# CHEMICAL STUDIES ON *THEVETIA PERUVIANNA*

**BY**

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**A thesis submitted to the Postgraduate School, Ahmadu Bello University, in Partial fulfilment of the requirements for the degree of Master of Science in Chemistry.**

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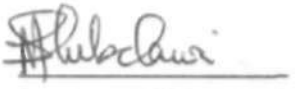
**Zaria**

**Nigeria**

**APRIL, 1995**

## DECLARATION

This is to declare that the work reported in this thesis is original and was carried out by myself. It has not been accepted in any previous application for higher degree, and all information cited from published and unpublished sources are specifically acknowledged in the text by means of references.



A. M. SHIBDAWA

## CERTIFICATION

This Thesis "Chemical Studies on ***Thevetia Peruviana***" by A.M. Shibdawa meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to Scientific knowledge and literary presentation.

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## DEDICATION

To my beloved son Sherif Muhammad Shibdawa

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Most of all; I am grateful to Almighty Allah for sparing my life and also giving me the courage to face all the challenges and vicissitude of life.

May God increase our knowledge.

**ABSTRACT**

The pulp and seed of ***"Thevetia Peruviana***" plant were analysed for their proximate composition, mineral elements and some properties of the tacky latex were investigated. The protien content of te seed is higher than that of the pulp. The seed could be a god source of protein as it compares well with those obtained in some legumes.

The extraction of protein from the defatted seed cake is found to be a function of flour: Solvent ratio, pH, type of solvent and extraction time.

The Kernel has as much as 60% of a yellow non-drying oil. Some physico-chemical properties of the oils *are* reported and these compare with those of edible and useful oils like Olive, Soybean, Sunflower and Sesame oils.

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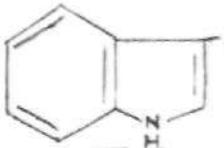
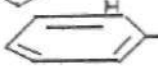
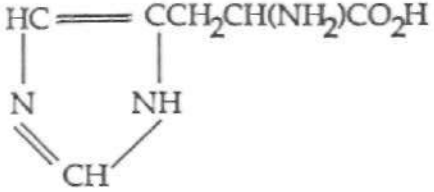
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| CLASS | AMINO ACIDS | FORMULAR | FIRST ISOLATION AS PRODUCT OF PROTEIN HYDROLYSIS | PROTEIN SOURCE OF FIRST ISOLATION |
|---|---|---|---|---|
| 1. Heterocyclic amino acid | Tryptophan |  $CH_2\,CH\,(NH_2)\,CO_2\,H$ | Hopkins and Cole 1902 | Casein |
| 2. Aromatic amino acid | Phenyl-alanine |  $CH_2\,CH(NH_2)CO_2\,H$ | Schulze and Berbieri 1881 | Lupine seedlings |
| 3. Aliphatic-mono amino mono carboxylic acid | Leucine | $(CH_3)_2\,CH\,CH_2\,CH\,(NH_2)\,CO_2\,H$ | Braconnot 1820 | Muscle fibre, wool |
| 4. " " | Isoleucine | $CH_3CH_2CH(CH_3)CH(NH_2)CO_2H$ | Ehrlich 1904 | Fibrin |
| 5. | Threonine | | | |
| 6. Sulfur containing amino acids | Methionine | $CH_3SCH_2CH_2CH(NH_2)CO_2H$ | Mueller 1922 | Casein |
| 7. Heterocyclic amino acids | Histidine |  | Kossel and Hedin 1896 | Sturine and Various protein hydrolysates |
| 8. Diamino mono carboxylic acid | lysine | $CH_2(NH_2)CH_2CH_2CH_2CH\,(NH_2)CO_2H$ | Drechesel 1889 | Casein |
| 9. Aliphatic mono amino mono Carboxylic acid | Valine | $(CH_3)_2CH\ \ CH(NH_2)COOH$ | | |
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# CHAPTER ONE

## INTRODUCTION

### 1.0 Proteins in Nutrition

The scientific approach to nutrition began in the late eighteenth and early nineteenth centuries with the development of analytical techniques by French chemists. Perhaps the first attempt to relate the results of these analytical approaches to nutritional studies was that of Magendie (1816) who reported that dogs fed a diet completely lacking in nitrogen shortly died, and that the addition to such diet of nitrogen - containing protein materials prevented this early mortality.

The requirements of the animal body for nitrogen organic combination was demonstrated and later influenced Boussingault (1836) to analyse food stuff for nitrogen, on the assumption that a direct relation might exist between the nutritive value of a food stuff and its nitrogen content.

This implied a dominating position for the protein in nutrition, a position which, with some modification is held to the present time.

## 1.1 Structure and Classification of Protein bound Amino Acids

Any organic acid which possesses one or more amino substituents may be classified as an amino acid. Such a definition would include a variety of substances of diverse structure. From this vast array of compounds, those singled out for exhaustive study have been confined to the relatively small group of α-amino acids which form the constituents of proteins, in addition to slightly larger number of related synthetic and naturally occurring compounds. With few exceptions, all possess the general structure $NH_2CHRCO_2H$, where the amino group occupies a position on the carbon atom alpha to that of the carbonyl group, and where the side chain R may be of diverse composition and structure.

Man and possibly all vertebrates are dependent upon the plant kingdom for certain of the amino acids which are characteristics of his own tissue. The following amino acids (Fig. 1) and others amounting to about twenty five different amino acids have been demonstrated by DeGroot (1955) and Wood (1953) to occur in the proteins of mammalian tissues and all have been isolated from hydrolysates of protein. The amino acids appear to be necessary to maintain nitrogen equilibrium in the adult dog and with the exception of histidine, in adult man as well. Citrulline but not ornithine can apparently be employed to satisfy the arginine requirements in the chick, Klose and Almquist (1940).

## 1.2 Protein Degradation Products

The concept of the intact protein as both a chemical and a nutritional entity had begun to weaken when Braconnot (1820) hydrolysed protein with acids and isolated glycine and leucine while Bopp (1849) isolated tyrosine in the same way. As the amino acids were isolated from acids or alkaline hydrolysates of protein, the concept that proteins might indeed be conglomeration of some sort of these smaller components took increasing hold. Not until the turn of the nineteenth century, however, was it possible through the efforts of Kossel and Kutscher (1899) to determine any of these components in a quantitative manner. A similar conclusion was also made from the biological type of evidence drawn from a lot of studies bearing on the digestive enzymes of the gastro-intestinal tract (Fig. 2).

By the end of the $19^{th}$ century it was apparent that the ingestion of protein foods by higher animals is followed successively by a degradation of the protein to smaller and more soluble aggregates by the gastric mucosa, and finally by the degradation of these smaller aggregates to amino acids by the action both of the pancreatic secretion and of the intestinal mucosa.

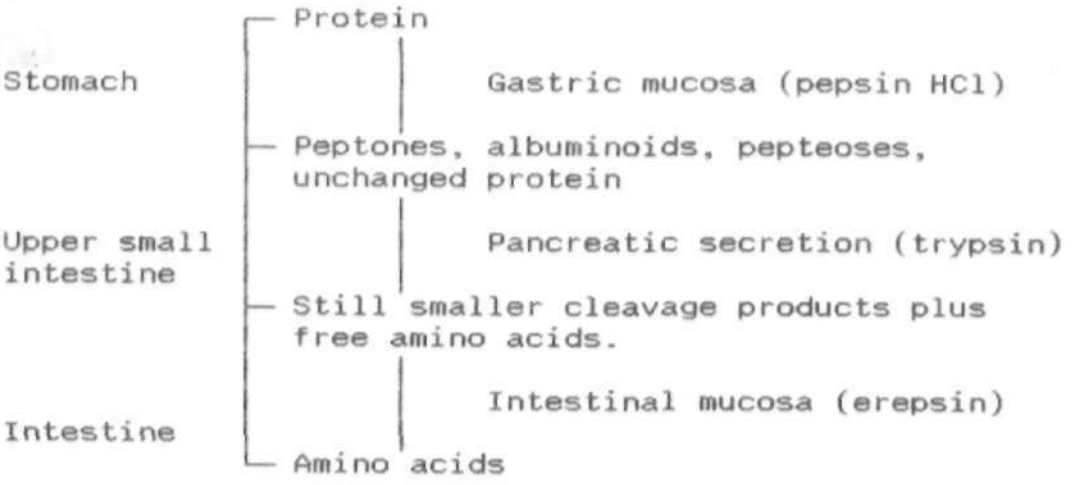



Fig. 2

Whether the amino acids that are the final products of protein digestion are absorbed as such into the blood stream or resynthesized prior to such absorption into protein was again a question which literally rage for many fruitless years and was finally settled when Folin and Denis (1912) independently showed that amino acids exits in the blood and increase markedly in amount during the course of protein digestion.

## 1.3 Classification and Uses of Polysacharides and Gums

Polysaccharides are complex carbohydrates (Whistler, 1973) which are condensation products or close derivatives of the monosaccharides. They have high molecular weight. Polysaccharides differ from the sugars in lacking sweetness and being insoluble in water although many of them form hydrophilic colloids. They are further classified as shown in figure below (Fig. 3).

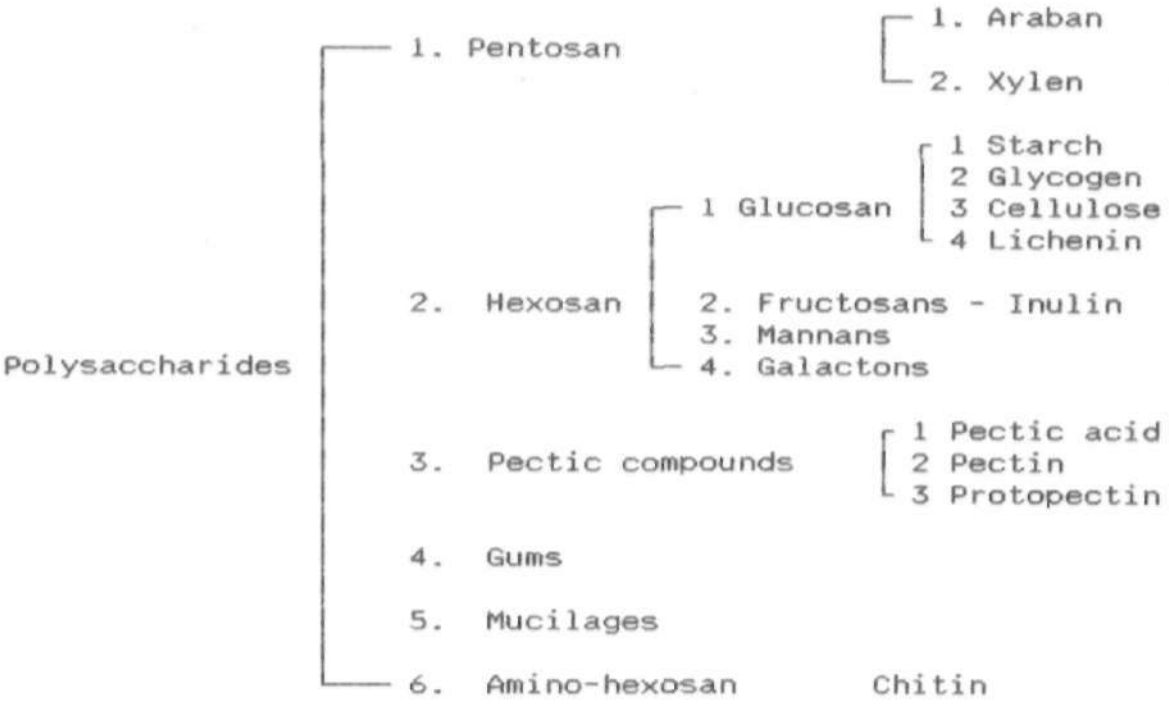



Fig. 3

In practical terms, gums are either hydrophobic or hydrophilic high molecular weight molecules, usually with colloidal properties, that in an appropriate solvent or swelling agent produce gels, which are highly viscous suspensions or solutions at low dry substance content.

1.4 Composition of Vegetable Oils

True fats are triglycerides of fatty acids (Miller, 1957). If the compound is a solid at ordinary temperatures it is usually called a fat; if liquid at ordinary temperatures it is called an oil. On hydrolysis the triglyceride yield three molecules of fatty acid and one of glycerol. According to (Hilditch and Williams, 1963) naturally fats consists of fatty acids esterified on the basis of maximum heterogeneity of acid.

Component fatty acids in fat may be two kinds; saturated. Unsaturated fatty acids are so called because they contain one, two or more ethylenic linkages example include oleic acid ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$).

Some commonly occurring saturated fatty acids are:-

| **Fatty Acids** | **Chemical Formula** |
|---|---|
| Butyric | $CH_3(CH_2)_2COOH$ |
| Caproic | $CH_3(CH_2)_4COOH$ |
| Caprylic | $CH_3(CH_2)_6COOH$ |
| Capric | $CH_3(CH_2)_8COOH$ |
| Lauric | $CH_3(CH_2)_{10}COOH$ |
| Myristic | $CH_3(CH_2)_{12}COOH$ |
| Palmitic | $CH_3(CH_2)_{14}COOH$ |
| Stearic | $CH_3(CH_2)_{16}COOH$ |
| Arachidic | $CH_3(CH_2)_{18}COOH$ |
| Behemic | $CH_3(CH_2)_{20}COOH$ |
| Lignoceric | $CH_3(CH_2)_{22}COOH$ |
| Cerotic | $CH_3(CH_2)_{24}COOH$ |

In the recovery of oil from plant materials, its refining, processing and conversion to finished products, workable techniques are available and are widely applied. Many of the processes have been modified and improved in recent years by the application of sound chemical engineering principles.

## 1.5 Mineral Elements and their Importance

Humans and animals depend on plant as ultimate source of food and mineral requirement. Plants in turn, obtain the

bulk of the mineral elements they require from the soil via the roots (Schroeder, 1976).

These minerals in the forms of ions in aqueous solution, such as those of sodium, potassium, calcium, magnesium, iron and zinc are absorbed and transported by a process called translocation to the various regions in the plant where they are required. These elements are considered to be essential to animal and human physiology.

**Table 1.0**

The daily requirement of essential mineral in food and water for adult human being (Schroeder, 1976).

| Elements | Amount (g) |
|---|---|
| Sodium | 4.400 |
| Potassium | 3.300 |
| Calcium | 1.100 |
| Magnesium | 0.300 |
| Iron | 0.013 |
| Zinc | 0.013 |
| Copper | 0.005 |

Both plant and animals require many minerals for their life processes. Certain essential processes in the body are catalysed by many elements either alone or in conjunction with others (Osborne, 1978).

Potassium and Sodium play crucial role in the body by maintaining equilibrium between extra and intracellular fluids, influencing contractility of smooth skeletal cardiac muscle, affecting the excitability of nerve and muscle

tissue controlling the optimum acid-base balance as well as intracellular metabolism (Stare and McWilliams, 1973). They are essential for cell synthesis and water balance, in plants. Iron and to a lesser extent copper are essential for many enzyme systems and synthesis in both plant and animals (Carrel and Price, 1965).

Calcium and magnesium are essential for the formation of teeth, bones and blood clot. They constitute the main components of the structural developments in both plants and animals. They are also important in regulation of nerve tissues, cardiac control, muscular activity regulation and also regulation of body fluids (Stare and McWilliams, 1973).

Zinc appears to be necessary for the building of protein which makes up most of the solid component of the cells. It is a component of metabolic enzymes. Zinc deficiency results in retarded growth, anaemia, poor wound healing and impaired sense of taste (Fox, 1971) and coronary heart disease (Feldman, 1976). Lead, mercury, cadmium, nickel and tin are widely distributed in nature, found in our soil and foods but have known or confirmed physiologic function (Stare and McWilliams, 1973).. Generally therefore, it implied conclusively that minerals absorbed from food are of importance to man and animals.

## 1.6 Relevance of Study and Research Objective

The effort to reduce the nations dependence on imported agricultural products in order to save the scarce foreign

exchange has made it imperative for Nigerians to exploit the available crops in this country that could be used to feed industries as well provide food for human and animals.

The determination of food composition is fundamental to theoretical and applied investigations in food science and technology and is the basis of establishing the nutritional value and overall acceptance from the consumer stand point. The analysis may establish the amount of one or several components or the overall composition.

Fruits and vegetables are sources of essential nutrients in most developing countries. For adequate use of these cheap sources to be made our knowledge of the nutritional values of the fruit trees, that are largely available in Nigeria should be broadened. This is because energy and nutritional values of plants as food depend on their chemical make-up. These plant source can also be studied with the view of compounding animal feeds from local materials. The presence of toxic substances however limit the nutritive value of plants. These should also be accorded intensive study when assessing plant materials as feed.

It is with these in view that this study was undertaken. The plant *Thevetia peruviana* is used as border plant and usually the fruits are allowed to drop away as waste. The study is to assess the multidimensional potential of the plant. Thus, some chemical studies of the

plant are undertaken to evaluate its domestic and industrial uses.

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The *Thevetia Peruviana*

*Thevetia peruviana* plant belongs to the family of *Apocynaceace* (Dalziel, 1937). it is named after the genus *Apocynum* (a name referring to the fruits of 2 separate carpels, which was thought to resemble 2 dog tails) and the order *Gentiales*.

It is known as 'yellow oleander' in English and 'olomiojo' in Yoruba 'gembush' in Hausa, exile tree in India cabalanga in Puerto Rico "ahonai" in Guyana.

It is a native of Central Tropical America from Mexico and the West Indies to Brazil. It is widely distributed by man. Occurring in the West Africa region as garden cultivate. It was taken to Europe in 1735 and cultivated as ornamental shrub because of its yellow flower.

The plant is large glaborous poisonous shrub or small tree. The leaf is alternate, linear,, sessile and 4-6 inch long with a strong mid-rib. The flower is yellow, the corolla is funnel shaped 2-3 inch long. The flower is hermaphroditic and radially symmetrical or actinomorphic. The fruit ripens to a black color due to the presence of a chromogen called aucuboside in the plant (Raymond, 1966). The seeds are 2-4 in number encased in a stony endocarp with orbicular cotyledons that has an oily albumen. The foliage is not grazed by stock. The plant makes a useful enclosure

hedge. It stands clipping, endures draught and suppresses plants from its shade.

## 2.2 Medicinal and Other Uses of *Thevetia Peruviana* plant

In the West Indies; the leaf and some tincture of the bark is known as an emetic and purgative (Evenkata, 1976). In Senegal a macerate of the bark and leaves may occasionally be used with precautions for amenorrhoea. In Indonesia it is reportedly used as a fish poison. The fruit have shown some insecticidal activity and especially the kernel (Jean, 1949). All parts of the plant are said to be poisonous including the milky juice which has been used as a vesicant and despite this it is used in Yucatan as a local application for toothache (Hurst, 1942). It has been used in Southern Africa as an arrow or ordeal poison. The oil from the kernel has numerous applications such as in contact insecticide and external applications to skin diseases.

A number of glycosidal derivatives of cardenolide have been detected of which thevetin is present throughout except in the leaves and the fruit pulp. The leaves contain several other glycosides including neriantin which is cardio-toxic acting like digitalis intolerance (Lee, 1970). The kernel contains another and more toxic bitter principle which causes tetanic convulsion and speedy death for which it is commonly used in India for suicide and murder. In Southern Africa it has acquire the name "bestill nut".

The aglycone of thevetin is an epimer of digitoxigenin (Walter, 1948). Thevetin is slowly and irregularly absorbed from the human digestive track, large doses frequently causes diarrhea and sometimes vomiting. An intravenous injection of thevetin produces digitalis - like effects on human heart very rapidly. Full effect develop in 6 minutes and the effects disappear almost completely after 2-3 hours (Walter, 1948).

This suggest possible use of thevetin injected intravenously for treatment of acute heart failure and other critical heart condition where speed is important. The elimination provides an important safety factor against overdose.

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

All chemical were of analytical grade unless otherwise stated. Distilled water was used throughout.

### 3.1 Sources of samples

The green fruits of yellow oleander plant were obtained from different locations in the Ahmadu Bello University (A.B.U) Zaria, main campus.

### 3.2 Sample Preparation

The fleshy pulp of the green fruit was carefully separated from the stony epicarp by means of a scapel. The kernel were obtained by cracking the stony epicarp of the seed. The pulp and the kernel were treated as two different samples. All the pulp and the kernel obtained from fruit at various locations were then properly mixed up for homogeneity. The sample were grinded into fine particles in Pifco-blender and were stored in large glass sample bottles after drying in an oven at a temperature of $70^{o}C$ so as not destroy carbohydrate in the samples.

### 3.3 Moisture and Drymatter Determination (AOAC, 1980)

Procedure:

A clean porcelain crucible was heated at $105^{o}C$ for 30 minutes, cooled to room temperature in a desicator and weighed. A known weight (about 5gm) of the sample was taken into the crucible, thinly spread and dried to constant

weight in an oven maintained at a temperature of 105$^{o}$C for 5 hours. The crucible with its content was removed and placed in a desiccator, cooled to room temperature and the weight was recorded. This was done in triplicate for both pulp and seed sample. The weight loss in each case represented the weight of the moisture and was computed from the following expression.

$$\text{Percentage Moisture} = \frac{\text{Loss in weight on drying}}{\text{Initial weight of sample (g)}} \times 100$$

$$\text{Dry matter} = \frac{\text{Oven dry weight (g)}}{\text{Initial weight of sample g)}} \times 100$$

## 3.4 Determination of Ash Content and Organic Matter (AOAC, 1980)

### Procedure

A clean porcelain crucible was heated at 100$^{o}$C for 30 minutes, cooled in a desiccator and weighed. About 5g of the sample was accurately weighed into the crucible and was ignited in a muffle furnace at 550$^{o}$C for about 7 hours and 16 hours for the pulp and seed respectively. This was done in triplicate for both the pulp and the seed.

The percentage of ash was calculated as follows:-

$$\text{Percentage Ash} = \frac{\text{weight of Ash (g)}}{\text{weight of sample g)}} \times 100$$

## 3.5 Determination of Mineral Elements

The determination of these elements are based on the interaction of the elements with electromagnetic radiation. Potassium and sodium were determined by the flame photometry, calcium, magnesium, copper, iron, zinc and nickel were determined by atomic absorption spectrometry and phosphorus by colorimetry.

In the flame photometry, the atoms of the elements are excited by flame and on returning to the ground state, they emit radiations characteristic of the elements concerned. The intensity of the radiation emitted is directly proportional to the concentration of the element present. A calibration curve is normally plotted and the concentration of the element in the sample, corresponding to its intensity is read from the curve.

In atomic absorption spectroscopy a solution of the element of interest is passed into a cool flame where the bulk of the atoms remain in the ground state. Radiation of a characteristic length from a hollow cathodelamp is passed through the flame. The atoms absorb some of these radiation and become excited. The decrease in intensity of the radiation is then measured using a monochromator and a detector system. The extent of absorption is dependent on the number of atoms in the ground state, and the decrease in intensity is therefore related to the concentration of the element in solution.

3.5.1 Sample Preparation

The mineral elements of the pulp and seed sample were taken into solution by wet digestion method.

3.5.1.1 Mixed Acid Digestion Method (Allen, 1974)

Reagents:

Perchloric acid 60%

Concentrated Nitric acid

Concentrated Sulphuric acid

Procedure:

Accurately weighed 0.50g of the dried sample was placed in a $100cm^3$ Kjeldahl flask and to it were added $1cm^3$ 60% Perchloric acid, $5cm^3$ nitric and sulphuric acids respectively. The mixture was digested slowly initially at moderate heat, and later more vigorously for 15 minutes after the appearance of white fumes. The cooled digest was diluted with water before filtering into a $100cm^3$ volumetric flask and was made up to the mark with distilled water. Blank determinations were taken through the same process and triplicate digestions were carried out on each sample. The solution was used for the determination of phosphorus.

3.5.2 Preparation of the Standard Solutions (Whiteside, 1975

i) Sodium Stock Solution (100ppm) Prepared by dissolving 0.254g of dry sodium chloride in 1000$cm^3$ distilled water. Working standards contained 0 to 5 ppm.

ii) Potassium Stock Solution (1000ppm) This was prepared by dissolving 1.9068g of potassium chloride in distilled water and making up the volume to 1000$cm^3$ with water in a litre volumetric flask. Working standard range between 0 to 10ppm.

iii) Lanthanum Stock Solution (5000ppm) This was prepared by dissolving 6.6837g of lanthanum chloride ($LaCl_3.7H_2O$) in water containing 1$cm^3$ of 2M hydrochloric acid and the solution was made up to 500$cm^3$ with water.

iv) Calcium Stock Solution 1000ppm) Prepared by dissolving 2.500g of dry anhydrous calcium carbonate in about 200$cm^3$ of distilled water containing 5$cm^3$ concentrated hydrochloric acid. The mixture was warmed to drive off carbon dioxide gas, cooled and made up to 100$cm^3$ with water. A working range of standard solutions of concentrations between 0 to 10ppm calcium were prepared. However sufficient stock lanthanum

chloride was added to these working standards to bring the concentration of lanthanum to 400ppm in each of them. Sufficient quantities of the stock lanthanum, were also added to the sample and blank solutions and appropriate quantity of 10% acid was then added to produce a final acid concentration of 1% (v/v).

v) Magnesium Stock Solution 1000ppm) This was prepared by dissolving 3.9106g magnesium chloride in 200cm$^3$ water and diluting the solution to 1000cm$^3$. Working standard, contained 0 to 3ppm magnesium. Sufficient stock lanthanum (5000ppm) were added to the standard, sample and blank solutions to obtain 400ppm in each. Appropriate quantity of 10% sulphuric acid were also added to sample and blank solutions to obtained acid concentration of 1% (v/v)

vi) Copper Stock Solution (1000ppm) 0.3930g copper sulphate was dissolved in water and the solution made up to 1000cm$^3$ in a litre volumetric flask. Working concentrations were between 0 to 1ppm.

vii) Zinc Stock Solution (1000ppm) 1.0g of zinc metal was dissolved in 30cm$^3$ 5M hydrochloric acid and making up to 1000cm$^3$ with water. Working standards ranged between 0 to 1ppm.

viii Iron Stock Solution (1000ppm) This was prepared by dissolving 7.0213g of iron (II) ammonium sulphate in 200cm$^3$ distilled water and making it up to 1000cm$^3$ in a litre volumetric flask. Working standards concentration range between 0 to 20ppm were prepared.

ix) Nickel Stock Solution (1000ppm) This was prepared by dissolving 4.0503g of nickel chloride in 1000cm$^3$ of water and making up to 1000cm$^3$ in a litre volumetric flask concentrations range between 0 to 10ppm

3.5.3 Determination of the Element in Sample Solutions

Procedure:

The various instrumental conditions necessary for maximum out-put of the instrument used were adjusted as recommended for each element (Whiteside, 1975). The absorbance readings of the sample solutions were subsequently read under the same conditions as the standards. The concentrations of the element present in the sample solutions (Cppm) were read off from the calibration curves (Appendix D - L). The amount of each element present in the sample is calculated from the expression:-

$$\text{Element (mg/1000g) (ppm)} = \frac{\text{Conc. of Std. X\% A (sample) X D F (cm}^3\text{) of flask}}{\text{\% A (standard) X Sample weight (g)}}$$

3.5.4 Phosphorus Determination (Allen, 1974)

Reagents

i) Phosphorus Standards

A 100ppm stock solution ($1cm^3$ = 0.1mg P) was prepared by dissolving 0.4393g dry potassium di-hydrogen orthophosphate in distilled water and diluting to $1000cm^3$ in a litre volumetric flask. An intermediate working standard ($1cm^3$ = 0.002mg P) was freshly prepared when required from the stock solution.

ii) Ammonium Molybdate - Sulphuric Acid Reagent

Ammonium molybdate (25.0g) was dissloved in about $200cm^3$ water in a beaker and warmed slightly, to dissolve. $280cm^3$ of concentrated sulphuric acid was added to about $400cm^3$ water and the molybdate solution was then filtered into the acid solution with thorough mixing. The solution was made up to $1000cm^3$ after cooling and stored in the dark.

iii) Stannous Chloride Reagent

Stannous chloride (0.50g) was dissolved in $250cm^3$ 2% (v/v) hydrochloric acid. This reagent was always prepared fresh before use.

Procedure:

Working standards containing 0 to 0.3mg phosphorus was prepared in 50cm$^3$ volumetric flask. Suitable aliquots of the sample solution were also pipetted into 50cm$^3$ volumetric flask and these were filled to two third capacity with water, 2cm$^3$ ammonium molybdate reagent was added to each and mixed followed by 2cm$^3$ stannous chloride reagent. The flask contents were again mixed, made up to volume and the absorbance readings were taken after 30 minutes at 700mm using water as reference. A calibration curved was plotted using the standard solution (appendix). The amount of phosphorus in the sample was read off from the graph and the mg/100g (ppm) calculated as below:-

$$\text{Phosphorus (mg/100gm) (ppm)} = \frac{\text{Conc. of Std. X\% A (sample) X D F X vol. of flask}}{\text{\% A (standard) X Sample weight (g)}}$$

Where,

% A = Absorbance
D.F. = Dilution factor

3.6 Determination of pH of Plant Latex

Procedure

The pH matter was allowed to warm up for 15 minutes. It was then calibrated using the buffer solutions of pH 4, 9.15 and at 25$^{o}$C prepared by dissolving the pH

table in appropriate volume of water following the method prescribed by the manufacturers. The electrode was then thoroughly washed with water and immersed in the fresh latex and the pH recorded when the needle drift had ceased (about 2 minutes after immersion). This was done in triplicate.

3.7 Determination of Nitrogen and Crude Protein Content

(Pearson, 1976). The semi-Micro Kjeldahl method was used

Principle:

The crude protein content of a particular sample is the figure obtained usually by multiflying the nitrogen content by the factor of 6.25. Sometimes the factor could vary depending on the percentage of nitrogen that is known to be present in the protein of sample.

The method involves the conversion of nitrogenous components of the sample to ammonium sulphate by digesting with concentrated sulphuric acid. Ammonium gas is distilled from the ammonium sulphate by heating with 40% sodium hydroxide trapped into the 2% boric acid solution. This was then titrated with standard hydrochloric acid solution. The nitrogen content, hence the crude protein content is then estimated. It must however be noted that this assumes that all nitrogen comes from protein and that proteins contain

16% nitrogen except where a conversion factor other than 6.25 is used.

Reagents:

i) Mixed Catalyst

This contained anhydrous sodium sulphate, copper sulphate and selenium dioxide in the weight ratio 96.0 : 3.5 : 0.5.

ii) Screened Methyl Red Indicator

Containing 0.016% (w/w) metyl red plus 0.083% (w/v) bromocresol green in ethanol.

iii) Boric Acid Indicator (2% )

Twenty grammes of boric acid was weighed and dissolved in 200$cm^3$ ethanol by vigorous shaking. 10$cm^3$ of the screened methyl red indicator was then added and the volume made up to 1000$cm^3$ with water.

iv) Standard hydrochloric acid solution, 0.1M.

v) Concentrated sulphuric acid.

vi) Sodium hydroxide solution, 40% (w/v).

Procedure:

About 0.5g of the well ground powder of the sample was accurately weighed into the digestion flask containing 4gm of the catalyst mixture and a few grains of anti-bumping granules. 10$cm^3$ of the flask and set up in the digestion rack in the fume cupboard. The flask was

heated gently for one hour to avoid charring and then it was heated strongly until a clear solution was obtained. The heating was continued for four hours after obtaining a clear solution. At the end of the digestion, the flask with its contents was cooled to room temperature, $5cm^3$ of water added and then the contents were poured gently into a $100cm^3$ volumetric flask.

The digestion flask was rinsed three times with $2cm^3$ portions of distilled water into the volumetric flask and the volume was made up to the mark with distilled water.

Ten ($10cm^3$) of the digest was pipetted into the Markham distillation apparatus, $10cm^3$ of the 40% sodium hydroxide solution was added and the mixture was distilled into $10cm^3$ of the boric acid solution containing 3 drops of the screened methyl red indicator. After $30cm^3$ of the distillate had been collected, it was titrated with 0.012M hydrochloric acid solution.

Triplicate determination were made for each of the samples.

3.7.1 'True' Protein Determination

3.7.1.1 Lowry Method (1951)

Reagents:

i) Solution A: 2% Sodium carbonate in 0.10M Sodium hydroxide solution.

ii) Solution B: 0.5% copper sulphate in 1% sodium potassium tartrate solution.

iii) Solution C: This solution was prepared just before use by mixing $50cm^3$ of solution A with $1cm^3$ of solution B.

iv) Folin-Ciocalteau reagent:

The commercial reagent was diluted in the ratio 1:2 with distilled water first before use.

v) Standard stock solution of bovine serum:

Albumin (BSA) of concentration $1mg/cm^3$ solution was also prepared immediately before use.

vi) Sodium chloride solutions of concentration range of 1% to 20%.

Procedure:

A) Calibration Curve

From the stock solution were prepared $1cm^3$ portion of solutions containing 0 to 1.0mg BSA. $5cm^3$ of solution C was added to each of these solutions which were then allowed to stand for 15 minutes at room temperature. After this time, $0.5cm^3$ of folin-ciocalteau reagent was added while stirring

and the absorbance readings of these solutions were taken after 30 minutes at 700nm wavelength with water as the reference.

The standard curve is shown in the appendix. The protein content of each extract was read off from the standard curve.

B) Extraction of the Seed Protein:

Samples of deffated seed flour were weighed into conical flasks. An appropriate amount of the extractant was measured into each flask to give the required meal: solvent ratio. Extractions were carried out at room temperature by using mechanical shaker for a stipulated time. The suspensions were filtered under pressure and the volume of the filtrates were noted. One $cm^3$ aliquot of the supernatant of each extract was further diluted and were then taken for protein determination. The extractant investigated were potassium hydrogen phosphate, distilled water, sodium chloride, sodium sulphate and sodium hydroxide.

3.7.2 Determination of the Isoelectric point (pI) of the seed Protein

Procedure:

The pH meter was allowed to warm up for about 15 minutes. It was then calibrated using buffer solutions of

pH 4.0, 6.0 and 7.0. The electrode was properly washed with distilled water before it was immersed in the protein solution obtained by filtering one of the protein extracts. The pH value when the protein in the solution started precipitating was recorded. Triplicate determinations were carried out.

3.8 Carbohydrate Determination

3.8.1 Determination of Total carbohydrate (Clegg, 1956)

Reagents:

i) Anthrone Reagent:

Accurately weighed 1.0 anthrone was dissolved in 1000cm$^3$ of13.6M sulphuric acid solution. Fresh reagent was prepared daily.

ii) Perchloric acid(v/v) This was preapred by adding 270cm$^3$ of concentrated perchloric acid to 100cm$^3$ of water.

iii) Ethanol (80%)

iv) Standard Glucose Solution (1mg/cm$^3$)

Procedure:

Extraction of Sugars from Sample

To 0.2g of the dried sample in a centrifuge tube was added two drops of 80% ethanol to aid mixing, then 5cm$^3$ of water and the whole mixture was thoroughly stirred. 25cm$^3$ of hot ethanol was then added, stirred thoroughly and set aside for 5 minutes after which it was centrifuged. The alcoholic solution was decanted and extraction procedure repeated with

$30cm^3$ hot ethanol. The alcoholic extract were combined and made up to $50cm^3$ with water. This aqueous solution of sugars was then ready for analysis with the reagent and was marked thus. Triplicate extractions were carried out on each example.

Extraction of Starch from Sample

Distilled water ($5cm^3$) followed by $6.5cm^3$ of 52% Perchloric acid wereb added to the sample residue above after extraction with alcohol.

After stirring for about 20 minutes, $20cm^3$ of water was added, the mixture was then centrifuged and the supernatant decanted into $100cm^3$ volumetric flask. Another $5cm^3$ of water was again added to the residue and extraction with Perchloric acid was repeated with occasional stirring for 3 minutes. The supernatant was again decanted into the $100cm^3$ volumetric flask and was made up to mark with water. This was filtered and the first $5cm^3$ of the filtrate was diluted to $10cm^3$ with water. This extract was used for analysis with the anthrone reagent.

Analysis of the Extracts.

For each determination the following were prepared in boiling tubes:-

i) "The blank" - Containing $2cm^3$ of distilled water prepared in duplicate.

ii) "Test extract alone" - Containing $1cm^3$ of the diluted extract (sugar or starch) prepared in triplicate.

iii "Test extract plus glucose standard" - Containing $1cm^3$ of the diluted extract (sugar or starch) and $1cm^3$ of glucose standard (100mg) prepared in triplicate.

To each of these tubes was added $10cm^3$ of the anthrone reagent and the contents were properly mixed before covering them with wooden corks and heated in a water bath for exactly 12 minutes, cooled to room temperature under running tap and the developed colour intensities were read at 630nm wavelength. The reagent blank was used as reference. The green colour developed was stable for at least 3 hours. The quantity of glucose in the test extract was calculated as follows:-

i) $$\text{Milligram glucose in sample (Amg)} = \frac{ZX0.1mg \times D.F. \times \text{volume of supernatant}}{(Y - Z)}$$

Where:-

Z = absorbance due to test extract only minus the absorbance due to the blank.

Y = absorbance due to the test extract and glucose standard minus the absorbance due to the blank.

D.F. = diltion factor of sample extract.

ii) $$\text{Percent Sugar in sample} = \frac{A(mg) \times 100}{\text{Weight of sample (mg)}}$$

For the starch, the expression (ii) was multiplied by a factor of 0.9 to give the starch in the original sample. The total carbohydrate was given as the sum of the percent sugars and the percent starch in the sample.

3.9 Determination of Crude Lipid

Soxhlet extraction method (A.C.O.C.S. oil and soap, 1940)

Reagent: Petroleum ether 40 - 60$^{o}$C.

Procedure: The dried sample (20.0gm) was accurately weighed into a preweighed fat-free thimble. 350cm$^{3}$ of petroleum ether was poured into a previously weighed 500cm3 round bottom flask containing antibumping granules.

The Soxhlet extractor containing the thimble with its content was then fitted into the round bottom flask and the extraction continued for 12 hours. The petroleum ether in the flask was distilled off using a vacuum rotary evaporator. The last traces of the solvent were removed by drying the flask in an oven to constant weight. The flask was cooled, weighed and amount of the lipid extracted was obtained from the difference between the weight of the flask before and after extraction.

$$\text{Crude lipid (\%)} = \frac{\text{Weight of lipid (g)} \times 100}{\text{Weight of dry sample (g)}}$$

## 3.10 Determination of Some Properties of the Extracted Lipid

### 3.10.1 Saponification Value (Pearson, 1976)

Reagents:-

i) Alcoholic solution of potassium hydroxide was prepared by dissolving 35g of potassium hydroxide in 20cm$^3$ of water and making up to 1000cm$^3$ with 95% ethanol. The solution was left to stand over night before carefully decanting off the clear aqueos layer.

ii) Phenolphthalein (1%).

iii) Hydrochloric acid 0.5M.

Procedure:-

Accurately weighed quantity (2.0g) of the extracted lipid was placed in a conical flask and exactly 25cm$^3$ of the alcoholic potassium hydroxide solution was added to it. The flask was heated under reflux in boiling water for 1 hour and 1cm$^3$ of phenolphthalein indicator was added to the hot solution. The excess alkali was the titrated with 0.5M hydrochloric acid (titre = b - acm$^3$).

Blank determination was carried out on each sample and saponification value of the lipid in milligram was calculated from the expression

$$\text{Saponification Value (mg)} = \frac{(b-a)\text{cm}^3 \times 28.05}{\text{Weight of sample (g)}}$$

3.10.2 Iodine Value (Dam's Method)

Reagents:-

i) Chloroform

ii) Sodium thiosulphate solution 0.025M

iii) Potassium Iodide solution (10%)

iv) Dam's reagents:

This was prepared by mixing 41cm$^3$ pyridine and 30cm$^3$ (98%) concentrated sulphuric acid with a solution of 13cm$^3$ bromine solution in 100cm$^3$ of glacial acetic acid. The solution was made up to 2,500cm$^3$ in acetic acid and was stored in a brown bottle.

Procedure:

Portions of chloroform and Dam's reagent, 5cm$^3$ each, were measured into a dry conical flask and allowed to stand for 10 minutes in the dark. Then 5cm$^3$ of 10% potassium iodide and 20cm$^3$ of water were added. The solution was quickly titrated against 0.025M sodium thiosulphate shaking vigorously to ensure proper mixing of the organic and aqueous layers. The titrant was added dropwise after the mixture had turned pale yellow until both phases just turned colourless. This was a blank titration to determine the iodine equivalent of bromine in the reagent.

The measurement of the iodine equivalent of the bromine not absorbed by the lipid was then carried out as follows:-

To $5cm^3$ portion of 1% lipid solution in chloroform was added $5cm^3$ of Dam's reagent and the mixture was left to stand in the dark for 10 minutes. $5cm^3$ of 10% potassium iodide and $20cm^3$ of water were then added, swirled and titrated with 0.025M sodium thiosulphate solution just as was done with blank.

Calculation:

$1cm^3$ $0.025cm^3$ $Na_2S_2O_3$ = 0.00317gm of iodine, iodine equivalent of the reagent = volume($cm^3$) required for the titration X 0.00317gm.

Triplicate determinations were carried out for the blank and samples.

3.10.3 Unsaponifiable Matter (Pearson, 1976)

Reagents:

i) Potassium hydroxide 0.5M and 3M

ii) Diethyl ether

Procedure:

The neutralized liquid obtained after titration for saponification value was again made alkaline with $1cm^3$ of 3M aqueous potassium hydroxide. The solution was then transferred to a separatory funnel, washed with water and extracted thrice, while still warm, with $50cm^3$ portions of diethyl ether. The ether extract were poured into another separatory funnel and washed with three $20cm^3$ portions of water. The extract was then further

washed with two $20cm^3$ portions of water until the wash water was no longer alkaline to phenolphthalein.

The extract was transferred into a pre-weighed flask ($w_1$) and the solvent was evaporated off using a rotary evaporator. The flask and the residue were then dried at $78^oC$ to constant weight ($w_2$). Tripilicate determinations were carried out on each sample.

$$\text{Unsaponifiable Matters (\%)} = \frac{(w_2-w_1) \times 100}{\text{Weight of sample (g)}}$$

3.10.4 Acid Value and Free Fatty Acid Value (Pearson, 1976)

Reagents:

Diethyl ether

Ethanol 95%

Sodium hydoxide 0.1M

Phenolphthalein 1%

Procedure:

The solvent mixture consisting $25cm^3$ each of diethyl ether, ethanol and $1cm^3$ of phenolphthalein solution was carefully neutralised with 0.1M sodium hydroxide. The lipid extract (1gm) was then dissolved in the neutral solvent mixture and titrated with aqueous 0.1M sodium hydroxide with

constant shaking until a pink colour which persisted for 15 seconds was obtained.

$$\text{Acid Value (\%)} = \frac{\text{Titre Value (cm}^3\text{) X 5.61}}{\text{Weight of sample (g)}}$$

Free fatty acid (FFA) value was calculated as oleic acid and; $1cm^3$ of 0.1M sodium hydroxide = 0.0282gm oleic acid in which case, the acid value is twice the free fatty acid.

3.10.5 Peroxide Value (Pearson, 1976)

Reagents:

Potassium iodide (powdered and 5% solution)

Glacial acetic acid

Chloroform

Sodium thiosuphate 0.002M

Starch indicator

Glacial acetic acid and

Chloroform mixture in the ratio 2:1 (v/v).

Procedure:

This determination was carried out in subdued daylight. Accurately weighed 0.5gms of the extracted lipid was mixed with 1gm of powdered potassium iodide and $20cm^3$ of the solvent mixture and boiled briefly. The mixture was quickly

transferred to a conical flask containing 20cm$^3$ of 5% of potassium iodide solution. The mixture was titrated with 0.002M sodium thiosulphate solution using starch as indicatior. A blank determination was carried out at the same time and triplicate determination were carried out on the samples. The peroxide value was reported as milliequivalent of peroxide oxygen per milligram of sample (cm$^3$) per Kg was calculated as; 2 X the volume (cm$^3$) of 0.002M sodium thiosulphate per gram of sample.

3.11 Solubility Study of the Fresh Plant Latex

Reagent:

Benzene, Chloroform, Methanol, Ethanol, But-1-ol, Water, Formaldehyde, Diethyl ether, Petroleum ether.

Procedure:

A few drops of the fresh latex was taken into a clean dry testube and to it few cm$^3$ of the solvent was added. The mixture was shaken for a few minutes and the observation noted. This was carried out with both hot and cold solvent.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Relative composition of the ripe and unripe fruits

The relative composition of the different parts of the ripe and unripe fruit of *Thevetia peruviana* plant are shown in Table 4.1. A wide range of weights was observed for the different parts of the fruit as a result of their different sixes and weights. The average weight of the different parts of the ripe fruits are higher than those of the unripe fruit, This observation may be as a result of the ripe fruit having reached its peak of maturity and thus contain the optimum amount of nutrients, water and other materials in its composition than the unripe fruit.

The average weight of the pulp for the ripe (19.22g) and unripe fruit (12.08g) are higher than that of other parts of the fruit because it constitutes a greater portion of the fruit. This is followed by the average weight of the seed with values (5.01g) and (4.06g) for the ripe and unripe respectively. The stony epicarp constitutes most part and weight of the seed. The average weight of the epicarp is (3.79g) and (3,28g) for the ripe and unripe fruit respectively. These are higher than those of the kernel 91.22g) and (0.78g) for the ripe and unripe fruit respectively.

TABLE 4.1 Relative composition of the ripe and unripe fruit.

| Different parts of the fruits | RIPE FRUIT | | UNRIPE FRUIT | |
|---|---|---|---|---|
| | Average wt. | % of each | Average wt. | % of each |
| Whole fruit | 24.23 | 100.00± 0.00 | 16.14 | 100.00± 0.00 |
| Pulp | 19.22± | 79.33±13.64 | 12.08 | 74.83±11.96 |
| Seed | 5.01 | 20.67± 3.89 | 4.06 | 25.15± 5.00 |
| Kernel | 1.22 | 5.04± 1.36 | 0.78 | 4.82± 1.73 |
| Stony-epicarp | 3.79 | 15.63± 2.66 | 3.28 | 20.35±3.25 |

The percentage of the kernel in the ripe (5.04%) and unripe fruit (4.82%) are fairly comparable; hence the increase in constitution of the kernel from unripeness to ripeness is not appreciable unlike for the pulp, the ripe (79.33%) and unripe fruit (74.83%). This may be as a result of the rapid increase in water content. The percentage of seed in the ripe fruit (20.67%) was found to be lower than that of the unripe fruit (25.17%). This may be due to the lower moisture content of the ripe fruits seeds when compared with that of the unripe fruit. The difference in the moisture contents of the seeds explained why the epicarp of the seeds for the ripe fruit is harder and drier than that of the unripe fruit. The stony epicarp serves as a protection for the kernel.

## 4.2 Moisture Content/Drymatter.

The moisture content of both the pulp and seed with their respective drymatter contents are given in the Table 4.2. The moisture content of the seed (23.70%) is higher than that of the pulp (16.40%). Moisture determination is one of the most important and widely used measures in the processing and testing of foods (Makower, 1950). Since the amount of dry matter in a food is inversely related to the amount of moisture it contains, moisture content is of direct economic importance to the processor and the consumer. food that contains too much water is subject to rapid deterioration from mould growth, heating, insect damage and sprouting (Martens and Hlynka, 1965).

The moisture content of foods generally vary widely. After commercial drying, fruits contain 10-12% water while dried vegetables have 7-10%. When fresh the value would be higher as in the above case.

## 4.3 Ash Content and Mineral Composition

The ash content of the pulp (7.57%) was higher than that of seeds 92.42%). This value obtained on the seeds compared fairly with those reported in literature for millet, groundnut and coconut pulp with values of 2.13%, 2.22% and 2.34% respectively (Oyenuga, 1968; Abede, 1981). it has also been reported that most nuts contain 1.5 to 2.5% ash (Pomeranz and Melon, 1971).

TABLE 4.2 Proximate Composition (%)of the Pulp, Seed and Latex of the yellow oleander plant

| | Pulp | Seed | Sap |
|---|---|---|---|
| Moisture | 16,40±0.80 | 23.70±2.10 | - |
| Ash | 7.57±0.28 | 2.42±0.07 | - |
| Crude Protein | 7.25±0.54 | 22.05±0.53 | - |
| Sugar | 1.33±0.07 | 0.48±0.07 | 0.61±0.07 |
| Starch | 50.38±1.13 | 18.18±0.37 | 23.11±0.51 |
| Total Carbohydrate | 51.71 | 18.66 | 23.72 |
| Lipid | - | 60.0±2.16 | - |
| pH | - | - | 5.4±0.08 |
| Isoelectric Point (pI) of protein extract | - | 3.3 ± 0.05 | - |

The ash content of the pulp (7.57%) was found to be higher than those reported for most fruits like orange, avocado, grape and apple with values 3.0%, 4.0%, 2.0% and 2.0% respectively, tomato has a higher ash content value of 10.0% (Blanck, 1955).

Except for experimental error, the ash content is a direct measure of the total mineral content of any food material.

### 4.3.1. Mineral Composition

The mineral composition of the samples are as shown in Table 4.3.

#### 4.3.1.1. Calcium

The calcium contents of the pulp and seed were 200 mg/100g and 240mg/100g compare favourably with the value obtained for soybean 240mg/100g sample. The value is however higher than the values reported for wheat, corn, rice and cow pea with values 45mg/100g, 20mg/100g. 70mg/100g and 180mg/100g respectively.

The calcium content of the pulp (200mg/100g) is higher than the values obtained for fruits like orange, Apple, grape and tomato with values 90mg/100g, 40mg.100g, 95mg/100g and 180mg/100g respectively. The value is lower than those of strawberry, squash and avocado with values 270mg/100g, 300mg/100g and 290mg/100g respectively (Blanck, 1955).

**TABLE 4.3** Mineral content of the pulp and seed of *Thevetia peruviana* plant in mg/100g.

| Elements | Pulp | Seed |
|---|---|---|
| Calcium | 200.00±4.05 | 240.00±7.60 |
| Phosporus | 146.70±0.50 | 234.71±1.27 |
| Magnesium | 136.00±0.45 | 280.00±1.50 |
| Potassium | 3,312.00±2.85 | 480.00±3.55 |
| Sodium | 24.00±0.28 | 24.00±0.95 |
| Copper | 1.60±1.50 | 2.80±2.16 |
| Iron | 1.00±0.34 | 10.00±0.87 |
| Zinc | 7.00±0.07 | 15.60±0.23 |
| Manganese | 0.80±0.02 | 2.00±0.53 |

Calcium is necessary for the growth of all green plants with the exception of some green algae. It is believed by some investigators of that one of the functions of calcium consists in combining with proteins of the cell nucleus and plastids. It is now known that though calcium is added to agricultural soils for the purpose of raising the pH, it's most important role is as a plant nutrients. In fact it is considered to be very important in the production of food high in nutritive value and also, of prime importance is its function in mobilizing nitrogen and phosphorus, thus producing proteinanceous rather than carbonaceous vegetation (Miller, 1957).

Calcium and phosphorus are required for bone formation and are incorporated into the bone by vitamin D, Inadequate supply of calcium in young animals results in bone deformation. The diets of man, poultry and pigs are generally low in calcium because of the high cereal intake. Calcium supplements for the latter two species are usually required (Mitchell and McClure 1937).

4.3.1.2. Phosphorus

The phosphorus content of *Thevetia* fruit pulp and seed were 146.6mg/100g and 234.7mg/100g respectively. The value for the seed is higher in line with the observation that nuts are among phosphorus - rich foods (Pomeranz, and Melon 1971). The phosphorus content of the seed (234.7mg/100g) is much lower than those reported for other seeds like wheat,

corn, rice and common beans with values 420mg/100g, 450mg/100g 350mg/100g and 500mg/100g respectively. The value for the pulp (14.6mg/100g) is lower than that of orange 160mg/100g) but lower than the value reported for tomato (460mg/100g) (Blanck, 1955).

Considerable evidence has been obtained on the action of phosphorylating enzymes in the trans formation of one carbohydrate to another or in the degradation of carbohydrates during respiration in cells and some of these enzymes have been identified in both plants and animal tissues (Dutcher, 1951).

4.3.1.3: Magnesium

The magnesium contents of the pulp and seed obtained were 136mg/100g and 80mg/100g respectively. The values are in line with the report of Pomeranz and Melon, (1971) and Miller (1957) that magnesium is found in high concentrations in nuts, cereals and legumes. The magnesium content of the seed (280mg/100g) is higher than those reported for seeds like wheat, rice and peanut with values 160mg/100g, 160mg/100g and 220mg/100g respectively, but is lower in comparison with those of corn and soyabean with values 430 and 310mg/100g respectively.

4.3.1.4 Sodium and Potassium

The pulp and seed of *Thevetia* plant are good sources of potassium containing 3,312mg/100g and 480mg/100g respectively. However the value obtained for the sodium

24mg/100g is the same for both. The value for the sodium is lower than that of the potassium. The sodium content of the pulp and seed are much lower than those reported for wheat, corn, rice and soybean with values 70mg/100g, 50mg/100g, 100mg.00g, and 240mg/100g respectively (Blanck, 1955).

The potassium content compares with that of wheat (480mg/100g) but is higher than those of Corn and Rice with value 400mg/100g and 380mg/100g respectively.

The potassium content of peanut and soybean with values 800mg/100g and 180mg/100g are higher than the 480mg/100g obtained for the seed in this study.

4.3.1.5 Copper

The copper content for the pulp and seed were 1.6mg/100g and 2.8mg/100g respectively which are higher than those reported for seeds like wheat, corn, rice, peanut and soyabean with values 0.9mg/100g, 0.8mg/100g, 0.04mg/100g and 0.12mg/100g respectively. The body however requires copper in minute quantity 2.15mg daily for an average man of about 75kg weight (Ekepte, 1985). Copper is essential for the utilization of Iron in the haemoglobin synthesis. The Public Health service permits up to 0.2 ppm of copper (0.8ppm $CuSO_4$) for this purpose in drinking water.

Copper deficiency is not observed in man but it is common in cattle and sheep consuming forages low in copper. A copper content of 7.5ppm in herbage is believed to be

adequate and a daily intake of 5mg of copper for pregnant ewes has been suggested (Marston, 1952).

4.3.1.6 Iron

The *Thevetia* plant fruit pulp and seed contain an appreciable amount of iron 1.0mg/100g and 10.0mg/100g respectively. The value for the seed is about ten times that of the pulp. The Iron content of the seed 10.0mg/100g is higher than the values reported for Wheat, Corn, Rice, Peanut and Soybean with values of 5.0mg/100g, 3.0mg/100g, 2.0mg/100g, 2.0mg/100g and 8.0mg/100g respectively (Blanck, 1955).

Iron being a constituent of the respiratory pigment haemoglobin is vital for the functioning of all tissues and organs of the body. Approximately 5grams of Iron occurs in man, most of which is present in the red blood cells. These are destroyed at a rate which liberates approximately 25mg of iron daily and normally these are reutilized for new red blood cell formation.

4.3.1.7 Zinc

Zinc content of the *Thevetia* plant fruit pulp and seed were found to be 7.0mg/100 and 15.6mg/100g respectively.

Among the trace elements, Zinc content is higher and the value for the seed is more than twice that of the pulp. The zinc content of the seed is higher than those obtained for wheat, corn, rice, and soybean with values of 6.3mg/100g, 2.0mg/100g, 1.3mg/100g, and 1.1mg/100g

respectively, peanut however has a much higher zinc content of 41mg/100g.

Dietary requirement of zinc ranges from 1.25mg for infants to 5.45mg for lactating women daily (FAO/WHO 1973). Zinc is similarly essential for rats and other animals. it is required for the function of the enzyme, carbonic anhydrase. Naturally occurring deficiencies have not been found (Vallee and Altchule, 1949).

4.3.1.8 Manganese

The manganese contents of the pulp and seed were found to be 0.8mg/100g and 2.0mg/100g respectively and these are lower than the value reported for corn (3,5mg/100g). Manganese is of practical importance in poultry feeding where a deficiency produces the bone deformity called "perosis". The lesion is in the development cartilage of the bones. Eggs produced by hens fed diets low in manganese have a poor hatchability and the embryos have shortened leg and wing bones and distorted beaks.

Manganese activates the enzyme phosphatase in vitro and phosphatase level of the blood reduced in peratic chicks (Schaibe *et al*, 1938).

Manganese deficiency has also been produced experimentally in mice, rats and rabbits. Since deficiency in other farm animals has not been observed, their requirements are likely considerably lower than poultry (Smith *et al*, 1947).

4.3.1.9 Nickel and Chromium

The two micronutrients in both the pulp and seed samples were below detection limit of the instrument employed.

4.4. Protein Content

4.4.1 Crude Protein

The crude protein contents of thevetia fruit pulp and seed in this study were 7.25% and 22.05% respectively (Table 4.2). Although the pulp is low in protein, the seed is a good source of it and compares well with groundnut with a crude protein value in the range 22.7% (Oyenuga, 1968) and some other legumes like cowpeas, peas and soybean seeds eith protein content of 22.7%, 22.2% and 35.1% respectively.

4.4.2 Extraction of protein (Wu, 1922)

The amount of protein extracted by using different meal; solvent ratios at various time using various solvents are expressed as a percentage of 22.05%. The results are shown in the Tables 4.4 - 4.6.

**TABLE 4.4** Effect of different extractants at 0.10M concentration at two flour: solvent ratios (w/v) on the extraction of protein at 60 minutes extraction time.

| Extractant | 2% w/v | 4% w/v |
|---|---|---|
| $KH_2PO_4$ | 20.36 ± 2.21 | 21.70±2.59 |
| $H_2O$ | 24.27 ± 2.55 | 19.50±5.49 |
| NaCl | 28.22 ± 1.22 | 23.42±1.45 |
| $Na_2SO_4$ | 39.49 ± 1.97 | 37.90±4.09 |
| NaOH | 61.02 ± 2.95 | - |

#### 4.4.2.1 Effect of Different Extractant at 0.1M Concentration

The effect of varying extractant on the percent protein extracted for the defatted seed sample are shown in table 4.4. The best extractant is sodium hydroxide with 61.02% of the protein extracted at an optimum flour: Solvent ratio of 2% w/v. The reason for these observations are that plant proteins and globulines are more soluble in basic medium. Thus, increase in the pH of the extractant resulted in a corresponding increase in percent protein extracted.

#### 4.4.2.2. Effects of Different Concentrations of NaOH on Protein Extraction

This was studied at 2% w/v flour: solvent ratio with 60 minutes extraction time.

The result is given in Table 4.5. It is observed that increasing the sodium hydroxide concentration resulted in a corresponding increase in percent protein extracted up to a point where it levels thus indicating that the optimum concentration of the extractant as 0.50M. The increase in extraction might be attributed to the continuous hydrolysis of the polypeptide bonds of the protein until all the bonds are hydrolysed.

TABLE 4.5 Effect of different concentrations of Extractant (NaOH) on the protein extraction at 2%w/v meal: solvent ratio at 60 minutes extraction time.

| Different concentrations of NaOH | % Protein Extracted |
|---|---|
| 0.05M | 56.23±2.53 |
| 0.10M | 61.33±3.65 |
| 0.15M | 65.75±0.88 |
| 0.20M | 70.34±1.88 |
| 0.25M | 69.18±2.73 |
| 0.30M | 76.64±2.63 |
| 0.50M | 83.35±0.16 |
| 1.00M | 84.84±0.07 |

4.4.2.3 Effect of Time on Protein Extraction with 0.5M NaOH

The result of this study showed that time, after the initial 30 minutes, does not have any further significant effect on the percent protein extracted. This is shown in Table 4.6.

**TABLE 4.6** Effect of time on protein extraction with 0.50M NaOH

| Extraction time (minutes) | % Protein Extracted |
|---|---|
| 30 | 85.25±1.54 |
| 60 | 82.60±1.87 |
| 90 | 88.00±0.95 |
| 120 | 82.43±0.55 |

### 4.4.3. Isoelectric point (pI) determination for the protein in the sample

The Isoelectric point (pI) obtained for the protein is at pH 3.3. which is lower than the pI reported for proteins like carboxypeptidase, hen oval-bumin, human serum albumin, fibrinogen and thyroglobulin with values 6.0, 4.6, 4.8, 5.5. and 4.6 respectively. The value is higher than that of pepsin pI with a value of less than 1.0.

Proteins are amphoteric compounds, and are capable of possessing either a positive or a negative charge just like amino acids. They migrate in an electric field at a rate dependent on their net charge and the pH of the medium. Each is electrically neutral at only one pH, which is the Isoelectric point (pI). The pI is determined by the number and kind of charged R-groups in a protein; a protein molecule is positively charged at pH values lower than its pI and negatively charged at higher pH values. This point is important as it affects seperations.

## 4.5 Carbohydrates

The total sugar content obtain for the pulp, seed and latex are 1.33%, 0.48% and 0.61% respectively. The starch content of these are 50.38%, 18.18% and 23.11% respectively (Table 4.2).

The sugar content obtained was lower than starch content of the pulp seed and latex as can be seen in the Table 4.2. The pulp has the highest value of starch and sugar, 50.38% and 1.33% respectively.

Nuts are generally a poor source of carbohydrates as reported for seed nuts like almond, walnut, pecan, with carbohydrate contents of 21%, 16%, and 15% respectively, though chest nuts contain up to 33% starch (Blanck 1955).

The increase in starch is accompanied by a parallel decrease in cellulose, hemicellulose and pentosans (Pomeranz and Melon, 1971). Apart from cellulose, starch is the most abundant and widely distributed substance in vegetables. Starch occurs in the form of granules as reserve food in various parts of most plants.

## 4.6 Lipids

The seed sample contained as much as 60.0% crude lipid (Table 4.2).

Most nuts are rich in lipids (almond, 55%; beech nuts, 50%; pecan, 71%; and walnuts, up to 64%).

The lipid content of the seed compares favourably with the main seed used for extracting lipids on a commercial scale such as peanut 48%, sesame seed 50%, sunflower 47% and soybean 18% (Pomeranz and Melon 1971);.

### 4.6.1 Some physico-chemical properties of the extracted lipids

The physico-chemical properties of the extracted oil are variable and they are affected by factors such as the growth environment of the seeds and their conditions of storage (Duffus and Slaughter, 1980).

4.6.1.1. Saponification Value

Saponification value is a measure of the amount of alkali required to saponify a definite weight of fat and an indication of the average molecular weight of the oil. A high saponification value indicates a greater proportion of fatty acids of low molecular weight (Thorpe *et al*, 1970). The saponification value of the seed oil was 191.33mg KOH/gm (Table 4.7) which compares well with that of sesame oil (187-196mg/g) (Eckey, 1955). The value indicated that the oils are made up of a greater propertion of low molecular weight fatty acids.

**TABLE 4.7** Some physico-chemcial properties of the lipid extracted from the Olenader seed.

| | |
|---|---|
| Saponification value (mgKOH/g) | 191.33±2.91 |
| Iodine value | 70.90±0.66 |
| Unsaponifiable matter % | 0.40±0.04 |
| Acid value % | 0.10±0.02 |
| Peroxide value (mEq/kg) | 0.50±0.04 |

4.6.1.2. Iodine value

The Iodine value (number) is defined as the grams of iodine that add to 100gm of sample (Allen, 1951). Iodine number measures the degree of unsaturation and therefore the

reactivity of the oil. The iodine value obtained for the seed oil was 70.9 which compares with that of olive oil and castor oil both with values of 85 (Pearson, 1976; Ockerman, 1978).

Olive and castor oils are both classified as non-drying oils on the basis of their iodine number. Thus, it can be inferred that the thevetia seed oil is also non-drying.

Generally, non-drying oils have iodine numbers below 100 while drying oils have iodine numbers between 130-200 (Pomeranz and Melon, 1971).

4.6.1.3. Unsaponified Matter

Unsaponified matter in fats and fatty acids include those materials that cannot be saponified by potassium hydroxide like sterols, higher alcohols and some hydrocarbons, but it consists principally of sterols and terpenes that are originally present in the lipid sample (Christie, 1973).

The *Thevetia* seed oil contains 0.4% of unsaponifiable matter which compares favourably with that of sunflower oil (0.3 -1.3%) and peanut oil (0.2 to 0.8%). (Eckey 1955).

4.6.1.4. Acid Value

The acidity of fats and oils can be expressed either as the number of milligrams of potassium hydroxide required to neutralize 1g of fat or it can be expressed as millilitres of normal (1N) sodium hydroxide solution used to neutralize fatty acids in 100g fat.

The seed oil has an acid value of 0.1% which is less than the value reported for olive oil (0.2-6%) and soybean (0.3-3%) (Eckey, 1955).

Deterioration of grain and milled product is accompanied by increased acidity. The acid formed include free fatty acids, acid phosphates and amino acids; but at the early stages of deterioration, fat acidity increases at a greater rate.

Several workers suggested determining fat acidity as an index of grain soundness (Zelany and coleman, 1938; Anderson and Alcock, 1954; Baker et al, 1959; Pomeranz and Shellenberger, 1966).

4.6.1.5 Peroxide Value

The peroxide value of the seed oil is (0.5mEq/kg) as shown in Table 4.7. The value is low in comparison with those of other oils.

Oxidative rancidity is a type of spoilage in fats and oils. It results from the action of atmospheric oxygen on the fat and oil molecules and is characterized by a typical strong and disagreeable odour and flavour (Roy, 1955). The rancid products not only make the food unpalatable but also destroy certain vitamins. This is caused by the free radicals produced during the peroxide formation when atmospheric oxygen adds across the double bonds in unsaturated fatty acids in the presence of enzymes or certain chemical compounds (Roy, 1955).

## 4.7 Preliminary Solubility Study on the Fresh Latex

The result of the solubility study of the fresh latex in different solvents is shown in Table 4.8. The latex is insoluble in organic solvents lacking hydroxyl groups but it is fairly soluble in alcohols and solvents with hydroxyl groups (alcohols and water). It is slightly soluble in 0.1.-1.0M hydrochloric acid solution and is soluble in 0.1M-1.0M sodium hydroxide with an accompanied change in colour to yellow from the initial milky colour. The colour deepened and changed to orange as more sodium hydroxide solution was added.

The observation may be as a result of chain scission by the strong alkali to produce lactic acid, propanol (phyruvic aldehyde or methyl glyoxal) as an intermediate, Saccharinic acids are also formed (Frear, 1950).

## 4.8 pH of the Plant latex

The pH value obtained for the plant milky and tacky latex is 5.4, indicating it is acidic in nature, which is in line with the definition of gums (Miller, 1957) that gums are acidic substances whcih swell in water to form either a gel or a viscous, sticky solution.

This explains its insolubility in acidic and other neutral organic solvents. It reacts with basic medium like sodium hydroxide with an accompanied change in colour from milky through yellow to an orange solution with increase concentration of the base.

**TABLE 4.8** Solubility of the fresh latex in different solvents

| S.No | Solvents | Solubility in cold solvent | Solubility in Hot solvent |
|---|---|---|---|
| 1. | Benzene | Insoluble; it turns curdy and the particles settles. | Same as in cold |
| 2. | Chloroform | Fairly soluble turns milky | " " |
| 3. | Methanol | " " | " " |
| 4. | Ethanol | " " | " " |
| 5. | But-1-ol | " " | " " |
| 6. | Water | " " | " " |
| 7. | Formaldehyde | " " | " " |
| 8. | Diethylether | Insoluble and form Particles | " " |
| 9. | Petroleum ether | " " | " " |
| 10. | 0.1M, 0.2M 0.5M, 1.0M NaOH solution | Soluble, but turns yellow with more NaOH the colour Changes to orange | " " |
| 11. | 0.1M, 0.2M 0.5M, 1.0M, HCl solution | Insoluble and forms particles that became suspended. | " " |

# CHAPTER FIVE

## 5.0 CONCLUSION

The protein content of the *Thevetia peruviana* plant is high (22.05%). This prompted a detailed study of the extracting ability of different solvents for the protein value of the defatted seed sample.

This involves the study of parameters like effect of different extractants furnishing a result that sodium hydroxide (NaOH) 0.1M at 2 %w/v flour: solvent ratio extracted best (61.02% protein) of all the solvents used at 60 minutes extraction time. The effects of different concentration of the extractant gives an optimum concentration of sodium hydroxide as 0.50M with an extraction of 83.35%. An increase in concentration does not affect the protein extraction. The effect of the extraction time of 30 Minutes with a percent protein extraction of 85.25% and any increase in time of extraction does not affect the protein extraction.

Therefore, this may be a good source of protein concentrate for poultry, more especially that it contains appreciable quantities of calcium, magnesium, phosphorus, potassium, zinc, and copper which are necessary in their diets (appendix M).

The kernel yields as much as 60% of a yellow non-drying oil which can be obtained free from the toxic principles (Dalziel, 1937). Some of the physicochemical properties of the seed oil compare well with those of some edible and

useful oils like olive, soybean, sunflower and sesame oils (Blanck, 1955).

## 5.1 Suggestion for Further Research

Judging from the chemical analysis done so far on the *Thevetia peruviana* plant; it can be conclusively inferred that the plant has high potential as a supplement for human and animal feed. The seed protein is high (22.05%) and may serve as a good source of protein concentrate for poults, though a futher study on the protein quality of the seed such as the amino acid constituents and their bioavailability is required before it is being recommended as edible protein.

The anti nutritional factors need to be determined for both the pulp and seed because they affect the availability of the mineral elements and the proteins (Harris, 1955; Duffus and Slaughter, 1980). Since it is aimed for use in poultry feed; the seeds content of vitamins A, D, E. K, thiamine, riboflavin, pantothenic acid, niacin, biotin, choline, folacin, vitamin $B_{12}$ and iodine need to be investigated.

Further studies on the composition and characterisation of the gummy latex need to be done to assess its usefulness.

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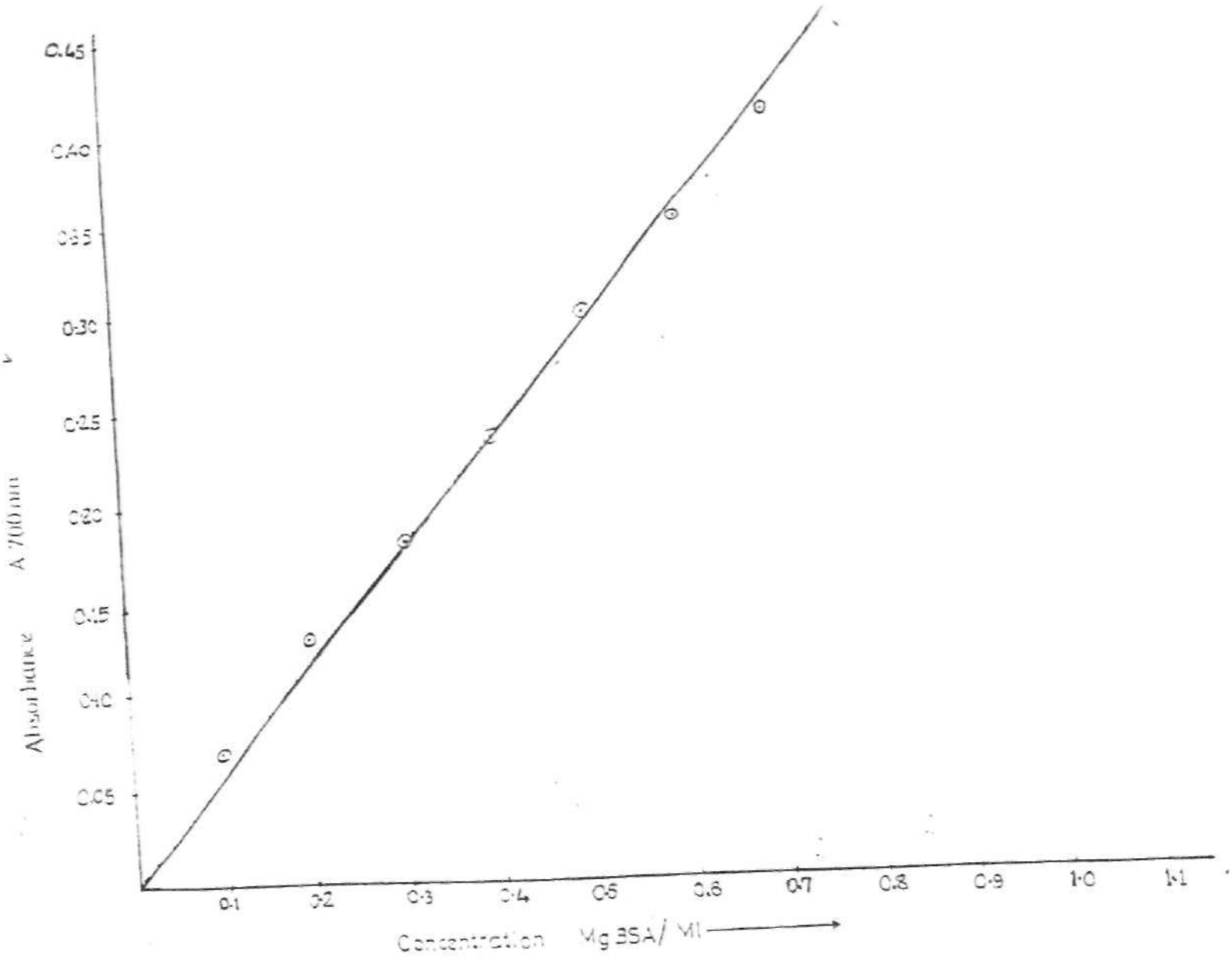
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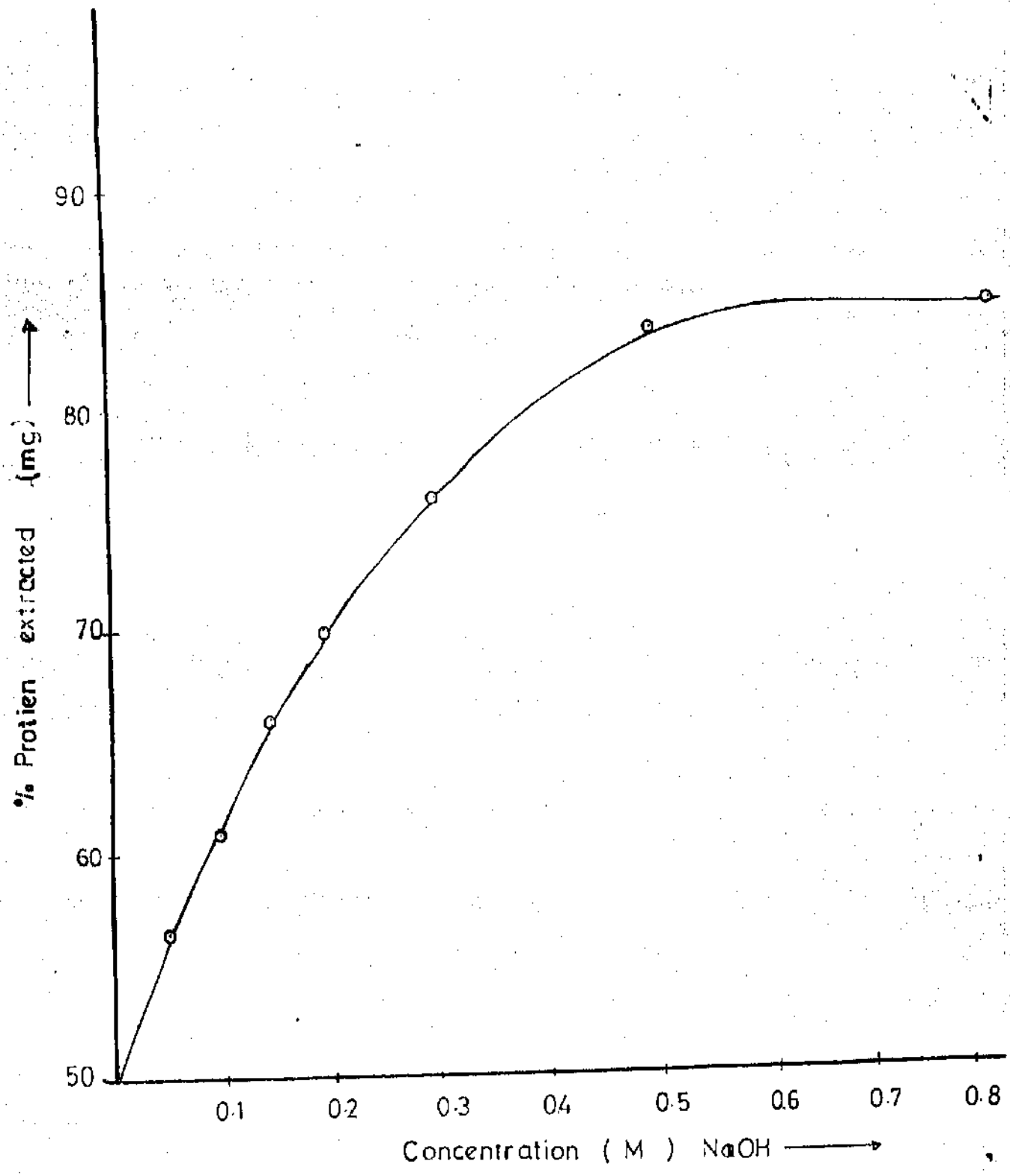
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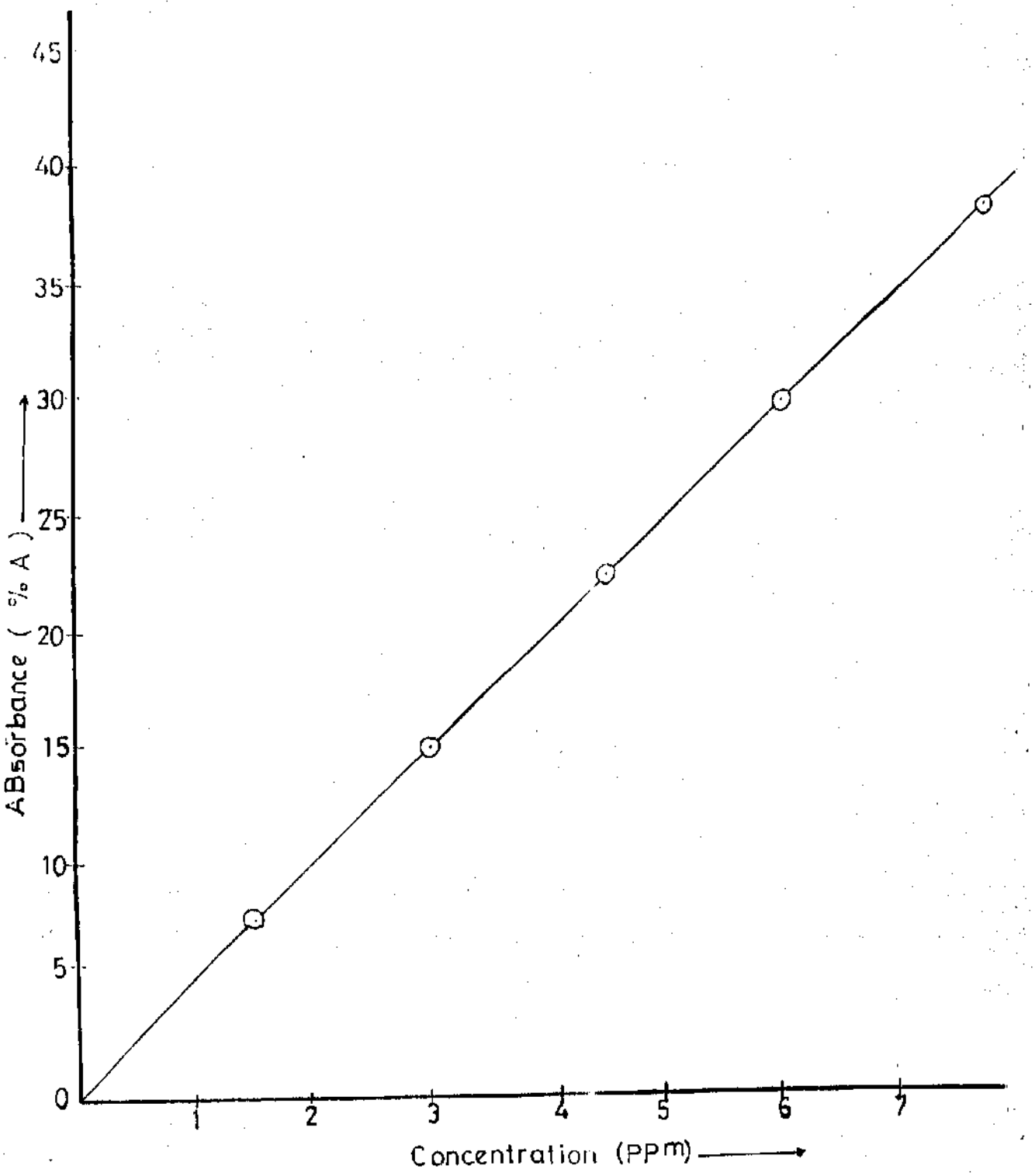
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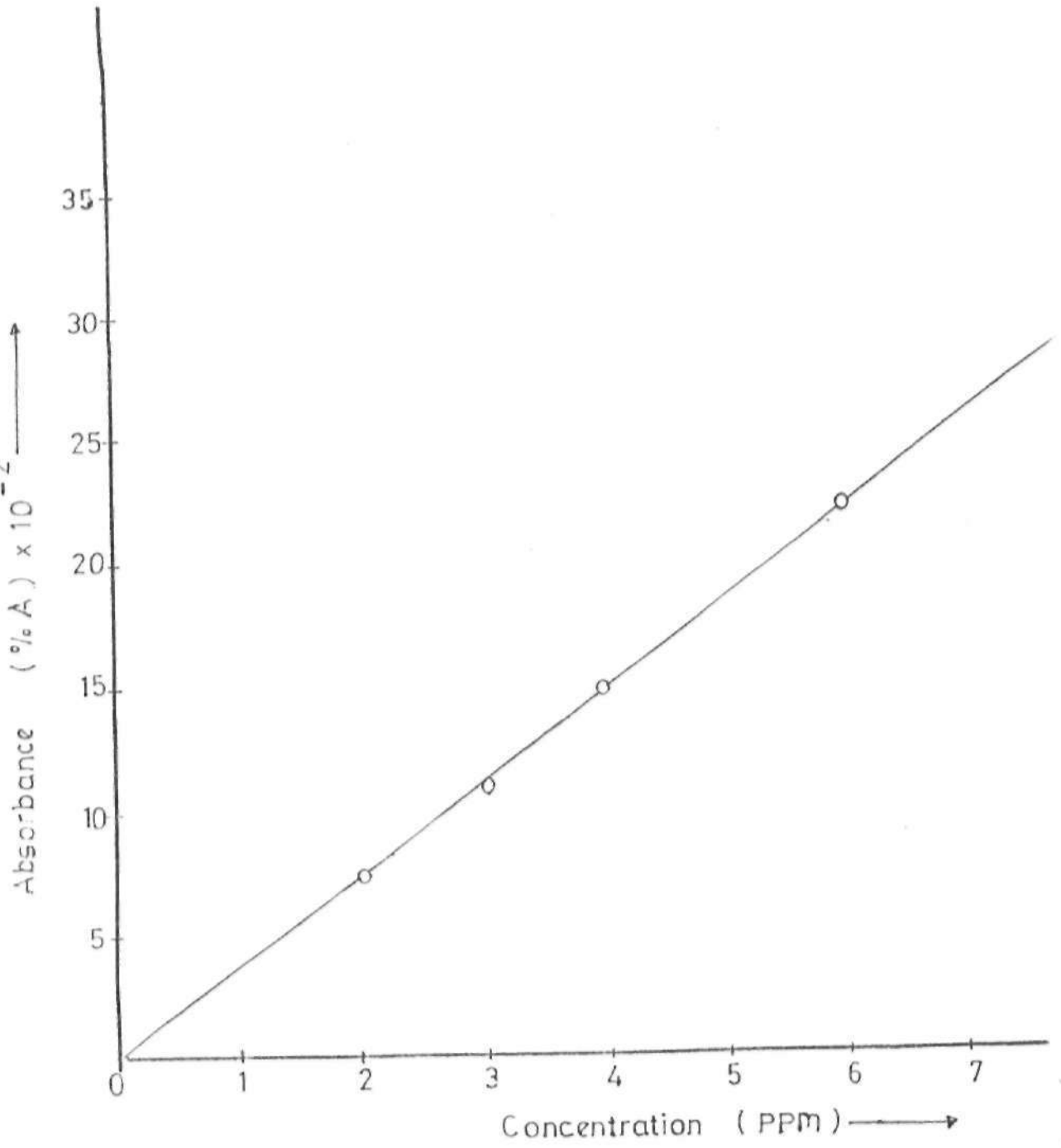

APPENDIX A True protein estimation by Lowry method.
Calibration Curve of BSA ( 1mg/cm3 ) in water.



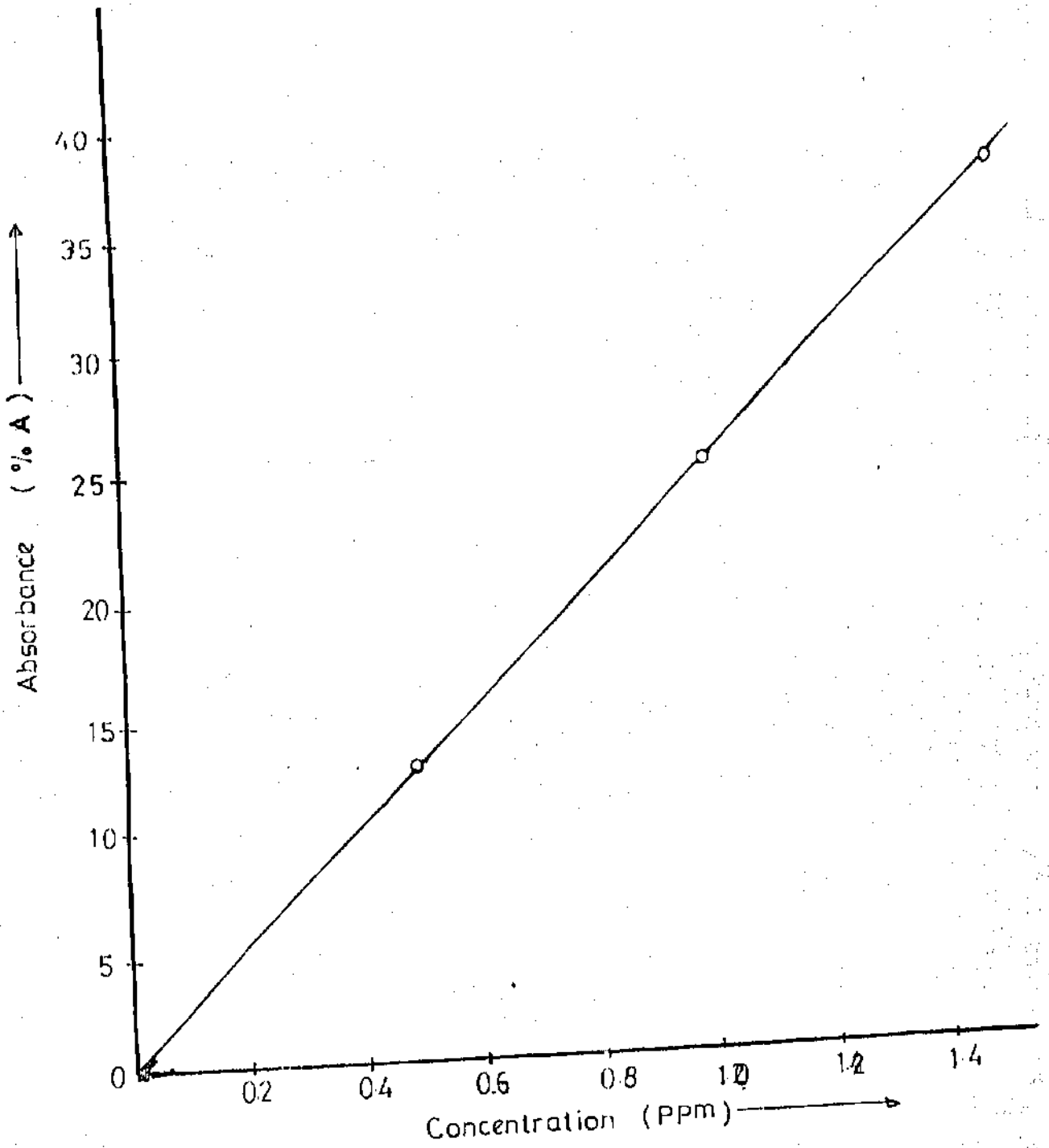

APPENDIX B Determination of the optimam concentration of the best extractant (NaOH) for the Protein extraction



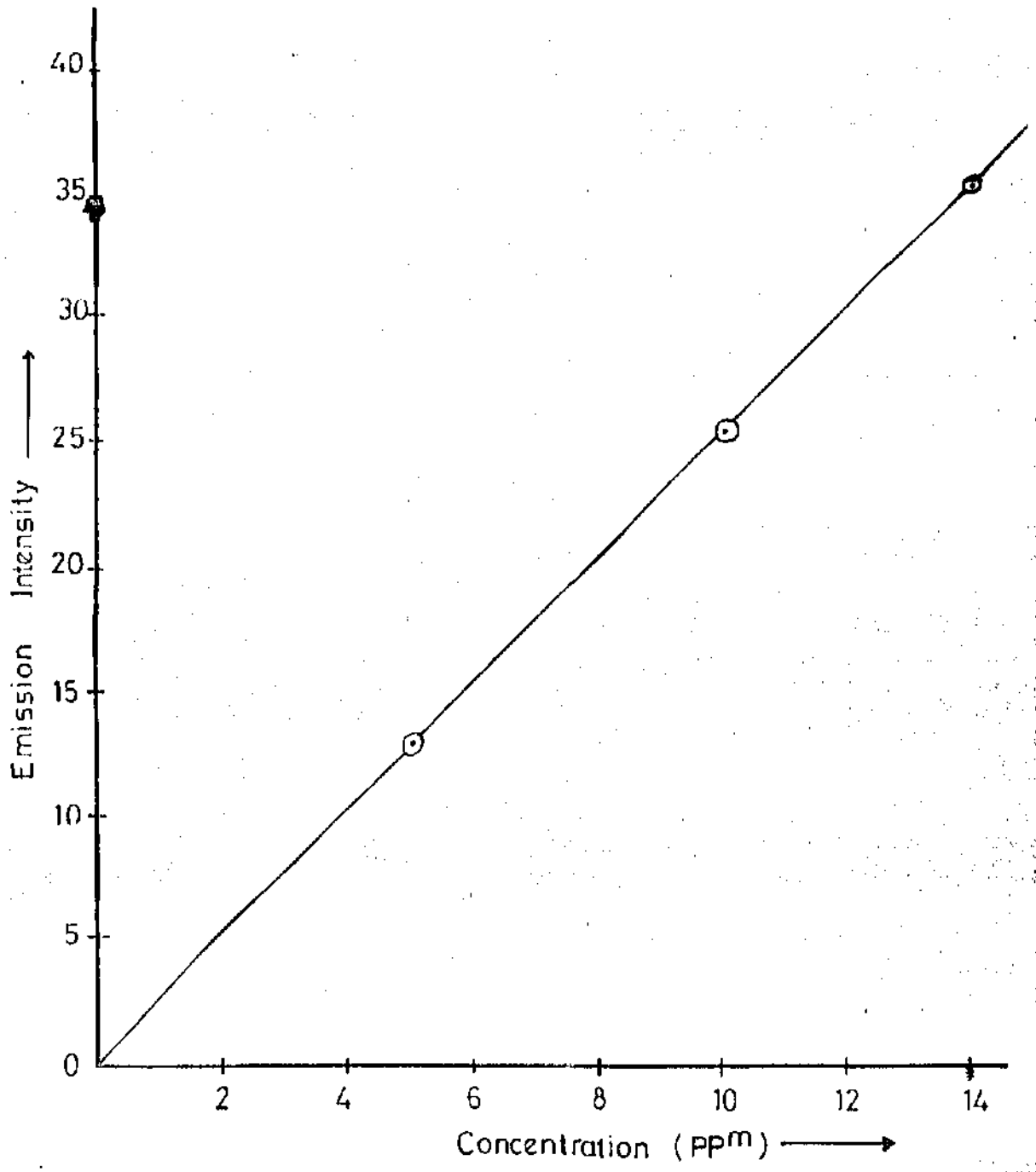

APPENDIX D Calibration curve for the determination of Calcium



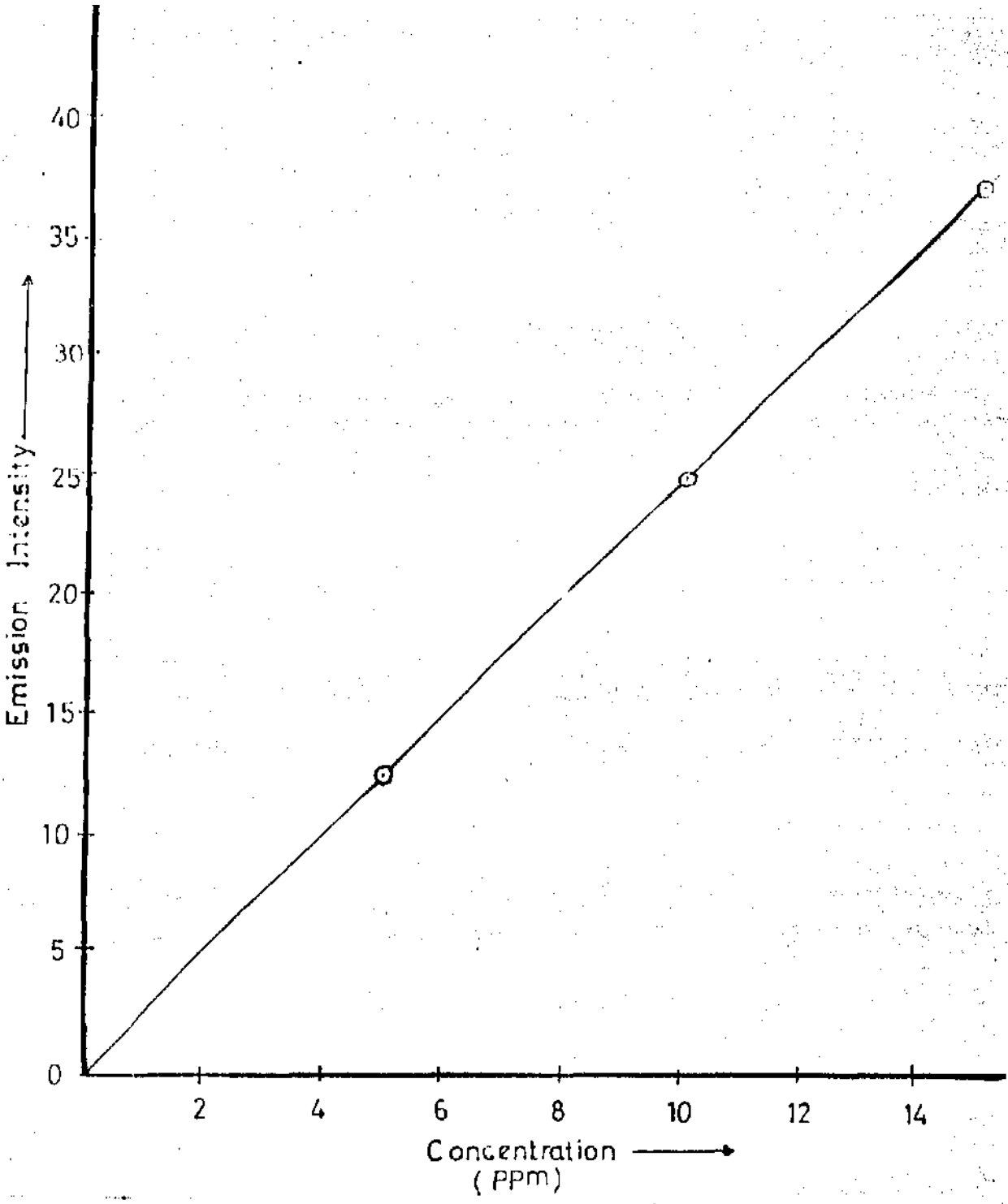

APPENDIX.E : Calibration curve for the determination of Phosphorous



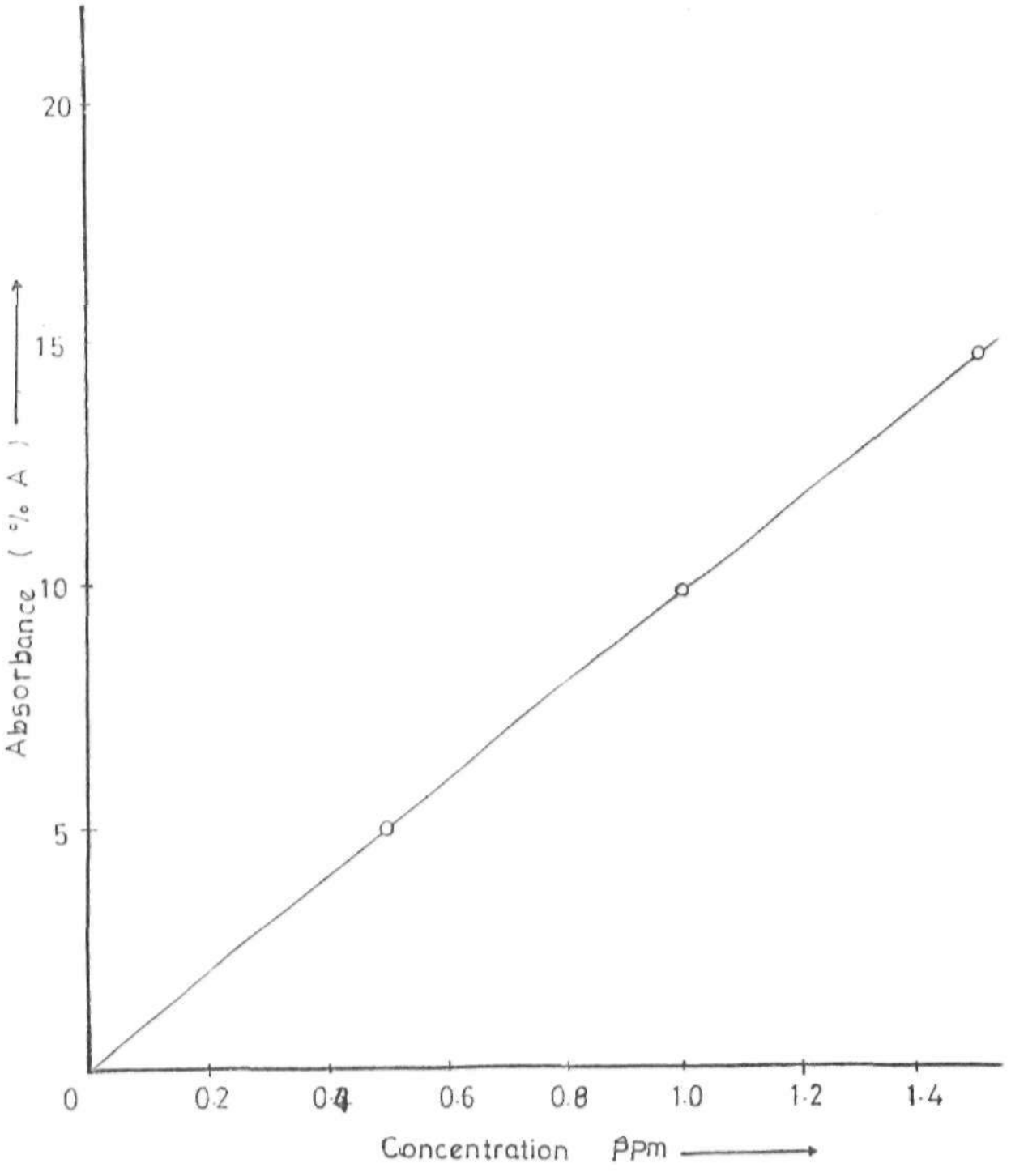

APPENDIX . F : Calibration curve for the determination of Magnesium



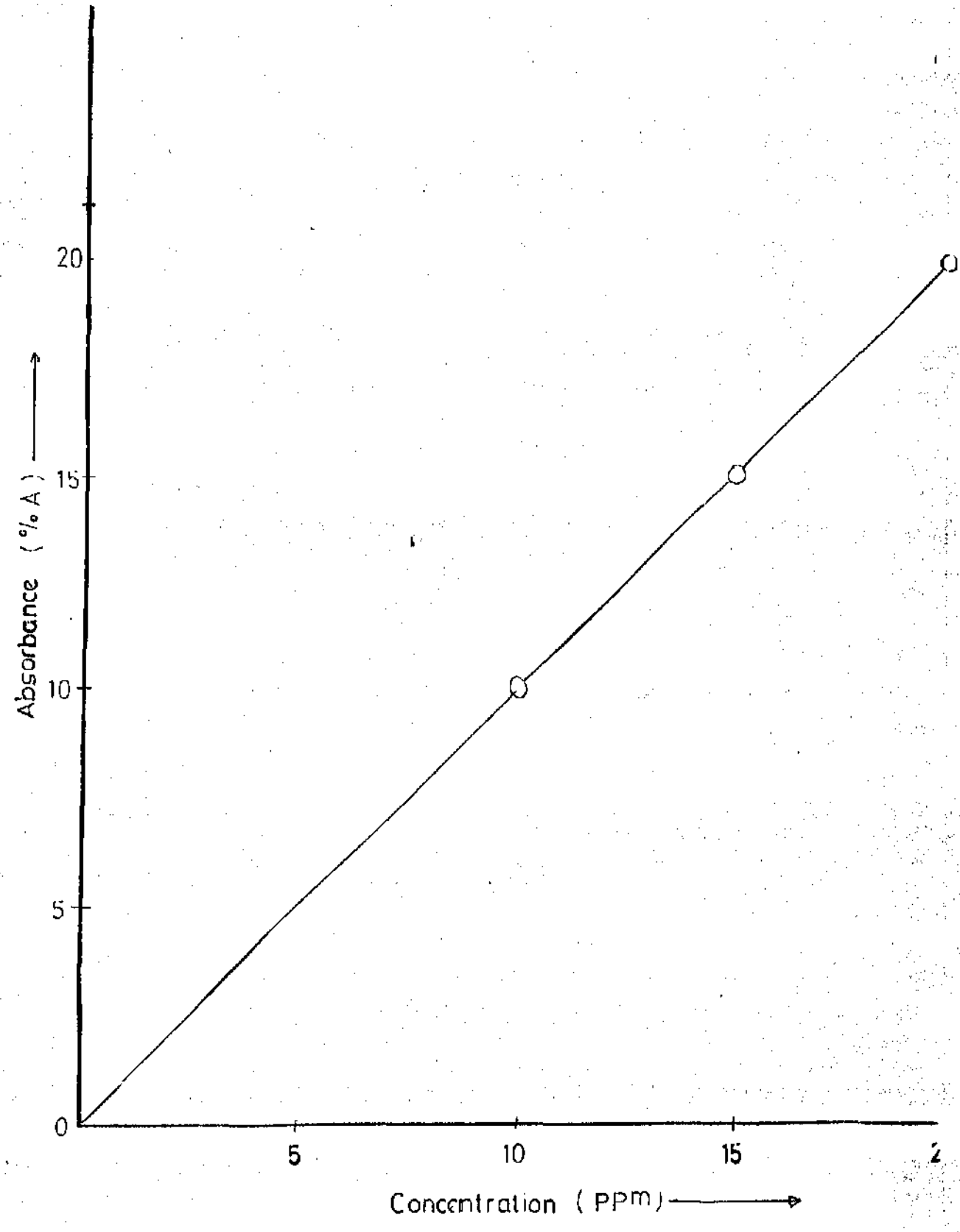

APPENDIX .G: Calibration curve for the determination of Potassium.



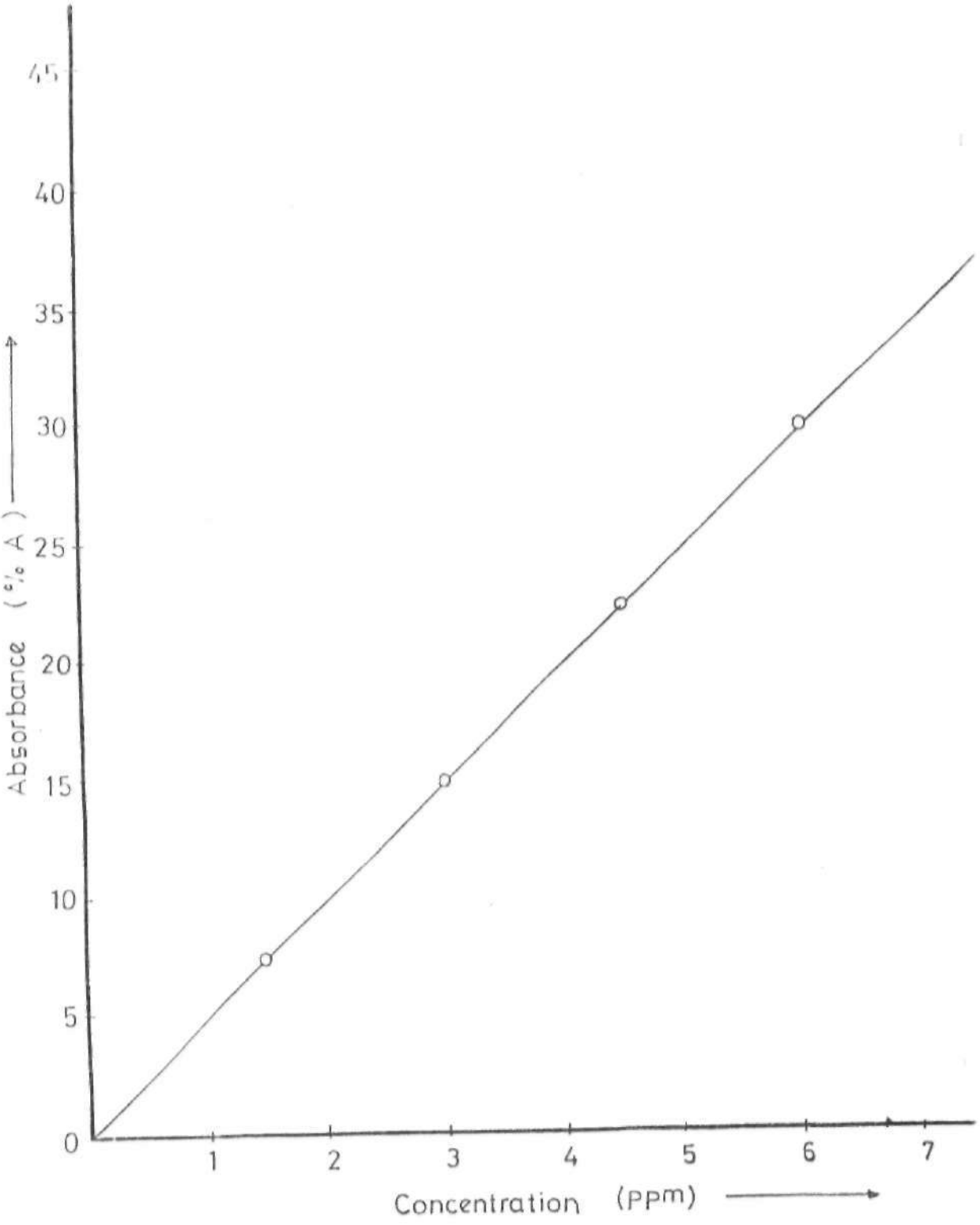

APPENDIX H: Calibration curve for the determination of Sodium.



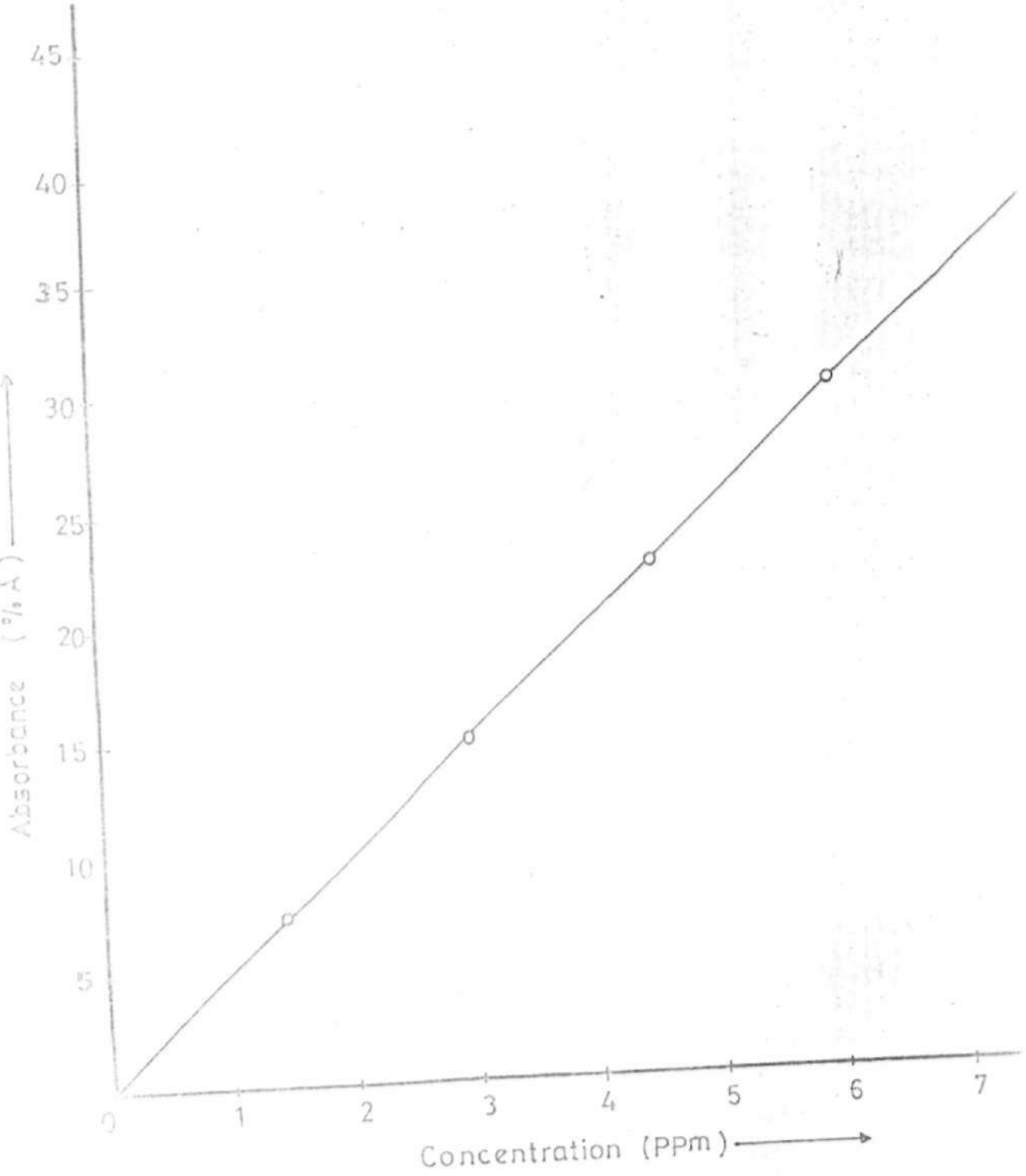

APPENDIX.I: Calibration curve for the determination of Zinc




APPENDIX J: Calibration curve for the determination of Iron




APPENDIX K : Calibration curve for the determination of Manganese




APPENDIX L: Calibration curve for the determination of Copper

APPENDIX M

Nutrient Requirement of Chicken

| NUTRIENT | 0 - 8 WEEKS CHICKS |
|---|---|
| Total Protein | 20% |
| Calcium | 1000mg/100g food |
| Phosphorous | 700 , , , |
| Sodium | 150 , , , |
| Potassium | 200 , , , |
| Iron | 4.8-8.0 , , |
| Copper | 0.40 , , |
| Zinc | 3.5-5.0 , , |
| Magnessium | 40-50 , , |

Ref: National Academy of Science/ National Res. Council - National requirements of Poultry 5th Ed. (19 66).