MAGNESIUM IN A BIOLOGICAL STANDARD MATERIAL

METHODS VARIABILITY FOR MAGNESIUM DETERMINATION IN A BIOLOGICAL STANDARD AND OTHER BIOLOGICAL MATERIALS

By

THOMAS PAUL MEYERHOF, M.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

July 1974

DOCTOR OF PHILOSOPHY (1974) McMASTER UNIVERSITY
(Chemistry) Hamilton, Ontario

TITLE: METHODS VARIABILITY FOR MAGNESIUM DETERMINATION IN A BIOLOGICAL STANDARD AND OTHER BIOLOGICAL MATERIALS

AUTHOR: Thomas Paul Meyerhof, B.Sc. (Dalhousie University),
M.Sc. (Dalhousie University).

SUPERVISOR: Dr. Klaus Fritze

NUMBER OF PAGES: ix, 159

SCOPE AND CONTENTS:

The use and analysis of biological standard reference materials has increased rapidly over the past few years with the mounting concern over the environment and the upsurge in health-related chemistry. Laboratory and method intercomparisons are an essential part of any program involved in the issuance or calibration of instrumental systems. It relies on the precision and accuracy of such analyses, often for vital decisions affecting public welfare. The lack of agreement among qualified analysts can thus be viewed with some dismay.

An attempt was made to examine in some detail the determination of magnesium in several biological materials using fundamentally different methods of analysis. Three procedures, a gravimetric precipitation with oxine, atomic absorption analysis and neutron activation, the latter both non-destructive and with chemical separation, were selected for the analysis in a biological standard (Bowen's kale). The latter two methods were then applied to a homogenized rat carcass and a feces sample.

Procedures were developed which gave reproducible results for the gravimetric and atomic absorption analysis. Discrepancies in the analysis by atomic absorption with and without standard addition were studied.

Neutron activation gave results which were in good agreement with the other methods provided that care was taken to account for flux variations within the pneumatic rabbit. A best value of 1663 ± 7 ppm was ascertained for the magnesium content of the dried kale.

Analyses of the rat samples by atomic absorption and neutron activation were also in close agreement, thereby helping to establish the overall accuracy of the procedures. For these samples, best values of 1550 ppm and 7370 ppm were obtained for the carcass and feces, respectively. An existing method discrepancy was resolved.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. K. Fritze for his assistance and encouragement throughout all phases of the work contained in this thesis.

The author is also indebted to the members of his supervisory committee, Dr. A. Corsini and Dr. J. H. Crocket for their helpful comments, as well as other members of the Chemistry Department and Reactor Staff who assisted.

In addition, he wishes to thank Dr. H. J. M. Bowen of Reading University, England for the generous supply of kale and Dr. O. Héroux of the National Research Council in Ottawa for the rat samples.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

"Try to find the method of analysis, for which the accuracy of the result least depends upon the skill of the chemist performing the analysis, and when this method has been selected, ponder which avoidable circumstances are present which cause an error in the result, and whether they produce a positive or negative effect."

J. J. Berzelius (1814) -- translated.

1.1 GENERAL

It is the intention of this thesis to investigate some of the difficulties inherent in three different methods; gravimetry, atomic absorption and neutron activation analysis, for the determination of magnesium in several biological materials. That there are discrepancies between various workers analyzing a supposedly homogeneous material for particular constituents has been recognized for some time and will be discussed in the next section of this chapter.

Interlaboratory comparisons typically yield discordant results, the reasons for which are not generally resolved. This disparity occurs despite the fact that every worker involved undertakes the analysis using a high degree of care, to obtain the best possible accuracy, using well-tested procedures. The values reported then, with possibly a small number of exceptions, represent the analyst's best work. The net result is thus disconcerting and deserves more attention than has been paid.

With the increase in automation and computer usage, resulting in ever greater quantities of analytical data being produced, the problem becomes increasingly critical.

To assist analysts in checking their performance, a well-calibrated standard is required. While there is a great variety of geological and metallic alloy standards to choose from,[46,91,92,260] the number of samples suitable for agriculture, health, nutrition, pollution and environmental studies, requiring an organic matrix, is much more limited. With the mounting concern in these areas, matrices such as organic and biological materials,[32,210,275] foodstuffs,[225] water[88,242] and air[3,141,284] are of increasing importance.

The first to develop a biological standard on a large scale was Bowen, who in 1965 issued the plant material, Brassica oleracea L. var. fimbriata Mill., more commonly known as marrow stem kale or kale.[29-31,35,36] Since that time, the National Bureau of Standards, Washington, D.C., in a continuing program has issued, or will issue, such biological standards as orchard leaves, tomato leaves, tuna fish, freeze-dried liver, and freeze-dried bovine serum, while at the same time expanding the number of standards suitable for the clinical chemist.[167,188] Other standards which have been proposed or prepared include flour, frozen fish, paper,[9] various dried fruit leaves,[149] bone, dried milk, dried blood and mollusc shells,[36] grasses, mixed plankton, kelp, sediments, algae and certain zoological materials.[181] No doubt the above list is incomplete, but it gives some indication of the variety of biological standard matrices available.

These various reference samples have been the subject of a number of interlaboratory studies.[10,36,147,149,166,280] Other studies

have been carried out using synthetic matrices.[9,16,60,65,84,266,267,293] The latter possibly offer a better check on the precision and accuracy of the various methods and laboratories involved. However, because of the much simpler matrices involved, the results obtained must be considered as only an indication of the true performance of the methods and laboratories.

Other more limited studies, too numerous to mention, have been undertaken using specific methods and self-prepared standards. Great care must be taken in selecting and evaluating any standard to be employed in such a study.[166,188,219,277] The limitations imposed by the use of such reference samples, as standards, have also been discussed.[219] Use of a reference sample as a standard can minimize systematic biases, but only fortuitously, random errors.[154]

Interlaboratory studies are designed to give information on one or more of the following; the accuracy and precision of an analytical method in several laboratories, comparison of various methods of analyses in several laboratories, or the establishment of a standard value for a measurement. To do this, a suitable amount of homogeneous material is issued to various collaborators who then perform the required analyses and return the results.

The analysis of this data can be undertaken in some detail. Two basic assumptions are required; that the material as issued was homogeneous and that all the methods of analysis used, have an equal likelihood of giving the most probably correct value. In the case of Bowen's kale, elaborate precautions were undertaken to ensure the homogeneity of the sample as prepared.[29] The second criterion must take into account the

differences in precision and sensitivity obtainable in the various methods used for the analyses.[161,210,239,265]

1.2 CHEMICAL STUDIES

Both geochemists and analytical chemists had been involved in "round-robin" testing long before the issuance of G-1 and W-1 standard rock samples in 1950 by the U. S. Geological Survey. However, these studies are mainly of historical interest. The first results from the granite and diabase study appeared in 1951.[83,233] Since that time, demand for these samples has depleted supplies and a further six standards were issued in 1964.[90] On the whole, with some notable exceptions, for example potassium, the precision of reported analyses has improved. Magnesium showed no change.[90] Since this thesis is concerned in particular with magnesium analysis in biological materials, a few pertinent studies will be mentioned in greater detail.

Several early collaborative studies involving elemental determinations in alfalfa and tobacco leaves were conducted by Mathis.[183,184] A total of 1184 determinations by 25 chemical methods and 3 instrumental methods -- arc and spark spectroscopy and flame emission -- were collected and statistically analyzed. Results were reported for potassium, calcium, phosphorus, magnesium and sodium. Mathis concluded that except for potassium, which was better determined by instrumental means, the instrumental methods were satisfactory, provided that great care was taken to match the standard matrix to the samples.[185]

In 1951, the American Society for Horticultural Science aided in setting up a collaborative program for the purpose of producing standardized

plant samples. Samples of prepared apple, cherry, peach and citrus leaves, as well as the "standard" alfalfa sample, were analyzed by 16 laboratories for various nutrient elements. Again routine chemical, spectrographic and flame emission methods were used. The results obtained showed satisfactory agreement for nitrogen, phosphorus, potassium, calcium and magnesium.[149] For calcium and magnesium, the results obtained by flame emission were higher than for the other methods.

In 1960, Ward and Heeney published the results of a Canadian Department of Agriculture collaborative study of methods for the determination of potassium, calcium and magnesium in plant materials.[280a] Thirteen laboratories participated in chemical and flame emission analysis of 5 types of prepared fruit leaves, as well as an acid extract of the ash of the dried samples sent each collaborator. The authors conclusions were somewhat different from those of Mathis.[183,184] Calcium and magnesium showed unacceptably high relative standard deviations by flame emission which could not be accounted for on the basis of experimental error alone. Based on the more satisfactory EDTA titrations, magnesium results appeared consistently low and calcium consistently high by flame emission.

Carpenter and coworkers published a study of the rapid spectrographic analysis of 7 plant tissue types for 11 elements including magnesium.[45] A statistical analysis of the results, all obtained in one laboratory, revealed a fairly high degree of accuracy using synthetic standards.

Isaac and Jones undertook a comparative study involving 91 samples of various leaves using atomic absorption and emission spectro-

scopy.[147] This study was conducted between two laboratories. For the eight elements investigated, significant differences (at the 1 percent level) were found for potassium and magnesium. The consistent difference between the laboratories was assumed to be due to differences in preparation and standardization techniques.

A later study by Jones of 18 laboratories with 7 similar plant species showed good agreement for potassium and magnesium among other elements.[146]

The above examples are typical of the results from interlaboratory studies. The complexity of biological and geological matrices make such comparisons both difficult and yet especially necessary.

The earliest chemical analysis of kale appears to have been conducted by Woodman, Evans and Eden.[292] Several other early studies of the approximate chemical composition of the whole plant, leaf and stem of various kales have also appeared in the literature.[81,82,235] Jones, however, was the first worker to publish a more complete study of the major kale varieties grown under controlled conditions with representative samples chosen from large populations.[144] For four marrow stem kale varieties, his average magnesium content was 2200 ± 200 ppm. Bowen's study of kale, which served as the impetus for this thesis, will be discussed separately in the next section of this chapter.

1.3 MAGNESIUM IN BOWEN'S KALE

Magnesium is an essential element in all living organisms. It has electrochemical, catalytic and structural functions, as well as activating numerous enzymes and being a constituent of all chlorophylls.[34,287]

Typically it is present in matter as a minor constituent (see Table 1.1).

TABLE 1.1 Concentration of Magnesium in Various Materials

Material	Range (ppm)
Plants	800-7000[33]
Soils	600-6000[33]
Rat Muscle	250[178]
Rat Bone	900[178]
Human Plasma	50[4]
Human Bone	1500,[33] 5000[179]

The chemistry of magnesium in plants has been extensively reviewed,[15,110,175,205] and the general principles of leaf analysis in relation to the nutritional status of plants have been discussed by various authors.[37,110,175,264,274] Because of its essentiality in living matter, the accurate determination of magnesium is of great importance.

In 1965, Bowen issued a carefully prepared biological material, kale, for use as a comparative reference material.[29,30] The results of this study were later summarized and showed numerous inconsistencies between laboratories, not only for trace elements, but for major constituents as well.[35,36] The results obtained by 18 laboratories for magnesium are shown in Table 1.2, page 8, together with the number of individual determinations in brackets. Bowen's weighted estimate of a best value is also given. It is unfortunate that the individual values and details of the analytical methods are not given in these papers for it is apparent that certain results differ greatly from the average.

TABLE 1.2 Collaborative Results Given by Bowen for Magnesium in Bowen's Kale[35,36]

Analytical Technique[1]	Results[2] (ppm)	Weighted Average[3] (ppm)	Bowen's Comments
Activation Analysis	1350 ± 186(6),1605(1)	1390(7)	
Atomic Absorption	1670(4),1600(4),1610(4),1700(5) 1570(4),1600(1),1560(9),1640(6) 1650(1)	1610 ± 72(31) 1615(38)	
Colorimetry	1540(4),1530(4)	1535 ± 142(8)	
Flame Photometry	3460(5)	3460(5)	too high
Spectrometry	1140 ± 155(5).	1140 ± 155(5)	too low
Volumetric Analysis	1700 ± 271(4),1450(3),1550(4)	1580(11)	
Bowen's Weighted Best Value	1604 ± 119(43),[35] 1600[36]		

1. As described in Bowen's compilations.
2. Bracketed values represent the number of determinations; standard deviation of a single determination is given where reported by Bowen.
3. Values calculated from Bowen's data. Those showing a standard deviation were taken from Reference 35.

The standard deviations shown give an indication of this. Indeed, various weighted "best" values are possible if one omits the flame photometry, spectrometry and neutron activation analysis results, as Bowen has done. An average based either on the method averages or on all the individual results may be calculated. Bowen failed to be more explicit in his descriptions of the individual techniques used. The method terminology given in Table 1.2 is that used by Bowen.

That the results are somewhat unsatisfactory can be seen by examining Table 1.3, page 10. The values given reflect the desired accuracy for a carefully conducted analysis. The allowable error figures given must be considered only approximate, since the accuracy obtainable will vary from element to element, with the complexity of the sample and the type of analysis. Still, the magnesium results have an error, based on two standard deviations, of about 15 percent, which is rather higher than the tabular value of 50 ppt.

Examining individual analytical methods used by Bowen's collaborators, several observations can be made. From Bowen's earlier report, showing individual laboratory codes for all results obtained to that time, it is apparent that no worker analyzed the kale for magnesium by more than one method. Furthermore, one technique conspicuously missing is gravimetry. This is probably due to the slowness of the method when compared to the more modern technique of atomic absorption which was, by far, the most popular procedure used. It is understandable when, in a study involving a large number of elements (over 50 were quantitatively analyzed in Bowen's report[36]), rapid methods are preferred. However, for major elements, including magnesium, the expected accuracy of the

TABLE 1.3 Allowable Errors for a Chemical Analysis[78,269]

Percentage of Constituent	Permissible Error (Parts per Thousand)
100	1-3
10	10
1	10-20
0.1	50
0.01	100
0.001	100-1000

gravimetric techniques is superior to comparative instrumental methods, and considering the importance of the study, this technique should have been applied.

The lack of agreement between the two sets of activation analysis results is disappointing. All other methods showed a much greater consistency between various workers. Bowen, in fact, rejected the lower result in calculating his best value. Atomic absorption, too, shows a larger range than might be expected, though Bowen deems the work satisfactory.

Colorimetric and volumetric results, presumably via EDTA titrations, both appear to give acceptable results. EDTA titrations would not be expected to produce as high a precision as some of the other methods, since the calcium to magnesium ratio is about 25 to 1 and magnesium is usually determined by difference, that is, the total alkaline earths minus the calcium titer.

Both the single flame emission set and the single spectrometric

set of results have been rejected by Bowen. This seems reasonable when it is considered how large the deviations are from the "best value", and that both of the analysts involved had more erratic results for all elements, than the average, as reported in the 1967 paper.

One source of error for the analysis of any hygroscopic material is the drying technique used. Bowen recommended oven drying at 90°C for 20 hours, with a resultant weight loss of about 5.5 percent for the kale issued by him.[35] A weight loss plot of various temperatures from 60°C to 100°C showed that at 60°C or 75°C samples reached approximate equilibrium after about 10 hours with a 4 and 5 percent weight loss, respectively, while at 90°C approximate equilibrium was reached after 6 hours with a 5.5 percent loss. At 100°C, while the major weight loss occurred during the first hour, there was a further linear decrease in weight, indicating some decomposition of organic matter.

Because kale contains about 0.16 percent magnesium, a variety of analytical techniques is available for its determination. For the present work, three techniques (atomic absorption, gravimetry and neutron activation analysis) were studied in detail. Gravimetry was selected because it is generally considered to be the most accurate method available for the determination of magnesium. Atomic absorption was chosen as it is the most widely used technique for magnesium. Neutron activation analysis was employed as it is unique in being free from chemical interference. The three methods will be described in greater detail in later subsections.

A brief summary will be given, of the commonly used methods which were rejected. Emission spectrography and colorimetric methods, widely used for magnesium in plant analysis, were both rejected on the

basis of lack of inherent accuracy. Emission spectrography is sensitive to matrix effects and while it apparently has been successfully used by several workers in a direct-reading mode,[12,45,145,147] it is generally agreed to be less reproducible than atomic absorption,[147] though its speed makes it attractive for routine work.[12]

Colorimetric techniques typically use the dye Titan Yellow (or the similar dye Thiazole Yellow also known as Clayton Yellow) to form an adsorption colloid with magnesium hydroxide.[38,39,43,51,56,74,124,151,165,281] While this method is popular for plant materials, the dye tends to form rather unspecific lakes with magnesium and itself consists of several more or less reactive components. The original method, formulated by Kolthoff,[157] has had many different modifications[38,39,51,56,124,151,165] and is claimed to be as precise as atomic absorption under certain conditions.[124] In general, it is difficult to obtain accurate and reproducible results with lake-forming compounds because of variations in dyestuff samples, impurities in protective colloids, precipitation of the lake, rapid fading of the color and the uncertain effect of other metals.[128,271]

The use of Eriochrome Black T (EBT) as a colorimetric reagent, has also been advocated.[186,189,211,212,247,299] Probably its main advantage is speed, with an accuracy of 4 percent claimed.[139] A variety of other colorimetric methods, not mentioned here, have also been proposed, but they do not appear to offer any real advantages in the present situation.[249]

Volumetric methods offer several possibilities for the determination of magnesium in kale. Probably the most common is the titration with ethylenediamine tetraacetic acid (EDTA).[70,95,215,282] For plant analysis, this usually involves determination of magnesium by difference;

that is, a titration for the total alkaline earths present at pH 10 and another titration at pH 12 with magnesium precipitated as the hydroxide, to determine calcium.[52,66,69,100,182,206,232,246,281,290] Because of the non-selectivity, various forms of removal of the interfering ions have been tried, including precipitation,[53,66,206,246] ion exchange[133,182,254] and solvent extraction,[53,100,206] in addition to the usual masking agents. Removal or masking of the large amounts of calcium (40,000 ppm) and phosphate (14,000 ppm) present, relative to the magnesium (1600 ppm) would possibly jeopardize the accuracy of the method.[5] Several preliminary experiments, by the author, showed that there were end-point uncertainties upon removing the phosphate with molybdate and the calcium with oxalate by several methods. Even then, at least one gram of kale would be required per titration, unless micro-titrations using a photometric end-point were to be used.[94,133,152] As the equivalent ratio of calcium to magnesium was 15 to 1, assuming 45 ml of EDTA were used for 1 g to obtain the total alkaline earth content; the magnesium determined by difference would have a standard deviation of 2 percent based on the buret reading error alone. Furthermore, a study of the accuracy of EDTA titrations for calcium and magnesium, revealed that the standard procedures[282] required modification and even then a comparison of magnesium results for NBS limestone and dolomite indicated slight differences when checked against a gravimetric method using pyrophosphate.[174] At low concentrations, gravimetric results appeared to agree more closely with the certified values.

1.3.1 Destruction of the Organic Matrix

For many analytical methods, it is first necessary to destroy

the matrix and dissolve the sample. In the case of biological samples, the most frequently used techniques are either wet digestion or dry ashing followed by dissolution of the inorganic residue.

Although both wet digestion and dry ashing are often interchangeably used for biological materials, the latter method is frequently precluded due to the volatility of certain elements.[209] While this is a problem for several elements, compounds of magnesium are generally regarded as non-volatile at ashing temperatures of around 500°C.[113] Gorsuch has noted that magnesium and the alkaline earths in general are the least demanding when considering methods for the removal of organic matter.[113]

Samples for magnesium analysis have been ashed by both wet and dry ashing techniques[64,115,261,262] and the relative merits of the two have been discussed by a number of workers.[2,57,108,112,132,263] Both methods were employed for the present work.

1.3.2 Gravimetric Analysis

Gravimetry is the oldest branch of quantitative analysis. It relies on the essentially complete precipitation of the component to be determined, as free as possible from any contaminants; a fundamentally simple though practically difficult objective. It is generally acknowledged that the most reliable results for magnesium are those obtained by gravimetric methods,[155,252] and for this reason this technique was selected as the standard reference method in the present work.

Two procedures are widely used for the gravimetric determination of magnesium; precipitation as magnesium ammonium phosphate followed by

ignition of the precipitate to magnesium pyrophosphate, and precipitation as magnesium 8-hydroxyquinolinate (oxinate) which is weighed either as the dihydrate or as the anhydrous form.[159] Both are standard methods for magnesium and were investigated carefully before deciding on the latter, which is acknowledged to be the most accurate for the determination of 1 to 20 mg of magnesium.[155]

1.3.2.1 Removal of Interfering Ions

Although determination of magnesium by precipitation as magnesium ammonium phosphate has been used since the nineteenth century, literature on the subject indicates that a pure precipitate is not easily obtained.[159] As in the case of precipitation with oxine, prior removal of all cations except the alkalis is essential, since both reagents form numerous other precipitates. As discussed later, however, the oxinate method has distinct advantages over the ammonium phosphate method.

For kale, the most serious interference is caused by calcium, which is ordinarily removed by an initial oxalate precipitation.[14,116] A procedure using an oxalate precipitation, however, tends to occlude slight amounts of magnesium and a double precipitation is required even under favorable conditions.[159] The extremely unfavorable calcium to magnesium ratio (25:1) tends to aggravate the situation. Furthermore, the excess oxalate, following calcium precipitation, must be completely destroyed prior to the magnesium precipitation to avoid incomplete precipitation of the latter owing to formation of complex magnesium-oxalate ions.[79,159]

These difficulties may be circumvented by use of a cation-exchange

column for separation of magnesium from calcium. This procedure has the added advantage that interfering anions pass through the column and are discarded with the first fractions.

Separation factors for cation-exchange chromatography of the alkaline earth elements with aqueous hydrochloric acid or other non-complexing acids as eluting agents are fairly small, however. In an effort to improve the separation, many complexing agents such as acetate,[80,136] formate,[272] citrate,[193] lactate,[173,193,213] glycolate,[191] malonate,[257] α-hydroxyisobutyrate,[213,291] ethylenediamine tetraacetic acid,[26] 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid,[259] ethylene-glycol bis (β-aminoethylether)-N,N,N',N'-tetraacetic acid,[191,213] 2,6-pyridinedicarboxylate,[17] acetylacetonate[173] as well as dimethylsulfoxide-water mixtures[143,248] have been suggested. However, use of complexing agents tends to complicate the subsequent measurement of the separated metal ions.[104]

The simplest system utilizes just hydrochloric acid. Campbell and Kenner applied the technique to the separation of calcium and magnesium in limestones using a Dowex 50 column and eluting with various concentrations of acid.[44] They found good separation of 1.5 mg magnesium from 4.5 mg calcium in 1.07 N hydrochloric acid or 1.21 N hydrochloric acid. At 2.0 N hydrochloric acid, only partial separation was obtained.

Milton and Grummitt investigated the use of hydrochloric acid at elevated temperatures for the separation of alkaline earths in both synthetic mixtures and milk ash.[193] While there is apparently an enhancement in the separation factor, generally in systems where ions of equal valence are involved, an increase in temperature causes a decrease

in selectivity.[23,162] In systems exhibiting a low degree of selectivity, only slight equilibrium displacements due to temperature changes are found.[23] For the present study, it was thus felt that an elevated temperature was not necessary to effect a sufficient separation.

Hence, it was decided to utilize the simplest effective ion exchange system; that is, Dowex 50 with hydrochloric acid as eluant.

1.3.2.2 Precipitation as the Oxinate

The oxine method for magnesium determination has been the subject of much investigation.[135] In 1910, Fox first described the preparation of the magnesium oxinate complex.[101] Later, in 1926, Hahn used oxine for the gravimetric determination of magnesium.[119] The following year, Berg described the quantitative precipitation using a solution of oxine added to an ammoniacal solution of the magnesium salt, and drying the precipitate at either 100-105°C as the dihydrate, or at 130-140°C as the anhydrous salt.[18] Hahn and Vieweg described a method employing addition of excess ammonia to a neutral or slightly acidic solution of magnesium containing oxine, and drying at 140-160°C.[120] These two methods were variously used over the following decade by a number of workers who claimed superiority for one or the other. Redmond and Bright studied both methods of precipitation.[224] They obtained high and low results for the first method, which they attributed to the joint effects of a tendency to incomplete precipitation and contamination of the precipitate with reagent, and low results for the second method owing to slow separation of the precipitate.

Miller and McLennan found in their experience that Berg's method

gave low results in the gravimetric procedure (and high results in the bromate volumetric procedure), while Hahn and Vieweg's method invariably gave high results.[192] Their investigation into these inconsistencies resulted in a slightly modified method which was found to give negligibly small errors (about 1 percent) for "technical" analyses of carbonate and silicate rocks, when compared to NBS values. It should be noted, though, that they attempted to obtain precipitates in the presence of oxalate, present as a result of calcium removal, which consistently gave negative errors. Results obtained for a standardized magnesium sulfate solution[77] agreed within ± 2 ppt. Their procedure, slightly modified, has been adopted as a standard method by Kolthoff et al.[159]

Precipitation of the magnesium oxinate is generally considered to be complete above a pH of about 9.[25,96,200] The precipitate may be obtained either with ammonium hydroxide or sodium hydroxide with added sodium tartrate. The latter system has been recommended for precipitation of magnesium in the presence of aluminum, though the separation from iron is not good.[137] Iron oxinates have been found to be very soluble in sodium hydroxide with tartrate added.[217] Erdy has suggested that the latter method must be used when magnesium is to be separated from metals such as iron and aluminum.[79] Still, thermograms run by him on precipitates obtained under both conditions showed a marked difference above 200°C.

The thermal behavior of the precipitate is far from resolved and a variety of drying temperatures have been recommended. Early workers who precipitated the complex near the boiling point, suggested that the tetrahydrate formed, which decomposed to the dihydrate on

drying at 100-110°C.[18,137] However, other workers have generally dismissed the possibility,[24,72] though Duval admits that the shape of the thermogram depends very much on the method of precipitation.[72] Chirnside et al., who investigated the oxine precipitates using X-ray diffraction, suggested that the dihydrate was reasonably stable after drying for 2.5 hours at 110°C or 3.5 hours at 98°C.[54] Even after 5.5 hours at 140°C, though, complete conversion to the anhydrous form had not taken place. Only 5 hours at 160°C, the method they recommended, was sufficient to produce a pure anhydrous precipitate. Borrel and Paris, on the other hand, found that the dihydrate was stable up to 122°C at which point dehydration occurred, producing the anhydrous form at 200°C.[24] Further confusion is added by Séguin, who states that the complex dried at room temperature is the tetrahydrate; at 120°C, the dihydrate, and at 130°C, the anhydrous form.[238]

No doubt the differing opinions are due to some extent to different thermal conditions used (e.g., different heating rates) and to the fact that excess oxine is required to ensure complete precipitation of the magnesium.[64,192] Because of the conflicting evidence, it was decided to run thermograms for the precipitate obtained by Kolthoff's method,[159] slightly modified, to obtain suitable drying temperatures for the present gravimetric work.

For the present study, the oxinate method has several advantages over the phosphate method. The fact that only volatile reagents are used is a distinct advantage in the oxinate method, relative to the ammonium phosphate method, in which excesses have to be carefully controlled and which usually requires that a double precipitation be

performed even under favorable conditions. A second advantage, especially where small amounts of magnesium are to be determined, is the higher gravimetric factor. A third advantage is the much lower ignition temperature required.

1.3.3 Analysis by Atomic Absorption Spectroscopy

With the advent of atomic absorption, the various gravimetric, colorimetric and volumetric methods assumed a position of lesser importance. Walsh was the first to successfully apply the techniques of atomic absorption.[279] Since the theoretical principles and practical applications have been thoroughly described in various monographs, they will not be further detailed here.[8,55,76,89,176,216,222,228,240]

1.3.3.1 Interferences in Magnesium Determination

Atomic absorption is subject to five types of interference: chemical interference, ionization interference, spectral interference, matrix interference and background absorption.

Chemical interference occurs when the element of interest combines with some other cation or anion in solution to form a species which changes the degree of formation of atomic vapor in the flame and hence alters the measured absorbance.

Cationic chemical interference in the case of magnesium can occur with a number of metals. The best known is aluminum, which interferes by producing an intermetallic compound in the flame after the solvent containing the two metal ions has evaporated.[190,230] For kale, where the aluminum-magnesium ratio is 1:40, this is not a problem.[36]

Anionic interference by sulfate, phosphate and silicate (all

present in the kale ash), due to the formation of refractory compounds with magnesium in the flame, may in many cases be controlled by the addition of strontium, lanthanum, disodium ethylenediamine tetraacetic acid, oxine or 5-sulfosalicyclic acid.[160]

The effect of the common acids (hydrochloric, nitric, perchloric, sulfuric, phosphoric, acetic and formic) in air-acetylene flames has been studied.[97,196] Up to approximately 1 M acid, no effect was found for 1 ppm magnesium except for sulfuric and hydrochloric acids. Another study has shown that hydrochloric acid concentrations above 0.12 N lowered magnesium absorbance even in the presence of lanthanum chloride.[187] Various acids used in the present work were investigated to affirm their effects on the magnesium absorbance.

Matrix interference occurs when the physical characteristics of the sample and standard differ. Such considerations as ionic strength, surface tension and viscosity are important.

Ionization interference in the case of magnesium is negligible. In air-acetylene flames at 2300°C no ionization has been reported, though in nitrous oxide-acetylene, 6 percent of the magnesium atoms are ionized.[7]

Spectral interference can occur when a resonance wavelength of an element present in the sample, but not being determined, falls within the width of the absorption line of the element of interest. For the magnesium-calcium-aluminum multielement lamp used, this was not a problem.

Background absorbance is a collective term referring to the combined effects of flame absorption, molecular absorption and light scattering in the flame due to a high concentration of dissolved salts.

Several studies involving interferences in the magnesium deter-

mination by atomic absorption should be mentioned, as they relate to the present work.

David, in 1958, pioneered the use of atomic absorption for the determination of zinc, iron, copper and magnesium in plant materials.[62] In the same year, Allan studied the determination of magnesium in some detail.[6] Since then a number of workers have reported the analysis of plant material for magnesium.[13,19,40,73,134,197,208,258,294] David found that using acetylene-air fuel mixtures, the method was relatively free from interference by sodium, potassium, calcium, phosphate, sulfate and aluminum. He suggested that standards for plant analysis could be prepared using just a solution of a magnesium salt in water. Allan, however, found that aluminum in equimolar or greater amounts caused depression of the magnesium signal, while calcium, potassium, sulfate and phosphate caused none. In a study of calcium and magnesium in soils, David noted that in the absence of calcium, aluminum and silicate caused a considerable decrease in the magnesium absorption, while the interference from sulfate and phosphate was only slight.[63] Furthermore, the presence of calcium afforded no protection against aluminum, slight protection against phosphate and considerable protection against silicon. Strontium, which had been previously shown to overcome the chemical interference of phosphate, sulfate, aluminum and silicon in calcium analysis, was then tried.[126,286,297] These same interferences for magnesium were controlled satisfactorily by 1500 ppm of strontium.

Roach et al. determined magnesium, copper and zinc in animal feedstocks and found the results in close agreement with those obtained by EDTA titrations.[227] Dry-ashing and simple dilution resulted in no

interferences from other elements present, due to the low concentrations involved. A lower relative standard deviation was also found for the atomic absorption results than for the titrations.

Many other studies on magnesium interferences have been conducted with contradictory results.[98,138,220,221,253,288]

The species sodium, phosphate, silicate, sulfate and aluminum among others, have been mentioned as possible interferences of magnesium by various workers, for air-acetylene flames. As these species are all known to be present in kale (sodium 2500 ppm, phosphate 13,500 ppm, silicate 510 ppm, sulfate 48,000 ppm, aluminum 37 ppm), and as the effect of a foreign species depends on the relative amounts of the analyte and the diverse ion,[87] a re-examination of the effect of these interferences was undertaken in the present work, on two different instruments. The importance of carrying out such a preliminary study for each type of atomic absorption instrument has been pointed out by Fleming and Stewart, who noted large effects dependent on the type of burner and aspiration system employed.[98]

McBroom, Lancaster and Weiss attempted to assess the effect of instrumentation for calcium, magnesium, sodium and potassium in rat tissue.[187] (Meinke has reported on a study in which samples of potassium nitrate and pyrene were sent to a number of laboratories for measurement on recording spectrophotometers.[188] A plot of the absorbances of potassium nitrate against pyrene for the 94 results yielded 11 different clusters of points, each corresponding to a particular make of instrument.) The study by McBroom *et al.* compared two atomic absorption instruments. Following dry ashing, the samples were digested in a mixture of nitric

and perchloric acids, evaporated to dryness and taken up in dilute hydrochloric acid. Aliquots of the ash solution and simulated tissue were compared on a Perkin Elmer 303 (PE) and a Beckman 979 (B) instrument. Addition of lanthanum chloride was found to cancel the positive interference of calcium, sodium and potassium in the synthetic tissue for the PE instrument while the results for the B machine were 5 percent low. The effects of acid on a calcium solution containing 1.5 percent lanthanum chloride, showed that the PE system was more sensitive to increasing concentrations of nitric acid than the B system, while the trend was reversed for sulfuric acid. With 1 N hydrochloric acid, the absorbance of a magnesium solution decreased by 6 percent for the PE system, but for the B system increased 2 percent.

1.3.3.2 The Use of Standard Addition

The standard addition method has been widely employed in emission spectroscopy and polarography and to a lesser extent in flame emission and atomic absorption. Using this method, no external standards are required and the sample matrix is often of relatively little importance. In the general method of standard addition, a sample and a sample containing a known "spike" of the analyte are prepared and analyzed identically and the concentration of the analyte in the sample is then calculated by the ratio-proportion method. The assumption is made that the spike adds linearly to the amount of analyte present in the sample so that the signal-increase is linear in the region under consideration. This is by no means the rule and a modified version of this method is often employed. In this latter version, a calibration plot is obtained using

a series of standards. The analyte concentrations of the sample and spiked sample, corresponding to their output signals, are read off this graph and the difference in calculated concentration is ideally equal to the amount of spike added. If this is not the case, an empirical correction can be obtained, based on the difference in the spike concentrations, and this correction can then be applied to the sample. A somewhat more complicated modification of the basic method can also be used. Here, equal volumes of the sample are added to a series of solutions containing known and varying amounts of the element of interest. A plot is made of signal against added analyte and is extrapolated back to zero-added-analyte. The concentration of the sample is then obtained by dividing this extrapolated reading by the slope of the line. For this purpose, the extrapolated portion may be made either linear or some other shape, by inspection or calculation of a best-fit curve.

Superficially, the general method appears quite simple, but it is not without pitfalls. For atomic absorption, it will correct for matrix effects only if the ratio of analyte to interference concentration has no effect on the observed absorbance, or if great care is taken to form a suitable extrapolation. Furthermore, it cannot correct for light-scattering or broad non-specific molecular absorption. In the latter case, use of a dual-channel instrument is necessary to perform the correction and even then, the wavelength used for the correction must be close to the analytical line and of suitable intensity, a requirement often not adequately fulfilled. Under optimum conditions an accuracy of 3 to 5 percent is claimed.[241]

Other theoretical problems also play a role in the use of standard addition for atomic absorption. The most important is the ratio of spike to analyte present. Typically, the recommendation is made to add two or three times the amount present in the sample, provided curvature of the graph does not become a problem. This usually restricts the absorbance to values below about 0.7 absorbance units in the case of magnesium. Since the relative error varies with the absorbance, an optimum lower value may be fixed. For magnesium this is approximately 0.1 based upon the data of Roos.[229] The slope or sensitivity of the analysis will also vary with the element and the instrumental conditions, and will have a bearing on the accuracy obtainable.

1.3.4 Neutron Activation Analysis

Neutron activation is a widely recognized and accepted analytical technique. It was first used by Hevesy in 1936, although it did not gain prominence until quite recently with the development of sophisticated electronic equipment and reactors.[130] The theoretical principles and practical considerations can be found in a number of monographs and will not be further discussed.[1,27,67,85,117,163,171]

Magnesium has a number of known major isotopes, as shown in Table 1.4, page 27. For the analysis of magnesium, use is made of the short-lived magnesium-27 isotope.

Magnesium-27 is produced by an (n,γ) reaction on magnesium-26 and so can be used for activation analysis. Two prominent gamma rays, at 0.8438 MeV and 1.0145 MeV, can be used for the determination of magnesium. However, because of the short half-life (9.46 min) of magnesium-27, its low cross-section (27 mb) and the relatively low natural abundance of the

TABLE 1.4 Known Major Isotopes of Magnesium[172]

Isotope	Half-Life (approx.)	Common Modes of Production
Mg^{23}	12 sec	$Na^{23}(p,n)$
Mg^{24}	stable, 78.6%	
Mg^{25}	stable, 10.1%	
Mg^{26}	stable, 11.3%	
Mg^{27}	9.46 min	$Mg^{26}(n,\gamma)$; $Si^{30}(n,\alpha)$; $Al^{27}(n,p)$
Mg^{28}	21.2 hr	$Mg^{26}(t,p)$; $Mg^{26}(\alpha,2p)$; $Al^{27}(\alpha,3p)$
Al^{28}	2.3 min	daughter of Mg^{28}

target isotope (11.29%), any separation of magnesium from the irradiated sample requires speed. Magnesium-27 can also be produced by an (n,α) reaction on silicon-30 and an (n,p) reaction on aluminum-27. These alternate modes of production, however, do not lead to an appreciable error in the analysis of kale or rat tissue. Calculations by the author indicated that for kale, the relative error incurred by ignoring the alternate production modes was about 10^{-2} percent for the aluminum reaction and 10^{-4} percent for the silicon reaction for the McMaster University reactor. These values are also in general agreement with those obtained experimentally by Bowen, who irradiated samples of pure aluminum oxide and silica and counted the magnesium activity produced.[28]

Applications of neutron activation analysis for magnesium have covered a wide range of materials including rocks,[36,234,295,296] lunar material,[42,102] water samples,[170,250] teeth,[226] hair,[218] air particulates,[61] bone,[59,111] blood serum,[20,21,127] biological materials[41,150,199,278]

and plant materials.[107]

Two approaches are possible for neutron activation analysis, in general. The first, and older, of the methods involves chemical separation of the element of interest either before or after irradiation and prior to counting. The alternate method is to perform a purely instrumental neutron activation analysis (INAA). In the present study, both approaches were investigated.

1.3.4.1 Instrumental Neutron Activation Analysis

With the development of sophisticated counting equipment and because of minimal chemical manipulations, INAA is rapidly gaining popularity. The relative speed and the number of elements that can be determined simultaneously using a computer for spectrum analysis are certainly advantages. Despite these advantages, however, there are limitations; for example, it is commonly recognized that the precision and sensitivity obtainable vary considerably with the overall sample composition.[22,273]

Manganese, due to its favorable nuclear properties, is a major interference for the determination of magnesium in many organic materials. A purely instrumental approach can give poor precision, as the 0.844 MeV magnesium-27 gamma peak is incompletely resolved from the 0.847 MeV manganese-56 gamma peak. Furthermore, the alternate 1.014 MeV magnesium-27 gamma peak has approximately one-third the relative intensity.

Few analyses appear to have been done for magnesium in kale using INAA. Garrec obtained a value of 1209 ppm for the magnesium content in kale using the $Mg^{24}(n,p)Na^{24}$ reaction.[107] The determinations by Bowen's collaborators (1350 ppm and 1605 ppm) were probably also by INAA.

1.3.4.2 Neutron Activation Analysis with Chemical Separation

The number of studies of biological significance, utilizing the alternate approach, namely a chemical separation of magnesium prior to counting, has been quite limited.

Ohely, Schmitt and Bethard determined magnesium in human blood.[203-205] Pressure elution was used following irradiation to separate the magnesium from copper, cobalt, zinc and manganese on Dowex 1. The samples were ashed prior to irradiation.

Bowen studied the determination of both calcium and magnesium in biological material using sodium chlorate to precipitate the manganese, followed by further sulfide precipitations.[28] Results for magnesium in tomato seeds and a mollusc shell showed a variation of ± 7 percent at about 3000 ppm.

Souliotis, Belkas and Grimanis used a similar separation scheme for determining 20 ppm magnesium in lake water.[250]

Hahn, Tuma and Quaife utilized an extraction procedure for rat liver to remove interfering activity.[121,122] This method, while apparently reproducible and having a 99 percent recovery of added magnesium spike, suffered from the fact that pre-irradiation extractions were required.

Hahn, Tuma and Sullivan in a later paper modified the method so that only post-irradiation extractions were required for removal of the manganese.[123] However, less than 100 percent extraction yield necessitated the use of an empirical correction factor which was also assumed to hold for the liver samples. Probably the greatest potential source of error in both procedures is the use of an extraction correction factor, although post-irradiation extraction is certainly to be favored over pre-irradiation

extraction where this is feasible.

Smathers, Duffey and Lakshmanan employed a derivative neutron activation procedure for magnesium using the chelating agent 5,7-dibromo-8-hydroxyquinoline.[243-245] By selective solvent extraction, the magnesium complex and excess chelate were isolated from interfering elements. The amount of magnesium present was determined from the bromine activity after irradiation of the isolated chelate. This allowed the sensitivity to be increased five-fold and permitted counting 36-hour bromine-82. Again, this method suffers from the requirement of several pre-irradiation steps. Since the standard was only carried through the post-extraction steps of the procedure and the extraction technique developed for a synthetic sample, further errors may have been introduced into the results.

From the foregoing, it can be seen that a number of approaches have been taken to eliminate interferences for magnesium determination by neutron activation.

1.4 MAGNESIUM IN RAT CARCASS AND FECES

This part of the project was undertaken as an independent assessment of the discrepancy between atomic absorption and neutron activation results in a rat metabolism study carried out by Dr. O. Héroux of the National Research Council of Canada with outside analyses by Dr. D. J. Evans of Atomic Energy Canada Ltd.

The essential nature of magnesium in animal nutrition has been established for over 40 years.[50,283] Because of the widespread occurrence of this element in both plant and animal tissue, only under extreme conditions is there an inadequate intake. The result in animals commonly

produces a tetany.[99,276] In rats, for example, a low magnesium tetany was produced by feeding a diet purified to such an extent that it contained only 1.8 ppm magnesium.[153,164] In contrast, the low magnesium diet, mentioned below, contained about 100 ppm.

In the present study, a number of rats at the National Research Council laboratories had been placed on 4 different diets; lab chow, control, low magnesium and high fat, over a period of time. The animals were then sacrificed and the diets, feces and homogenized carcasses analyzed for magnesium and calcium using atomic absorption. As an independent check on the results, samples of the various materials were submitted to an external organization for neutron activation analysis. An assessment of the results obtained by the two techniques, revealed that the atomic absorption values were approximately 1.5 times higher than those obtained by neutron activation analysis for both the tissue and feces. The results obtained for the rat samples discussed in this thesis are shown in Table 1.5, page 31.

TABLE 1.5 Collaborative Results Obtained for Magnesium in Rat Samples

Sample	Method	Values (mg/g)
Carcass	Atomic Absorption	1.00[1]
	Instrumental Activation	.7[2]
Feces	Atomic Absorption	7.14[1], 7.2[3]
	Instrumental Activation	3.7[2]
	Emission Spectroscopy	7-8[3]

1. Obtained by analyst A.
2. Obtained by analyst B.
3. Obtained by analyst C.

Results for the lab chow diet were similar for both methods while the other three diets were not determined by neutron activation.

Dr. Héroux submitted aliquots of all the samples as well as a copy of the results obtained by both methods.[129] Because of the obvious similarity posed by the implications of this study, to the results from Bowen's kale study, one rat sample and the corresponding feces were analyzed in the present work by atomic absorption and neutron activation using the same procedures as had been developed for the kale.

CHAPTER II

EXPERIMENTAL EQUIPMENT AND PROCEDURES

2.1 MATERIALS AND REAGENTS

The bulk of the experimental work was performed on kale. A less detailed investigation was also undertaken on a rat carcass and feces sample.

The kale, as dispatched by Bowen, was said to have the following properties.[29-31] It consisted of irregular discs of tissue of a maximum size of 220μ, 60 percent between 53μ and 220μ. It was pale green in color and had a moisture content, depending on atmospheric humidity, of between 3 percent and 5 percent. Drying for 20 hours at 90°C was recommended prior to analysis.[35] It had a bulk density of 0.56 g/cm^3 and an ash content of about 17.5 percent. While Bowen concluded that the sample was homogeneous at 0.01 g, samples between 0.1 g and 1 g were recommended to increase the analytical precision.

The kale was received as a 100 g sample in a snap-cap plastic container. For all work, samples were dried for 24 hours at 90°C and then used immediately.

Less information was available on the rat samples. They were received as approximately 5 g samples in plastic screw-cap glass bottles. The feces was a fine brown powder while the rat carcass appeared to consist of hair, bone chips up to 5 mm long and connective tissue pieces. No attempt was made to further homogenize the samples. Samples marked

#60 (lab-chow diet) were used for all analyses. Both feces and carcass samples were dried 24 hours at 90°C prior to further use. The feces was found to contain 6.1 percent moisture and the carcass 1.3 percent. They were similarly stored in a dessicator.

Unless otherwise noted, all reagents were either analytical grade and used without further purification, or of sufficient purity for the purpose for which they were employed. Only distilled, deionized water was used in all procedures.

Oxine (BDH analar reagent, lot #33412) was recrystallized from ethanol prior to use as a precipitating agent for the magnesium. Infrared spectra before and after purification appeared unchanged and agreed with a standard reference spectrum.

For preliminary experimentation, a synthetic kale solution was prepared containing 23 cations added as soluble salts at the concentrations given by Bowen. No magnesium was added. Sulfur and silicon were not added as they tended to form precipitates under the conditions employed.

A stock magnesium solution was prepared by dissolution of magnesium ribbon (BDH lot #480400, 99.9 percent pure, manganese less than 0.02 percent) in a minimum volume of 2 N hydrochloric acid. Two liters of solution were prepared at 3200 ppm and stored in a polyethylene bottle. For neutron activation analysis, another magnesium stock solution (320 ppm) was prepared for use as a standard, by dissolution of clean magnesium ribbon in dilute nitric acid rather than hydrochloric acid to avoid chlorine-38 production during irradiation.

Stock ethylenediamine tetraacetic acid (EDTA) was prepared by dissolution of 37.223 g reagent grade ethylenediamine tetraacetic acid,

disodium salt (Eastern Chemical Co., lot #41075), previously dried 3 days at 80°C, in deionized water and diluting to the mark. It, too, was stored in a polyethylene bottle.

A 1 l solution of 854 ppm zinc was prepared by dissolution of a zinc pellet (Cominco 69 grade, lot #HPM5205) in 2 ml of 6 N hydrochloric acid and diluting to the mark.

Standardization of the EDTA using both magnesium and zinc stock solutions agreed within 0.2 percent. Restandardization of the EDTA, 16 months later, gave a value 0.2 percent lower for each metal indicating no apparent deterioration using this method of storage.

For separation of the magnesium in the gravimetric work and some atomic absorption analyses, the ash solution was passed over a Dowex 50 x 12 column (2.1 x 28 cm) prepared as follows. Sufficient Dowex 50 x 12, 100-200 mesh (Bio-Rad Analytical Grade, lot #5596-41B-1603, dated 9-4-62) was washed twice with deionized water, allowed to settle and the fines decanted. A slurry was then prepared and transferred to a buret. About 400 ml of 6 N hydrochloric acid were allowed to pass over the column via a reservoir system to remove extraneous ions and the column was equilibrated with 200 ml of 0.4 N hydrochloric acid. The column was regenerated by passing over 750 ml of 4 N hydrochloric acid followed by 200 ml of 0.4 N hydrochloric acid.

All irradiations were conducted using a pneumatic rabbit system. The same rabbit, having internal dimensions of about 5.3 x 2.0 cm was employed for all irradiations.

Three kinds of irradiation capsules were used. For purely instrumental analysis, 3 ml snap-cap plastic vials (Sherwood Medical

Industries Inc., St. Louis, Mo.) were used. Both solids and liquids could be irradiated by heat-sealing the caps and the samples were counted without transfer. For irradiation followed by wet digestion, 1.0 x 1.3 cm quartz vials were taken. These were initially cleaned by soaking in aqua-regia for 24 hours and then rinsing with deionized water. The vials were sealed with parafilm (American Can Co., Neenah, Wisc.), which suffered no apparent damage for up to 2 minutes irradiation. Prior to reuse, the capsules were cleaned with a hot sulfuric-nitric acid mixture. Liquid standards were prepared by heat-sealing samples in 5 ml polyethylene bottles (Canus Equipment Ltd., Ottawa, Ont.) prior to irradiation.

Except for the purely instrumental analysis, samples were counted in 25 ml plastic safety vials (Ampak Ltd., Ville St. Pierre, Que.) using a lucite holder to ensure geometric reproducibility.

2.2 INSTRUMENTATION

Atomic absorption spectrophotometry was conducted using a Jarrell-Ash model 82-800 instrument with a 10 cm path length premix burner for the air-acetylene flame and a 5 cm premix burner for nitrous oxide-acetylene. As well, a Heath model EU-703 instrument with a 5 cm Varian Techtron premix burner was employed as an alternate system. The same Westinghouse aluminum-calcium-magnesium hollow cathode lamp, model #WL22930 was used with both.

The former instrument, containing dual-beam, dual-channel circuitry, was employed in the single-channel mode. This was satisfactory for the present purpose as with a 75μ extrance slit, the background

absorption for samples was quite low and the reading was identical in both the compensated and noncompensated modes.

The latter, a single-channel, dual-beam instrument, was used both with a standard photometric output as well as a photon counting arrangement.

Most results were obtained using the parameters shown in Table 2.1, which were found to be optimum.

TABLE 2.1 Operating Conditions for Atomic Absorption Equipment

Specification	Jarrell-Ash	Heath
Monochromator	0.4 m Ebert	0.35 m Czerny-Turner
Grating (lines/mm)	2360	1180
Lamp Current (ma)	10	10
Wavelength (Å)	2852	2852
Gas Flows (scfh)		
acetylene	5	3
air	7	13
auxiliary	15	7
Burner Height (mm)	12	10
Slit (μ)		
entrance	75	50-100
exit	100	
Sample Flow Rate (ml/min)	4	3

Irradiations were carried out using the McMaster University reactor with a thermal flux of about 2×10^{13} n $cm^{-2} sec^{-1}$ and a cadmium ratio of about 20. The reactor output was later increased to approximately

4×10^{13} n $cm^{-2} sec^{-1}$. The flux in the rabbit position was lower by approximately a factor of 2 under both conditions.

For counting the activity, the following equipment was used: a coaxial Ge(Li) solid state detector (active volume 40 cm^3, peak efficiency 7.3 percent relative to a 7.5 x 7.5 cm NaI(Tl) detector at 25 cm, resolution 4.1 Kev and a peak to Compton ratio of 15:1; the latter three specifications at 1.33 Mev) coupled to an Ortec model 118A preamplifier; a Hewlett-Packard model 5582A linear amplifier and a Nuclear Data 2200 series 1024 channel analyzer. The system was later modified to incorporate Canberra model 1510 digital stabilizers and model 1501 stabilization pulsers for zero and gain shift suppression. As well, the memory was increased from 1024 to 2048 channels. Gamma spectra were displayed on a Fairchild model 701 oscilloscope and could be printed out in whole or in part by an IBM typewriter.

For obtaining complete spectral plots, the system was coupled via a suitable interface to a paper tape output unit. A computer plot was then made using the paper tape.

2.3 GRAVIMETRIC PROCEDURE

Samples were dry ashed in a Lindberg Hevi-Duty furnace model 56622 with control console model 59344. This enabled the ashing procedure to be reproducibly controlled for 48 hours at 500°C. They were placed in a platinum crucible enclosed in a quartz beaker with a loose-fitting quartz cover. This was placed in a Petrie dish and covered with a 250 ml beaker. The whole assembly was placed in the cold furnace with the door left ajar to permit air circulation.

Upon removal from the furnace, the grey-white ash was transferred

to a 100 ml tall-form beaker. Ten ml of 6 N hydrochloric acid were added drop-wise down the side of the covered beaker, the effervescence being allowed to subside between additions. The solution was then digested at about 60°C for 1 hour and allowed to stand overnight. The solution, containing a few flakes of silica and carbon, was filtered through a 20 ml glass Millipore filter with 0.45μ Gelman filter discs. It was transferred back to the tall-form beaker and taken to dryness. Ten ml of 0.4 N hydrochloric acid were added. The solution was warmed again for about 1 hour at 60°C and allowed to stand overnight, leaving some undissolved calcium sulfate.

The supernatant, following centrifugation, was transferred to a 30 ml beaker using a Pasteur pipet while the residual precipitate, consisting mainly of calcium sulfate (about 0.1 to 0.2 g) was put in another 10 ml platinum crucible. The solid was fluxed with 0.2 g sodium carbonate, following which 5 ml of a 1 percent sodium carbonate solution were added to the melt. The melt was crushed with a glass rod and the platinum crucible warmed several minutes prior to centrifugation. The supernatant was discarded and the fluxing procedure repeated with a further 0.1 g sodium carbonate. The resulting melt was dissolved in a minimum amount of 6 N hydrochloric acid and added to the bulk of the ash solution. This was evaporated to dryness and 10 ml of 0.4 N hydrochloric acid added to redissolve the solid.

The sample solution was transferred to the Dowex 50 column and eluted with 2 N hydrochloric acid. Nine ml fractions were collected in test tubes using a Packard Automatic Fraction Collector (Packard Instrument Co. Inc., La Grange, Ill.). Fractions 20 to 40, containing the magnesium

as well as the iron, some sodium and potassium, were transferred to a 200 ml volumetric flask. The selection of the elution parameters was based on a series of preliminary experiments and typical elution curves are shown later (page 53).

For each kale sample, two 75 ml aliquots were transferred to 150 ml beakers. Ten drops o-cresolphthalein were added and then concentrated ammonium hydroxide, with vigorous stirring, until the indicator changed from colorless to violet. An additional 3 ml were added (pH ~ 10) and the solution digested at 80°C for 3 hours to precipitate ferric hydroxide. A visible precipitate began to form after about 1 hour. The beakers were then placed in a refrigerator overnight before filtering through medium porosity glass frit filters into 150 ml beakers. After rinsing the precipitate with water, the filtrate was warmed and allowed to evaporate to about 50 ml and another 5 drops o-cresolphthalein added. Concentrated ammonium hydroxide was added to the solution, at about 80°C, until a violet color was again obtained.

To precipitate the magnesium, a freshly prepared oxine solution (1 percent in 0.4 N acetic acid) was added dropwise via a Pasteur pipet, while stirring vigorously with a glass rod. About 3 ml (125 drops), were added at the rate of about 1 drop per second. The solution was continuously stirred, care being taken to avoid scratching the walls, until a fine yellow suspension appeared. The solution was then digested at 80°C for 1 hour, allowed to cool overnight in a refrigerator and filtered cold through a preweighed 3 ml, fine porosity glass frit filter. The crystals were washed with cold water and dried 1 hour at 105°C before being weighed as the dihydrate. They were then dried 1 hour at 150°C,

weighed as the anhydrous form, and finally dried another hour at 150°C and reweighed to check for possible incomplete conversion.

The suitability of these drying temperatures was checked by means of thermogravimetric analysis of the precipitate. About 1 g of the dihydrate was prepared in two batches by adding a fresh, 5 percent oxine solution in 2 N acetic acid dropwise to 32 mg of magnesium. Both batches were then dried overnight at 80°C before being combined.

A number of thermograms were then run on a Stanton model HT-D thermobalance (Stanton Instruments Ltd., London, England) equipped with an alumina sample crucible and a platinum-rhodium thermocouple. For most work, a heating rate of 0.5 C° per minute was employed up to 150°C and 6 C° per minute above this temperature. An experimental buoyancy curve for the crucible indicated an apparent weight gain of 0.5 mg on heating from 250°C to 950°C. A correction was thus made for the weights calculated based on the thermogram.

Infrared spectra were taken of the precipitate dried at 105°C and 150°C. All spectra were run as nujol mulls on a Perkin-Elmer model 337 grating instrument (Perkin-Elmer Corp., Norwalk, Conn.) against an air reference.

2.4 ATOMIC ABSORPTION PROCEDURES

In order to study a number of inter-related effects, four approaches were taken for the atomic absorption analysis of kale; dry ashing followed by ion exchange, wet digestion of the sample, both followed by analysis using external standards, wet digestion using standard addition and photon counting.

2.4.1 Basic Atomic Absorption

For the first approach, from the 200 ml of eluant collected from the ion exchange column, and used for the gravimetric analysis (page 38), separate 5 ml aliquots were taken and diluted with deionized water to 100 ml. Standards were prepared from the stock 3200 ppm magnesium solution to contain from 0.32 ppm to 3.2 ppm magnesium in very dilute hydrochloric acid.

A second set of kale samples was directly prepared for atomic absorption analysis. Each sample, weighing between 50 mg and 200 mg, was digested separately in a 30 ml Kjeldahl flask. Three ml of fuming nitric acid were added, followed by 1.5 ml perchloric acid when the former had evaporated to about 1 ml. The solution was taken to fumes of perchloric acid and 10 ml of water added. The sample solution was then filtered through a 20 ml glass Millipore filter and a 0.45μ Gelman filter disc to remove the few white silica flakes which appeared. The solution was made up to 100 ml with deionized water and run against the same standards as for the first procedure.

The rat samples were analyzed using the same wet digestion procedure as for the kale. Weighed 100 mg samples were ashed and in the case of the carcass samples, simply diluted to 100 ml. The feces samples, which contained a slight amount of white crystalline material following digestion, were first filtered before dilution to 500 ml. Both sets of samples were run against standards prepared from dilutions of the stock magnesium solution.

2.4.2 Standard Addition

For this procedure, a 250 mg and a 500 mg kale sample were separately

wet ashed using nitric and perchloric acids, with the acid volumes commensurately increased. Each sample was prepared in a 100 ml volumetric flask and various aliquots transferred to 25 ml volumetric flasks. To each 25 ml flask, a spike was added from a 8 ppm magnesium solution using a 5 ml "A"-type buret. Seven sets of solutions, each containing five different-sized spikes, were prepared.

2.4.3 Photon Counting

Photon counting experiments were performed using two Nuclear Chicago model 8703 single-channel scalar-timers coupled to the photomultiplier output of the Heath 703 via a homemade interface. In this way, the number of photons impinging on the phototube over a 12 second period was counted. With this procedure, a measure could be obtained of both the emission and the absorbance plus emission for the samples, using a mechanical light chopper. With a scanning monochromator it was also possible to record the change in lamp output with wavelength under various conditions.

2.5 NEUTRON ACTIVATION PROCEDURES

The kale and rat samples were analyzed via both non-destructive and destructive neutron activation using external standards and standard addition. Since irradiation times were short, a rabbit system was employed.

2.5.1 Rabbit Flux Homogeneity

To study the effect of flux variations across and along the length of the rabbit, a lucite rod was machined to fit the rabbit. The rod contained four grooves along the length, spaced 90 degrees apart.

Pieces of lanthanum-aluminum wire (0.47 percent lanthanum), 1 cm long, were weighed to the nearest microgram (buoyancy error +0.3 percent) and placed end to end, 5 to each groove. They were secured for the irradiation by pieces of tape. Following a 5 minute irradiation, the assembly was allowed to cool 45 minutes to permit the decay of aluminum-28. The wire sections were then sequentially counted for 400 seconds each at 10 cm from the detector, in a standard geometry. Peak areas for the 0.487 MeV and 1.596 MeV lines were plotted after adjusting for time and weight.

2.5.2 Instrumental Neutron Activation

Dried 100 mg samples were weighed into 3 ml snap-cap plastic vials. A weighed strip of magnesium ribbon was taped around the circumference outside the container to act as a monitor, and the whole assembly wrapped in tissue paper and placed in the rabbit. Following a 1 minute irradiation, the monitor was removed and the outside of the sample vial rinsed with 2 N hydrochloric acid followed by water, to remove surface activity. The magnesium ribbon was rinsed in water, methylene chloride (to remove any tape adhesive) and water again.

The sample vial was placed 10 cm away from the detector on a lucite shelf assembly and counted for 1000 seconds live time after 300 seconds cooling (geometry A). The room background was subtracted for another 1000 seconds.

The monitor ribbon was dissolved in 5 ml of 2 N hydrochloric acid and diluted to 25 ml. A 10 ml aliquot was transferred to a plastic safety vial and counted on the detector surface for 200 seconds at 3000 seconds following irradiation (geometry B). Both the sample and

the monitor were recounted after approximately 3 hours to correct for manganese activity at the 844 KeV peak. By recording all times to the nearest second, time correction errors were minimized throughout all procedures.

Standards containing 160 mg of magnesium in solution, were separately irradiated in plastic vials surrounded by a strip of magnesium ribbon. Two standards were run for each of the kale, rat carcass and rat feces samples, using a liquid volume equivalent to the solid sample volume; that is, 0.25 ml, 1.5 ml, and 0.10 ml, respectively. The standards were counted at equivalent times and positions to the samples.

A time sequence for the procedure described in this section is shown in Table 2.2, page 45.

TABLE 2.2 Time Sequence for the Instrumental Neutron Activation Analysis Procedure

Operation	Starting Time (sec)[1]	Time Employed (sec)	Comments
Standards:			
Irradiation	0	60	Magnesium standard with magnesium ribbon monitor.
Cooling	60	300	
γ-Counting	360	1000	Live time count for magnesium standard solution in geometry A, followed by subtraction of room background.
γ-Counting	3060	200	Live time count for dissolved ribbon monitor in geometry β.
γ-Counting	3 hours + [2]	200	Recount of ribbon monitor to correct for manganese activity.

(Continued)

TABLE 2.2 (Continued)

Samples:			
Irradiation	0	60	Sample with magnesium ribbon monitor.
Cooling	60	300	
γ-Counting	360	1000	Live time count for sample in geometry A, followed by subtraction of room background
γ-Counting	3060	200	Live time count for dissolved ribbon monitor in geometry B.
γ-Counting	3 hours + [2]	1000	Recount of sample to correct for manganese activity.
γ-Counting	3 hours + [2]	200	Recount of ribbon monitor to correct for manganese activity.

1. All times were subject to an error of one second, as recorded.
2. Exact times were taken, subject to the error noted in Footnote 1.

2.5.3 Destructive Neutron Activation

Dried 100 mg samples were weighed into clean quartz vials and sealed with parafilm. Originally, a small package of magnesium sulfate, sealed in plastic was irradiated with the samples to serve as a flux monitor, though this was later changed to a piece of magnesium ribbon taped around the vial. Each sample was irradiated for 1 minute and then transferred in the vial, after removal of the monitor, to a 12 ml Kjeldahl flask to which exactly 16 mg magnesium and approximately 5 mg manganese carriers had previously been added and evaporated to dryness. Three ml of 90 percent nitric acid were added and the sample digested until about 1 ml of liquid remained. Perchloric acid (1.5 ml of 12 N) was added and the sample taken to fumes. The Kjeldahl flask was cooled in an ice bath, 1 ml water added and then 15 N ammonium hydroxide, dropwise, to pH 10. Enough water was added to dissolve the salts which formed, followed by 2 drops of a 20 percent ammonium sulfide solution. After

warming for about 1 minute, the sample was filtered through a 20 ml glass Millipore filter into a 15 ml centrifuge tube. Fifteen drops of 25 percent disodium hydrogen phosphate were added to precipitate the magnesium and the sample centrifuged. The supernatant was discarded, another 10 ml water added and the sample recentrifuged. This procedure was repeated for a third time and finally 2 ml of 2 N hydrochloric acid was added to dissolve the precipitate. The sample was transferred to a plastic safety vial, diluted to 10 ml and counted on the detector for 1000 seconds live time at 1300 seconds after irradiation. The magnesium sulfate monitor was counted at 360 seconds for 400 seconds in a constant geometry. Samples were recounted 3 hours later to check for manganese activity.

To determine the chemical yield for the samples, the following procedure was adopted. After cooling for 1 day, the samples were quantitatively transferred to a 125 ml separatory funnel and 30 ml water added. Two ml of concentrated hydrochloric acid and 20 ml of a water-saturated 1:1 n-butanol-chloroform solution were added, followed by 10 ml of a 20 percent solution of sodium molybdate to form the phosphate complex which was extracted. Immediately following the sodium molybdate addition, the separatory funnel was shaken 1 minute and after standing 2 minutes, the yellow organic layer removed and discarded. After another extraction with 20 ml of 1:1 n-butanol-chloroform mixture, three further 10-ml extractions were performed (or 1 extraction following the first absence of a yellow complex in the organic layer). Finally, the aqueous layer was removed and made up to 100 ml. Two 40-ml aliquots were removed for titration against standardized 0.01 EDTA. Titrations were run

for magnesium plus calcium at pH 10, and calcium alone at pH 12, adding triethanolamine, tartaric acid and potassium cyanide with 3-hydroxy-4-(2-hydroxy-5-methylphenylazo)-1-naphthalenesulfonic acid (calmagite) and 2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid (HHSNN) indicators, respectively.

Aqueous standards were prepared at four concentrations using a 320 ppm magnesium nitrate stock solution. Eppendorf pipets were used to transfer the standards to 5 ml polyethylene bottles which were heat-sealed prior to irradiation. Following a 1 minute irradiation together with a magnesium sulfate monitor, the standards were transferred to plastic vials and counted in the same manner as the samples.

The time sequence for the destructive activation analysis is shown in Table 2.3, page 49.

2.5.4 Standard Addition

Six 100 mg kale samples were prepared with spikes ranging from 0.064 mg to 1.60 mg of magnesium and each spiked sample was irradiated together with an unspiked sample. Using a magnesium ribbon wrapped around each sample vial, which served the dual purpose of flux monitor and standard, it was possible to calculate the amount of magnesium present in the kale using individual ratios of spiked to non-spiked sample counts (method one), using a least-squares curve obtained for the series (method two) and using the magnesium ribbons as standards (method three).

To try to further minimize the flux effects, a sample and spiked sample were placed one above the other in the rabbit and their relative positions reversed for each consecutive irradiation of the series.

TABLE 2.3 Time Sequence for the Destructive Activation Analysis

Procedure

Operation	Starting Time (sec)[1]	Time Employed (sec)	Comments
Standards:			
Irradiation	0	60	Aqueous standard with monitor.
Cooling	60	1300	Transferred to counting vial.
γ-Counting	420	400	Live time count for monitor in geometry A.
γ-Counting	1360	1000	Live time count for magnesium standard in geometry B.
Samples:			
Irradiation	0	60	Sample with monitor.
Cooling	60	1300	Sample ashed, carriers added, manganese activity removed and sample prepared for counting.
γ-Counting	420	400	Live time count for monitor in geometry A.
γ-Counting	1360	1000	Live time count for sample in geometry B.
γ-Counting	3 hours + [2]	1000	Recount of sample to check for manganese activity
Chemical Yield	1 day + [3]		Phosphate removed, aliquots titrated with EDTA for total alkaline earths and calcium. Magnesium obtained by difference

1. All times were subject to an error of one second, as recorded.
2. The exact time was taken, subject to the error noted in Footnote 1.
3. The major proportion of activity had decayed after one day.

The same ashing, chemical and counting procedures were used as in the previous experiments.

CHAPTER III

RESULTS AND DISCUSSION

This thesis is concerned with three different procedures for determining magnesium. The results obtained will be discussed separately to highlight some of the features of each method, before directly comparing them. It should be noted that all methods were initially developed for the analysis of kale and were then used without further modification, except where necessary, for examination of the rat samples.

3.1 GRAVIMETRIC DETERMINATION OF MAGNESIUM IN THE KALE

As has been mentioned in the Introduction, the gravimetric analysis of magnesium offers potentially the most accurate method for its determination. In the present work, the procedure given was developed after experimentation with the synthetic kale solution spiked with known amounts of magnesium.

A dry ashing procedure was used. Despite air circulation through the furnace, slight carbon (less than 2 mg for a 2.5 g sample) invariably remained and had to be filtered off, together with the silica (about 1 mg), prior to further treatment of the calcium sulfate residue.

It had originally been hoped that, following dissolution of the residue in 6 N hydrochloric acid and evaporation to dryness, the entire sample would be soluble in dilute acid for the ion exchange procedure. However, while early work using the synthetic solution had shown this

to be the case, the real kale proved somewhat more refractory. The difference in solubilities was due to the lack of sulfate in the synthetic solution.

The solubility problem was overcome by converting the calcium sulfate to calcium carbonate using the method of Hillebrand et al.[131] Two fluxings, each using 0.2 g sodium carbonate, were required for the approximately 0.1 g of material which was insoluble in 0.4 N hydrochloric acid. Less than 0.05 percent of the magnesium was lost for the combined fluxing procedures (determined by atomic absorption) and the remaining precipitate was readily soluble in dilute hydrochloric acid.

Investigations were limited to Dowex 50 cation exchange columns as retention of the alkaline earths is insignificant for anion exchangers in hydrochloric acid media. While it is possible to separate the alkaline earths on Dowex 1[201] in the citrate form or in the EDTA form,[202] an additional step would have been required to destroy the chelating agent prior to the gravimetric determination.

Preliminary experimentation to optimize the separation conditions indicated that the degree of resin cross-linking had a large effect on the mutual separation. Use of 4 percent divinylbenzene resin resulted in incomplete separation while higher cross-linking improved separation, with 12 percent divinylbenzene being optimum. This was in keeping with Mann's findings.[180] Size of the resin beads had much less of an effect, with 100-200 mesh being selected for all quantitative work. This combined with the long column required, 28 cm, resulted in a slow flow rate and good separation, with only slight peak broadening. Smaller columns such as 15 x 0.8 cm were found to result in very poor separation and

severe column breakthrough on applying the kale ash solution.

The kale ash solution was applied to the column in 0.4 N hydrochloric acid. Both alkaline earth ions were more strongly adsorbed at low concentrations, while the insolubility of the sulfate and phosphate increased rapidly as the acidity decreased. Thus, a 0.4 N acid concentration was chosen as a compromise. At this normality the magnesium and calcium still had acceptable distribution coefficients (about 150 and 240, respectively, as calculated from data by Strelow for a Dowex 50 x 8 column[256]) to prevent column bleeding before addition of the eluting agent.

A number of experiments had determined that the mutual separation of calcium and magnesium reached an acceptable level if 2 N hydrochloric acid was used as the eluant. At higher normalities, the peaks tended to overlap while at lower concentrations, although the separation further improved, the peaks became progressively broader, resulting in an unnecessarily large fraction being collected for magnesium. The iron-magnesium separation did not improve from 1 N to 4 N hydrochloric acid.

The elution curves shown in Figures 3.1, page 53, were obtained by analyzing the individual 9 ml fractions for magnesium and calcium on the Jarrell-Ash atomic absorption instrument. The calcium content of the fractions was checked until the instrument output went off scale after which the remaining fractions were not checked. For 1.5 N hydrochloric acid, calcium was not observed even in the last fraction collected. Iron was determined using a thiocyanate spot test which was sensitive to about 3 ppm.[48]

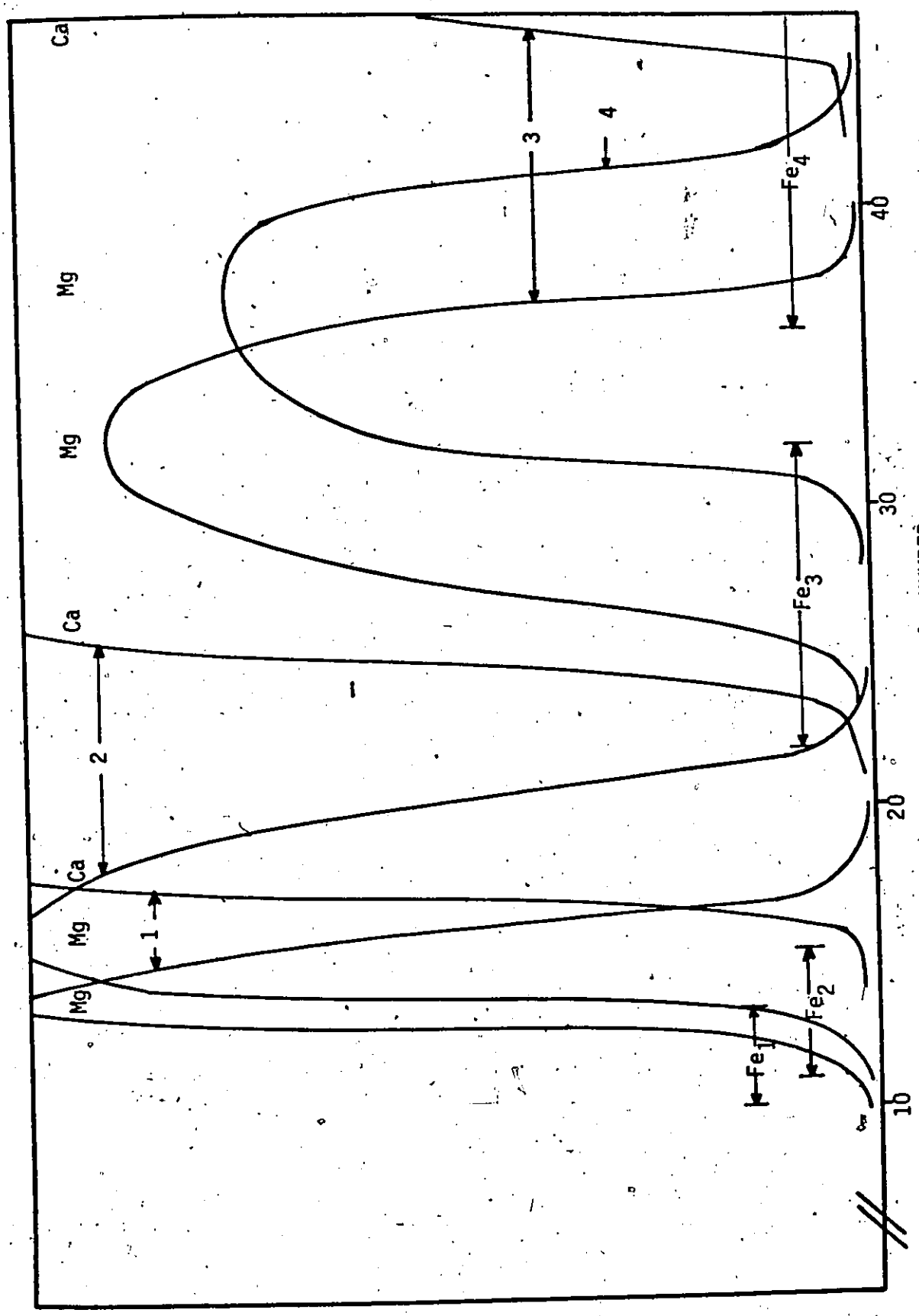
As shown, the column separation resulted in magnesium being collected together with sodium, iron and slight potassium in the same

Figure 3.1 Elution Curves for a Dowex 50 x 12 Column with a Magnesium-Calcium Mixture at Different Hydrochloric Acid Concentrations

Column Dimensions: 28 x 2.1 cm
Column Load: 20 mg Mg, 500 mg Ca
Eluant Flow Rate: 1 min/ml
Fraction Size: 9 ml

Graph 1: Elution with 3.0 N HCl
Graph 2: Elution with 2.5 N HCl
Graph 3: Elution with 2.0 N HCl
Graph 4: Elution with 1.5 N HCl

The fractions containing iron for each pair of curves are shown using the notation Fe_1 to indicate the fractions for Graph 1, etc. The relative magnesium and calcium concentrations were determined by atomic absorption.


Ca
Mg
Mg
Ca
Ca
Mg
Mg
1
2
3
4
Fe1
Fe2
Fe3
Fe4
10
20
30
40
FRACTION NUMBER
RELATIVE CONCENTRATION

fractions. Since iron oxinate precipitated semi-quantitatively under the conditions employed to precipitate magnesium oxinate, its complete sequestering was imperative. With an iron to magnesium ratio in kale of about 1:13, failure to separate the iron quantitatively, resulted in an experimentally determined positive error of 8 percent in the weight of the final precipitate. Several different approaches were possible at this stage. Iron could be masked using complexation with tartrate, by passage of the 180 ml of solution collected from the ion exchange column over a Dowex 1 column, provided the acidity was increased, or by precipitation as the hydroxide, followed by filtration. All three methods were investigated with the last approach proving the most satisfactory.

The iron hydroxide, collected on a glass frit, was dissolved using 2 N hydrochloric acid and checked against magnesium standards by atomic absorption. 0.1 percent or less of the magnesium was lost due to occlusion in the precipitate. A duplicate analysis of a pure magnesium solution with and without iron initially added (at 120 ppm), using this precipitation method, showed no significant differences in yield. In all four cases, yields of 100 ± 1 percent were obtained indicating that any losses were within experimental error.

The oxine precipitation procedure itself was derived from one first employed by Miller and McLennan.[192] The procedure, given in Section 2.3, was found to be the only one that consistently gave yields of 100 percent with the synthetic kale solution. The success of the magnesium precipitation procedure was found to depend markedly on the age, amount and speed of addition of the oxine solution. As

recommended by Miller and McLennan, a 1 percent oxine solution in 0.4 N acetic acid was used, rather than a 5 percent solution in 2 N acetic acid as advocated by Kolthoff et al.[159] This solution was made up not more than a few hours prior to use, as it was found that using an older solution (a few days to a few weeks old) resulted in a dark yellow-brown precipitate and the precipitation was sometimes less than 100 percent complete when checked with a known amount of magnesium.

As has been mentioned, the amount of reagent added to precipitate the magnesium was found to be critical, since oxine itself is only soluble to the extent of about 360 mg per 100 ml of water.[79] Thus, a large excess tended to give high results. On the other hand, magnesium could not be quantitatively precipitated, in the present work, without a 65 percent excess being added.

The speed of reagent addition was important. To avoid local supersaturation and the occlusion of large amounts of oxine, a dilute solution was added slowly. The slow formation of magnesium oxinate was also found to aid in obtaining quantitative precipitation. Addition of reagent at a rate of approximately 0.02 ml per second with vigorous stirring resulted in no production of precipitate during the addition itself and the first visible crystals typically began to form 1 to 5 minutes later. Stirring was then stopped and the heating allowed to continue the agitation via convection.

The results of a number of preliminary experiments conducted to obtain consistent 100 percent precipitation yields of magnesium are given in Table 3.1, page 56.

Digestion conditions following oxine addition were varied both

TABLE 3.1 Precipitation of Magnesium Oxinate

Experiment	Method[1]	Oxine Excess (%)	Digestion Time (hr)	Iron Present[2]	Percent Recovery[3]	Percent Recovery[4]
1	A	33	0.5	-	74.7	
2	"	33	0.5	-	73.7	
3	"	33	0.5	+	24.0	
4	"	33	0.5	+	33.3	
5	"	33	2	-	60.0	
6	B	0	1	-	75.5	
7	"	0	1	-	66.6	
8	"	65	1	-	99.3	
9	"	65	1	-	99.0	
10	"	65	1	-	100.1	100.9
11	"	65	1	-	99.7	100.1
12	"	65	1	+	102.2	
13	"	65	1	+	99.7	
14	"	65	1	+	102.9	100.8
15	"	65	1	+	101.0	99.8
16	"	65	1	+	102.9	100.9

1. Method A used a 5 percent oxine solution in 2N acetic acid, method B used a 1 percent solution in 0.4 N acetic acid.
2. Presence at 120 ppm indicated by "+".
3. Based on weighing as the dihydrate after drying the precipitate 1 hour at 105°C.
4. Based on weighing as the anhydrous form after drying an additional 1 hour at 150°C.

with respect to the length of time the solution was heated at 80°C and the length of cooling, either at room temperature or in a refrigerator (not shown in the table). It was apparent that drying the precipitate at 150°C rather than 105°C resulted in approximately a 1 percent change in the yield in most cases. Values obtained by drying for 3 hours at 150°C resulted only in an insignificant change in the calculated recoveries. Experiments 8 to 16 were judged to be satisfactory, based on the recoveries of added magnesium and the lack of apparent iron interference at 120 ppm. This procedure was then selected for the actual kale analyses.

As a check on precipitation losses, the filtrates for all experiments were measured by atomic absorption for magnesium. Losses of about 0.5 percent were found for experiments 8 to 16. The slightly high yields were thus probably due to a compensation of errors.

The overall analysis was estimated to have a total error of about ± 1.5 percent based on losses for the fluxed portion, coprecipitation during the iron removal, unprecipitated magnesium and the final weighing error.

The values presented in Table 3.2, page 58, represent the results obtained for 9 kale samples. Since two aliquots were taken for analysis from the column eluant collected for the precipitation procedure, two values are given for each experiment. Thus, a measure of the method precision (experiment to experiment) as well as sample to sample variation was possible. From an inspection of the data, several observations may be made. First, in most cases, the experiment to experiment variations were greater than those from sample to sample within a given experiment.

TABLE 3.2 Gravimetric Results for Magnesium in Kale

Experiment	Sample[1]	Value Calculated as Dried (ppm)[2] 1 hour at 105°C	1 Hour at 150°C	2 hours at 150°C	Weight[3] Loss (%)
No Magnesium Observed in Filtrate:					
1	B	168(0)	163(8)	162(9)	12.5
2	A	165(7)	165(0)	165(2)	11.1
	B	163(9)	163(6)	164(0)	10.9
3	A	165(9)	163(9)	162(5)	11.7
	B	167(3)	165(3)	164(1)	11.7
4	A	175(7)	174(5)	172(3)	15.6
	B	172(5)	171(7)	168(7)	11.5
5	A	169(7)	167(2)	168(7)	11.0
	B	168(3)	165(2)	168(7)	11.3
6	A	166(7)	162(7)	163(2)	12.3
	B	166(6)	163(7)	163(7)	11.9
Average		168(2)	166(1)	165(8)	11.9
Standard Deviation		12.6	12.8	15.2	1.31
Standard Deviation of the Mean		3.8	3.9	4.5	0.39
Magnesium Observed in Filtrate:[4]					
1	A	169(9)	167(1)	167(1)	11.8
7	A	166(9)	166(4)	165(2)	11.0
	B	167(9)	166(5)	165(0)	11.5
8	A	168(8)	170(4)	169(0)	11.2
	B	169(2)	170(9)	169(4)	10.9
9	A	163(9)	162(4)	163(7)	11.1
	B	164(0)	164(7)	166(1)	10.0
Average		167(2)	166(9)	166(5)	11.1
Standard Deviation		11.0	8.9	9.5	0.56
Standard Deviation of the Mean		4.2	3.4	3.6	0.21
Overall Average[5]		166(3)			
Overall Standard Deviation		19.5			
Overall Standard Deviation of the Mean		4.6			

(Continued)

TABLE 3.2 (Continued)

1. Two magnesium determinations were performed using separate aliquots of the column eluant for each kale sample.
2. Fourth calculated figure in brackets subject to uncertainty.
3. The theoretical weight loss on conversion of the dihydrate to the anhydrous form is 10.3 percent.
4. The calculated values were adjusted, based on the unprecipitated magnesium as found by atomic absorption (2 to 8 percent).
5. See text for method of calculation.

This indicated that, as might be expected, greater variation was found in the initial drying, ashing and separation procedures than for the final determination. Secondly, in almost all cases, the values calculated based on the anhydrous form were approximately 1 to 2 percent lower than those for the dihydrate. This clearly showed that in most cases the precipitate was contaminated with a slight amount of reagent despite the slow precipitation, long digestion and careful washing of the precipitate. Evidence for this was confirmed by the percent weight losses which were almost all 1 percent higher than the theoretical calculated for conversion from the dihydrate to the anhydrous form.

No additional weight loss apparently occurred on drying the precipitate for 2 hours at 150°C rather than 1 hour, as half the samples gained and half the samples lost about 0.5 percent in weight.

The values given in Table 3.2 are divided into two groups; those for which no magnesium was apparent on examining the filtrate following the oxinate precipitation, and those for which magnesium absorbance was noted. In the former case, this was equivalent to approximately 0.005 mg of magnesium or less remaining unprecipitated, while in the latter case, from 2 to 8 percent of the magnesium was not precipitated. In this case,

the amount found by atomic absorption was added to that calculated for the precipitate, and the sum is shown in the lower portion of the table. As can be seen, good agreement was found between the two sets of results, both for the means and the calculated standard deviations.

As the dihydrate was apparently contaminated with excess oxine, only the values obtained after drying at 150°C were used in calculating a final mean value. Using the "t" test, no significant difference was found among the means of all four sets of results for the anhydrous form. Furthermore, the precision of all four sets of results was shown to be similar by means of the "F" test. As a result, a final overall value was calculated from the combined data from these analyses. The mean value and standard deviations are given in the last three lines of the table.

The results for the gravimetric analysis of kale could be summarized as follows. The data on magnesium presented in Table 3.2 reflected a reasonable degree of precision, showing a standard deviation for a single determination of about 1.2 percent. An important contributing term to this error was the original weighing of the kale powder, which has an uncertainty of up to 1 percent associated with it according to Bowen.[35] Those values obtained by drying at 105°C were just slightly higher than those at 150°C, indicating some oxine co-precipitation. Thus, the overall mean for the gravimetric analysis was calculated using only those results obtained at the higher temperature. This gave a mean magnesium content for the kale of 1663 ± 5 ppm.

3.1.1 Thermogravimetric Analysis of the Precipitate

It has been pointed out in the Introduction that a variety of

drying temperatures have been suggested for magnesium oxinate prior to weighing. Since this step is critical in obtaining the correct value for magnesium, a reinvestigation of the drying procedure was undertaken.

Thermograms were run for magnesium oxinate obtained by the previously described experimental procedure, using a magnesium stock solution and oxine recrystallized from ethanol. Suitable drying temperatures could then be inferred from the chart recording of weight loss against temperature; the horizontal plateaus indicating stable species. In the present case, these were expected to be magnesium oxinate dihydrate, magnesium oxinate and at still higher temperatures, magnesium oxide. The results of two typical thermograms are shown in Table 3.3, page 61.

TABLE 3.3 Thermogravimetric Analysis of Magnesium Oxinate

Experiment[4]	Stable Range (°C)[1]	Theoretical Weight (mg)[2]	Experimental Weight (mg)	Species Present
1	25-112	50.0	50.0	Magnesium oxinate dihydrate
	135-370	44.8	45.3	Magnesium oxinate
	550-1000	5.8	6.3	Magnesium oxide
2	25-112	25.0	25.0	Magnesium oxinate dihydrate
	180-380	22.4	22.5	Magnesium oxinate
	450[3]-1000	2.9	3.1	Magnesium oxide

1. Temperatures given are accurate to ± 2°C; exact range varied with heating rate.
2. Corrected for buoyancy effect.
3. Temperature program was interrupted at 450°C for 2 hours.
4. Same crucible used for both experiments, but heating rate different.

Several conclusions could be drawn from this table. The stable temperature range varied with the rate of heating. In experiment 1, a heating rate of 0.5C° per minute was maintained up to 150°C, while for experiment 2 the rate was increased above 60°C to 6 C° per minute. Furthermore, for this latter experiment, the temperature program was disabled at 450°C, towards the start of the rapid weight loss. After about 70 minutes at this temperature, decomposition of the organic material was complete, with 95 percent of the weight loss occurring within 35 minutes. Another experiment, employing a 5 mg sample and using the same temperature program as for experiment 2, above, showed complete transition from magnesium oxinate to magnesium oxide within 15 minutes at 450°C. Loss of the two water molecules above 112°C required about 30 minutes at a heating rate of 0.5 C° per minute. The other noticeable feature of this table was the lack of agreement between the theoretical and experimental weights recorded for experiment 1. In fact, a check of the final weight of the crucible and contents on an analytical balance gave a value of 5.6 mg (theoretical weight 5.8 mg) rather than 6.3 mg obtained on the thermobalance. The other runs showed no such deviations suggesting that the thermobalance was in error on this occasion. No reason is known for this discrepancy.

From the point of view of the present study, the most interesting part of the thermogram was the behavior of the precipitate between 100°C and 112°C. A close examination revealed that in the 24 minutes required to cover this range, a 50 mg sample lost less than 0.1 mg. The thickness of the recorded line itself corresponded to 0.05 mg and slight oscillations of the recording pen gave a final uncertainty of approximately ± 0.05 mg.

This data, extrapolated to a 20 mg sample dried for 1 hour, would suggest that any weight loss at 112°C due to decomposition would be insignificant. Thus, it was concluded that a final drying temperature of 105°C offered an ample margin of safety.

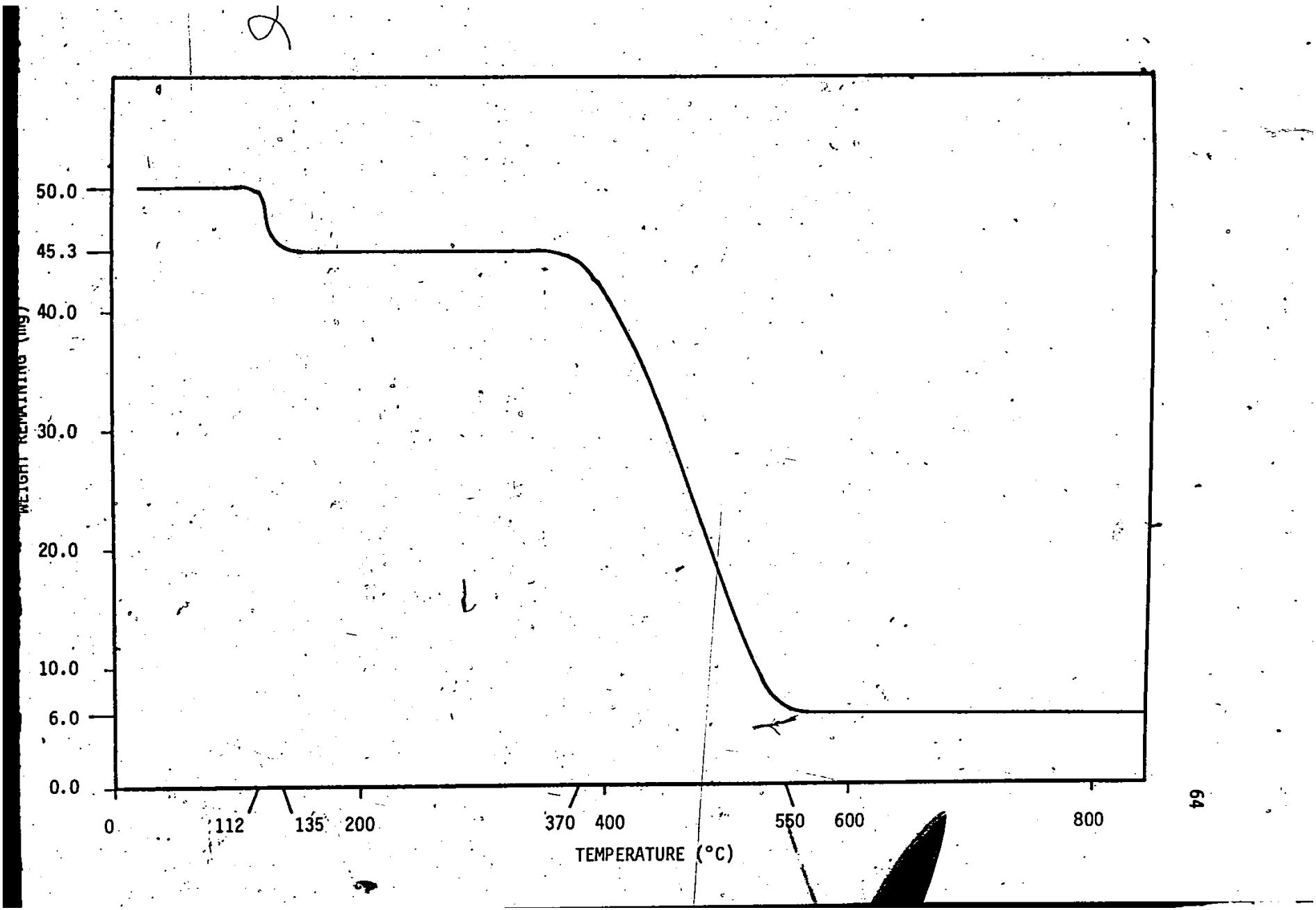
A typical thermogram is plotted in Figure 3.2, page 64. The sharp breaks in the curve are indicated on both axes and show the presence of 3 distinct species; magnesium oxinate dihydrate, magnesium oxinate and magnesium oxide. The thermogram showed no further weight loss up to 1000°C (the highest recorded temperature), though the plot shown here extends only to 850°C. The curve shows the same general features as those obtained by Duval[72] and Erdey.[79]

Duval found that at a heating rate of 1.5 C° per minute, the first break occurred at 126°C, while at 3.5 C° per minute the break occurred at approximately 105°C. He also found that the weight loss was generally slightly higher than the stoichiometric factor, indicating the presence of excess oxine. At the former heating rate, hydrated water was lost up to 206°C while the anhydrous form started to decompose at between 440°C and 660°C, at which temperature only pure magnesium oxide remained. The temperature of the breaks in the curve depended greatly upon the sample weight. For example, a 300 mg sample would require 30 minutes heating at 960°C to completely destroy the organic material, while a 55 mg sample would obey the above description.

The curve obtained by Erdey, on the other hand, revealed a more complex behavior above 200°C dependent on the method of oxine formation. Using an ammonium hydroxide buffer, as in the present case, the first break occurred at less than 100°C, with the stable anhydrous salt being

Figure 3.2 Typical Thermogram of Magnesium Oxinate.

Sample Weight: 50.0 mg
Heating Rate: 0.5 C°/min to 150°C
6.0 C°/min above 150°C


WEIGHT REMAINING (mg)
50.0
45.3
40.0
30.0
20.0
10.0
6.0
0.0
0
112
135
200
370
400
550
600
800
TEMPERATURE (°C)

obtained between 155°C and 210°C. Decomposition occurred above 230°C and the thermogram revealed a number of irregularities up to 850°C, at which temperature the anhydrous form was obtained. No weight or heating rate were recorded in his description. He advocated drying at 160°C to 180°C only and weighing the precipitate in the anhydrous form.

The data contained in this thesis indicated that temperatures of 105°C and 150°C were quite satisfactory. The discrepancy in drying temperatures probably arises because of the large amount of precipitate taken for experiments, typically 0.5 g up to 1 g and the high heating rates which tend to alter the stable temperature ranges. It has been acknowledged that precise measurement of the sample temperature is one of the most difficult problems in thermogravimetric analysis and the chief source of error.[118] Clearly, a small sample size and a slow heating rate will minimize this error.

3.1.2 Infrared Characterization of the Precipitate

As a further check on the loss of water on heating the precipitate at 150°C, several infrared spectra were taken on samples of the precipitate dried 1 hour at 105°C and an additional hour at 150°C, as well as recrystallized oxine itself. These are shown in Figure 3.3, page 66.

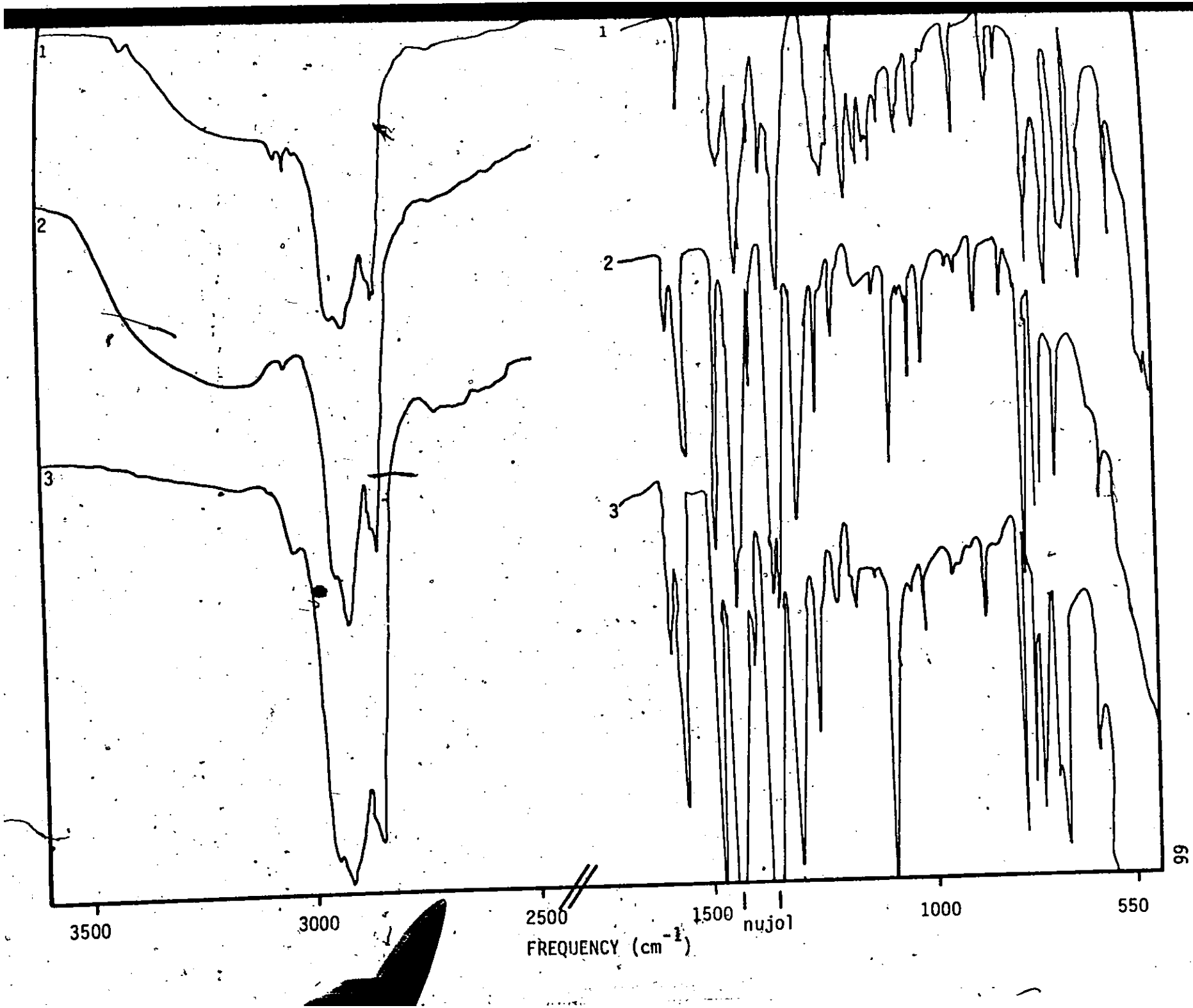
The most obvious feature is the loss of the broad band centered around 3200 cm^{-1} on drying the precipitate at 150°C. This band and the loss thereof has been assigned to the loss of water.[177] This was also confirmed by a decrease in the intensity of the band at about 1210 cm^{-1}.[177]

The three spectra conformed very closely to the spectra obtained

Figure 3.3 Infrared Spectra of Oxine and Its Magnesium Chelates

Spectrum 1: Recrystallized Oxine
Spectrum 2: Magnesium Oxinate Dihydrate
Spectrum 3: Magnesium Oxinate -- Anhydrous Form

All spectra were run as nujol mulls.
Spectrum 1 shows oxine recrystallized from ethanol and air-dried overnight.
Spectrum 2 was obtained after drying the precipitate 1 hour at 105°C.
Spectrum 3 was obtained after further drying the same sample 1 hour at 150°C.


1
2
3
1
2
3
3500
3000
2500
1500
nujol
1000
550
FREQUENCY (cm-1)
99

by Stone with a prism instrument and nujol mulls,[255] though the 3200 cm^{-1} band was shifted to a higher frequency when compared to a potassium bromide disc spectrum for oxine.[231] Charles et al., who studied the infrared spectra of a number of metal chelates of oxine found the general features of all spectra quite similar.[47] Since all their spectra were obtained for anhydrous salts, it was not possible to confirm the assignment of the 1210 cm^{-1} band in general to the presence of a water molecule.

Other slight differences can also be noted in Figure 3.3 for the three spectra at lower frequencies, but these were not ascribed to particular stretching or bending bands due to the difficulty of interpretation. Since the accuracy of infrared spectroscopy is commonly given as ± 1 percent under ideal conditions,[58] it is probably safe to say that not more than 4 percent of water contamination could be present and not appear in the spectra. These findings, combined with the results for the thermogravimetric analysis, made a good argument for complete conversion of the dihydrate form to the anhydrous form after drying 1 hour at 150°C.

3.2 ATOMIC ABSORPTION APPLIED TO KALE

It is ironic that while atomic absorption is now probably the most popular instrumental technique for the analysis of many common metals, the theoretical principles underlying both the flame processes themselves and the interelemental interferences, are little understood.

It has been stated that magnesium analysis is the most sensitive (expressed as aqueous concentration in µg/ml necessary to produce 1 percent absorption[241]) analysis obtainable by atomic absorption and that,

since magnesium is difficult and tedious to determine by other means, atomic absorption is almost always the method of choice.[8,240] Furthermore, the determination is said to be easy and subject to very few interferences. These statements are, however, an oversimplification, for the literature abounds with conflicting results as pointed out in the Introduction.

3.2.1 Preliminary Interference Studies

Before commencing the analysis of kale, experiments were undertaken to study possible interferences in the magnesium determination.

To make the results of such a study meaningful in the present context, all interferences were added at approximately their concentrations in kale, as given in Bowen's 1967 compilation.[35] The results, therefore, specifically apply to the problem at hand, since the behavior of diverse ions is far from simple.

Table 3.4, page 69, shows the effect of a number of foreign ions on the absorbance of a solution of 1.60 ppm magnesium at about pH 5 in very dilute hydrochloric acid, obtained by dilution of the 3200 ppm stock solution. The values shown were obtained over a period of days on the Jarrell-Ash unit and represent the average of a number of sets of readings. The synthetic kale solution used, contained both sulfate and silicate for these experiments, since at the low concentrations used for atomic absorption work, no precipitation occurred. Because it was impossible to keep the absorbance readings constant from day to day, due to changes in the lamp current and the exact lamp and burner positions, the values presented in all tables have been adjusted to that of an appropriate magnesium standard run in all series. In all cases the

TABLE 3.4 Effect of Some Ions on Magnesium Absorbance[1]

Ion Added[2]	Added Species	Ion Concentration (ppm)	Absorbance[3]	Percent Change[4]	Number of Analyses
Mg^{2+} alone			.469	--	10
Ca^{2+}	$CaCO_3$/HCl	40	.490	+5	10
SO_4^{2-}	Na_2SO_4	48	.495	+3	10
SiO_3^{2-}	$Na_2SiO_3 \cdot 9H_2O$	.65	.318	-30	10
PO_4^{3-}	$NH_4H_2PO_4$	14	.451	-4	10
Fe^{3+}	$FeCl_3$	.12	.479	+2	3
K^{1+}	KNO_3	25	.485	+3	3
Na^{1+}	NaCl	2.5	.483	+3	3
	$NaNO_3$	2.5	.480	+2	3
Sr^{2+}	$SrCl_2$	1500	.470	0	1
EDTA	Na_2EDTA	3700	.485	+3	1
Fe^{3+}, Sr^{2+}	as above		.473	0	1
K^{1+}, Sr^{2+}	as above		.472	0	1
Na^{1+}, Sr^{2+}	as above		.466	0	2
Fe^{3+}, K^{1+}, Na^{1+}	as above		.484	+3	1
Fe^{3+}, K^{1+}, Na^{1+}, Sr^{2+}	as above		.466	0	1
synthetic kale			.424	-10	10
synthetic kale, Sr^{2+}			.470	0	2

1. Results were obtained on the Jarrell-Ash unit with an air-acetylene flame. Values were recorded over a period of time. See text.
2. All solutions contained 1.60 ppm magnesium.
3. Mean of all analyses, normalized as discussed in the text. Standard deviation ± 0.004 absorbence units for a single determination.
4. Relative to magnesium alone.

precision was approximately ± 2 percent (two standard deviations).

The results of this initial study indicated that silicate and phosphate both lowered the absorbance, while calcium enhanced it slightly. To check whether the very slight effects exhibited by iron, sodium and potassium were real, various mixtures were examined both with and without added strontium and disodium EDTA, both of which have proven successful in nullifying diverse ion effects.[220] The results obtained in this case, showed that within experimental error, no effect was apparent (Table 3.4). The synthetic kale exhibited a lower absorbance, presumably due to a combination of these effects. The releasing effect of the strontium is apparent on examining the last row in the table. Synthetic kale and real kale solutions passed over a Dowex 50 column, as for the gravimetric analysis, showed no difference with and without added strontium on either the Jarrell-Ash or Heath instruments.

On the other hand, the real kale when wet ashed, filtered and diluted showed no difference in magnesium absorbance with or without added strontium. In the present case, where both silicate and sulfate were present in the synthetic kale solution, the difference would appear to be related to the removal of a mineral phase by filtration, in the real kale. In the synthetic kale solution, where no visible precipitation was observed, filtration produced no effect on the magnesium absorbance.

Table 3.5, page 71, presents the results of a more comprehensive examination of the effects of iron, sodium and potassium on the observed magnesium signal. These ions were studied in more detail as they would be collected from a cation-exchange column in the magnesium fraction. As anions are not absorbed on the Dowex 50 column, the eluate would only

TABLE 3.5 Effect of Iron, Sodium and Potassium on Magnesium Absorbance

Ion Added[1]	Conc.[2] (ppm)	Heath				Jarrell-Ash			
		Mean Abs.	Percent Change	Std. Dev.	Std. Dev. of the Mean	Mean Abs.	Percent Change	Std. Dev.	Std. Dev. of the Mean
Fe^{3+},Na^{1+}	.10,100								
K^{1+} alone	20	.001	--	.00081	.00025	.001	--	.00031	.00010
Mg^{2+} alone	1.6	.469	--	.00117	.00036	.469	--	.00319	.00101
Fe^{3+}	.025	.475	+1.3	.00367	.00115	.470	+0.2	.00266	.00083
	.050	.476	+1.5	.00172	.00055	.470	+0.2	.00262	.00083
	.10	.479	+2.1	.00176	.00056	.467	-0.4	.00278	.00088
	.50	.482	+2.8	.00186	.00058	.471	+0.4	.00378	.00119
Na^{1+}	1	.479	+2.1	.00445	.00140	.468	-0.2	.00262	.00083
	5	.478	+1.9	.00128	.00040	.473	+0.8	.00289	.00091
	10	.478	+1.9	.00319	.00100	.475	+1.3	.00403	.00127
	50	.482	+2.8	.00362	.00114	.483	+3.0	.00413	.00130
	100	.486	+3.6	.00347	.00110	.493	+5.1	.00377	.00119
K^{1+}	5	.484	+3.2	.00312	.00098	.477	+1.7	.00257	.00080
	10	.485	+3.4	.00133	.00042	.476	+1.5	.00252	.00079
	20	.487	+3.8	.00265	.00083	.474	+1.1	.00226	.00071
	50	.487	+3.8	.00233	.00073	.479	+2.1	.00245	.00077
Fe^{3+},Na^{1+}	.10,50	.495	+5.5	.00251	.00079	.483	+3.0	.00363	.00114
Fe^{3+},K^{1+}	.10,20	.492	+4.9	.00119	.00037	.479	+2.1	.00173	.00054
Na^{1+},K^{1+}	50,20	.484	+3.2	.00379	.00119	.482	+2.6	.00335	.00106
Fe^{3+},Na^{1+}	.10,50								
K^{1+}	20	.491	+4.7	.00218	.00068	.485	+3.4	.00177	.00055

1. All solutions, except for the first one listed, contained 1.60 ppm magnesium.
2. Concentrations relate to ions added, in the order given in column 1.

contain chloride ions from the hydrochloric acid eluant.

A range of concentrations was chosen to cover the expected values found in kale and absorbence measurements were made on both atomic absorption instruments. All measurements were made with the instruments optimally adjusted (Table 2.1, page 37), after allowing 1 hour for the lamp and electronics to stabilize. Ten consecutive measurements were made for each solution, aspirating water in-between for 10 seconds. A separate magnesium solution was used to monitor instrumental drift. This monitor solution was aspirated after every fourth solution. No drift was noted for the Heath instrument, while a 2 percent jump in absorbance was found about two-thirds of the way through the series on the Jarrell-Ash. In this case, the first 12 solutions were again aspirated. No further drift was found.

The results showed that the Jarrell-Ash had a slightly lower precision than the Heath instrument, as reflected by the larger standard deviation for the absorbance of most solutions for the Jarrell-Ash unit. For every solution, the relative standard deviation of the mean was 0.5 percent or less (5 percent level) for both instruments.

Interpretation of the effect of the three ions on the magnesium absorbance was complicated by the fact that the two instruments did not appear to exhibit the same sensitivity towards the foreign ions. This general phenomenon has also been noted by other workers, without explanation.[187,188] A maximum difference of about 0.1 percent in absorbance between any two solutions on the same instrument could have been accounted for by the use of different 100 ml volumetric flasks for the 19 solutions. However, this error was obviously masked by the up to 1 percent scatter exhibited by consecutive absorbance readings

of the same solution.

The overall results obtained on the Jarrell-Ash instrument for this study, compared semi-quantitatively with those found earlier for the preliminary study, shown in Table 3.4. The lack of close agreement was not too surprising in view of the fact that the preliminary results were obtained over a period of a few days, so that burner height, gas flows and other critical parameters could not be maintained at as constant a level.

It was difficult to explain satisfactorily the causes for the enhancement by the alkali metals of the magnesium absorbance. Suppression of ionization, due to the presence of a more easily ionized species, namely sodium or potassium, would have explained the increased absorbance in the presence of the alkali metals. However, the degree of ionization for magnesium in an air-acetylene flame has been given as "approximately" zero, in a paper by Amos and Willis.[7] Furthermore, Ramirez-Munoz found that at ratios for sodium to magnesium of less than 5000:1, no "appreciable" decrease in absorbance occurred.[223] In this instance, the depressed absorbance was thought to be due to a change in the magnesium diffusion rate and a shifting of the evaporation equilibria for the particles reaching the burner, conditions prevailing at high sodium concentrations which would reduce the atomization efficiency. Unfortunately the words "approximately" and "appreciable" limited the usefulness for the present discussion and no absorbance data was presented in either paper. If indeed, ionization was invoked to explain the absorbance change upon addition of either alkali metal, the different behavior between the Jarrell-Ash and Heath instruments became plausible in terms of different burner geometry, fuel mixture and light path through the flame. The

possibility of unresolved sodium emission reaching the detector, was quite unlikely, as both instruments were dual-beam models. Also, the enhancement due to potassium would have required an alternate explanation as potassium does not possess a nearby emission line.

The iron interference was equally difficult to explain. The most plausible reason was probably in terms of competing flame reactions. It was impossible to compare the different results obtained by a number of workers for iron-magnesium interference, since not only were the burner and flame types different, but the relative amounts of iron and magnesium differed and it has been shown as well that the freedom from interference depends on the relative position of the light path through the flame.[7] Formation at low iron concentrations of a mixed refractory oxide, followed at higher concentrations by formation of additional free atoms and their subsequent reaction has been postulated.[125] This would be in keeping with the results obtained by Frank and coworkers who found considerable enhancement of the magnesium absorbance at or above a 1 to 1 ratio with magnesium.[103]

Iron has two lines very close to the 2852.1 Å magnesium absorption line, namely 2852.1 Å and 2851.8 Å.[195] While Frank and coworkers used the former iron emission line as a spectral source for magnesium, no reference was made to the latter atomic line which might be a resonance line. A typical atomic absorption instrument would be unable to resolve these two lines completely and their relative emission intensities have been given as 150 and 200, respectively, compared to 300 for the 2852.1 Å magnesium line.[195]

To check whether these lines were really absorption lines, a

number of iron chloride solutions were prepared. Using a magnesium lamp, no absorbance was observed even at 500 ppm iron at an instrumental resolution of 1 Å FWHM, suggesting that neither iron line was, in fact, a resonance line.

In the present case, the effect was quite small. Real kale samples which had been passed over an ion exchange column showed no difference with or without strontium added, indicating that the presence of a releasing agent was not required, or that any effect was masked by other reactions.

In conclusion, the aforementioned studies have shown that the presence of iron, sodium, or potassium might lead to up to a 3 percent increase in the magnesium absorbance, depending upon the exact ratio(s) of the interfering ion(s) to magnesium and the atomic absorption instrument employed. The net result would be that the magnesium levels calculated for the kale and rat samples might have a positive bias of several percent, due to the presence of interfering ions in the solutions used for the absorbance measurements.

Although strictly not an interference, a brief look was taken at the effect of sample temperature on absorbance for both stock magnesium solutions as well as a wet ashed kale sample. This work was carried out on the Heath instrument at a burner height of 12 mm. The two solutions, containing about 1.6 ppm magnesium each were warmed to about 60°C on a hot plate and then checked for magnesium absorbance every few degrees as they cooled. An unheated control sample, kept at ambient room temperature, was used to correct for any instrumental drift. In each case, a roughly parabolic curve was obtained with the absorbance in-

creasing with temperature. The absorbance was enhanced at the rate of approximately 0.004 absorbance units per 1 C° from 15°C (placed in a refrigerator) to 25°C and then at about 0.008 absorbance units per 10 C° above that for the standard magnesium solution. The kale showed a slightly greater effect, about 0.005 and 0.010 absorbance units, respectively. This slight difference in behavior probably was a direct result of the much higher ionic strength of the kale. Temperature effects, commonly ignored, are thus obviously important if high accuracy is required, especially as the temperature decreases.

3.2.2 Photon Counting

The use of photon counting can provide information about the relative emission and absorption intensities in the flame under given conditions. In the present work, it provided a simple means of obtaining the hollow cathode spectral output around the 2852 Å magnesium line.

Measurements were made for a number of magnesium-containing solutions on the Heath 703, using the chopped absorbance mode. Flame emission was found to vary from 15 to 30 percent of the absorbance signal depending on the slope of the calibration plot and the net counts obtained for a water blank. Thus, for a given solution, a larger absorbance was calculated for a decreased net transmittance, obtained by dividing the net photon count for the water blank by the net count for the sample. These counts, in turn, depended on the sample aspiration rate and the gas flow. No emission difference was observed for water or solutions containing magnesium or kale, or kale plus 1500 ppm strontium, under identical conditions. The relatively low emission for all samples meant that flame background was acceptably low and

unlikely to interfere with the absorbance readings. The flame background was found to be much higher for an oxy-hydrogen flame than for the cooler air-hydrogen or air-acetylene flames, due to the decreased production of hydroxyl radicals in the latter which absorb strongly in this region of the spectrum. For a given solution, however, the ratio of absorbance to absorbance plus emission remained relatively constant regardless of flame type.

The observed spectrum around the magnesium analytical line is shown in Figure 3.4, page 78. The relative constancy of the spectra as viewed by the phototube, regardless of the aspirated solution, can be seen. This constancy would suggest minimal spectral interference. The degree of line broadening was also apparently quite low at a resolution of 1 Å FWHM. Thus, minimum curvature of the calibration plot was assured. The constancy of the spectrum under a variety of conditions provided satisfactory evidence that the light source was unlikely to introduce an error under the analytical conditions employed.

3.2.3 Studies Employing the Basic Technique

As has been indicated, all results for kale discussed in this subsection were obtained using calibration curves for standard solutions run together with the samples. This method is the basic technique of atomic absorption.

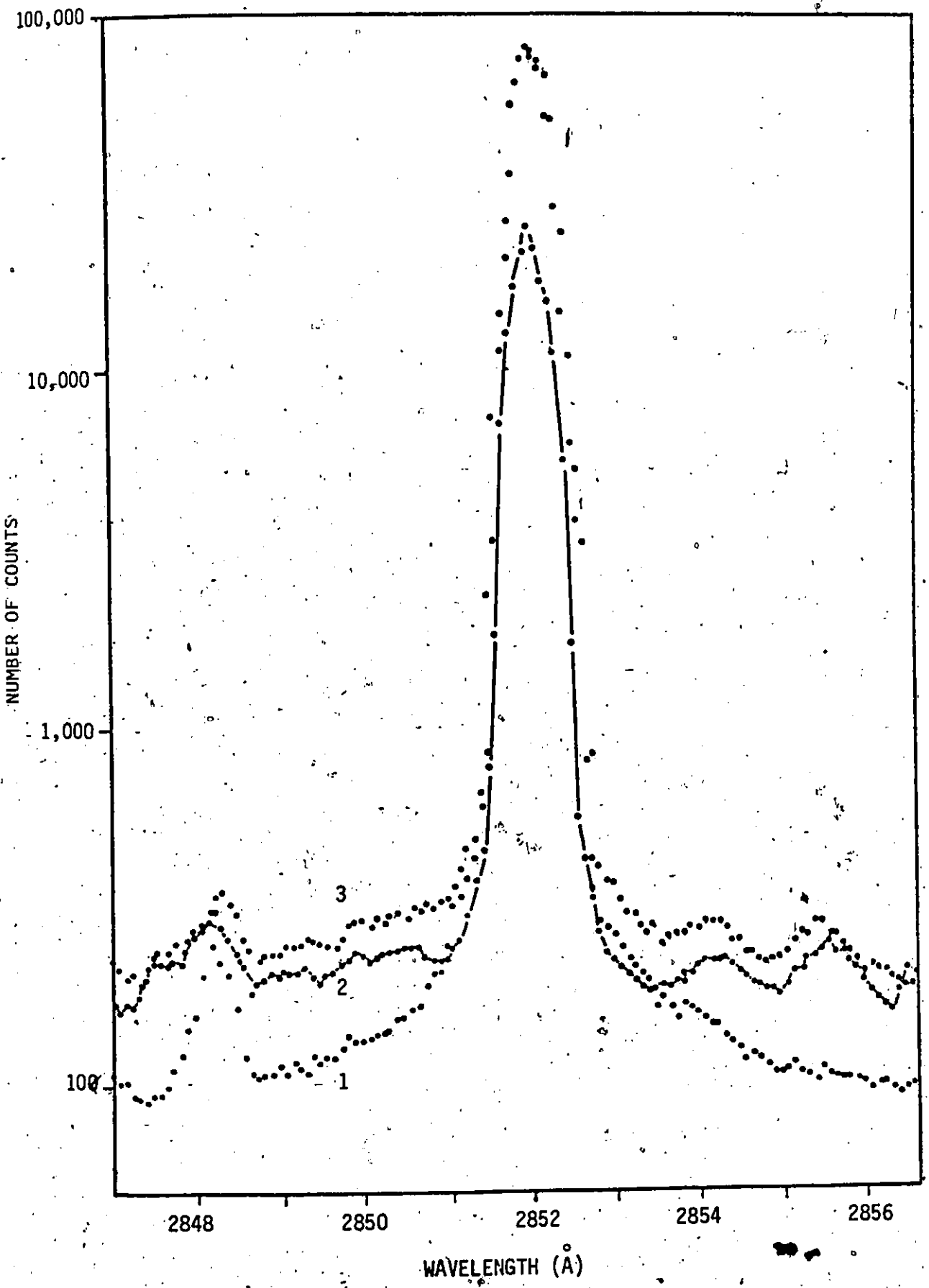
An attempt was made to ensure that the measured absorbance fell between about 0.3 and 0.6. Generally, it was found that the calibration plot began to curve above an absorbance of 0.7. In all cases, a graph was first plotted to check for curvature and points falling more than

Figure 3.4 Observed Spectrum Around the 2852 Å Magnesium Absorption Line

Lamp:	Westinghouse Ca-Mg-Al, Model #W122930, serial #C56289, neon fill gas, operated at 10 ma
Instrument:	Heath EU-703 with a 5 cm Varian burner assembly
Slit Width:	50 μ
Resolution:	1 Å FWHM (calculated)
Scan Rate:	0.1 Å/sec
Analyzer Resolution:	0.08 Å/channel

Spectrum 1: Spectral output of lamp
Spectrum 2: Lamp with air-acetylene flame, aspirating 1.60 ppm magnesium standard in hydrochloric acid
Spectrum 3: Lamp with air-acetylene flame, aspirating water

Other spectra obtained by aspirating synthetic kale with added perchloric acid, synthetic kale to which both perchloric acid and strontium (1500 ppm) had been added and real kale, all appeared virtually identical to Spectrum 2.


100,000
10,000
1,000
100
NUMBER OF COUNTS
3
2
1
2848
2850
2852
2854
2856
WAVELENGTH (Å)

3 standard deviations from a straight line, fitted by eye, were rejected prior to calculating a least-squares fit. The concentration of the samples was then found using the slope and intercept obtained for the straight line. The importance of making such a preliminary graph was critical for analysis by standard addition, as will be shown later.

The magnesium values obtained for 9 kale samples using a preliminary dry ashing and separation on Dowex 50 are given in Table 3.6, page 80. These values were obtained using standards prepared in a hydrochloric acid matrix. Various aliquots of the column eluant were prepared and run over a period of time on both instruments. New dilutions were prepared for each analysis and each value given in the table represents a separate aliquot.

The values had a wider scatter than the gravimetric results, reflected in the larger calculated standard deviations. No particular trend was apparent on comparing the result to those given in Table 3.2, page 58. No corrections have been made for the effects of iron, sodium or potassium (described on page 70), on the magnesium values given in Table 3.6. However, that the overall average value was 1.6 percent higher than that obtained for the gravimetric analysis (shown in Table 3.2) would be in accordance with the previously described finding that these three ions increased the absorbance of magnesium up to roughly 3 percent.

Values for experiments 5 and 6, obtained on the Heath instrument, were about 5 percent higher than those determined using the Jarrell-Ash unit. However, this difference was not generally noted for either the preliminary interference studies nor for the other kale samples.

TABLE 3.6 Atomic Absorption Results for Dry Ashed Kale Following Ion Exchange Procedure[1]

Experiment[2]	Calculated Values (ppm)	Average
1	1710 1700 1640 1670	1680
2	1650 1630	1640
3	1720 1720	1720
4	1670 1670	1670
5	1650 1620 1740[3] 1740[3] 1750[3]	1700
6	1680 1660 1760[3] 1750[3] 1750[3]	1720
7	1730 1760 1730[3] 1760[3]	1740
8	1690[3] 1700[3] 1670[3] 1670	1680
9	1680 1675 1675	1670
Overall Average[4]	1690 ± 35	

1. Values were obtained on the Jarrell-Ash instrument unless otherwise indicated.
2. Experiment numbers correspond to those in Table 3.2, page 58.
3. Obtained on the Heath instrument. See text regarding averaging.
4. Standard deviation of a single measurement. Standard deviation of the mean was 6.

Furthermore, for both experiments 5 and 6, all data for the Heath instrument were obtained on the same day using the same calibration curve. As the standard deviation of a single measurement was approximately 2 percent, as shown in Table 3.6, retention of this data was felt to be justified at the 2σ level.

A separate set of kale samples was analyzed by wet digestion followed by direct dilution of the ash solution. Six samples were

separately ashed with a nitric-perchloric acid mixture and run over a period of time on both atomic absorption units.

Table 3.7, page 82, gives the results for the nitric-perchloric acid digestion. Again each value was calculated from a series of magnesium standards run simultaneously, except for experiment 6, where the values given were based on one standard, because the absorbances measured were in the curved region of the calibration plot. In this case, a standard was used whose absorbance was within 3 percent of the measured kale value. Photon counting experiments showed that the emission for this sample was similar to all the other samples, so it is unlikely that flame emission caused the significantly lower value. Similarly, light scatter seems improbable since no difference was seen between the values obtained with the 10 cm and 5 cm burners and since high sodium and strontium concentrations had no effect on the absorbance of magnesium standard solutions. A more probable cause was some form of molecular absorption or variation in the refractive index within the flame caused by particle vaporization. This type of flame occurrence has been postulated by Koirtyohann and Pickett.[156] Diluting the sample by a factor of two, resulted in a 2.5 percent increase in the calculted magnesium concentration, supporting this theory.

It is germane at this point to compare the results obtained from the wet ashed and dry ashed samples. In all statistical tests used in this thesis, unless otherwise mentioned, a 5 percent level of significance was taken. The result of a test was then said to be critical, that is, the hypothesis rejected, if the value obtained for the test exceeded this value. Since the selection of a level of signi-

TABLE 3.7 Atomic Absorption Results for Wet Ashed Kale[1]

Experiment	Calculated Values (ppm)	Average
1	1700[2] 1620 1790[2] 1720 1560 1720 1700 1670 1800 1690[2] 1740 1670[2]	1700
2	1710[2] 1580 1710[2] 1640 1570 1650 1680 1690 1750 1700[2] 1660[2] 1710	1670
3	1690 1700 1720 1790 1720[2] 1740 1690[2]	1720
4	1640 1680 1740 1650[2] 1700 1690[2]	1690
5[3]	1660 1820 1750 1780[2] 1830 1760[2]	1760
6[4]	1620 1660 1630[2] 1610 1620[2]	1630
Overall Average[5]	1700 ± 50	

1. Values were obtained on the Jarrell-Ash instrument unless otherwise indicated; ashed with nitric plus perchloric acids.
2. Obtained on the Heath instrument.
3. Sample weight 50 mg.
4. Sample weight 200 mg. Values calculated based on one standard.
5. Standard deviation of a single measurement. Standard deviation of the mean was 7.

ficance is quite arbitrary, this level was taken in keeping with common experimental practice.

A measure of agreement between means can be obtained by use of the "t" test, while a comparison of variances is possible with the "F" test.

In order to utilize these tests, each value, as shown in Tables 3.6 and 3.7, was assumed to be an independent measure of the same quantity. This hypothesis was checked by use of Bartlett's test, which permits a measure of the homogeneity of variances of the individual sets of data.[298] When this was done for the values in Table 3.6, a chi-squared value of 19.23 was obtained, greater than the critical value of 15.52 for 8 degrees of freedom.[71] Thus, the distribution of dry ashed results was not homogeneous at the 5 percent level, though it was at the 1 percent level (critical value 20.08).

Cochran's test allows an estimation of the inhomogeneity of a variance, based upon the variance of one value being much larger than the sum of the remainder of the variances. This would invalidate an analysis of variance test for the means.[71] As the critical value for this test was exceeded for experiments 5 and 6, these contributed excessively to the overall pooled variance. Eliminating these results, Bartlett's test was reapplied. A chi-squared value of 9.20 was obtained, less than 12.54, the critical value for 5 degrees of freedom. Nonetheless, for a comparison of wet and dry ashed analyses, values from experiments 5 and 6 were retained using the 1 percent level of significance for Bartlett's test.

The wet ashed results, shown in Table 3.7, gave a chi-squared value of 6.09 using Bartlett's test, less than the critical value of 11.05 for 5 degrees of freedom. Thus, at the 5 percent level, no differences were observed in the parent populations.

Using the pooled standard deviation calculated from the individual results for each ashing method, a "t" value of 0.36 was obtained,

which was much less than the critical value of 2.00 for 64 degrees of freedom. Thus, no difference was detected in the means. Again, using the individual results for both methods, the variances gave an "F" value of 2.15. Since this was greater than the critical value of 1.94, at the 5 percent level of significance, the means obtained by the two methods did not apparently have the same precision. This value, however, was not significant at the 1 percent level, which has a critical value of 2.58.

The difference in precision arose from a number of sources, those previously mentioned, as well as possibly the difference in sampling error due to the different weights employed. Wilson has derived an expression relating the relative standard deviation of sampling to the concentration of analyte present, its density and the number of particles forming the sample itself.[289] While this work was developed for rock powders, it has also been experimentally shown to hold for graphite powders used in emission spectroscopy and provides a good theoretical approximation, especially for platelet-type particles,[114] of which kale is composed. A calculation can be made in the present case using values from Bowen's work,[29] the only unknown equation variable being the number of particles per gram. Two estimates were made, based on 10^5 and 10^6 particles per gram, the order of magnitude expected for the kale mesh size. A 0.1 g sample gave values of 44 and 14 percent, respectively, while a 2.5 g sample gave values of 9 and 3 percent. These differences dramatically illustrate the improvement in sampling precision with increasing sample size. The difference in the precision of the means was thus strongly affected

by the sample size in the present work.

In summary, while both wet ashing and dry ashing procedures gave the same result within experimental error; the dry ashing procedure had a greater degree of precision, despite the fact that the latter method involved the additional step of ion exchange on a Dowex 50 column. The improved precision appeared to be related to sample size, since preliminary experiments on pure and complex magnesium solutions had shown atomic absorption procedures to be relatively unaffected by the presence of foreign ions.

3.2.4 The Use of Standard Addition

Several approaches were taken for the analysis of magnesium in kale by standard addition. Initially, two 100 mg samples were wet ashed and a 0.160 mg magnesium spike added. In one case, the spike was added prior to ashing and in the other, after ashing and transferring the solution to a 200 ml volumetric. Both solutions were run together with standards and the value of magnesium in the kale calculated from the calibration curve obtained for the standards. Each solution, together with standards, was analyzed three times over a period of time. The calculated magnesium concentrations are shown in Table 3.8, page 85. Values were obtained on the Jarrell-Ash except where shown.

TABLE 3.8 Magnesium in Kale -- Standard Addition Using a Calibration Curve

Sample	Calculated Values (ppm)	Average
1[1]	1660[2] 1750 1620	1680
2	1710[2] 1780 1640	1710

1. Spike added prior to ashing.
2. Obtained on the Heath instrument.

It was apparent from this table that the values agreed well with those obtained by direct analysis and that, as expected, no difference was observed whether the magnesium spike solution was added prior to or after ashing.

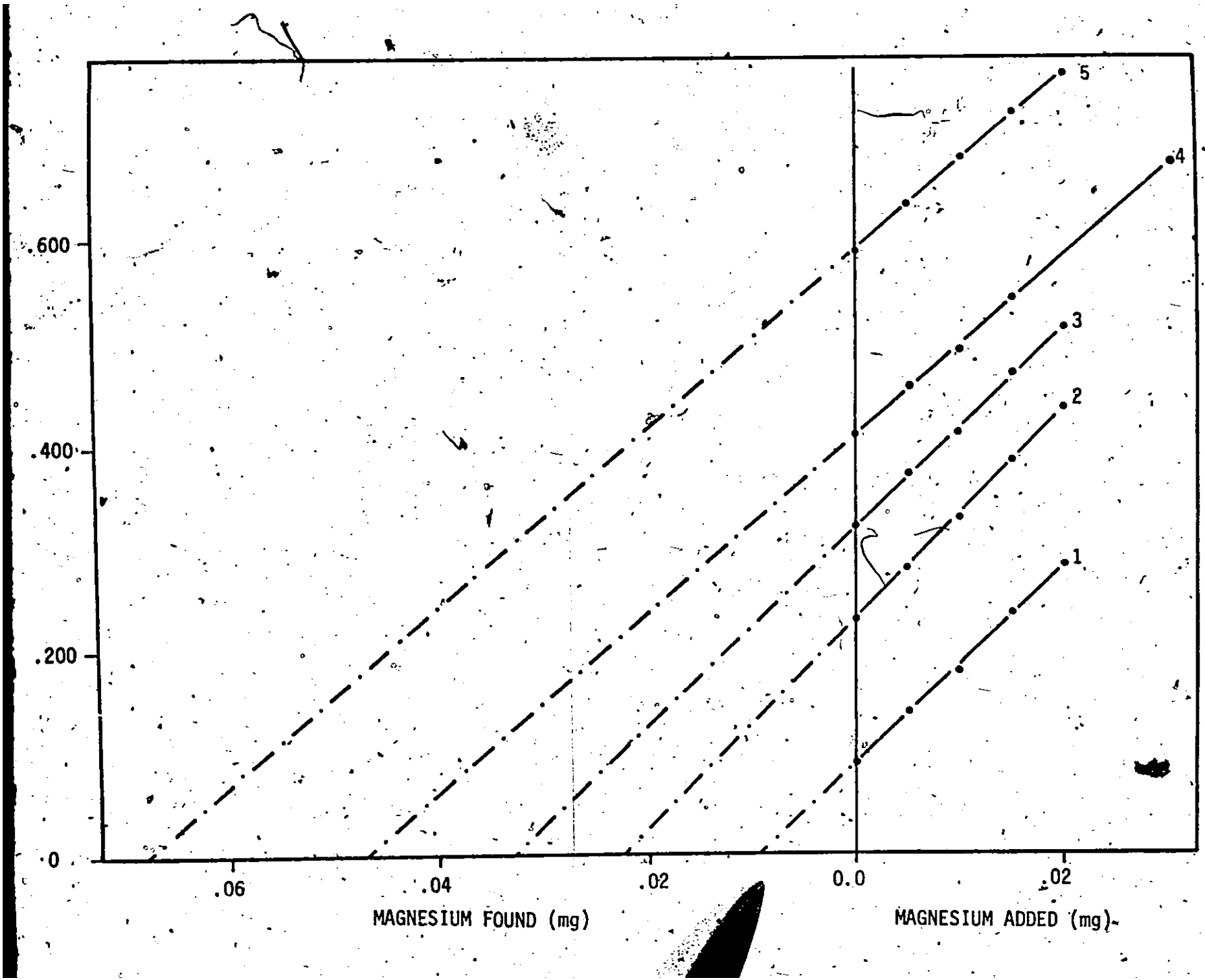
The possibility of analyzing the kale without the use of external standards was also examined. For this purpose, two kale samples weighing 250 mg and 500 mg were separately wet ashed, diluted to known volumes and five series of solutions prepared for analysis using various aliquots of the ash solution to which various amounts of spike were added. The 25 samples thus prepared were run three times over a period of time. The magnesium content was calculated using a least-squares fit for the individual sets of data. A typical family of curves is shown in Figure 3.5, page 87.

The aliquots and additions were chosen in such a manner as to cover a wide range of spike to sample ratios. This also enabled an estimate of the error in extrapolation. While the slopes of the lines remained fairly constant for any given set of curves as can be seen (relative standard deviation 0.9 percent), the variation from run to run was significant at the 5 percent level, a reflection on the inability to sufficiently control the instrumental parameters. It was apparent from this figure that the slope of a given line did not vary with the ratio of spike to sample present, suggesting that interferences were not detected in the range covered by these solutions. To try to improve the precision of these results, the solutions in each series were run first in the order of ascending spike concentrations and then in descending order, thus obtaining two values for each spike.

Figure 3.5 Typical Family of Standard Addition Curves for Kale

Graph 1: Effective weight of kale 5.01 mg
Graph 2: Effective weight of kale 12.5 mg
Graph 3: Effective weight of kale 20.3 mg
Graph 4: Effective weight of kale 25.0 mg
Graph 5: Effective weight of kale 37.6 mg

Graphs 1, 3 and 5 were obtained from different dilutions of a 500.7 mg sample.
Graphs 2 and 4 were obtained from different dilutions of a 250.2 mg sample.
All solutions were prepared in 25 ml volumetric flasks.


.600
.400
.200
0
.06
.04
.02
0.0
.02
MAGNESIUM FOUND (mg)
MAGNESIUM ADDED (mg)
5
4
3
2
1

The values calculated for the magnesium content in kale for the various series are shown in Table 3.9, page 88. The five samples correspond to the lines shown on Figure 3.5 for a single run. The goodness of fit for each of the lines on Figure 3.5 can readily be seen. For example, values for curve 1 span a concentration range of about a factor of 4 and since the line is generated by 5 points, a minimum correlation coefficient of 0.63 is required.[75] The calculated

TABLE 3.9 Magnesium in Kale -- Standard Addition Using Self-Standardization

Series[1]	Sample Weight (mg)	Calculated Values (ppm)	Average
1	5.01	1810[2] 1740 1730	1760
2	12.5	2210[3] 1830 1870	1840
3	20.3	1870 1670 1750	1750
4	25.0	1830 1720 1620	1720
5	37.6	1900 1910 1810	1870
Overall Average			1790

1. Series 2 and 4 were obtained from aliquots of the 250 mg sample, the rest from the 500 mg sample.
2. Values in this column were obtained on the Heath 703.
3. Not used in calculating the average.

correlation coefficient was better than 0.998 in all cases, indicating a low degree of random error. The standard error of the regression was also calculated for several lines, using the method of Larsen.[168] The 5 percent limits for the intercept of the extrapolated line were found to be about ± 3 percent, again showing the good fit. Yet, it

was quite apparent on examining the results in Table 3.9 that they were significantly higher and more variable than those obtained by analysis using an external calibration curve. This suggested the possibility of a constant or a proportional error. The former would be expected to show up as a change in the axes intercepts and the latter in the slope of the line.[265]

Using a scatter diagram, plots were made of the absorbance for the unspiked sample against samples spiked with 0.01 mg and 0.02 mg for each series and a slope and intercept calculated. In all cases, the intercept differed from zero, an indication of possible systematic error.[75] However, when the fit of the line was measured using the "F" test to compare the intercept calculated with the theoretical zero intercept, the value for "F" was less than the critical value in all cases and so it was concluded that the intercept did not differ from zero more than could be accounted for by experimental error. Thus, the presence of a constant error was ruled out.

The presence of proportional errors would affect the slope of the standard addition curve. This was checked by preparing another two series of kale samples spiked with various quantities of magnesium and comparing the absorbances with those from a set of solutions containing similar quantities of the spike alone. It was found that the line obtained with the pure magnesium solutions had a greater slope than that of the spiked kale solutions indicating that, in fact, the spike did not add linearly to the magnesium present in the kale, thus invalidating the basic assumption of standard addition. Using the ratio of the slopes of the two lines, an empirical correction factor of

0.91 ± 0.02 (2σ) was calculated. When this factor was applied to the results in Table 3.9, a mean value of 1630 ppm was obtained.

The adjusted values, obtained by applying the correction factor were approximately 4 percent low when compared to those obtained for samples analyzed against external standards. However, considering the error in the empirical correction factor itself, the results were considered acceptable. The cause for this interference was shown to be chemical in nature, rather than due to molecular absorption or light scatter. A series of measurements on spiked and unspiked samples was made using the dual-channel mode for the Jarrell-Ash. No change in absorbance was found on using a second wavelength of 2844 Å for background correction or employing an iron lamp at 2719 Å for light scattering correction.

A further two identical sets of wet ashed kale solutions were prepared for standard addition to examine reproducibility under given conditions and the effect of very slight curvature on the calculated result. To minimize extrapolation error, the additions were chosen to give a relatively steep slope. Both solutions were run twice on the Jarrell-Ash. The absorbance results are shown in Table 3.10, page 91.

The calculated results for the extrapolated values were seen to show a marked difference based on very slight changes in the measured absorbance value. Furthermore, very slight curvature in the graph was found to result in a sizeable change in the calculated value. This large magnification of error, due to the extrapolation procedure, is probably the greatest drawback for standard addition methods. It cannot

TABLE 3.10 Reproducibility of Standard Addition for Kale

Magnesium Added (mg)	Series A Run 1	Series A Run 2	Series B Run 1	Series B Run 2
0	.095	.096	.096	.101
.0100	.210	.209	.212	.215
.0200	.322	.324	.327	.330
.0300	.433	.438	.433	.441
.0400	.542	.542	.538	.541
.0500	.645	.645	.648	.644
.0600	.740	.741	.743	.742
.0700	.835	.840	.828	.843
.0800	.917	.925	.932	.929
Calculated Magnesium Concentration (ppm) a.[1]	1730	1740	1810	1880
Calculated Magnesium Concentration (ppm) b.[2]	1790	1810	1840	1900

1. Calculated based on the linear portion of the graph, that is to the addition of 0.040 mg magnesium.
2. Calculated based on additions including 0.050 mg magnesium.

be significantly reduced for atomic absorption as the scatter of the data shown in Table 3.10 is not easily reduced.

The following comments can be made based on the results obtained for kale by standard addition. Great caution must be exercised when using the method and the method cannot be advocated for use where a high degree of precision is required. Through use of an emiprical correction factor, improved accuracy was obtained, though accuracy without precision is a meaningless concept. Even so, use of an empirical

correction factor is questionable and the overall standard deviation of the calculated result will be increased. In the case of magnesium, matrix interference could not be satisfactorily eliminated by use of standard addition. Thus, the greatest advantage of the method was not realized for the present analysis.

3.3 NEUTRON ACTIVATION ANALYSIS OF KALE

Neutron activation analysis provided a third independent method for the determination of magnesium in kale. Procedures were developed which produced a fair degree of precision though flux inhomogeneities in the rabbit were found to be much more significant than is commonly realized and ultimately contributed to the limited precision of the activation methods.

3.3.1 Flux Variations Within the Rabbit

As the variation of flux within the rabbit was found to be an important consideration in attempting to obtain an accurate result for magnesium by neutron activation analysis, a study was made of the inhomogeneity. Possible variation in the epithermal-to-thermal flux was not examined, as any effect was expected to be very small for the present application. For the present analysis, use of a pneumatic rabbit which was situated in a position of high flux gradient produced conditions that tended to enhance flux variations.

To measure the degree of flux inhomogeneity, a series of 5 minute irradiations was performed using 20 lanthanum-aluminum alloy wires situated around a lucite holder which just fitted the rabbit.

A graph was plotted of the specific activity against the position for each of the four rows around the diameter. A typical diagram is shown in Figure 3.6, page 94. The flux gradient is seen to be quite linear along the length of the rabbit. All points fell within 1 standard deviation of the least-squares line except for positions 6 and 16 which were within 2 standard deviations. The variation around the circumference is given by the vertical difference in counts between the points on adjacent lines. The flux difference along both the rabbit length and across the diameter was found to be about 10 percent. A maximum difference of 30 percent was found between positions 6 and 20. The flux gradient was thus approximately 2 percent per centimeter along the rabbit length. A similar difference was found for all shim rod positions checked and the plots obtained were identical to that shown, indicating that the amount of exposed fuel rod did not appreciably affect the flux inhomogeneity.

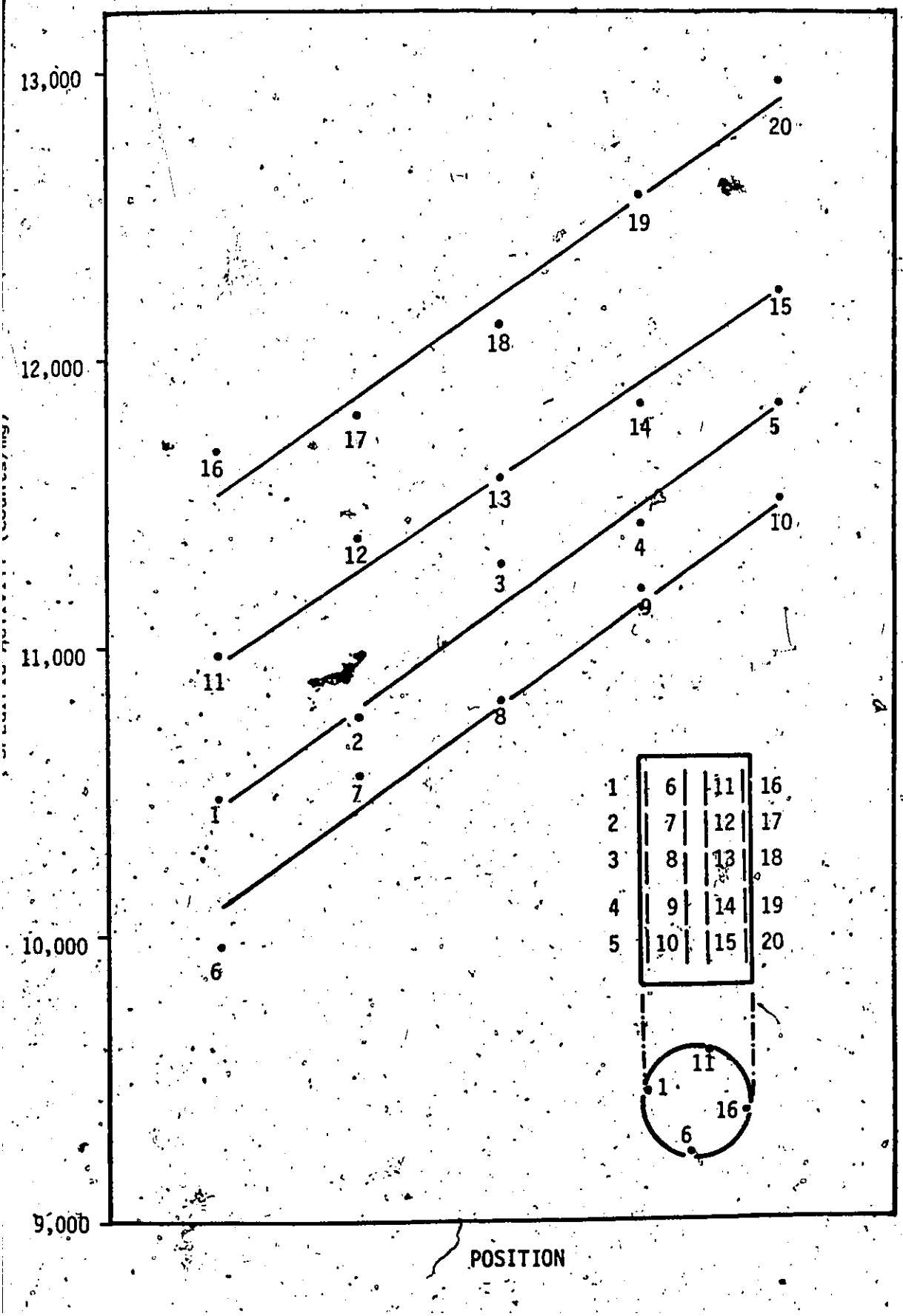
The magnitude of the inhomogeneity found for the McMaster reactor was larger than had been expected, especially around the circumference of the rabbit. For this reason, it was apparent that anything less than a flux monitor which completely encircled the sample to be irradiated, would only give an approximate indication of the flux, since no control was possible over the radial orientation during the actual irradiation.

The smaller the sample volume, the more critical the exact placement in the rabbit would become and irradiating a 1 cm^3 sample and a standard together, without flux monitors, could be expected to give an error as large at 10 percent in the calculated sample magnesium concentration.

Figure 3.6 Flux Variations Within the Rabbit

5 minute irradiation of 1 cm pieces of lanthanum alloy wire mounted on a lucite rod. The holder dimensions were 52 mm x 20 mm diameter.

Each piece of wire was numbered as shown in the lower right-hand corner.


13,000
12,000
11,000
10,000
9,000
POSITION
1 6 11 16
2 7 12 17
3 8 13 18
4 9 14 19
5 10 15 20
1
11
16
6

From these experiments it became clear that the precision expected for rabbit irradiations could not equal that for longer in-core irradiations where flux variation is much less a problem. Unfortunately, the half-life of magnesium is such that this was not possible.

3.3.2 The Instrumental Method

For the analysis by INAA, a series of four 100 mg samples was irradiated together with weighed magnesium ribbon monitors.

Self-shielding was not regarded as a problem in the present analysis due to the low effective neutron cross-section of the organic material itself and its low density. Fast flux moderation due to the use of aqueous standards was also expected to exert a negligible influence on the total thermal flux seen by the standards. Moderation of the epithermal and fast flux for a thermal reactor would increase the thermal flux by less than 1 percent, since moderation is a function of volume and the sample volume was approximately 1 ml.

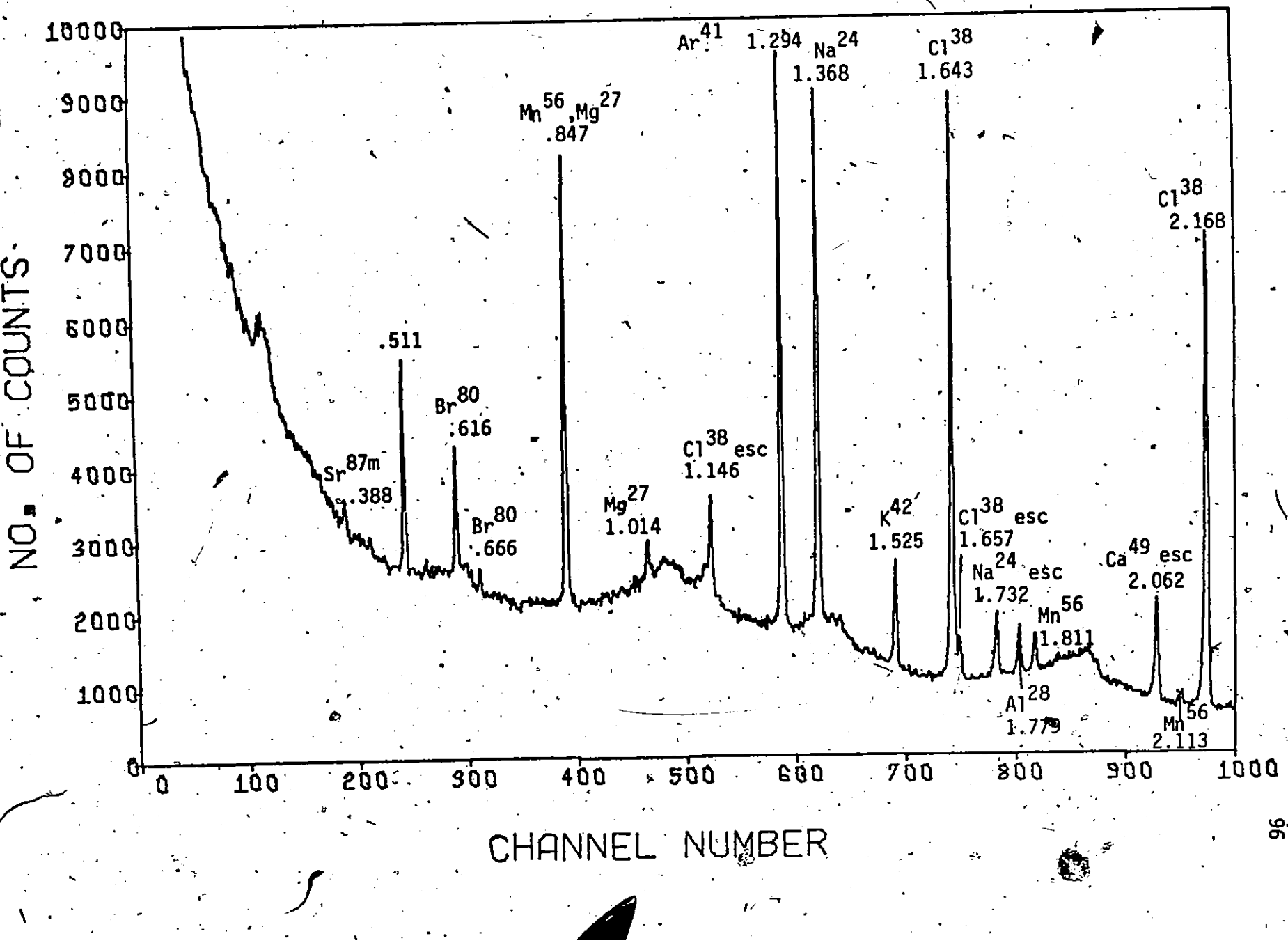
A typical gamma ray spectrum for an irradiated kale sample is shown in Figure 3.7, page 96, with the various peaks labelled. The resolution shown in this gamma ray spectrum, as well as the other spectra, was approximately half that of the spectra used for analytical purposes, due to technical problems involved in recording the data for the computer plotter.

The low sensitivity and high background of the 1.014 MeV magnesium peak were readily apparent. The 0.844 MeV peak has about three times the relative intensity of the higher energy peak.[106] However, for the kale spectrum shown, the activity in this energy region

Figure 3.7 Gamma Ray Spectrum of Irradiated Kale

Irradiation time: 1 minute
Cooling time: 300 seconds
Counting time: 1000 seconds, background subtracted
Sample size: 100 mg

The prominent peaks are identified with energies in MeV. The magnesium concentration in kale is approximately 1660 ppm.


NO. OF COUNTS
CHANNEL NUMBER
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
100
200
300
400
500
600
700
800
900
1000
Ar41 1.294
Na24 1.368
Cl38 1.643
Mn56,Mg27 .847
Cl38 2.168
.511
Br80 .616
Sr87m .388
Br80 .666
Mg27 1.014
Cl38 esc 1.146
K42 1.525
Cl38 esc 1.657
Na24 esc 1.732
Mn56 1.811
Ca49 esc 2.062
Al28 1.779
Mn56 2.113

consists mainly of manganese-56 so that the net magnesium concentration was derived from the difference of two large numbers, which would result in a low degree of precision. The presence of argon could be traced to the failure to transfer the irradiated sample to another container prior to counting. Some of the chlorine activity was undoubtedly due to the plastic container.

By counting the samples and flux monitors in a known time sequence, flux corrections and subtraction of manganese activity were performed. The results obtained for the duplicate standards and the four kale samples are shown in Tables 3.11 and 3.12; pages 98 and 99, respectively. The last column in Table 3.11 shows that the aqueous volume had an effect on the number of counts. Since the volume of the kale samples was about 0.25 ml, the sample activity was compared to a standard of similar volume.

The calculations involved in obtaining a value as shown in the tables can be summarized as follows. After printout of the peak channels plus 20 additional channels on either side, the unadjusted peak area was obtained by summing the peak channels and subtracting the background. This background was obtained by finding the mean counts per channel for 10 channels on either side of the peak and multiplying by the number of peak channels, which typically was 15 and 10 for the 0.844 MeV and 1.014 MeV peaks, respectively. This method of obtaining a peak area is only one of at least seven methods commonly used. The choice of method was based on a study of the relative precision of seven methods under different conditions by Baedecker, who found this simple method and another more complex one to be the most advantageous.[11]

TABLE 3.11 Standards Used for Instrumental Neutron Activation Analysis

Standard[1]	Volume (ml)	Original Monitor Counts	Normalized Monitor Counts[2]	Original Standard Counts	Normalized Standard Counts[3]	Average Value (Counts/100 mg)
1	.10	64515[4] 18720	43055 13764	4717 1221	3513 853	3446
2	.10	66049 18698	44251 13748	5663 1385	3378 969	911
3	.25	67383 19076	43416 14026	4781 1317	3530 903	3432
4	.25	64299 18422	43220 13683	4495 1417	3335 996	950
5	1.5	68549 20038	45661 14734	4435 1134	3126 740	3177
6	1.5	63066 18152	41667 13250	4195 1134	3228 830	785

1. All standards contained 0.160 mg of magnesium in dilute nitric acid.
2. Counts per 100 mg ribbon, corrected for manganese-56 activity.
3. Adjusted to a sample weight of 100 mg and a standard monitor count. A 2.6 percent dead time correction has also been applied. (See Appendix, equation 5.7).
4. The upper row values were obtained for the 0.844 MeV peak, the lower row values for the 1.014 MeV peak.

TABLE 3.12 Instrumental Neutron Activation Analysis for Magnesium in Kale

Sample	Weight (mg)	Original Monitor Counts	Normalized Monitor Counts[1]	Original Sample Counts	Manganese Corrected Counts[2]	Normalized Sample Counts[3]	Magnesium Concentration[5] (ppm)
1	101.7	68984[4]	49052	16566	4896	4908	1430
		19469	14638	1468	1622	1634	1720
2	102.2	61899	42756	16277	4960	5675	1650
		17860	13633	1344	1485	1599	1680
3	98.5	48151	43065	16416	5464	6441	1870
		13831	13694	1286	1457	1620	1700
4	98.3	46671	43741	16143	4878	5672	1650
		13532	13532	1298	1470	1658	1760
Average[6]							1650 ± 91(180)
							1710 ± 13(30)

1. Counts per 10 mg ribbon, corrected for manganese-56 activity.
2. Corrected for manganese-56 activity as well as 13.3 percent dead time correction. (See Appendix, equation 5.7).
3. Adjusted to a standard sample weight and monitor count.
4. The upper row values for each sample represent the values obtained for the 0.844 MeV peak, the lower row, the values for the 1.014 MeV peak.
5. Based on the counting statistics for the figures given in columns 5 and 6 and the gross peak counts, the calculated values for the 0.844 MeV and 1.014 MeV peaks had standard deviations of about 8 percent and 10 percent, respectively.
6. Standard deviation of a single result given in brackets.

Following a recount of the sample at a later, known time, generally about 2 or 3 hours later, the net counts obtained for the 0.844 MeV peak due to manganese (corrected for decay), were subtracted. The magnesium counts following this subtraction were adjusted for the high count dead time.[68] This latter correction was necessitated due to the high overall sample activity for the kale and rat samples. The dead time, about 20 percent for the kale samples, required that the count values be multiplied by an empirical factor of 1.133.

This dead time correction factor could strictly only be applied to a pure isotope, since the form of the dead time decay curve will be complex if a large number of short-lived species are present, as in the present case.[148] Chance summing, the capture of two gamma rays simultaneously so that they appear as one event in the analyzer, would tend to lower the magnesium photopeak in a complex material such as kale. Since chance summing is a function of the analyzer dead time, the dead time factor could only result in an approximate correction. However, it did enable an estimate and based on the instrumental results obtained, must be considered quite acceptable in this case.

Following dead time correction, the magnesium counts were normalized to a standard flux monitor activity. Formulae for various count corrections are given in the Appendix. Finally, the counts were adjusted to a standard sample weight. Similar count corrections were made for the standards, permitting a direct comparison of the magnesium activity and thus the relative quantities of magnesium.

Values are given in Table 3.12 for the magnesium content calculated from both gamma-ray peaks. Although there was no significant

difference at the 5 percent level in the mean calculated for either of the two energies, there was a critical difference in the precision. The difference in precision could not be accounted for on the basis of counting statistics, which gave standard deviations of about 8 percent and 11 percent for the 0.844 MeV and 1.014 MeV peaks, respectively. A similar difference in precision was not found for the rat samples using the same procedure. All values were pooled to give a mean magnesium concentration of 1680 ± 46 ppm.

This work appears to be the first report in which the results of an INAA determination of magnesium in kale were not low. For example, Garrec obtained a value of 1209 ppm, using the $Mg^{24}(n,p)Na^{24}$ reaction.[107] This may have been due to the failure to sufficiently recognize the possibility of errors due to flux and dead time variations.

3.3.3 The Destructive Method

Destructive neutron activation analysis, while requiring more complex sample treatment than INAA, enabled separation of the activity of the species of interest from that of the rest of the matrix. For the kale analysis, this task proved difficult in view of the high manganese activity and the short time available for separation.

For the destructive analysis, the greatest potential source of error arose from the interference of manganese. Since the high manganese-56 activity, due to its favorable nuclear properties, tended to obscure the lower energy magnesium-27 peak, as shown in Figure 3.7, the possibility of complete removal of the manganese activity, prior to counting, was investigated. Because of the 9.46 minute half-life for magnesium-27,

the most promising methods appeared to be those involving precipitation or ion exchange.[169]

A number of separation procedures were investigated, including precipitation of manganese as the sulfide[194,237] or dioxide[49,158,236,268,270] from a basic solution, and as the dioxide using chlorate[93,142,198] or persulfate[140,251] from an acid solution. The possibility of separation as the sulfide on an ion exchange column was also examined.[105,109] Of these procedures, separation of manganese as the sulfide from a basic solution proved the most successful and is fully described in Section 2.5.3.

The rapid separation of manganese as the sulfide resulted in manganese contributing an average of 4 percent to the activity of the 0.844 MeV peak, based on 21 kale analyses. A typical spectrum of a kale sample following separation of the manganese activity is shown in Figure 3.8, page 103. This spectrum, obtained for a 100 mg sample, showed the improvement obtained for destructive analysis. Strontium and calcium were not separated due to a phosphate precipitation procedure being employed and the sodium peak resulted from the presence of occluded sodium in the phosphate precipitate. This might have been avoided by reprecipitating the magnesium phosphate. However, any gain obtained by this approach would have been offset by decreased magnesium activity. The gamma-spectrum of this particular sample, recounted after 5 hours, is shown in Figure 3.9, page 104, and revealed the complete absence of manganese.

To obtain quantitative data for the analysis, four series of 4 standards each, were prepared as dilute solutions, sealed in polyethylene

Figure 3.8 Gamma Ray Spectrum of Irradiated Kale after Separation of Magnesium -- I

100 mg sample, wet ashed, with magnesium separated as magnesium ammonium phosphate.

Irradiation time: 1 min.
Cooling time: 1400 sec.
Counting time: 1000 sec.

The prominent peaks are identified with energies in MeV.

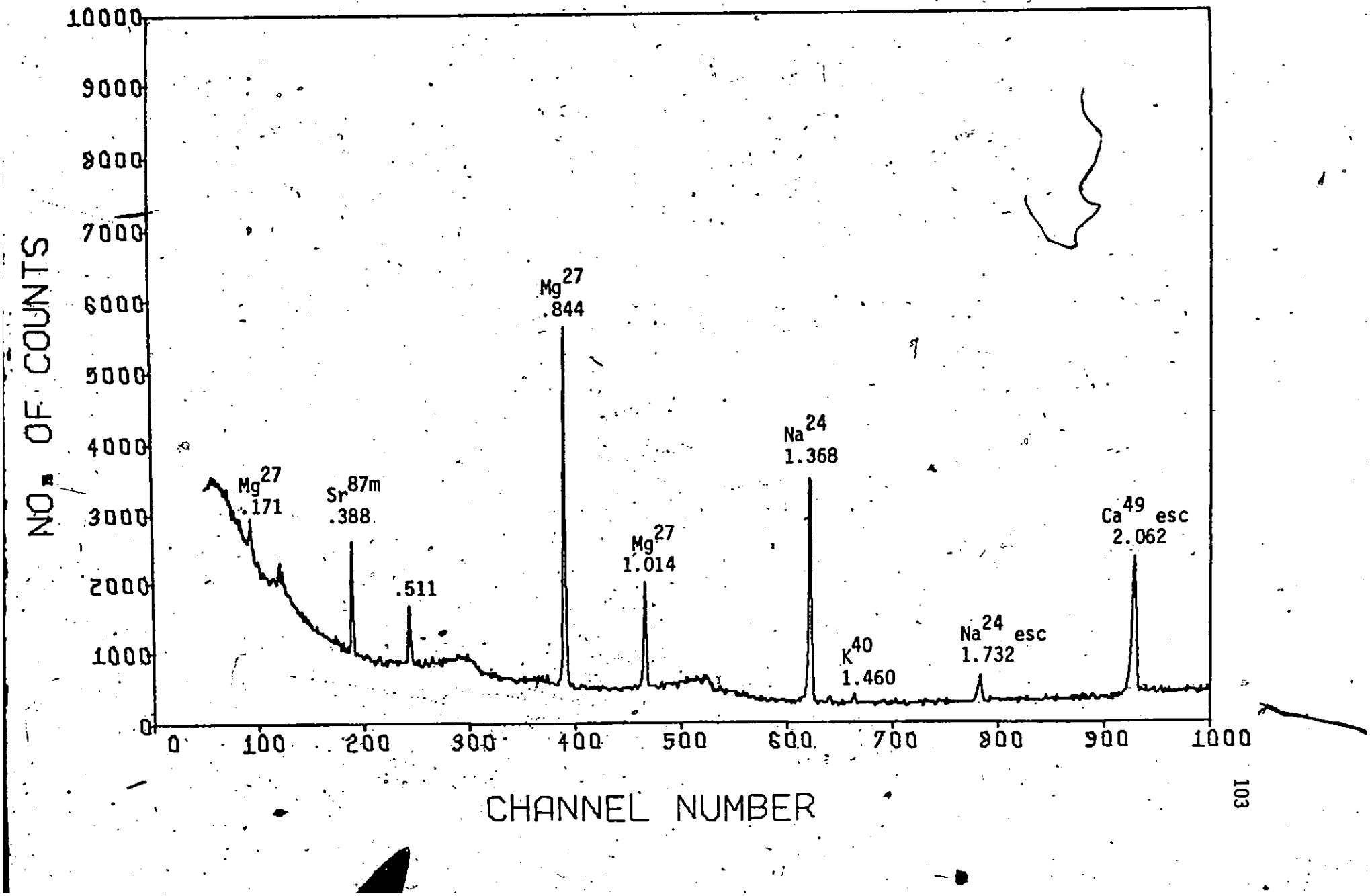
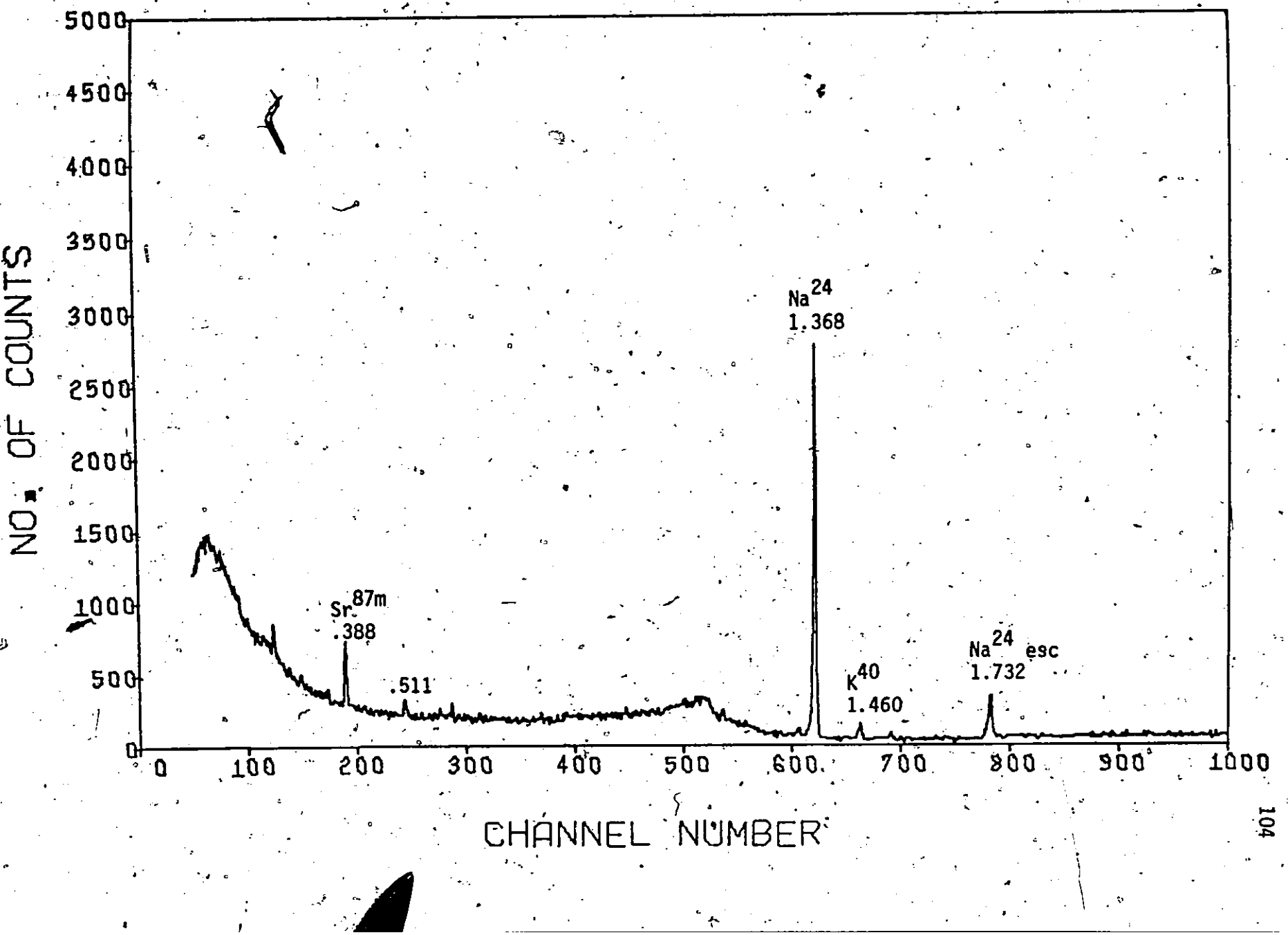

NO. OF COUNTS
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
100
200
300
400
500
600
700
800
900
1000
CHANNEL NUMBER
Mg27 .171
Sr87m .388
.511
Mg27 .844
Mg27 1.014
Na24 1.368
K40 1.460
Na24 esc 1.732
Ca49 esc 2.062

Figure 3.9 Gamma Ray Spectrum of Irradiated Kale After Separation of Magnesium -- II

Same sample as Figure 3.8, recounted after 5 hours.


NO. OF COUNTS
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
100
200
300
400
500
600
700
800
900
1000
CHANNEL NUMBER
Na24
1.368
Sr87m
.388
.511
K40
1.460
Na24 esc
1.732

vials and irradiated separately with a flux monitor. The two magnesium peaks were each normalized to an arbitrary flux monitor count and a calibration curve was drawn based on a linear, least-squares fit of the data. The reproducibility of this procedure may be seen from Table 3.13, page 106. The plot, Figure 3.10, page 107, showed a systematic negative error of 80 counts which was difficult to explain. Had the water or nitric acid contained a small amount of magnesium, the extrapolated line should have had a positive intercept on the ordinate. This slight bias was also apparent on calculating the specific activity for the magnesium, which increased 8 percent as the amount of magnesium present increased from 0.08 mg to 2.8 mg. The same effect was noted for both lines. Possibly the net counts calculated for each peak tended to overestimate the background, or chance summing distorted the spectrum slightly resulting in curvature at low concentrations. However, the fact that the curve did not go through the origin was of no practical consequence as the normalized sample counts fell in the central portion of the graphs shown in Figure 3.10.

To determine possible systematic errors in the chemical yield procedure, a series of solutions containing known amounts of calcium, magnesium and phosphate was taken through the extraction and titration procedure described on page 46. The results are shown in Table 3.14, page 106. A high degree of accuracy was evident from these results.

For the actual magnesium analysis, 14 kale samples were irradiated for 1 minute each, the magnesium activity separated and the samples counted, employing the same counting geometry and time sequence as for the standards, so that a direct comparison of the induced activity was possible. The calculations and final values are shown in Table 3.15, page 108.

TABLE 3.13 Aqueous Standards for Neutron Activation Analysis

Energy (MeV)	Magnesium (mg) .0829	.1391	.2042	.2869
	Normalized Counts			
0.844	3394[1] 3357[2]	6140	9228	13582
	3603 3565	6098 6151	9198 9302	13487 13548
	3437 3435	6079 6152	9166 9199	13146 13047
	3496 3528	6183 6226	9058 9060	13090 13060
Average[3]	3480 ± 32	6150 ± 19	9170 ± 34	13320 ± 95
1.014	822 832	1377	2004	2960 2954
	798 831	1382 1402	2008 2033	2948 2960
	816 823	1349 1332	1939 1941	2901 2915
	812 805	1353 1350	2221 2181	3059 3072
Average[3]	820 ± 30	1360 ± 8	2050 ± 38	2970 ± 21

1. Without room background correction.
2. Room background subtracted.
3. Average of all values; with 1 standard deviation of the mean shown.

TABLE 3.14 Accuracy of the Chemical Yield Determination

Present in Solution	Condition	Volume of Titrant (ml) Theoretical Magnesium	Theoretical Calcium	Actual Magnesium	Actual Calcium
Mg	no extraction	26.52		26.55	
Mg	extraction	26.52		26.35	
Mg,PO_4	extraction	26.52		26.45	
Mg,Ca	no extraction	26.52	4.02	26.45	4.05
Mg,Ca,PO_4	extraction	26.52	4.01	26.38	3.98

Figure 3.10 Calibration Curves for Destructive Neutron Activation Analysis Using External Standards

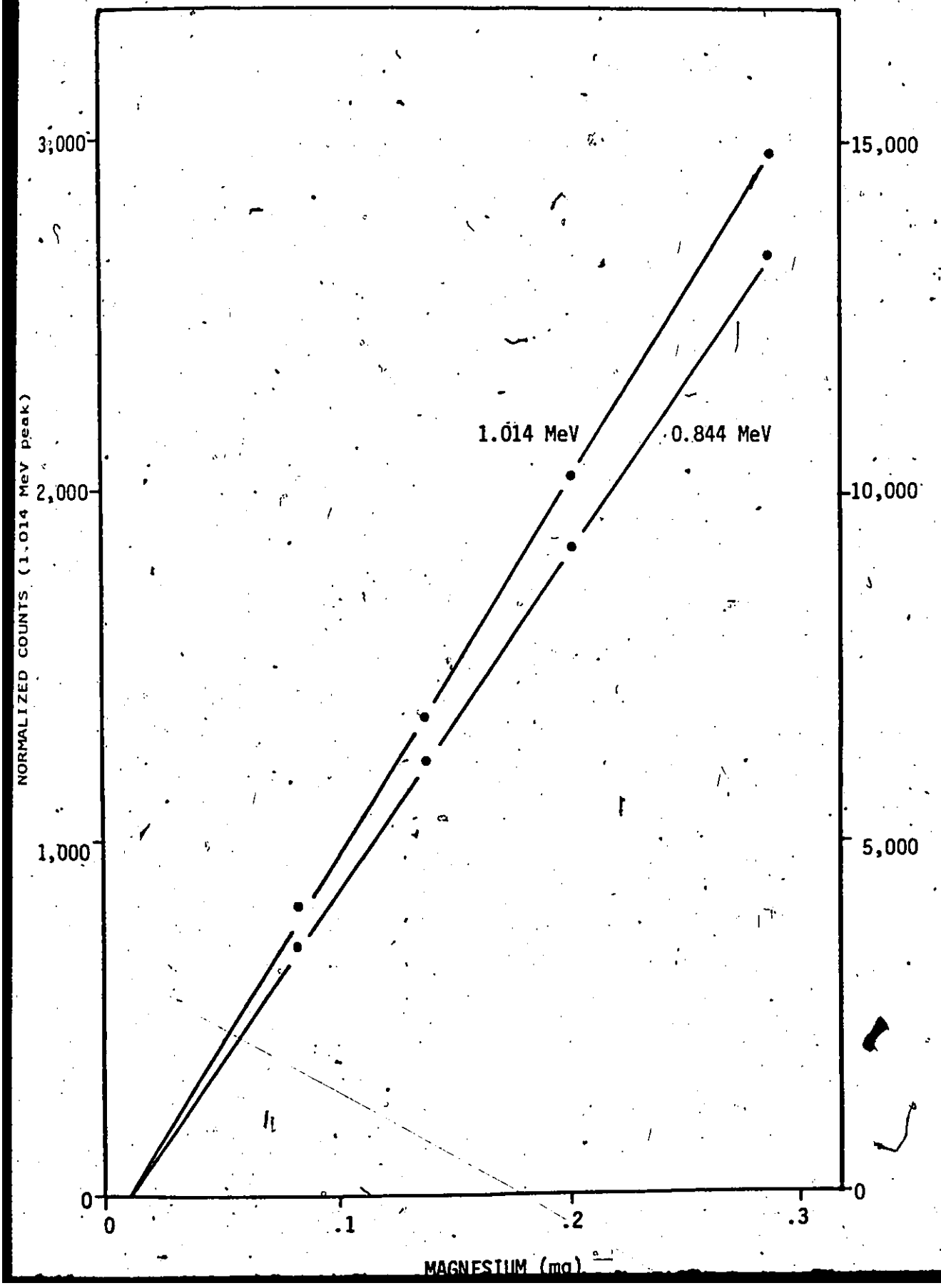

3,000
2,000
1,000
0
15,000
10,000
5,000
0
1.014 MeV
0.844 MeV
NORMALIZED COUNTS (1.014 MeV peak)
NORMALIZED COUNTS (0.844 MeV peak)
0
.1
.2
.3
MAGNESIUM (mg)

TABLE 3.15 Destructive Neutron Activation Analysis for Magnesium in Kale

Sample	Weight (mg)	Flux Monitor Counts	Original Sample Counts	Manganese Corrected Counts[1]	Chemical Yield (%)	Normalized Sample Counts[2]	Magnesium Concentration (ppm)
1	98.2	27626[3] 8523	5602 1485	5003 1485	81.4	7145 1592	1600 1590
2	101.8	40652 12660	10244 2350	8124 2350	88.1	6805 1475	1500 1450
3	103.8	30328 9403	6594 1740	5706 1740	86.8	6503 1492	1410 1440
4	98.4	21516 6583	4773 1341	4773 1341	92.8	7170 1536	1630 1560
5	101.4	20940 6623	4576 1467	4133 1467	87.9	6736 1764	1490 1720
6	102.2	31356 9864	6996 1791	6125 1791	88.1	6652 1443	1470 1420
7	100.6	22688 7205	5038 1469	5038 1469	89.6	7435 1593	1650 1580
8	100.1	23571 7439	5255 1550	5255 1550	91.5	7310 1594	1630 1590
9	48.5	22093 6865	2177 526	2177 526	91.0	3248 590	1630 1320[4]
10	201.0	22520 6996	9509 2921	9509 2921	86.5	14644 3379	1570 1630
11	101.4	91581 29926	17901 5703	20226 6444	85.5	7749 1763	1680 1700

(Continued)

TABLE 3.15 (Continued)

Sample	Weight (mg)	Flux Monitor Counts	Original Sample Counts	Manganese Corrected Counts[1]	Chemical Yield (%)	Normalized Sample Counts[2]	Magnesium Concentration (ppm)
12	99.9	113386	17441	25166	88.5	7522	1660
		36162	5090	7342		1606	1580
13	102.3	97842	28968	21867	94.8	7073	1530
		31520	5304	7286		2357	1640
14	100.0	125734	21018	25634	82.5	7414	1630
		40643	9485	8031		1677	1650
Average[5]							1580 ± 22(80) 1590 ± 28(100)

1. Corrected for residual manganese activity and a constant cooling time.
2. Corrected for chemical yield and for a normalized flux monitor.
3. The upper row values were obtained for the 0.844 MeV peak, the lower values for the 1.014 MeV peak.
4. Too low, omitted from average.
5. Average of all values with 1 standard deviation of a single determination in brackets.

The flux monitor used for this series was a sealed package of magnesium sulfate placed just below the sample vial. The same packet was used for each irradiation and the large variations shown for the count rates between samples were due to changes in the reactor operating power during the course of the study.

The degree of precision obtained and the differences between the values calculated, based on the two energies, in some cases was disappointing. However, the difference in the means was not significant. The uncertainty due to counting statistics alone, was about 2 percent and 5 percent for the 0.844 MeV and 1.014 MeV peaks, respectively. However, on calculating the precision of the means obtained for the two energies using the "F" test, a value of 1.45 was found, which was less than the critical value of 2.60. Thus, no significant error was introduced due to the manganese correction. This was confirmed by comparing the samples which showed no manganese activity, on recounting them several hours later, to those with residual manganese activity. The seven samples without manganese activity did not have a significantly different mean compared with the other seven. The low value obtained for sample 9 was probably due to the poor counting statistics (relative standard deviation 8 percent) for the higher energy gamma peak. The overall mean magnesium concentration, omitting this value, was about 6 percent lower than that found by atomic absorption, gravimetry and INAA. Because of this difference, the method of standard addition was investigated.

3.3.4 Analysis by Standard Addition

To avoid the use of a comparator method, a series of analyses was performed by standard addition. This method allows detection of

relative systematic errors such as local flux inhomogeneities and neutron shielding differences between the sample and comparator. However, such absolute errors as the presence of a foreign nuclide with a similar gamma-ray energy as the element of interest, in the present case the interference of manganese-56 with the 0.844 MeV magnesium-27 line, cannot be eliminated.

A series of standard addition samples was irradiated with a piece of magnesium ribbon attached around the circumference of each sample vial. Each spiked sample was irradiated together with an unspiked sample. Three methods were used to calculate the magnesium concentration for this series of samples.

For the first method, values were calculated for each pair of samples irradiated together, and are shown in Table 3.16, page 112. The low value for sample 2 was attributed to the very high manganese correction on this one occasion. (Manganese contributed 65 percent of the 0.844 MeV activity.) The low value for sample 3A was a result of poor resolution due to a zero shift in the multichannel analyzer during the count. The average, 1680 ppm, calculated without these values, was in good agreement with the gravimetric value (1663 ppm).

As the spike size for each sample was different, it was possible to construct a calibration curve for this series, shown in Figure 3.11, page 114. The net counts due to the spike were plotted against the weight of spike added. The best least-squares fit for the 0.844 MeV line was taken omitting points for the 0.160 mg and 0.960 mg spike as these showed a fairly large deviation from the straight line. All points were retained for the 1.014 MeV calibration curve.

TABLE 3.16 Neutron Activation with Standard Addition -- Method One

Sample	Spike (mg)	Mg Monitor Counts	Normalized Monitor Counts[1]	Original Sample Counts	Manganese Corrected Counts[2]	Chemical Yield (%)	Normalized Sample Counts[3]	Magnesium Concentration Individual Ratio (ppm)[4]
1	0	25032 6084	1633 489.0	12911 4031	12911 4031	83.3	15419 4948	1700
1A	0.0640	18018 3894	1742 495.0	19097 5608	19097 5608	84.0	21208 6743	1760
2	0	28522 6109	2830 889.2	28048 6861	22855 7205	90.4	14513 4481	1110[5]
2A	0.160	18912 3482	1677 847.7	16056 13046	50486 13046	86.5	35422 8894	1620
3	0	13512 2798	1583 503.5	12324 2465	12892 4066	89.6	14772 4506	1600
3A	0.320	11973 2089	1701 506.2	6166 1918	41421 14588	89.3	44310 16134	1240[5]
4	0	27908 6699	1628 494.0	15291 4311	13752 4311	92.2	14886 4732	1620
4A	0.640	18444 4076	1676 517.5	19532 4716	69165 20694	90.9	73774 21996	1760
5	0	10988 5257	1512 486.7	16231 3979	13093 3979	86.3	16303 4736	1670
5A	0.960	16105 3520	1701 531.5	28520 6463	96041 28709	83.5	109848 32344	1650

(Continued)

TABLE 3.16 (Continued)

Sample	Spike (mg)	Mg Monitor Counts	Normalized Monitor Counts[1]	Original Sample Counts	Manganese Corrected Counts[2]	Chemical Yield (%)	Normalized Sample Counts[3]	Magnesium Concentration Individual Ratio (ppm)[4]
6	0	24701 5895	1647 590.0	16117 4304	14241 4309	87.7	16016 4821	1800
6A	1.60	18526 3942	1640 499.0	33392 10037	137706 45688	86.3	158131 53047	1600
Average[6]								1680 ± 33(80) 1680 ± 33(80)

1. Counts per 10 mg magnesium ribbon, corrected for manganese-56 activity.
2. Corrected for manganese-56 activity and a constant cooling time.
3. Corrected for chemical yield and for a normalized flux monitor count.
4. Values based on count ratios obtained for each sample and spiked sample irradiated together. See text.
5. Not included in averages.
6. Standard deviation of a single determination given in brackets.

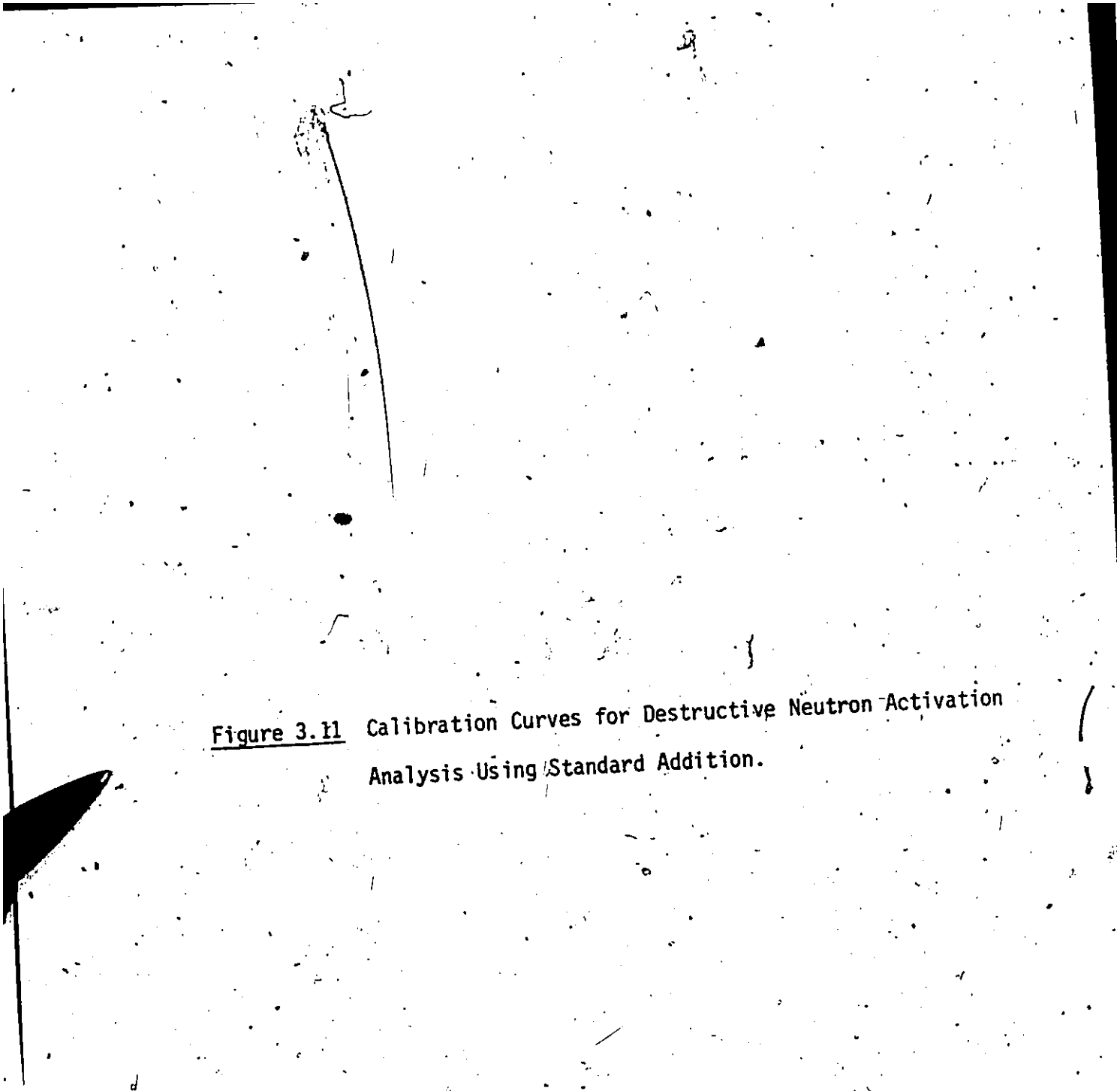
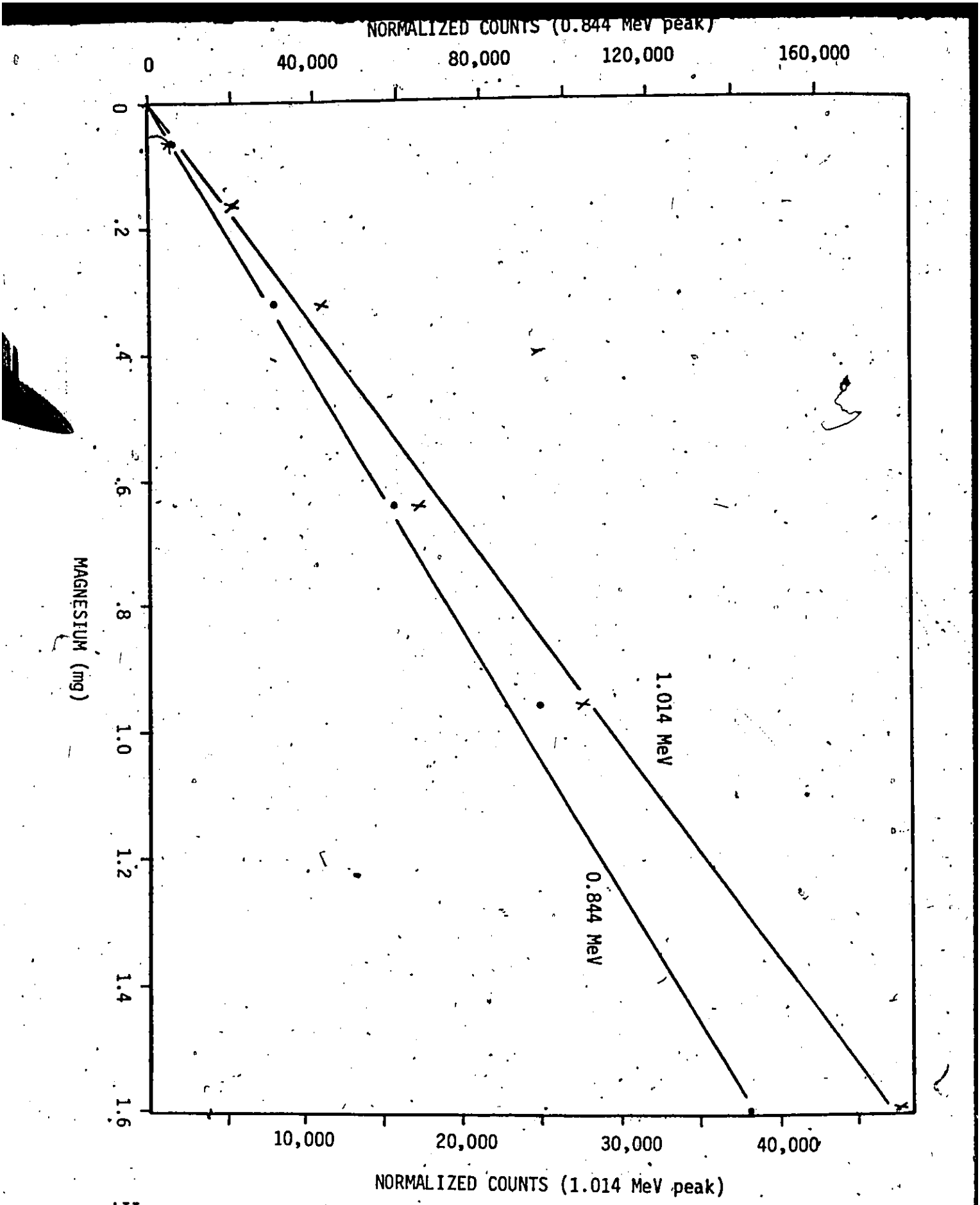


Figure 3.11 Calibration Curves for Destructive Neutron Activation Analysis Using Standard Addition.


NORMALIZED COUNTS (0.844 MeV peak)
0
40,000
80,000
120,000
160,000
MAGNESIUM (mg)
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.014 MeV
0.844 MeV
10,000
20,000
30,000
40,000
NORMALIZED COUNTS (1.014 MeV peak)

Values for each unspiked sample could be calculated, based on the slope and intercept of these curves. This set of values is shown in Table 3.17, column 5, page 116. The mean value, 1640 ppm, obtained by this method was significantly lower than that obtained for the individual ratios.

A third method of obtaining the net magnesium concentration was also available from this standard addition series. Since each sample was irradiated together with a piece of magnesium ribbon, this effectively served as both a flux monitor and a standard. Using the correction formula given in the Appendix, equation 5.1, the count values obtained for the monitors were adjusted to the time interval employed for the unspiked samples. A direct comparison was then possible, as the solutions of the magnesium ribbons were counted in the same geometry as the samples. Obtaining the specific activity for the ribbon enabled a comparison with the unspiked sample counts, corrected for chemical yield and manganese activity. The values obtained by this procedure are shown in the last column of Table 3.17, page 116. A mean value of 1560 ppm was obtained. On attempting similar calculations for the spiked kale samples using the magnesium ribbon monitor-standard, the values were found to be badly scattered and bore little relation to the unspiked samples. The precision was found to become worse as the size of the spike was increased, due to the error in obtaining the difference of two large numbers.

The use of the magnesium ribbon as both a monitor and standard would not be advocated, based on the present experiments, if great accuracy is to be obtained. One unexpected source of error was found

TABLE 3.17 Neutron Activation with Standard Addition -- Methods Two and Three

Sample[1]	Spike (mg)	Net Spike Counts[2]	Normalized Sample Counts[3]	Magnesium Concentration (ppm)[4]	Corrected Monitor Counts[5]	Magnesium Concentration (ppm)[6]
1	0.0640	5789	15499	1630	99574	1580
		1248	4839	1750	29808	1620
2	0.160	20909	25282	1550	172509	1470
		4672	7970	1580	54203	1470
3	0.320	29538	14388	1570	96477	1490
		11626	4538	1590	30692	1480
4	0.640	58888	16336	1730	99251	1650
		17264	4676	1660	30113	1550
5	0.960	93545	15171	1690	92186	1650
		27608	4610	1660	29668	1550
6	1.60	142115	16238	1690	100427	1620
		48226	4913	1690	31027	1580
Average[7]						1570 ± 37(90) 1530 ± 20(50)

1. Samples are those shown in Table 3.16.
2. The count differences for the spiked and unspiked samples; column 8, Table 3.16.
3. Corrected for chemical yield and manganese activity only.
4. Based on the standard curve generated from Column 3, see text.
5. Adjusted to the counting conditions used for the kale samples.
6. The value in Column 6 divided by the value in Column 4.
7. Standard deviation of a single determination shown in brackets.

by taking the ratio of the counts for the upper sample ribbon to the lower sample ribbon for each of the six irradiations for both the 0.844 MeV and 1.014 MeV lines. The ratio of these two energy values should be unity. Instead, a mean difference of +2 percent was found in favor of the 1.014 MeV line, with individual ratios having a range of -2 to +7 percent. This difference, while masked by other factors for the final calculated magnesium values, is indicative of the type of bias that contributed to the overall standard deviation of a single result by neutron activation analysis.

A comparison of the results obtained by these last three methods is relevant. Based on the standard deviation for an individual result shown in Tables 3.16 and 3.17, none of the three methods would appear to be preferred with respect to precision. This was also borne out by the results of an "F" test, which in no case showed a significant difference (Table 3.23, page 130). Further, there was no significant difference in the means for results obtained from the individual ratios of spiked to unspiked sample (Method 1) and from the standard addition graph (Method 2). However, the mean shown in Table 3.17 for the ribbon monitor-standard (Method 3), did show a significant difference from the other two means. This was not unexpected, since the results obtained with separately irradiated aqueous standards also gave a significantly lower mean than the values obtained for standard addition. The correction factor used for the adjustment of the ribbon counts probably had less than a ± 3 percent error, based on an error for the counting time of under 5 seconds. However, an error in the half-life of 1 ppt would alone contribute an error of ± 2.5 percent. Hence, the correction factor was subject to a certain inherent error. Based on the mean values obtained by gravimetry and atomic absorption, the results obtained using

the magnesium ribbon as both a flux monitor and a standard, gave consistently low results. Use of a standard addition calibration curve or simply taking the ratio of individual spiked and unspiked kale samples gave a value in general agreement with the other methods and was considered the most advantageous.

3.4 ANALYSIS OF RAT SAMPLES FOR MAGNESIUM

Using the knowledge gained from the investigation of the analytical difficulties associated with the determination of magnesium in kale, an examination of its determination in rat carcass and feces was undertaken. These samples would provide a check on the generality of the analytical problems observed and their solutions.

Only atomic absorption and neutron activation analysis were employed, as these were the two main methods by which the samples had previously been analysed. Also, the quantity of sample received did not allow a replicate oxinate determination.

A brief description of the methodology employed for the atomic absorption analysis was received, but not before the work contained in this section had been completed. As a result, the magnesium values obtained on the inhomogeneous carcass sample received, could only be semiquantitatively compared with those shown in Table 1.5, page 31, which had been obtained by using a 4 g sample per determination. (The total freeze-dried rat carcass would probably have had a freeze-dried weight of the order of 30 g or so.) The feces sample, which was a finely divided powder, was directly comparable.

Prior to commencing any analyses, the carcass and feces samples

were dried 24 hours at 90°C to obtain a dry weight. The drying temperature was arbitrarily selected. It was later learned that this was also the procedure used for the original analyses by other workers.

3.4.1 Atomic Absorption Results

For the atomic absorption work, six 100 mg samples of both the rat carcass and rat feces were wet ashed. The results for the atomic absorption analysis are shown in Table 3.18, page 119.

The average of the results obtained by this technique were found to agree within 3.5 percent for the feces with those shown in Table 1.5, with a slight positive bias, possibly due to a difference in the exact

TABLE 3.18 Magnesium in Rat Samples by Atomic Absorption

Sample	Weight (mg)	Value Obtained (ppm) Jarrell-Ash	Heath
Carcass	105.5	2240	2220
	95.7	840	860
	102.3	740	780
	99.6	2180	
	98.2	880	
	99.8	1800	
Average[1]		1390 ± 230(690)	
Feces	98.9	7300	7450
	99.7	7240	7390
	98.2	7430	7620
	101.4	7440	
	96.0	7390	
	101.9	7380	
Average[1]		7400 ± 33(100)	

1. One standard deviation for a single determination given in brackets.

drying procedure. The original procedure, employed by Héroux, called for the addition of 0.5 percent lanthanum chloride for the dilution, as a releasing agent for both feces and carcass samples. Had this releasing agent had any marked effect on the magnesium free-atom concentration, lower results would have been expected for the present analyses. Since this was not found, the lanthanum additions appeared unnecessary.

The inhomogeneity of the carcass sample was quite apparent from the above results. Even use of 4 g samples appeared to result in some inhomogeneity, for the original procedure suggested that repeat analyses be performed on separately dried fractions if large variations were found in the final magnesium result. The original frozen rat had been chopped into four pieces prior to homogenization and then samples prepared from individually blended sections. A more homogeneous final product would have resulted, had the whole carcass been freeze-dried and blended prior to bottling aliquots. Also, a much finer mesh size would have been desirable from the point of view of final sampling. Even had the 4.6 g of carcass received been very finely ground prior to analysis, deviations between this fraction and the rest of the carcass could still have been expected.

3.4.2 Instrumental Activation Results

For the instrumental neutron activation, four 100 mg samples of both the carcass and feces were analyzed. Typical spectra for the carcass and feces are shown in Figures 3.12 and 3.13, pages 121 and 122, respectively. The very large manganese-56 activity in the feces meant that analysis

Figure 3.12 Gamma Ray Spectrum of Irradiated Rat Carcass

Irradiation time: 1 minute
Cooling time: 300 seconds
Counting time: 1000 seconds, background subtracted
Sample size: 100 mg

The prominent peaks are identified with energies in MeV.

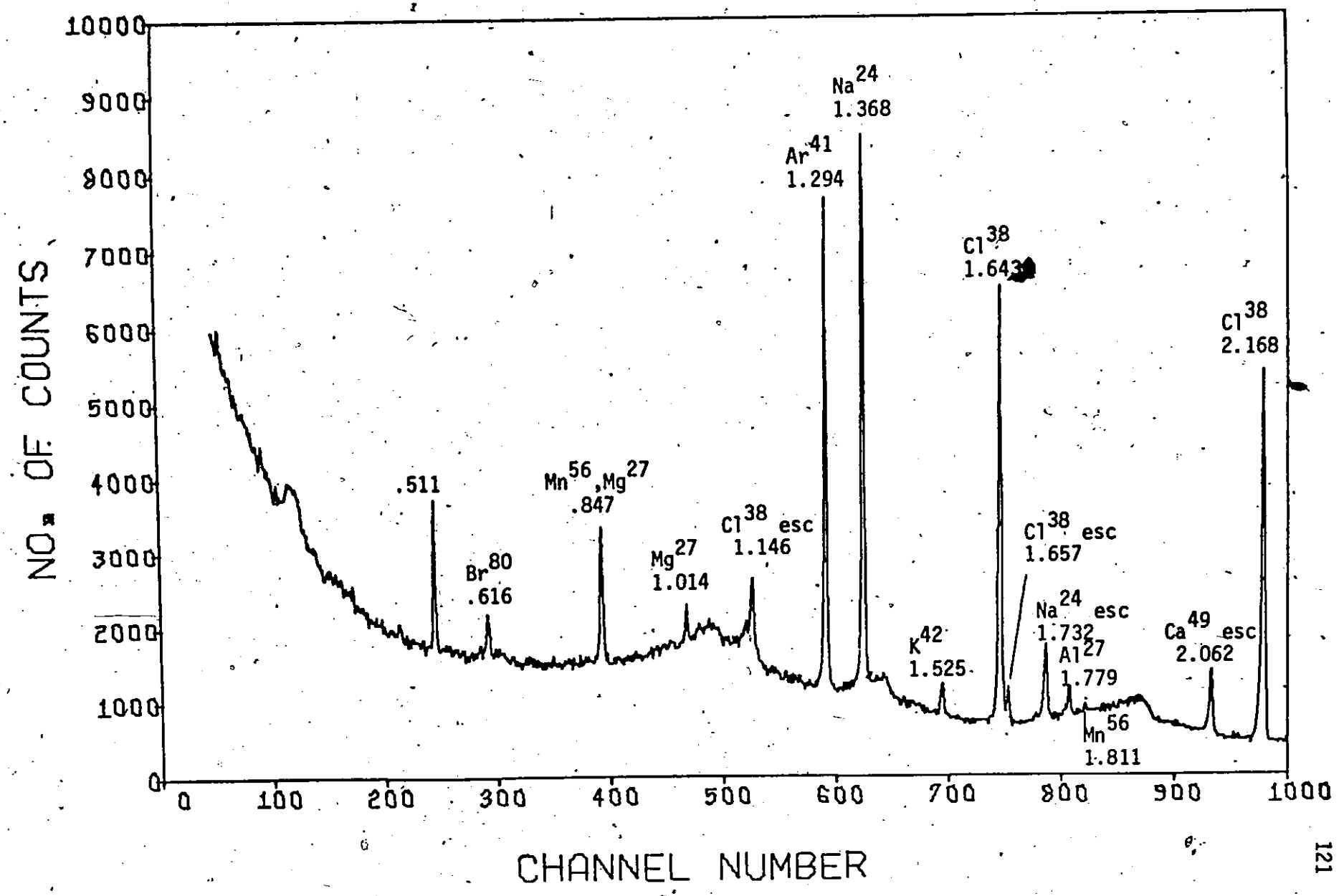
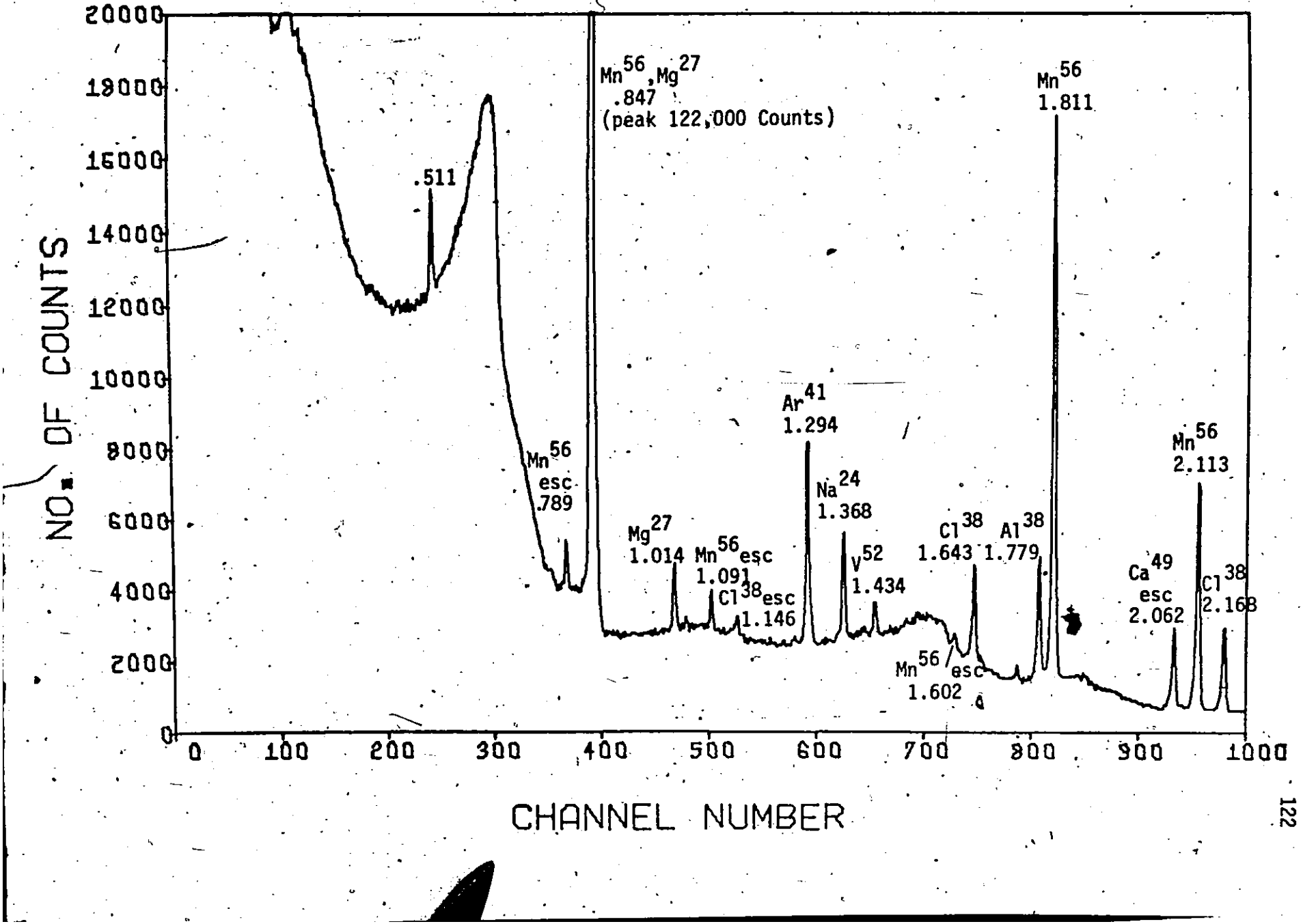

NO. OF COUNTS
CHANNEL NUMBER
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
100
200
300
400
500
600
700
800
900
1000
.511
Br80 .616
Mn56,Mg27 .847
Mg27 1.014
Cl38 esc 1.146
Ar41 1.294
Na24 1.368
K42 1.525
Cl38 1.643
Cl38 esc 1.657
Na24 esc 1.732
Al27 1.779
Mn56 1.811
Ca49 esc 2.062
Cl38 2.168

Figure 3.13 Gamma Ray Spectrum of Irradiated Rat Feces

Irradiation time: 1 minute
Cooling time: 300 seconds
Counting time: 1000 seconds, background subtracted
Sample size: 100 mg


NO. OF COUNTS
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
100
200
300
400
500
600
700
800
900
1000
CHANNEL NUMBER
.511
Mn56, Mg27
.847
(peak 122,000 Counts)
Mn56
esc
.789
Mg27
1.014
Mn56 esc
1.091
Cl38 esc
1.146
Ar41
1.294
Na24
1.368
V52
1.434
Cl38
1.643
Al38
1.779
Mn56
1.811
Mn56 esc
1.602
Ca49
esc
2.062
Mn56
2.113
Cl38
2.168

122

based on the 0.844 MeV peak would be subject to a large error. It was actually found to be impossible to obtain meaningful results from this peak, due to the manganese interference. A small vanadium-52 peak was also observed in the feces. The spectrum for the carcass sample, on the other hand, revealed a very small manganese-56 contribution to the 0.844 MeV peak. Argon-41 and chlorine-38 peaks occurred because the sample was counted in its original container following the irradiation.

Employing the same procedure as for the INAA determination of kale, described in subsection 2.5.2, page 44, the magnesium concentration in the carcass and feces samples was calculated using the separately irradiated standards shown in Table 3.11, page 98. The carcass volume was roughly 1.5 ml and feces 0.1 ml on irradiation and the samples were compared to standards of approximately equal volumes.

The calculated results for the carcass and feces are shown in Tables 3.19 and 3.20, pages 124 and 125, respectively. Satisfactory results were found for the feces samples, in good agreement with those from atomic absorption. On the other hand, the carcass samples showed poor reproducibility as expected. The large difference in the two values obtained for carcass sample 4 was attributed to instability in the analyzer, resulting in the production of double peaks which were difficult to integrate accurately because of the poor signal to background ratio.

3.4.3 Destructive Activation Results

For the activation analysis with radiochemical separation, three 100 mg samples of both the feces and carcass were separately irradiated with magnesium ribbon monitor-standards, using the same analytical pro-

TABLE 3.19 Instrumental Neutron Activation Analysis for Magnesium in Rat Carcass

Sample	Weight (mg)	Original Monitor Counts	Normalized Monitor Counts[1]	Original Sample Counts	Manganese Corrected Counts[2]	Normalized Sample Counts[3]	Magnesium Concentration (ppm)
1	99.2	48590[4]	45047	7499	5298	5923	1720
		14188	13910	1471	1615	1757	1930
2	102.8	65583	44419	4112	2104	2303	670
		19357	14233	NP[5]			
3	98.4	47257	45149	8582	5032	5663	1640
		13631	14318	1388	1524	1622	1780
4[6]	101.3	46452	43683	6006	4044	4297	1250
		13500	14062	1460	1603	1688	1850
Average							1550

1. Counts per 10 mg magnesium ribbon, corrected for manganese-56 activity.
2. Corrected for manganese-56 activity as well as a 9.8 percent dead time correction.
3. Adjusted to a standard sample weight and monitor count.
4. The upper row values were obtained for the 0.844 MeV peak, the lower row values for the 1.014 MeV peak.
5. No peak observed.
6. Zero shift in multichannel analyzer during count.

TABLE 3.20 Instrumental Neutron Activation Analysis for Magnesium in Rat Feces

Sample	Weight (mg)	Original Monitor Counts	Normalized Monitor Counts[1]	Original Sample Counts[2]	Dead Time (%)	Corrected Counts[3]	Normalized Sample Counts[4]	Magnesium Concentration (ppm)
1	98.9	14171	14171	4111	40	5299	5670	7000
2	100.1	12196	12396	4165	30	5031	5916	7530
3	100.8	10947	10839	4073	25	4765	6542	8070
4	99.3	13750	14031	4230	40	5452	5866	7240
Average[5]								7460 ± 230

1. Counts per 10 mg magnesium ribbon.
2. Only values for the 1.014 MeV energy are listed. High manganese-56 activity made the correction at 0.844 MeV meaningless.
3. Corrected for analyzer dead time.
4. Adjusted to a standard sample weight and monitor count in accord with Table 3.11.
5. Standard deviation of the mean given. Standard deviation of a single result was ± 460 ppm.

TABLE 3.21 Destructive Neutron Activation for Magnesium in Rat Samples

Sample	Weight (mg)	Mg Monitor Counts	Normalized Monitor Counts[1]	Original Sample Counts	Manganese Corrected Counts[2]	Chemical Yield (%)	Normalized Sample Counts[3]	Magnesium Concentration[4] (ppm)
Carcass								
1	96.2	54976[5] 15911	111365 35574	12021 3927	12021 3927	74.0	16886 5516	1520 1550
2	102.1	51986 15335	107037 34888	10987 3169	10071 3169	82.6	11942 3758	1120 1080
3	99.5	54322 15915	112079 36208	20496 6704	20496 6704	74.2	27761 9080	2480 2510
Average								1710 ± 260
Feces								
1	98.8	52280 15522	107486 35314	63400 17788	56518 17787	71.2	80343 25285	7470 7160
2	99.9	52952 15304	110712 35126	70868 20471	64160 20471	83.1	77285 24659	6980 7020
3	103.0	53275 15339	108886 34594	70172 21399	66342 21399	79.3	81223 26125	7460 7550
Average								7280 ± 100[6]

1. Counts per 1 mg magnesium ribbon, corrected for manganese-56 activity and count time.
2. Corrected for manganese-56 activity and a standard cooling time.
3. Adjusted to a 100 percent chemical yield and a standard sample weight.
4. The value in column 8 divided by column 4.
5. The upper row values were obtained for the 0.944 MeV peak, the lower row values for the 1.014 MeV peak.
6. Standard deviation of a single result was ± 250 ppm.

cedure as for the kale, described in subsection 2.5.3, page 46. The results are given in Table 3.21, page 126.

A comparison of the relative precision and mean variation for the two activation methods for the feces samples, showed that while the means were not significantly different, the precision of the destructive method was significantly better than for the purely instrumental method.

As an independent check on the analytical methods initially developed for kale, the different matrix of the samples did not present any problem for the methods and gave no reason to believe that the use of separate aqueous standards should give any difficulties for activation analysis.

3.5 COMPARISON OF THE METHODS

Any decision based on the relative merits of several different procedures for a particular determination calls for an expert opinion. There is rarely a unique solution, for the choice of method is typically biased by considerations of the accuracy required, the time and equipment available, the number of samples, the amount and quality of technical assistance and the possibility of interference by the presence of other constituents in the material under examination.

Table 3.22, page 128, presents a summary of the results obtained by the three methods for kale. Omitted from this table are the atomic absorption results obtained by standard addition shown in Table 3.9 and Figure 3.5, pages 88 and 87, respectively. The mean value obtained by this procedure (1790 ppm) was shown to exhibit a proportional error; that is, the magnesium spike did not add linearly to that present in the kale itself.

TABLE 3.22 Summary of Results Obtained in the Present Work for Kale

Method	Procedure	Mean (ppm)	Std. Dev. Mean	Std. Dev. Single Det'n.	Number of Det'ns.
Gravimetry		1663	5	19	18
Atomic Absorption	dry ashing followed by ion exchange	1690	6	35	31
	wet ashing without chemical separation				
	-external standards	1700	7	50	48
	-standard addition	1690	29	70	6
Neutron Activation Analysis	non-destructive	1680	46	130	4
	destructive				
	-external standards				
	-separately irradiated aqueous standards	1580	17	90	14
	-magnesium ribbon monitor employed as standard[1]	1560	12	70	6
	-standard addition				
	-based on ratio of spiked to unspiked samples irradiated simultaneously	1680	22	70	6
	-based on graph of net spike activity to size of spike	1640	12	70	6
Overall Mean Average		1645	4.2	55	

1. Not included in overall mean value.

The overall mean value shown at the bottom of Table 3.22 was calculated based on the means for all individual procedures shown, with the exception of that obtained for the destructive neutron activation analysis using a magnesium ribbon both as a flux monitor and as an external standard. The mean for this method (1560 ppm) appeared to be too low and the procedure itself was found to give very scattered results when used for spiked kale samples. The overall mean value was obtained by summing the products of the method means and the degrees of freedom for the individual methods and dividing this sum by the sum of the degrees of freedom. Thus, the individual method means were suitably weighted.

A comparison was made of the means and precisions for the gravimetric, atomic absorption and neutron activation procedures. The "t" and "F" values calculated for individual pairs of procedures are shown in Table 3.23, page 130. The critical 5 percent values are shown in brackets.

The gravimetric procedure, while time-consuming, showed the highest degree of precision with the major source of error being the inability to completely precipitate the magnesium. The error from this source was shown to be about 0.5 percent based on atomic absorption analysis of the supernatant.

Atomic absorption was found to be surprisingly free from severe interference when applied to kale, though preliminary interference studies had shown that silicate, phosphate and sulfate might be expected to cause a strong depression in the magnesium absorption signal which could not be markedly reversed by addition of the common releasing agents. Sodium, potassium and iron were individually and in combination

BLE 3.23 Statistical Values for the Comparison of the Means and Precisions of the Procedures Used for the Kale Analysis

Method[1,2]	1	2	3	4	5	6	7	8
2	3.92(1.99) 10.30(1.67)							
3	6.12(2.00) 4.78(1.80)	0.36(2.00) 2.15(1.94)						
4	2.70(2.01) 18.82(2.59)	0.00(2.01) 1.83(2.60)	0.21(2.06) 3.93(2.82)					
5	1.47(2.00) 16.24(2.06)	0.83(2.01) 1.58(2.08)	1.18(2.04) 3.40(2.30)	0.44(2.15) 1.16(3.48)				
6	2.44(2.00) 21.55(2.06)	3.09(2.01) 2.09(2.08)	3.51(2.04) 4.50(2.30)	1.54(2.15) 1.14(5.96)	1.47(2.09) 1.33(2.98)			
7	9.86(2.00) 17.13(2.06)	7.34(2.01) 1.66(2.08)	8.34(2.04) 3.58(2.30)	3.85(2.15) 1.10(3.48(	4.30(2.09) 1.05(2.98)	2.54(2.09) 1.26(2.98)		
8	6.17(2.00) 30.13(1.77)	6.66(2.00) 2.93(1.76)	6.19(2.01) 6.30(2.02)	2.43(2.05) 1.60(5.77)	3.38(2.03) 1.86(2.74)	1.82(2.03) 1.40(2.74)	0.77(2.03) 1.76(2.74)	
9	1.26(2.01) 62.62(2.32)	1.47(2.01) 6.08(2.34)	0.69(2.05) 13.09(2.55)	0.20(2.23) 3.33(6.16)	0.18(2.12) 3.85(3.22)	2.02(2.12) 2.90(3.22)	4.57(2.12) 3.66(3.22)	4.37(2.04) 2.08(2.49)

1. Code:
   1. Gravimetry
   2. Atomic Absorption -- wet ashing without chemical separation
   3. Atomic Absorption -- dry ashing followed by ion exchange
   4. Atomic Absorption -- standard addition
   5. Neutron Activation Analysis -- standard addition, based on ratio of spiked to unspiked samples irradiated simultaneously.
   6. Neutron Activation Analysis -- standard addition, based on graph of net spike activity to size of spike.
   7. Neutron Activation Analysis -- destructive, magnesium ribbon monitor employed as standard.

(Continued)

TABLE 3.23 (Continued)

1. Code:
   8. Neutron Activation Analysis -- destructive, separately irradiated aqueous standards.
   9. Neutron Activation Analysis -- non-destructive.

2. Upper row gives "t" values, with critical 5 percent values shown in brackets.
   Lower row gives "F" values, with critical 5 percent values shown in brackets.

found to enhance the magnesium absorption signal approximately 2 percent, though the degree of interference was affected by the relative concentrations and the atomic absorption instrument employed. The significant difference in the mean values for gravimetry and the atomic absorption procedures could be accounted for by this enhancement. These cations were not eliminated by the dry-ashing-ion-exchange procedure. The mean magnesium value obtained for the dry-ashing-ion-exchange procedure was found not to be significantly different from that obtained by wet digestion followed by dilution. The fact that the degree of precision attainable through the use of ion-exchange was greater than for the directly wet ashed and diluted samples, suggested that interaction processes were quite complex. The very high magnesium content for both the kale and rat samples was felt to account to a large degree for the lack of difficulty with the method. Atomic absorption employing standard addition based on a calibration curve is also seen to have a similar mean and precision to that for the wet ashing procedure.

Neutron activation procedures showed the greatest mean standard deviations. This was partly due to the large flux variations found within the irradiation capsule. The half-life of magnesium-27 did not permit the use of the less flux-variable in-core reactor position and this was thus a fundamental weakness of the method. Since this parameter varies widely from reactor to reactor, the limitation imposed by this variable is related to the irradiation facilities available. Another source of error stemmed from the difficulty in reproducing the counting geometry exactly.

Two procedures, the non-destructive neutron activation analysis

and the standard addition procedure based on the ratio of spiked to unspiked samples irradiated simultaneously, gave similar means to those obtained for the gravimetric and atomic absorption procedures. The precision of both procedures was not significantly different from atomic absorption employing standard addition or several other neutron activation analysis procedures. A major source of error for the non-destructive procedure was introduced by the counting statistics which were calculated to give a standard deviation of approximately 8 percent and 11 percent for the 0.844 MeV and 1.014 MeV peaks, respectively, based on the gross peak counts, the manganese-56 contribution to the 0.844 MeV peak, the peak background and the flux monitor correction uncertainties. Not included was the error in the dead time correction factor which was taken as constant for all irradiations. The counting errors associated with the destructive analysis were 2 percent and 5 percent for the 0.844 MeV and 1.014 MeV gamma peaks, respectively.

Other neutron activation analysis procedures, with the exception of the graphical standard addition method which gave a similar mean to that for atomic absorption using standard addition, showed significantly lower mean values. The two different standard addition procedures for obtaining the magnesium content had similar precisions and means. This was not unexpected, as the values were derived from the same irradiations and were just different methods of calculating the magnesium value.

Table 3.24, page 134, presents a summary of the best results obtained for kale. The values are derived from those shown in Table 3.22. A 2 ± 1 percent reduction has been applied to the atomic absorption results to correct for the absorption enhancement of magnesium in the presence of

TABLE 3.24 Summary of Best Results Obtained in the Present Work for Kale

Method	Procedure	Mean (ppm)[1]	Std. Dev. Mean
Gravimetry		1663	5
Atomic Absorption	dry ashing followed by ion exchange	1659	18
	wet ashing without chemical separation		
	-external standards	1663	18
	-standard addition	1659	34
Neutron Activation Analysis	non-destructive	1683	46
	destructive		
	-standard addition		
	-based on ratio of spiked to unspiked samples irradiated simultaneously	1678	22
	-based on graph of net spike activity to size of spike	1635	12
Best Value		1663	6.9

1. Fourth significant figure for information only, except in the case of gravimetry.

sodium, potassium and iron as demonstrated on page 70. The uncertainty in this correction factor is reflected in the higher mean standard deviations in Table 3.24 when compared with those given in Table 3.22. The neutron activation analysis results obtained using external standards have been rejected in this compilation of best results as the two procedures in question gave low results.

The value 1663 ± 7 ppm was arrived at as the most probably correct value for the magnesium content in kale. When the individual values shown in Table 3.24 were compared with those in Table 1.2, listing the values obtained by Bowen's collaborators, the improvement in precision of the present value was apparent. Furthermore, the agreement between the three methods presented here, was considerably better than that previously obtained. The other notable feature was the higher value obtained in the present work. A calculation based on all the results shown in Table 1.2, gives a mean of 1670 ppm for 64 determinations. If the values obtained by the flame photometry and spectrophotometry procedures were eliminated, a weighted average of 1580 ppm was obtained. Since Bowen's weighted best value of 1600 ppm fell in-between, the weighting scheme was apparently derived using an unpublished procedure. A drying error of 4 percent would account for this discrepancy. However, this seems unlikely since Bowen himself limited this source of error to about 1 percent and for the present work, various portions of the total kale sample received, were dried separately, further decreasing the probability of a constant bias. The difference thus appears to be real.

For the present work, three independent methods were employed

with a good degree of agreement being attained between the mean values found for each method. A high degree of confidence could thus be placed in the best value obtained. The best value reported by Bowen was therefore felt to be low by about 4 percent.

For the rat samples, good agreement was attained between atomic absorption and neutron activation analysis, both instrumental and with radiochemical separation. A best estimate of 1550 ppm was obtained for the carcass, though due to the inhomogeneity of the sample as received, the uncertainty was quite high. For the feces, a best estimate of 7370 ppm was obtained. This value was in fairly good agreement with those shown in Table 1.5, having a positive bias of about 3 percent. The agreement for the rat samples between the two methods gave additional support to the validity of the methods as used, since not only was the biological matrix quite different, but the magnesium content covered a wide range.

With all three methods, an acceptable degree of precision was attained, so that the choice for the analyst would be determined mostly by the considerations set forth at the outset of this subsection. For the highest accuracy work, the gravimetric method proved superior to the instrumental methods. For standard processing of many samples, the atomic absorption method would have the greatest advantage. In general, the results obtained by activation analysis were disappointing, but this was not totally unexpected in view of the unfavorable nuclear properties of magnesium-27 and the highly favorable properties of its concommitant interference, manganese-56.

CHAPTER IV

CONCLUSIONS

Three independent methods (gravimetry, atomic absorption spectrometry and neutron activation analysis) were developed for the analysis of magnesium in a biological standard, namely Bowen's kale. Sources of error in these methods were studied, including those for standard addition, commonly employed to avoid corrections for matrix effects. Good agreement was obtained among all three methods for the magnesium content of the kale. To check the general applicability of two of these methods, namely atomic absorption and neutron activation analysis, the most successful methodologies were applied to the analysis of a rat carcass and feces sample for magnesium. Again, agreement between the methods was good. The major findings obtained during the course of the work embodied in this thesis are set forth below.

1. The magnesium content of Bowen's kale is 1663 ± 7 ppm.
2. The gravimetric oxinate procedure is the most precise method for the determination of 1.6 mg of magnesium. The magnesium oxinate precipitate should be weighed as the anhydrous form after drying at least 1 hour at 150°C.
3. Determination of magnesium by atomic absorption is subject to about a 2 percent positive bias in the presence of sodium, potassium and iron, singly or in combination. The exact effect is both concentration and instrument dependent. The common releasing agents have no effect on the enhanced magnesium

absorbance.

4. The difference in the means of the results obtained for dry ashing followed by ion exchange on Dowex 50 and wet ashing without chemical separation, as methods of preparing kale for atomic absorption, was not found to be significant at the 5 percent level. The dry ashing procedure gives the more precise results. The difference in precision, however, is not significant at the 1 percent level.
5. Standard addition for atomic absorption employing an external calibration curve is an acceptable means of obtaining the magnesium content of kale though the precision is poorer than for the previously mentioned methods. Use of self-standardization, that is, no calibration curve, results in a 6 percent positive error and poor precision.
6. The mean magnesium value for kale obtained by instrumental neutron activation analysis is in agreement with the gravimetric and atomic absorption results. The precision is much lower, with a standard deviation of the mean approximately 8 times higher.
7. The mean magnesium value for destructive neutron activation analysis of kale employing external standards is 5 percent lower than those obtained by other methods, due to the difficulty of obtaining accurate flux measurements for the rabbit. Differences as large as 30 percent are found over a 5 cm distance in the rabbit for the McMaster University reactor.
8. Destructive neutron activation employing standard addition

gives a magnesium value for kale in agreement with gravimetry and atomic absorption. The standard deviation of the mean is about 3 times higher than for the latter methods.

9. Analysis for magnesium in a rat carcass and rat feces sample by atomic absorption and neutron activation analysis revealed good agreement between the two techniques, thereby resolving a previous discrepancy.

10. The methods in order of precision are ranked from highest to lowest; gravimetry, atomic absorption and neutron activation analysis.

In view of the good agreement found for the different methods in this study, the limiting factor in collaborative studies appears to be the length of time the individual participants can spend on the analysis. When it is realized that the National Bureau of Standards in Washington, with its vast resources, has to-date spent well over six man-years in preparation for the issue of six botanical standard reference materials, the magnitude of the problem can readily be seen. (Work on four of these standards is still continuing at the present time.) The work embodied in this thesis can form but a small part in the effort to better understand the fundamental interactions that characterize an analytical method and affect its practical usefulness.

CHAPTER V

APPENDIX

Formulae Employed in the Neutron Activation Analysis

The following formulae were employed on various occasions in the present work to adjust the counts obtained for a given photopeak, for the purposes of comparison and standardization of magnesium data. Only the final forms of the equations will be listed here.

1. To adjust an integrated number of counts to a different time interval where the two time intervals differ in length.

$$N_{(0,t_3)} = N_{(t_1,t_2)} \frac{(1 - e^{-\lambda t_3})}{(e^{-\lambda t_1} - e^{-\lambda t_2})} \qquad (5.1)$$

where

$N_{(0,t_3)}$ is the number of counts over the desired time interval 0 seconds to t_3 seconds.

$N_{(t_1,t_2)}$ is the number of counts obtained for the time interval t_1 to t_2 where $t_3 < t_1 < t_2$ and all times are relative to 0 seconds at the start of the desired time interval.

λ is the radioactive decay constant, equal to 1.221×10^{-3} sec^{-1} for magnesium.

2. To adjust an integrated number of counts to a different time interval where the two time intervals are the same length.

$$N_{(0,t_3)} = N_{(t_1,t_2)} e^{\lambda t_1} \qquad (5.2)$$

where the symbols have the same meanings as in equation (5.1).

3. To estimate the counting error incurred for various counting times at a fixed sample to background count ratio.

The following preliminary formula was employed to calculate the initial count rate, A_o.

$$A_o = \frac{\lambda N_s}{(1 - e^{-\lambda t})} \tag{5.3}$$

where N_s is the net integrated number of counts for the sample over time t.

Assuming then a certain gross integrated number of counts for the sample, N_g and a net background, N_b, under the peak, the net number of counts for the sample will have the value

$$N_s = N_g - N_b \pm (N_g + N_b)^{\frac{1}{2}} \tag{5.4}$$

and the percent error (%E) incurred will be

$$\%E = \frac{(N_g + N_b)^{\frac{1}{2}}}{N_s} \times 100 \tag{5.5}$$

Using equation (5.5), the following table was set up, assuming a constant background count rate of 2 cps and an initial net magnesium count rate of 10.4 cps, typical values for the present experimental work with kale.

TABLE 5.1 Counting Error for Various Counting Times with a Fixed Background and Initial Sample Count Rate

Count Time (sec)	500	700	1000	1200	1500	2000	2500
N_s	3885	4888	6000	6537	7141	7761	8101
%E	1.97	1.79	1.67	1.63	1.61	1.62	1.66

From this table, it was seen that the counting error was minimized, under the prevailing conditions for a counting conditions for a counting time of 1500 seconds.

To correct for the extra counting time at high analyzer dead times for a pure short-lived isotope, the following formula was applied.

$$C = C' \frac{(e^{\lambda DT} - 1)}{\lambda DT} \qquad (5.6)$$

where C is the true integrated number of counts,

C' is the integrated number of counts found,

DT is the analyzer dead time.

This equation may be rewritten as

$$C = C'F \qquad (5.7)$$

where

$$F = \frac{(e^{\lambda DT} - 1)}{\lambda DT}$$

For magnesium, the correction factors shown in Table 5.2 should be applied to correct for the analyzer dead time.

TABLE 5.2 Dead-Time Correction Factors for Magnesium

Dead Time (sec)	Factor
10	1.006
50	1.031
100	1.064
200	1.133
300	1.208
400	1.289

5. To calculate the standard addition results the following formulae were employed,

$$\frac{C_u}{C_s} = a = \frac{x}{x + b} \qquad (5.8)$$

where C_u is the normalized number of counts for the unspiked sample,

C_s is the normalized number of counts for the spiked sample,

x is the mg of magnesium per unit weight for the unspiked sample,

b is the mg of spike added per unit weight.

Since the chemical yield and the flux differed for the spiked and unspiked sample, equation (5.8) was modified to

$$x = \frac{bC_u m_s y_u}{C_s m_u y_u - C_u m_s y_s} \qquad (5.9)$$

where m_s is the net number of counts for the spiked sample monitor,

m_u is the net number of counts for the unspiked sample monitor,

y_s is the chemical yield for the spiked sample,

y_u is the chemical yield for the unspiked sample.

By normalizing the data, that is, adjusting the sample counts to a 100 percent chemical yield and a constant flux monitor value, equation (5.9) simplified to equation (5.8). A plot of "x" against "a", the latter being related to the amount of magnesium added, revealed that for both samples, the error in "x" decreased as the ratio "a" decreased. Thus, the ratio of spike to magnesium present should be as large as possible, limited, of course, by the requirement of similar dead time for both samples if a correction is not to be made.

BIBLIOGRAPHY

1. "Activation Analysis -- Principles and Applications", J. M. Lenihan, S. J. Thomson (Eds.), Academic Press, New York, 1965.
2. W. J. Adrian, Analyst, 98, 213 (1973).
3. "Air Polution Control", W. Strauss (Ed.), J. Wiley and Sons, New York, 1971.
4. N. W. Alcock, I. MacIntyre and I. Radde, Nature, 206, 89 (1965).
5. N. W. Alcock, N. Y. Acad. Sci. Annals, 162, 707 (1969).
6. J. E. Allan, Analyst, 83, 466 (1958).
7. M. D. Amos, J. B. Willis, Spectrochim. Acta, 22, 1325 (1966).
8. "Analytical Flame Spectroscopy", R. Mavrodineanu (Ed.), MacMillan and Co., London, 1970.
9. D. H. Anderson, J. J. Murphy, W. W. White, Anal. Chem., 44, 2099 (1972).
10. O. J. Attoe, Soil Sci. Soc. Am. Proc., 23, 460 (1959).
11. P. A. Baedecker, Anal. Chem., 43, 405 (1971).
12. D. E. Baker, G. W. Gorsline, C. B. Smith, W. I. Thomas, W. E. Grube, J. L. Ragland, Agron. J., 56, 133 (1964).
13. J. H. Baker, T. Greweling, J. Agr. Food Chem., 15, 340 (1967).
14. A. Bashar, A. Townshend, Talanta, 13, 1123 (1966).
15. W. Baumeister in "Encyclopedia of Plant Physiology", W. Ruhland (Ed.), Vol. 4, pp. 5, 482, Springer Verlag, Berlin, 1958.
16. W. P. Belk, F. W. Sunderman, Am. J. Clin. Path., 17, 853 (1947).
17. W. E. Bennett, D. O. Skovlin, Anal. Chem., 38, 518 (1966).
18. R. Berg, Z. Anal. Chem., 71, 23 (1927).
19. W. L. Berry, C. M. Johnson, Appl. Spectros., 20, 209 (1966).
20. W. F. Bethard, R. A. Schmitt, D. A. Olehy in "L'Analyse par Radioactivation et Ses Applications aux Sciences Biologiques", D. Comar (Ed.), p. 379,

Presses Universitaires de France, Paris, 1964.

21. Ibid., J. Nucl. Med., 7, 917 (1966).

22. P. Biloen, J. Dorrepaal, H. B. van der Heijde, Anal. Chem., 45, 288 (1973).

23. O. D. Bonner, P. R. Pruett, J. Phys. Chem., 63, 1420 (1959).

24. M. Borrel, R. Paris, Anal. Chim. Acta , 4, 267 (1950).

25. Ibid., 6, 393 (1952).

26. J. J. Bouquiaux, J. H. Gillard, ibid., 30, 273 (1964).

27. H. J. M. Bowen, D. Gibbons, "Radioactivation Analysis", Oxford Press, London, 1963.

28. H. J. M. Bowen, P. A. Cawse, M. Daglish, Analyst, 89, 266 (1964).

29. H. J. M. Bowen, "Proceedings of the SAC Conference, Nottingham, England, 19-23 July 1965", P. W. Shallis (Ed.), p. 25, W. Heffer and Sons, Cambridge, 1965.

30. H. J. M. Bowen, P. A. Cawse in "Activation Analysis -- Principles and Applications", J. Lenihan, S. Thomson (Eds.), p. 149, Academic Press, New York, 1965.

31. H. J. M. Bowen in "Modern Trends in Activation Analysis", p. 58, College Station, Texas, 1966.

32. Ibid., "Trace Elements in Biochemistry", p. 65, Academic Press, New York, 1966.

33. Ibid., Chapter 5.

34. Ibid., Chapter 8.

35. Ibid., Analyst, 92, 124 (1967).

36. Ibid., "Advances in Activation Analysis", J. Lenihan, S. Thomson (Eds.), Vol. 1, p. 101, Academic Press, New York, 1969.

37. D. Boynton, O. C. Compton, Soil Sci., 59, 339 (1945).

38. E. G. Bradfield, Analyst, 86, 269 (1961).

39. Ibid., Anal. Chim. Acta , 27, 262 (1962).

40. E. G. Bradfield, D. Spincer, J. Sci. Food Agr., 16, 33 (1965).

41. D. Brune, H. E. Sjoberg, Anal. Chim. Acta , 33, 570 (1965).

42. A. O. Brunfelt, K. S. Heier, E. Steinnes, B. Sundvoll, Earth Planetary Sci. Lett., 11, 351 (1971); CA75:120286t.

43. A. Bussmann, Z. Anal. Chem., 148, 413 (1956).

44. D. N. Campbell, C. T. Kenner, Anal. Chem., 26, 560 (1954).

45. P. N. Carpenter, A. Ellis, H. E. Young, Maine Agr. Exptl. Stn. Tech. Bull. #30 (1968).

46. "Catalog of Standard Reference Materials", Natl. Bur. Std. (U.S.), Spec. Publ. #260, July 1970.

47. R. G. Charles, H. Freiser, R. Friedel, L. E. Hilliard, W. D. Johnson, Spectrochim. Acta , 8, 1 (1956).

48. G. Charlot, "Qualitative Inorganic Analysis", p. 161, J. Wiley and Sons, New York, 1954.

49. Ibid., p. 188.

50. D. B. Cheek, J. E. Graystone, J. B. Willis, A. B. Holt, Clin. Sci., 23, 169 (1962).

51. E. Chenery, Analyst, 89, 365 (1964).

52. K. L. Cheng, R. H. Bray, Soil Sci., 72, 449 (1951).

53. K. L. Cheng, S. W. Melsted, R. H. Bray, ibid., 75, 35 (1953).

54. R. C. Chirnside, C. F. Pritchard, H. P. Rooksby, Analyst, 66, 399 (1941).

55. G. D. Christian, F. J. Feldman, "Atomic Absorption Spectroscopy", J. Wiley and Sons, New York, 1970.

56. J. Ch. van Wesemael, Anal. Chim. Acta , 25, 238 (1961).

57. R. E. Collier, Hilger J., 10(2), 52 (1967).

58. R. T. Conley, "Infrared Spectroscopy", 2nd Ed., p. 232, Allyn and Bacon Inc., Boston, 1972.

59. H. H. Conway, G. W. Leddicotte, A. D. Kenny, Anal. Biochem., 39, 218 (1971).

60. G. B. Cook, M. B. A. Crespi, J. Minczewski, Talanta, 10, 917 (1963).

61. R. Dams, J. A. Robbins, K. A. Rahn, J. W. Winchester, Anal. Chem., 42, 861 (1970).

62. D. J. David, Analyst, 83, 655 (1958).

63. Ibid., 85, 495 (1960).

64. J. Davidson, ibid., 77, 263 (1952).

65. H. De La Roche, K. Govindarajo, Analusis, 2, 59 (1973).

66. M. D. Derderian, Anal. Chem., 33, 1796 (1961).

67. D. De Soete, R. Gijbels, J. Hoste, "Neutron Activation Analysis", J. Wiley and Sons, New York, 1972.

68. Ibid., Chapter 10.

69. H. Diehl, C. A. Goetz, C. C. Hach, J. Am. Water Works Assoc., 42, 40 (1950).

70. H. Diehl, "Calcein, Calmagite and O,O'-Dihydroxyazobenzene. Titrimetric, Colorimetric and Fluorometric Reagents for Calcium and Magnesium", G. F. Smith Chemical Co., Columbus, Ohio, 1964.

71. W. J. Dixon, F. J. Massey, Jr., "Introduction to Statistical Analysis", McGraw-Hill, New York, 1969.

72. C. Duval, "Inorganic Thermogravimetric Analysis", Chapt. 12, Elsevier Publ. Co., New York, 1953.

73. I. Dvork, Chem. Listy, 61, 390 (1967); CA66:1023972.

74. S. N. Edson, R. H. Mill, Chemist Analyst, 46, 4 (1957).

75. G. Ehrlich, R. Gerbatsch, Z. Anal. Chem., 209, 35 (1965).

76. W. T. Elwell, J. A. Gidley, "Atomic Absorption Spectrophotometry", 2nd Ed.,

Pergamon Press, New York, 1966.

77. A. W. Epperson, J. Am. Chem. Soc., 50, 321 (1928).

78. L. Erdey, "Gravimetric Analysis", Pt. 1, p. 165, MacMillan Co., New York, 1963.

79. Ibid., Pt. 2, Chapter 42.

80. G. Eulitz, Z. Anal. Chem., 178, 360 (1961).

81. T. W. Fagan, R. Phillips, R. O. Davies, Welsh J. Agr. 17, 97 (1943); CA28:771.[9]

82. Ibid., 18, 75 (1945); CA40:1427.[8]

83. H. W. Fairbairn et al., U.S. Geol. Survey Bull. #980 (1951).

84. H. W. Fairbairn, J. F. Schairer, Am. Min., 37, 744 (1952).

85. R. A. Faires, B. H. Parks, "Radioisotope Laboratory Technique", J. Wiley and Sons, New York, 1973.

86. R. H. Filby, W. A. Haller, K. R. Shah, J. Radioanal. Chem., 5, 127 (1970).

87. R. J. Firman, Spectrochim. Acta , 21, 341 (1965).

88. M. J. Fisherman, B. P. Robinson, Anal. Chem., 41, 323R (1969).

89. "Flame Emission and Atomic Absorption Spectroscopy", J. A. Dean, T. C. Rains (Eds.), Marcel Dekker, New York, 1971.

90. F. J. Flanagan, Geochim. Cosmochim. Acta , 31, 289 (1967); 33, 81 (1969).

91. F. J. Flanagan, M. E. Gwyn, ibid., 1211 (1967).

92. Ibid., 34, 121 (1970).

93. H. Flaschka, R. Puschel, ibid., 71 (1955).

94. H. Flaschka, P. O. Sawyer, Microchem. J., Symp. Ser., 2, 783 (1962).

95. H. Flaschka, "EDTA Titrations", 2nd Ed., Pergamon Press, New York, 1964.

96. H. R. Fleck, A. M. Ward, Analyst, 58, 388 (1933).

97. H. D. Fleming, Spectrochim. Acta , 23B, 207 (1967).

98. L. W. Fleming, W. K. Stewart, Clin. Chim. Acta , 14, 134 (1966).

99. H. R. Follis, Jr., "Deficiency Disease", p. 35, Charles C. Thomas, Springfield, Illinois, 1958.

100. W. A. Forster, Analyst, 78, 179 (1953).

101. J. J. Fox, J. Chem. Soc., 97, 1119 (1910).

102. F. Frana, M. Vobecky, Z. Randa, J. Benada, J. Kuncir, Radiochem. Radioanal. Lett., 8, 97 (1971).

103. C. W. Frank, W. G. Schpenk, C. E. Meloen, Anal. Chem., 38, 1005 (1966).

104. J. S. Fritz, M. A. Peters, Talanta, 16, 575 (1969).

105. S. Gaddis, J. Chem. Ed., 19, 327 (1942).

106. "Gamma Ray Energy Tables for Neutron Activation Analysis", R. H. Filby, A. I. Davis, K. R. Shah et al. (Eds.), Washington State Univ., publ. #WSUNRC-97(2), 1970.

107. J. P. Garrec, Centre d'Etudes Nucleaires de Grenoble Rept. CEA-R-3636, Saclay, France, 1968.

108. H. C. Giron, At. Abs. Newsletter, 12, 28 (1973).

109. E. Glueckauf, P. J. Roberts, "Precipitation Separations Without Filtration -- A New Procedure Employing Anion Exchange Resins", AERE Harwell, June 1954.

110. D. W. Goodall, F. G. Gregory, "Chemical Composition of Plants as an Index of their Horticultural Status", Tech. Comm. #17, Imp. Bur. Hort. Plantation Crops, England, 1947.

111. G. C. Goode, C. M. Howard, A. R. Wilson, V. Parsons, Anal. Chim. Acta , 58, 363 (1972).

112. T. T. Gorsuch, Analyst, 84, 135 (1959).

113. Ibid., "The Destruction of Organic Matter", Pergamon Press, New York, 1970.

114. C. L. Grant, 4th Int. Conf. on Atomic Spectroscopy, Nov. 1973, personal communication.

115. J. D. Grier, J. Assoc. Offic. Anal. Chem., 49, 291 (1966).

116. R. Grzekowiak, T. A. Turner, Talanta, 20, 35 (1973).

117. "Guide to Activation Analysis", W. S. Lyon, Jr. (Ed.), D. Van Nostrand Inc., New York, 1964.

118. "Guide to Modern Methods of Instrumental Analysis", T. H. Gouw (Ed.), p. 466, J. Wiley and Sons, New York, 1972.

119. F. L. Hahn, Z. Angew. Chem., 39, 1198 (1926).

120. F. L. Hahn, K. Vieweg, Z. Anal. Chem., 71, 122 (1927).

121. K. J. Hahn, D. J. Tuna, M. A. Quaife, Anal. Chem., 39, 1169 (1967).

122. Ibid., Trans. Am. Nucl. Soc., 10, 62 (1967).

123. K. J. Hahn, D. J. Tuna, J. L. Sullivan, Anal. Chem., 40, 974 (1968).

124. R. J. Hall, G. A. Gray, L. R. Flynn, Analyst, 91, 102 (1966).

125. D. J. Halls, A. Townshend , Anal. Chim. Acta , 36, 278 (1966).

126. H. C. Hanig, M. H. Aprison, Anal. Biochem., 21, 169 (1967).

127. M. C. Haven, G. T. Haven, A. L. Dunn, Anal. Chem., 38, 141 (1966).

128. F. W. Heaton, J. Clin. Path., 13, 358 (1960).

129. O. Héroux, National Research Council of Canada, personal communications, Aug. 1972, May 1973, Oct. 1973.

130. G. Hevesy, H. Levi, Det. Kgl. Danske Vid. Selsk. Math. fys. Medd., 14, 24 (1936); CA31:2507.[5]

131. W. F. Hillebrand, G. E. F. Lundell, H. A. Bright, J. I. Hoffman, "Applied Inorganic Analysis", 2nd Ed., p. 714, J. Wiley and Sons, New York, 1953.

132. D. J. Hiscox, J. Sci. Agr., 27, 136 (1947).

133. M. Hofer, Experimentia, 19(7), 367 (1963).

134. F. Hoffman, Albrecht-Thaer-Arch, 11, 405 (1967); CA67:114 239y.

135. R. G. Hollingshead, "Oxine and Its Derivatives", Vol. II, p. 323, Butterworths Sci. Publ., London, 1954.

136. M. Honda, Japan Analyst, 3, 132 (1954).

137. Hopkin and Williams, "Organic Reagents for Metals",4th Ed., p. 81, Hopkin and Williams, Ltd., London, 1944.

138. C. Iida, K. Fuwa, W. E. Wacker, Anal. Biochem., 18, 18 (1967).

139. S. Impedovo, A. Traini, P. Papoff, Talanta, 18, 97 (1971).

140. C. O. Ingamells, N. H. Suhr, Geochim. Cosmochim. Acta , 27, 897 (1963).

141. H. B. Jacobs, "The Chemical Analysis of Air Pollutants", Interscience Publ., New York, 1960.

142. L. H. James, Ind. Eng. Chem., Anal. Ed., 3, 31 (1931).

143. G. E. Janauer, Mikrochim. Acta., 6, 111 (1968).

144. D. J. C. Jones, J. Agr. Sci., 52, 230 (1959).

145. J. B. Jones, Jr., C. R. Weaver, Jarrell Ash Co., Reprint #68 (1967).

146. J. B. Jones, Jr., J. Assoc. Offic. Anal. Chem., 52, 900 (1969).

147. J. B. Jones, Jr., R. A. Isaac, Agron. J., 61, 393 (1969).

148. E. Junod, Report CEA-R2980, Paris, 1966.

149. A. L. Kenworthy, E. J. Miller, W. T. Mathis, Proc. Am. Soc. Hort. Sci., 67, 16 (1956).

150. C. K. Kim, W. W. Meinke in "Modern Trends in Activation Analysis", p. 316, College Station, Texas, 1968.

151. H. G. King, G. Pruden, Analyst, 92, 83 (1967).

152. T. Kiss, F. F. Gaal, T. M. Suranzi, Anal. Chim. Acta , 43, 340 (1968).

153. H. Kline, E. R. Orent, E. V. McCollum, Am. J. Physiol., 112, 256 (1935).

154. M. R. Klugerman, J. H. Boutwell, Jr., Clin. Chem., 7, 185 (1961).

155. K. Kodama, "Methods of Quantitative Inorganic Analysis", p. 396, Interscience Publ., New York, 1963.

156. S. R. Koirtyohann, E. E. Pickett, Anal. Chem., 38, 1087 (1966).

157. I. M. Kolthoff, Biochem. Z., 185, 344 (1927).

158. I. M. Kolthoff, E. B. Sandell, E. J. Meehan, S. Bruckenstein, "Quantitative Chemical Analysis", 4th Ed., p. 594, Macmillan Co., Toronto, 1969.

159. Ibid., Chapter 29.

160. J. Komarek, J. Jambor, L. Sommer. Z. Anal. Chem., 262, 91 (1972).

161. I. Korenman, "Analytical Chemistry of Low Concentrations", p. 41, Israel Program for Scientific Translations, Jerusalem, 1968.

162. K. A. Kraus, R. J. Raridon, D. L. Holcomb, J. Chromatog., 3, 178 (1970).

163. P. Kruger, "Principles of Neutron Activation Analysis", J. Wiley and Sons, New York, 1971.

164. D. Kruse, E. R. Orent, E. V. McCollum, J. Biol. Chem., 96, 519 (1932).

165. J. Lacoeuille, P. Martin-Prevel, C. Egoumenides, Fruits (Paris), 19, 539 (1964); CA64:18010g.

166. R. H. Laessig, Anal. Chem., 43(8), 18A (1971).

167. P. D. LaFleur, National Bureau of Standards, personal communication, May 1971.

168. I. L. Larsen, N. A. Hartmann, J. J. Wagner, Anal. Chem., 45, 1511 (1973).

169. G. W. Leddicotte, "The Radiochemistry of Manganese", USAEC Publ. #NAS-NS 3018 (1960).

170. Ibid., CONF-651108-2, Urbana, Illinois (Nov. 1965).

171. Ibid., "Methods of Biochemical Analysis", D. Glick (Ed.), Vol. 19, p. 345, Interscience Publ., New York, 1971.

172. C. M. Lederer, J. M. Hollander, I. Perlman, "Tables of Isotopes", 6th Ed., J. Wiley and Sons, New York, 1968.

173. M. Lerner, W. Rieman, Anal. Chem., 26, 610 (1954).

174. L. L. Lewis, L. M. Melnick, ibid., 32, 38 (1960).

175. H. Lundegardh, "Leaf Analysis", Hilger and Watts, Ltd., London, 1951.

176. B. V. L'vov, "Atomic Absorption Spectrochemical Analysis", J. H. Dixon (trans.), Adam Hilger, London, 1970.

177. W. Lyon, E. L. Kinsey, Phys. Rev., 61, 482 (1942).

178. I. MacIntyre, D. Davidsson, Biochem. J., 70, 456 (1958).

179. I. MacIntyre, "Scientific Basis for Medicine, Annual Reviews", p. 216, Athlone Press, London, 1963.

180. C. K. Mann, Anal. Chem., 32, 67 (1960).

181. W. F. Marlow, P. D. LaFleur in "Nuclear Techniques in Environmental Pollution", p. 91, (Proc. Symp. Salzburg, 1970), IAEA, Vienna, 1971.

182. A. C. Mason, Analyst, 77, 529 (1952).

183. W. T. Mathis, J. Assoc. Offic. Agri. Chem., 34, 604 (1951).

184. Ibid., 35, 406 (1952).

185. Ibid., 36, 441 (1953).

186. R. Mauston, Anal. Biochem., 16, 65 (1966).

187. M. J. McBroom, Y. J. Lancaster, A. K. Weiss, ibid., 42, 178 (1971).

188. W. W. Meinke, Anal. Chem., 43(6), 28A, (1971).

189. V. P. Menon, M. S. Das, Analyst, 83, 434 (1958).

190. A. C. Menzies, Anal. Chem., 32, 818 (1960).

191. M. Milan, K. L. Cheng, ibid., 42, 652 (1970).

192. C. C. Miller, I. C. McLennan, J. Chem. Soc., 656 (1940).

193. G. M. Milton, W. E. Grummitt, Can. J. Chem., 35, 541 (1957).

194. R. F. Milton, W. A. Waters, "Quantitative Micro-Analysis", p. 294, Arnold and Co., London, 1949.

195. "M.I.T. Wavelength Tables", G. R. Harrison (Ed.), J. Wiley and Sons, New York, 1939.

196. C. Monder, N. Sellis, Anal. Biochem., 20, 21 (1967).

197. P. Morard, J. C. Gullo, M. Couvrand, Ann. Agron., 21, 229 (1970); CA 74:39017p.

198. A. G. Morris, Anal. Chem., 33, 509 (1961).

199. G. H. Morrison, N. M. Potter, ibid., 44, 839 (1972).

200. H. V. Moyer, W. J. Remington, Ind. Eng. Chem., Anal. Ed., 10, 212 (1938).

201. F. Nelson, K. A. Kraus, J. Am. Chem. Soc., 77, 801 (1955).

202. F. Nelson, R. A. Day, Jr., K. A. Kraus, J. Inorg. Nucl. Chem., 15, 140 (1960).

203. D. A. Olehy, R. A. Schmitt, W. F. Bethard, USAEC Rept. #GA-2803 (1962).

204. Ibid., "L'Analyse par Radioactivation et Ses Applications aux Sciences Biologiques", D. Comar (Ed.), p. 385, Presses Universitaires de France, Paris, 1964.

205. Ibid., J. Nucl. Med., 7, 917 (1966).

206. V. P. Padhye, Analyst, 82, 634 (1957).

207. K. Paech, M. V. Tracey, "Modern Methods of Plant Analysis", Springer-Verlag, Berlin, 1955.

208. V. K. Panday, A. K. Ganguly, Anal. Chim. Acta , 52, 417 (1970).

209. J. Pijck, J. Gillis, J. Hoste, Int. J. Appl. Rad. Isotopes, 10, 149 (1961).

210. M. Pinta, "Detection and Determination of Trace Elements", p. 19, Ann Arbor-Humphrey Science Publishers, Ann Arbor, 1970.

211. H. Pohl, Z. Anal. Chem., 155, 263 (1957).

212. F. H. Pollard, J. U. Martin, Analyst, 81, 348 (1956).

213. F. H. Pollard, G. Nickless, D. Spincer, J. Chromatog., 10, 215 (1963).

214. P. Povondra, R. Pribil, Talanta, 10, 713 (1963).

215. R. Pribil, "Analytical Applications of EDTA and Related Compounds", Pergamon Press, New York, 1972.

216. W. J. Price, "Analytical Atomic Absorption Spectroscopy", Heyden and Sons, New York, 1972.

217. W. Prodinger in "Organic Reagents Used in Quantitative Inorganic Analysis", S. Holmes (Ed.), 2nd Ed., p. 111, Elsevier Publ. Co., New York, 1940.

218. P. Quittner, E. Szabo, G. Perneczki, A. Major, J. Radioanal. Chem., 5, 133 (1970).

219. N. Radin, Clin. Chem., 13, 55 (1967).

220. T. V. Ramakrishna, J. W. Robinson, P. W. West, Anal. Chim. Acta , 36, 57 (1966).

221. Ibid., 40, 350 (1968).

222. J. Ramirez-Munoz, "Atomic Absorption Spectroscopy and Analysis by Atomic Absorption Flame Photometry", Elsevier Publ. Co., New York, 1968.

223. Ibid., Anal. Chem., 42, 517 (1970).

224. J. C. Redmond, H. A. Bright, J. Res. Nat. Bur. Stand., 6, 113 (1931).

225. K. M. Reese, Anal. Chem., 42, 26A (1970).

226. D. H. Retief, P. E. Cleaton-Jones, J. Turkstra, J. Dent. Assoc. S. Africa, 25, 188 (1970); CA75:72302w.

227. A. G. Roach, P. Sanderson, D. R. Williams, Analyst, 93, 42 (1968).

228. J. W. Robinson, "Atomic Absorption Spectroscopy", Marcel Dekker, New York, 1966.

229. J. T. H. Roos, Spectrochim. Acta , 24B, 255 (1969).

230. I. Rubeska, B. Moldan, Anal. Chim. Acta , 37, 421 (1967).

231. Sadtler Catalog of Standard Spectra, S. P. Sadtler and Sons, Philadelphia, Pa.

232. A. Sapek, T. Zminkowska, Pamiet. Pulawski, 42, 159 (1971); CA75:115820h.

233. W. G. Schlect, Anal. Chem., 23, 1568 (1951).

234. R. A. Schmitt, T. A. Linn, Jr., H. Wakita, Radiochim. Acta , 13, 200 (1970).

235. B. H. Schneider, "Feeds of the World", West Virginia University, Morgantown, 1947.

236. "Scott's Standard Methods of Chemical Analysis", N. H. Furman (Ed.), 6th Ed., Vol. I, p. 642, Van Nostrand Co. Inc., Toronto, 1966.

237. Ibid., p. 641.

238. M. Séguin, Comptes Rendu Acad. Sci. Paris, 222, 952 (1946).

239. D. A. Skoog, D. M. West, "Principles of Instrumental Analysis", Holt, Rinehart, Winston, Inc., Toronto, 1971.

240. W. Slavin, "Atomic Absorption Spectroscopy", Interscience Publ., New York, 1968.

241. Ibid., Chapter 3.

242. K. G. Sloman, A. K. Foltz, J. A. Yeransian, Anal. Chem., 41, 63R (1969).

243. J. B. Smathers, D. Duffey, S. Lakshmanan, Trans. Am. Nucl. Soc., 10, 448 (1967).

244. Ibid., Nucl. Appl. Tech., 7, 84 (1969).

245. Ibid., Anal. Chim. Acta , 46, 9 (1969).

246. A. M. Smith, E. S. McCallum, Analyst, 81, 160 (1956).

247. P. Smith, C. H. Kurtzman, M. E. Ambrose, Clin. Chem., 12, 418 (1966).

248. R. Smits, K. Larsen, D. L. Massart, J. Chromatog., 59, 237 (1971).

249. F. D. Snell, C. T. Snell, "Colorimetric Methods of Analysis", 3rd Ed., Vol. IIa, p. 528, D. Van Nostrand Inc., New York, 1959.

250. A. G. Souliotis, E. P. Belkas, A. P. Grimanis, Analyst, 92, 300 (1967).

251. G. Staats, H. Bruck, Z. Anal. Chem., 223, 185 (1966).

252. "Standard Methods of Chemical Analysis", F. Welcher (Ed.), 6th Ed., Vol. IIB, p. 2439, D. Van Nostrand Ind., Princeton, New Jersey, 1963.

253. W. K. Stewart, F. Hutchinson, L. W. Fleming, J. Lab. Clin. Med., 61, 858 (1963).

254. W. J. A. Steyn, J. South Afr. Chem. Soc., 9, 39 (1956).

255. K. G. Stone, J. Am. Chem. Soc., 76, 4997 (1956).

256. F. W. Strelow, Anal. Chem., 32, 1185 (1960).

257. F. W. Strelow, C. R. van Zyl, C. R. Nolte, Anal. Chim. Acta , 40, 145 (1968).

258. J. Stupar, J. Furlan, I. Glazer, Landwirt. Forsch., 20, 12 (1967); CA 67:105894f.

259. Z. Sulcek, P. Povondra, R. Pribil, R. Stangl, Talanta, 9, 467 (1962).

260. S. R. Taylor, P. Kolbe, Geochim. Cosmochim. Acta , 28, 447 (1964).

261. R. E. Thiers in "Trace Analysis", J. H. Yoe, H. J. Koch, Jr. (Eds.), p. 637, J. Wiley and Sons, New York, 1957.

262. Ibid., "Methods of Biochemical Analysis", D. Glick (Ed.), Vol. 5, p. 284, Interscience Publ., New York, 1957.

263. A. D. Thomas, L. E. Smythe, Talanta, 20, 469 (1973).

264. W. Thomas, Plant Physiol., 12, 571 (1937).

265. G. Tolg, Talanta, 19, 1489 (1972).

266. D. B. Tonks, P. H. Allen, Can. Med. Assoc. J., 72, 605 (1955).

267. D. B. Tonks, Clin. Chem., 9, 217 (1963).

268. F. P. Treadwell, W. M. Hall, "Analytical Chemistry", Vol. II, p. 189, J. Wiley and Sons, New York, 1963.

269. "Treatise on Analytical Chemistry", I. M. Kolthoff, P. J. Elving, E. B. Sandell (Eds.), Pt. I, Vol. 1, p. 22, Interscience Publ., New York, 1961.

270. Ibid., Pt. II, Vol. 3, p. 57.

271. Ibid., p. 79.

272. H. Tsubota, Y. Kitano, Bull. Chem. Soc. Japan, 33, 770 (1960).

273. J. Turkstra, P. J. Pretorius, W. J. de Wet, Anal. Chem., 42, 835 (1970).

274. A. Ulrich in "Diagnostic Techniques for Soils and Crops", American Potash Institute, Washington, 1948.

275. E. J. Underwood, "Trace Elements in Human and Animal Nutrition", Academic Press, New York, 1971.

276. B. L. Vallee, W. E. C. Wacker, D. D. Ulmer, New England J. Med., 262, 155 (1960).

277. J. C. van Loon, Chem. Can., 25(5), 12 (1973).

278. M. Wald, B. Kaysser, Mikrochim. Acta , 1137 (1970).

279. A. Walsh, Spectrochim. Acta , 7, 108 (1955).

280. G. M. Ward, H. B. Heeney, Can. J. Plant Sci., 40, 589 (1960).

281. G. M. Ward, F. Johnson, Can. Dept. Agriculture Publ. #1064, Feb. 1960.

282. F. J. Welcher, "The Analytical Uses of EDTA", D. Van Nostrand Co., Princeton, New Jersey, 1958.

283. E. S. West, W. R. Todd, H. S. Mason, J. T. van Bruggen, "Textbook of Biochemistry", 4th Ed., p. 1386, Macmillan Co., New York, 1966.

284. P. W. West in "Air Pollution", Vol. 2, A. C. Stern (Ed.), Academic Press, New York, 1970.

285. J. O. Westgard, M. R. Hunt, Clin. Chem., 19, 49 (1973).

286. C. H. Williams, Anal. Chim. Acta , 22, 163 (1960).

287. R. J. P. Williams, Quart. Rev., 24, 331 (1970).

288. J. B. Willis, Spectrochim. Acta , 16, 273 (1960).

289. A. D. Wilson, Analyst, 89, 18 (1964).

290. A. E. Wilson, Anal. Chem., 22, 1571 (1950).

291. L. Wish, ibid., 33, 53 (1961).

292. H. E. Woodman, R. E. Evans, A. Eden, J. Agr. Sci., 26, 212 (1936).

293. I. D. P. Wootton, Clin. Chem., 2, 296 (1956).

294. A. Wuensch, K. Teicher, Z. Pflanzenernaehr. Dueng. Bodenk., 97, 101 (1962); CA 57:10136e.

295. A. Wyttenbach, H. Dulakas, Chimia, 22, 484 (1968).

296. A. Wyttenbach, Helv. Chim. Acta , 52, 2458 (1969).

297. J. Yofe, R. Finkelstein, Anal. Chim. Acta , 19, 166 (1958).

298. W. J. Youden, "Statistical Methods for Chemists", J. Wiley and Sons, New York, 1964.

299. A. Young, T. R. Sweet, B. B. Baker, Anal. Chem., 27, 356 (1955).