UNIVERSITY OF NAIROBI
CHIROMO LIBRARY

DETERMINATION OF IODINE IN ASSORTED SAMPLES USING ENERGY-DISPERSIVE X-RAY FLUORESCENCE ANALYSIS (EDXRFA).

THIS THESIS HAS BEEN ACCEPTED FOR
THE DEGREE OF MSc (1992
AND A COPY MAY BE PLACED IN THE
UNIVERSITY LIBRARY

Karugu Mwaura.
B.Sc (Hons).

UNIVERSITY OF NAIROBI
CHIROMO LIBRARY

A thesis submitted in partial fulfillment of the degree of Master of Science in the University of Nairobi.

July 1992.

This thesis is my original work and has not been presented for a degree in any other University.

Signature :

Name : Mwaura Karugu.

This thesis has been submitted for examination with our approval as University Supervisors.

Signature :

Dr. D. G. S. Narayana

Dept. of Physics

University of Nairobi.

Signature :

Mr. A. M. Kinyua

C. N. S. T.

Faculty of Engineering

University of Nairobi.

ACKNOWLEDGEMENTS.

I wish to express my gratitude to Dr. D.G.S. Narayana and Mr. A.M. Kinyua for supervising this research. My thanks also go to the staff of the Department of Physics, and to the staff of the Center for Nuclear Science Techniques (CNST), University of Nairobi, for their continued cooperation during the carrying out of this research. Thanks also to Professer Kariuki of Chemistry Department for his constructive critizism. Thanks also to the German Academic Exchange (DAAD) and the University of Nairobi for their funding, and the Government of Kenya for allowing me to conduct the research.

My gratitude also goes to the staff and pupils of Kammorou, Nasokol, Nangrotum and Makutano primary schools for their help in sample collection. Special thanks to Mr. Marango Wanyonyi of Nasokol for showing me how to make kids co-operative, and Thomas Mukwana for his unfailing support.

Lastly, I am grateful to my parents, brothers and sisters, the family of Mr. J. K. Kaguthi, Lucy, Nancy, Rebecca, Njambi and the three Wanjirus, for being with me all the way. And I also can't forget my colleague Mr. J. M Munyithya who has been my partner me on this drive, and P. W. Wambugu and J. A. W. Maina for their moral support.

A B S T R A C T.

A method for the analysis of iodine in biological and other samples was developed. After digestion of the sample with chromic acid, iodine was precipitated from the sample as palladium iodide by the addition of a solution of palladium chloride. The precipitate was analysed using X-ray Fluorescence Analysis (XRFA), with ^{241}Am (114 mCi) as the excitation source. The technique was found to be sensitive to concentrations as low as 10 part per billion (ppb) or 1.0 microgram per decilitre (µg/dl). The samples analysed were; urine (102), water (5), cooking and table salt (13).

The data for the urine samples indicate a median urine iodine concentration in the range 2.5 - 3.0 µg/dl, much lower than the safe value of 5.0 µg/dl. 66% of the sample population had iodine content lower than 5.0 µg/dl.

Water samples had iodine content lower than 0.4 ug/dl. For a goitre-free area, the water iodine content should be at least 1.5 µg/dl.

Salt samples were found to have iodine content much lower than that labelled by the manufacturers.

C O N T E N T S . PAGE

List of tables. i

List of figures. ii

Introduction. iv

CHAPTER ONE : LITERATURE REVIEW ON IODINE

1.1 : Iodine deficiency disorders. 1

1.2 : Iodine requirements of the human body. 3

1.3 : Iodine in foods. 4

1.4 : Iodine metabolism. 5

1.5 : Iodine supplementation. 6

1.6 : The IDD situation in Kenya. 7

1.7 : Iodine supplementation in Kenya. 8

1.8 : Effect of iodine supplementation in Kenya. 9

CHAPTER TWO : THEORY OF X-RAY FLUORESCENCE SPECTROMETRY

Introduction 11

2.1 : Atomic structure. 12

2.2 : Interaction of X-rays with matter. 14

2.3 : X-ray absorption phenomena. 16

2.4 : Absorption edges. 17

2.5 : Production of characteristic X-rays. 21

2.6 : Spectral line notation. 26

2.7 : Excitation of characteristic X-rays. 27

2.8 : The basic equation for quantitative XRFA. 28

CHAPTER THREE : ENERGY-DISPERSIVE X-RAY FLUORESCENCE SPECTROMETRY.

Instrumentation. 35

3.1 : Excitation source. 35

3.2 : Specimen presentation apparatus. 36

3.3 : The Energy-Dispersive X-Ray fluorescence (EDXRF) spectrometer. 37

3.3.1 : The detector. 38

3.3.2 : The amplifier. 40

3.3.3 : The Multichannel Analyser (MCA). 40

3.3.4 : The computer. 43

CHAPTER FOUR : EXPERIMENTAL PROCEDURES.

4.1 : Method development. 44

4.1.1 : Reagents. 45

4.1.2 : pH of optimal recovery. 46

4.1.3 : Time of optimal precipitation. 47

4.1.4 : Digestion procedure. 48

4.1.5 : Reduction of iodine and iodate. 50

4.1.6 : Summary. 52

4.2 : Detection limit. 53

4.3 : Sampling. 54

4.4 : Summary of experimental procedures. 57

4.5 : Errors associated with the method. 61

CHAPTER FIVE : RESULTS.

5.1 : Urine samples. 63

5.1.1 : Kammorou primary school. 64

5.1.2 : Nasokol primary school. 67

5.1.3 : Nangrotum primary school. 69
5.1.4 : Makutano primary school. 71
5.1.5 : Discussion. 73
5.2 : Water samples. 78
5.3 : Salt samples. 78

CHAPTER SIX : CONCLUSIONS.
6.1 : The method. 81
6.2 : Urine samples. 81
6.3 : Water samples. 82
6.4 : Salt samples. 83

REFERENCES. 84

LIST OF TABLES.

Table 4.1 : The recovery of iodine from standard solutions having concentrations varying from 0 to 20 parts per million. 53

Table 5.1 : Iodine concentration in samples from Kammorou primary school. 64

Table 5.2 : Iodine concentration in samples from Nasokol primary school. 67

Table 5.3 : Iodine concentration in samples from Nangrotum primary school. 69

Table 5.4 : Iodine concentration in samples from Makutano primary school. 71

Table 5.5 : Iodine concentration in water samples. 78

Table 5.6 : Iodine concentration in salt samples. 79

LIST OF FIGURES.

Figure 2.1 : Block diagram of the processes involved when X-rays interact with matter. 14

Figure 2.2 : Typical X-ray absorption curve. 18

Figure 2.3 : X-ray absorption curves for copper, zinc, tin and lead. 19

Figure 2.4 : Binding energies and dorminant X-rays used in X-ray fluorescence analysis. 22

Figure 2.5 : Possible electron transitions which follow the creation of a K-shell vacancy. 23

Figure 2.6 : The relationship between the fluorescence yield and atomic number. 25

Figure 2.7 : Geometry of excitation source, sample and detector in an XRF experiment. 29

Figure 3.1 : A cross-section of a radioisotope excitation source having an annular geometry. 35

Figure 3.2 : Arrangement of sample, excitation source and detector. 36

Figure 3.3 : Block diagram of an Energy-Dispersive X-Ray Fluorescence spectrometer. 37

Figure 3.4 : Schematic diagram of the sensitive volume of the Si(Li) detector crystal. 38

Figure 3.5 : A typical spectrum observed on the MCA. 42

Figure 4.1 : Intensity versus pH for a 10 ppm standard filtered after five hours. 47

Figure 4.2 : Intensity versus time before filtration of a mixture. 48

Figure 4.3 : Intensity of iodine versus volume of saturated sodium sulphite solution added to a 10 ppm standard solution. 51

Figure 4.4 : Intensity of iodine versus the volume of saturated sodium sulphite solution added to urine samples. 51

Figure 4.5 : Map of Kenya showing the sampling region. 55

Figure 4.6 : Log count - Channel number plot of a sample spectrum. 58

Figure 4.7 : Spectrum of figure 4.6 after fitting using QXAS. 59

Figure 5.1 : A spectrum of a urine sample from Kammorou. 65

Figure 5.2 : Distribution of urine iodine in samples from Kammorou school. 66

Figure 5.3 : Distribution of urine iodine in samples from Nasokol school. 68

Figure 5.4 : Distribution of urine iodine in samples from Nangrotum school. 70

Figure 5.5 : Distribution of urine iodine in samples from Makutano school. 72

Figure 5.6 : A comparison between the iodine distributions in the four schools. 74

Figure 5.7 : Distribution of urine iodine in the pooled samples. 75

Figure 5.8 : Comparison between the iodine distribution in males and females. 77

INTRODUCTION.

Trace elements are important in biological systems. Elements such as calcium, iron and iodine are just some of the many elements that are necessary for the proper functioning of the body. Calcium is used in bone synthesis, iron is a normal constituent of blood while iodine is crucial to the function of the thyroid gland. The thyroid gland controls a wide range of metabolic processes in the body (Underwood, 1962).

Most of the trace elements enter the body through diet and drinking water. Diet and drinking water are in turn influenced by geochemical and soil conditions (Mills, 1990). Thus an area will have a specific balance of trace elements. In the developing countries, a lot of the food grown in a certain region is consumed within the same area. As such, if the region is not environmentally favoured as regards some trace elements, the deficiency disorders associated with such elements can rise to endemic proportions (Golden and Golden, 1991). For corrective measures such as supplementaion to be undertaken in an area, it is therefore necessary to determine the trace element intake of the people in that area.

Since the trace elements are usually in low concentrations, trace element analysis requires techniques which are sensitive to such small concentrations. One of the methods used in trace element analysis is Energy-Dispersive X-Ray Fluorescence Spectrometry (EDXRFS). This method

provides a versatile tool for rapid multielemental analysis of many types of samples. The method has been used in such diverse fields as enviromental monitoring, medicine, archaelogy, geology and criminology (Goulding and Jaklevic, 1973).

This work deals with the quantitative analysis of the element iodine in samples collected in an area known to have endemic goitre. Goitre is said to be endemic when it's prevalence exceeds ten per cent of the population in an area. Various surveys carried out in the late nineteen sixties and early seventies determined that iodine deficiency disorders were a potential problem for the people living in the highland areas of Kenya. The principle strategy for preventing these disorders is the fortification of salt with potassium iodate (Alnwick, 1988). Although a salt fortification programme was started in the early seventies, goitre is still prevalent in some parts of Kenya, such as parts of West Pokot District (Wouters, 1991). This work involves the development of a rapid method for the determination of iodine using EDXRFS, and the application of the method in the analysis of urine, water and salt samples obtained from the Kapenguria Division of West Pokot District. The urine and water data would help in determining the prevalence of goitre, while salt data would determine the efficacy of the supplementation programme.

Some aspects of iodine nutrition, and the theory and principles of X-Ray Fluorescence Spectrometry have been discussed.

CHAPTER ONE.

LITERATURE REVIEW ON IODINE.

1:1. IODINE DEFICIENCY DISORDERS

Iodine is an essential constituent of body tissues. Its use in the biosynthesis of triiodothyronine and thyroxine hormones have been known for a long time (Underwood, 1977). These hormones are manufactured by the thyroid gland. It is estimated that a normal thyroid gland contains about 400 μg of iodine per gram of its weight (Ahlgren et al., 1982). The triiodothyronine and thyroxine hormones are involved in a wide range of metabolic processes in the body.

For efficient operation of the thyroid gland, and hence of the metabolic processes, the body requires a certain amount of iodine daily. When the dietary intake of iodine fails to reach the level required by the body, physiological feedback mechanisms result in the enlargement of the thyroid in an attempt to enhance its iodine-trapping activity (Obel,1982). This enlargement of the thyroid is called goitre, and is observed in people not consuming enough iodine. When the goitre prevalence in an area exceeds ten per cent of the population, the population is said to suffer from endemic goitre (Ingbar and Woeber, 1977).

Goitre is just one among the many disorders associated with iodine deficiency. In severe iodine deficiency cases, other disorders are observed. These include endemic cretinism characterised by mental deficiency, spastic diplegia and lesser degrees of neurological defects related to fetal iodine

deficiency and impaired mental function in children and adults associated with reduced levels of circulating thyroxine (Hetzel, 1990). Inadequate iodine during pregnancy is associated with an increased risk of abortion, stillbirths, perinatal and infant mortality. Children receiving inadequate iodine during their early development may suffer from learning disabilities, impaired psychomotor performance and high school dropout rates even if they do not exhibit the classic signs of cretinism (Stanbury, 1986). In 1983, Hetzel (1983) introduced the concept of Iodine Deficiency Disorders (IDD) to encompass this spectrum of disorders.

Iodine deficiency principally occurs due to two factors;

(i) the environment may be deficient in iodine,

(ii) there may exist certain substances in natural food which inhibit proper uptake of iodine by the thyroid.

The mountains and flooded river valleys of Africa and Asia are some of the areas deficient in iodine. It is estimated that at present, more than one billion people resident in these areas are at risk of suffering from IDD's (Hetzel, 1990; HIE, 1990).

The substances which inhibit proper uptake of iodine by the thyroid are called goitrogens. Some of the goitrogenic substances so far identified are thiouracil (Chensey et al., 1928), 1-5-vinyl-2-thiooxazolidone (Astwood,1949) and thiocyanate (Ermans et al., 1983). Fluorine has also been suspected of having goitrogenic activity due to its being more active than iodine, and hence blocking the iodine uptake (Obel, 1982). However, it has been observed that these

goitrogens are usually effective when the iodine intake is marginal, and that goitre produced by these agents can be reversed by iodine supplementation, except for the thiouracil type (Underwood, 1962).

1.2 : IODINE REQUIREMENTS OF THE HUMAN BODY.

To avoid damage to the thyroid gland, the physiological requirement of iodine must at least equal the daily amount of hormonal iodine degraded in the peripheral tissues and unrecovered by the thyroid i.e. 40-100 ug/day (Delange, 1985).

In 1974 the Food and Nutrition Board of the National Academy of Sciences, National Research Council of the United States (Mitchell, 1974) recommended the following daily iodine intakes;

35 µg for children aged 0-6 months

45 µg " " " 6-12 months

60-110 µg " " " 1-10 years

100-115 µg for anybody over 11 years old

125 µg and 150 µg for pregnant and lactating mothers respectively.

Although there is some disagreement about these values most authorities agree on at least 100 µg/day for adults. The World Health Organisation (WHO) recommends that an adult man requires 150 - 300 µg/day (WHO, 1973).

1.3 : IODINE IN FOODS

The main source of iodine for human consumption is food. Drinking water constitutes less than 10% of the daily iodine intake (Delange, 1985)

The highest concentration of iodine is found in seafoods (about 800 ug of iodine per kilogram). Other main dietary sources are eggs, meat, milk and cereals. Food additives like the iodate in dough conditioner and iodised cooking salt also increase the amount of iodine in food.

Some data has been collected on iodine content of foods (CIEB, 1952; Underwood, 1962). Most of this data deals with the iodine content of raw foods, which is not equal to the iodine content of the food as on the plate.

A good determination of the iodine content of foods would involve analysis of ready-to-eat food samples. Such a study carried by Vought at al. (1964) in the United States showed a higher than average consumption of iodine. A similar study by Koutras et al. (1974) in Greece showed that the iodine content of food produced in goitrous areas is on the whole lower than that obtained in non-goitrous areas. They concluded that even in areas without endemic goitre, the iodine content of natural food is barely adequate. As such, iodine supplementation should be universal.

1.4 : IODINE METABOLISM

In vegetables, the iodine appears as inorganic iodide. In foods of animal origin, the iodide partially exists in inorganic form and partly in organic combination. Human beings therefore ingest iodine as organic and inorganic iodide. Absorbed iodine disappears from the blood at an exponential rate which is the sum of the individual rates of removal by the thyroid, the kidneys and other tissues (Underwood, 1962). The main pathway of excretion of iodine is the kidneys. Iodine is excreted as the iodide. The quantity of iodine excreted in the urine thus generally reflects the amount which is not taken up by the thyroid gland. Indirect estimation of the thyroid function can therefore be made by determining the urinary excretion of iodine. This method was used by Hetzel (1990) to categorize IDD into three levels of severity ;

(i) Mild IDD with goitre prevalence in the range 5-20% (school children) with median urine iodine levels in the range 3.0 - 5.0 µg/dl.

(ii) Moderate IDD with goitre prevalence upto 30% and some hypothyroidism. Median urine iodine levels in the range 2.0 - 3.5 µg/dl.

(iii) Severe IDD indicated by high prevalence of goitre (30% or more), endemic cretinism (1 - 10%), with median urine iodine less than 2.0 µg/dl.

1.5 : IODINE SUPPLEMENTATION.

Ever since it was established that goitre (and hence IDD) can be prevented by iodine supplementation (Abott, 1932), various methods have been tried for increasing human iodine intake. Some of the early methods involved applying iodine rich fertilizer to farm and grazing land in order to increase iodine content of farm and animal produce, and addition of iodine to municipal water supplies (Underwood,1962). The three methods currently in use, with their merits and demerits are;

(i) The administration of iodine as candy to school children - this has the advantage of providing known doses of iodine. However, its success depends on continous co-operation between a number of individuals and the authorities. It is rarely successful.

(ii) The use of iodized salt - this is the most commonly used method. It's main drawback is that it does not take into consideration individual food habits; some people do not take any salt, or if they do, they take it in insufficient quantities.

Hetzel (1990) recommends salt iodisation levels of 10 - 25 mg per Kg for mild IDD areas, and 25 - 40 mg of iodine per Kg of salt for moderate Iodine Deficiency Disorders (IDD) areas.

(iii) Iodized oil, either given orally or by injection.

This method is used in areas with severe goitre and endemic cretinism. Iodized oil is recommended for the definite correction of iodine deficiency, particularly in women of child- bearing age whenever the efficacy of other corrective measures such as salt iodization is uncertain (Butterfield et al., 1965).

1.6 : THE IDD SITUATION IN KENYA

Ever since a goitre survey was carried out by the WHO team under Munoz (Bohdal et al., 1968), goitre has been accepted as one of the diseases we have to contend with in Kenya. Over 28,000 children in the age group 6-15 years were examined from 108 schools spread over 14 districts. Goitre rates varying from 15-72% were found, the highest being recorded from the highlands of the Rift Valley, Central, Nyanza and Western Provinces.

Various other surveys (Hanegraaf et al., 1988; CRSP, 1984; Jansen et al., 1987; Gitau, 1988) have gone further in confirming Munoz's findings that goitre is prevalent in Kenya, particularly in the highlands regions. So far, there have been no reported cases of endemic cretinism anywhere in Kenya.

This study centers on the Kapenguria Division of West Pokot District, Rift Valley province. Geographically, the area is hilly and lies in a rift-like trough, gradually rising eastwards to the Cherangani Hills and westwards towards Mount

Elgon. Goitre is prevalent, particularly in the older population, but a case has been reported of a five year old child from the Kishaunet area of the division who had operable goitre (Wouters, 1991). Most of the cases of operable goitre observed at the Kapenguria District Hospital are from the Mount Elgon region of the neighbouring Trans - Nzoia district. Goitre is considered operable if its size interferes with normal breathing mechanisms.

1.7: IODINE SUPPLEMENTATION IN KENYA

Iodisation of salt started voluntarily in Kenya around 1970. The salt was fortified with 33.7 mg of potassium iodate per kg salt. This was aimed at providing 20 parts per million (ppm) iodine, a value which had been recommended by Bohdal et al.(1969). Following a study on the impact of the consumption of iodised salt on goitre prevalence in 1974, a recommendation was made to double the prescribed concentration of iodine (Sehmi, 1974).

In 1978, legislation was passed (Laws of Kenya, Cap.254) stipulating that all household salt should contain 33.7 mg of potassium iodate per kg of salt, or if it didn't, it should be clearly labelled as not providing a necessary nutrient.

A study by Mannar(1987) estimated that over 60% of the salt consumed in Kenya was iodised . Another study by

Alnwick (1988) on the iodine content of salts sold in rural areas showed that some contained close to the specified amount, while some had less than half the specified amount.

The 1978 legislation was revoked on October 10, 1988 (Legal Notice No. 189). After this date, no manufacturer was to be allowed to sell uniodised salt. At present, Kenyan salts are fortified with 168.5 mg potassium iodate per kilogram of salt, equivalant to 100mg I/kg or 100 parts per million (ppm).

1.8 : EFFECT OF IODINE SUPPLEMENTATION IN KENYA.

Studies on the effect of the salt iodisation programme on goitre have shown results which were not very encouraging (Stanbury et al., 1974; Hanegraaf et al., 1974 and 1977; Jansen et al., 1987). The disappointing results were mainly attributed to the loophole in the 1978 legislation, the long storage times of the salt and the packaging and storage conditions.

Gitau (1988) showed that goitre is still common in some regions, including Nairobi. Although his study was carried out prior to the 1988 legislation, his results raises two questions; Is the public sufficiently informed on the advantages of eating iodised salt and is the amount of salt eaten enough to supplement the iodine intake? The second question raises the issue of whether the salt contains

sufficient iodine to meet the required levels of supplementation by the time it reaches the consumer.

Since the potassium iodate in cooking salt is known to degrade with time and storage conditions, the best way of answering the second question is to check the iodine content of salt at the consumer level. In this study, this will be done by determining the iodine content of the salts available at Kapenguria. A further check will be conducted on some salts sold in different parts of Kenya. Some salts of foreign origin will also be analysed for comparison purposes.

CHAPTER TWO.

THEORY OF X-RAY FLUORESCENCE SPECTROMETRY.

INTRODUCTION

X-ray fluorescence spectrometry is based on the emissions from electron transitions which take place between various atomic sub-levels when a vacancy is created in one of the inner levels. When an electron falls from a higher energy level to fill a vacancy in a lower level, the difference in energy between the two levels can be emitted as a spectral line. The emitted spectral line has a particular frequency. The Moseley relation (Moseley, 1912) relates the frequency of the spectral line, ν, with atomic number Z as;

$$\nu = k\,(Z-\sigma)^2 \qquad \text{......... 2.1}$$

where k and σ are constants which vary with the spectral series.

The frequency of the spectral line is related to its energy by;

$$E = h\nu \qquad \text{......... 2.2}$$

where h is Planck's constant.

From equations 2.1 and 2.2, the spectral line is

characteristic of the energy difference between the energy levels involved in the transition and hence is a distinctive property of the element producing it.

The emitted X-rays may be analysed using energy dispersive semiconductor spectrometers. This is called Energy-Dispersive X-ray Fluorescence Analysis (EDXRFA).

In EDXRFA, the characteristic X-rays are converted into peaks in a digitized spectrum of counts per channel versus energy. Each channel in the spectrum represents a certain energy, thus the peaks appearing in the spectrum correspond to certain energies. There are charts relating the peak energy to the element producing the peak. Using these tables, the elements in the spectrum can be identified according to the peaks present. The intensity of each spectral line is determined by calculating the area under the peak. The concentration of each element is calculated from these intensities.

2.1: ATOMIC STRUCTURE.

To understand the nature and origin of characteristic X-ray line spectra, a brief review of the atomic structure is discussed as follows.

Every atom consists of a dense central nucleus

containing Z protons and (A-Z) neutrons, with Z electrons orbiting around this nucleus. Z is the atomic number, and A is the mass number.

The electrons are grouped in shells designated K, L, M, etc in order of increasing distance from the nucleus. The electrons in each shell are classified further with respect to angular momentum and direction of spin. Each of these parameters: shell, momentum and spin is designated by a quantum number which may take only certain values. No two electrons in an atom may have the same quantum number (Pauli's exclusion principle).

The quantum numbers are:

(i) Principal quantum number **n**. It indicates the shell. **n** can take values 1,2,3, . . .,n. Common shell designations are K for n=1, L for n=2, M for n=3, etc.

(ii) Azimuthal quantum number ℓ. It represents orbital angular momentum and determines the shape of the orbital. ℓ can take values 0,1,...,(n-1).

(iii) Spin quantum number **s**. This number indicates the direction of spin. **s** can only take two values, +1/2 or -1/2.

(iv) Inner precession quantum number j. j is the vector sum of ℓ and **s**. It can take $\ell \pm s$ values, excluding the case where j = 0 - 1/2.

On the basis of n,l and j quantum numbers, atomic electrons fall into orbitals or energy levels; one for K, three for L, five for M, etc. The number of sublevels in each shell corresponds to the allowed values of j in that shell.

The significance of these sublevels to the characteristic X-ray line emission process will be discussed later.

2.2 : INTERACTION OF X-RAYS WITH MATTER.

If a beam of X-rays interacts with matter, the X-ray beam is scattered and attenuated. Several distinct types of interactions occur as shown in figure 2.1.

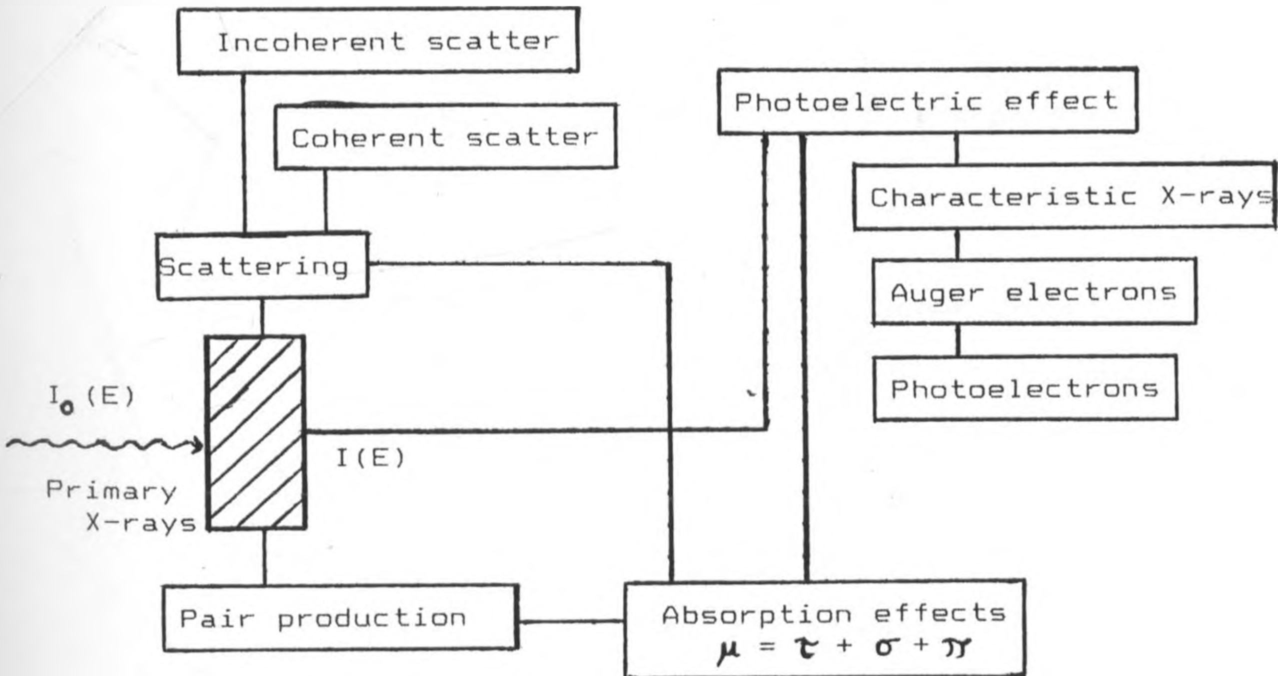


Fig.2.1 : Block diagram of the processes involved when X-rays interact with matter.

(i) Scattering : There are two types of scattering;

(a) Coherent or Rayleigh scattering in which the scattered photon has the same energy as the incident photon.

(b) Incoherent or Compton scattering in which the energy of the scattered photon is altered.

(ii) Pair production : In this case the energy of the incident photon is used to create electron-hole pairs. This process requires high energies of the order of 1.02 MeV.

(iii) Photoelectric effect : If the energy of the incident X-ray photon is greater than the energy binding the electon in its shell, the electron may absorb this energy and be ejected from its shell. The ejected electron is called a photoelectron, the process is called photoelectric effect.

These three processes result in absorption of the X-ray intensity.

2.3 : X-RAY ABSORPTION PHENOMENA.

As discussed above, when a beam of X-rays passes through a material, it is attenuated. The attenuation is described by the Beer - Lambert law (Marshall, 1980), which says that the intensity I of a beam of X-rays which has passed through a material of thickness x and linear absorption coefficient μ, is given by :

$$I = Io \exp(-\mu x) \qquad \text{.........2.3}$$

where Io is the intensity of the incident beam.

The linear absorption coefficient $\mu = \tau + \pi + \sigma$ where τ, π, and σ represent losses by photoelectric, scattering and pair production processes respectively.

A more useful form of this equation is

$$I = Io \exp\{-(\mu/\rho)\rho x\} \qquad \text{.........2.4}$$

where (μ/ρ) is the mass absorption coefficient (cm^2/g) of the absorber, ρ is the density of the absorber and ρx is the area density (g/cm^2).

The mass absorption coefficient (μ/ρ) is an atomic property of chemical elements and is a measure of their X-ray opacity or "stopping power".

For a specified element Z, (μ/ρ) is different at every energy disregarding absorption edges (section 2. 4); (μ/ρ) decreases as the energy increases, i.e the X-rays become more energetic and hence more penetrating.

At a specified energy, (μ/ρ) is different for every element and, disregarding absorption edges, increases as Z

increases, i.e. X-ray penetration decreases as atomic number Z increases.

The linear and mass absorption coefficients are measures of that portion of the incident X-ray beam which does not appear in the emergent beam.

2.4 : ABSORPTION EDGES.

The absorption edge is the minimum energy that can expel an electron from a specified orbital in a given atom of a specified element. Each element has as many absorption edges as it has subgroups; one K, three L, five M, etc. Figure 2.2 shows a typical absorption curve.

The relationship between absorption and photon energy for a specified element is best expressed in the form of a log - log plot of mass absorption coefficient versus X-ray photon energy. Absorption curves for copper, zinc, tin and lead are shown in figure 2.3.

From figure 2.3, it is evident that absorption cannot be estimated simply on the basis of atomic number and photon energy. There are departures from the Bragg - Pierce law (Bertin, 1978) predictions that

$$\frac{\mu}{\rho} \propto Z^{4} \lambda^{3} \qquad \text{.........2.5}$$

or in terms of energy,

$$\frac{\mu}{\rho} \propto \frac{Z^{4}}{E^{3}} \qquad \text{..........2.6}$$

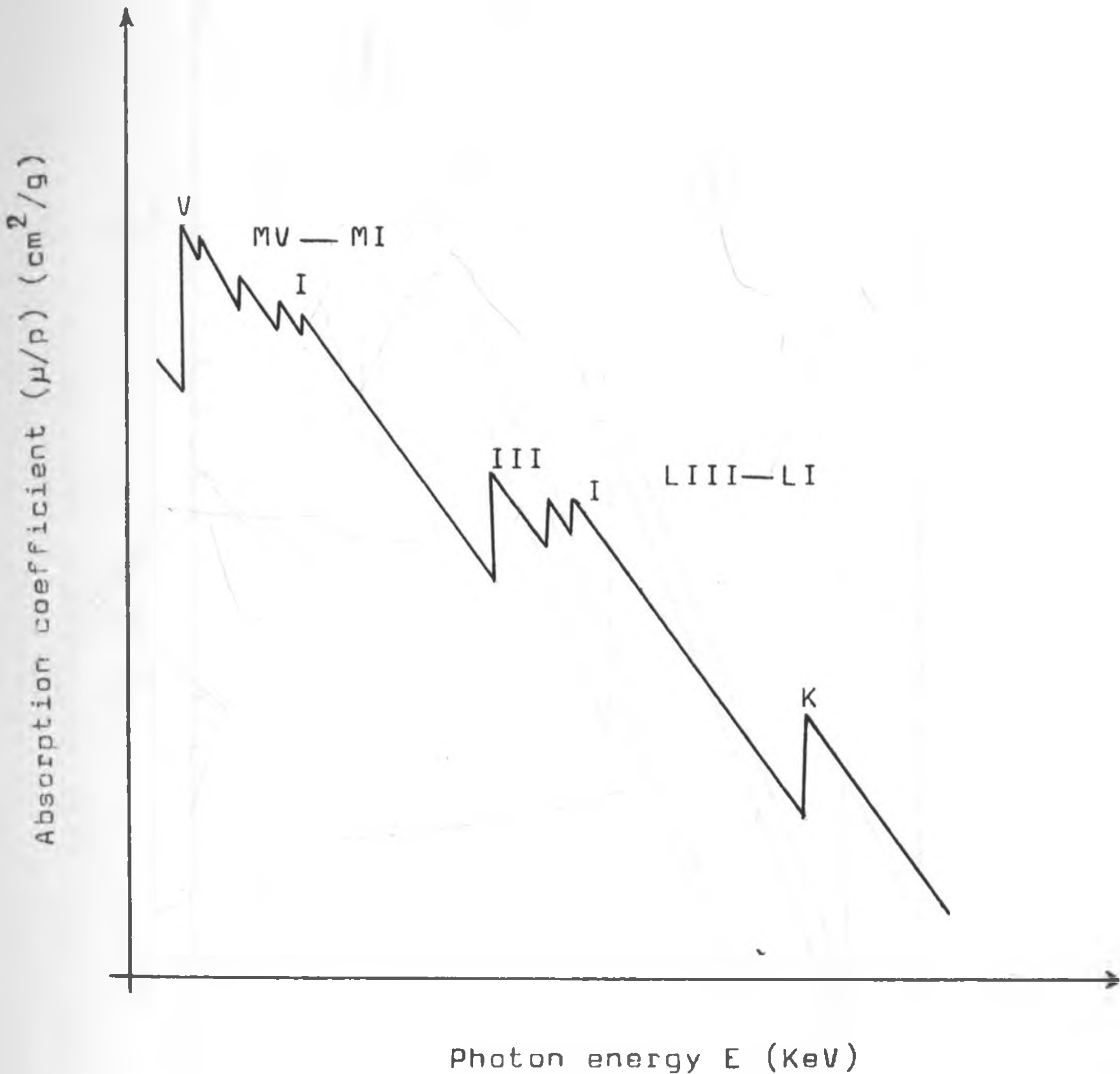


Fig. 2.2 : A log-log plot of a typical X-ray curve showing X-ray "stopping power" as a function of X-ray photon energy. It illustrates the fact that as photon energy increases, X - ray penetration increases and the stopping power of the absorber decreases except at the absorption edges.
(Note : The figure shows the general features of the absorption edges and is not for any particular element, hence the absence of a scale).

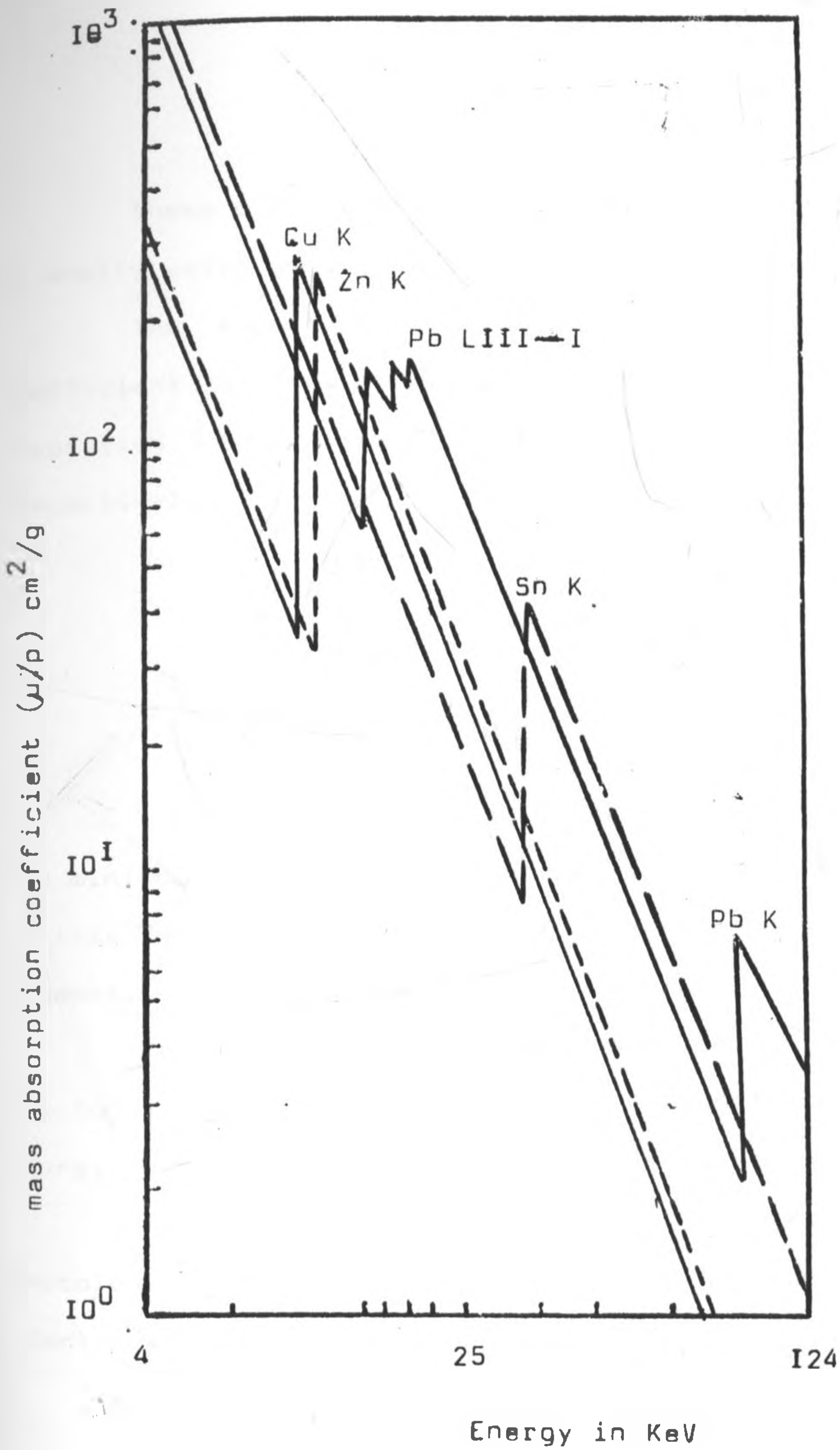


Fig. 2.3 : Log-log plot of the X-ray absorption curves for copper, zinc, tin and lead (atomic numbers 29, 30, 50 and 80 respectively. (adapted from Bertin (1978)).

These predictions are essentially realized and would be wholly valid were it not for the absorption edges.

The magnitude of the change in mass absorption coefficient at the absorption edge is expressed as the absorption edge jump ratio J and jump difference d respectively ;

$$J = \frac{(\mu/\rho)_h}{(\mu/\rho)_l} \qquad \text{.........2.7}$$

$$d = (\mu/\rho)_h - (\mu/\rho)_l \qquad \text{.........2.8}$$

where h and l refer, respectively, to the maximum and minimum values of (μ/ρ) at the edge. A pair of equations of this form can be written for each absorption edge of each element.

The values of J and d are measures of that portion of the total absorbed X-radiation that is absorbed by the atomic energy level associated with a specific edge.

The actual fraction of the total number of photoionizations that occur in a specified shell (Bertin,1978) is

$$\frac{(\mu/\rho)_h - (\mu/\rho)_l}{(\mu/\rho)_h} = 1 - \frac{1}{J} = \frac{J - 1}{J} \qquad \text{...2.9}$$

Between the absorption edges, log - log plots of (μ/ρ) versus E are linear and mutually parallel as predicted by the Bragg - Pierce law.

2.5 : PRODUCTION OF CHARACTERISTIC X-RAYS.

The ejection of inner shell electrons by photoelectric interaction of an incident photon requires that the photon energy exceed the binding energy of the appropriate shell. Figure 2.4 shows the binding energies and dominant X-rays used in X-ray fluorescence analysis.

X-ray fluorescence analysers generally measure the effects of K-shell vacancies in the light elements ($Z < 55$) and L-shell vacancies for the heavy elements (Goulding and Jaklevic, 1973).

To understand the processes involved in the production of characteristic X-rays, consider the case where an electron is ejected from an atom via the photoelectric effect process. Ejection of electrons follows the order of increasing energy of the energy levels, i.e., K, L, M, and so on. For example, consider the case where an electron is ejected from the K-shell of an atom. A succession of spontaneous electron transitions follow, each filling a vacancy in a lower level, with the resultant emission of an X-ray photon. Figure 2.5 shows some of the transitions which follow the creation of a K-shell vacancy. The arrows indicate electron transitions giving rise to spectral lines of intensity of order 1 or more. Each transition is labelled with the symbol of the corresponding absorption edge or spectral line. A discussion on spectral line notation and absorption edges will be given later.

Since the energy involved in each electron transition

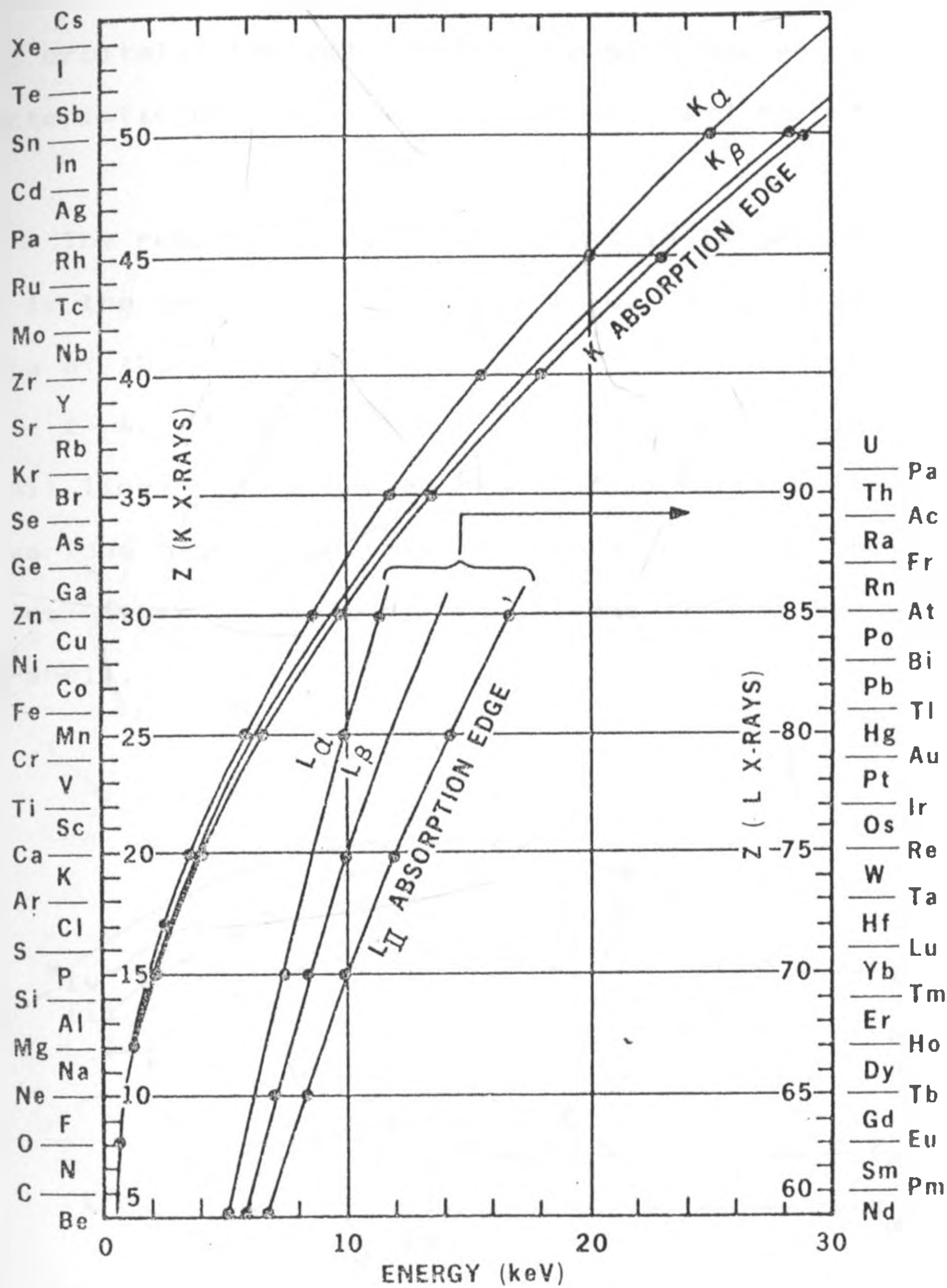


Fig. 2.4 : Binding energies and dominant X-rays used in X-ray fluorescence analysis (Goulding and Jaklevic, 1973)

corresponds precisely to the difference in energy between two atomic orbitals, the emitted X-ray photon has energy characteristic of this energy difference and therefore of the atom.

The result of such transitions in large numbers of atoms is the generation of the K, L, M, etc, series of the spectra of that element.

X-ray spectral lines are grouped in series K, L, M, etc. All lines in a series result from electron transitions from various higher orbitals to the indicated shell, e.g. K-series is as a result of transitions from higher shells to the K-shell.

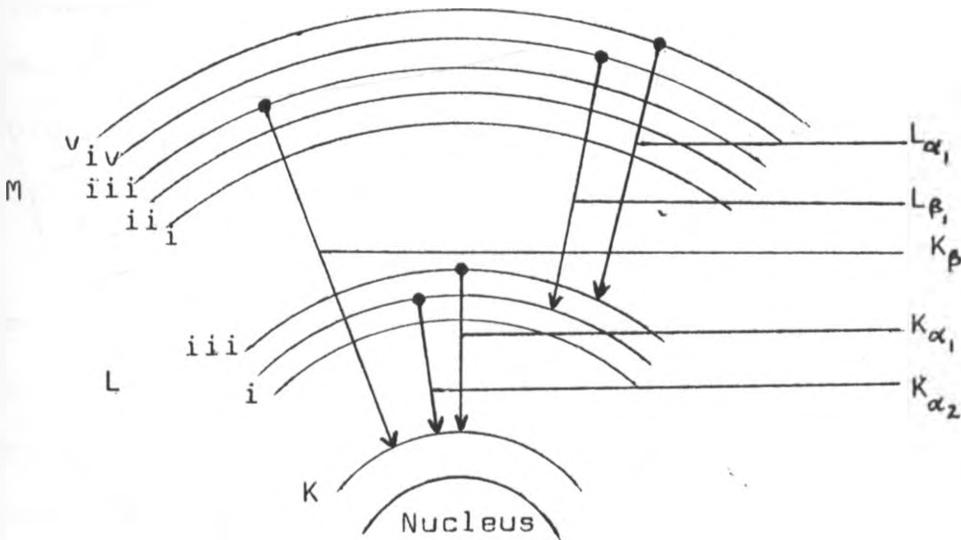


Fig. 2.5 : Possible electron transitions which follow the creation of a K-shell vacancy.

Electron transitions cannot occur from just any higher orbital to any lower orbital. Only the transitions which obey the selection rules are allowed. Selection rules are the rules which govern the changes that the quantum numbers can undertake between the orbitals involved in the transitions (Bertin, 1978).

The selection rules are;

$\Delta l = -1$ or $+1$

$\Delta j = 0, -1$ or $+1$

where n is the principal quantum number, l is the azimuthal quantum number and j is the inner precision quantum number.

Spectral lines produced as a result of transitions which obey the selection rules are called diagram lines. These are the lines of interest in X-ray fluorescence spectrometry. A discussion on the other types of lines which can also be produced during the electron transition process, i.e. the forbidden lines, satellite or non-diagram lines and the spectral band can be found in Bertin (1978).

Not all the characteristic X-rays produced escape the atom. In elements of low atomic number, an internal interaction may occur. The X-ray photon produced during the electron transition may be used to knock off an electron from a higher orbital. The electron emitted in this process is called an Auger electron.

The Auger process reduces the number of characteristic spectral lines emitted. The fraction of electron interactions which result in the emission of characteristic X-rays is known as the fluorescent yield ω. ω lies between 0 and 1. The fluorescent yield strongly depends on the atomic number.

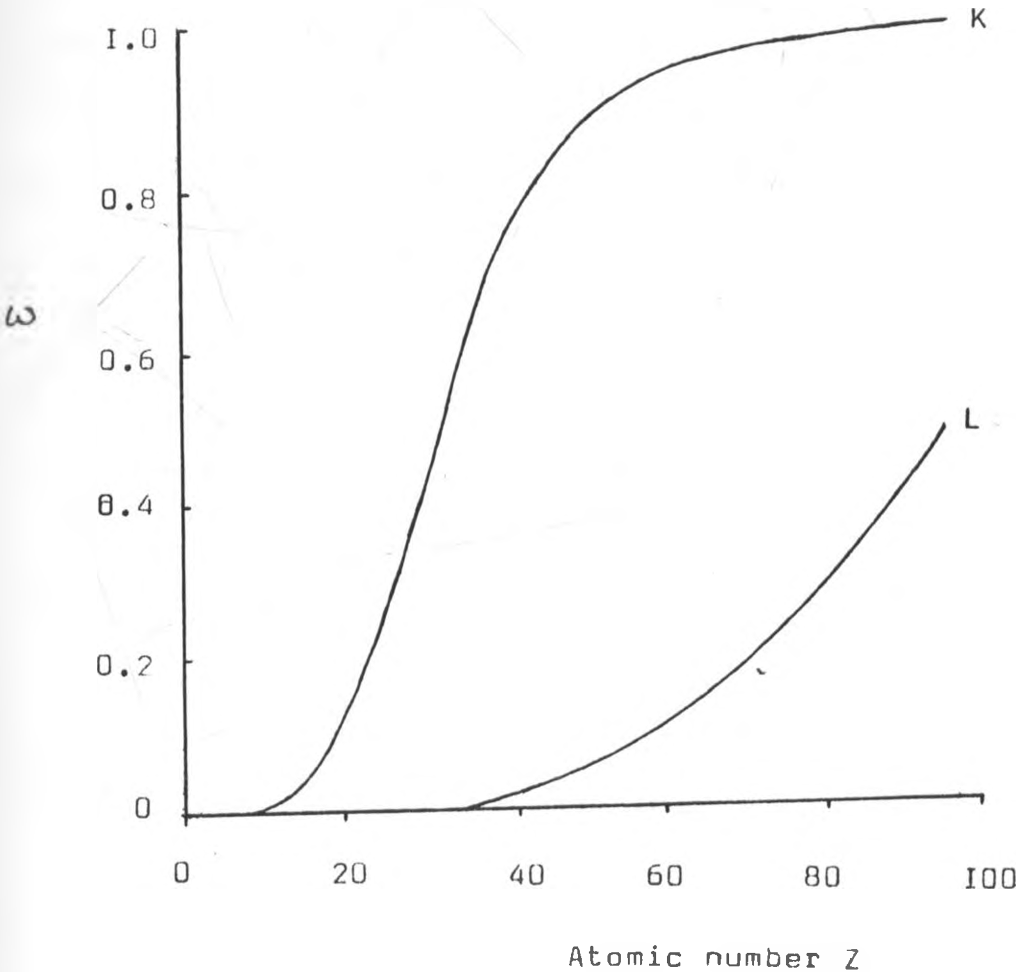


Fig. 2.6 : Relationship between fluorescent yield and the atomic number (Marshall, 1980).

Since scattering is more acute for the light elements (Andersen, 1966), it implies that a large fraction of the incident X-rays are scattered by the elements in the lower region of the periodic table. For those photons which are actually absorbed, a large fraction results in the emission of Auger electrons (Marshall, 1980), while the rest cause the emission of characteristic X-rays. These processes result in low fluorescence yields for the low Z elements, with ω rising gradually as Z increases, since scattering and Auger electron production decrease with increasing atomic number. This variation of ω with Z can be observed in figure 2.6.

2.8 : SPECTRAL LINE NOTATION.

In the Siegbahn notation system (Bertin, 1978) the symbol of an X-ray spectral line, for example Zr K_{α} consists of:

(i) the symbol of the chemical element.

(ii) the symbol of the series, K, L, M etc. The line originates from an electron transition to the indicated shell.

(iii) a lowercase Greek letter, usually with a numerical subscript;

> the α is the strongest line in a series. α lines arise from $\Delta n = 1$ transitions. The β and γ lines usually arise from $\Delta n = 1$ or 2 transitions.

Other systems of notation such as the Level designation system and Jenkins notation system are discussed by Bertin (1978).

2.7 : EXCITATION OF CHARACTERISTIC X-RAYS.

X-ray line spectra arise when vacancies occur in the inner electron orbitals of atoms. X-ray excitation consists of the creation of these vacancies in large numbers. This can be achieved by;

(i) bombardment with electrons.

(ii) bombardment with protons, deuterons, α-particles and heavier ions from particle accelerators.

(iii) irradiation with α-, β-, γ- or X-rays from radioisotopes.

(iv) irradiation with X-rays from high- and low- power X-ray tubes.

(v) indirect excitation by X-rays from secondary emitters, themselves excited by X-ray tubes or radioisotopes.

The excitation modes commonly used at the Centre for Nuclear Science Techniques, University of Nairobi, are indirect excitation by X-rays excited by an X-ray tube, and irradiation with radioisotopes.

Some of the radioisotopes used are Fe-55 which produces Mn K_α X-rays, Cd-109 which produces Ag K_α X-rays, and Am-241 which undergoes α- decay, producing Np L X-rays with energy in the range 14 - 21 KeV; and γ- rays of energy 59.6 KeV.

2.8 : THE BASIC EQUATION FOR QUANTITATIVE X-RAY FLUORESCENCE ANALYSIS (XRFA).

Quantitative analysis depends on correlation of elemental concentrations with observed fluorescence x-ray intensities. This correlation is represented by basic equations derived using fundamental parameters. Derivation of these equations by Fundamental Parameters Technique (FPT) is based on the following assumptions (Kinyua, 1982; Sparks, 1975):

(i) a monochromatic primary radiation source is used to excite characteristic X-rays from the sample.

(ii) the sample is homogeneous, i.e., the density is well defined and constant throughout the sample.

(iii) a fixed geometry of sample, excitation source and detector orientation is maintained.

To calculate the intensity of fluorescence radiation from an element i within the sample excited by primary radiation of energy E_1, a geometry of source, sample and detector as shown in figure 2.7 is assumed.

The probability of exciting and detecting K X-rays of energy Ei from element i in a layer of thickness dx at adepth x in the sample is represented by the product of three probabilities, P_1, P_2 and P_3 where

$$P_1 = I_0 \Omega_1 \exp - [\mu(E_1) \rho x \operatorname{cosec} \phi_1] \quad ....2.10$$

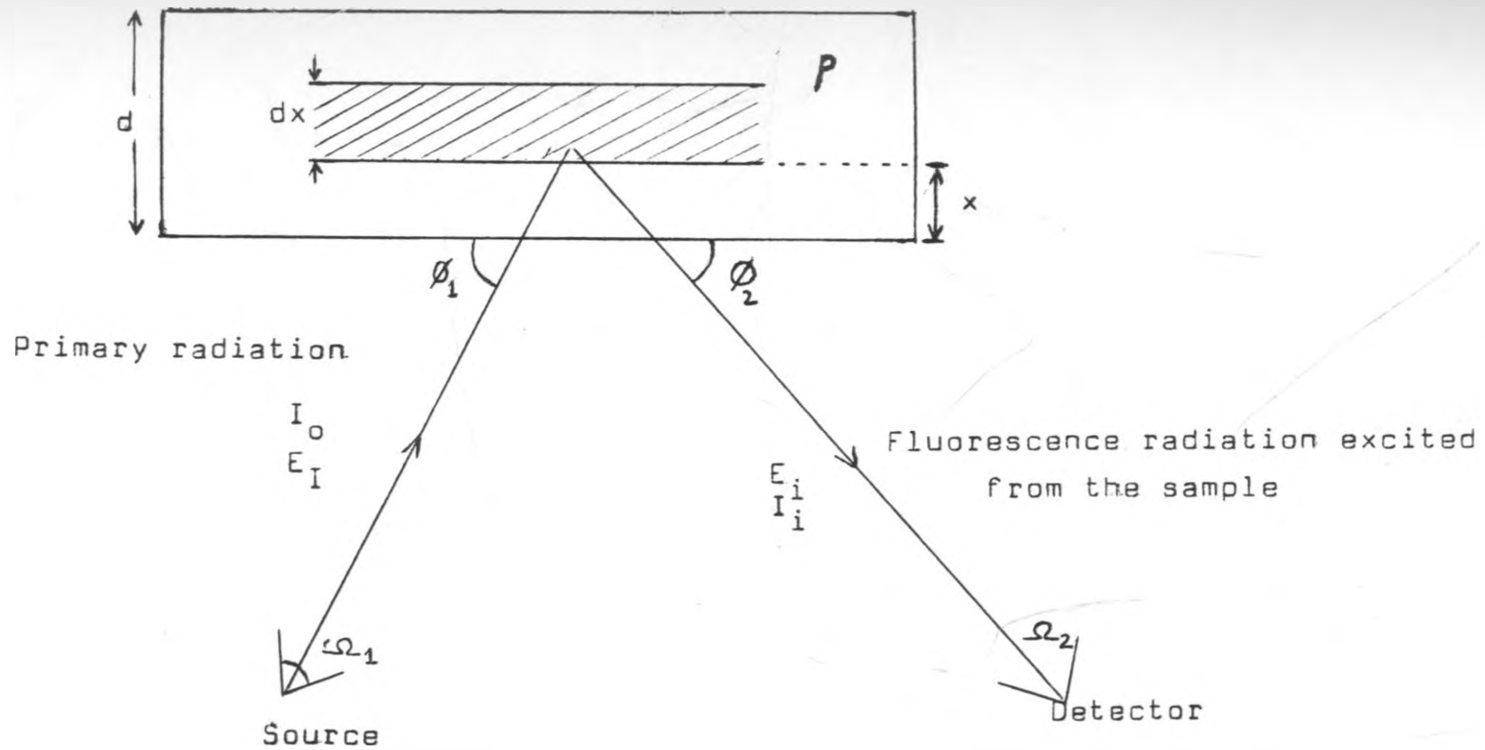


Figure 2.7 : Geometry of excitation source, sample and detector in an XRF experiment (Kinyua, 1982).

$$P_2 = \sigma_i^{ph}(E_1)\, \rho_i \, dx \, \mathrm{cosec}\, \phi_1 \left(1 - \frac{1}{J_k^i} \right) w_k^i \, f_\alpha^i \quad \ldots 2.11$$

$$P_3 = \Omega_2 \exp - [\mu(E_1)\, \rho\, x \, \mathrm{cosec}\, \phi_2] \, . \, E(E_i) \quad ..2.12$$

P_1 is the probability that primary radiation will reach a distance x, P_2 is the probability that element i will absorb primary radiation in the layer of thickness dx and emit a K_α X-ray of energy E_i, while P_3 is the probability that the K_α X-ray will penetrate out of the sample and be detected.

In equations 2.10, 2.11 and 2.12 the symbols are as defined below.

I_0 is the primary radiation in counts per second.

Ω_1, Ω_2 are the solid angles subtended at the sample by the source and detector respectively.

ϕ_1, ϕ_2 are the angles formed by directions of primary and fluorescent (characteristic) radiations with the sample surface.

ρ and ρ_i are the density of sample and partial density of element i within the sample respectively.

$\sigma_i^{ph}(E_1)$ is the photoelectric cross - section of element i for primary radiation.

$\mu(E_1)$ is the total mass absorption coefficient for radiation of energy E_1.

J_k^i is the K absorption jump.

$\left(1 - \frac{1}{J_k^i}\right)$ is the relative probability for the photoelectric process to occur in K-shell of element i.

w_k^i is the fluorescence yield for K_α X-rays of element i.

f_α^i is the relative transition probability for K_α X-rays of element i.

$E(E_i)$ is the relative efficiency of the detector for X-rays of energy E_i.

$\mu(E_1)$, $\sigma_i^{ph}(E_1)$, J_k^i, w_k^i and f_α^i can be obtained from tables (Goulding and Jaklevic, 1973). $E(E_i)$ must be evaluated for the detector used.

The fluorescence intensity dI_i, which is a contribution from atoms of element i at a depth x can be written as

$$dI_i = I_0 \Omega_1 \Omega_2 \operatorname{cosec} \phi_1 \, \sigma_i^{ph}(E_1) \left(1 - \frac{1}{J_k^i}\right) w_k^i f_\alpha^i E(E_i) \exp -(a \rho x) \, dx \qquad \text{...........2.13}$$

where a, the combined absorption coefficient for primary and fluorescence X-rays in the sample is defined as;

$$a = \mu(E_1) \operatorname{cosec} \phi_1 + \mu(E_i) \operatorname{cosec} \phi_2 \qquad \text{.....2.14}$$

Integrating expression 2.13 over the thickness d of

the sample, we obtain the contribution of the total sample for element i as;

$$I_i = I_0 \Omega_1 \Omega_2 \operatorname{cosec} \phi_1 \, \delta_i^{ph}(E_1)\left(1 - \frac{1}{J_k^i}\right) w_k^i f_\alpha^i E(E_i)(\rho_i d) \frac{1 - \exp-(a\rho d)}{a\rho d} \qquad ....2.15$$

Equation 3.16 can be further reduced to :

$$I_i = Go \cdot K_i \cdot (\rho_i d) \frac{1 - e^{-a\rho d}}{a\rho d} \qquad ......2.16$$

where $G_0 = I_0 \Omega_1 \Omega_2 \operatorname{cosec} \phi_1$ (counts per second) ..2.17

called the geometrical constant.

$$K_i = \delta_i^{ph}(E_1)\left(1 - \frac{1}{J_k^i}\right) w_k^i f_\alpha^i E(E_i) \; (cm^2/g) \qquad ..2.18$$

K_i is called the relative excitation detection efficiency.

Equation 2.16 is the basic equation in X-ray Fluorescence Analysis using the Fundamental Parameters Method.

The factor $\frac{1 - e^{-a\rho d}}{a\rho d}$ is called the absorption correction factor. It is used to correct for the absorption component of matrix effects.

Matrix effects consist of the influence of the

chemical combination (matrix) of the sample on the fluorescence radiation intensity of the elements in the sample. There are two types; enhancement and absorption (Mangala, 1987) .

(i) Enhancement effects consist of additional excitation of the elements in the specimen by the fluorescence radiation of heavier elements in the matrix.

(ii) Absorption effects consist of the absorption of primary X-rays by the sample, and absorption of fluorescence X-rays on their way out of the sample.

The absorption correction factor strongly depends on the value of the product a d. There are three special cases;

(i) $a\rho d \ll 1$. Equation 2.16 becomes

$$I_i = G_O \cdot K_i \cdot (\rho_i d) \qquad \ldots\ldots 2.19$$

This is the equation for thin samples.

(ii) $a\rho d \gg 1$. Equation 2.16 transforms to

$$I_i = Go \cdot K_i \; \frac{\rho_i}{\rho} \cdot \frac{1}{a} \qquad \ldots\ldots 2.20$$

This is the equation for thick samples. As can be seen from the equation, measured fluorescence intensity does not depend on sample thickness d.

(iii) $0.1 < a\rho d < 2$. This characterises transparent samples, and equation 2.16 then transforms to

$$I_i = G_o \cdot K_i \cdot \frac{\rho_i}{\rho} \cdot \frac{1 - e^{-a\rho d}}{a} \qquad ...2.21$$

Quantitative analysis by FPT is performed on thin and transparent samples only. For thin samples enhancement effects are negligible. Absorption effects are also neglected as can be seen from equation 2.19.

Relative excitation detection efficiency, K_i for different elements and for a particular excitation source are determined using tabulated values of the fundamental parameters (Storm and Israel, 1970).

Geometric constant G_o is determined using single element thin samples with known (ρ_i d) amount of element spread homogeneously on a substrate. From measured intensity I_i, G_o is calculated using equation 2.19.

G_o includes activity or emission rate of the radioactive source. Since radioactive decay is characterised by half-life $T_{1/2}$, the constant also decreases with time as

$$G_o(t) = G_o(t=0)\ 2^{-t/T_{1/2}} \qquad ....2.22$$

Corrections for G_o must be performed from time to time depending on the half-life of the radioisotope used.

CHAPTER THREE.

ENERGY-DISPERSIVE X-RAY FLUORESCENCE SPECTROMETRY.

INSTRUMENTATION.

A X-ray Fluorescence Spectrometer consists of three main parts;

(a) a source of primary X-rays for exciting the specimen.

(b) specimen presentation apparatus.

(c) the readout components.

3.1 : EXCITATION SOURCE.

The radioisotope excitation sources used were annular in geometry. Figure 3.1 shows a cross-section of an annular radioisotope source.

UNIVERSITY OF NAIROBI
CHIROMO LIBRARY

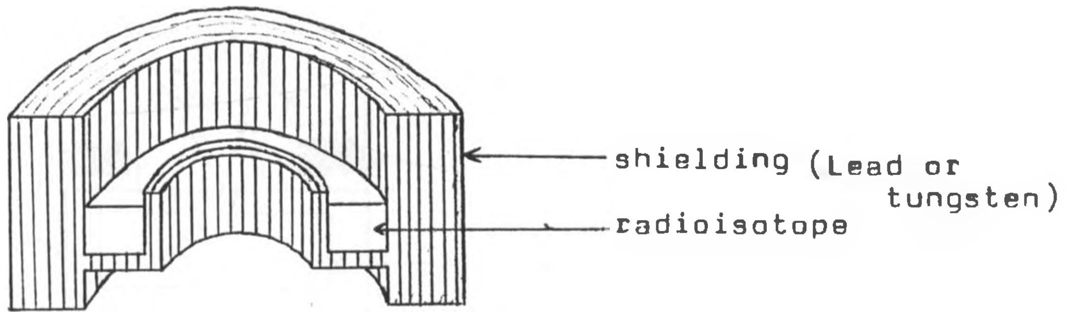
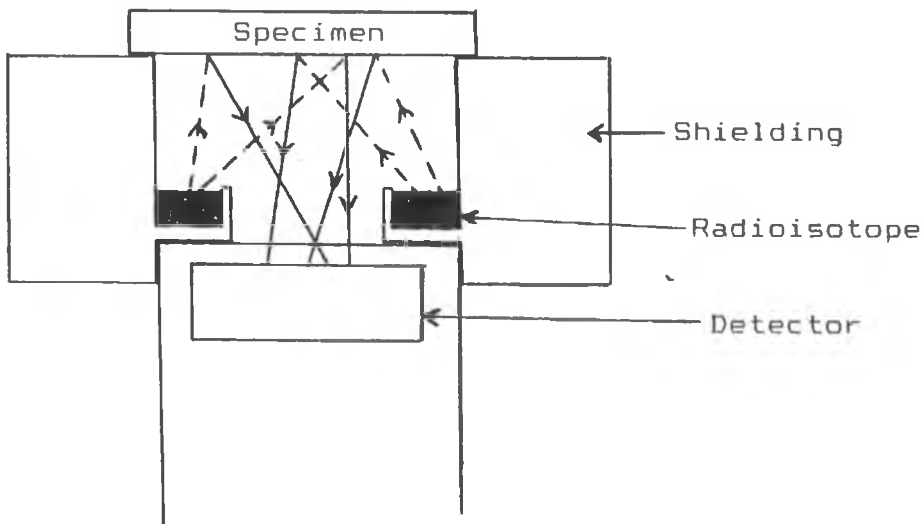


Fig. 3.I : A cross section of an radioisotope excitation source having an annular geometry.

The radioisotope is shielded on three sides; the inner and lower parts so that X-rays from the source do not irradiate the detector directly, and the outside for the safety of the analyst. Only the upper part is open to allow X-rays to excite the specimen.

3.2 : SPECIMEN PRESENTATION APPARATUS.

The specimen, as a precipitate on a membrane filter paper, was placed between two Mylar papers. These papers were then clamped between two aluminium rings to ensure fixed geometry of the sample. The arrangement of the specimen, excitation source and detector were as shown in figure 3.2.



- - - - -> - - primary X-rays from radioisotope.

———> fluorescence and scattered X-rays from the sample.

Fig. 3.2 : Arrangement of sample, excitation source and detector.

3.3 : THE ENERGY-DISPERSIVE X-RAY FLUORESCENCE (EDXRF) SPECTROMETER.

Figure 3.3 shows the main components of the Energy-Dispersive X-ray Fluorescence Spectrometer.

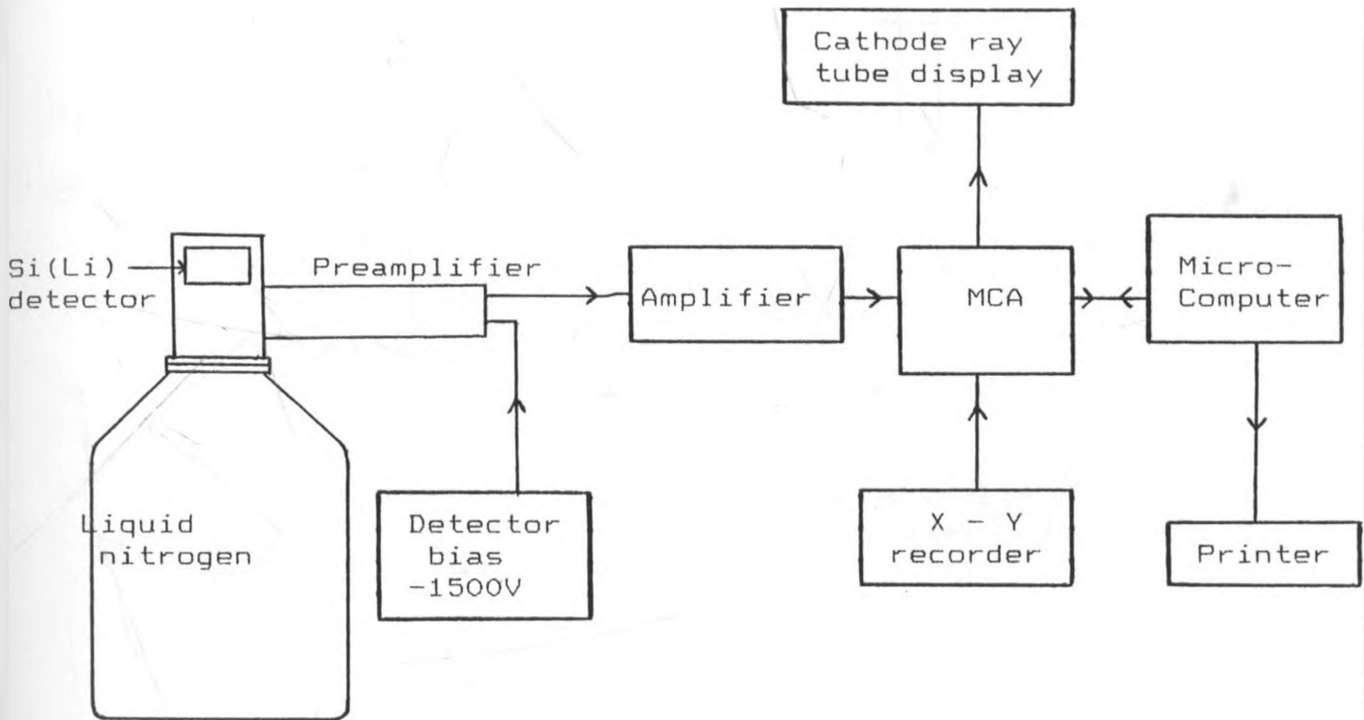


Fig. 3.3 : Block diagram of an Energy-Dispersive X-ray Fluorescence (EDXRF) spectrometer.

The EDXRF spectrometer consists of;

(i) a detector.

(ii) preamplifier.

(iii) main amplifier.

(iv) multichannel analyser (MCA).

(v) computer with associated accessories.

3.3.1 : THE DETECTOR.

The detector is a Si(Li) semiconductor crystal. The detector goemetry is shown in figure 3.4.

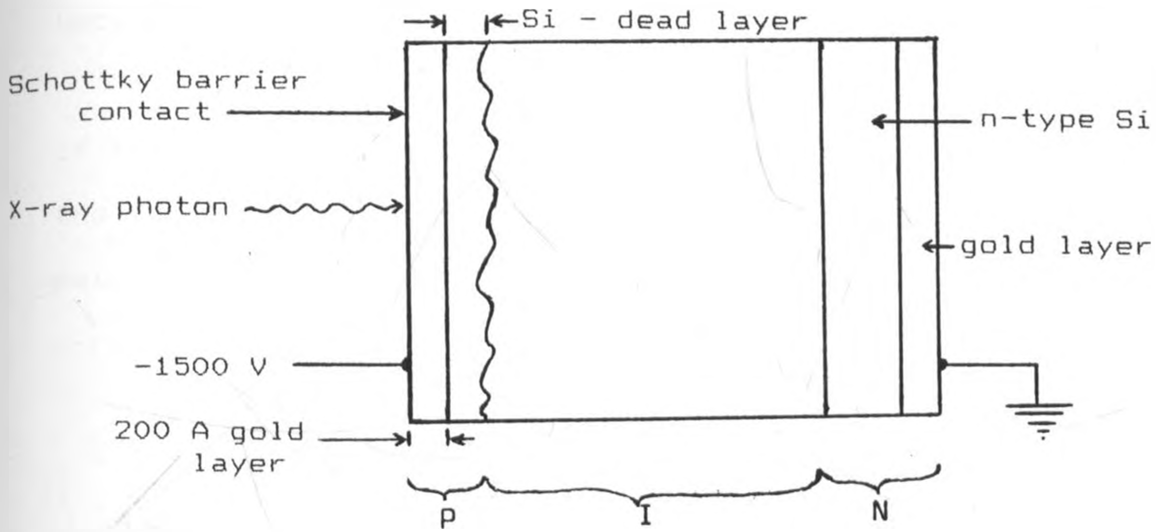


Fig. 3.4 : Schematic diagram of the sensitive volume of the Si(Li) detector crystal (Jenkins et al., 1981).

To increase electrical resistivity, the detector is compensated with lithium. A high concentration of lithium near the rear contacts creates an n-type region over which a layer of gold is deposited to fabricate a non-rectifying contact to the n-type region. To the front of the detector, a Schottky barrier contact is applied to produce a p-i-n type diode. The front of the crystal is also coated with a layer of gold, to which is connected a bias of - 1500 V. The other side of the crystal is connected directly to a Field Effect Transistor

(FET), the first stage of the signal amplification process (Jenkins et al., 1981). A detailed background on the use of semiconductors as radiation detectors can be obtained from Knoll (1979).

When operating under the reverse bias of - 1500 V, the diode is depleted of the remaining charge carriers and becomes a solid state ionization chamber.

When an X-ray photon is stopped in the sensitive volume of the detector diode, a cloud of ionization is generated in the form of electron-hole pairs. The number of electron-hole pairs produced, n, is proportional to the energy, E, of the x-ray photon;

$$n = \frac{E}{e} \qquad \text{........3.1}$$

where e = 3.8 eV, the average energy required to produce one electron-hole pair in silicon.

The electrons produced are swept to the rear contact of the diode by the high negative bias applied across the crystal, while the holes drift to the front contact.

The total charge collected by the rear contact is;

$$Q = \frac{E}{e} q \qquad \text{........3.2}$$

where $q = 1.6 \times 10^{-19}$ C is the charge of an electron.

The rear contact of the detector is connected

directly to the Field Effect Transistor (FET) (the preamplifier) input so that the charge is stored on a feedback capacitor Cf to produce a voltage pulse of magnitude

$$V_o = \frac{Q}{C_f} = \frac{E}{e} \frac{q}{C_f} \qquad \text{........3.3}$$

To minimise electronic noise added to the signal during this process, the Si(Li) detector along with the first stage and feedback elements of the preamplifier is mounted in a light-tight vacuum cryostat operated at the boiling temperature of liquid nitrogen (77° K). In front of the crystal is a thin beryllium window that seals the vacuum cryostat and prevents the crystal from contamination.

3.3.2 : THE AMPLIFIER.

The signal from the preamplifier is small and has a low signal-to-noise ratio. It is in the form of a step signal in the millivolt range. In the main amplifier, this signal is amplified to produce positive pulses upto 10 V in amplitude.

The main amplifier is the heart of the analytical procedure. It is concerned with the linear amplification of the preamplifier output, pulse shaping or noise filtering, pulse pile-up resolution, base line restoration and live-time correction. These procedures are reviewed by Marshall (1980).

3.3.3 : THE MULTICHANNEL ANALYSER (MCA).

The MCA consists of three main sections;

(i) the analogue-to-digital converter (ADC).

(ii) the memory.

(iii) the display.

Upto the input of the MCA, the system has an analogue response. The purpose of the multichannel pulse height analyser is to measure the height of each amplifier output and represent this amplitude by an integer number. This is an analogue to digital conversion process.

The spectrum is digitized in the ADC which is the first part of the MCA. Digitizing is done via a high frequency clock which measures the time taken for a capacitor to discharge after being charged by a pulse from the amplifier. Each pulse in the spectrum is given a value in terms of clock pulses. This value corresponds to the pulse height or energy of the spectral pulse. The number of clock pulses is determined by a scaler and this number is used as a memory channel address.

Each channel in the memory can be associated with a particular energy interval. The display is represented with the x-axis calibrated in terms of the mean energy interval. The y-axis of the display gives the number of photons counted in each energy interval (counts) during the data accumulation period (Jenkins et al., 1981).

When a pulse corresponding to a particular memory address is detected, the number in that memory is increased by 1. This process proceeds on a pulse by pulse basis untill the histogram representing the X-ray energy spectrum is built up.

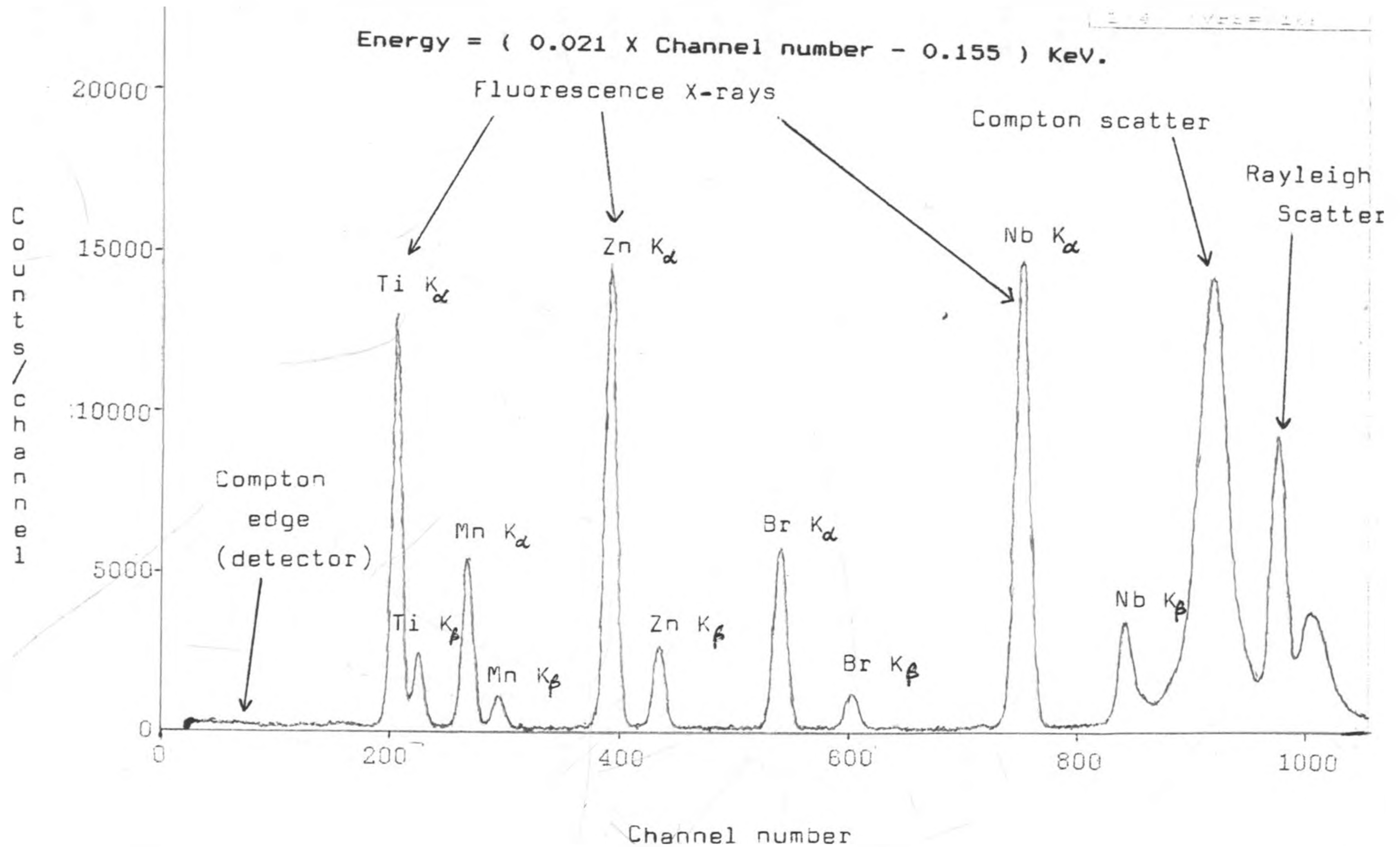


Fig. 3.5 : A typical spectrum observed on the Multichannel Analyser (MCA). The spectrum was obtained by running a multi-element target containing Ti, Mn, Zn, Br and Nb, using 109...

The result is a spectrum as shown in figure 3.5. The principal features of the spectrum are the two high energy peaks caused by Compton and Rayleigh scattering from the sample into the detector and the characteristic fluorescence lines of elements in the sample, superimposed on a generally flat background throughout the whole spectrum, and a plateau in the low energy region caused by the compton scatter of photons out of the detector, leaving only low-energy electrons in it to produce signals (Goulding and Jaklevic,1973).

Such a spectrum is used for the identification of the elements in the specimen. The scale of the spectrum is in counts/per channel versus the spetral line energy. By means of an MCA cursor, the energy of a peak is determined. The element is then identified by referring to the tabulated data on spectral line energies. From these tables, the peak energies of the element are checked.

3.3.4 : COMPUTER.

The X-ray Fluorescence Spectrometer includes a small computer for storage and handling of the spectral data. The computer analyses the spectrum from the MCA, calculates the background and computes the raw element intensities. Software in the computer can also be used to correct for interelement effects and calculation of the concentration of each element.

CHAPTER FOUR.

EXPERIMENTAL PROCEDURES.

4.1 : METHOD DEVELOPMENT.

INTRODUCTION.

Various methods for the quantitative determination of halides are documented. Some of the methods are titrimetric, using silver nitrate as the precipitating agent, with a variety of end-point indicators such as adsorption indicators (Fajan's method), iron(III) in Volhard's method (Laititen, 1960), or potentiometric determination of the end point. The principal methods used in the determination of halides, e.g. volumetric, potentiometric, amperometric (Belcher, 1960), coulometric (Biffen and Seaman, 1956), polarographic, gravimetric, chronometric, X-ray fluorescence or absorption, radiochemical and chromatographic methods, are summarised by Bailer et al., (1973). In practice, the anions are usually determined volumetrically, but measurements of the highest precision rely on the classical gravimetric procedures (Bailer et al., 1973).

Most of the above methods suffer the short-coming that they are usually for the determination of the halides in comparatively simple matrices, for example in inorganic salts. In cases where the matrix is complex, for example in biological materials, separation methods such as digestion,

followed by distillation, have to precede these determinations. Such procedures usually take a lot of time.

There are two gravimetric procedures for the determination of halides. The first and most common uses silver nitrate as the precipitating agent. This method has been discussed by a number of authors (Hillebrand, 1953, Cummings, 1956, Vogel, 1961 and Nagj et al., 1985). The second method uses palladium chloride as the precipitating agent. Palladium chloride selectively precipitates iodine as palladium iodide. Precipitation with palladium chloride has been discussed by Scot (1939), Hillebrand, (1953) and Vogel, (1961).

The main drawback with the silver nitrate method is that precipitation has to be done under ultraviolet lighting. Under normal light, the silver halide decomposes (Nagj et al, 1985). Unavailability of ultraviolet light can give rise to a lot of analytical problems. Palladium iodide, however, poses no such problem and is stable at temperatures of up to 140 C and is not sensitive to light. It was therefore decided to try and develop a protocol for the determination of iodine using palladium chloride without undergoing through the long distillation procedure given by Seki et al., (1990).

4.1.1 : REAGENTS.

i) Water - double distilled.

ii) Potassium iodide - analytical grade.

iii) Hydrochloric acid - analytical grade.

iv) Sulphuric acid - " "

v) Sodium sulphite - " "

vi) Potassium dichromate - " "

vii) Palladium Chloride - General Purpose Reagent(GPR) (due to unavailability of the analytical grade).

4.1.2 : pH OF OPTIMAL RECOVERY.

According to Hillebrand (1953) and Vogel (1961) precipitation of iodine by palladium chloride solution takes place in presence of an excess of chloride ions. The first step was therefore to determine the amount of chloride ions required. This was done by determining the pH at which the recovery of iodine from a standard solution was maximum.

A 10 ppm iodine standard solution was prepared by dissolving potassium iodide in water. The pH of 10ml quantities of the standard solution was adjusted to various values using 1N hydrochloric acid. 2 ml of a 500 ppm palladium chloride solution were added. The mixture was filtered through Nucleopore (0.4 micron pore diameter) membrane filter paper after a timed interval. After drying, the samples were analysed using ^{241}Am as the excitation source.

The results obtained were fitted into a curve shown as figure 4.1

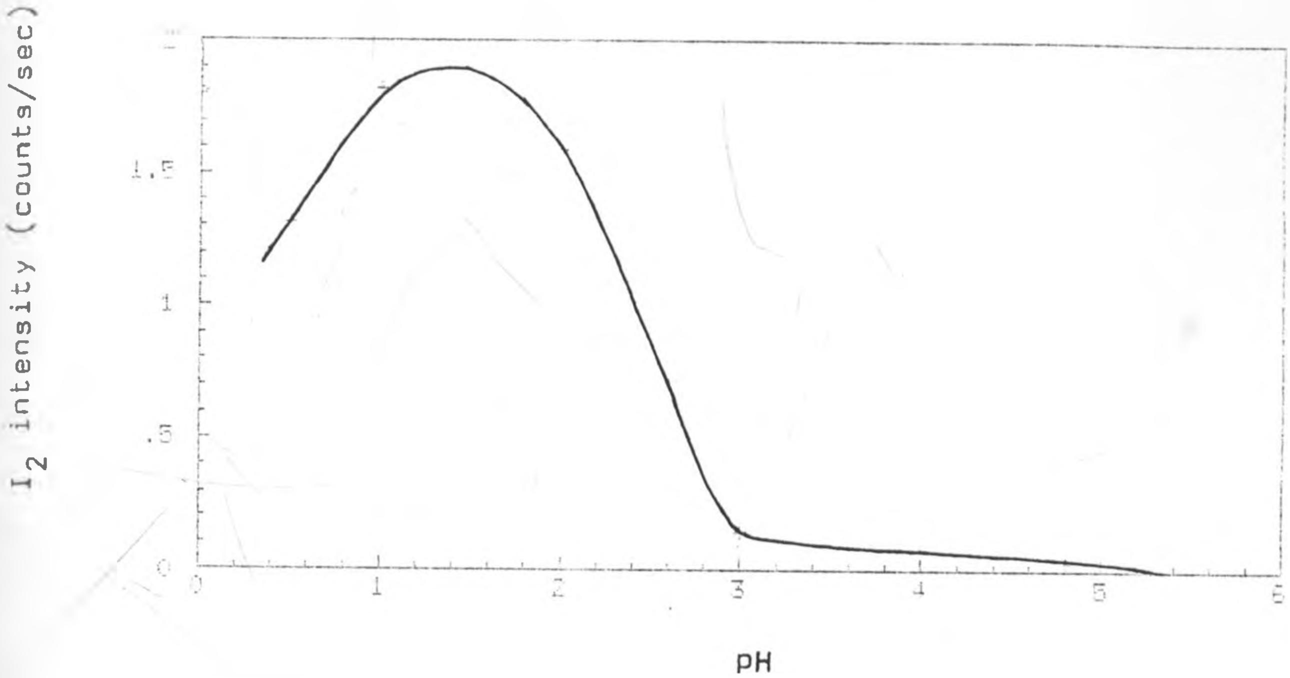


Fig. 4.1 : Intensity versus pH for a 10 ppm standard solution filtered after 5 hours.

As can be seen from figure 4.1, maximum recovery of iodine occurs was at a pH of around 1.4. Since it was observed that the observed intensity was much lower than expected, a test was carried out to determine the effect of the time allowed for precipitation on the recovery of iodine.

4.1.3 : TIME OF OPTIMAL PRECIPITATION.

To investigate the effect of time before filtration on the recovery of iodine, six 10 ml quantities of the standard solution were adjusted to a pH of 1.4. 2 ml of the palladium

chloride solution were added to each sample. The mixtures were filtered after different time intervals.

The results are shown as figure 4.2.

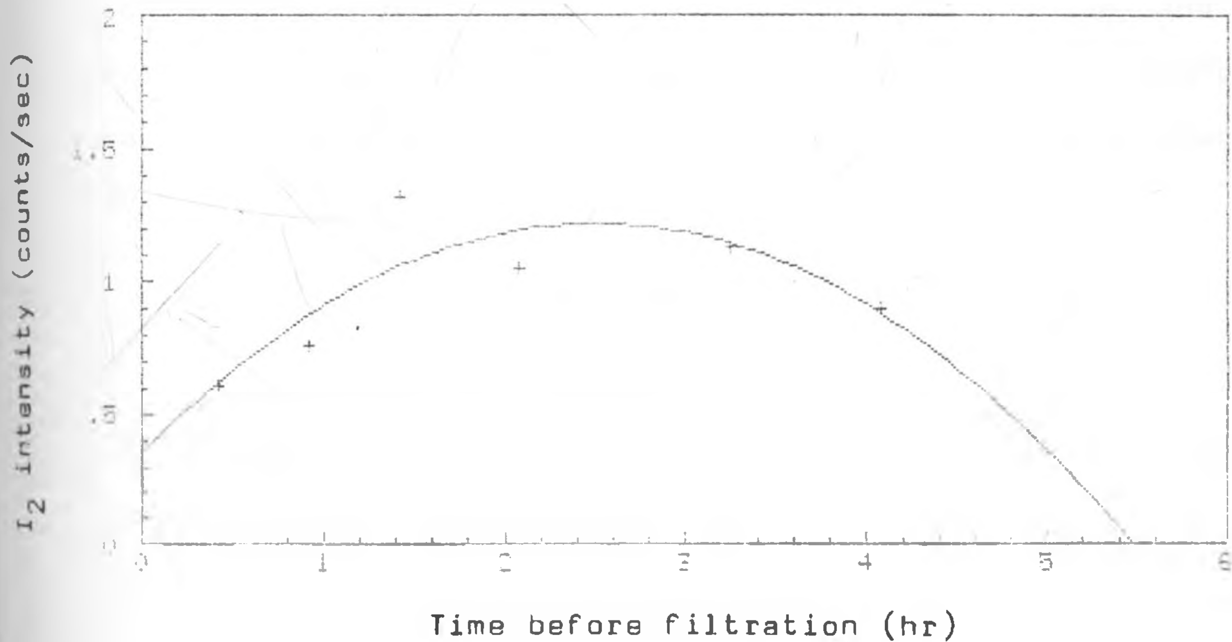


Fig. 4.2 : Intensity versus time before filtration of a mixture.

From figure 4.2 it can be deduced that maximum recovery of iodine occurs when the sample is filtered two and a half hours after addition of the palladium chloride solution.

4.1.4 : DIGESTION PROCEDURE.

The sample digestion procedure used was a combination of methods outlined by Hillebrand (1953), Bock (1979) and Seki

et al (1990).

The samples were digested using chromic acid mixture. The chromic acid mixture was prepared by adding 2g of potassium dichromate to every 30 ml of concentrated sulphuric acid. The reaction mechanism may be as follows (Vogel, 1954) :

$$K_2Cr_2O_7 + H_2SO_4 + \text{Substrate} \longrightarrow I_2 + Cr_2(SO_4)_3 + K_2SO_4 + H_2O$$

Due to the high acidity of the digestion mixture, the iodine reacts with hydroxyl ions to form iodide and the extremely unstable hypoiodate, the latter being transformed rapidly into iodate and iodide by self oxidation and reduction (Vogel, 1961) :

$$I_2 + 2\,OH^- \longrightarrow I^- + IO^- + H_2O$$

$$3\,IO^- \longrightarrow 2\,I^- + IO_3^-$$

The iodine or iodate in the solution can be reduced to iodide by adding a strong solution of sodium sulphite (Hillebrand, 1953). The reaction mechanism for this reduction may be (Jacobson, 1959) ;

$$I_2 + SO_3^{2-} + H_2O \longrightarrow 2\,I^- + SO_4^{2-} + 2\,H^+$$

$$IO_3^- + 3\,SO_3^{2-} \longrightarrow I^- + 3\,SO_4^{2-}$$

The iodide can then be precipitated using palladium chloride solution.

To 50 ml of the sample, 30 ml of chromic acid mixture was added. The mixture was boiled for 10 minutes. The digested sample was then adjusted to a convenient volume.

4.1.5 : REDUCTION OF IODINE AND IODATE.

To determine the amount of saturated sodium sulphite required for the complete reduction of the iodine and iodate in the digested sample, the following experiment was carried out.

To portions of the 10 ppm standard solution which had undergone the digestion procedure, different volumes of a saturated solution of sodium sulphite were added. 2 ml of 1N HCl and 2 ml of the palladium chloride solution were added. The mixture was filtered after two and a half hours, the determined time of optimal precipitation.

A sample of the results are shown as figures 4.3 and figure 4.4.

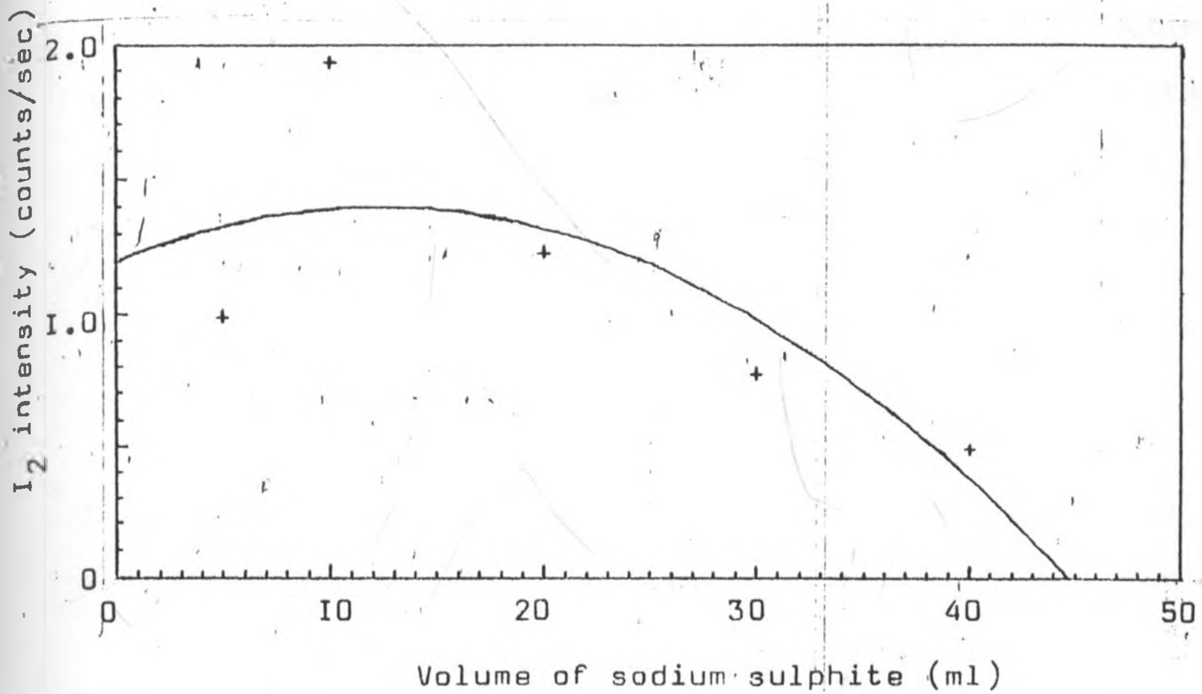


Fig. 4.3 : Intensity of iodine versus the volume of saturated sodium sulphite added to a 10 ppm standard solution.

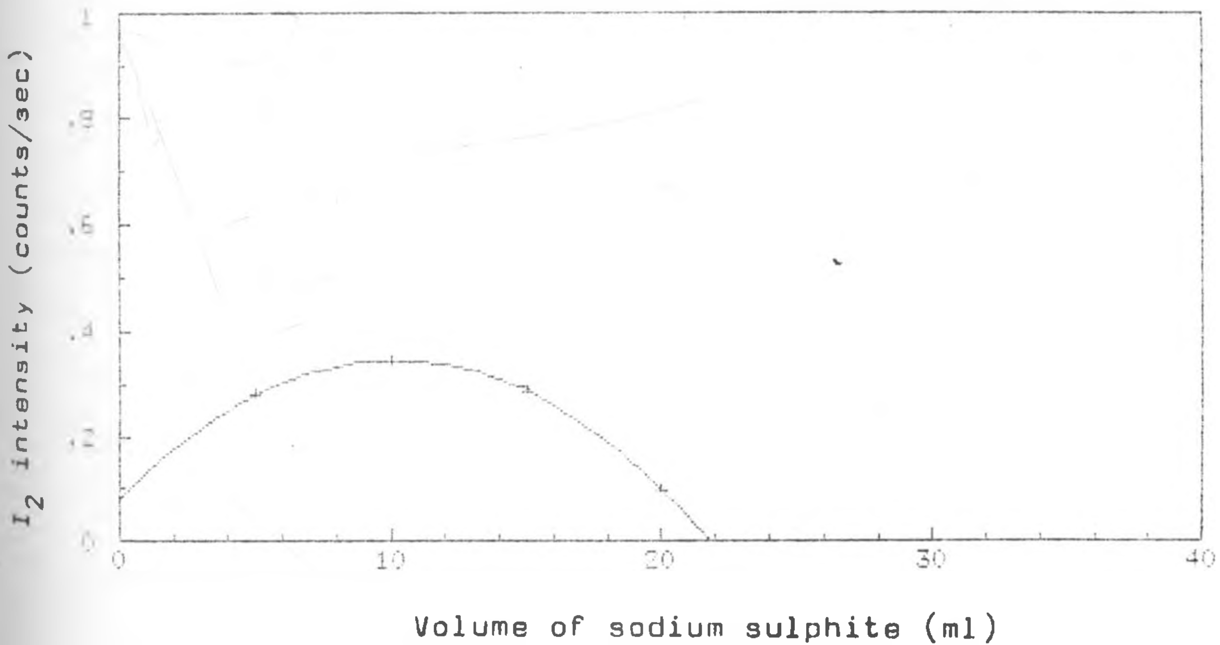


Fig. 4.4 : Intensity of iodine versus the volume of saturated sodium sulphite solution added to urine samples.

The two graphs show that for the standard solutions as well as the real urine sample, 10 ml of the sodium sulphite solution were sufficient to reduce the iodine and iodate in the mixture to iodide. This was equivalent to one third the volume of the chromic acid used in the digestion.

4.1.6 : SUMMARY.

Based on the results of the previous sections, the following procedure for the treatment of samples was adopted;

(i) 30 ml of the chromic acid mixture was used to digest each 50 ml of sample. The sample was then made up to a suitable volume.

(ii) To a fraction of the digested sample, an equal volume of water was added, followed by a volume of the sodium sulphite solution equivalent to one-third the amount of chromic acid present in that fraction.

(iii) 2 ml of 1N HCl and 2 ml of palladium chloride solution were added.

(iv) The mixture was filtered through Nucleopore (0.4 micron pore diameter) membrane filter paper after not less than 2.25 and not more than 2.75 hours.

(v) The filters were dried in clean air, preferably by being left overnight in closed petri dishes.

The residue was analysed using ^{241}Am as the excitation source and a Si(Li) crystal as the detector.

4.2 : DETECTION LIMIT.

To determine the lowest amount of iodine detectable by the above method, standard solutions containing different concentrations of iodine were treated as per section 4.1.6 above. Low iodine samples were run for 2000 seconds while high iodine samples were run for 500 seconds.

Table 4.1 shows the recoveries for the range 0 to 20 parts per million (ppm).

Table 4.1 : The recovery of iodine from standard solutions having concentrations varying from 0 to 20 parts per million (ppm).

Concentration in ppm	Recovered amount (ppm) (n=5)	% recovery (n=5)
0.005	0.0044 ± 0.0024	88 ± 48
0.01	0.0102 ± 0.0054	102 ± 54
0.03	0.0292 ± 0.0095	97 ± 32
0.05	0.0479 ± 0.0129	96 ± 26
0.1	0.0913 ± 0.0183	91 ± 18
0.3	0.287 ± 0.0307	96 ± 10
0.5	0.428 ± 0.0557	86 ± 11
0.8	0.703 ± 0.0767	88 ± 10
1.0	0.981 ± 0.0788	98 ± 8
5.0	4.754 ± 0.184	95 ± 4
10.0	9.331 ± 0.225	93 ± 2
15.0	13.835 ± 0.335	92 ± 2
20.0	19.214 ± 0.286	96 ± 1

In table 4.1, column 2 is given as the recovered amount +/- the standard deviation. The standard deviation was calculated from the counting statistics and is the combined value of the counting statistics of the five samples. The standard deviation in the counting statistics was used instead of the statistical deviation between the five samples because it was the major source of error in the results. The variation between the concentrations obtained for the five samples was below 10 %. Column 3 gives the % recovery +/- the standard deviation in the recovery. It can be seen from column 3 that although the recovery is high for samples with iodine concentrations below 0.05 ppm, the standard deviations in these recoveries are also high, varying between 30 and 60 %. This is expected since these concentrations are within the calculated detection limit (Kinyua, 1982) of 0.01 ppm. These standard deviations could have been improved by running the samples for a longer time. The average recovery was 93 %. The loss was mostly due to some of the precipitate adhering to the walls of the containers during specimen preparation.

4.3 : SAMPLING.

Urine samples were collected from four primary schools in the Kapenguria division of West Pokot district, namely Kammorou, Nasokol, Nangrotum and Makutano. Of these, only Makutano lies in an urban area. The distribution of these sampling points was such that they would give a fair representation of the whole division. Figure 4.1 is a sketch

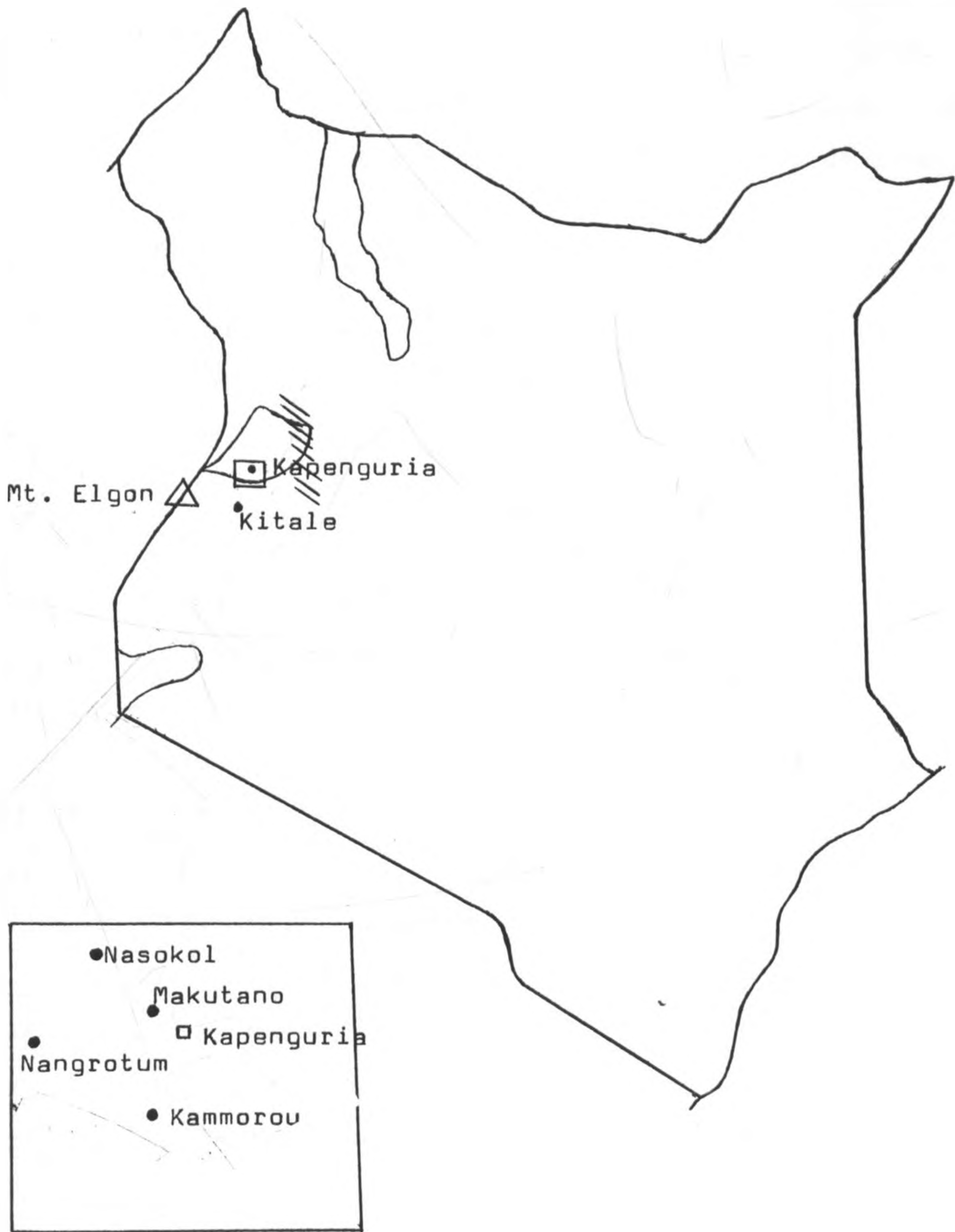


Fig.4.5 : A map of Kenya showing the West Pokot District. The shaded region is the Cherangani Hills. The squared region was the sampled region. This region has been expanded to show the distribution of sampling points. The expansion is not to scale.

map of the sampled region, indicating the sampling points.

In each of the schools, standard four pupils were picked at random, given a container and a piece of paper on which was written the pupils name, age and sex. The plastic, opaque containers had been thoroughly cleaned with detergent, then soaked in dilute nitric acid for 24 hours, and then rinsed with copious amounts of double distilled water. All the samples were collected between 10 and 11 a.m. Only one sample was collected from each pupil. Over 90 % of the samples had a deep yellowish colour. The age distribution in the sample population was 8 to 14 years. After collection, 10 ml of the digestion mixture was added to each sample. This was done to ensure that any decomposed iodine would be converted to iodate in the sample. A sample of the researcher was also taken for testing of preservation of the samples. The sample was divided into two, one portion was treated with the digestion mixture while the other was left raw.

Samples of drinking water were also taken from the water sources available around each of the sampling points, i.e., spring water from Kammorou and Nangrotum, tap water from Nasokol, while from Makutano both spring and tap water were collected.

Salt was bought at Makutano market. Since this is the supplying centre for the whole region, the assumption was that this would give a representative sample of the salt consumed in the region.

4.4 : SUMMARY OF EXPERIMENTAL PROCEDURES.

Urine samples were treated as per section 4.1.6. For sample amounts less than 50 ml, 30 ml of chromic mixture was used. For samples larger than 50 ml, a volume of the chromic mixture was added as to make the final ratio of sample : acid be 50:30.

For salt samples, a weighed amount of the sample was dissolved in 50 ml of water and then treated as for urine samples.

Water samples were treated as for the urine samples.

The excitation source used was ^{241}Am. This source has sufficiently energetic γ-rays capable of exciting the K-series of iodine. The detector was an ORTEC Si(Li) crystal with a resolution of 205 eV (Mn K_{α} peak). Spectral data was collected using a Canberra Series 40 Multichannel Analyser, and was stored on a DEC Professional 350 microcomputer. Some samples were also analysed using the Canberra S100 MCA coupled to an IMC computer.

Area under the peak was determined using the Qualitative X-ray Analytical Spectrometry (QXAS) software (IAEA, 1985). Figure 4.6 shows a sample spectrum before analysis, while figure 4.7 shows the same spectrum after analysis using QXAS. In figure 4.6, the shaded area was the actual area of the iodine peaks. The region between the lower line of this shadowed area represents the background. This feature can be seen more clearly in figure 4.7, where there is a lower line for the background radiation. In QXAS, the

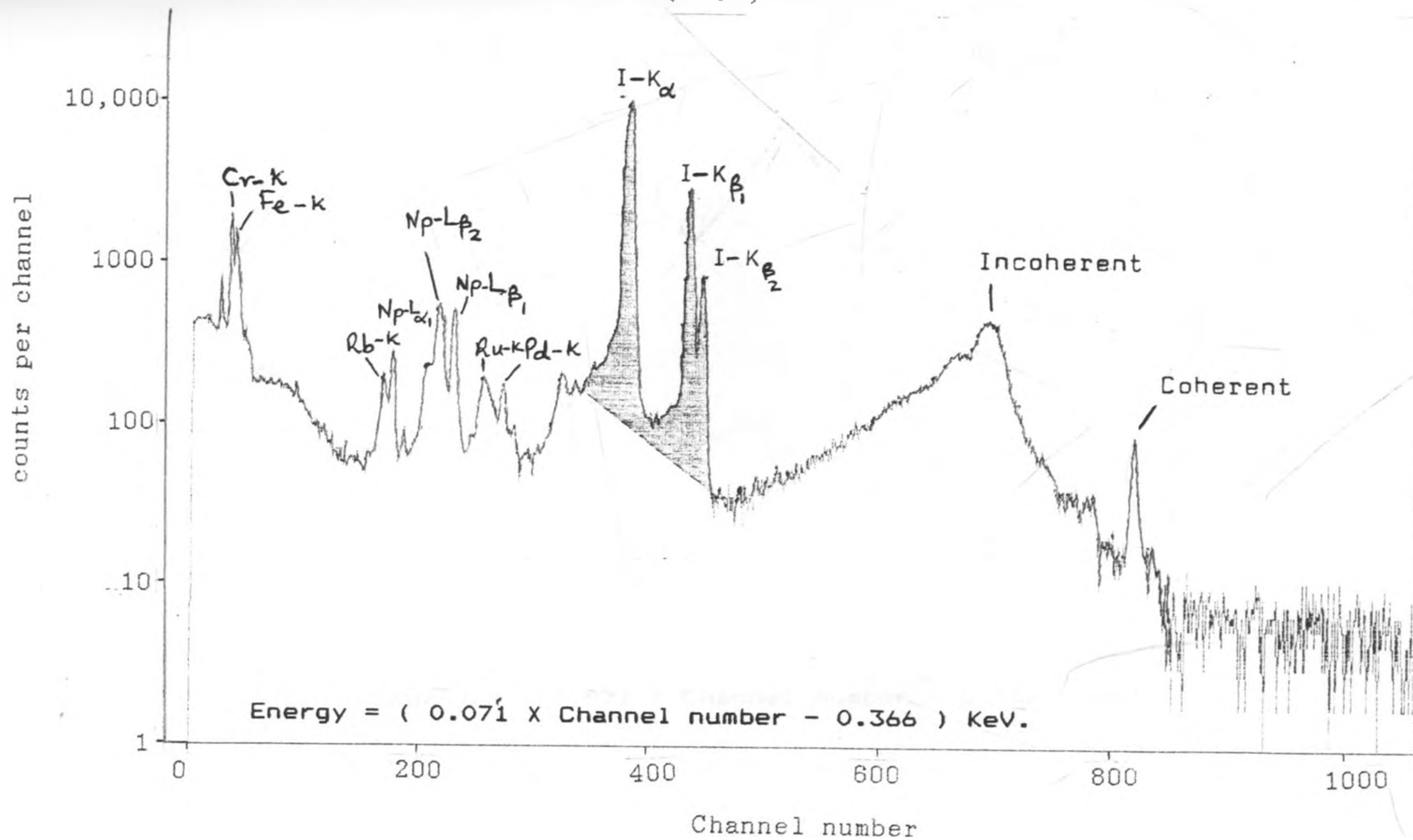


Fig. 4.6 : Log count Channel number plot of a sample spectrum.

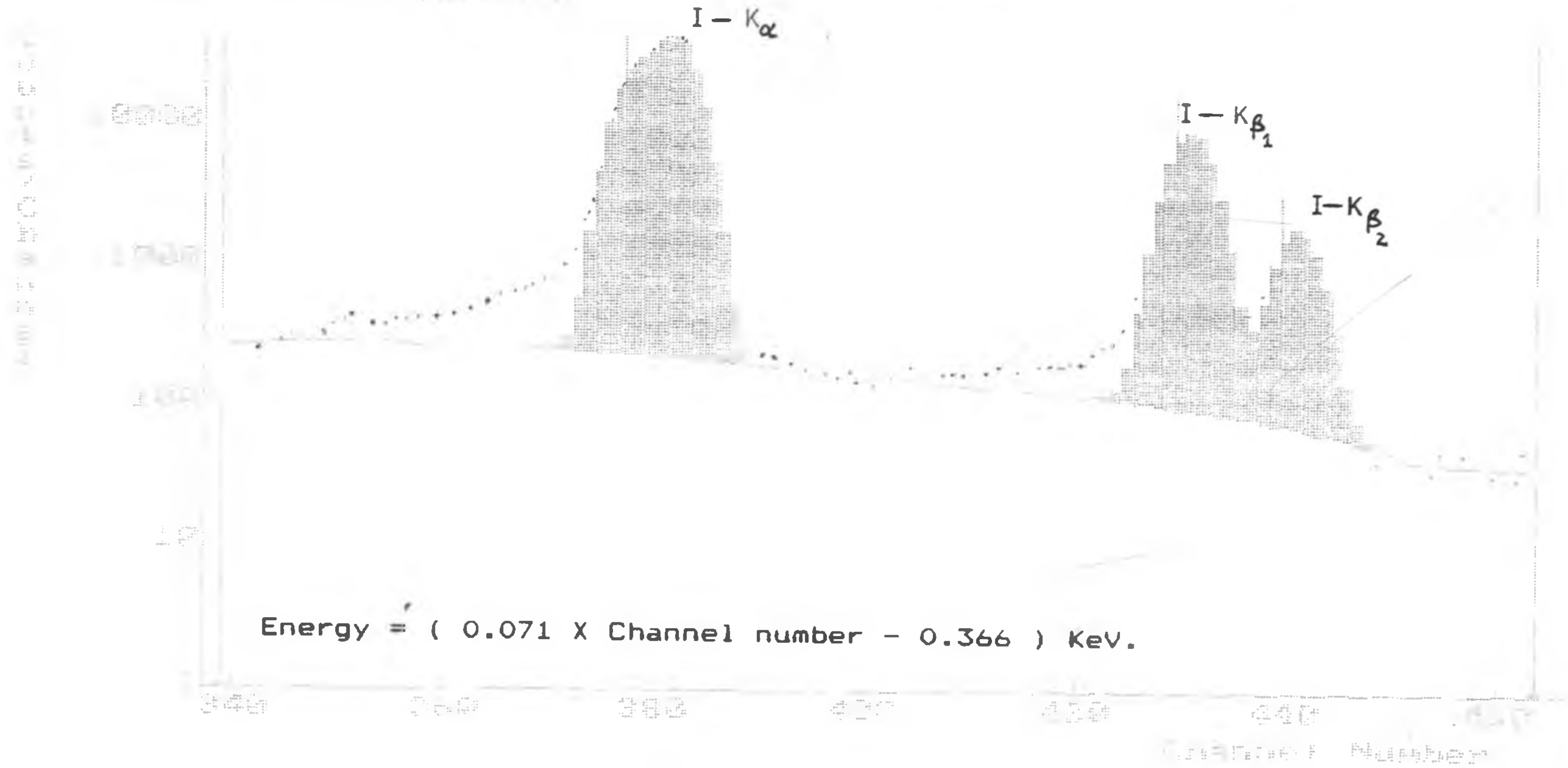


Fig.4.7 : The spectrum of figure 4.6 after fitting using the QXAS software. The shaded regions are the calculated areas of the I-K_α and K_β peaks.

background is shape is calculated first, then the area of any peak which projects above this background is calculated, hence in figure 4.7, the area given for the iodine peaks will only cover the shaded region, the background having been calculated and substracted from the spectrum by the computer.

The area was converted to intensities. Calculation of the concentrations was done using the equation

$$\text{Conc (ppm)} = \frac{I_i \cdot A}{G_O \cdot K_i \cdot V} \qquad \text{..........4.1.}$$

where I_i is the observed intensity.

A is the area of the sample exposed to the primary radiation. The diameter of the exposed area has been calculated as 3.5 cm.

G_O is the geometric constant and a value of 1.67457×10^5 counts per sec was used. This value was not adjusted daily, since with ^{241}Am having a half-life of 453 years, the daily variation of G_O is negligible.

K_i is the relative excitation detection efficiency. A K_i value of 3.754 cm^2/g was calculated from equation 2.18.

V is the volume of the sample used in the preparation of the filter being analysed.

4.5 : ERRORS ASSOCIATED WITH THE METHOD.

There are two main types of errors associated with this method;

i) the analyst's error.

ii) the error arising from the X-ray Spectrometer.

The analyst's error or random error arise during specimen preparation, for example in the sample preparation technique used in this study, the sample digestion may have been incomplete, or the recovery of iodine from the solution may have been incomplete. This type of error was minimised by analysing samples in duplicate. A higher number of replicates would have been better, but time and the volume of the samples prevented this.

The sources of errors in EDXRFA, as given by Bertin (1975) are;

a) Statistical counting errors: - these were minimised by running the samples for 2000 seconds. In this work, the counting errors were between 30 and 50 %. A higher counting time could have reduced the statistical counting error, but sufficient reduction would have necessitated a counting time of over 10,000 seconds, a time that the project logistics could not allow.

b) Instrumental errors like short-term and long-term variation, instability and drift of instrumental components, conditions and parameters. These were minimised by keeping the

the instruments permanently "ON", and calibration of the instruments prior to any analysis.

c) Specimen errors like absorption-enhancement effects (matrix effects) and position effects like specimen plane, take-off angle, position, orientation and flatness. Matrix effects were neglected according to the condition of equation 2.19. The design of the radioisotope excitation source and sample holder ensure minimal errors in positioning.

Except for the high error due to the counting statistics, there was good reproducibility in the results, even in the samples with very low concentrations of iodine. This indicates that the method is usable for determination of iodine so long as the run-time is sufficiently increased.

RESULTS.

5.1 : URINE SAMPLES.

The results for the urine samples are given in tables 5.1 to 5.4 as follows;

Table 5.1 : Kammorou primary school.

Table 5.2 : Nasokol primary school.

Table 5.3 : Nangrotum primary school.

Table 5.4 : Makutano primary school.

In tables 5.1 to 5.4, the results are given as the assayed amount +/- the arithmetic deviation from the mean of two samples. The cases where no arithmetic deviations are given are for those samples which got spoiled during preparation and which, due to reasons of volume, could not be repeated. It can be seen from the tables that there is good agreement between each of the two samples analysed per each case.

The error in each of the values, as calculated from the counting statistics, was between 30 and 50 %. The large error was due to the fact that in most of the samples, iodine concentration was within the detection limit of 1.0 micrograms per decilitre ($\mu g/dl$). The counting statistics could have been improved by running the samples for a longer time, for example, a 30 % decrease in the standard deviation was observed when a sample was run for 15,000 seconds instead of 1,000 seconds. However, such an increase in run time would have greatly increased the time and cost of the project, so a compromise time of 2,000 seconds was adopted.

In the preservation test, there was no difference between the samples which had been treated with the digestion mixture and those which had not been treated.

5.1.1 : Kammorou primary school.

Figure 5.1 shows the spectrum for one of the samples from Kammorou primary school. The results for the 24 samples obtained from the school are given in table 5.1.

Table 5.1 : Iodine concentration in the samples from Kammorou primary school.

Sample Code	Sex M-male F-female	Av. concentration iodine (µg/dl). n=2.
KAM1	F	0.99 ± 0.48
KAM2	F	1.60 ± 0.92
KAM3	M	1.85 ± 0.24
KAM4	F	1.82 ± 0.40
KAM5	M	2.04 ± 0.05
KAM6	M	2.59 ± 0.55
KAM7	M	4.76 ± 0.85
KAM8	M	6.62
KAM9	M	2.87 ± 0.29
KAM10	M	1.40
KAM11	M	4.70 ± 0.07
KAM12	F	3.61 ± 0.59
KAM13	M	1.66 ± 0.36
KAM14	F	5.10 ± 0.12
KAM15	F	2.30 ± 0.15
KAM16	M	3.71 ± 0.42
KAM17	F	nd
KAM18	M	3.80 ± 0.35
KAM19	M	5.17 ± 0.59
KAM20	M	6.33 ± 0.35
KAM21	F	9.22
KAM22	M	1.44
KAM23	F	nd
KAM24	F	nd

nd - iodine not detected in the sample.

Figure 5.2 shows the frequency distribution of urine iodine for the samples from Kammorou.

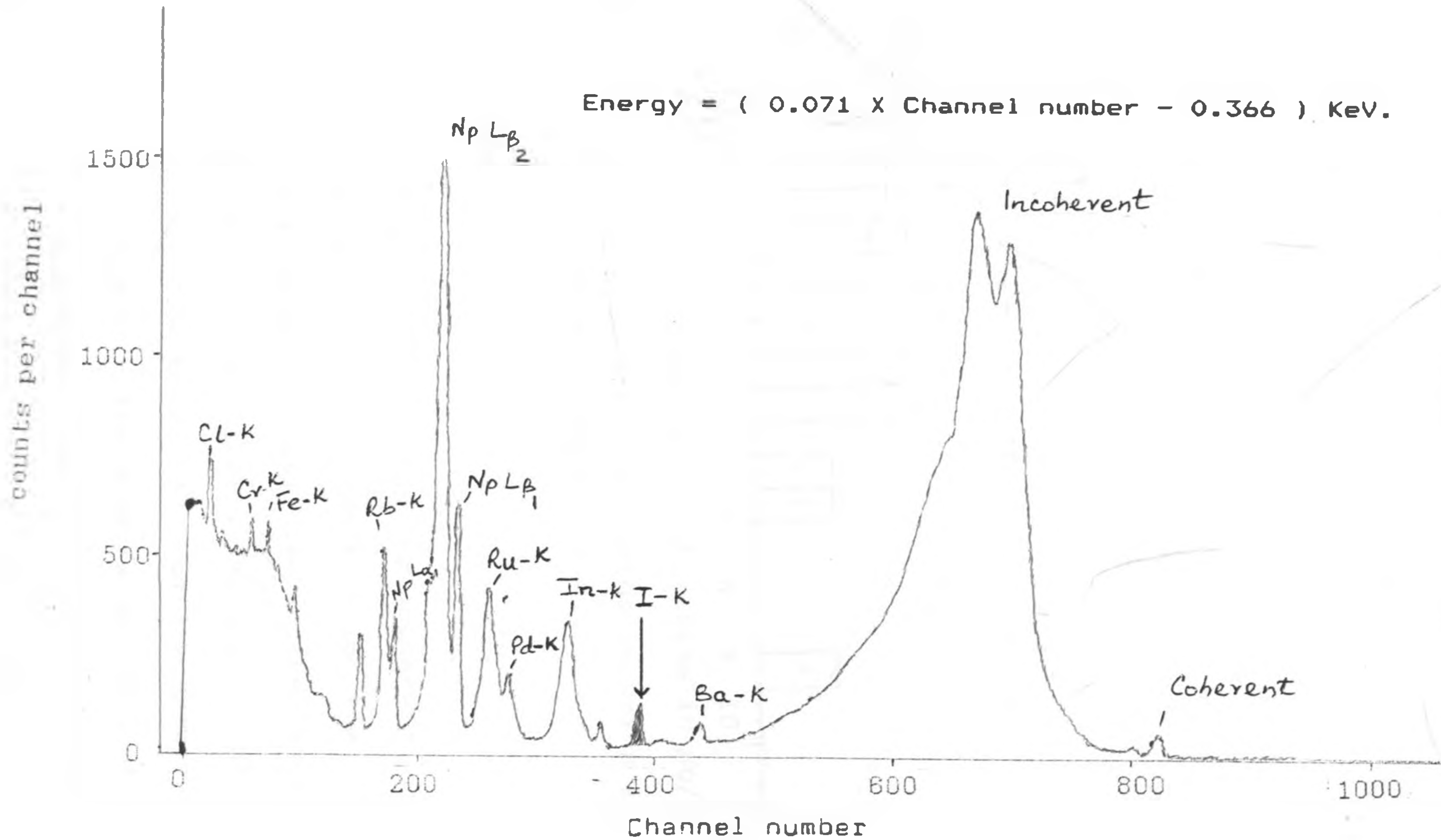


Fig. 5.I : A spectrum for one of the samples from Kammorou Primary School.

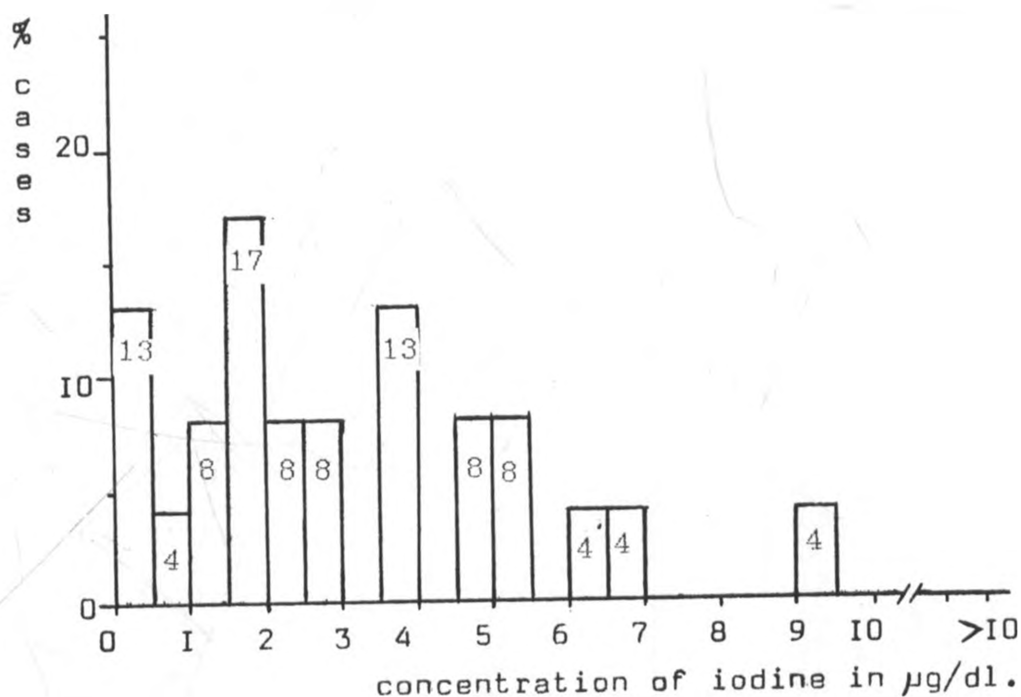


Figure 5.2 : Distribution of iodine in urine samples from Kammorou school. n=24.

From figure 5.2, it can be observed that for the samples from Kammorou, the median lies in the range 2.0 to 2.5 µg/dl. The mode of the data lies in the range 1.5 to 2.0 micrograms per decilitre (µg/dl). The mean urine iodine concentration was 3.06 µg/dl. 17 % of the samples had urine iodine concentrations in the mode range. 80 % of the samples had iodine concentrations lower than the safe value of 5.0 ug/dl. 20 % had concentrations in the range 5.0 - 10.0 µg/dl. No sample had iodine concentration above 10.0 µg/dl.

5.1.2 : Nasokol primary school.

Table 5.2 gives the results for the 28 samples obtained from Nasokol primary school.

Table 5.2 : Iodine concentration in the samples from Nasokol Primary School.

Sample Code	Sex M-male F-female	Av. concentration of iodine (μg/dl). n=2.
NASO1	M	2.32
NASO2	F	0.33 ± 0.04
NASO3	F	1.60 ± 0.42
NASO4	F	2.26
NASO5	F	1.70
NASO6	F	1.21 ± 0.33
NASO7	F	0.83 ± 0.11
NASO8	M	1.95
NASO9	F	3.32
NASO10	F	3.18
NASO11	F	1.51
NASO12	M	0.97 ± 0.12
NASO13	M	1.59
NASO14	F	2.61
NASO15	M	8.47 ± 1.79
NASO16	F	57.58 ± 4.95
NASO17	M	4.99
NASO18	F	7.10
NASO19	M	4.57 ± 0.30
NASO20	F	8.18
NASO21	M	1.67 ± 0.35
NASO22	F	4.84
NASO23	F	7.37 ± 0.64
NASO24	M	33.28
NASO25	M	4.05
NASO26	F	2.93
NASO27	F	15.42
NASO28	F	2.52 ± 0.07

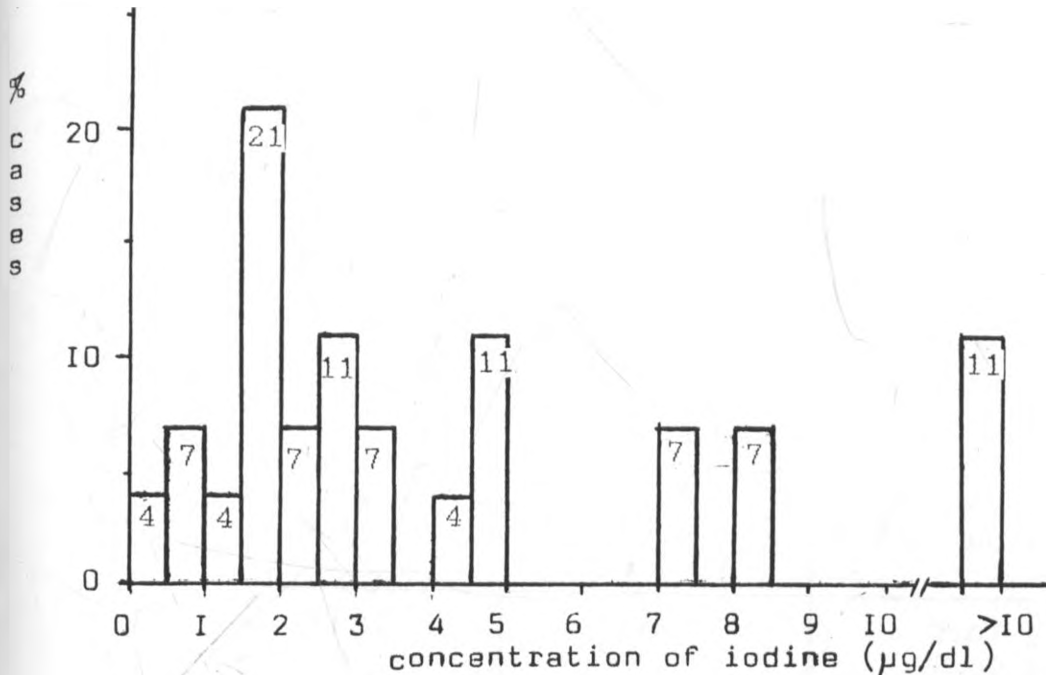


Figure 5.3: Distribution of urine iodine in samples from Nasokol school. n=28.

Figure 5.3 shows the frequency distribution of urine iodine concentrations in the samples from Nasokol. It can be noted that the median range for this group of samples is the 2.5 - 3.0 µg/dl range. The mode of the data is the 2.5 - 3.0 µg/dl range, which has 21 % of the cases. The mean was 6.73 µg/dl. However, if the three cases with extra high iodine values (15.42, 33.28, 57.58) are disregarded, the mean value becomes 3.28 µg/dl, which is a more representative value for the sample population. 75 % of the samples had urine iodine values below 5.0 µg/dl, 14 % between 5.0 and 10.0 µg/dl, while 11 % of the samples had iodine values higher than 10.0 µg/dl.

5.1.3 : Nangrotum primary school.

The results for the 27 samples obtained from Nangrotum primary school are given in table 5.3. Figure 5.4 shows the frequency distribution for these samples.

Table 5.3 : Iodine concentration in samples from Nangrotum Primary School.

Sample Code	Sex M-male F-female	Av. concentration of iodine (μg/dl). n=2
NANG1	M	2.07 ± 0.13
NANG2	F	2.05 ± 0.35
NANG2	F	2.36 ± 0.08
NANG4	M	5.36 ± 0.18
NANG5	F	1.81
NANG6	F	4.39 ± 0.36
NANG7	M	5.52 ± 0.09
NANG8	F	1.60 ± 0.43
NANG9	F	3.51 ± 0.23
NANG10	F	2.60
NANG11	F	2.99
NANG12	M	0.17
NANG13	F	1.74
NANG14	M	1.94
NANG15	F	1.64
NANG16	F	2.05 ± 0.40
NANG17	F	7.72
NANG18	M	nd
NANG19	M	6.79
NANG20	F	nd
NANG21	M	2.62 ± 0.58
NANG22	M	2.09 ± 0.03
NANG23	F	3.72 ± 0.46
NANG24	F	6.08
NANG25	F	4.27 ± 0.25
NANG26	F	12.39 ± 0.17
NANG27	M	15.79 ± 1.19

nd - iodine not detected

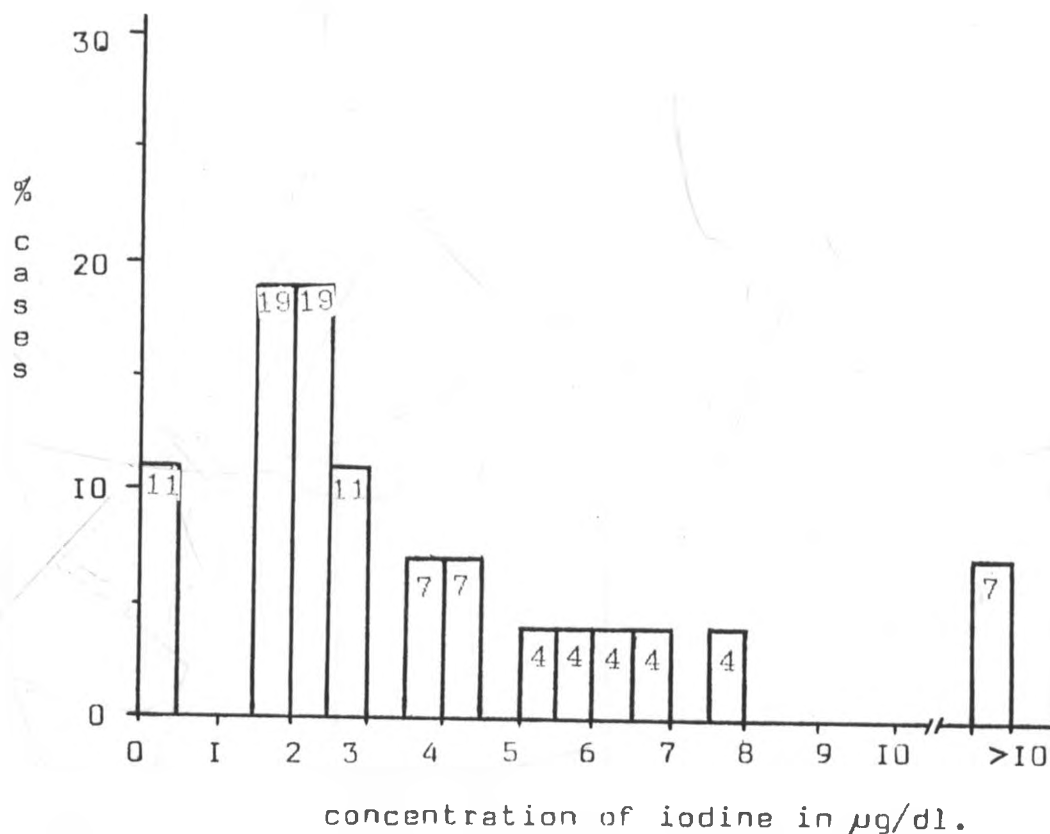


Figure 5.4 :Distribution of urine iodine in samples from Nangrotum school. n=27.

From figure 5.4, it can be observed that the median urine iodine concentration for this group of samples lies in the 2.5 - 3.0 µg/dl range, while the mode lies in range 1.5 - 2.5. The mean value was 3.82 µg/dl. 38 % of the samples fall into the mode range. 74 % of the samples had iodine values less than 5.0 µg/dl, while 7 % had values higher than 10.0 µg/dl.

5.1.4 : Makutano primary school.

The results for the 23 samples obtained from Makutano primary school are given in table 5.4. Figure 5.5 shows the frequency distribution for these samples.

Table 5.4 : Concentration of iodine in samples from Makutano Primary School.

Sample Code	Sex M-male F-female	Av. concentration of iodine (μg/dl). n=2.
MKT1	F	0.42
MKT2	M	0.26
MKT3	M	3.45
MKT4	M	1.83 ± 0.10
MKT5	M	7.94
MKT6	M	1.40 ± 0.04
MKT7	M	1.70
MKT8	F	5.54 ± 0.00
MKT9	M	5.45
MKT10	F	34.64 ± 7.47
MKT11	F	nd
MKT12	F	6.75 ± 1.46
MKT13	M	10.87
MKT14	M	14.43
MKT15	F	58.51
MKT16	M	15.33 ± 0.34
MKT17	M	4.82 ± 3.64
MKT18	M	5.17 ± 0.53
MKT19	F	19.00 ± 2.13
MKT20	M	22.21
MKT21	M	7.07
MKT22	F	18.78 ± 0.00
MKT23	F	25.91

nd - iodine not detected.

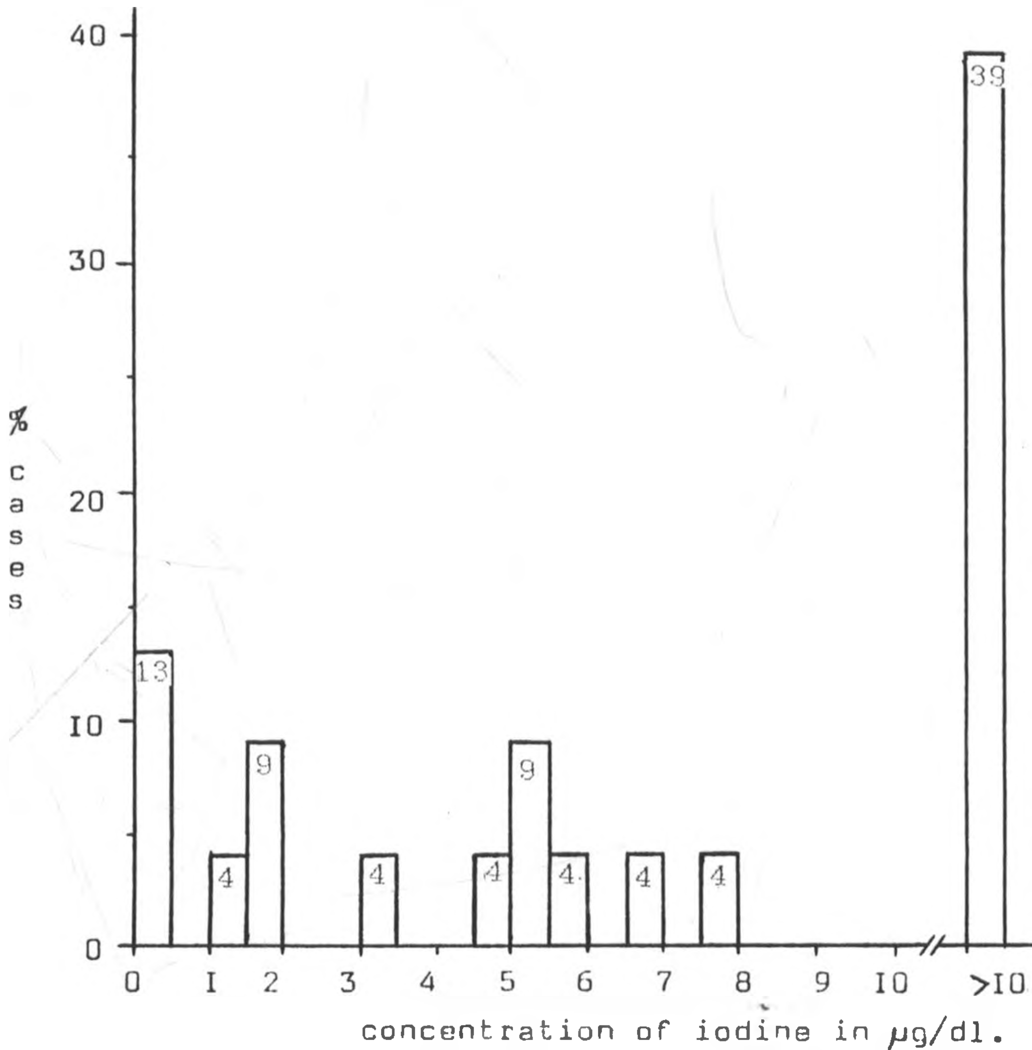


Figure 5.5 : Distribution of urine iodine in samples from Makutano school. n=23.

It can be observed that for the samples from Makutano, the median urine iodine is in the 5.5 to 6.0 µg/dl range, while the mode lies above 10.0 µg/dl. The mean iodine concentration was 11.80 µg/dl. Only 34 % of the samples had urine iodine values lower than 5.0 µg/dl. 39 % of the samples had iodine values higher than 10.0 µg/dl.

5.1.5. DISCUSSION.

Figure 5.6 shows a comparison between the data from the four primary schools. For most of the samples, the iodine value was below 3.0 µg/dl, with the exception of samples from Makutano Primary School.

Makutano lies in a semi-urban area. The population is mixed, with the larger fraction being from communities from other parts of the country. Only a small fraction of the Makutano residents are Pokots, the tribe indigenous to this region. The other three schools have a high percentage of local people. Since the feeding habits of the "foreigners" are not necessarily the same as those of the local residents, this might account for the good iodine values in the samples obtained from Makutano Primary.

There was no significant difference ($p < 0.01$) between the samples from the other three schools, i.e. Kammorou, Nasokol and Nangrotum. Both Nasokol and Nangrotum had median urine iodine levels in the range 2.5 - 3.0 µg/dl. Kammorou had a median urine iodine concentration in the range 2.0 - 2.5 µg/dl.

Iyengar et al. (1978) gives values ranging from 6.6 to 38.8 µg/dl. These values were for healthy adult specimens. Based on these values, it would seem that the urine iodine values for this region are low. However, it should be noted that in this work, Fraser's T index, or extra renal disposal rate index (Underwood, 1962) was not used. In Fraser's

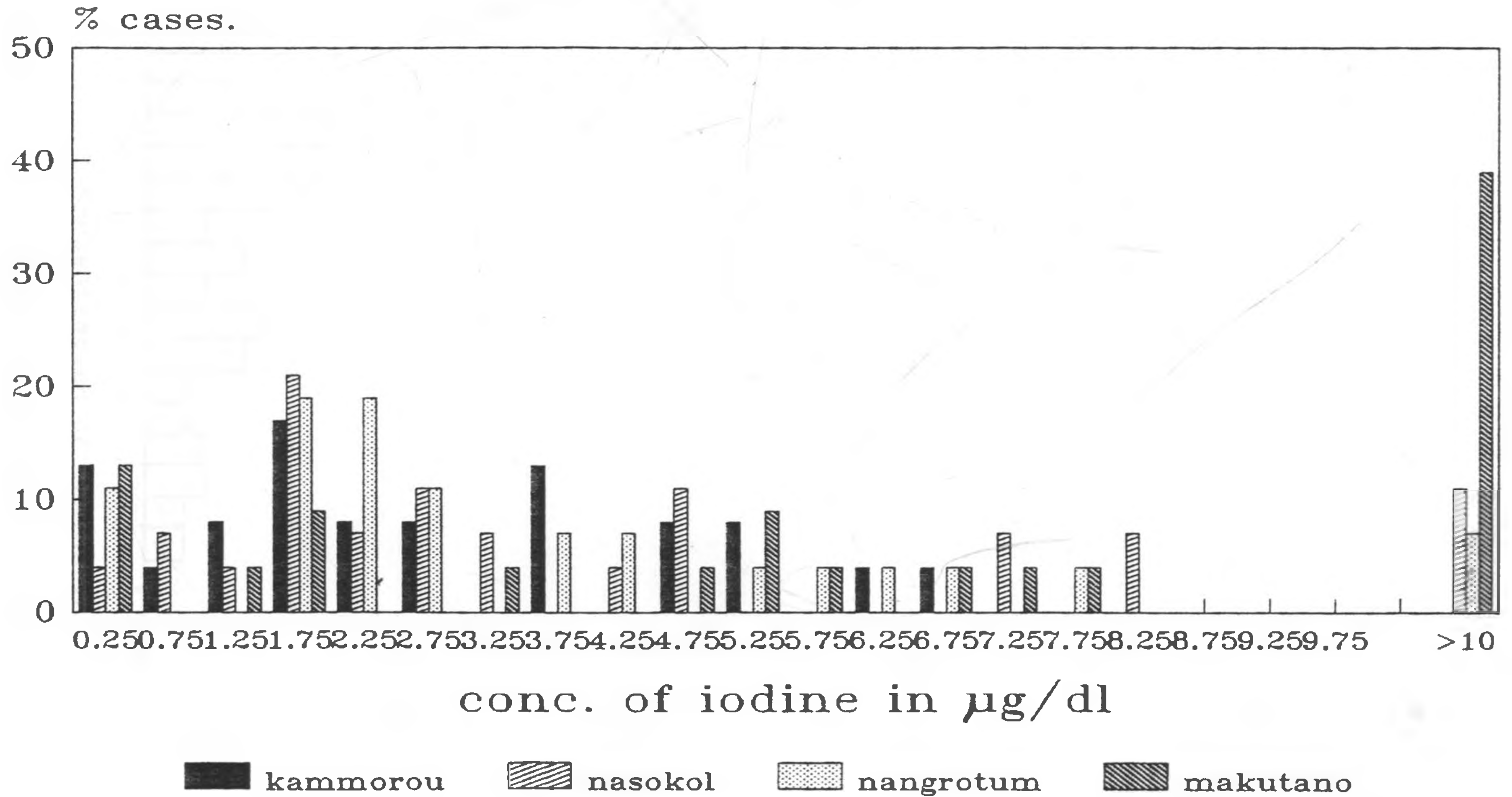


Fig. 5.6 : A comparison between urine iodine distributions in the four schools. (The conc. axis is labelled in terms of the mid-range value).

technique, consecutive urine samples are collected from each specimen for a period covering 24 hours. Due to reason of cost and logistics, this procedure was not followed, and only one sample was collected from each specimen. As such, the results as given only give a rough idea of the iodine situation in the area, but they do indicate that the iodine values of the subjects in this area are low.

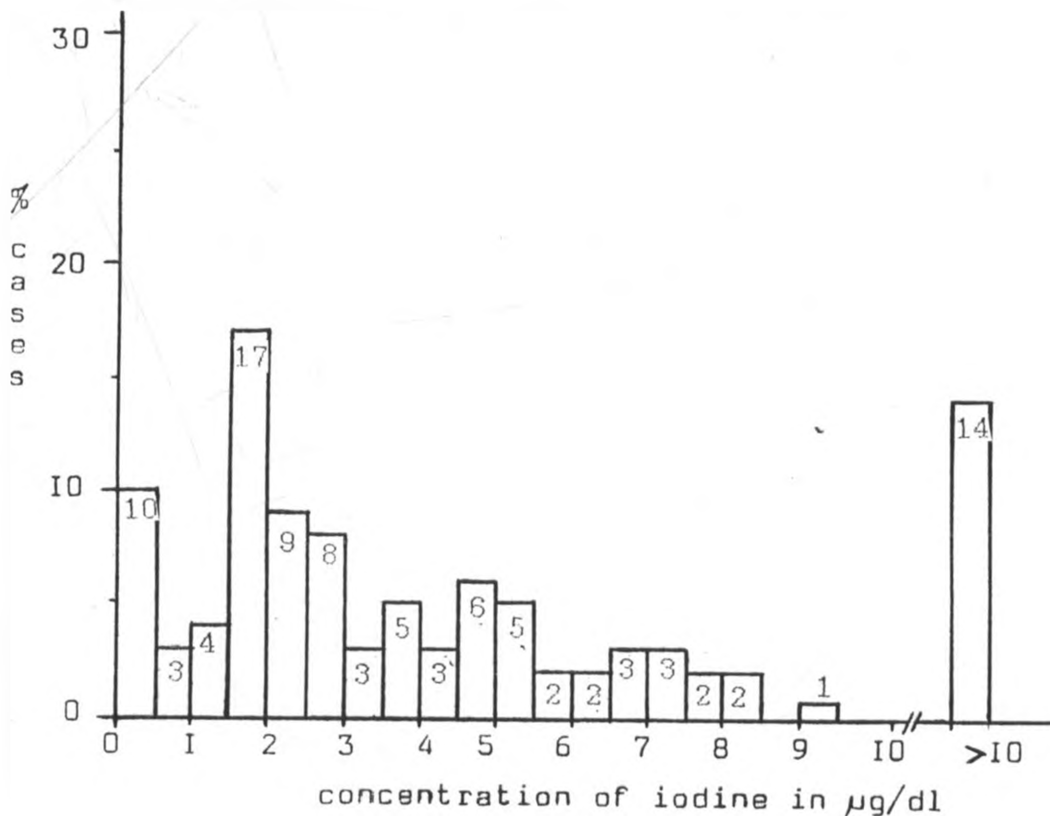


Figure 5.7 : Distribution of urine iodine in the pooled samples. n=IO2.

Figure 5.7 has been derived from the pooled data for the four primary schools. It can be noted that the median urine iodine concentration for the region lies in the range 2.5 - 3.0 µg/dl. 66 % of the cases had iodine values below 5.0 µg/dl.

Figure 5.8 shows a comparison between the data for males and that for the females. For males, the median urine iodine concentration lies in the 3.5 - 4.0 µg/dl range. For females, the median concentration lies in the 2.5 to 3.0 µg/dl range. 63 % of the males had iodine value less than 5.0 µg/dl, compared to 70 % for the females. This shows that within the age group sampled, there was a slight difference ($p < 0.01$) between the iodine values for the males and for the females, with the males having more iodine in their urine.

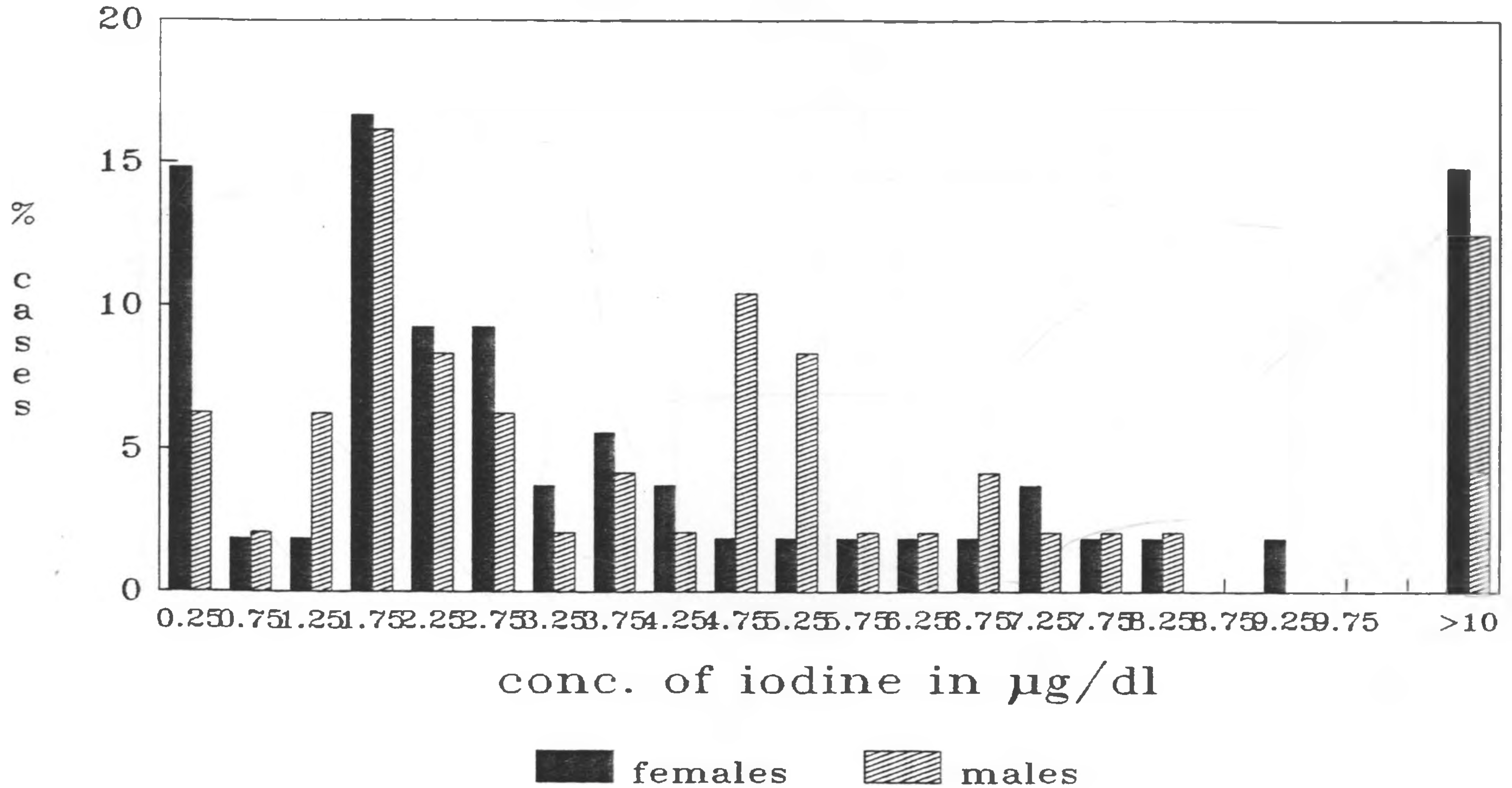


Fig.5.8 : Comparison between iodine distribution in males and females.
(The conc. axis is calibtrated in mid range values).

5.2 : WATER SAMPLES.

Table 5.5 gives the results for the water samples obtained from the sources available at each of the four primary schools.

Table 5.5 : Iodine concentration in water samples.

Name of school	Concentration of iodine in micrograms/dl		
	Sample A	Sample B	Av. conc
Kammorou (spring)	0.13±0.20	0.12±0.26	0.125±0.33
Nasokol (tap)	0.35±0.22	0.35±0.23	0.35 ± 0.32
Nangrotum (spring)	0.10±0.20	0.16±0.25	0.13 ± 0.32
Makutano (spring water)	0.29±0.19	0.33±0.23	0.31 ± 0.30
Makutano (Tap water)	0.19±0.28	0.18±0.28	0.185±0.40

There was no significant difference ($p < 0.01$) between the iodine values of the five water samples. All the samples fall short of the minimum value of 1.5 µg/dl indicated by Underwood (1962) for goitre-free areas.

5.3 : SALT SAMPLES.

Table 5.6 gives the iodine concentrations in parts per million for salt samples. The name in brackets below the trade name indicates the point of purchase of the salt. The

samples X1 to X5 were obtained from passengers flying in various Airlines. The Airline salts were in sealed satchets of about 2 grams. All the other samples were in 500g packets.

Table 5.6 : Iodine concentration in salt samples.

Trade name	Concentration of iodine in ppm			Label claim (ppm)
	Sample A	Sample B	Av. conc.	
Malindi (fine) (Makutano)	2.26±0.35	2.42±0.05	2.34±0.35	100
Malindi(coarse) (Makutano)	1.73±0.06	2.44±0.14	2.08±0.15	100
Ishwa (Makutano)	4.40±0.27	4.02±0.01	4.21±0.27	100
Kensalt (Nairobi)	4.20±0.27	5.00±0.27	4.60±0.38	100
Barit (Nairobi)	3.87±0.27	4.30±0.27	4.09±0.38	100
Jodsalz (Germany)	2.99±0.11	2.95±0.14	2.97±0.18	ni
Solana (Yugoslavia)	2.58±0.08	3.18±0.24	2.88±0.25	ni
Giant (USA)	6.30±0.05	6.73±0.41	6.52±0.41	100
X1	73.91±2.97	64.20±3.05	69.05±4.26	ni
X2	49.08±1.83	38.51±1.53	43.79±2.39	ni
X3	12.57±2.16	12.57±2.43	12.57±3.25	ni
X4	48.38±2.79	43.06±2.86	45.72±4.00	ni
X5	29.44±1.63	33.47±3.70	32.46±4.04	ni

ni - iodine content not indicated on the label.

From table 5.6, it can be observed that all the Kenyan salts have iodine concentrations below 5 parts per million (ppm), much lower than the labelled 100 ppm. The foreign salts which were in large packets also had a low iodine value. The only samples with slightly high iodine values were those obtained from the Airline satchets. This difference can be attributed to the method of packaging, storage and duration of stay of the sample before it was analysed. The Airline salt satchets have packaging which is different from that of the other samples.

There is a possibility that not all the iodate in the salt was converted to iodide during specimen preparation, and hence was not precipitated. This might explain the generally low iodine values observed above. Further tests are therefore necessary to confirm the results for the salt samples.

CHAPTER SIX.

CONCLUSION.

6.1 : THE METHOD.

A method was developed for the determination of iodine in various kinds of samples. After digestion of the sample with chromic acid, iodine was precipitated from the solution using a solution of palladium chloride. The precipitate was analysed using Energy - Dispersive X-Ray Fluorescence Analysis (EDXRFA). The method was found to be sensitive to concentrations as low as 1.0 micrograms per decilitre (μg/dl) or 10 parts per billion (ppb). The method was found to have an accuracy of between 50 and 80 % for iodine concentrations below 5.0 μg/dl, but for concentrations higher than 5.0 μg/dl, the accuracy was above 90 %.

The method was used to analyse urine samples (102), water samples (5) and salt samples (13).

6.2 : URINE SAMPLES.

A total of 102 urine samples were analysed for iodine. 24 of the samples were from Kammorou, 28 from Nasokol, 27 from Nangrotum and 23 from Makutano primary schools.

The median urine iodine levels were obtained as follows;

School	Median Range (μg/dl)
Kammorou	2.0 - 2.5
Nasokol	2.5 - 3.0
Nangrotum	2.5 - 3.0
Makutano	5.5 - 6.0

The combined data had a median range of 2.5 - 3.0 μg/dl.

Using urine iodine concentration data, Hetzel (1990) categorizes Iodine Deficiency Disorders (IDD) into three groups;

(i) mild IDD for areas with median urine iodine levels in the range 3.5 - 5.0 μg/dl.

(ii) moderate IDD for areas with median urine iodine levels in the range 2.0 - 3.5 μg/dl.

(iii) severe IDD for regions with median urine iodine levels below 2.0 μg/dl.

From this classification, it can be concluded that Kammorou, Nasokol and Nangrotum fall in the second category i.e. moderate IDD areas. The median for the pooled data indicates the region as falling under the moderate IDD category.

6.3 : WATER SAMPLES.

All the five water samples analysed had iodine levels lower than 0.4 μg/dl. The Kammorou spring water had an iodine content of 0.125 μg/dl, Nasokol tap water had 0.35 μg/dl, Nangrotum spring water 0.13μg/dl, Makutano tap water

0.185 $\mu g/dl$, while the Makutano spring had water with an iodine content of 0.31 $\mu g/dl$.

Underwood (1962) indicates that for goitre free areas, the iodine content of drinking water should be above 1.5 $\mu g/dl$. Thus judging from the water iodine levels, the area can be considered as goitrous.

6.3 : SALT SAMPLES.

All the five Kenyan salts analysed had an iodine content lower than 5 parts per million. The manufacturers claim the salt to be containing 100 parts per million. The salts of foreign origin also had an iodine content lower than 5 ppm, with the exception of Giant salt from the United States, which had 6.5 ppm, 6.5 % of the label claim. Only the Airline salt satchets had a high concentration of iodine. The low iodine values may have been due to incomplete conversion of the iodate in the salt to iodide before precipitation.

The Airline salt satchets were in double layered paper bags. The foreign salts were in tins made of hard paper (with the exception of Solana which was in a plastic container). Kenyan salt were in polythene bags. The results tend to give the impression that the mode of packing the salt is crucial to the retention of iodine. The decrease of the iodine content of salt with time should be studied comprehensively, with the aim of establishing better packaging methods, or establishing a " Use By " period for the salt.

REFERENCES

Abott (1932) : Quoted in : Axelrad, A.A., Leblond, C.P. and Isler,H. Endocrinology 56 : 387 (1955).

Ahlgren, L., Christofferson, J.O.,Gronberg,T and Mattson, S. (1982) : In-vivo determination of iodine in the thyroid. In : X-ray Fluorescence (XRF & PIXE) in medicine. Field Educational, Italia.

Alnwick, D.J.(1988) : Prevention of iodine deficiency disorders in Kenya - a preliminary investigation of the iodine content of salt on sale in Western Kenya. E. Afr. Med. J. 65/11/723.

Andersen, C.A.(1966) : Electron probe microanalysis of thin layers and small sections with emphasis on light element determination. In: The Electron Microbe. McKinley, T.D., Heinrich, K.F.J. and Wittry, D.B. (Eds). John Wiley, New York.

Astwood, E.B. (1946): Ann. Int. Med. 30 : 1087.

Bailer Jr, J.C., Emeleus, H.J., Nyholm, R., Trotman-Dickenson, A. F.(1973) : Comprehensive inorganic chemistry. Pergamon Press, London.

Belcher, R. (1956) : New methods in analytical chemistry. Reinhold Publishing, New York.

Belcher, R. (1960) : Quantitative inorganic analysis. Butterworths, London.

Bertin, E.P. (1975) : Principles and Practices of X-Ray Spectrometric Analysis. Plenum Press, New York.

Bertin, E.P. (1978) : Introduction to X-ray Spectrometric Analysis. Plenum Press, New York.

Biffen, F.M. and Seaman, W. (1956) : Modern instruments in chemical analysis. McGraw-Hill, New York.

Bock, R. (1979) : A Handbook of Decomposition Methods in Analytical Chemistry. International Textbook Company, New York.

Bohdal, M., Gibbs, N.E. and Simmons, W.K.(1968) : Nutrition survey and campaign against malnutrition in Kenya, 1964 - 1968 : Report to the Ministry of Health of Kenya, Nairobi.

Bohdal, M., Gibbs, N.E. and Simmons, W.K.(1969) : Nutrition survey and campaign against malnutrition: Report to the Ministry of Health of Kenya on the WHO / FAO / UNICEF assisted project. Mimeo, Nairobi.

Bohdal, M., Gibbs, N.E. and Simmons, W.K.(1968) : Incidence of endemic goitre in Kenya and its prevention : Report to the Ministry of Health of Kenya on the WHO / FAO / UNICEF assisted project, Nairobi.

Butterfield, I.H., Black, M.C., Hoffman, M.J., Mason, E.K. and Hetzel, B.S. (1965) : Correction of iodine deficiency in New Guinea natives by Iodized Oil Injection. Lancet ii : 767.

Chensey, A.M., Clawson, T.A. and Webster, B. (1928) : Bull. John Hopkins Hosp. 43 :261.

CIEB, (1952) : Chilean Iodine Educational Bureau : Iodine content of foods, London.

CRSP study, Embu, (1987) : Final Report - University of California, Los Angeles.

Cummings, A.C. (1956) : Quantitative chemical analysis. 11th Edition. Oliver and Boyd, London.

Delange, F. (1985) : Physiopathology of iodine. In : Trace Elements in Children. R.K. Chandra (Ed). Nestle Nutrition. Vevey / Raven Press, New York.

Ermans, A.M., Bourdoux, P., Kinthaert, J., Lagasse, R., Luvivila, K., Mafuta, M., Thilly, C.H. and Delange, F. (1983) : Role of cassava in the etiology of endemic goitre and cretinism. In : Cassava toxicity and thyroid : Research and Public Health Issues". Proceedings of a workshop held in Ottawa, Canada, 31 May - June 2, 1982. (Eds) Delange F. and Ahluwalia H. IDRC - 207c.

Gitau, W. (1988) : A review of iodine deficiency disorders in Kenya. E. Afr. Med. J. 65 : 727.

Golden, B.E. and Golden, M.H.N. (1991) : The detection of trace element deficiencies in the Third World. In : Trace Elements in Man and Animals 7 (TEMA 7). Momcilovic, B.(Ed). IMI, Zagreb.

Goulding, F.S. and Jaklevic, J.M. (1973) : Photon excited Energy Dispersive X-Ray Fluorecence Analysis for trace elements. In : Annual Review of Nuclear Science Vol.23. Annual Reviews Inc., Palo Alto, California.

Hanegraaf, Th. A.C., and McGill, P.E. (1970) : Prevalence and geographical distribution of endemic goitre in Eastern Africa. E. Afr. Med. J. 47 : 61.

Hanegraaf, Th. A.C., McGill, P.E. and Taylor J.R. (1971) : Endemic goitre and its prevention in Kenya. In : Health and Disease in Africa. East African Literature Bureau, Nairobi.

Hanegraaf, Th. A.C. (1974) : Population based studies of endemic goitre. In : Health and Disease in Kenya. (Ed) L. C. Vogel. East African Literature Bureau, Nairobi.

Hanegraaf, Th. A.C. (1977) : Endemic goitre in Kenya : An intermediate evaluation of an experimental control programme. E. Afr. Med. J. 64 : 40.

Hetzel, B.S. (1983) : Iodine Deficiency Disorders (IDD) and their eradication. Lancet 2 : 1126.

Hetzel, B.S. (1990) : The effects of iodine deficiency at the population level. Paper presented at the conference on "Trace Element Research in the Third World". 16 - 18 May 1990. Inter-University Centre, Dubrovnik.

Hetzel, B.S. (1990) : The effects of iodine deficiency during fetal development.Paper presented at the conference on "Trace Element Research in the Third World". 16 - 18 May 1990. Inter-University Centre, Dubrovnik.

Hillebrand, W.F. (1953) : Applied Inorganic AnaLysis. John Wiley, New York.

Hindu International Edition (HIE), October 19, 1990.

Ingbar, S.H. and Woeber K.A. (1977) : Disease of the thyroid. In : Harrisons Principles of Medicine. G. W. Thorn, R.D. Adams, E. Braunwald, K.J. Isselbacher and R.G. Petersdorf (Eds). McGraw - Hill, Tokyo.

International Atomic Energy Agency (IAEA) (1985) : Quantitative XRFA Software. IAEA, Vienna.

Iyengar, G.V., Kollmer, W.E. and Bowen, H.J.M. (1978) : The elemental composition of human tissues and fluids. Verlag Chemie Weiheim, New York.

Jacobson, C.A.(Ed)(1956) : Encyclopedia of Chemical Reactions. Volume III. Reinhold Publishing, New York.

Jansen, A.A.J., Horelli, H.T. and Quinn, V.J.(Eds) (1987) : Food and Nutrition in Kenya : A historogical review. Dept. of Community Health, Faculty of Medicine, University of Nairobi.

Jenkins, R., Gould, R.W. and Gedke D. (1981) : Quantitative X-ray Spectrometry. Marcel Dekker, New York.

Kenya Subsidiary Legislation Part XXII - Salt, Para. 299,1978.

Kinyua, A.M.(1982) : Multielemental analysis of solid and liquid samples by X-ray Fluorescence Analysis. MSc Thesis, University of Nairobi.

Knoll, G.F.(1979) : Radiation detection and measurement. John Wiley, New York.

Koutras, D.A., Papapetrou, P.D., Yataganas X. and Malamos, D. (1974). Am. J. Clin. Nutrition 23 : 870.

Laititen, H.A. (1960) : Chemical analysis : An advanced text and reference. McGraw-Hill, New York.

Laws of Kenya Cap. 254, 1980 : Republic of Kenya, Food, Drugs and Chemical Substances (General Regulations), rev. 1980, paras 398 - 400.

Legal Notice No.189 (1988) : Republic of Kenya, Kenya Gazette, 8th April 1988.

Mangala, M.J. (1987) : Multielemental Energy Dispersive X-ray Fluorescence Analysis of Kerio Valley Ores and Mrima Hill Soil sediments. MSc. Thesis, University of Nairobi.

Mannar, V.M.G.(1987) : Control of iodine deficiency disorders through iodation of salt. Report to the World Health Organisation, Nairobi.

Marshall, A.T.(1980) : Principles and Instrumentation. In : X-ray Microanalysis in Biology. M.A. Hayat (Ed). University Park Press, Baltimore.

Mills, C.F. (1990) : The predictive value of geochemical and soils data for detecting trace element deficiency and excess. Paper presented at the conference on "Trace Element Research in the Third World", 16 - 18 May 1990. Inter-University Centre, Dubrovnik.

Mitchell, H.S. (1974) : Recommended dietary allowance up to date. J. Am. Diet. Assoc. 64 : 149.

Moseley, H.G.J. (1912) : The high frequency spectra of the elements. Phil. Mag. 26 : 1024.

Nagj, M., Jaksic, M., Orlic,I. and Valkovic, V. (1985) : Sample preparation techniques in trace element analysis of water. Nuclear Instruments and Methods in Physics Research, A236 : 563. North - Holland, Amsterdam.

Obel, A.O.K., (1982) : Goitre and Fluorosis in Kenya. E. Afr. Med. J. 59 : 363.

Scot, W.W.(Ed) (1920) : Standard Methods of Chemical Analysis. D. van Nostrand, New York.

Sehmi, J.K.(1974) : Report on the iodine content of salt and fluoride content in water in Kenya, 1971-1974. National Public Health Laboratories Document, Nairobi.

Seki R., Kimura, E., Takahashi, T., Ikeda, N. (1990) : J. Radioanal. Chem. Articles 138 : 17.

Stanbury, J.B., Ermans, A.M., Hetzel, B.S., Pretell, E.A. and Querido A. (1974) : Endemic goitre and cretinism : Public health significance and prevention. Wld. Hlth. Org. Chron. 28 : 220.

Stanbury, J.B. (1986) : The iodine deficiency disorders : Introduction and general aspects. In : Prevention and control of iodine deficiency disorders. (Eds) Hetzel, B.S., Dunn, J.T., and Stanbury, J.B. Elselvier, Amsterdam.

Storm, E. and Israel, H.I. (1970) : Nuclear Data Tables. A7.

UNIVERSITY OF NAIROBI
CHIROMO LIBRARY

Sparks Jr. C.J. (1975) : Quantitative XRFA using FPT . In : Advances in X-ray Analysis. Gould, R. W. (Ed). Plenum Press, New York.

Task Force for the Prevention and Control of Iodine Deficiency Disorders in Africa. Report on second meeting, Addis Ababa, December 1987.

Underwood, E.J. (1962) : Trace Elements in Human and Animal Nutrition. Academic Press, New York.

Underwood, E.J. (1977) : Trace Elements in Human and Animal Nutrition. Academic Press, New York.

Vogel, A.I. (1954) : Macro- and semi-micro qualitative inorganic analysis. 4th Ed. Longmans, London.

Vogel, A.I. (1961) : A textbook of qualitative inorganic analysis. 3rd Ed. Longmans, Green and Company, London.

Vought, R.L., London, W.T., Brown, F.D., Eckloff, J.C. and Murphy, R.S. (1964) : Iodine intake in healthy non-hospitalised subjects. Am. J. Clin. Nutr. 15 : 124.

World Health Organisation (WHO) (1973): Trace Elements in Human Nutrition. Technical Report Series No. 532. WHO, Geneva.

Wouters, J.S.M. (1991) : MOH, Kapenguria District Hospital. Personal Communication, February 1991.

UNIVERSITY OF NAIROBI
CHIROMO LIBRARY