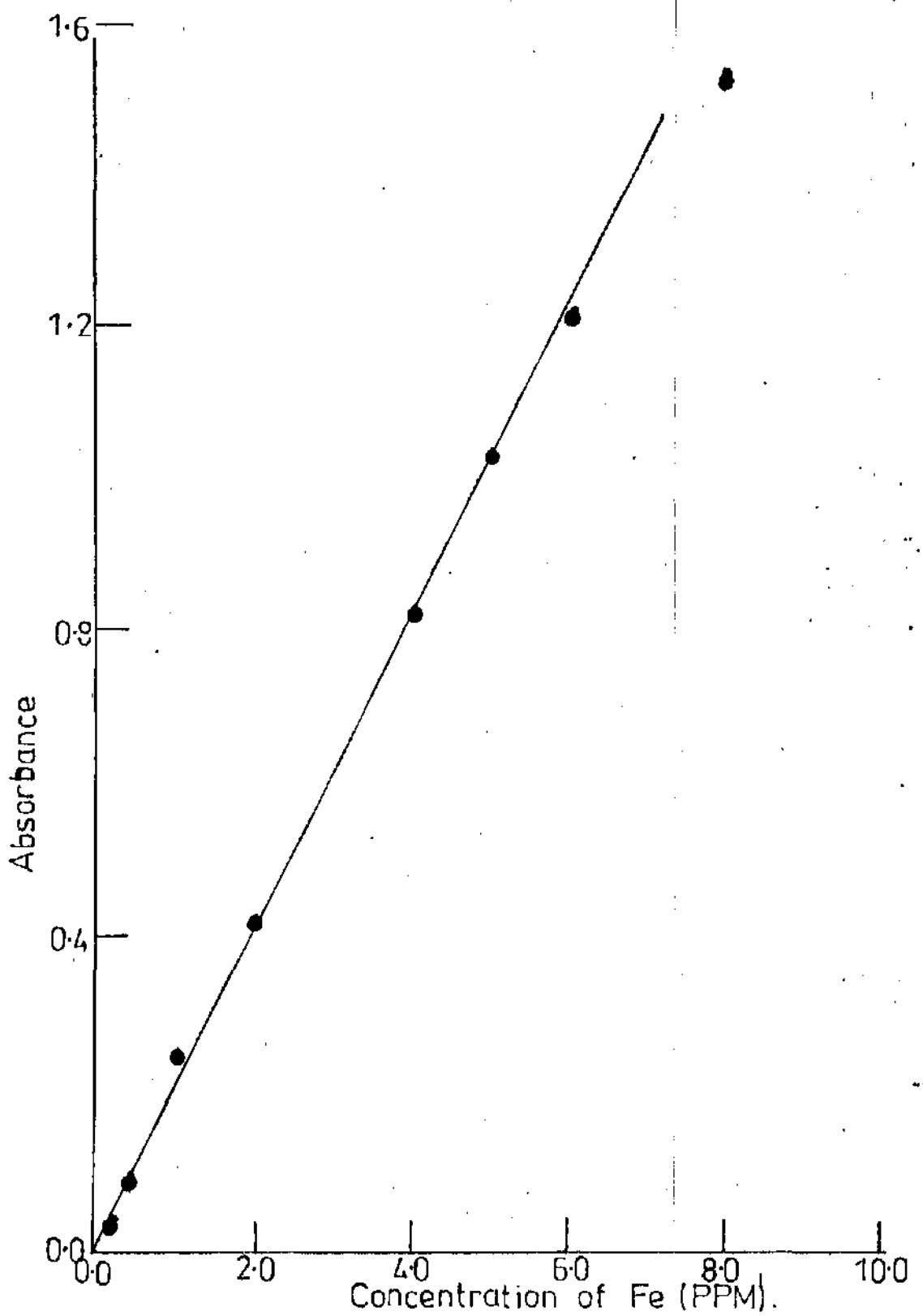
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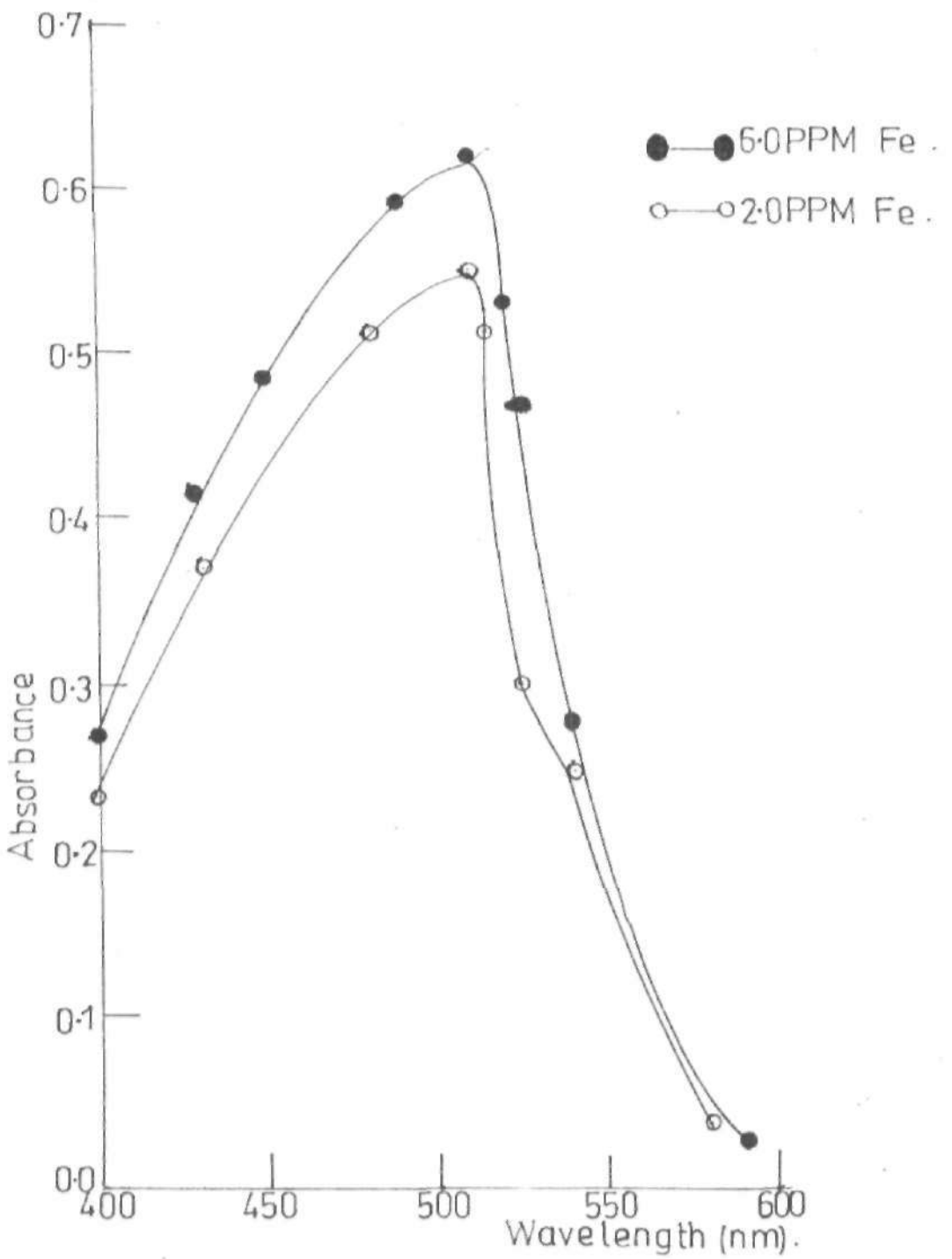
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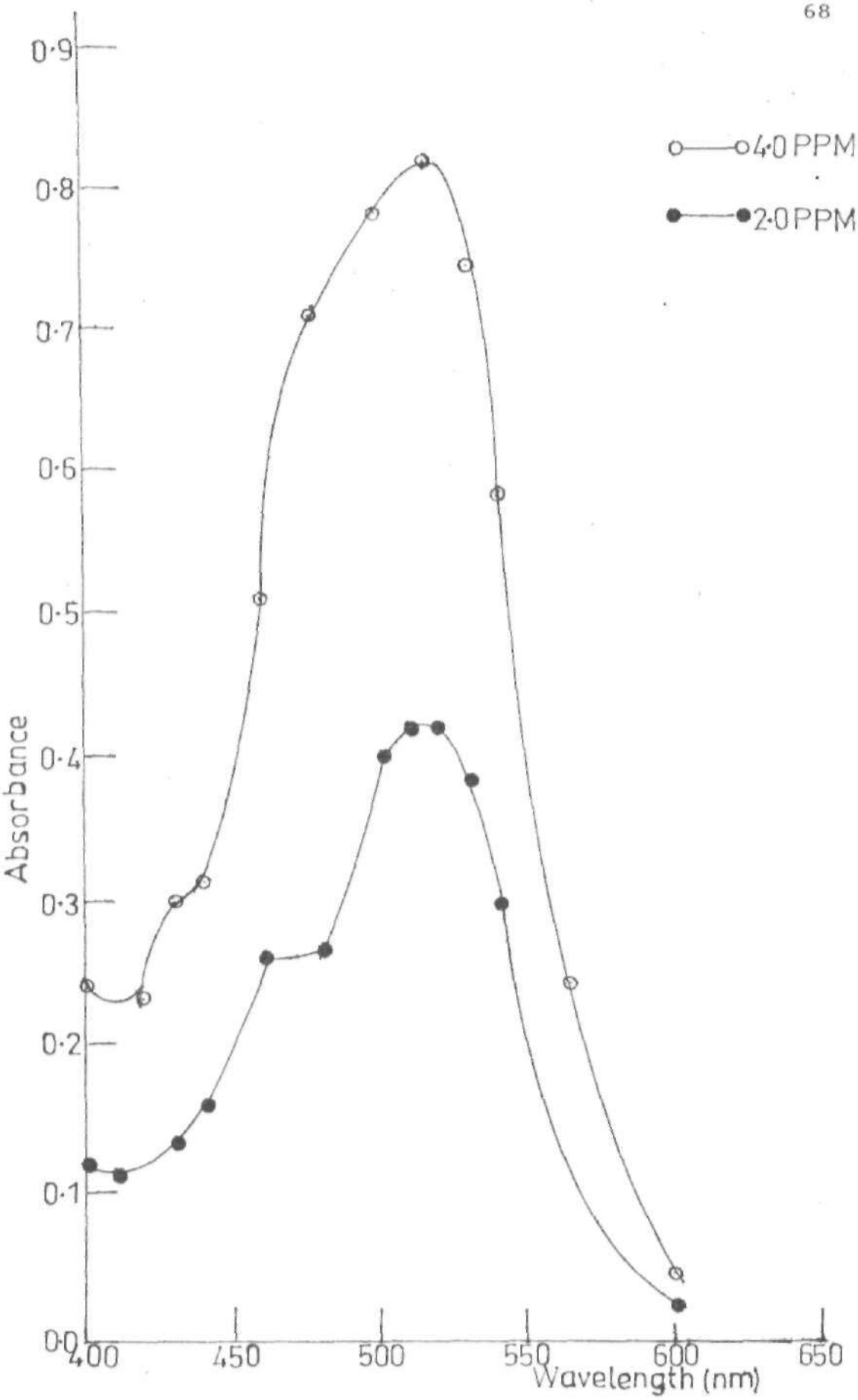
Appendix A(I): Calibration graph for Fe determination using 1,10-Phen. (λ_{max} = 510nm).



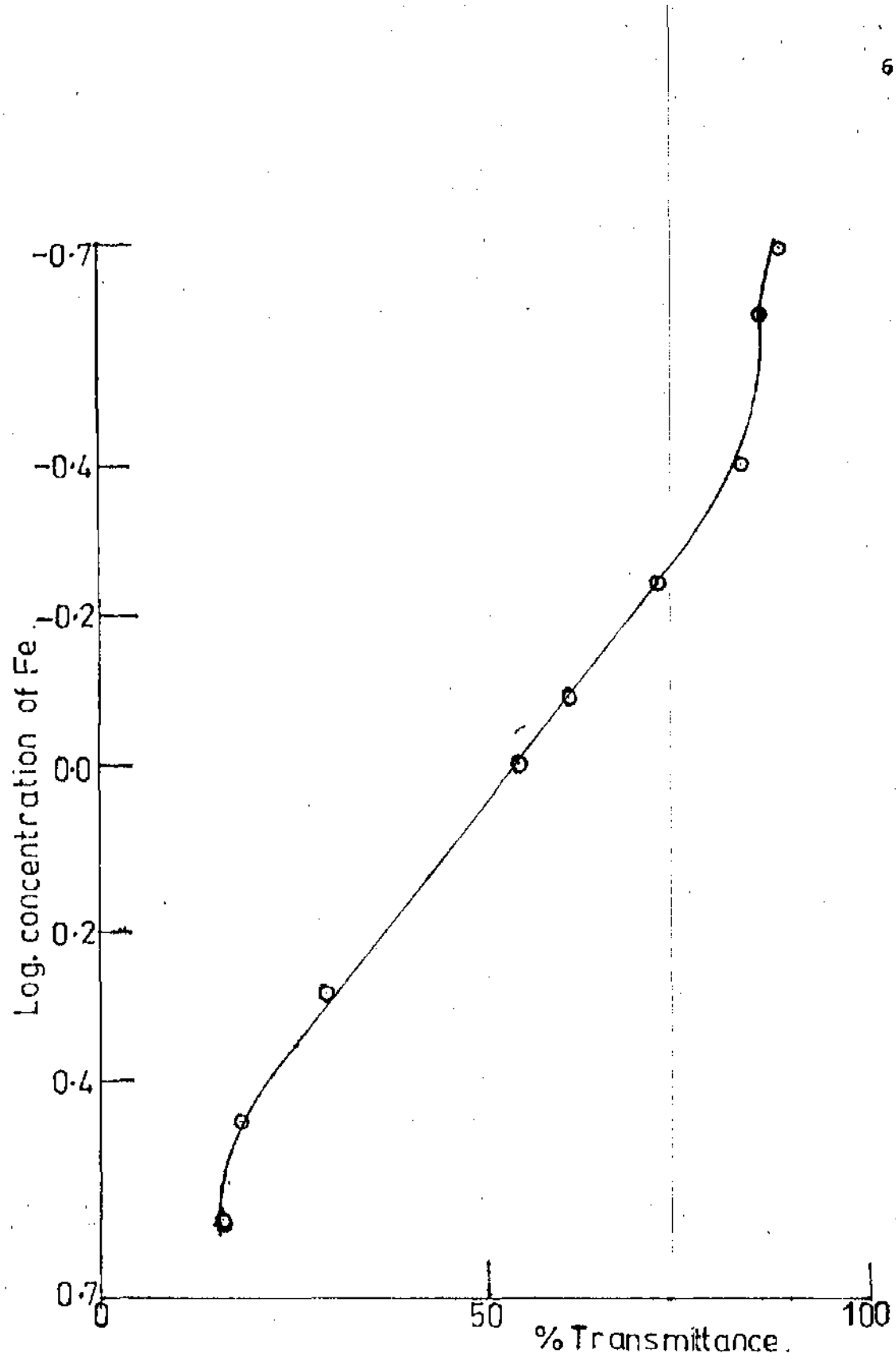
Appendix A(II): Calibration graph for Fe determination using 2,2-Bipy. (λ_{max}=520nm)



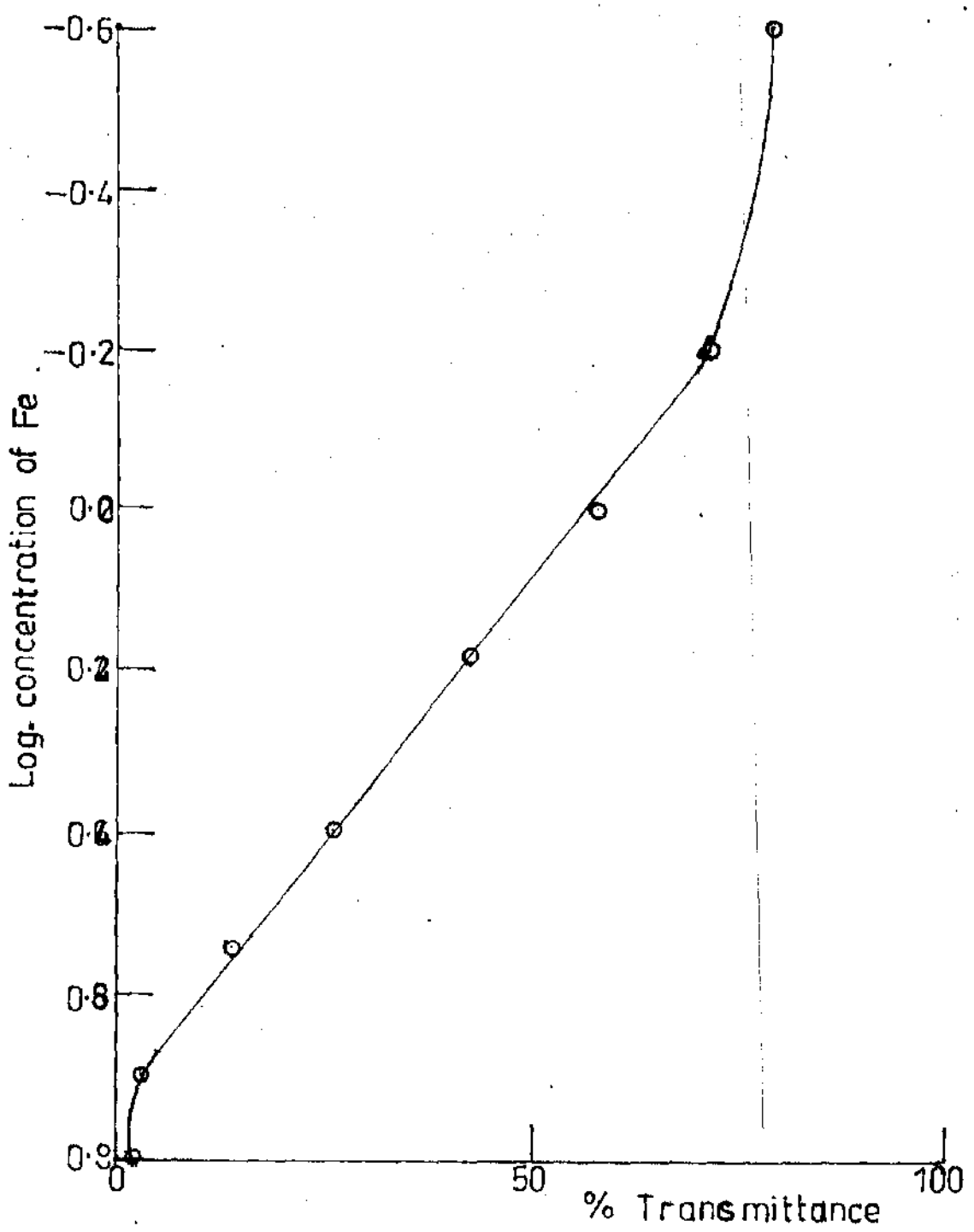
Appendix A(III): Absorption spectra for Fe determination using 1,10-Phen.



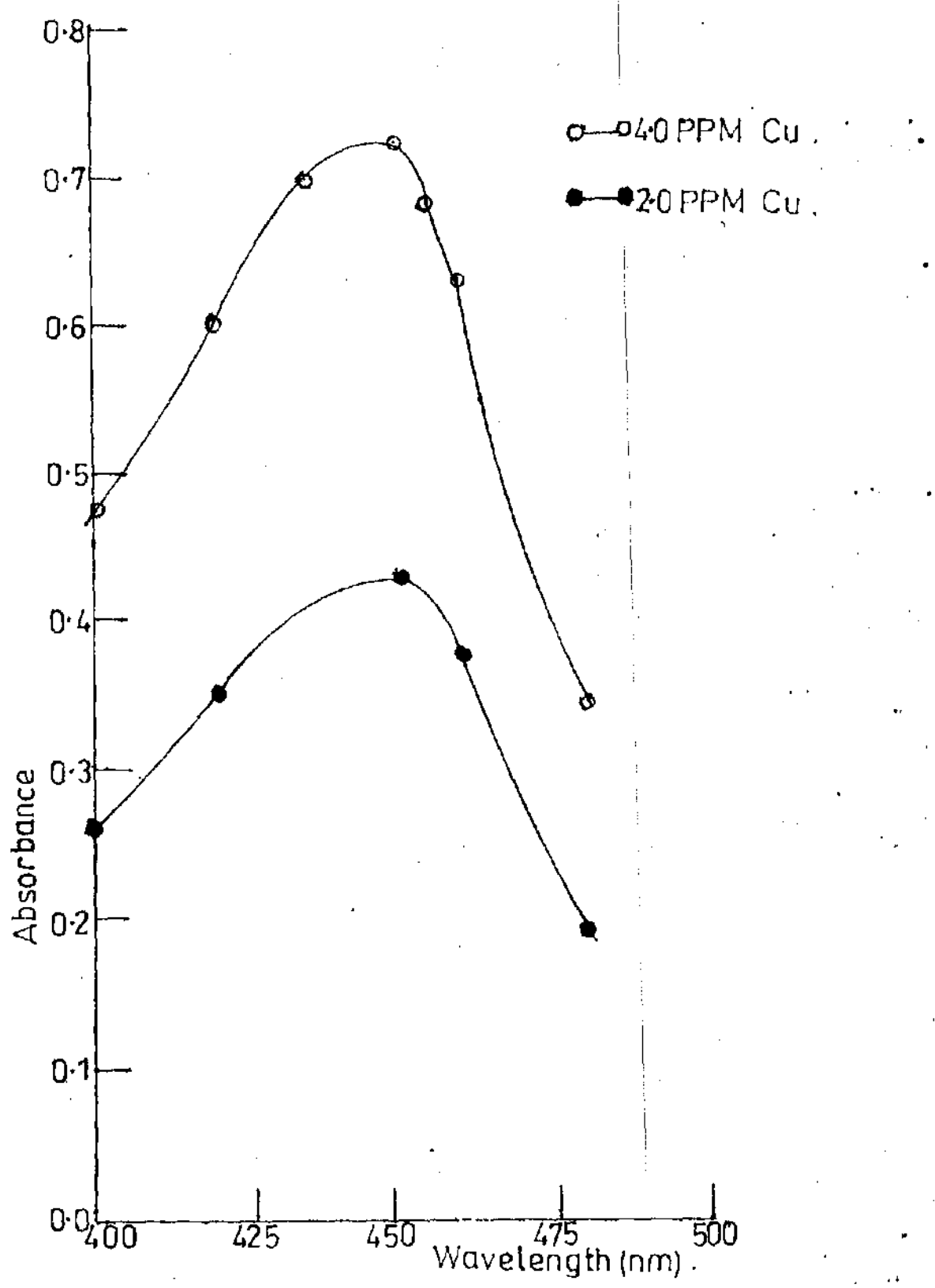
Appendix A(IV): Absorption spectra of Fe-2,2-Bipy. complex.



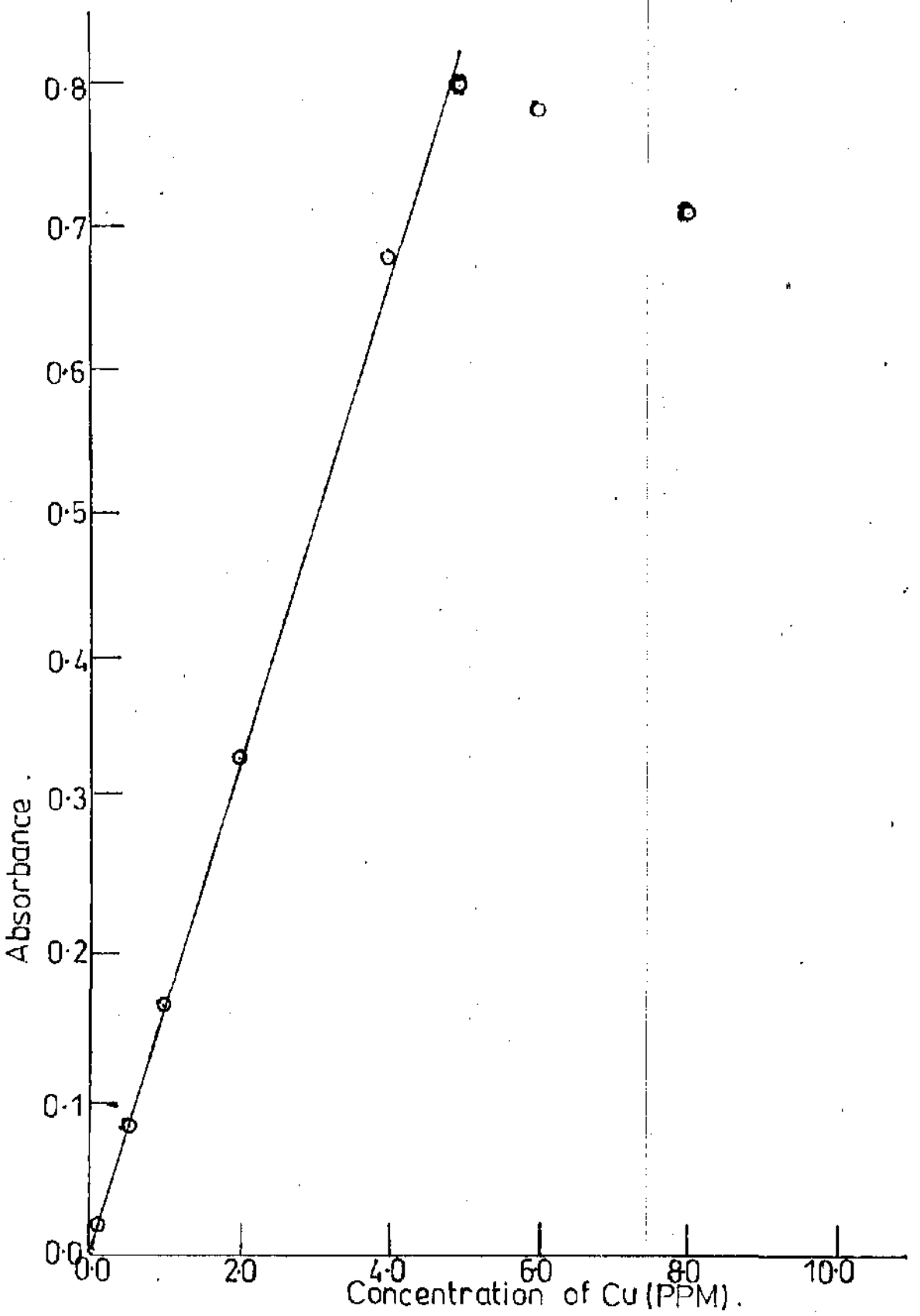
Appendix A(V): Rigbom plot for Fe determination using 1,10-Phen.



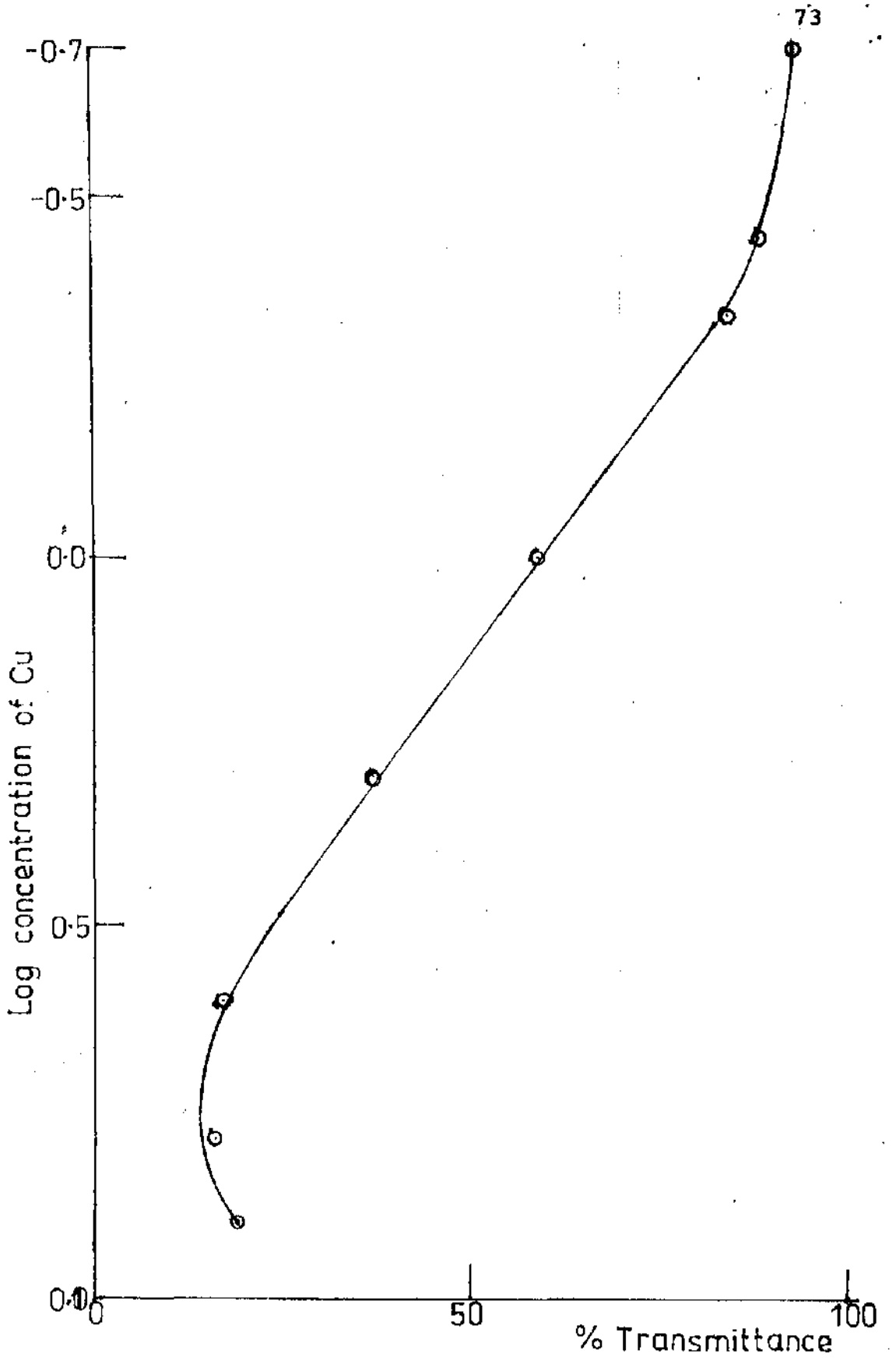
Appendix A(VI): Rigbom curve for Fe determination using 2,2-Bipy.



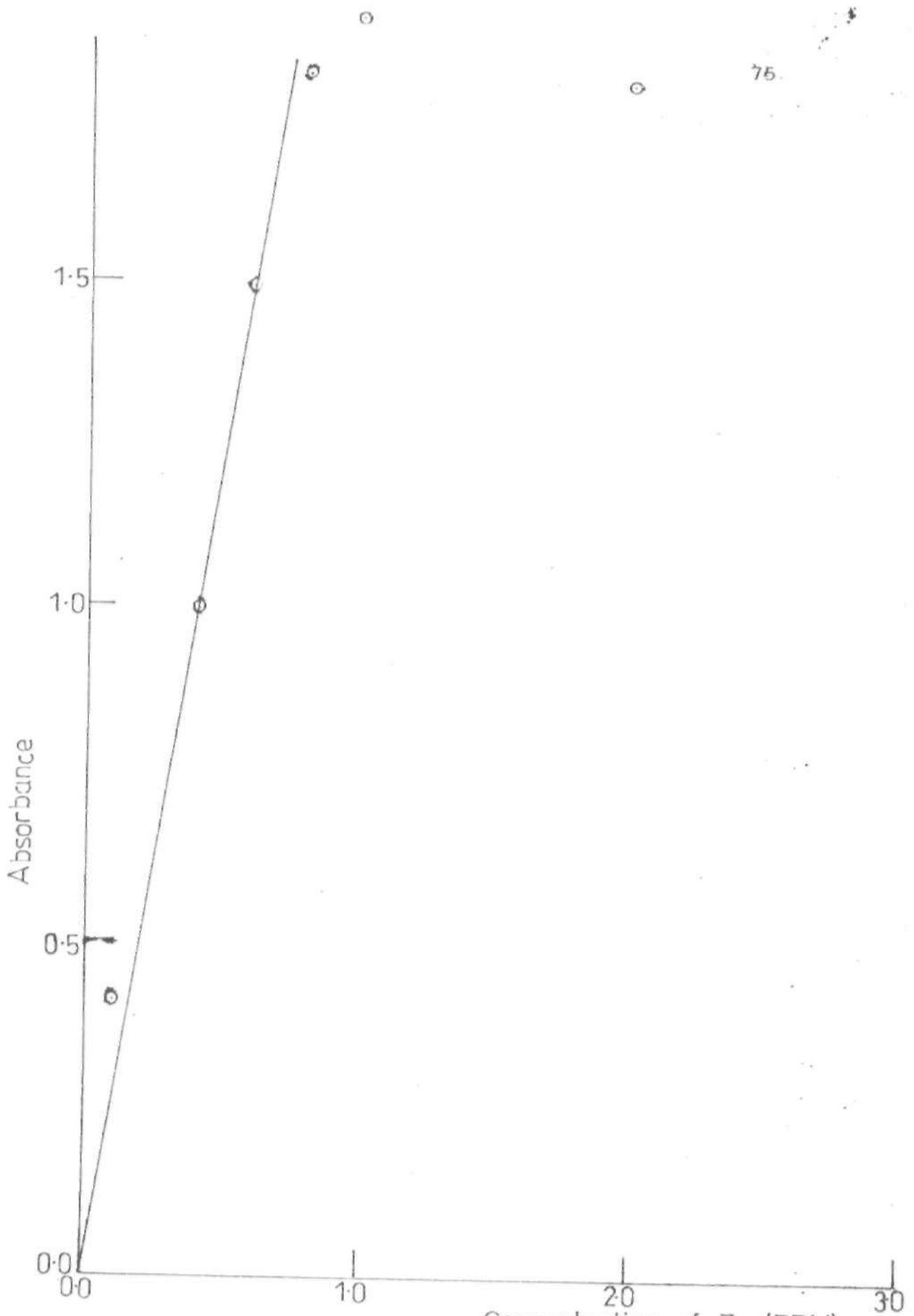
Appendix B(I): Absorption spectra of Cu-2,9-Dimethyl-1,10-Phen. complex.



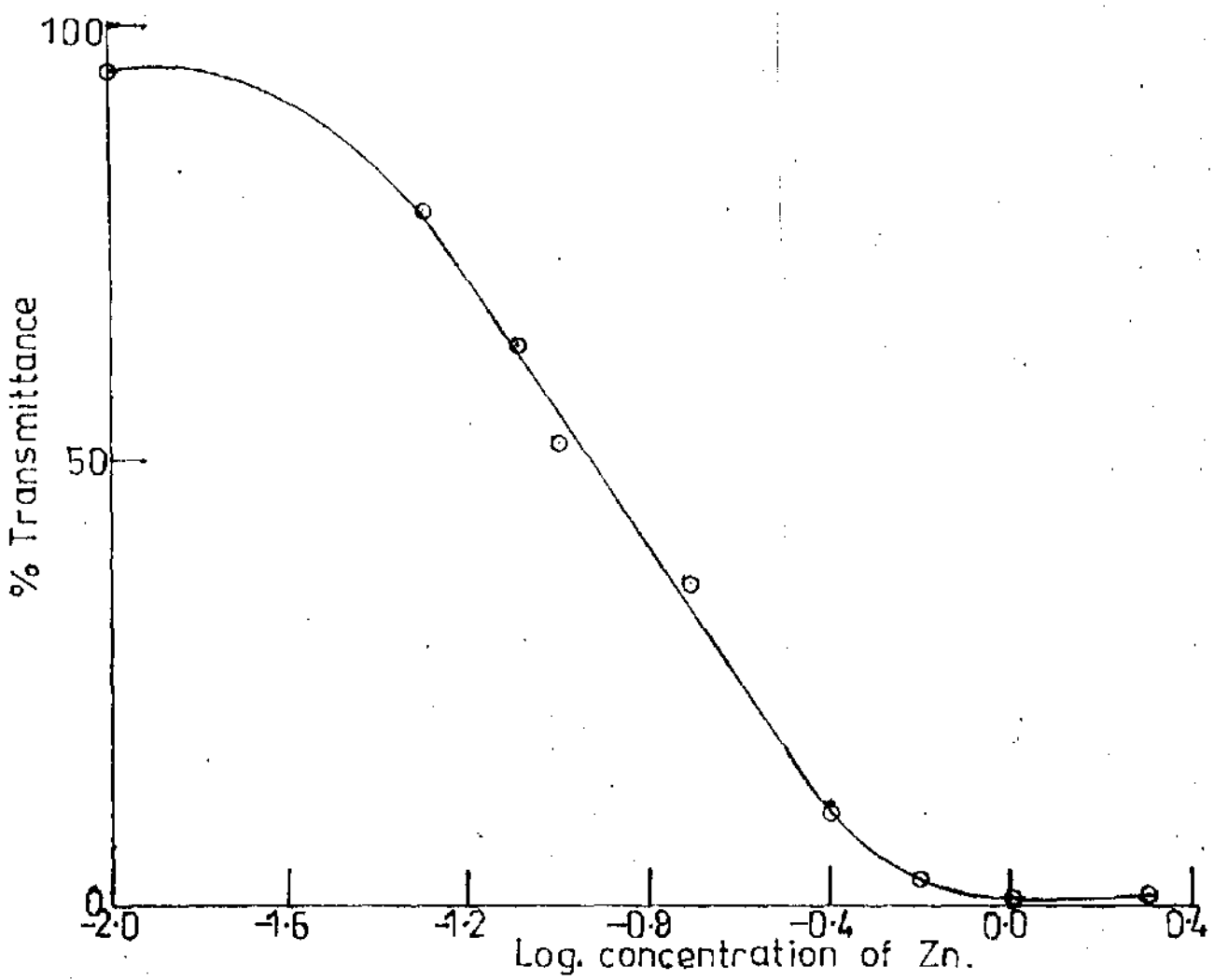
Appendix B(II): Calibration graph for Cu determination using 2,9-Dimethyl-1,10-Phen. (λ_{max} = 450nm).



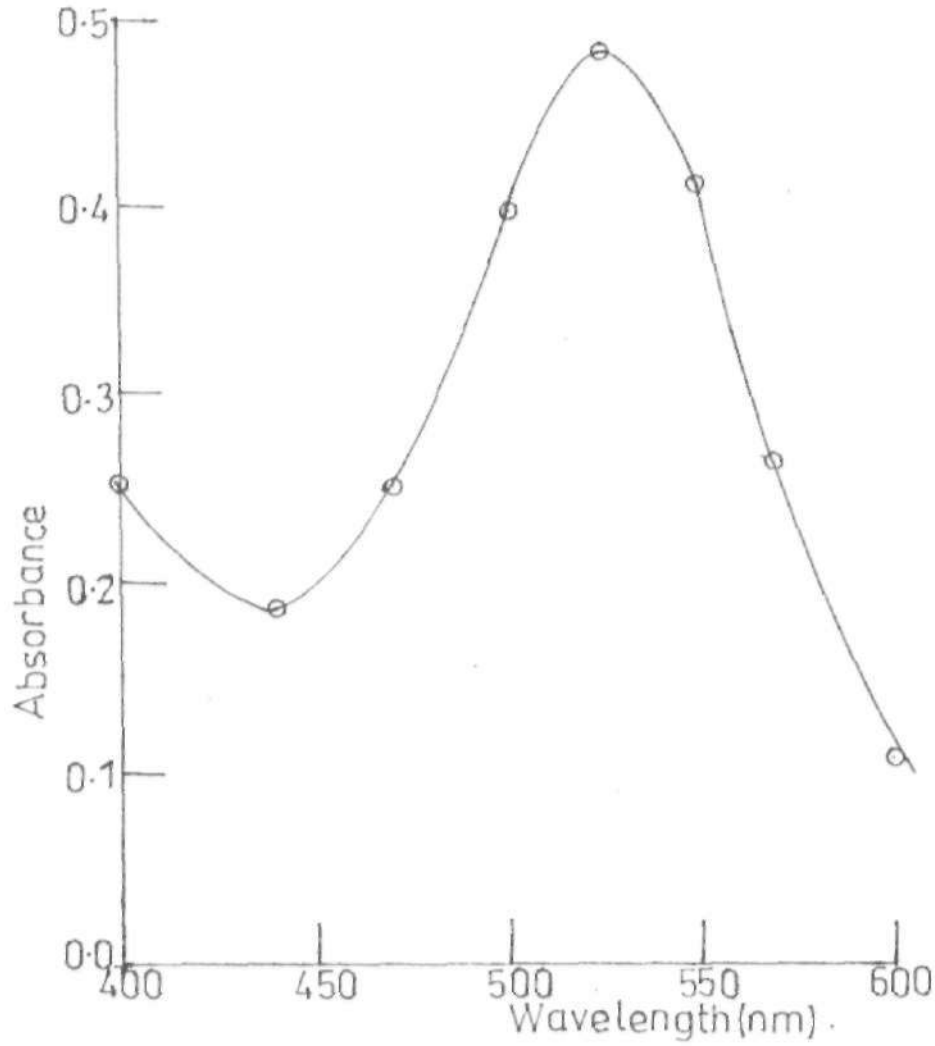
Appendix 3(III): Rigbom curve for Cu determination using 2,9-Dimethyl-1,10-Phen. at (λ_{max}=450nm).



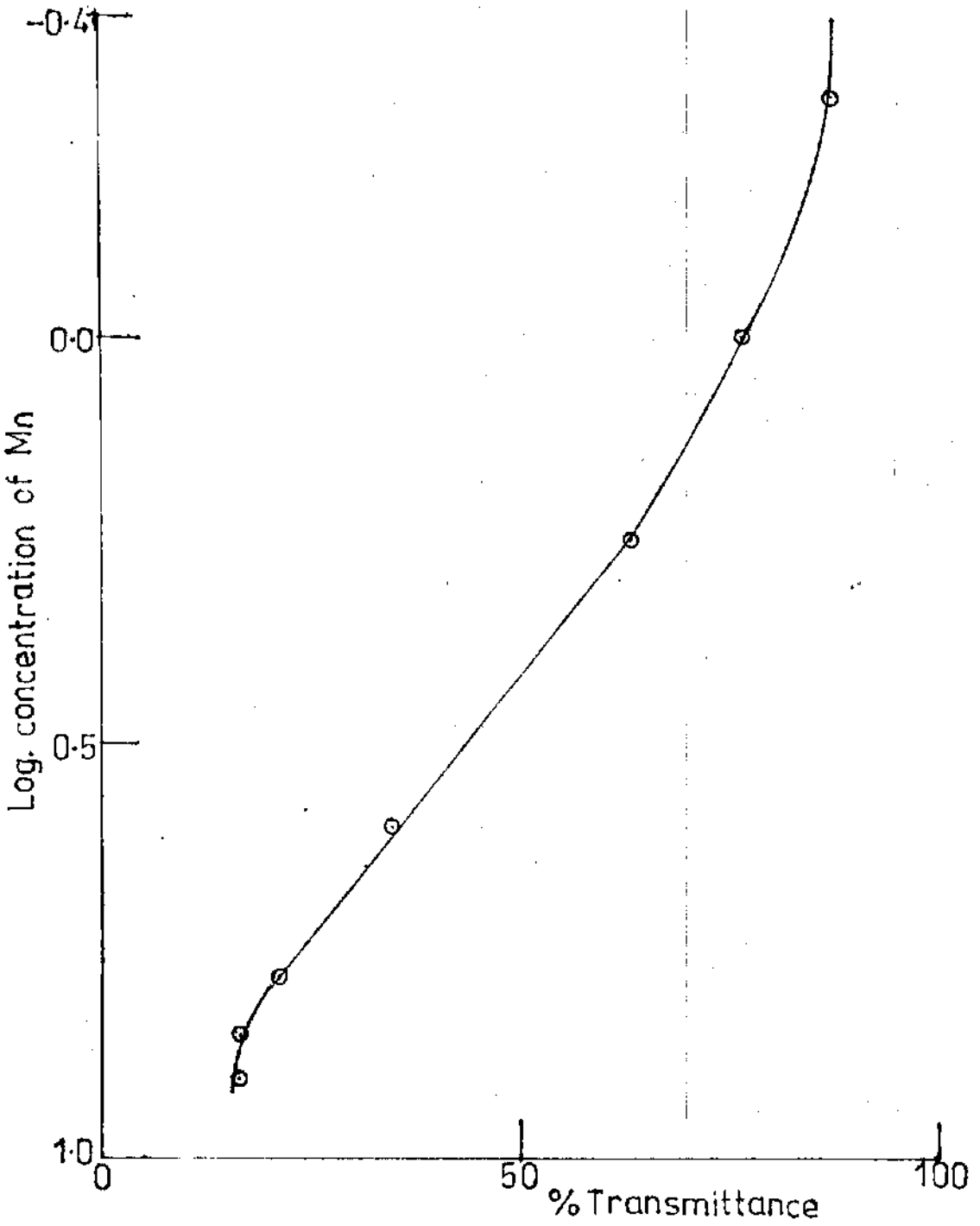
Appendix C(II): Calibration curve for Zn determination using Dithizone (λ_{max}=535nm).



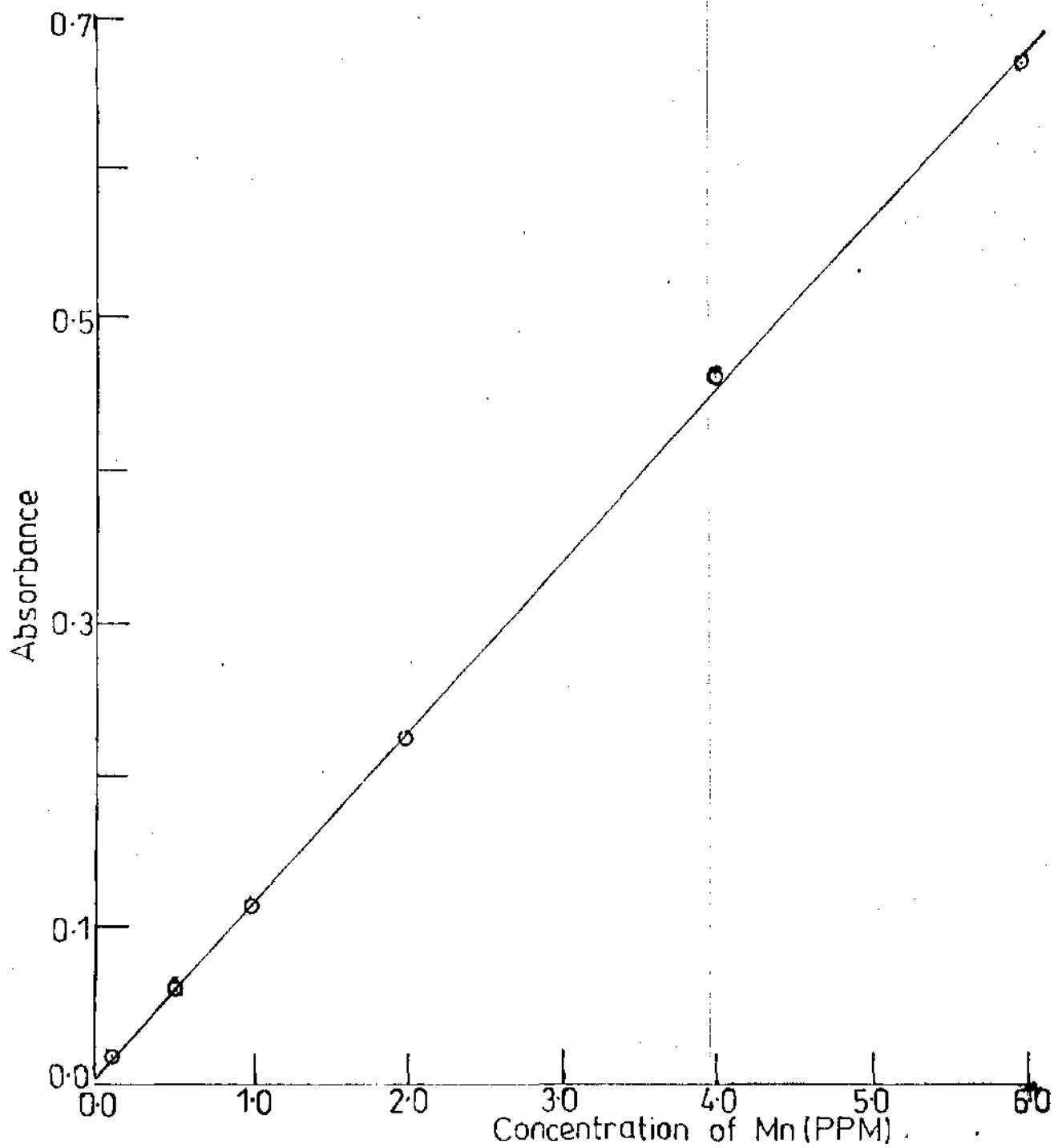
Appendix C(III): Rigbom curve for Zn determination using Dithizone at (λ_{max}=535nm).



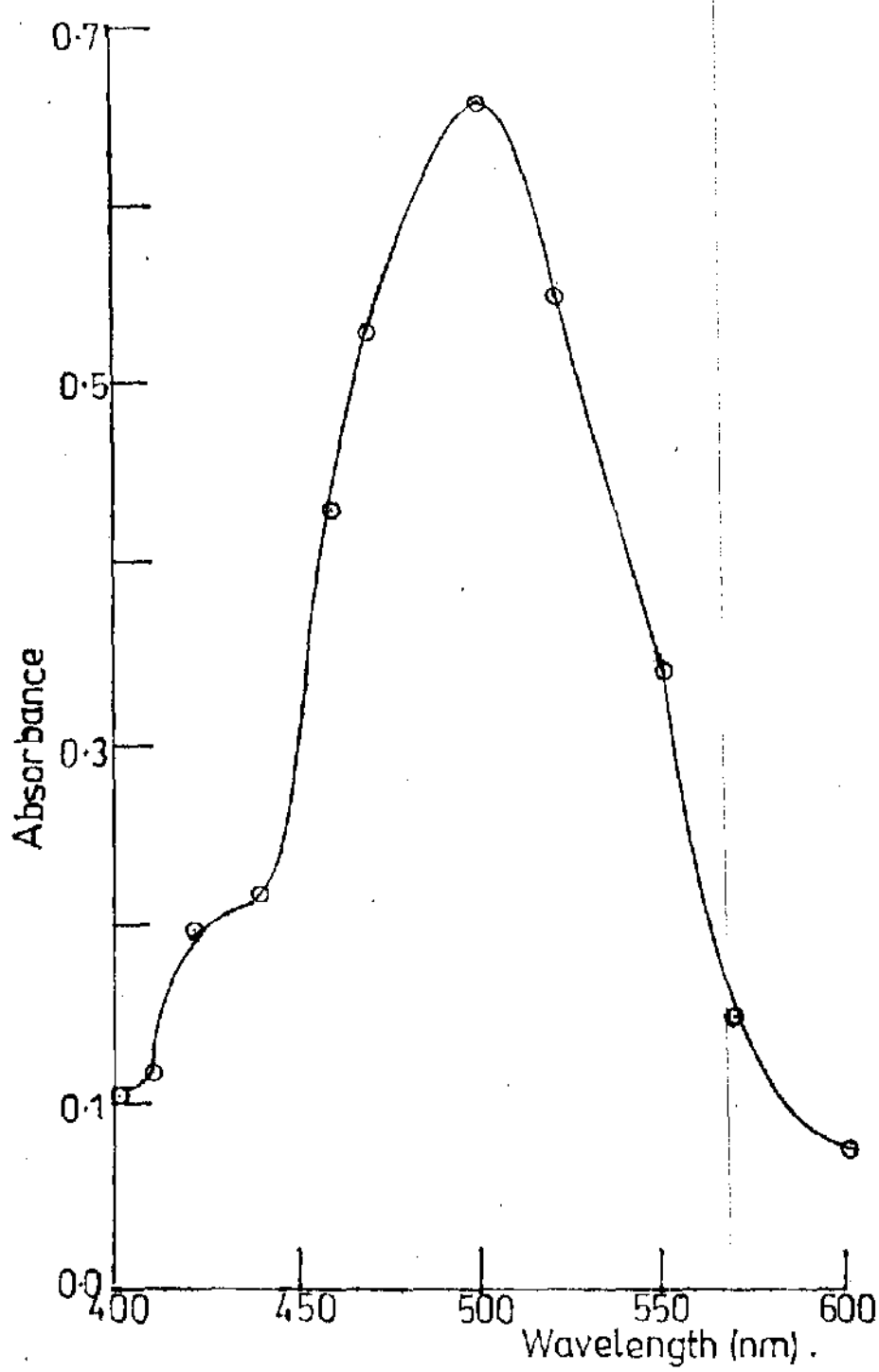
Appendix D(I): Absorption spectrum for Mn determination using potassiumperiodate .



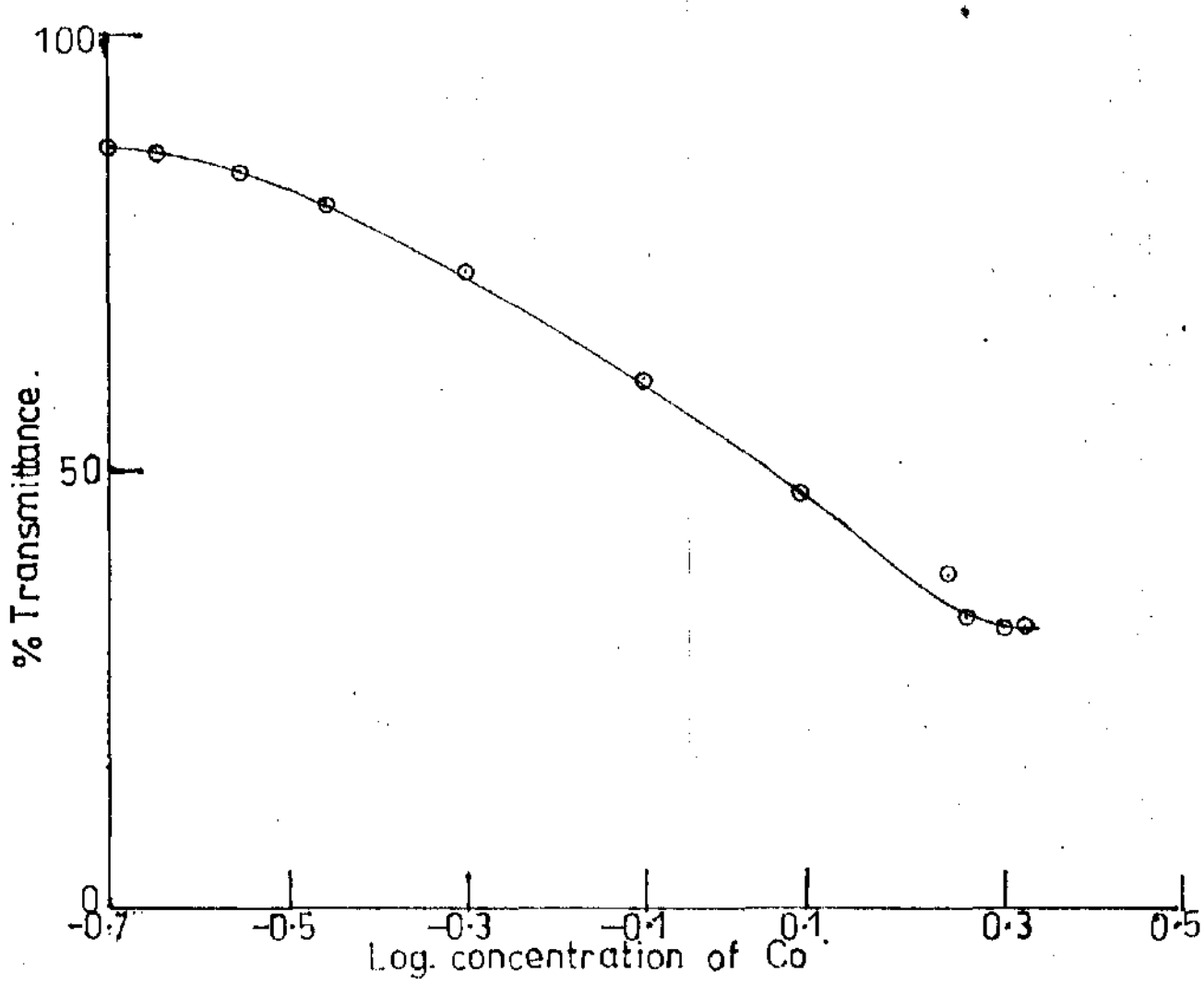
Appendix D(II): Rigbom curve for Mn determination using potassiumperiodate at (λ_{max}=525nm)



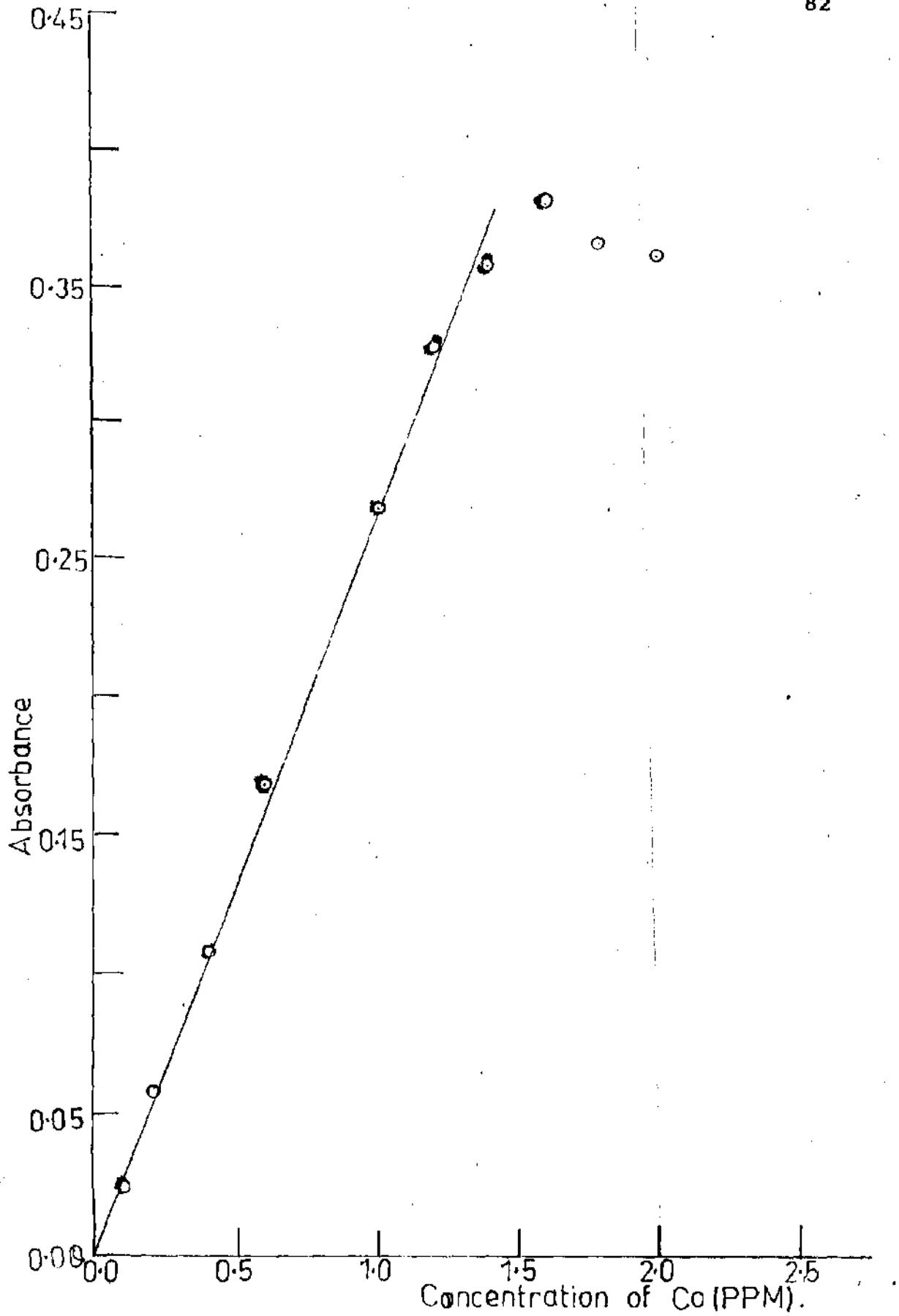
Appendix D(III): Calibration graph for Mn determination using potassiumperiodate at (λ_{max}=525nm).



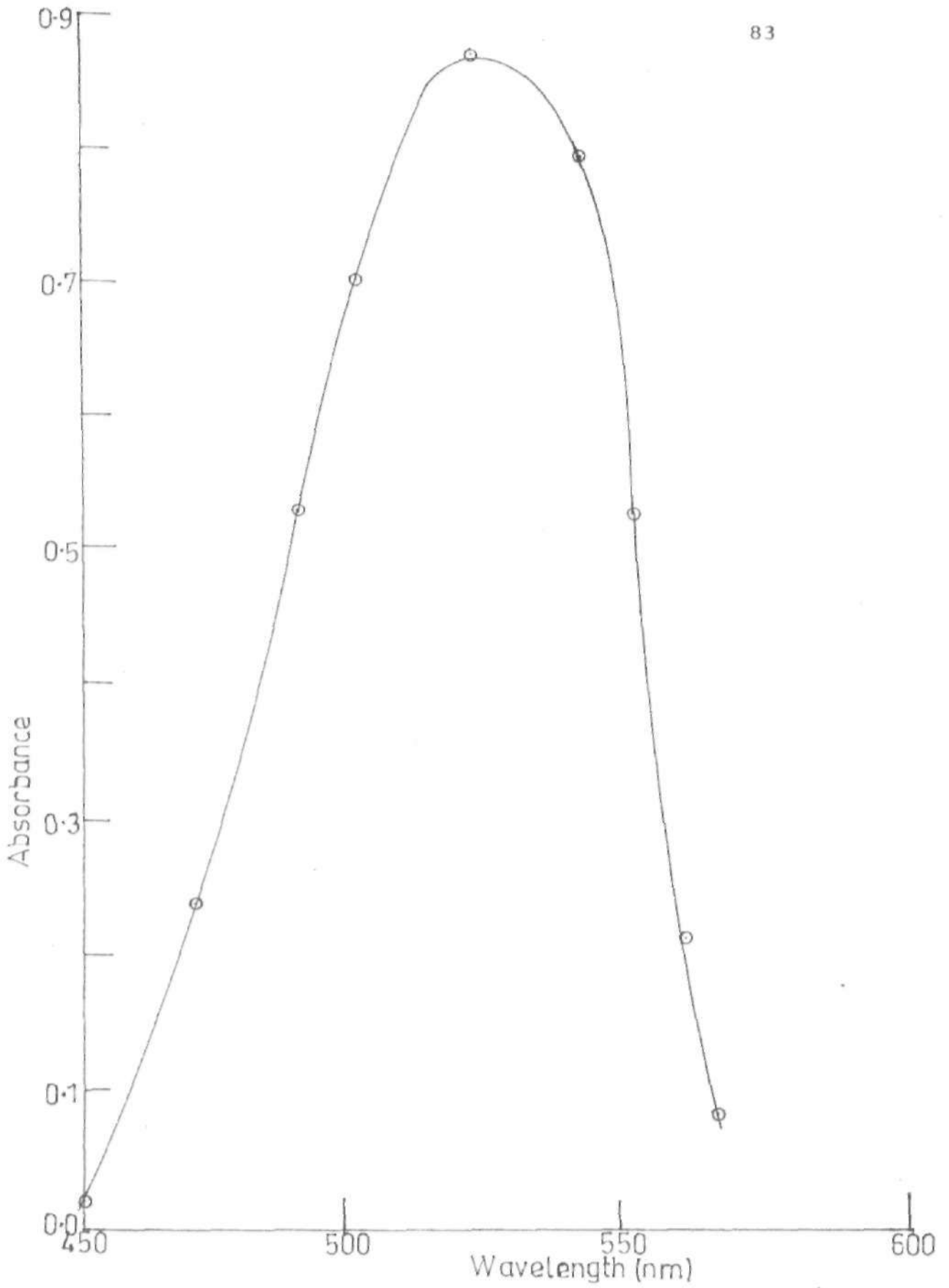
Appendix E(I): Absorption spectrum of Co-Nitroso-R-salt complex.



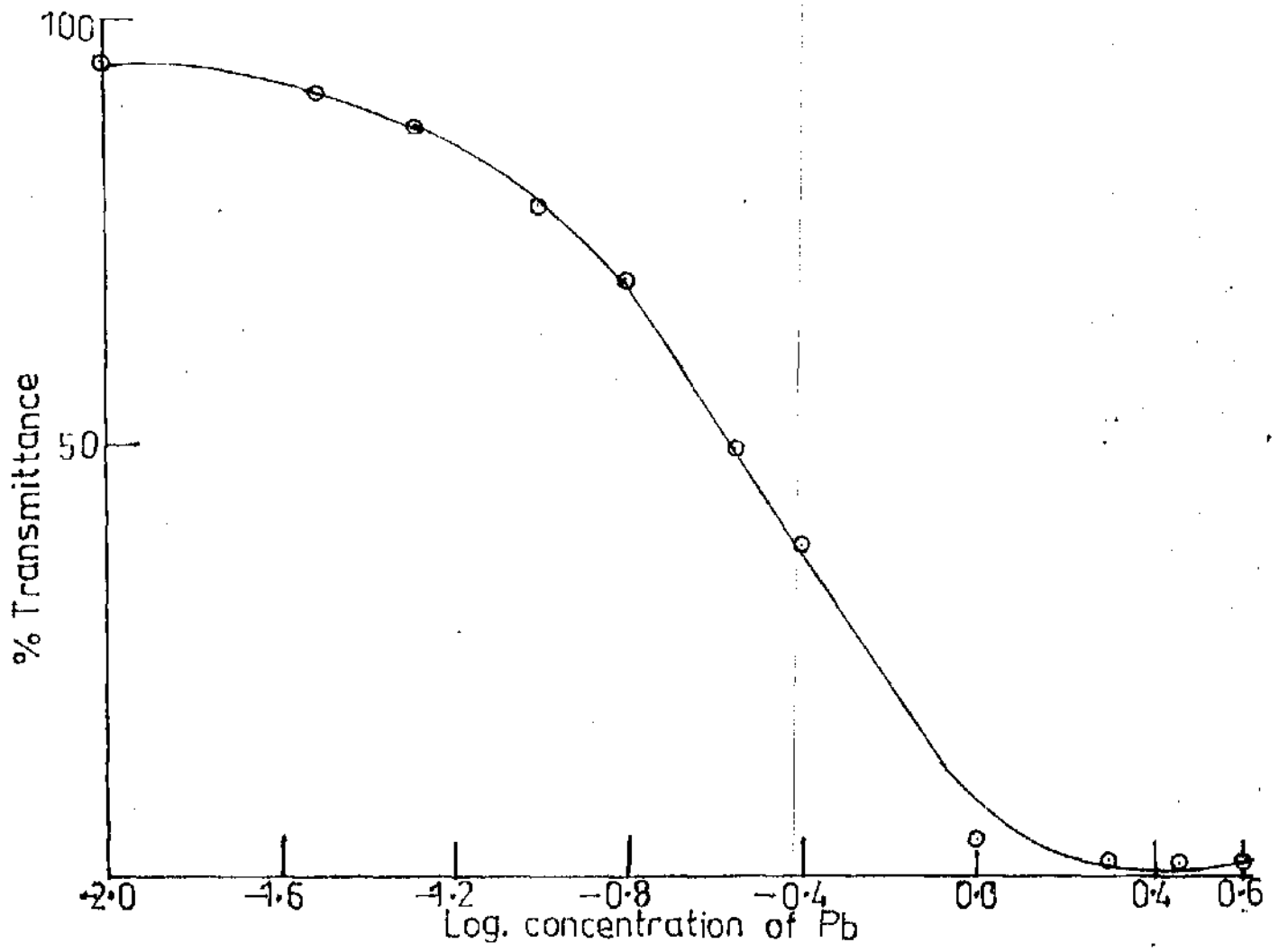
Appendix E(II): Rigbom curve for Co determination using Nitroso-R-salt at (λ_{max}=500nm).



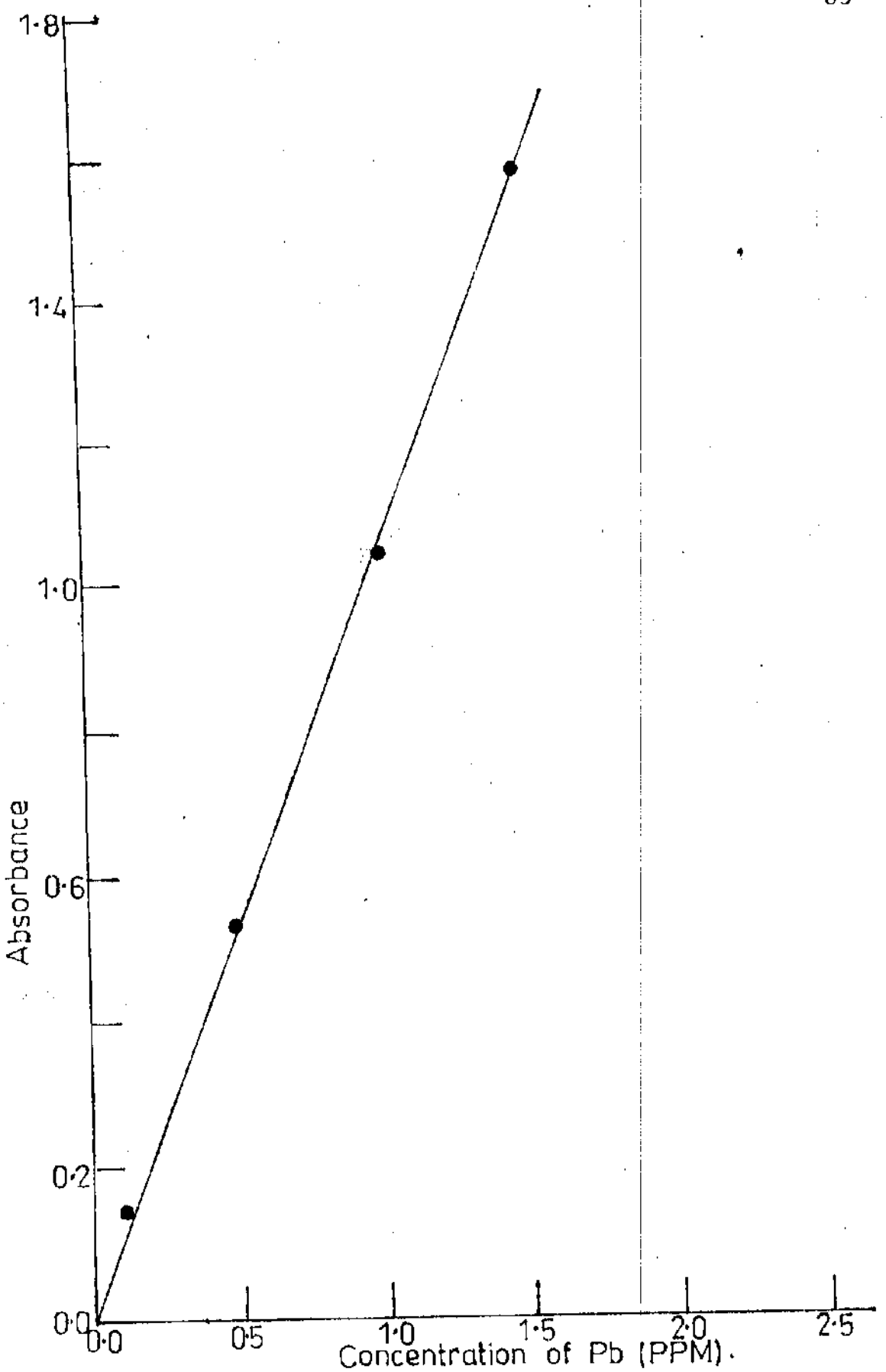
Appendix E(III): Calibration graph for Co determination using Nitroso-R-salt at (λ_{max}=500nm).



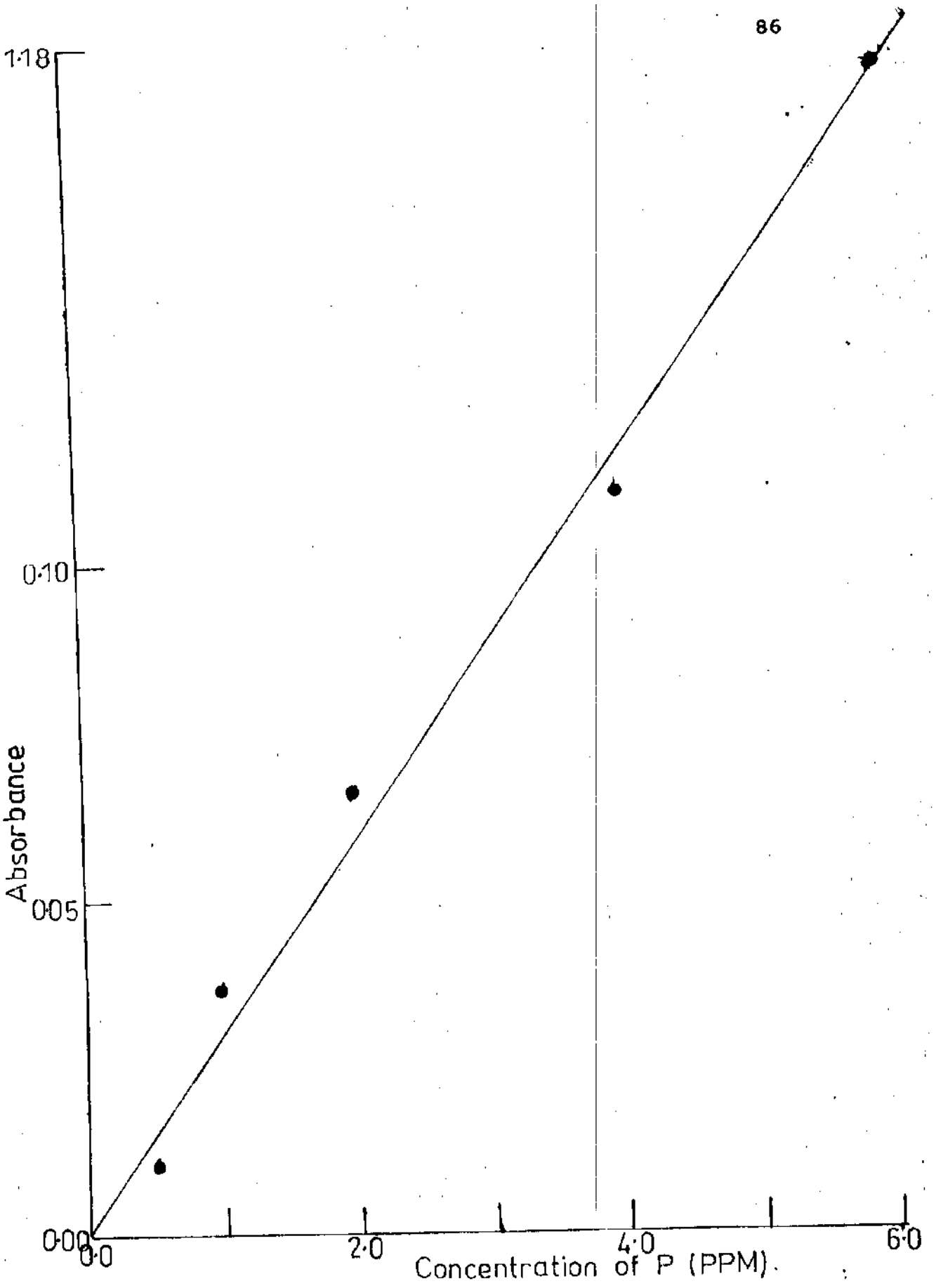
Appendix F(I): Absorption spectrum of Pb-Dithizone complex.



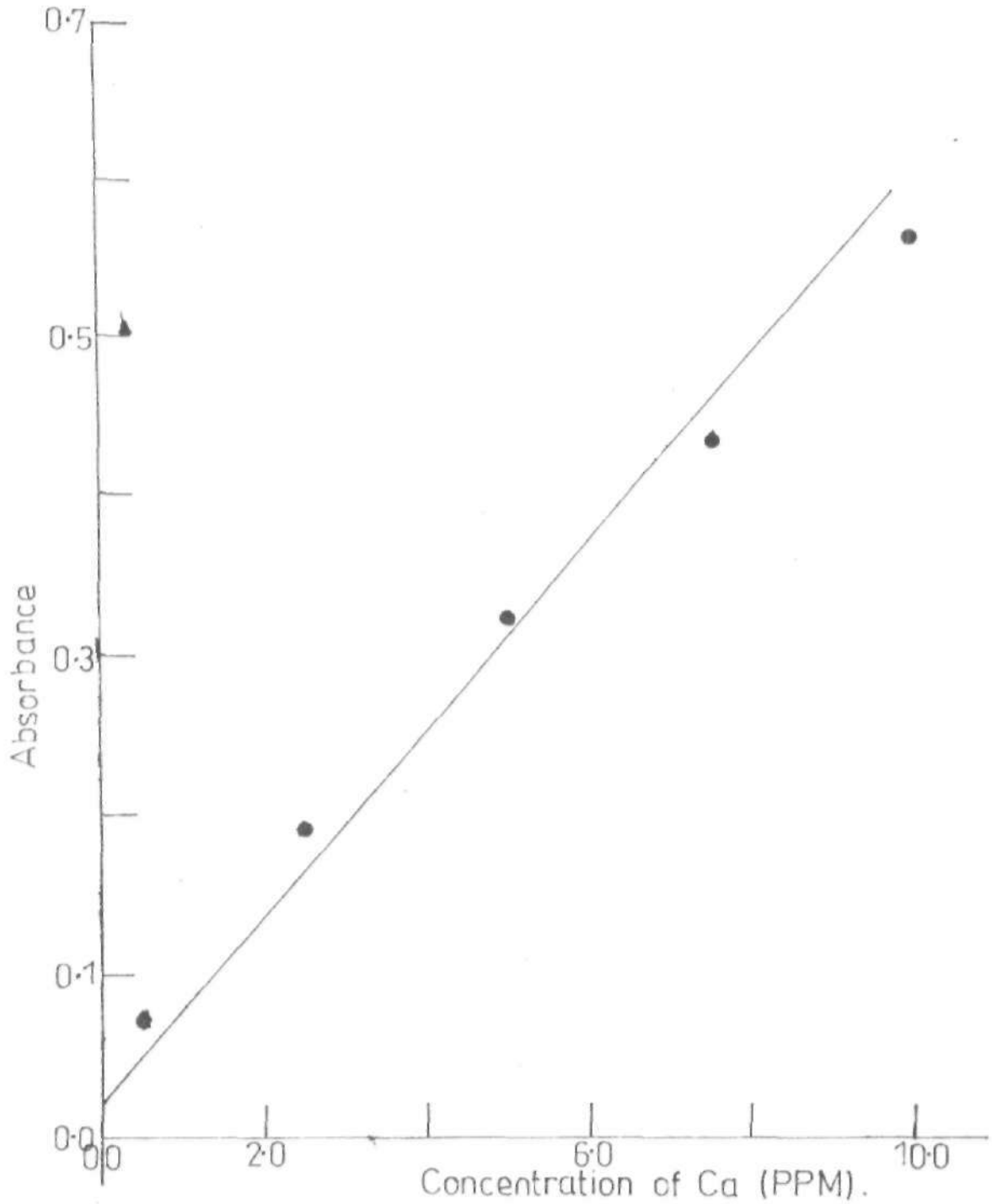
Appendix F(II): Rigbom curve for Pb determination using Dithizone at (λ_{max}=510nm).



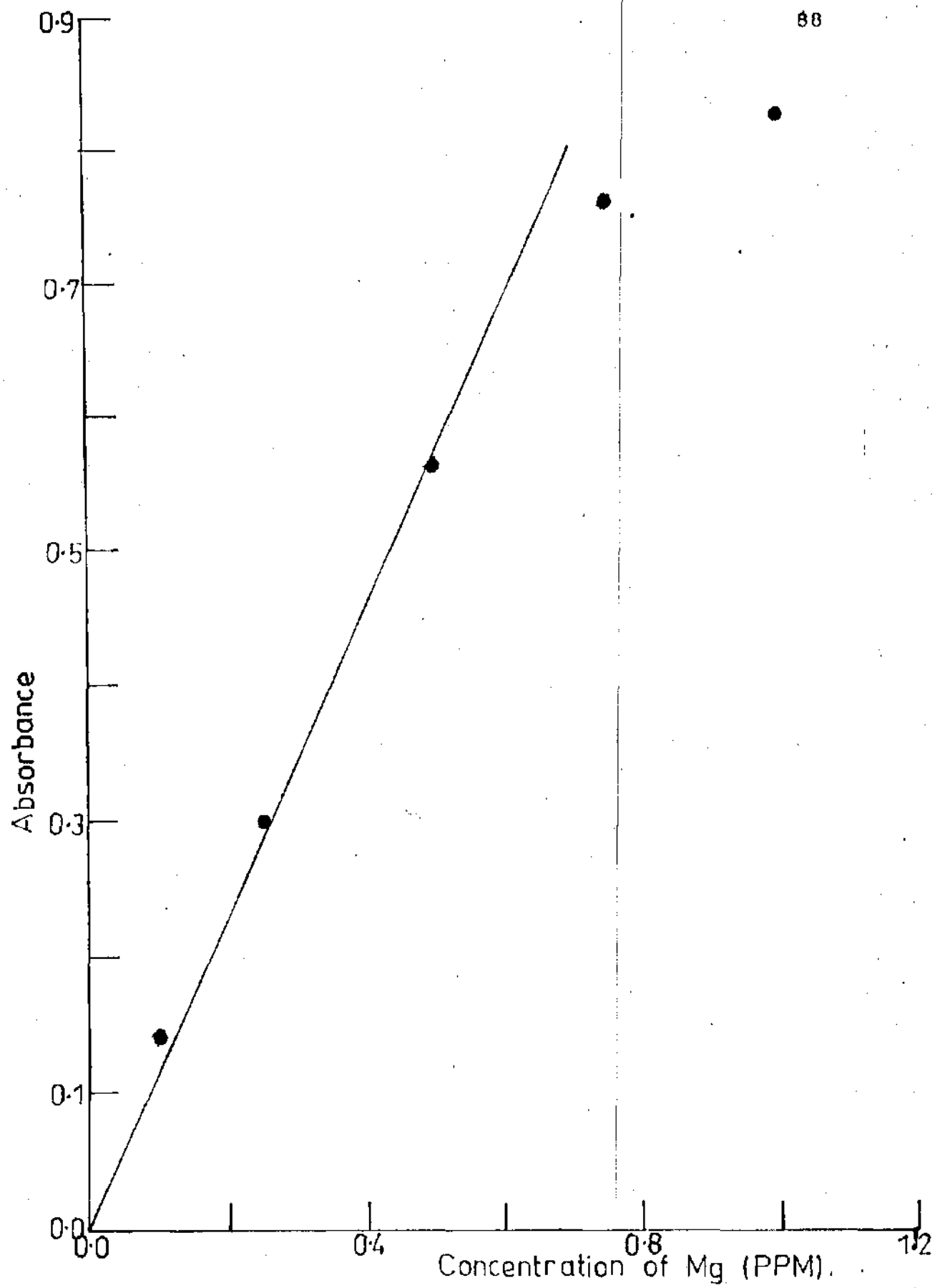
AppendixF(III):Calibration curve for Pb determination using Dithizone (λ_{max}=510nm).



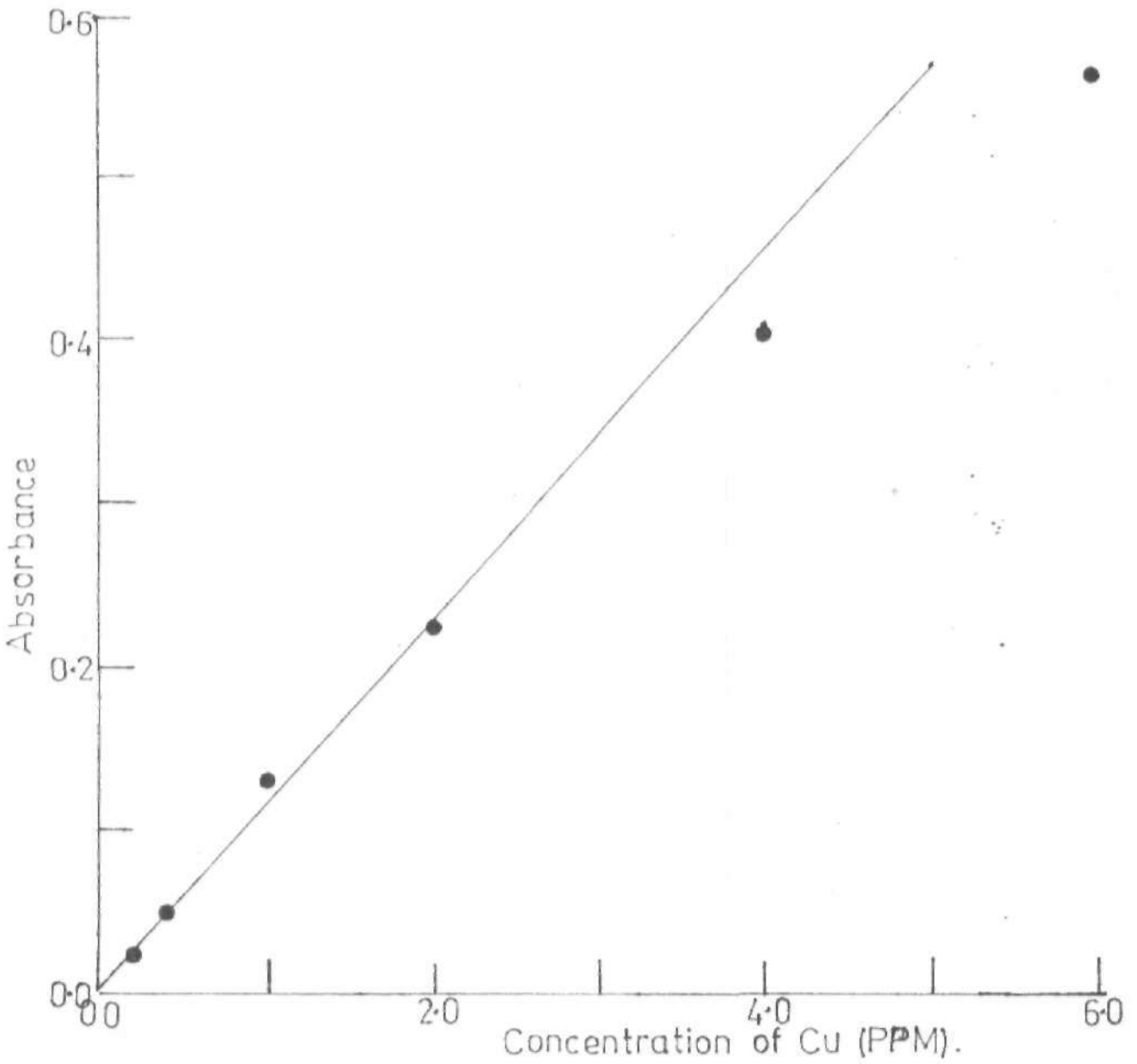
Appendix G: Calibration graph for the colorimetric determination of P at (λ_{max} = 460nm).



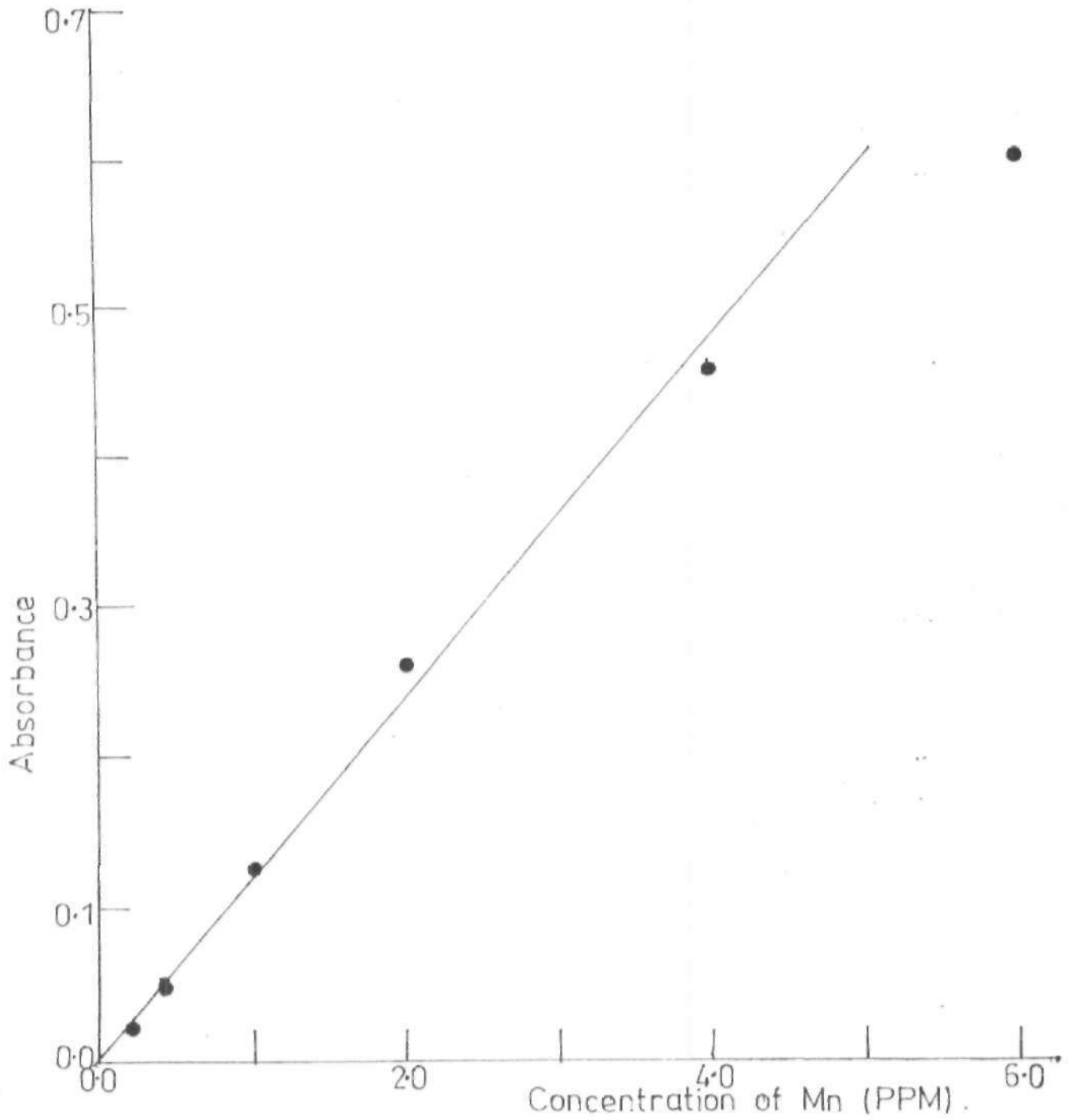
Appendix H(I): Calibration graph for the A.AS. determination of Ca .



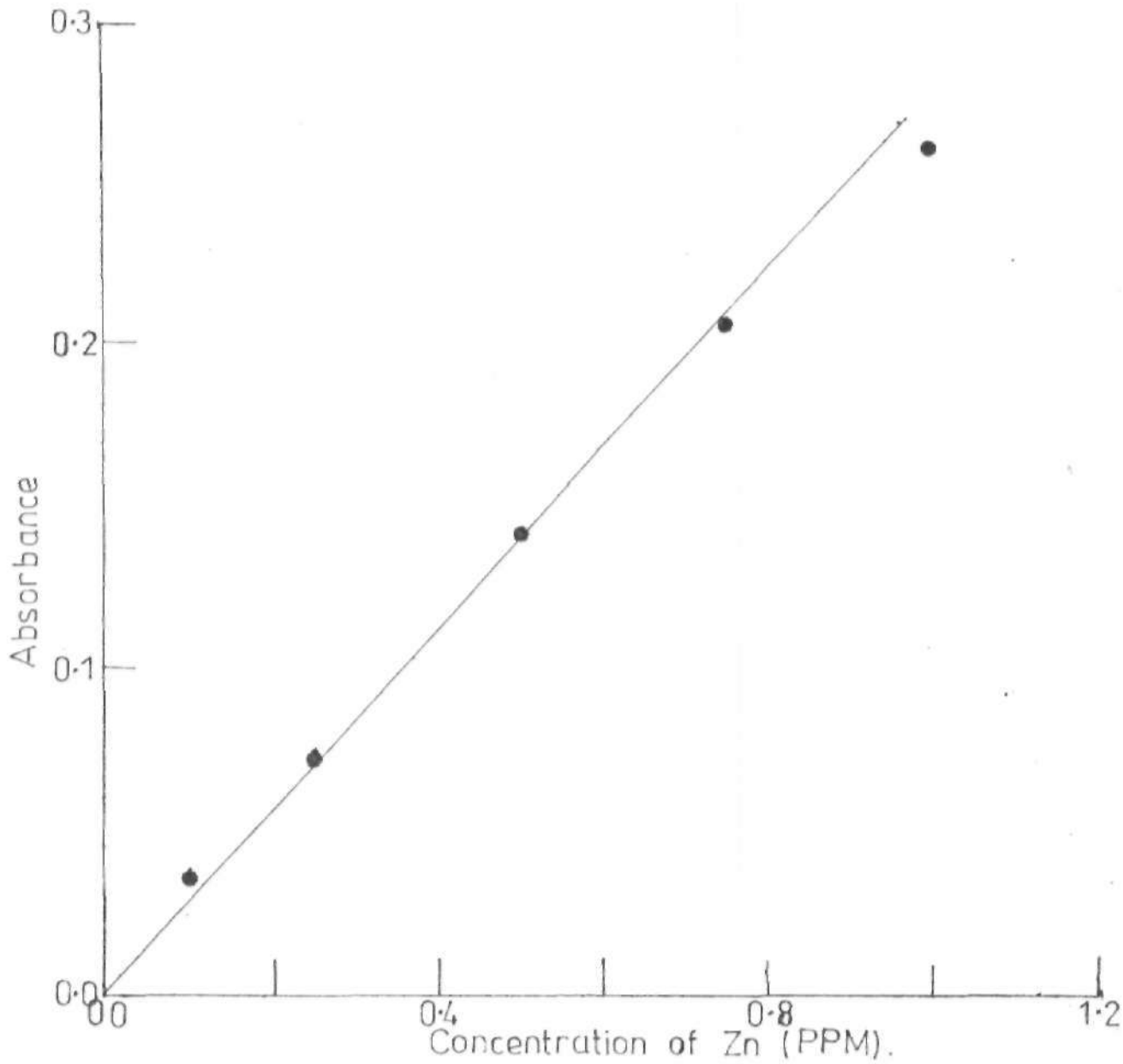
Appendix H(II): Calibration graph for Mg determination using A.A.S.



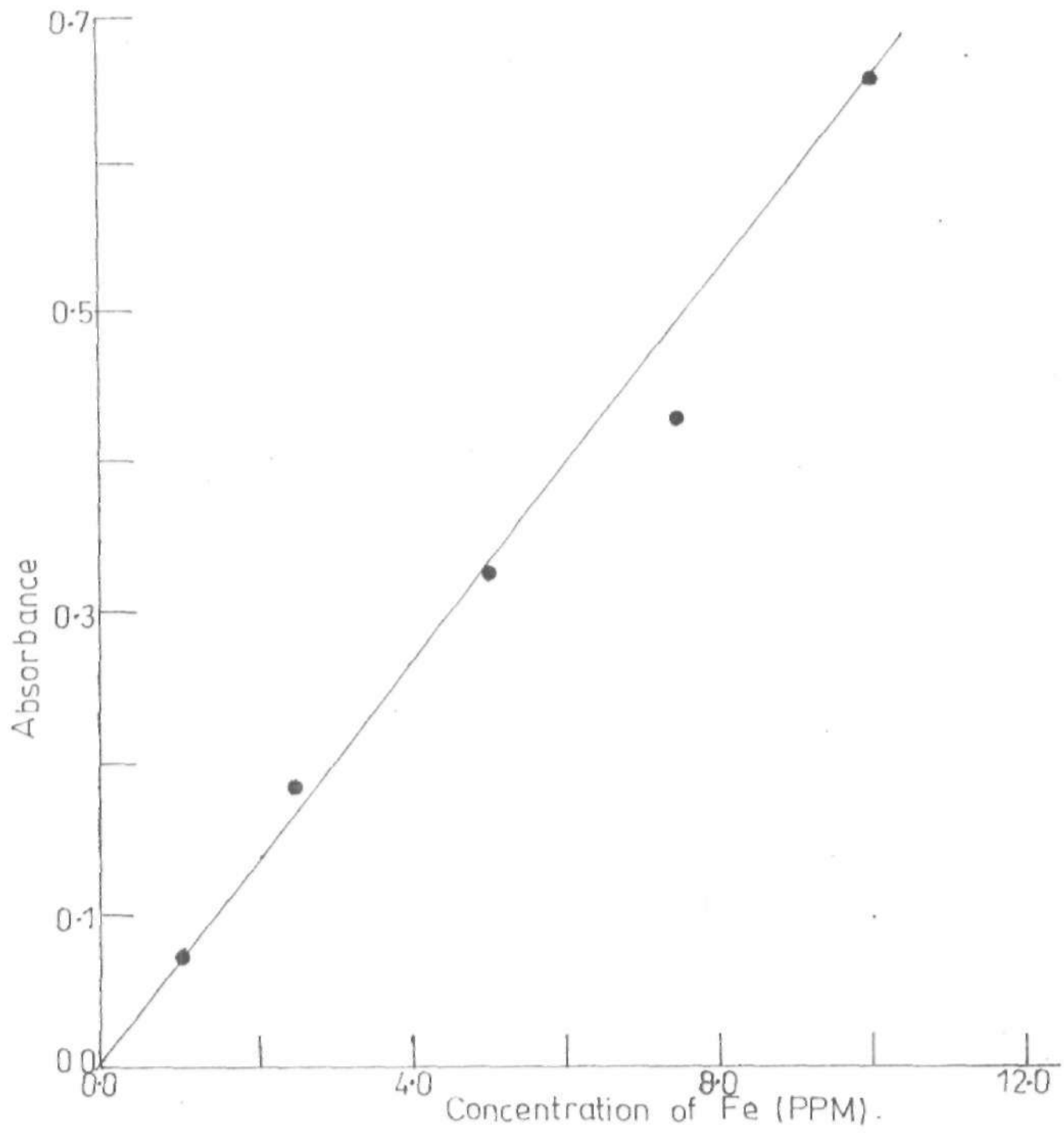
Appendix H(III): Calibration graph for AAS determination of Cu.



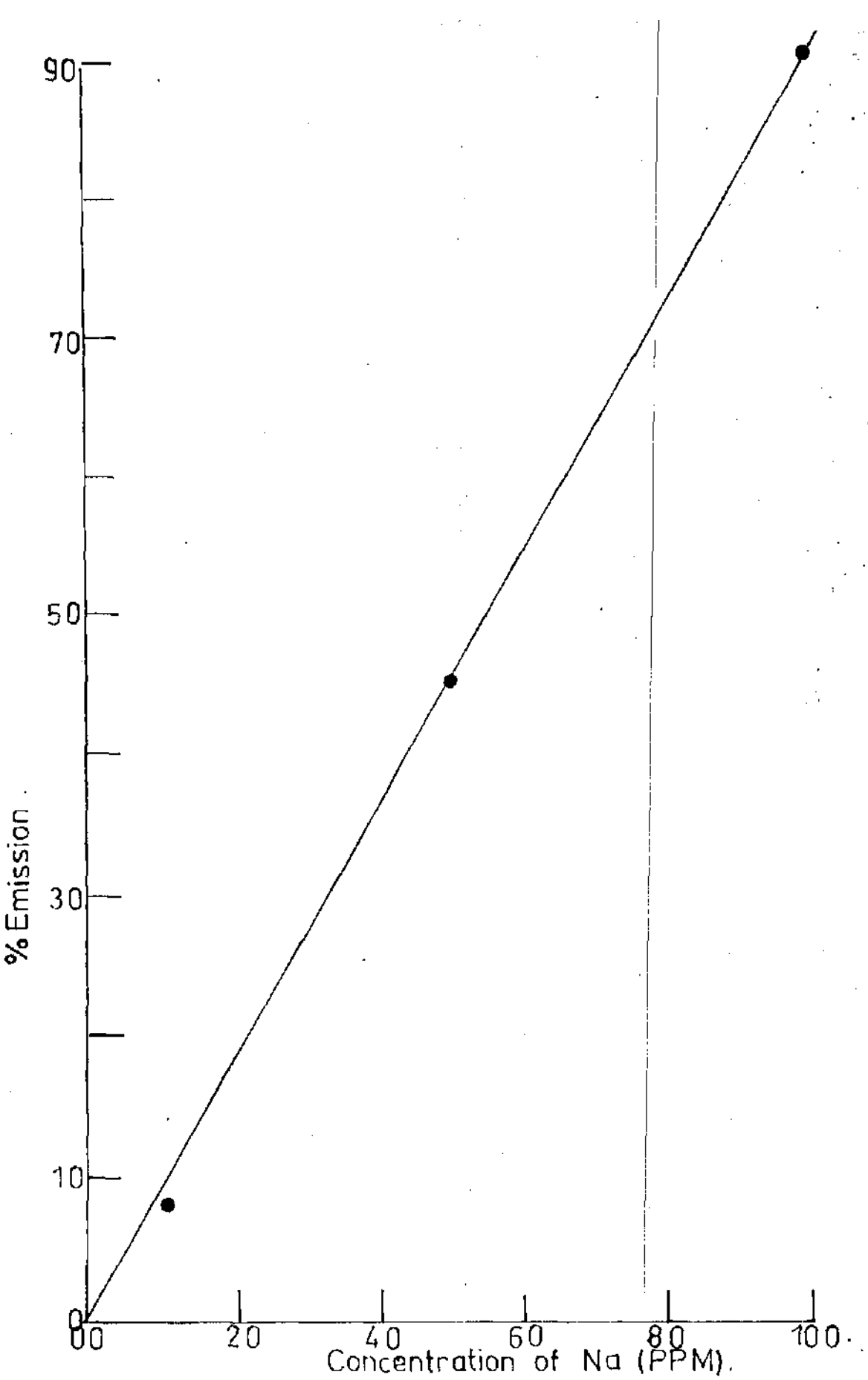
Appendix H(IV) :Calibration graph for AAS determination of Mn .



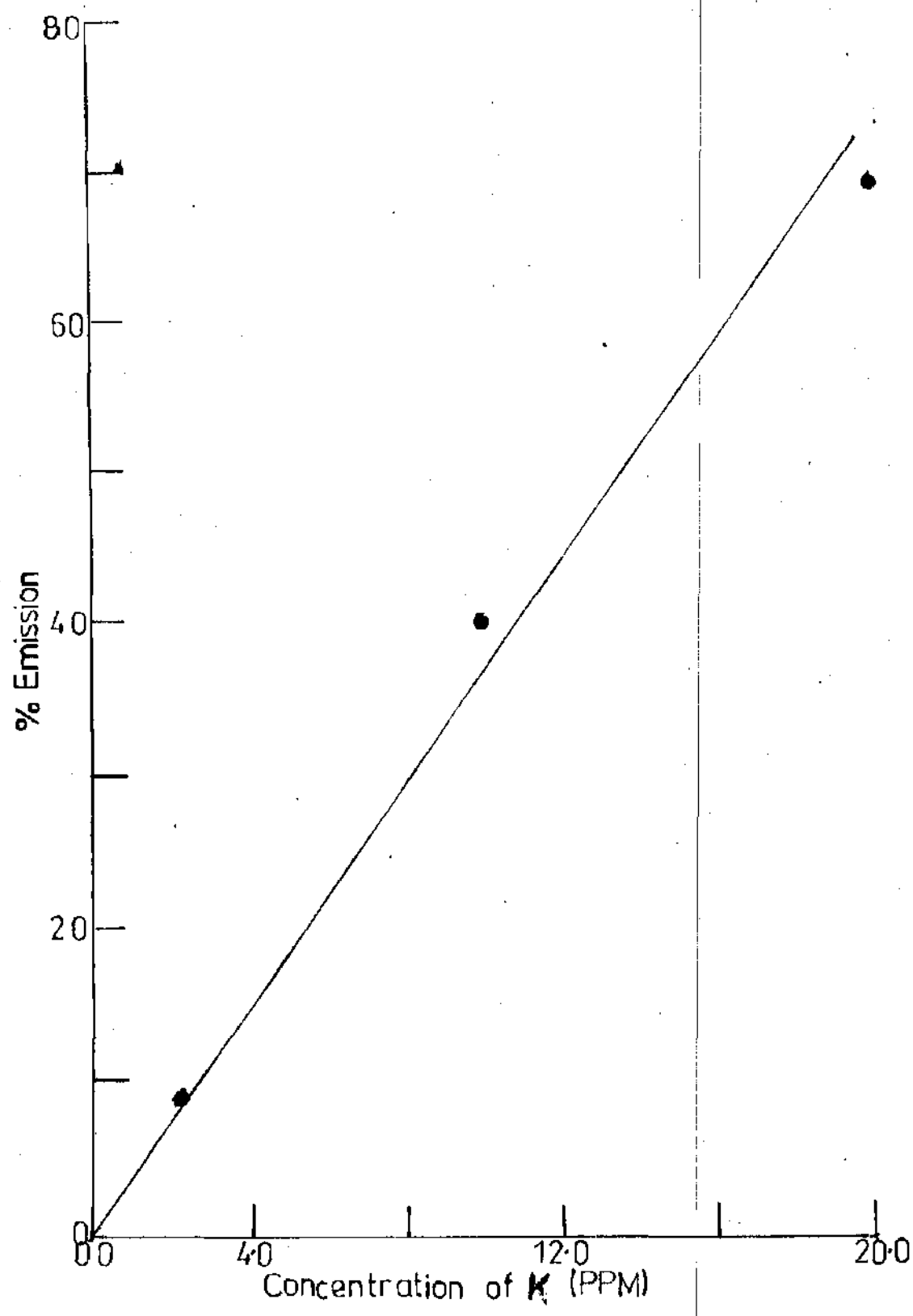
Appendix H(V): Calibration graph for AAS determination of Zn.



Appendix H(VI): Calibration graph for AAS determination of Fe.



Appendix H(VII) :Calibration graph for Na determination using FAES.



Appendix H(VIII): Calibration graph for potassium determination using FAES.

APENDIX

APENDIX I (I) Statistical Comparison of the Methods for Fe with AAS method at 95% Confidence limit. (X ⇒ Difference between pair of data: *t* ⇒ Student - *t* - stastistic)

	Wet Method				Furnace Method				TCAA Method			
Formula	1,10-Phen Vs AAS		2,2'-Bipy Vs AAS		1,10-Phen Vs AAS		2,2'-Bipy Vs AAS		1,10-Phen Vs AAS		2,2'-Bipy Vs AAS	
	X	$(X - \bar{X})$	X	$(X - \bar{X})$	X	$(X - \bar{X})$	X	$(X - \bar{X})$	X	$(X - \bar{X})$		$(X - \bar{X})$
NAN	0.15	-0.75	-0.11	-0.88	0.49	-0.77	0.89	-0.66	0.02	-0.768	-0.00	-0.84
COWBELL	0.59	-0.31	0.71	-0.06	3.02	1.76	3.43	1.88	0.21	-0.58	0.31	-0.53
BEBELAC	0.31	-0.59	-0.65	-0.14	0.64	-0.62	0.89	-0.66	1.96	1.17	2.65	1.83
SMA	2.69	1.79	2.92	2.35	2.11	0.85	2.27	0.72	1.86	1.07	1.34	0.30
I-ER	0.76	-0.14	0.96	0.19	0.03	-1.23	0.25	-1.30	-0.11	-0.90	0.09	-0.75
$\Sigma X =$	4.50		3.83		6.29		7.73		3.94		4.21	
$\bar{X} =$	0.90		0.77		1.26		1.55		0.79		0.84	
$\Sigma(X - \bar{X})^2 =$	4.23		5.47		6.31		6.61		4.25		4.99	
t	± 1.96		± 1.47		± 2.24		± 2.69		± 1.79		± 1.69	

APENDIX

APENDIX I (I) Statistical Comparison of the Methods for Fe with AAS method at 95% Confidence limit. ($X \Rightarrow$ Difference between pair of data; $t \Rightarrow$ Student - t - stastistic)

	Wet Method				Furnace Method				TCAA Method			
Formula	1,10-Phen Vs AAS		2,2'-Bipy Vs AAS		1,10-Phen Vs AAS		2,2'-Bipy Vs AAS		1,10-Phen Vs AAS		2,2'-Bipy Vs AAS	
	X	$(X - \overline{X})$	X	$(X - \overline{X})$	X	$(X - \overline{X})$	X	$(X - \overline{X})$	X	$(X - \overline{X})$		$(X - \overline{X})$
NAN	0.15	-0.75	-0.11	-0.88	0.49	-0.77	0.89	-0.66	0.02	-0.768	0.00	-0.84
COWBELL	0.59	-0.31	0.71	-0.06	3.02	1.76	3.43	1.88	0.21	-0.58	0.31	-0.53
BEBELAC	0.31	-0.59	-0.65	-0.14	0.64	-0.62	0.89	-0.66	1.96	1.17	2.67	1.83
SMA	2.69	1.79	2.92	2.15	2.11	0.85	2.27	0.72	1.86	1.07	1.14	0.30
I-ER	0.76	-0.14	0.96	0.19	0.03	-1.23	0.25	-1.30	-0.11	-0.90	0.09	-0.75
$\Sigma X =$	4.50		3.83		6.29		7.73		3.94		4.21	
$\overline{X} =$	0.90		0.77		1.26		1.55		0.79		0.84	
$\Sigma(X - \overline{X})^2 =$	4.23		5.47		6.31		6.61		4.25		4.99	
t	±1.96		±1.47		±2.24		±2.69		±1.79		±1.69	

APENDIX I (II)

Statistical Comparison of the Methods for Cu with AAS method at 95% confidence limit (X $\Rightarrow$ Difference between pair of results, t $\Rightarrow$ Student - t - stastistic)

	Wet Method		Furnace Method		TCAA Method	
Formula	Colorim Vs AAS		Colorim Vs AAS		Colorim Vs AAS	
	X	$(X - \bar{X})$	X	$(X - \bar{X})$	X	$(X - \bar{X})$
NAN	0.00	-0.19	-0.52	-0.51	-0.11	-0.05
COWBELL	0.07	-0.12	-0.05	-0.03	0.00	0.06
BEBELAC	-0.12	-0.31	-0.01	-0.01	-0.01	0.05
SMA	-0.29	-0.48	0.24	0.26	-0.04	0.03
I-ER	-0.62	-0.81	0.26	0.28	-0.16	0.10
ΣX	0.96		-0.08		-0.32	
$\bar{X}$	0.19		-0.02		-0.07	
$\Sigma(X - \bar{X})^2$	0.30		0.40		0.02	
t	± 0.84		± 0.11		± 2.08	

APENDIX I (III)

Statistical Comparison of the Metods for Zn with AAS method at 95% Confidence limit (X => Differnce between pair of results, *t* = Student - *t*)

	Wet Method		Furnace Method		TCAA Method	
Formula	Colorim Vs AAS		Colorim Vs AAS		Colorim Vs AAS	
	X	$(X - \bar{X})$	X	$(X - \bar{X})$	X	$(X - \bar{X})$
NAN	-1.70	-1.20	0.07	-0.30	0.19	0.15
COWBELL	0.54	0.04	1.03	0.66	-0.10	-0.14
BEBELAC	-1.90	-2.40	0.12	-0.25	-0.11	-0.16
SMA	0.44	-0.07	0.62	0.25	0.37	0.33
I-ER	0.10	-0.40	0.00	-0.37	-0.13	0.17
ΣX	-2.52		-1.84		0.22	
$\bar{X}$	-0.504		0.37		0.044	
$\Sigma(X - \bar{X})^2$	7.38		0.79		0.20	
t	±0.83		±1.86		±0.44	

APENDIX I (IV)

Stastistical comparison of the Methods for Fe Using Pyrogallol with AAS Methods at 95% Confidence (X ⇒ Difference between pair of results; *t* ⇒ Student - *t* - Statistic)

	Wet Method		Furnace Method		TCAA Method	
Formula	Colorim Vs AAS		Colorim Vs AAS		Colorim Vs AAS	
	X	$(X - \bar{X})$	X	$(X - \bar{X})$	X	$(X - \bar{X})$
NAN	0.12	-0.22	0.34	-0.24	0.80	-0.33
COWBELL	1.33	0.99	0.77	0.19	1.18	0.05
BEBELAC	-1.74	-2.08	-1.04	-1.62	0.06	-1.07
SMA	1.67	1.34	1.62	1.04	2.29	1.16
I-ER	0.29	-0.04	1.19	0.61	1.32	0.19
ΣX	1.67		2.88		5.62	
$\bar{X}$	0.334		0.58		1.13	
$\Sigma(X - \bar{X})^2$	7.13		4.17		2.64	
t	±0.56		±1.27		±3.11	

APENDIX I (V)

Student - *t* - values obtained by comparison of Experimental and Manufacturers' mean values.

Formula	Protein	Fat	Lactose	Moisture	Ash	Ca	K	Na	Mg	P	Cl-	Fe	Cu	Zn	Mn
NAN	± 5.20	± 14.43	± 31.10	± 561.18	± 15.40	± 1.42	± 22.86	± 0.89	± 19.48	± 35.27	± 38.97	± 34.64	± 3.46	± 4.16	± 0.69
COWBELL	± 28.86	± 3.98	± 34.15	-	-	± 86.72	± 10.46	± 57.86	± 70.32	± 6.31	± 3.46	± 4.62	± 1.73	±2 .17	± 34.64
BEBELAC	± 18.19	± 130.39	± 187.06	-	± 7.62	± 41.65	± 99.58	± 123.96	± 15.58	± 23.25	± 12.82	± 56.91	± 1.73	± 0.62	± 8.66
SMA	± 20.78	± 43.87	± 43.30	± 87.18	± 3.71	± 19.49	± 3.82	± 16.82	± 197.80	± 34.17	± 0.35	± 79.67	± 2.94	± 3.03	± 2.43
I-ER	± 22.52	±1.25	± 1.39	± 42.43	± 8.66	± 15.95	± 3.53	± 9.26	± 246.68	± 18.29	± 3.75	± 11.69	± 1.73	± 3.46	± 2.31

APPENDIX I (VI) Statistical Analysis of Variance for Iron Determination at 5% point of F.

Source	df	ss	ms	F-calculated	F-table
Replication	3	8.18	2.73	5.74	2.76
Method	2	3.18	1.59	3.35	3.23
Variety	4	6.01	15.25	32.11	2.61
M x V	8	8.31	1.04	2.16	2.18
Error	42	19.95	0.48	-	-
Total	59	100.63	21.098		

Deduction: The Calculated F-Values exceeded the tabulated F- values in all cases, expcept in the meethod Variety interaction, showing significant Variations in the results. The Variations are shown in LSD table below.

Source	LSD	Mean - Ranking
Replication	0.42	r_4 r_3 r_1 r_2 6.45 5.77 5.61 5.53
Method	0.37	wet TCAA Dry 6.08 5.90 5.55
Variety	0.47	SMA I-ER NAN COWBELL BEBELAC 7.34 6.34 5.83 5.04 4.57

df - degree of freedom; SS - Sum square

ms - mean square; *LSD - Least significance*

r_1, r_2, r3, and r_4 ⇒ 1,10-phen., 2,2- Bipy., Pyrogallol and AAS respectively.

APPENDIX I (VII) Statistical Analysis of Variance for Copper Determination, at 5% point of F.

Source	df	ss	ms	F-calculated	F-table
Replication	1	0.06	0.06	2.14	4.60
Method	2	0.07	0.03	1.14	3.74
Variety	4	0.03	0.01	0.24	3.34
M x V	8	0.22	0.03	0.93	2.70
Error	14	0.40	0.03	-	-
Total	29	0.77	0.16	-	-

Deducuction: The calculated - F in all cases was lower than the tabulated F values, showing that there are no significant variations in sources of the analysis.

MXV - Method - Variety intteraction

df - Degree of freedom, SS - sum square

ms - Mean square

APPENDIX 1 (VIII)

Statistical Analysis of Variance for Zinc Determination at 5% point of *F*

Source	df	ss	ms	F-calculated	F-Table
Replication	1	0.05	0.06	0.17	4.60
Method	2	1.01	0.51	1.70	3.74
Variedly	4	7.95	1.99	6.70	3.34
M X V	8	0.58	0.07	0.25	2.70
Error	14	4.16	0.30	-	-
Total	**29**	**13.75**	**2.91**	-	-

Source	LSD	Mean - Ranking				
Variety	0.55	BEBELAC, 4.22	SMA 3.72	NAN 3.50	1-ER, 3.47	COWBELL 2.63

Deduction; The calculated F Value exceeded the tabulated F value only in the results of the variety, implying significant variations in the results. This variation is shown in the LSD table above.