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UNIVERSITY OF ALBERTA

DETERMINATION OF TRACE METALS IN AGRICULTURAL MATERIALS BY DSI-ICP

BY

LISHI YING

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science**

DEPARTMENT OF CHEMISTRY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled DETERMINATION OF TRACE METALS IN AGRICULTURAL MATERIALS BY DSI-ICP submitted by LISHI YING in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

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DATE: 20 August 92

To

Mom and Dad

Thanks for your love and support

To

Barbara and Steve

Thanks for the opportunity to bring me to North America and

for your continuing encouragement and help

Abstract

The simultaneous multielement analysis of microgram portions of agricultural materials by direct insertion of a graphite sample cup into an inductively coupled plasma (ICP) has been investigated. For standardization microliter volumes of standard solutions were pipetted into a graphite cup, the solution dried and ashed below the ICP, and the cup then introduced into a continuously operating argon and oxygen mixed gas ICP for sample vaporization. Compromise operating conditions for the multielement determination of 5 elements, Al, Fe, Cu, Zn and Mn, were established by a univariate method. The method was tested by determining these elements in U.S. National Institute of Standards and Technology (NIST) reference materials Rice Flour (SRM1568a), Wheat Flour (SRM1567a), Citrus Leaves (SRM1572), Corn Kernel (RM8413) and Corn Stalk (RM 8412). The results were in good agreement with the certified values.

The variability in distribution of these five trace elements in Rice Flour (SRM 1568a) and Wheat Flour (SRM 1567a) from NIST was examined. The homogeneity of aluminum in Rice Flour is high at test portion sizes of 10 to 15 mg, but for Wheat Flour variability among test portions is significant.

The concentrations of Al, Fe, Cu, Zn and Mn in eight candidate agricultural reference materials from Agriculture Canada were also studied by mixed Ar-O_2 gas direct insertion ICP. Values were generally comparable with published data. An assessment of these materials for within vs. between bottle homogeneity was done by one-way analysis of variance. All were found to be homogeneous at test portion sizes of 5 to 15 mg except for aluminum in Durum Wheat flour 187 and iron in Cellulose 189.

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GLOSSARY OF ABBREVIATIONS

NIST	U.S. National Institute of Standards and Technology
SRM	Standard Reference Material
RM	Reference Material
CRM	Certified Reference Material
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
ETA-AAS	Electrothermal Atomizer Atomic Absorption Spectroscopy
FAAS	Flame Atomic Absorption Spectroscopy
FAES	Flame Atomic Emission Spectroscopy
NAA	Neutron Activation Analysis
INAA	Instrumental Neutron Activation Analysis
DCP	Direct Current Plasma
STPE	Stabilized Temperature Platform Furnace
EIE	Easily Ionized Element
SIMAAC	Simultaneous Multielement Atomic Absorption Spectrometer with a Continuum Source
DSI	Direct Sample Insertion
BLC	Below Load Coil
ADC	Analog to Digital Converter
NRC	National Research Council of Canada
NBS	U.S. National Bureau of Standards
RSD	Relative Standard Deviation
ANOVA	Analysis of Variance
PDA	Photodiode Array

CHAPTER 1

INTRODUCTION

1.1 Background

There is an increasing awareness and concern for improving data and measurement of both harmful and beneficial constituents in environmental, health related and biological matrices. This has generated a rapidly growing need for extensive measurement systems and methods for more " routine" analysis and methodology capable of fast, economic and precise analytical throughput. As our knowledge of environmental and health related processes becomes more detailed and extensive, creating a demand for more accurate and precise data, the resulting analytical measurement systems and methodologies become more complex and interrelated. To validate analytical methods and measurement systems, and to link both new and "routine" field methods to more definitive methodology requires quality control by using of appropriate reference materials.

1.2 Reference Materials

A reference material (RM) is a substance for which one or more properties are established sufficiently well to calibrate a chemical analyzer or to validate a measurement process(1). A certified reference material (CRM) is a reference material issued and certified by an organization accepted to be technically competent. CRM's are stable, homogeneous and well characterized reference materials prepared in quantity and having very similar matrices to test samples so as to minimize matrix effects (2).

Several steps are required in the production of RM's. First of all there must be a demonstrated need for a specific type of RM. The properties of a useful RM must undergo careful consideration. The kind and level of parameters certified, the matrix and other physical characteristics, homogeneity requirements and largest acceptable uncertainties for the certified values are important considerations(3).

The major suppliers of internationally available biological reference materials are Community Bureau of Reference (BCR) in Belgium, Behring Institute (BI) in Germany, Dr. Bowen (BOWEN) at the University of Reading in the United Kingdom, International Atomic Energy Agency (IAEA) in Austria, National Institute of Standards and Technology (NIST) in the USA, National Institute for Environmental Studies (NIES) in Japan, and National Research Council of Canada (NRC) in Canada.

1.3 Determination of Trace Metals in Reference Materials

R. M. Parr in IAEA did a survey and evaluation of available biological reference materials for trace element analysis (4). For several elements of biological interest there is a serious lack of RMs. These include Al, Mo, Sn and V. In addition, confidence intervals for the Al, As, Cr, Mo, Ni, and V values are generally large in these RM's.

As far as internationally available biological reference materials are concerned, a more modest assessment of future needs can be made by looking at the number of available materials for each element of interest and at the uncertainties in the quoted concentrations. A recent publication (4) points out that while there are 39 current biological reference materials certified for copper, 32 for iron, 29 for zinc and 26 for manganese, there are only 4 certified for aluminum.

Table 1.1 List of botanical reference materials for which aluminum concentration values are provided, arranged in descending order of concentration*

Material	Code	Concentration (μg/g)	Status
Aquatic plant	BCR-CRM-061	17,000	NC
Aquatic plant	BCR-CRM-060	6,400	NC
Tomato leaves	NIST-SRM-1573	1,200	NC
Pine needles	NIST-SRM-1575	545	C
Olive leaves	BCR-CRM-062	260	NC
Citrus Leaves	NIST-SRM-1572	92	C
Hay powder	IAEA-V-10	47	NC
Cotton cellulose	IAEA-V-9	44	NC
Kale	BOWEN's Kale	39.9	NC
Wheat flour	NIST-SRM-1567a	5.7	C
Rice flour	NIST-SRM-1568a	4.4	C
Corn kernel	NIST-RM-8413	4	NC
Rye flour	IAEA-V-8	3	NC

NC – not certified, value for information only

C – certified by NIST

* This table is modified from the data in reference 4 in 1987.

Thus we see that the need for developing Al biological reference materials is an urgent task. Table 1.1 lists the biological reference materials for which an aluminum value has been reported, along with the estimated concentration provided by the agency (for information only) or from the literature. These tables are based on the data in reference 4 in 1987. From a recent NIST catalog 1992-1993, we see that citrus leaves has been replaced by the two new botanical reference materials which are SRM 1515 Apple Leaves with aluminum concentration 286 μg/g and SRM 1547 Peach Leaves 1547 with aluminum concentration 249 μg/g.

It is clear that the accurate determination of trace aluminum in botanical materials is difficult. Problems include contamination or loss during sample collection and preparation for measurement, and bias and scatter in the measurement steps. As a result, only five of the botanical reference materials presently available from various agencies in the world are certified for aluminum.

For the last 5 to10 years the measurement of trace aluminum in biological samples has centered around detection of toxicity and monitoring in patients with chronic renal failure (CRF). A relationship between aluminum and senile dementia of the Alzheimer's type has frequently been considered throughout the same period(5). However, projects aimed at assessment of much lower aluminum exposure via food, drinking water, etc., and at evaluation of body burden and disposition, are now being undertaken. Other groups of subjects are exposed to aluminum at work (dust and fumes), or while on dialysis, and require monitoring. These developments have been matched by an increase in the number of laboratories measuring low concentrations of aluminum in samples such as serum, urine, water, dialysis fluid, brain and other tissues.

Although many analytical procedures for the measurement of aluminum in biological samples have been described, almost all laboratories now use graphite furnace -atomic absorption spectrometry (GFAAS). This technique ususally offers the required sensitivity and is widely employed in many centers for the measurement of lead and other trace elements. Despite the considerable experience and expertise that exists with this particular technique, attempts to establish methods for the determination of aluminum have, in many cases, been accompanied by immense problems. Laboratories with little or no previous experience with GFAAS have found even greater difficulty.

1.4 Survey of Reference Materials Certified for Trace Metals

The National Institute for Environmental Studies (NIES) of Japan has produced a variety of reference materials certified for elemental composition over the past several years (6). Recently, a tea leaves powder has been prepared and certified as a reference material to assist in the validation of analytical procedures for several metals in agricultural and environmental laboratories.

Several methods were used, including atomic absorption, ICP-AES, and NAA. A Hitachi 180-80 Zeeman atomic absorption spectrometer was used in the air-acetylene flame mode to determine Al, Mn, Fe, Cu and other metals. A Jarrell-Ash 975 direct-reading emission spectrometer was used with the following principal plasma operating parameters: r.f. power 1.1 kw, observation height 18 mm, and sample uptake rate 1.0 mL/min. The analytical lines (nm) for the measured elements were as follows: Al 298.2, Mn 257.6, Fe 259.9, Cu 324.8. A two-point calibration procedure was employed for standardization of the instrument. The low standard blank was 0.1 M nitric acid, and the high standards were 0.1 M nitric acid containing 10 µg/ml of each of Al, Mn, Fe, and Cu. The multi-element standard solutions were prepared from stock solutions of the individual elements (1000 µg/mL).

To assess the homogeneity of the tea leaves reference material, the variation of elemental content in several bottles was examined by AAS and ICP-AES analyses following acid digestion utilizing Teflon double-vessel bombs. Five bottles were randomly selected from the lot of 600 bottles and 5 aliquots(about 250 mg dry weight) were taken from each bottle to give a total of 25 samples.

Variations due to sample variability were estimated by one-way analysis of variance to be less than 1% (as relative standard deviation) for Al, Mn, and Cu, indicating that the prepared tea leaves satisfies the homogeneity criteria for a reference material. However, between-bottle variation was significant for Fe. The reason for this has not been clarified.

The certified values for the tea leaves reference material were: Al 775 ± 20 μg/g, Mn 700 ± 25 μg/g, Cu 7.0 ± 0.3 μg/g. The certified values are based on results of determinations by at least three independent analytical techniques. For Al and Mn these were AAS, ICP-AES, NAA; for Cu they were AAS, ICP-AES, and isotope dilution mass spectrometry.

Koch et al. analyzed the aluminum content in coffee beans and tea leaves (7). The aluminum level in coffee beans was so low that it could not be quantified satisfactorily using the method employed for tea leaves (wet ashing, then ICP-AES) as was reflected in the large relative standard deviations that they observed. No attempts were made to obtain more accurate values for the Al levels in coffee beans. It was found that wet ashing followed by ICP-AES allows for the reasonably accurate determination of Al in dry tea leaves. On the other hand, for tea/coffee infusions, which contain up to two orders of magnitude less Al, GFAAS is more suitable as a means of quantification. Further, it is evident that the Al concentrations found in tea infusions depend on the origin of the tea and infusion time in addition to the fineness of the tea. In contrast, coffee infusions prepared from ground coffee beans contain only small amounts of Al. Typical tea infusions contain between 10 and 20 times more Al per unit volume than do coffee infusions.

Sargasso seaweed was chosen as a new reference material to be prepared by NIES for use in the fields of marine and environmental sciences (8). In order to determine the homogeneity of the sargasso reference material, the variations of elemental content in several bottles were determined by ICP-AES and AAS after acid dissolution of the samples. Six bottles were randomly selected from the lot of 800 bottles and five aliquots of approximately 290 mg dry weight each were taken from each bottle for a total of 29 samples. The homogeneity of this reference material was determined using one-way analysis of variance. Variations due to sample heterogeneity were insignificant ($p<0.05$) and calculated to be less than 1% for Fe, Mn and Cu. These results indicate that the NIES Sargasso reference material satisfies the homogeneity criteria for a reference material. The certified values are: Al 209 µg/g, Mn 23.7 µg/g, Fe 187 µg/g, Cu 4.9 µg/g.

Certification of a Rice Flour-Unpolished reference material is underway at NIES. The elemental composition is essentially the same as the average concentration in Japanese unpolished rice and is similar to the levels found in NBS Rice Flour and Wheat Flour SRMs. The content of Al is 1.5 to 2.7 µg/g, of Fe 11to 13 µg/g, and of Mn 31 to 40 µg/g.

A Cabbage Leaves reference material has been prepared by the Institute of Physics and Nuclear Techniques (Department of Radiometric Analysis), Poland (9). The certified value for the concentration of Cu is 3.3 ± 0.25 µg/g, for Fe 58.4 ± 3.9 µg/g, and for Mn 57.6 ± 2.1 µg/g in cabbage leaves CL-1. The information value for Al is 23.83 to 27.60 µg/g.

A potato reference material was prepared for the analytical quality control program to be employed by the sub-network on trace elements in food of the FAO European Co-operative Network on Trace Elements (10). The recommended concentration values for Fe is 22.0 ± 2.0 μg/g, for Mn 8.1 ± 0.32 μg/g and for Cu 3.87 ± 0.18 μg/g.

Meat and meat products form a major part of the western diet and contribute significantly to the daily intake of both toxic and nutritionally important elements (11). A commission of the European Communities is developing legislation which will impose limits on toxic element concentrations in meats and meat products designed for human consumption within the EEC. It was in the context of this legislation that the BCR undertook the development of a series of reference materials which would provide a basis for ensuring uniformity in the determination of toxic elements and which would meet some of the needs concerning laboratory quality control of other elements commonly determined in these materials.

During this evaluation, it become evident that RM 185 (bovine liver) had proved more difficult to analyze for metals than either RM 184 or 186 (bovine muscle and pig kidney). This difficulty was reflected in greater between-method variations, particularly where different sample digestion techniques were used. This effect appears to be associated with the nature of the fat in liver, which is a complex mixture of mono-, di-, and triglycerides (the total fat content of liver and kidney materials are similar at about 11%). The certified values for Cu are 2.36 ± 0.06 μg/g in bovine muscle, 189 ± 4 μg/g in bovine liver, and 31.9 ± 0.4 μg/g in pig kidney; for Fe they are 79 ± 2 μg/g in bovine muscle, 214 ± 5 μg/g in bovine liver, and 299 ± 10 μg/g in pig kidney; for Mn they are 334 ± 28 ng/g in bovine muscle, 9.3 ± 0.3 μg/g in bovine liver, and 8.5 ± 0.3 μg/g in pig kidney.

The preparation of a bovine teeth powder and a bovine muscle powder has been reported; they are further presented as possible reference materials (12). The value of Al is 115 ± 2 mg/kg for bovine teeth and 675 ± 20 mg/kg for muscle powder by DCP; Fe is 4.7 ± 0.5 mg/kg for bovine teeth and 880 ± 28 mg/kg for muscle powder by DCP; Mn is 1.5 ± 0.04 mg/kg for bovine teeth and 28 ± 2 mg/kg for muscle powder by ICP; Cu is 0.17 ± 0.03 mg/kg for bovine teeth and 4.6 ± 0.5 mg/kg for muscle powder by ICP.

The NRC Canada biological reference material TORT-1 lobster hepatopancreas was analyzed for aluminum by instrumental neutron activation analysis (INAA) and graphite furnace absorption spectroscopy (GFAAS) (13). The methods gave similar results of 43 ± 3 and 42 ± 2 μg/g respectively for 200 to 290 mg test portions. Analyses of six portions from each of six bottles of TORT-1 showed no statistical difference at the 95% confidence level for between and within bottle variances. Therefore, the material can be considered homogeneous for aluminum if 200- to 290- mg test portions are taken. The variance was greater and the average lower when 29-mg test portions were analyzed for aluminum by GFAAS. The pattern of the results, together with the need for HF in the dissolution procedure, suggests the presence of aluminum-containing microparticle mineral matter in the material.

Ten agriculture materials, obtained from Agriculture Canada and the U.S. National Institute of Standards and Technology, were analyzed for their aluminum content (14). Graphite furnace atomic absorption spectroscopy (GFAAS) gave reproducible results that showed all of the materials to have a homogeneous distribution of aluminum at test portion sizes of 250 mg. Neutron activation analysis (NAA) gave reproducible results which were high compared to the GFAAS results due to phosphorus and silicon interference. Correction of the NAA aluminum results for phosphorous interference gave values that agreed reasonably well with the aluminum values by GFAAS. Details of the aluminum contents are listed in Appendix B.

1.5 Direct Analysis of Solid Samples

The most time-consuming aspect of the elemental analysis of biological materials is most certainly the sample preparation. There is little doubt that as sample preparation procedures become more tedious, involved and complicated, they also become error-prone, labour-intensive and time consuming. In addition, there are cases where a limited sample quantity is available, for example, in samples of archaeological, biological, biochemical, environmental, forensic and planetary origin. In other cases, even when a large sample quantity is available, conversion to a solution may result in small volumes after separation. Clearly, for AAS or ICP-AES continuous aspiration of small solution volumes via pneumatic nebulization (typically requiring 1-3 mL/min) is unrealistic. The best way to reduce sample preparation time is direct introduction of solids or slurries by methods such as FAAS, FAES, ETA-AAS, ICP-AES or DCP-AES.

The direct analysis of solids or slurries offers several advantages over conventional wet oxidation and dry ashing sample preparation procedures. These include : (1) reduced sample preparation time; (2) decreased possibility of analyte loss through volatilization prior to analysis; (3) reduced loss of analyte related to retention by insoluble residue; (4) reduced possibility of sample contamination. However, several limitations have prevented direct solid analysis from becoming more widely accepted. Researchers have repeatedly reported the following problems:(1) high background levels; (2) difficulties with calibration; (3) problems with micro-weighing and sample introduction; (4) difficulties with less volatile elements; (5) necessity for matrix-matched standards and/or matrix modifiers; (6) particle size dependence; (7) build-up of residual carbonaceous material in the graphite tube; (8) sample-dependent peak shapes; (9) sampling errors related to inhomogeneity; and (10) poor precision.

A prototype simultaneous multielement atomic absorption spectrometer with a continuum source (SIMAAC), consisting of a continuum source and an echelle polychromator modified for wavelength modulation, has been used with flame atomization as well as graphite furnace atomization (15-18). SIMAAC GFAAS detection limits are superior to ICP-AES detection limits and sample volume requirements are much smaller, typically 5 to 25 µL. ICP-AES direct reading polychromator systems are typically capable of determining more elements simultaneously. SIAAC GFAAS detection limits are in many instances comparable to ICP-MS detection limits.

1.6 Slurry Atomization

Slurry sample preparation for simultaneous multi-element GFAAS determination shows a great deal of promise. Slurries prepared using 5 to 10 mg of homogeneous freeze-dried NBS SRMs (significantly less than the recommended 0.25 to 0.50 g) with 5 mL of dilute HNO_3 were successfully analyzed using stabilized temperature platform furnace (STPF) conditions and calibrated with aqueous standards (17). The particles were kept from settling by ultrasonic agitation of the slurry preparation. This research together with literature reports from Kurfurst, et al (19) suggest that many of the NBS biological reference materials are suitably homogeneous (2 to 10% RSD) when 5 to 10 mg sample masses are used rather than the recommended 250 to 500 mg minimum masses. For NBS wheat flour(SRM 1567), however, the expected Fe solution concentration in the slurry preparations was 37 ng/mL, but analytical determinations provided results which were consistently low by about 25% (17). Subsequent experiments indicated that the low values for Fe resulted from the association of the Fe with small heavy particles that sedimented very rapidly.

A slurry of NBS spinach leaves (SRM 1570) has been used to evaluate the precision obtainable with this ultrasonic agitation method (17). Mn, Zn, Cu, and Ca values are extremely consistent (<11%). However, Fe and Al values show significant steadily decreasing values over ten replicate analyses, suggesting that these elements are associated with particulates which are falling out of suspension. The values for Fe decreased by 50% whereas Al values decreased by 77%. The relative precision for Fe and Al was 22.8% and 45% respectively.

The wavelengths used in the above analyses were Al (299.3 nm), Cu (324.7 nm), Fe (248.3 nm), and Mn (279.5 nm). In most cases, the most sensitive resonance lines are utilized. The Al value for Bovine Serum SRM 1598 was found to be 3.14 ± 0.89 µg/g compared to a certified value of 3.8 ± 0.9 µg/g, and for Bovine Serum RM 8419 8.04 ± 0.3 µg/g compared with a certified value of 13 ± 5 µg/g. The Al value obtained for Citrus Leaves was 80 ± 18 µg/g compared with a certified value 92 ± 15 µg/g.

Solid powder samples deposited in a graphite tube furnace can be vaporized and introduced into a magnetron rotating direct-current arc plasma (20). The arc plasma has the form of a radial current sheet that completely covers the end of the graphite anode. The sample vapor from the graphite furnace is forced to pass through the current sheet. This ensures adequate sample-plasma interaction and results in detection limits in the microgram per gram range. Analytical data were presented for pure solid powder samples and for a number of NIST biological reference materials. Percent error values for the determination of various metallic elements using the method of standard additions ranged from 1.2% to 19.6%. Absolute detection limits at the subnanogram level were reported. In this work the powder standards and NIST materials were weighed on a microbalance and suspended in 0.2% HNO_3. These suspensions were then mixed with an ultrasonic bath and a vortex mixer. Typically, 29-µL aliquots of suspensions were drawn and deposited into the graphite furnace while the suspension was still thoroughly mixed.

In all cases, peak area values were larger for solid powder samples than for solution samples. The solid powder peaks not only were higher but were also wider than the corresponding solution peaks. The NIST reference materials used here all contain significant amounts of easily ionized elements, and these elements are probably the cause of the poor results. The concentrations of concomitant species in these samples could easily account for the enhancement effects as well as the decrease in background intensity observed. The lowest enhancement factor of 1.1 was obtained for Zn in rice flour, while the highest enhancement factor of 3.6 was obtained for Mg (I) in bovine liver. Note that the concomitant EIE concentrations in the rice flour are very low, only 0.112% K and 0.014% Ca. These enhancement factors suggest that it would be difficult to use aqueous solutions as standards for NIST reference materials by this method. Two attempts were made by the authors to reduce the matrix enhancement effects so that it would be possible to use solution standards. The first attempt involved using the ashing step to volatilize selectively the concomitant species. The second involved matrix matching. It was found that most of the K solutions were volatilized with the ashing temperature used; however, the Ca and Na solutions were not. The ashing temperatures needed to clean the system of Ca and Na often led to a major loss of the analyte as well.

1.7 Direct Sample Insertion (DSI) ICP

Conventional sample introduction utilizing a pneumatic nebulization technique to generate and introduce an aerosol into the plasma has some limitations. For example, the sample should be a solution with less than 3% total solid content, and a solid sample must be converted to a solution by appropriate digestion procedures, which may lead to loss of some volatile elements or contamination from reagents and glassware. Moreover, dilution may reduce the detectability of the trace elements. To avoid transport losses, in which 97% of a hard earned sample literally "goes down the drain", it is desirable to introduce the solid sample directly into the plasma.

The working definition of direct sample insertion(DSI) is a system, device or technique in which a sample is placed into or onto a probe, with subsequent introduction of the sample carrying probe into the plasma. In 1979 Salin and Horlick (20) reported the use of a conventional undercut d.c. arc electrode as a means to introduce mg quantities of powders/solids and μL volumes of liquid samples into a medium power (<2.5 kW) ICP and coined the term direct sample insertion (DSI). An interrupted plasma operation (the ICP had to be reignited for each electrode insertion) was, perhaps, the most important disadvantage of their manually driven DSI device. At about the same time, Sommer and Ohis (22) reported on the development of a DSI system, dubbed SET (sample elevator technique), and achieved continuous plasma operation. In their device, a version of which is commercially available from Kontron(Kontron Instruments Gnbh, Div. of Spectralanalytic, Oskar-v, Miller-Strasse 1, D-8057, Eching Bei Munchen, FRG), a graphite cup is pneumatically driven into a 3 kW argon mixed-gas plasma.

Since then, DSI devices appear to have reached both a critical mass and a state of relative maturity. A number of papers dealing with DSI devices have been published. Furthermore, DSI devices are being studied in a number of laboratories around the world and are being developed for both ICP-AES and ICP-MS. The basic experimental set-up requires five building blocks: (1) the DSI device, (2) the ICP (RF generator and plasma box), (3) the spectrometer, (4) the readout and control electronics, and (5) the front end processor.

The insertion itself is accomplished either as one reproducible motion (e.g. one shot) or in a stepwise fashion utilizing either a series of mechanical stops (which can be under either manual or computer control) or via a computer-controlled motor. By positioning the sample holder at positions successively closer to the plasma discharge, thus taking advantage of the thermal gradient that exists underneath the plasma, sample drying and ashing can be achieved prior to atomization. These steps are analogous to the dry, ash and atomize cycles utilized in graphite furnace atomic absorption spectrometry (GFAAS). These steps, however, may have to take on new meanings when used in conjunction with a DSI device. Moreover, the analysis of samples with an organic matrix also necessitates the use of an Ar-O_2 mixed-gas plasma. The key advantages of DSI-ICP are its 100% sample introduction efficiency and elimination of sample pretreatment. The major drawbacks are that the graphite cup causes carbide formation, and the sample size must be small.

V. Karanassions and G. Horlick (23) wrote an extensive review on direct sample insertion devices for inductively coupled plasma spectrometry. The review covered about 100 papers world wide dealing with DSI-ICP. It described different instrument designs and detection limits, calibration curves, and applications. Advantages, limitations and criticisms were discussed in detail. There are three instrument designs specifically dealing with biological sample analysis. The following section reviews these designs and the studies done with them in some detail.

A computer-controlled graphite cup direct insertion device which allows ashing and atomization of 1 to 5 mg of powdered plant samples in an inductively coupled plasma has been developed for direct solid sample analysis (24-26). The use of a graphite lid on the top of the cup prevented loss of sample by spitting and allowed release of atomic vapor through a central hole in the lid into the plasma at the atomization stage. The system was optimized by a simplex method. The effects of ashing time and of Ca, K, Mg, and Na on the analytical emissions of other elements were examined. The absolute detection limits and the relative standard deviations for 18 elements with 2-mg amounts of sample were at the nanogram level and 1.4 to 13%, respectively, for all elements except Al, B, Ca, K, La and Ti. The system was then tested with a series of standard reference plant materials. The analytical results for NBS Orchard Leaves (SRM1571) showed good agreement with the certified values. However, elements such as Al, Ca, B, and Sr which form refractory carbides in the graphite cup could not be determined by the technique. These carbide deposits cause memory effects in subsequent burns. A cellulose powder was used to prepare synthetic solid standards. In order to minimize impurity levels, the cellulose powder was soaked in concentrated HCl for 1 day, washed with distilled water, then freeze dried for 2 days.

With conventional flow rates of external and intermediate argon gases, the plasma could be easily ignited by closing the argon gas flow stopper in the Teflon base at the bottom of the injector tube. Thereafter, the plasma could be sustained by keeping the bottom of the injector tube open. Normally the injector tube bottom was closed in order to prevent a downward flow of argon gas when the graphite cup was not inserted. Two milligrams of the solid sample was taken into the graphite cup, and the graphite lid was placed on top. The argon gas flow stopper was opened, and a Teflon slider attached to a timing belt was raised by a computer-controlled stepping motor to make the graphite rod enter the plasma torch via the injector tube. Ashing of the plant sample was performed inside the injector tube at a cup height of 11 ± 1 mm below the bottom of the plasma body for 5 min. The cup was then introduced into the plasma (2.2 mm above the load coil), and after a few seconds, vaporization of the analytes began.

However, in the case of plant samples, the plasma was extinguished when they were directly introduced. When ashing of the sample was performed prior to atomization, continuous operation of the plasma was possible, but the sample spattered out of the cup at the ashing stage, resulting in a large sample loss. The use of a graphite lid on the top of the cup effectively prevented loss of the sample by spitting and, at the atomization stage, helped in introducing the atomic vapor through the central hole of the lid into the plasma center. Considering the different vaporization times of the elements examined, an integration time of 29 s was employed for simultaneous multielement analysis of plant samples. Flow rates of the external and intermediate argon gas flow rate of about 0.1 L/min were necessary for continuous operation of the plasma during the drying and ashing of the samples. Practically no sample loss took place for Cd, Pb, and As, while Zn showed a small sample loss with increased ashing time. It was found that sample densities were different for each plant material and affected ashing rates. A less dense sample accelerated ashing and a more dense sample retarded it.

The effects of 2% Na, K, Ca, and Mg added as chlorides on the emission intensities of other elements in the cellulose matrix were investigated. Background correction for each analytical line was made by measuring the blank emission signal with cellulose powder and with 2% of the major element in the cellulose matrix. Enhancement of the analyte signal in the presence of KCl occurred for most elements studied except for Mg, Mn, P, Pb, and Sn. On the other hand, emission signals of some of the carbide forming elements such as Ba, La, and Sr were totally suppressed with $CaCl_2$ and $MgCl_2$. The effects caused by the major components may be ascribed to changes in analyte volatilization phenomena, but further investigation is required to elucidate the mechanisms by which NaCl and KCl significantly enhance the emission signals of carbide forming elements in the graphite cup direct insertion device. The results suggest that in the direct insertion analysis of plant materials which contain Na, K, Ca, and Mg as major components, calibration of the spectrometer must be done either with a reference standard having similar concentrations of the major components or with a synthetic standard prepared by matrix matching.

The calibration curves for Pb, As, and Mn covered a wide concentration range in NIES Pepperbush and in NIST-SRM's Spinach, Orchard Leaves, Tomato Leaves and Pine Needles. For these studies, the background correction was made by deducting the emission intensity at a wavelength 0.03 nm higher than the analytical line for each element. Similar calibration curves could also be obtained for other elements whose emission signals were not affected by the presence of the major components of the samples. However, at lower concentrations of the elements, precautions had to be taken to correct for the background emissions where the major compositions of the samples are similar.

Salin et al. (27) analyzed copper and zinc in human head hair by ICP-AES with a DSI device. A concentration of 10.2 ppm with an RSD of 21% was determined for the male donor. In this work the electrodes had a crater volume of 28 mm^3 and a depth of 4.0 mm. To form a large crater volume that would contain the sample sputtering and avoid sample loss, graphite rods of the same diameter as the electrodes were drilled to a depth of 20.0 mm to give a calculated volume of 106 mm^3. Increasing the electrode capacity by using a larger electrode was considered undesirable because the introduction of a large mass into the plasma would result in lower heating rates with a subsequent degradation of detection limits.

An automated direct sample insertion device for inductively coupled plasma-atomic emission spectrometry was built by Shao, Chan and Horlick (28-30). A graphite cup sample probe can be mechanically inserted into the plasma, and the insertion sequence is completely under computer control via a stepper motor. Multichannel high-speed electronics have been developed for a direct-reading polychromator that allow the real-time digitization of the transient spectral signals from up to six channels simultaneously. For volatile elements such as Zn and Cd, detection limits were in the pg range for a 10-μL solution sample.

1.8 Objectives of This Research

Due to the difficulty of obtaining accurate aluminum values for trace levels in biological matrices by present techniques, direct sample insertion inductively coupled plasma spectroscopy was selected as a possible new approach for the determination. Modifying the system and optimizing the conditions made up the first part of the research. Once reliable and stable instrument conditions were reached, the technique was then used to measure the aluminum content in botanical samples. Since the ICP is a multi-element technique, four other selected elements were also analyzed. Also, since the DSI-ICP can handle small samples, it becomes an excellent tool for sample homogeneity studies, and so the distribution of several elements in a series of proposed reference materials was examined. The final part of the research involved analyzing five trace metals in eight Canadian candidate agriculture reference materials.

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Chapter 2

Determination of Al, Fe, Zn, Cu, Mn in Certified Reference Materials by Direct Sample Insertion ICP-AES

2.1. Abstract

The simultaneous multielement analysis of microgram amounts of botanical samples by direct sample insertion of a graphite cup into an inductively coupled plasma was developed. Either microliter volumes of standard solutions or microgram portions of botanical sample powder were placed in a graphite cup, dried and ashed inside an injector tube below an ICP, and then introduced into a continuously operating argon and oxygen mixed gas ICP for sample vaporization. Standard solutions without matrix matching were used to prepare external calibration curves for the analysis of the botanical samples. Compromise operating conditions for the multielement determination of five elements were established. The method was applied to the analysis of the NIST reference materials Rice Flour (SRM1568a), Wheat Flour (SRM 1567a), Citrus Leaves (SRM1572), Corn Kernel (RM8413) and Corn Stalk (SRM 8412). The results were in good agreement with certified values.

2.2. Introduction

Since the introduction of a commercial ICP instrument for atomic emission spectrometry (AES) in 1974 (1), interest in their analytical applications has been growing steadily. According to one estimate (2), by 1987 there were about 30 vendors of ICP-based instrumentation world-wide, the number of installed units was approximately 6000 and was increasing at a rate of approximately 400/year. This represented a percentage growth rate higher than any other analytical instrument during that time (3). The areas in which the ICP has been applied are diverse, ranging from industrial, geological, environmental , and biological/clinical materials to agriculture, organic and petroleum products.

The success the ICP enjoys can be attributed to its desirable analytical characteristics. These include a wide linear dynamic range, relative freedom from interferences, acceptable accuracy, good precision, detection limits in the ppb range for ICP-AES and sub-ppb range for ICP-MS for some elements, and a capability for simultaneous determinations. However, from a more immediate and practical point of view, sample introduction into the ICP and, in particular, a capability for direct analysis with little or no sample pre-treatment of powdered/solid samples has not yet been either fully developed or exploited.

Because many materials of analytical interest naturally occur as powders or solids, they must be first quantitatively converted to a solution by appropriate digestion procedures before they can be introduced into a conventional ICP via pneumatic nebulization. As the number of sample pretreatment steps increases, sample preparation procedures become more complicated; also, as more reagents are added to the sample, the risk of contamination increases. As ICP detection limits approach sub-ppb levels, contamination becomes an increasingly important consideration because purified acids, matrix modifiers and solvents may contain elemental concentrations above this level. Moreover, dilution may reduce the detectability of trace elements. For these reasons, and to avoid transport losses during nebulization, which can amount to as much as 97% of the sample, it is desirable to introduce the solid sample directly into the plasma.

In 1979, Salin and Horlick (4) reported the use of a conventional undercut dc arc electrode as a means of introducing mg quantities of powder/solids and μL volumes of liquid samples into a medium power (<2.5 kw) ICP and coined the term direct sample insertion (DSI). Since then, a number of similar systems has been built in Japan (5-9), the U.K. (10), Germany (11), Israel (12), and Canada (13-16). Some of these systems are specifically designed for botanical powder sample analysis. In general, reasonably good agreement was found between experimental and certified values, but precisions (% RSD) reported ranged from 1 to 25%, and detection limits varied widely, often deviating by more than an order of magnitude. Differences in instrumentation and in sample and probe types appear to be the reason for the variation. However, in many cases, matrix matching was found to be important. The construction of calibration curves for powdered/solid samples from liquid standards was not possible. Because a broad range of elements form carbides, the determination of these elements becomes very difficult. Often elements such as Al, B, Ca, La, and Ti could not be determined in botanical samples. Refractory elements such as aluminum are difficult in many matrices.

A recent development in the determination of carbide forming and refractory elements is that of using an argon-oxygen mixture to increase the plasma temperature and energy density, and to burn off the solid sample along with the graphite cup (17). This chapter reports on the development of a method for determining aluminum, along with copper, zinc, iron and manganese, in botanical materials. Among the variables studied were ashing time and position, atomization time and position. Liquid standard calibration curves were successfully used to obtain results for five NIST standard reference materials that were in good agreement with certified values.

2.3. Experimental Design and Apparatus

2.3.1 Instrumentation.

The instrumental details and operating conditions are summarized in Table 2.1

Table 2.1 Hardware specification and typical operating conditions for Ar-O_2 mixed gas ICP DSI system

ICP	Plasma Therm ICP 5000
ICP Generator	HFS-5000D; 27.12 MHz; Max power: 5.5 kw
Spectrometer	A 1-m Pachen-Runge mount, 29-channel direct reader with a 1200 groove/mm concave grating
DSI system	The DSI device has been described in detail in refs. 13 and 15. No major modifications were made except that the device was controlled by a IBM PC computer instead of an Apple II
DSI Probe	Graphite electrodes of varying size and shape were used (Bay Carbon Inc., Bay City, MI).
DSI control board	PC Motion™ (Rogers Labs, 2727-E SO. Croddy Way, SANTA ANA, CA 92704) for IBM PC with BASIC callable machine language control subroutine.
Data acquisition	DT2801-A (Data Translation Inc., 100 Locke Drive, Marlborough, MA 01752-1192 USA) with a 12-bit ADC, two 12-bit DACs, two 8-bit I/O ports, a programmable interval timer/counter. Up to 8 differential or 16 single ended analog inputs can be multiplexed and read by the ADC. Programmable ADC frequency with max of 27.5 kHz. Programmable gain amplifier (PGA) supportive of 1, 2, 4, and 8 gains. ADC rate: 640 Hz/channel, 8 channel

Figure 2.1 shows a schematic diagram of the DSI-ICP-AES system. The torch, stepper motor, and mechanical probe driving system were built in the laboratory of Professor G. Horlick and have been described in references 13 and 15. The system was modified for this work so that both stepper motor and data acquisition can be controlled by an IBM PC. The operating program, which was written by X. Liu in quick-BASIC, is menu driven with a screen edit function that provides a convenient way to change operating parameters, such as sample weight, ADC gain, probe position for ashing and analyzing, and times for ashing and atomizing. Sample quantitation was done by using peak area integration. The integration area was choosed by selecting the lowest point in either side of the peak. For normal peak shape the lowest point was automaticlly choosed by the computer.

The graphite electrodes were purchased from Bay Carbon Inc., USA and modified by drilling a 3 mm diameter hole about 6 mm deep into the base so as to allow the cup to be mounted on a support rod. The graphite contained considerable metallic impurities, which were eliminated by inserting each cup into the argon-oxygen mixed gas plasma for 30 seconds prior to use as a sample carrying cup. The inner diameter and depth of the graphite cup was 3.17 and 4.78 mm respectively, giving a volume of 40 μL.

2.3.2 Chemicals

Solutions of 1000 ng/mL of each of the metals studied were prepared by dilution of stock commercial standards to the appropriate concentrations with distilled/ deionized water (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085, USA).
Argon and oxygen gases were from Linde, Union Carbide Canada, Limited, Toronto.

Cellulose filter paper (Pall Ultrafine Filtration Corporation, Glen Cove, New York 11542 USA) was soaked in concentrated HNO_3 for 2 days, washed with deionized water, then dried. This procedure reduced the trace aluminum levels to about one fifth of the original amount (2 ng per 2*20 mm strip). Of four papers tested, this paper was found to have the lowest background level of Aluminum. Nitric acid was double sub-boiling distilled in quartz from Seastar Chemical Inc, Sidney, B. C..

2.3.3 Sample Drying and Mixing

Rice Flour(SRM1568a), Wheat Flour (SRM 1567a), Citrus Leaves (SRM1572), Corn Kernel (RM8413) and Corn Stalk (SRM 8412) were purchased from the U.S. National Institute for Science and Technology, Gaithersburg, M.D. USA 20899. All samples were tumbled end-over-end for at least 2 hours. After tumbling, about 5-g portions were transferred to clean, dry glass weighing bottles and vacuum-dried at room temperature for 24 hours at a pressure not greater than 70 Pa. A liquid nitrogen cold trap was used to isolate the pump from the vacuum system..

2.3.4 Procedure

For the calibration curves a small strip of wet cellulose filter paper (about 2*20 mm) was packed into a graphite cup. Of four papers tested, this paper was found to have the lowest background level of aluminum. Levels of Al after HNO_3 treatment are about 2 ng per strip. After drying the paper under an infrared lamp, 10 µL of a known concentration of standard solution was pipetted onto the filter paper and the paper dried again. The paper was ashed by positioning the cup 18 mm below the load coil for 30 seconds, then inserting the cup into the plasma for 30 seconds for atomization.

The botanical powder samples were placed in preweighed graphite cups to half full. The cups were weighed again to give the weight of sample. Each cup was placed 18 mm below the load coil for 30 s to ash the sample, then positioned in the plasma for analysis. The observation time usually was 30 s. The emission signals acquired by the ADC were stored on floppy discs for data processing.

2.4 Results and Discussion

Unlike other methods of sample introduction, sample loss is minimal when a DSI device is utilized, therefore essentially 100% sample introduction efficiency is reached. In addition, because sample introduction can be done with little or no sample pretreatment by direct introduction of powders or solids, an added advantage is realized, that of reducing or eliminating sample preparation. The benefits of this are two-fold. First, sample introduction is more amenable to automation and second, the possibility of contamination is reduced or eliminated.

Automation of the graphite cup insertion operation enabled precise control of cup height and holding time for any desired set of operations. Inorganic powders could be easily introduced into the plasma for atomization. However, in the case of plant samples, the plasma was extinguished when they were directly introduced because of the gases produced by the organic matrix. When ashing of the sample was performed prior to atomization, continuous operation of the plasma was possible, but occasionally the sample would spatter out of the cup in the ashing stage, resulting in a large sample loss. Therefore optimizing the operating conditions was the first step in this study. In order to avoid sample spattering, only enough powdered sample was added to fill half of the cup. Also, the stepper motor was modified to reduce vibration. With these factors under control the important parameters, ashing position and ashing time, were studied.

Cup position is defined as the distance between the top of the cup to the top of the load coil. Rice flour was used as a test powder sample for the initial investigation of ashing position and time. When the cup was positioned too close to the plasma (too high), the sample started to burn, which could lead to major loss of sample or could extinguish the plasma. When the cup position was too far away from the plasma (too low), the temperature was not high enough to complete the ashing, which led to extinguishing of the plasma and/or sample loss upon subsequent insertion. This is because residual sample could produce large volumes of gases during atomization and thereby cause spattering. Therefore the optimum ashing position was selected as that which gave the highest signal upon atomization without the sample bursting into flame. Figure 2.2 shows that for rice flour the highest signal was obtained for an ashing step 18 mm below the load coil. For an ashing step 22 mm below the load coil, the plasma was extinguished in the atomization step.

Figure 2.3 shows the relationship between ashing time and signal intensity for Al, Zn, Cu, and Fe. In this study the ashing position was fixed at 18 mm below the top of the load coil. At ashing times of 5 and 10 seconds there was a very unstable situation and the plasma was often extinguished upon subsequent insertion. At 20 seconds, the situation became quite stable. Reasoning that ashing times for different samples may vary somewhat, 30 seconds was chosen as the ashing time. Another factor in choosing 30 seconds was that the main focus was on the determination of aluminum, which after a 30 second ashing time begins to show loss of signal. For zinc and copper a small signal loss was seen with an ashing time of 30 seconds, but since standards and botanical samples were put though the same ashing step, the loss was expected to be about the same.

Table 2.2 Working Conditions for Solid Botanical Samples

ICP Operating Conditions:

Forward power 1.8 kw; Reflected power <30 w
Outer gas 18 L/min with 20% O_2 in Ar; Intermediate gas 1 L/min with 20% O_2 in Ar; Central gas 2 L/min Ar with saturated water vapor
Observation Height: 14 mm above load coil

Typical DSI analysis conditions:

Ashing time: 30 s for most materials
Ashing Position: 18 mm below top load coil.
Observation Time: 30 s
Atomization Position: 2 mm above top load coil

Table 2.3 Channels and Wavelengths of ARL 34000 Spectrometer for Data Acquisition

Channel	Element	Wavelength	Order
1	Al	237.34 I	2
2	Fe	259.94 II	2
3	Cu	324.75 I	2
4	Mn	257.61 II	2
5	Zn	213.86 I	3

The working conditions are summarized in Table 2.2 and the channels and wavelengths used in Table 2.3.

The emission signal from a DSI-ICP system is transient, and different analytes have different vaporization / atomization time behavior, as is illustrated in Figures 2.4 to 2.8. The readout electronics and associated software of a conventional direct-reading ICP spectrometer are designed to provide a fixed integration time for all channels. Using a fixed integration time for the determination of multiple elements in a sample simultaneously with a DSI system could be a problem. A short integration time will result in the loss of the tail of the signal of nonvolatile elements, but a long integration time will increase the background for volatile elements.

In Figures 2.4 to 2.8, the Y-axis is in arbitrary intensity units and the X-axis is in time. From these plots, we see that the same element may have a different time profile in different materials and that different elements may show different time profiles in the same material. In general, aluminum has the largest peak width, and zinc has the sharpest peak. In some cases, such as citrus leaves, zinc was so volatile that some signal was lost before the computer started collecting data. The aluminum peak in citrus leaves was sharper than in the other materials. This probably was caused by the lower density of the leaves causing the material to atomize more rapidly. Iron showed a double peak, whereas the other four elements did not. It is possible that iron may exist in two chemically-bound forms in the leaves.

In general, the linear calibration range was from 1 µg/g to 50 µg/g for 10 µL standard solution, which can be seen in Figure 2.9(a). Undoubtedly, the applicability of calibration curves prepared from liquid standards for powder/solid samples is useful new information for this technique. Since this method saves time in preparing matrix matching solutions or using the standard addition method. Since oxygen was used in these experiments, the graphite electrode cup is burned off the insertion rod, therefore any elements in the pores of the graphite cup will be carried into the plasma. Log-log scale plots of these calibration curves are shown in Figure 2.9(b). The slopes of these plots are all greater than 0.91, which means that self-absorption is minimal. It was found that the standard addition method could not be performed by pipetting a standard solution into an electrode cup. The solution penetrated the electrode pores below the cup, and since only the cup part of the electrode burns off in the plasma, quantitative results are not possible. For agricultural materials, the results depend on whether the volume of standard solution can be absorbed by the material. Generally, therefore, the standard addition method was not used in this study.

Usually peak widths for atomization of botanical samples were quite similar to peak widths for solutions added to filter paper and dried. However, the aluminum peak width for standards was significantly broader and noisier than those in botanical samples when the concentration of the standard was over 20 µg/g. In Figure 2.10, it can be seen that the 50 µg/g and 100 µg/g aluminum standards had atomization peak widths of 15 seconds and 45 seconds respectively, whereas an aluminum concentration in citrus leaves of 90 µg/g gave a peak with a width of less than 5 seconds. As seen from Figures 2.11 to 2.14, the other four elements did not show increasing peak width with concentration. In addition to the difference in peak width, there was considerable spiking on the signals of the aluminum standards of high concentration. Fortunately, the peak areas were the same for the botanical samples and solution samples at the same concentrations. Therefore, the standard solution calibration curve could be used for analysis of the botanical samples.

For Zn, Cu and Mn the 100 µg/g points deviated from a straight line calibration curve. This probably is because for these volatile elements the peak front rises so rapidly for higher concentrations that the initial part of the peak profile is lost, and the integrated peak areas are smaller than they should be. Figures 2.11, 2.13 and 2.14 illustrate this point. No other reason for this lack of linearity is apparent.

Several potential interferences were examined. When 10 µL of a 1000 ppm solution of Na, K, Mg, or Ca was added to a filter paper, dried, then inserted into the plasma, no signal appeared at the Al, Mn, Fe, An and Cu channels, nor did 10 µL of 1000 ppm solutions of Al, Mn, Fe, Zn, or Cu give a signal at channels other than those for the expected emission lines. Therefore there were no interfering signals obtained between the trace element and major element channels.

Potential matrix interferences were also studied directly. Figures 2.15 to 2.19 show the effects of 2000 µg/g each of K, Na, Ca and Mg on 10 µg/g each of Al, Cu, Fe, Mn and Zn. There was no statistical difference in the trace metal results in the presence of K, Na and Ca, but Mg significantly reduced all five peak areas. In general, peak profiles became narrower when matrix elements were added and peak appearance times were delayed. This effect was probably caused by the fact that easily ionized elements take a significant part of the plasma energy at the beginning of insertion of the sample cup.

The NIST standard reference botanical samples Rice Flour (SRM1568a), Wheat Flour (SRM 1567a), Citrus Leaves (SRM1572), Corn Kernel (RM8413) and Corn Stalk (SRM 8412) were analyzed by the described method using the working conditions listed in Table 2.2 and the calibration curves of Figure 9. The results are listed in Table 2.4.

The overall average precision (%RSD) found in this study was about 15%. This is larger than the 2% routinely achieved with conventional pneumatic nebulizer systems. Although the causes of this imprecision are not well understood at this point, reproducibility of weighing, reproducibility of sample insertion, sample homogeneity (applicable to powdered/solid samples), the physical condition of the sample-carrying probe (i.e. cup porosity), the nature of the residue remaining in the cup after drying/ashing or at the initial stages of atomization, and matrix effects may all contribute to varying degrees. The accuracy of the determinations was satisfactory, however, with reasonably good agreement between experimental and certified values.

The design of the DSI system used here makes it capable of handling μL quantities of liquids or mg test portions of powdered/solid samples. Therefore, multi-element analysis can be performed even when sample quantities are limited. Special attention must be paid to sample homogeneity when analyzing powdered/solid samples because the rather limited sample size of DSI systems can lead to significant sampling errors when dealing with inhomogeneous powdered samples. This will be addressed in the next chapter.

It is interesting to note that the detection limit will be larger if the peak is broader. This is because a broader peak has a larger integration range. However, the background intensity is also larger, which leads to a higher standard deviation of the background. Therefore it is important to have a fast temperature rise and high sample cup temperature so that the peak of an analyte is as sharp as possible. The detection limits found for rice flour are listed in Table 2.5. These detection Limits (DL) are defined as equal to 3(SD)B/S, where (SD)B is the standard deviation of the base line to the side of the peak, and S is the peak height sensitivity.

Table 2.4 Analytical Results for Five Reference Materials by Direct Sample Insertion ICP

Element		Citrus Leaves SRM-1572	Rice Flour SRM-1568a	Wheat Flour SRM-1567a	Corn Kernel RM8413	Corn Stalk RM8412
Al	NIST	92 ± 15	4.4 ± 1.0	5.7 ± 1.3	(4 ± 2)	76 ± 4*
(µg/g)	Found	87 ± 13	4.9 ± 0.7	5.6 ± 2.0	5 ± 2	69 ± 3
Cu	NIST	16.5 ± 1.0	2.4 ± 0.3	2.1 ± 0.2	(3.0 ± 0.6)	(8 ± 1)
(µg/g)	Found	17 ± 2	2.7 ± 0.2	2.5 ± 0.2	2.9 ± 0.1	8.5 ± 0.5
Zn	NIST	29 ± 2	19.4 ± 0.5	11.6 ± 0.4	(15.7 ± 1.4)	(32 ± 3)
(µg/g)	Found	27 ± 8	22 ± 2	15 ± 1	18.9 ± 0.7	38 ± 6
Fe	NIST	90 ± 10	7.4 ± 0.9	14.1 ± 0.5	(23 ± 5)	(139 ± 15)
(µg/g)	Found	77 ± 8	8 ± 2	14 ± 2	21 ± 3	134 ± 15
Mn	NIST	23 ± 2	20.0 ± 1.6	9.4 ± 0.9	(4.0 ± 0.3)	(15 ± 2)
(µg/g)	Found	15 ± 2	17 ± 1	9.1 ± 0.7	3.7 ± 0.1	13 ± 1

Values in parentheses are not certified, but are given by NIST for information only.

* A value for aluminum in corn stalk is not available from NIST; this value is from reference #17

Uncertainties of NIST values represent 95% confidence interval

Uncertainties of Found values represent ± 1 standard deviation (n=4 for Citrus Leaves, 5 for Corn Stalk, 6 for Corn Kernel, and 33 for Rice Flour and Wheat Flour)

Table 2.5 Detection Limits for the Five Elements under Study Using 15 mg of NIST SRM-1568a Rice Flour as the Test Portion

Element	Detection Limit	
	in (ng)	in (µg/g)
Al	1	0.1
Fe	0.25	0.025
Cu	0.06	0.006
Mn	0.05	0.005
Zn	0.5	0.05

DL = 3(SD)B/S,where DL = Detection Limit; (SD)B = Standard deviation of off-peak base line and S = peak height sensitivity.

2.5 Conclusions

The computer-controlled graphite cup direct sample insertion device with an argon-oxygen mixed gas ICP has been successfully developed and applied to the direct determination of five elements in botanical samples by ICP-AES. Direct sample insertion ICP with its multi-element capability is a valuable tool for quick screening the trace metal content of botanical materials at test portion sizes of 1 to 20 mg. The concentrations of five elements, Al, Cu, Zn, Fe and Mn, were measured in the NIST reference materials. Rice Flour, Wheat Flour, Corn Kernel, Corn Stalk and Citrus Leaves. Results were within the tolerance ranges of the certified values in almost all cases; discrepancies could be caused by inhomogeneity of the materials at there small test-portion sizes.

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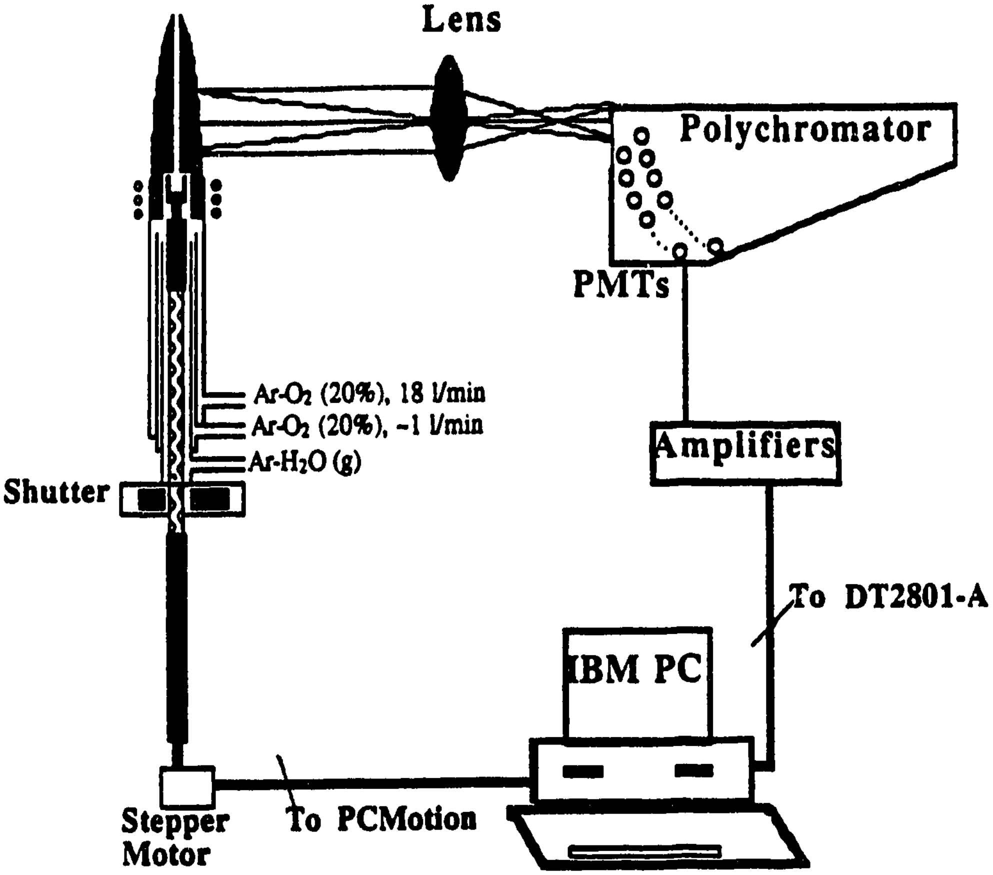

Figure 2.1 Schematic diagram of Ar-O_2 mixed gas Direct Sample Insertion System for Inductively Coupled Plasma Atomic Emission Spectrometry (DSI-ICP-AES)

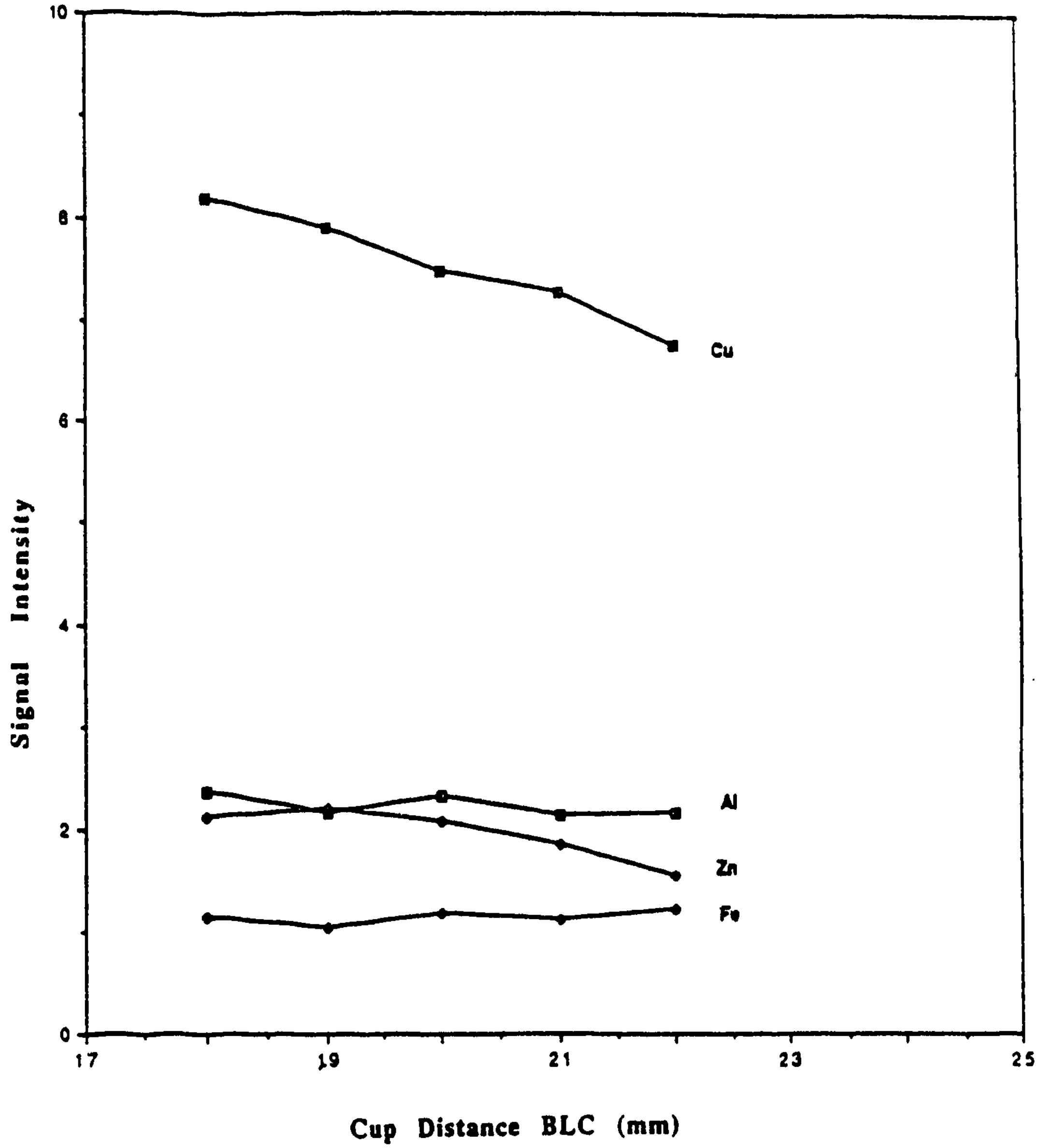

Figure 2.2 Effects of distance of sample cup below load coil (BLC) during ashing step upon signal intensity at atomization for Al, Zn, Cu and Fe. Sample is NIST Rice flour (SRM1568a)

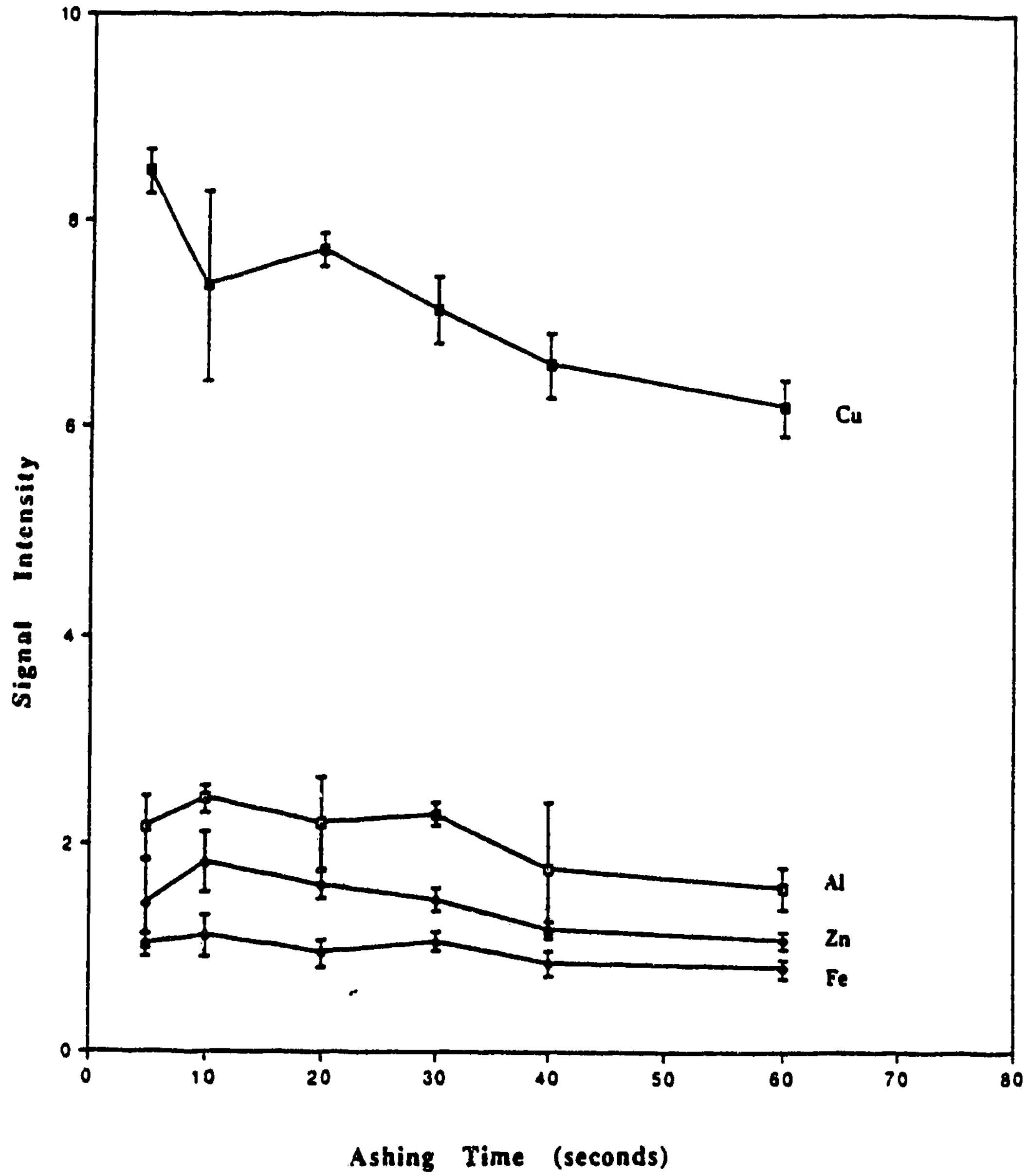

Figure 2.3 Effects of ashing time upon signal intensity for atomization of Al, Zn, Cu and Fe. Sample is NIST Rice flour (SRM1568a). Sample cup was fixed at 18 mm below load coil during ashing. The error bars refer to one standard deviation for three replicates.

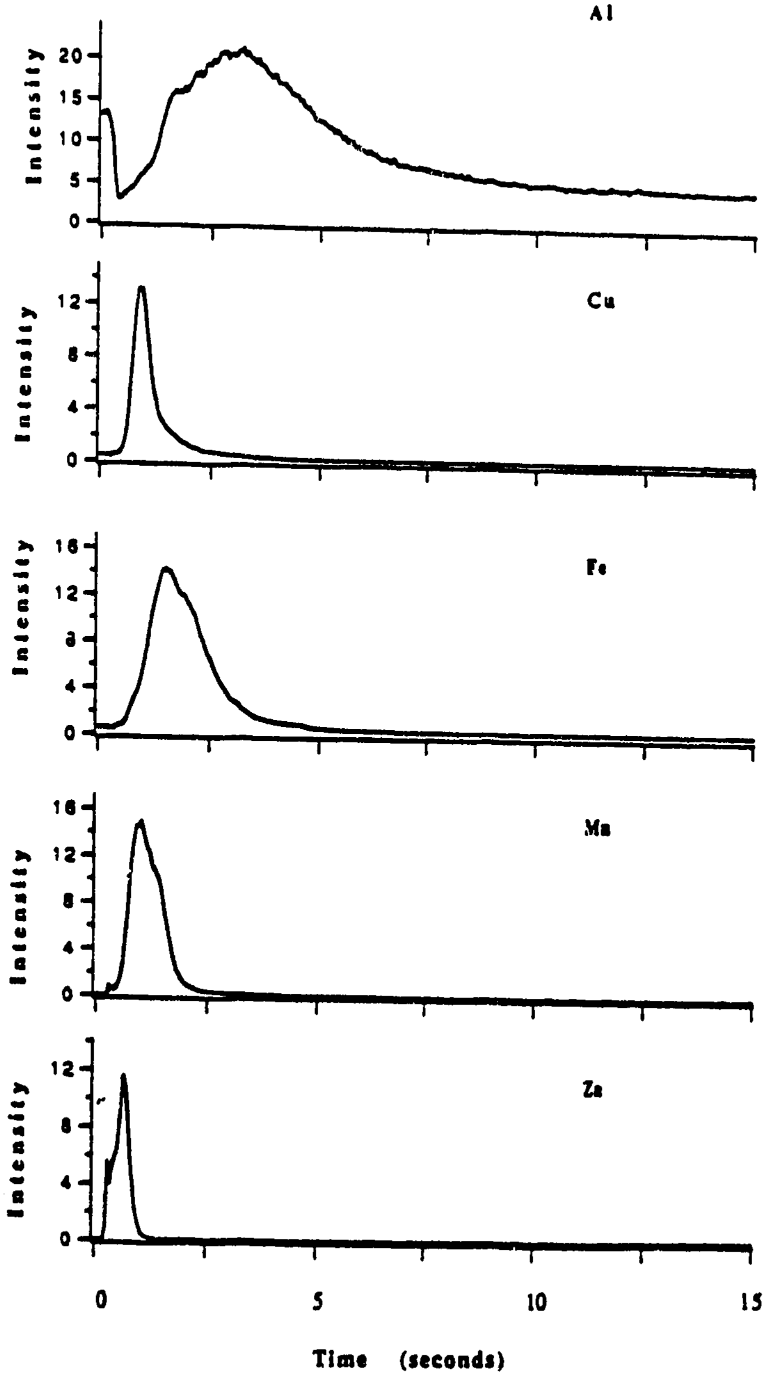

Figure 2.4 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: NIST Rice Flour (SRM 1568a)

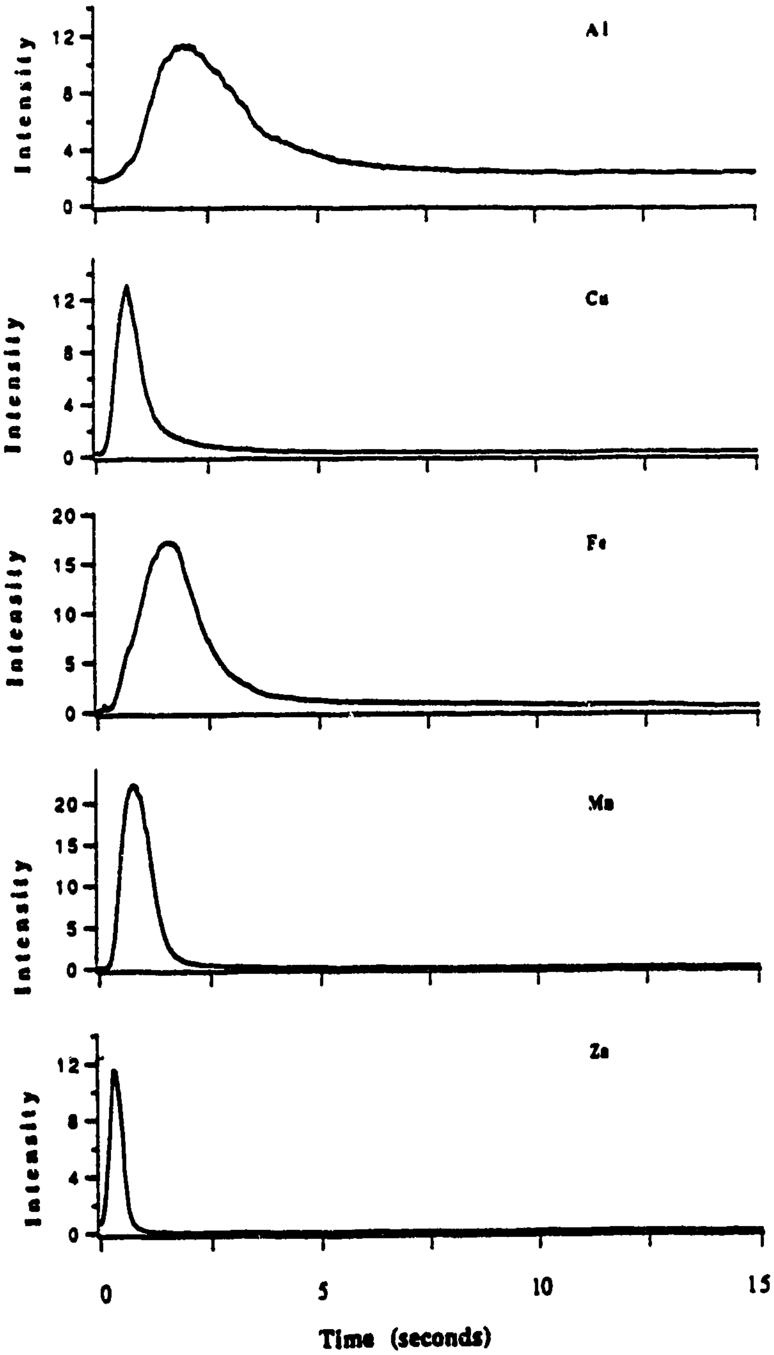

Figure 2.5 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: NIST Wheat Flour (SRM 1567a)

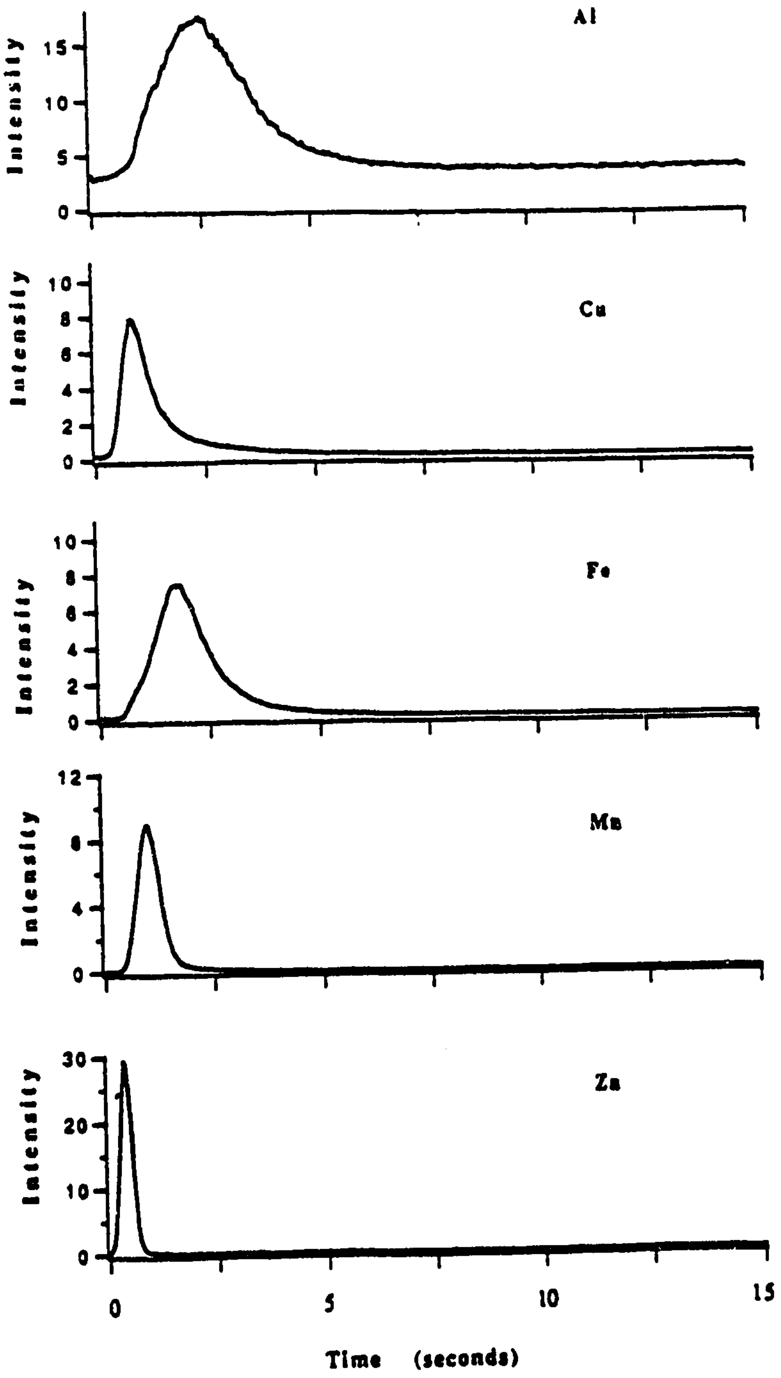

Figure 2.6 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: NIST Corn Kernel (RM 8413)

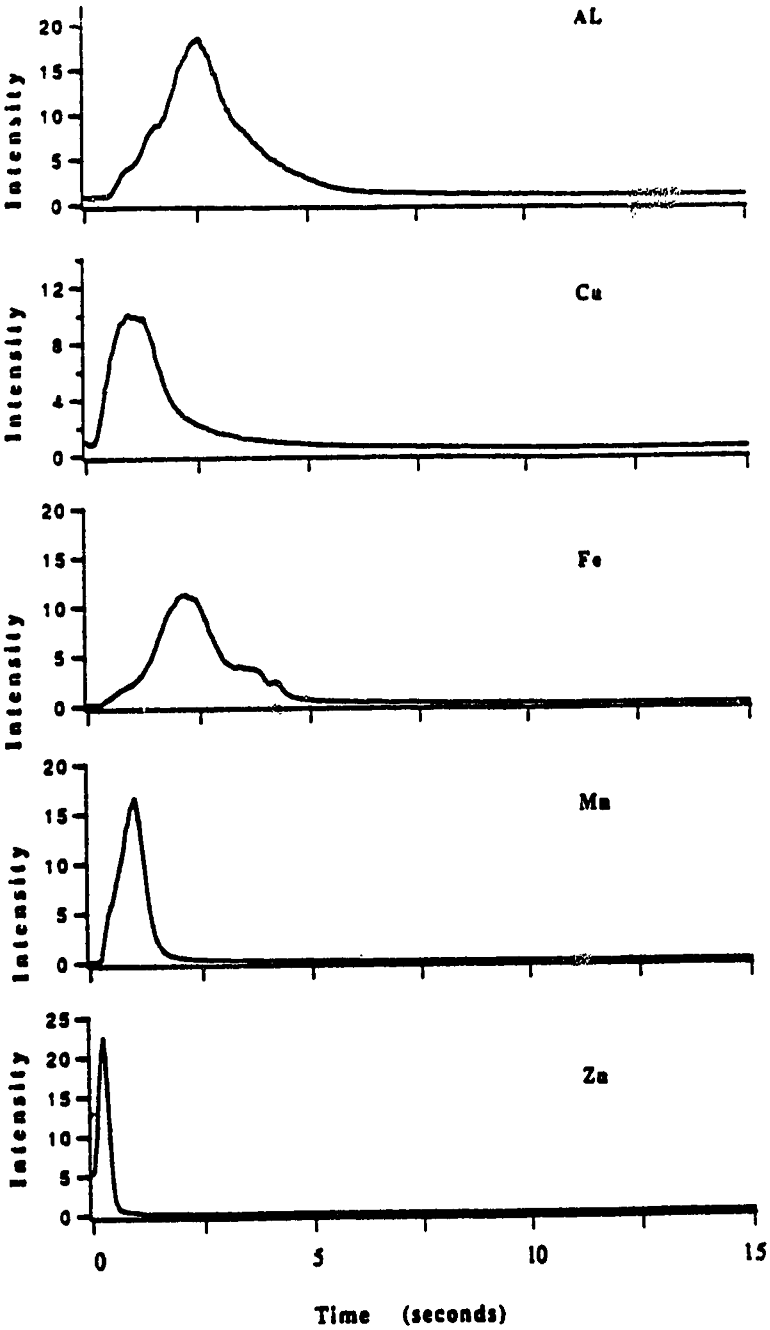

Figure 2.7 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: NIST Corn Stalk (RM 8412)

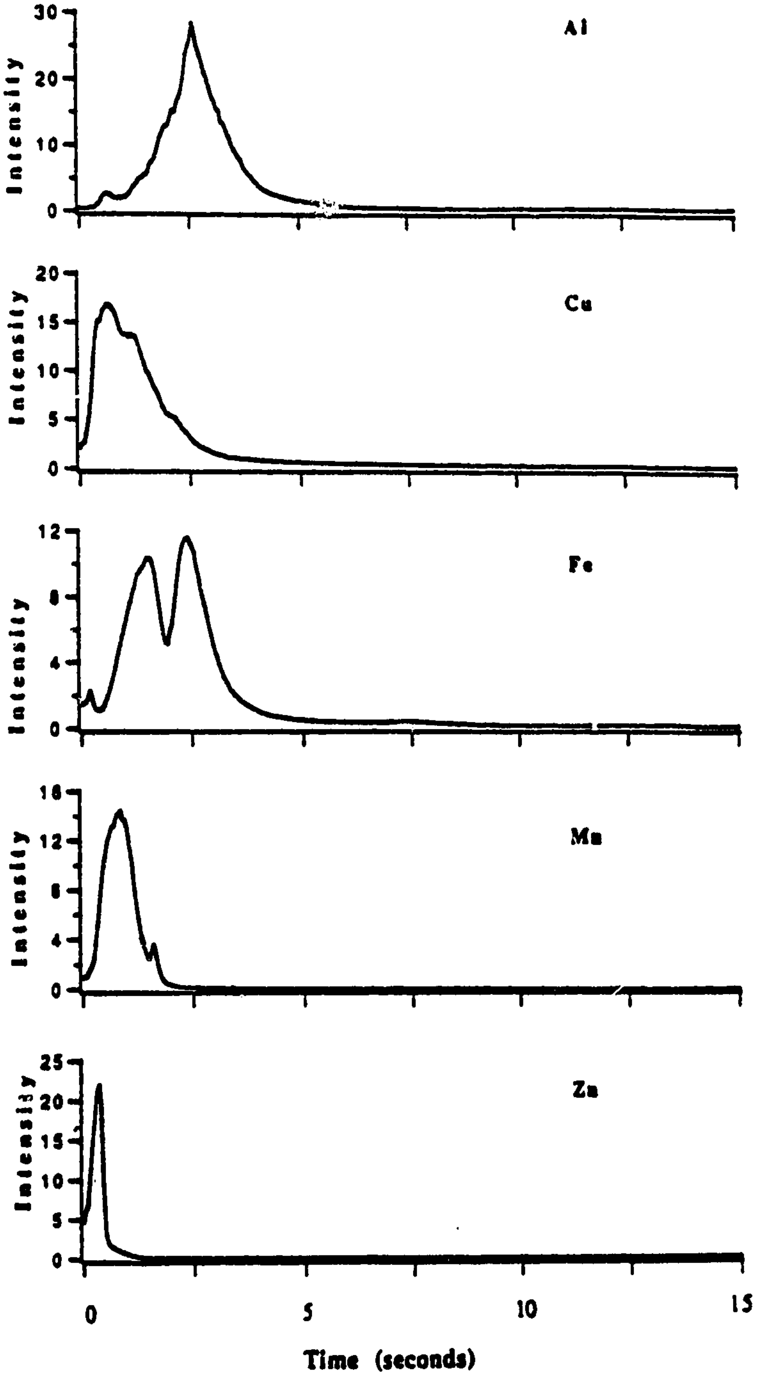

Figure 2.8 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: NIST Citrus Leaves (SRM 1572)

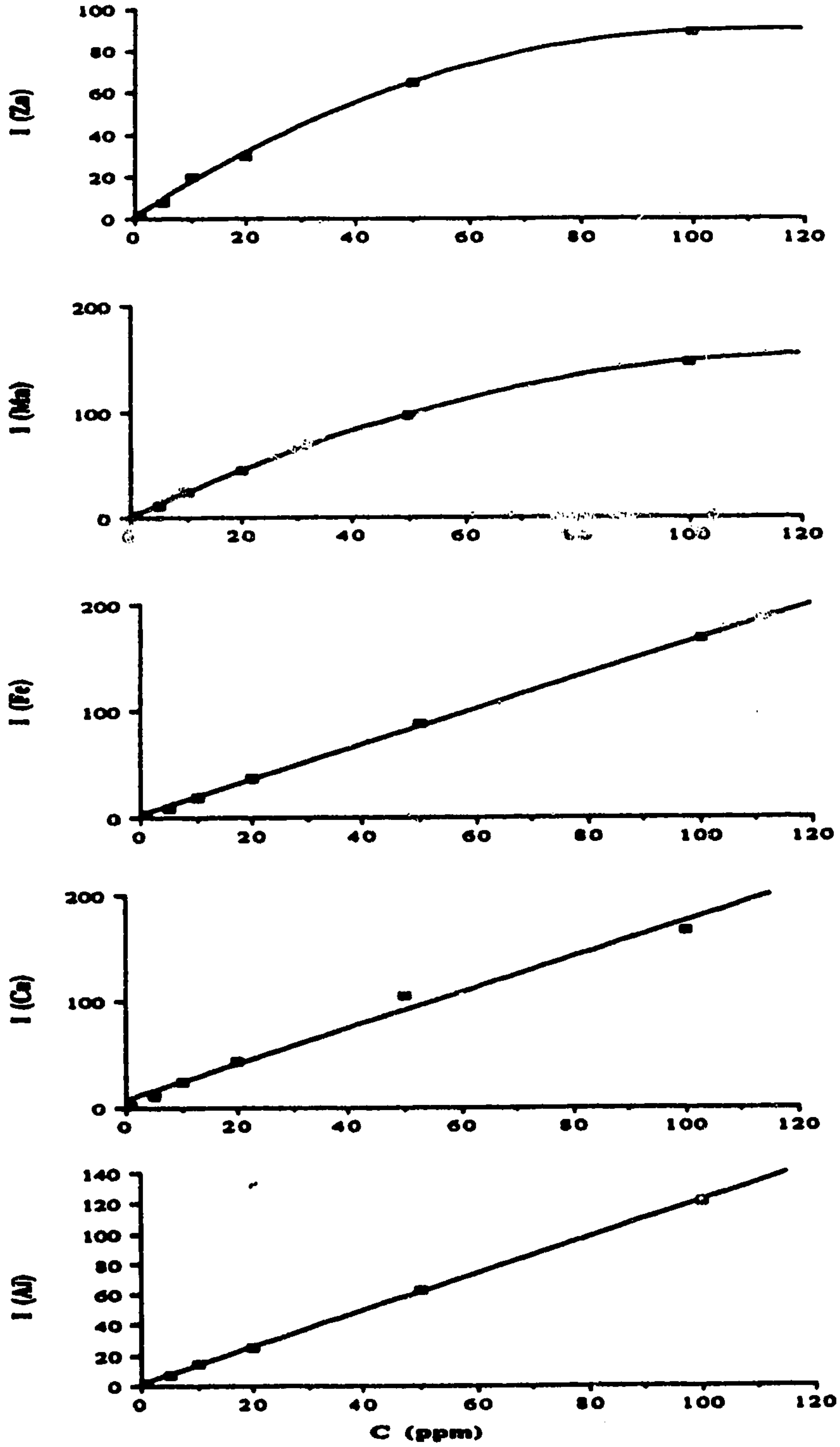

Figure 2.9(a) Calibration plots for Mn, Zn, Fe, Cu and Al using direct sample insertion system and aqueous solution standards. Instrument settings as in Tables 2.2 and 2.3

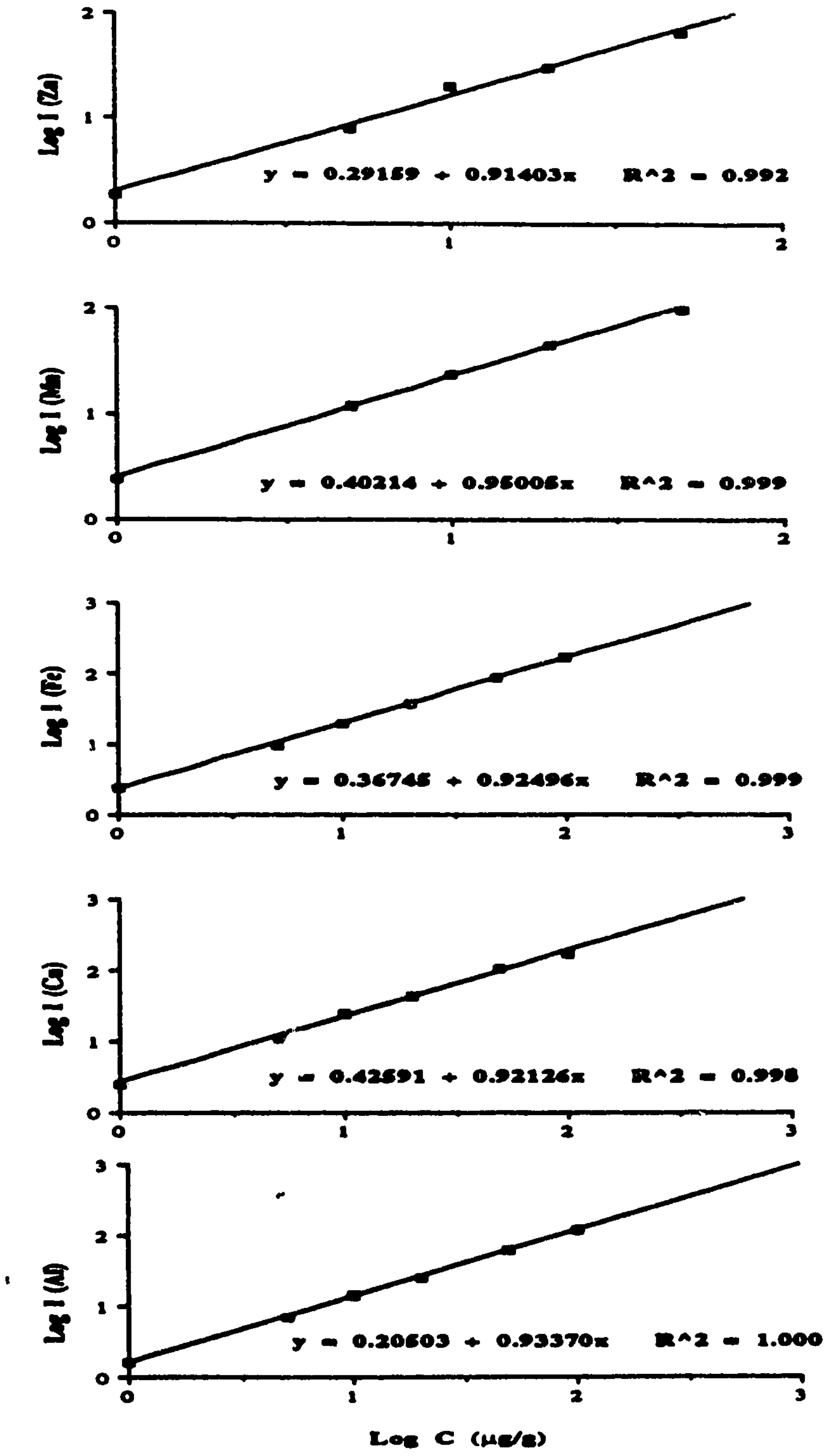

Figure 2.9(b) Calibration plots as in Figure 2.9(a), but on log-log scale

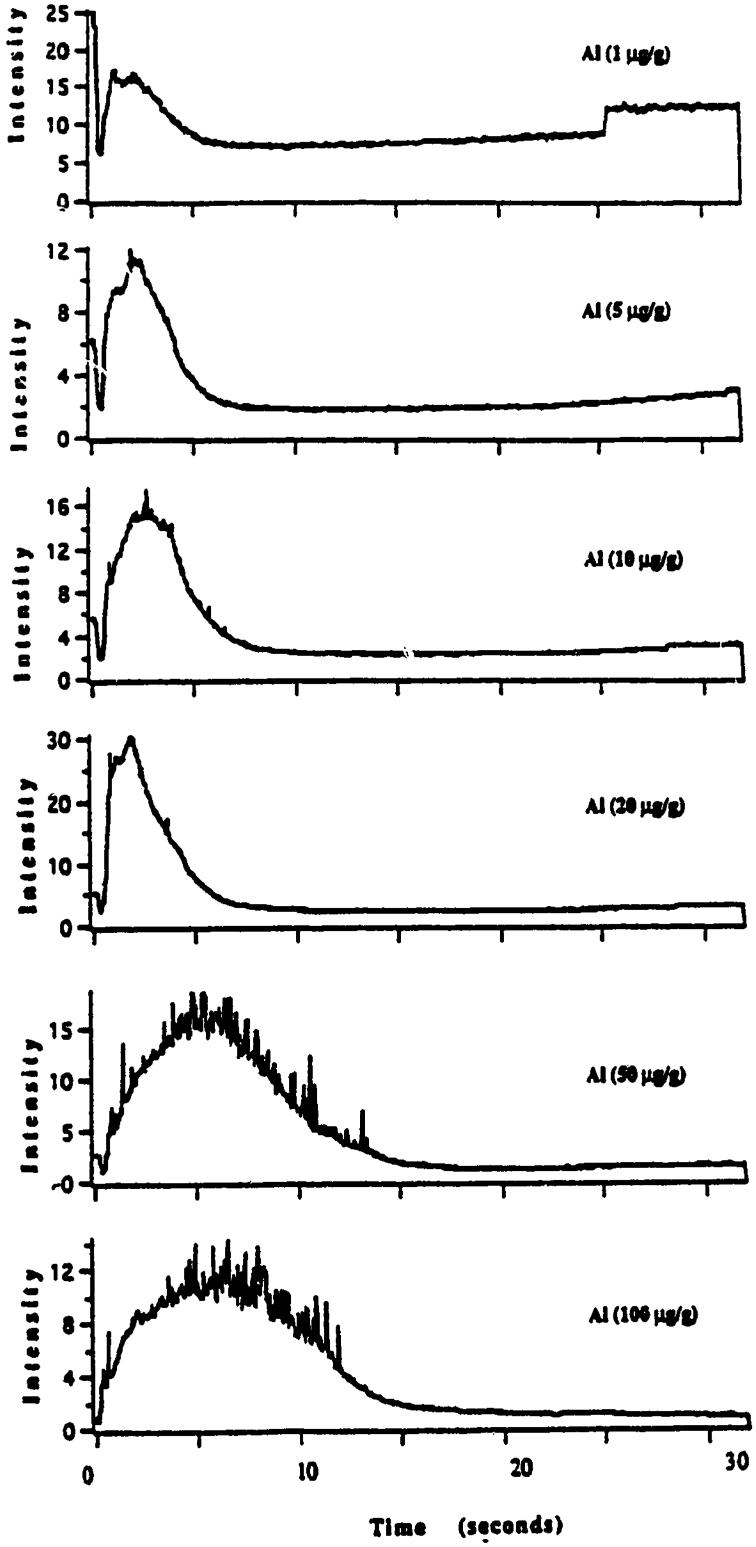

Figure 2.10 Effect of Al concentration on intensity - time profile

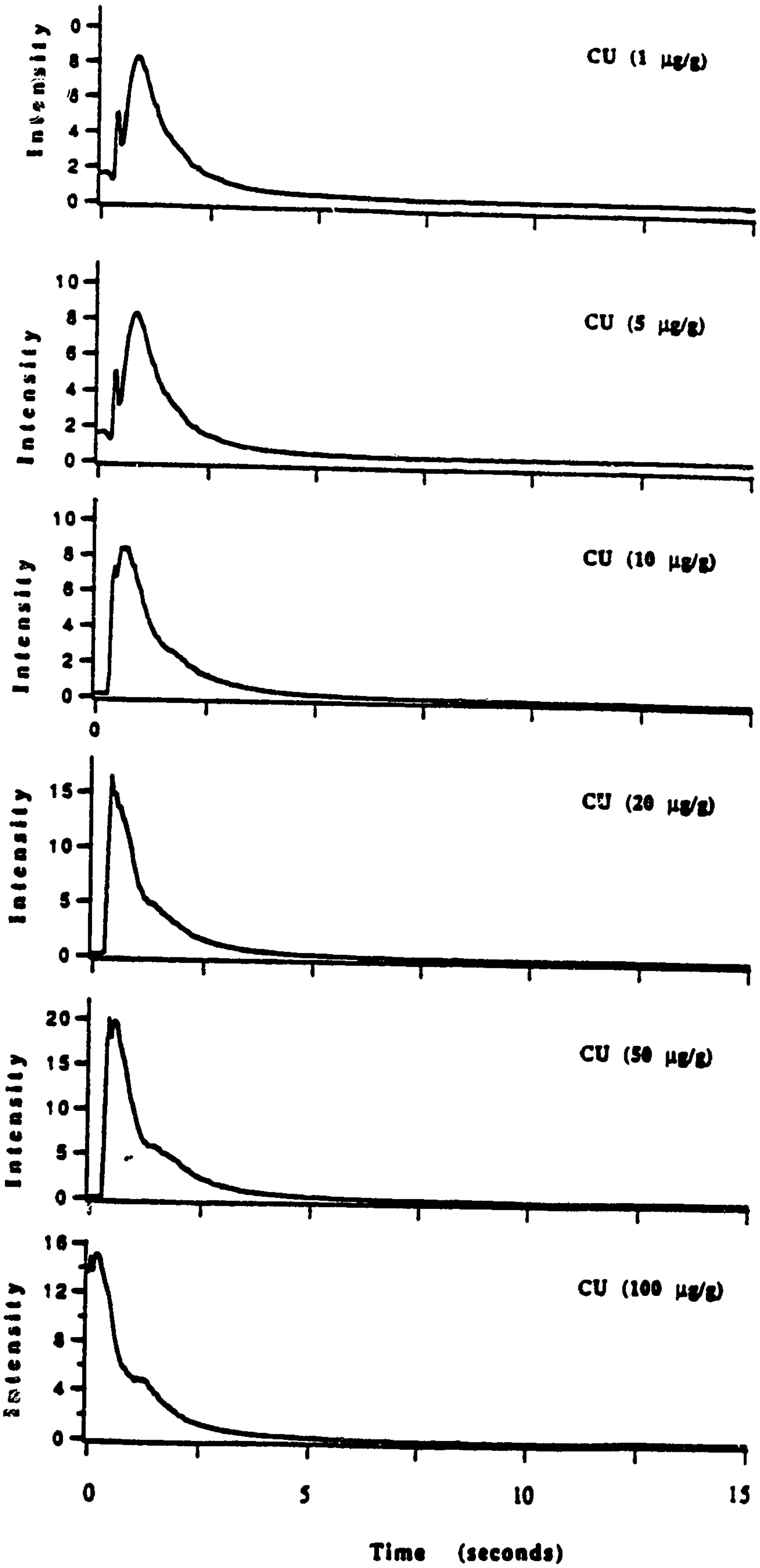

Figure 2.11 Effect of Cu concentration on intensity - time profile

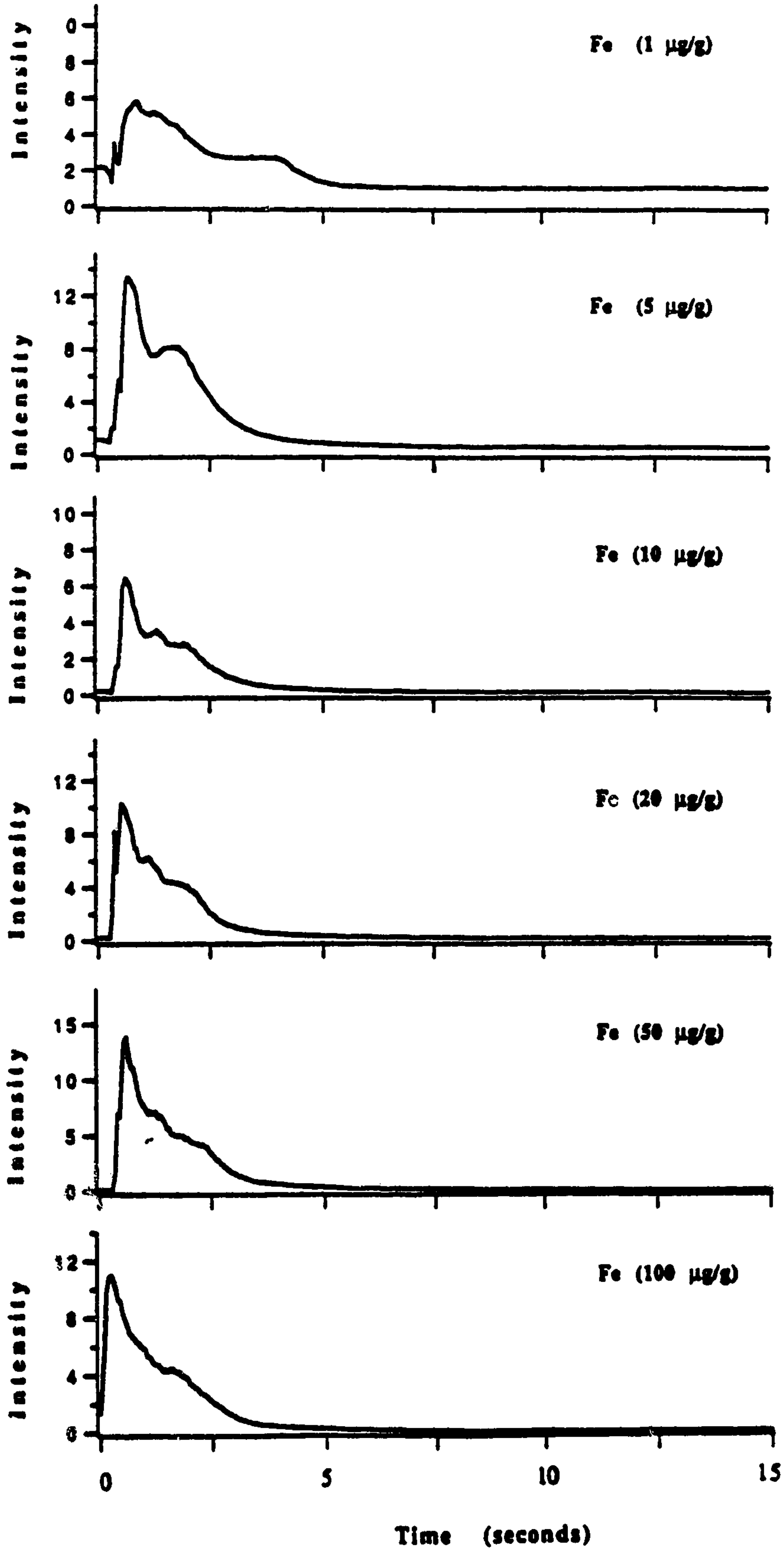

Figure 2.12 Effect of Fe concentration on intensity - time profile

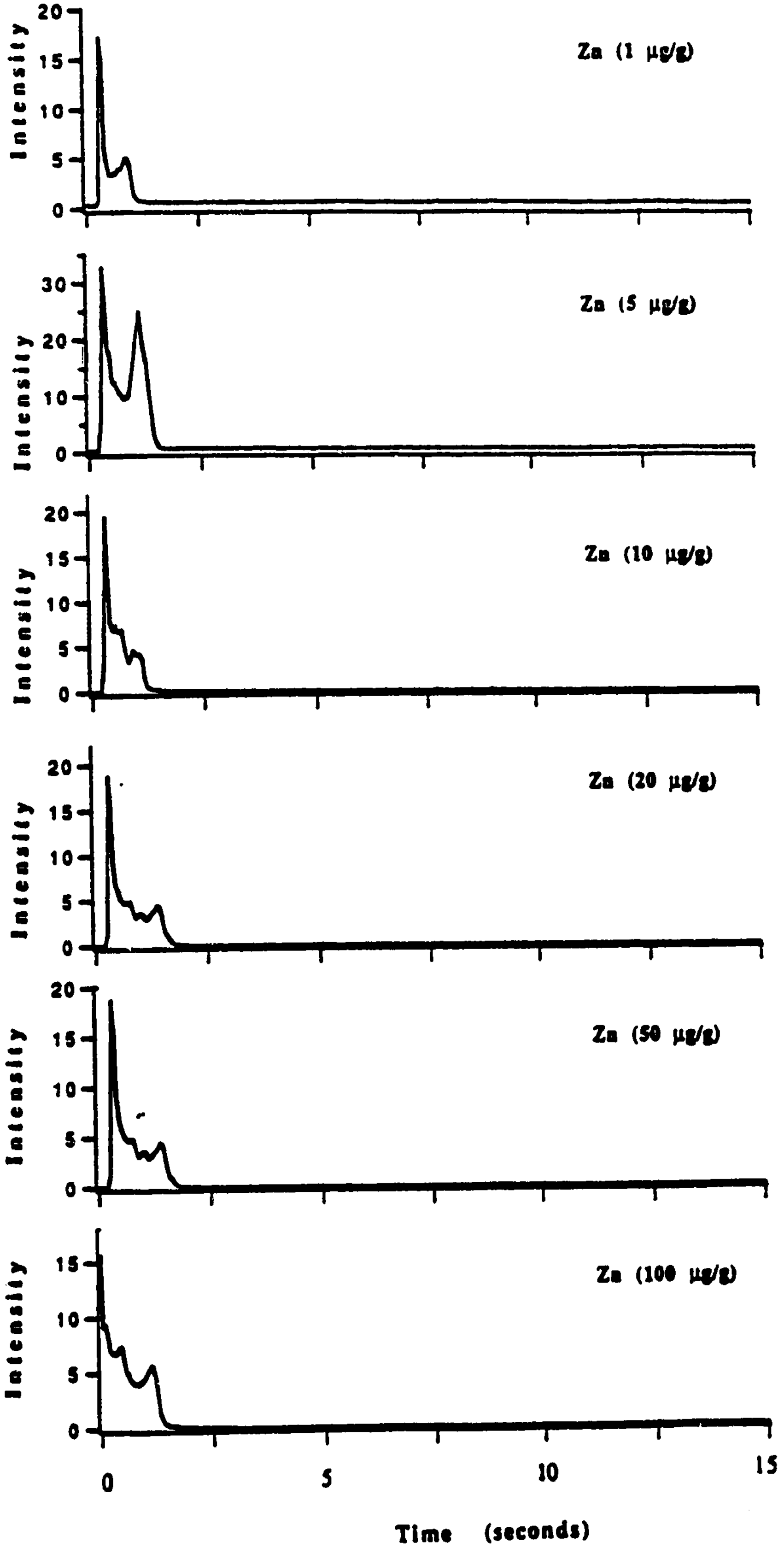

Figure 2.13 Effect of Zn concentration on intensity - time profile

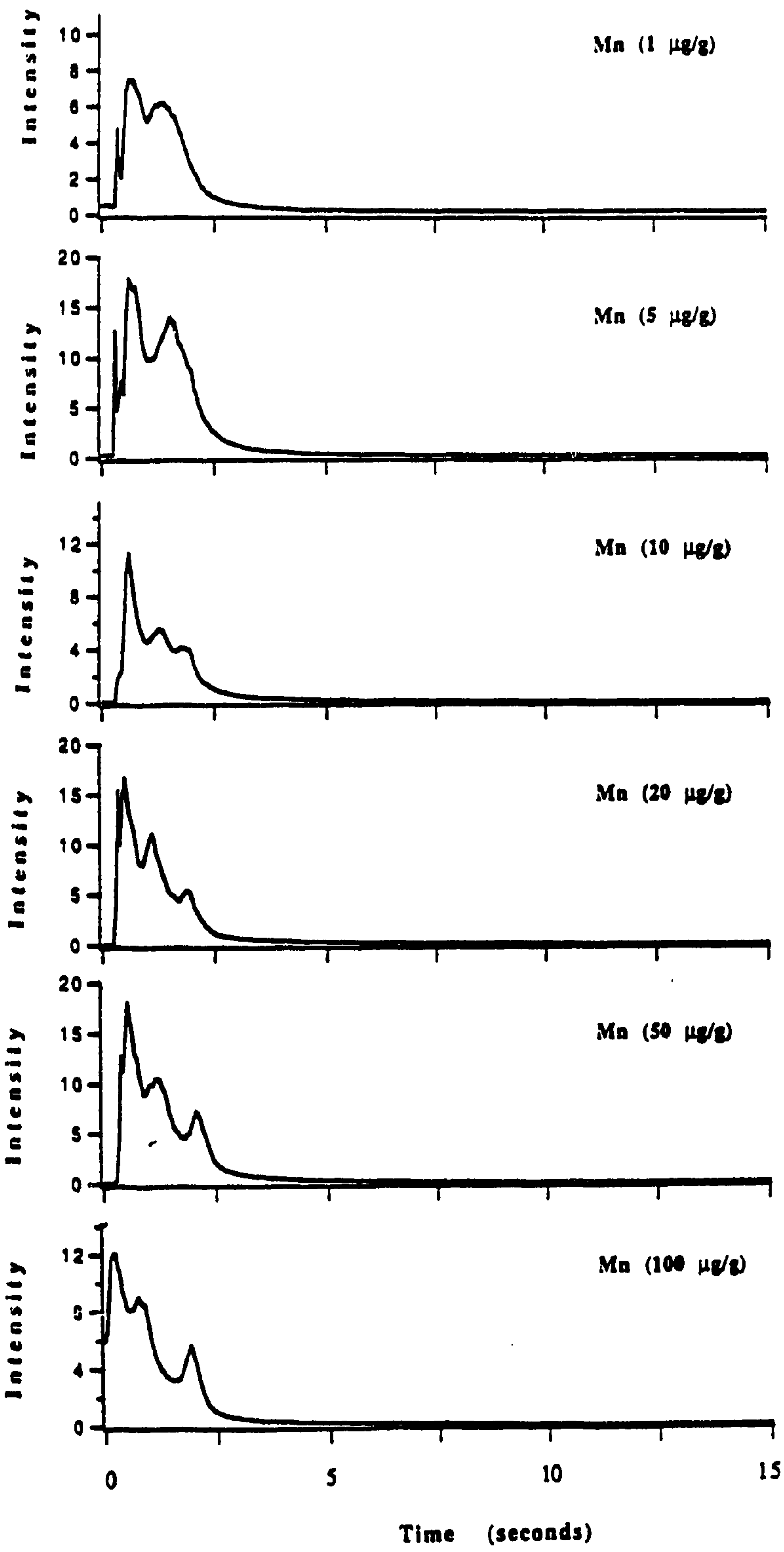

Figure 2.14 Effect of Mn concentration on intensit - time profile

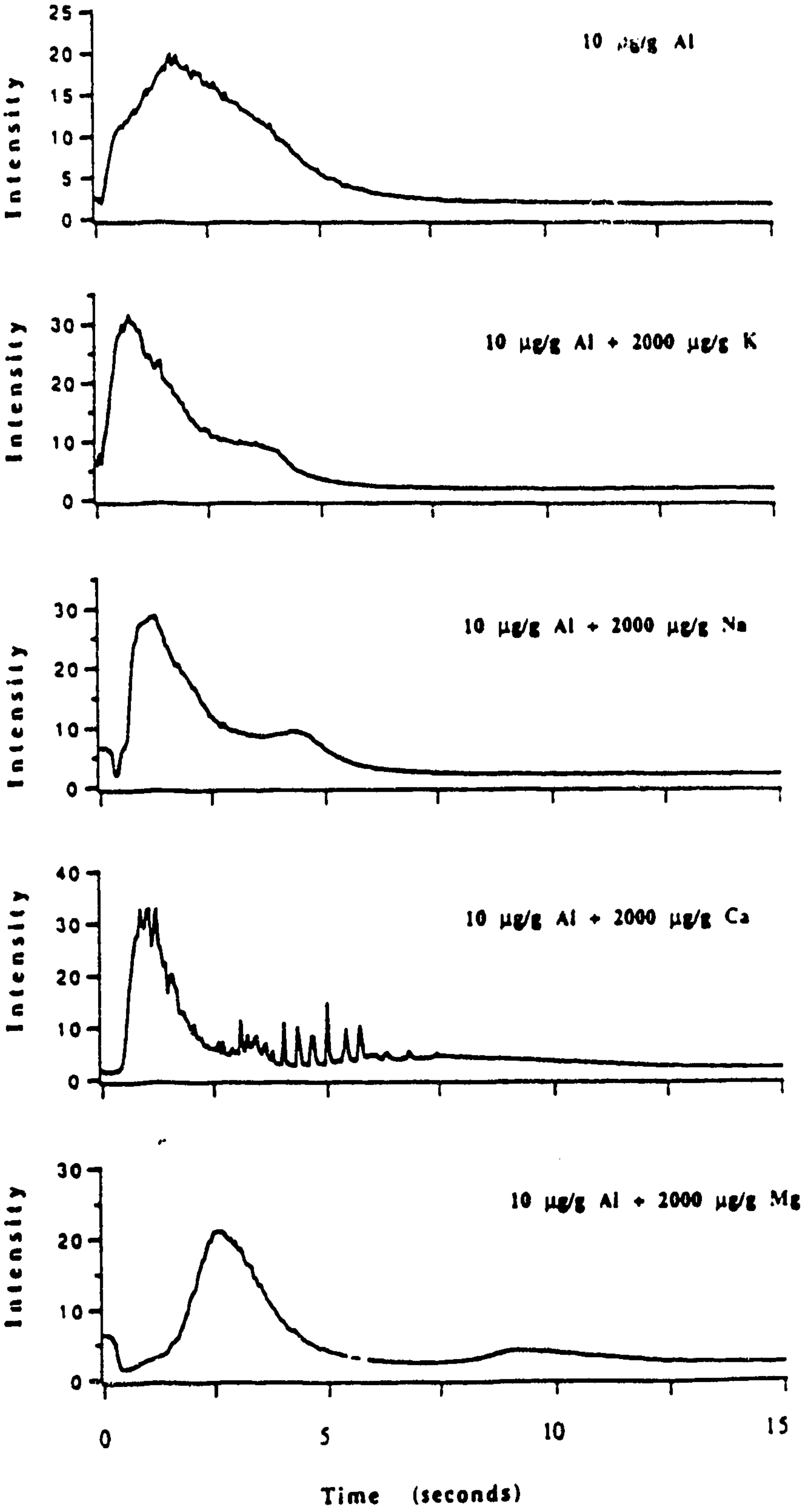

Figure 2.15 Effect of K, Na, Ca and Mg on the emission intensity-time profile of Al

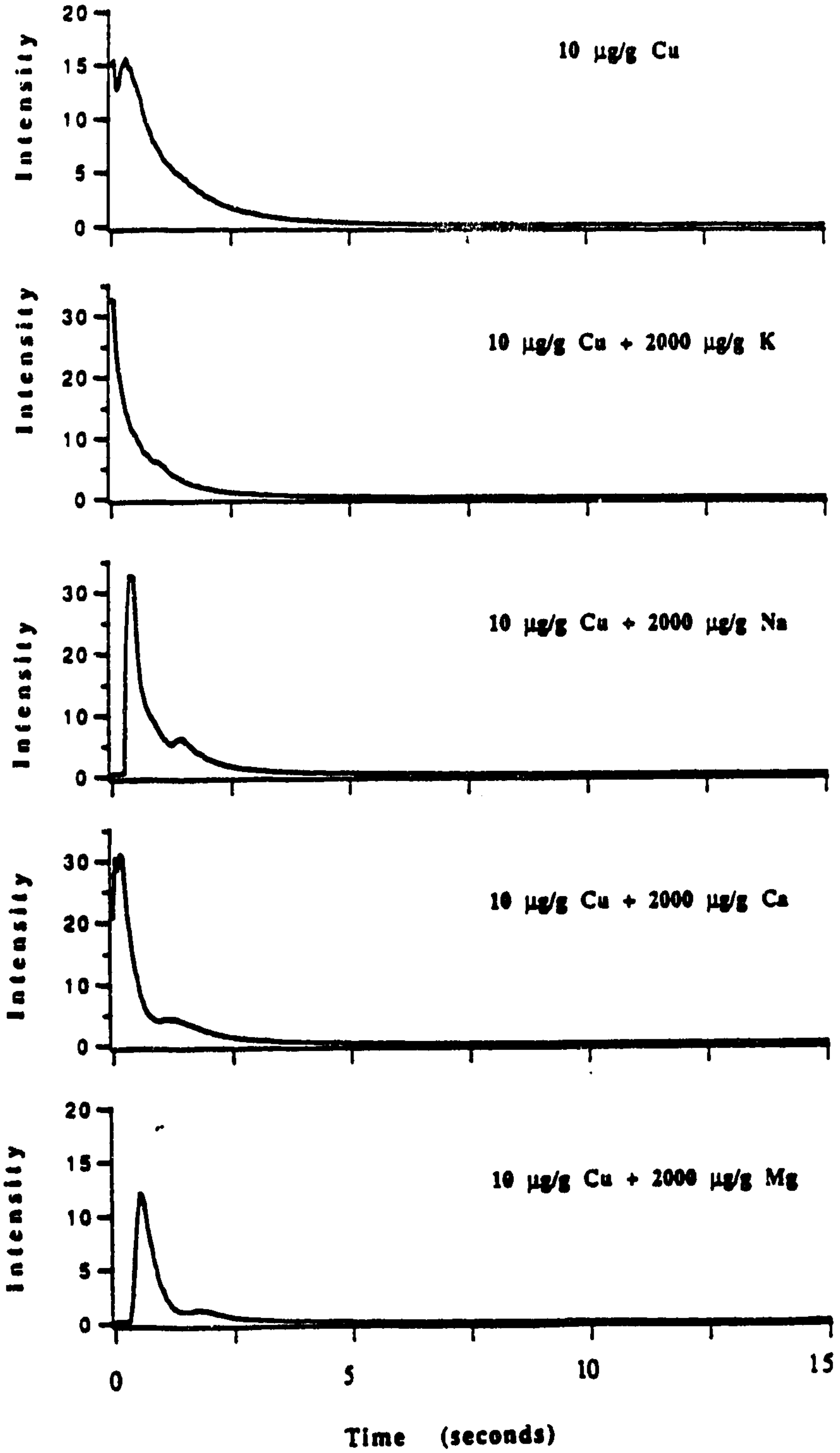

Figure 2.16 Effect of K, Na, Ca and Mg on the emission intensity-time profile of Cu

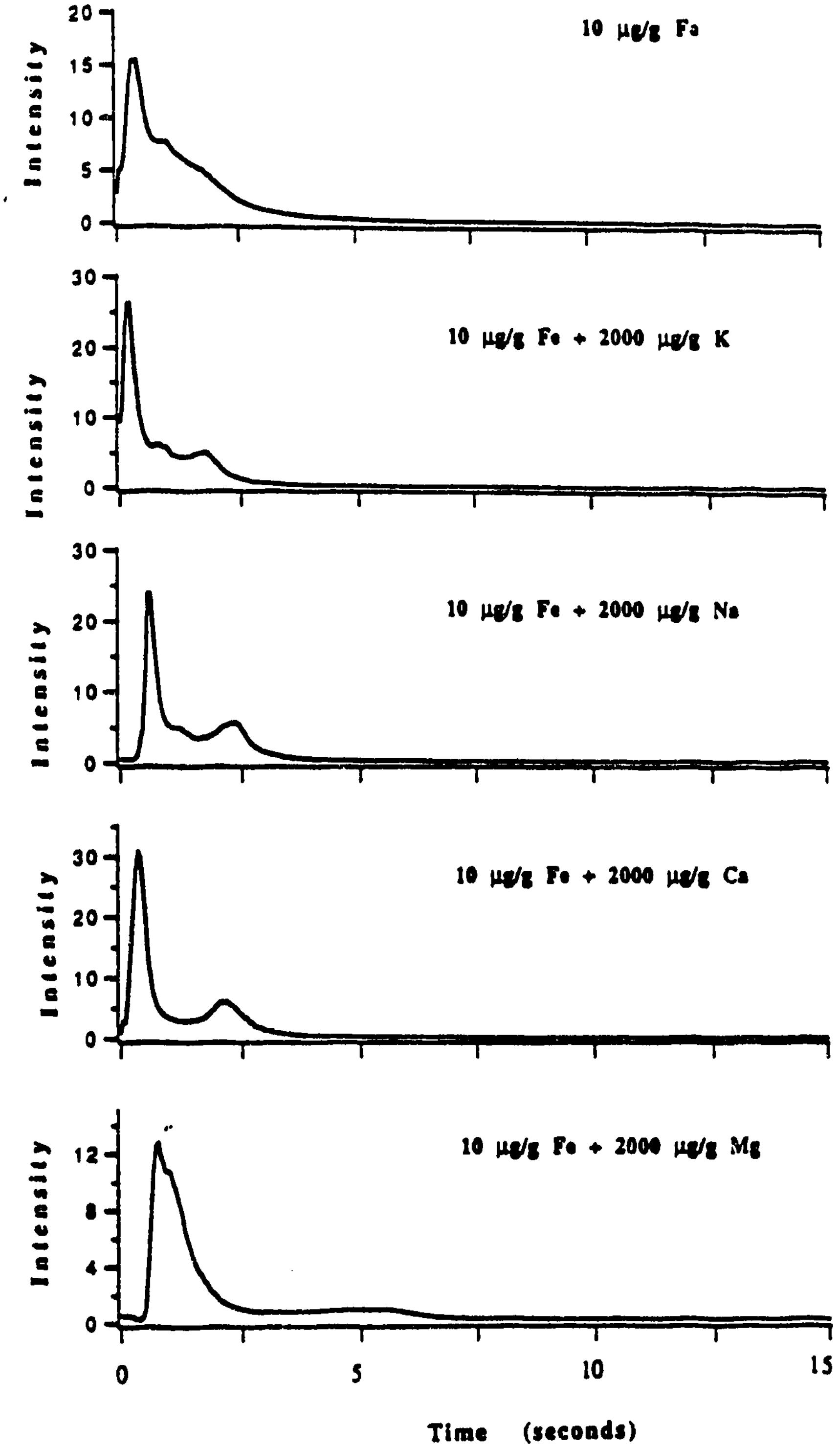

Figure 2.17 Effect of K, Na, Ca and Mg on the emission intensity-time profile of Fe

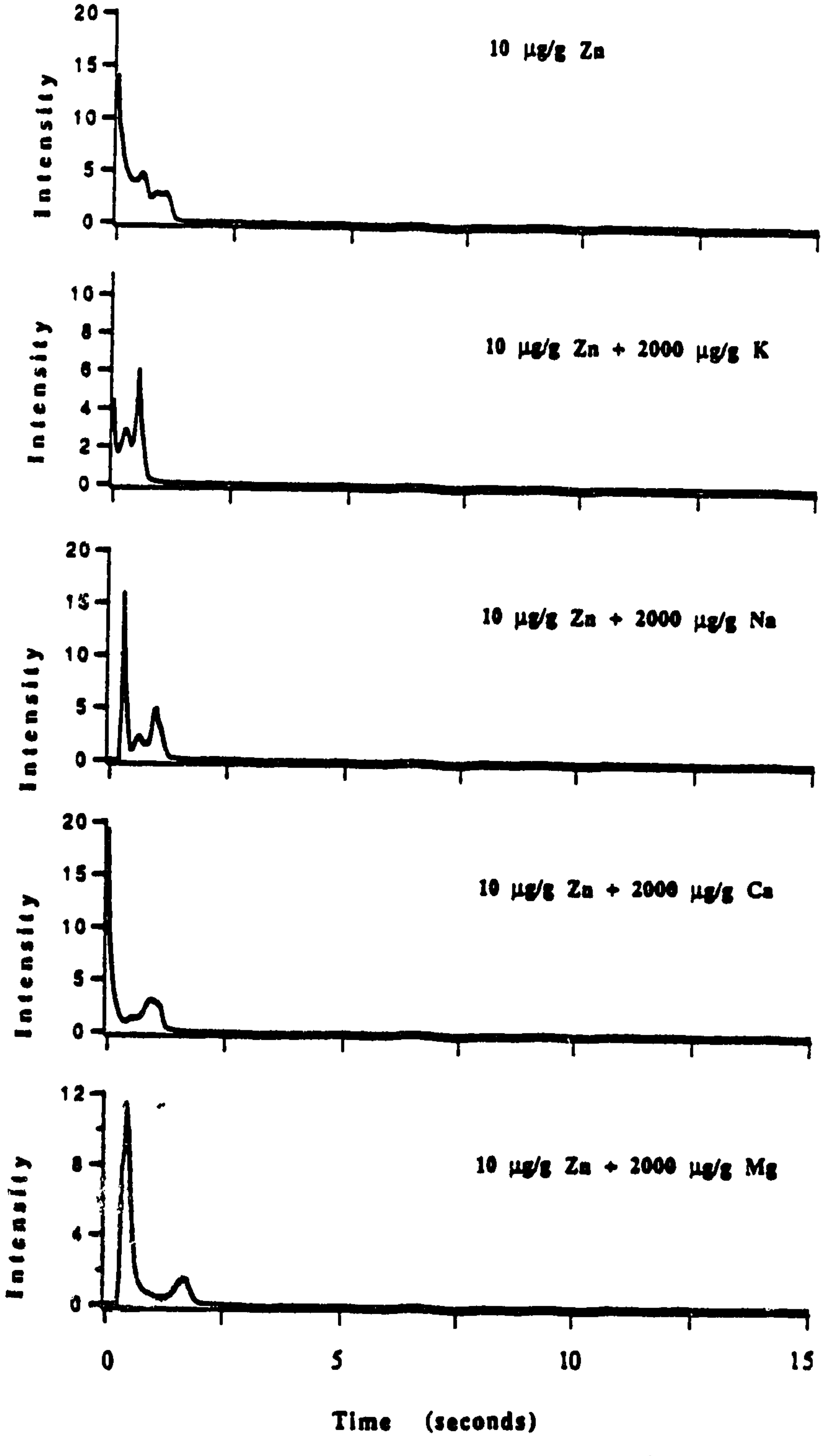

Figure 2.18 Effect of K, Na, Ca and Mg on the emission intensity-time profile of Zn

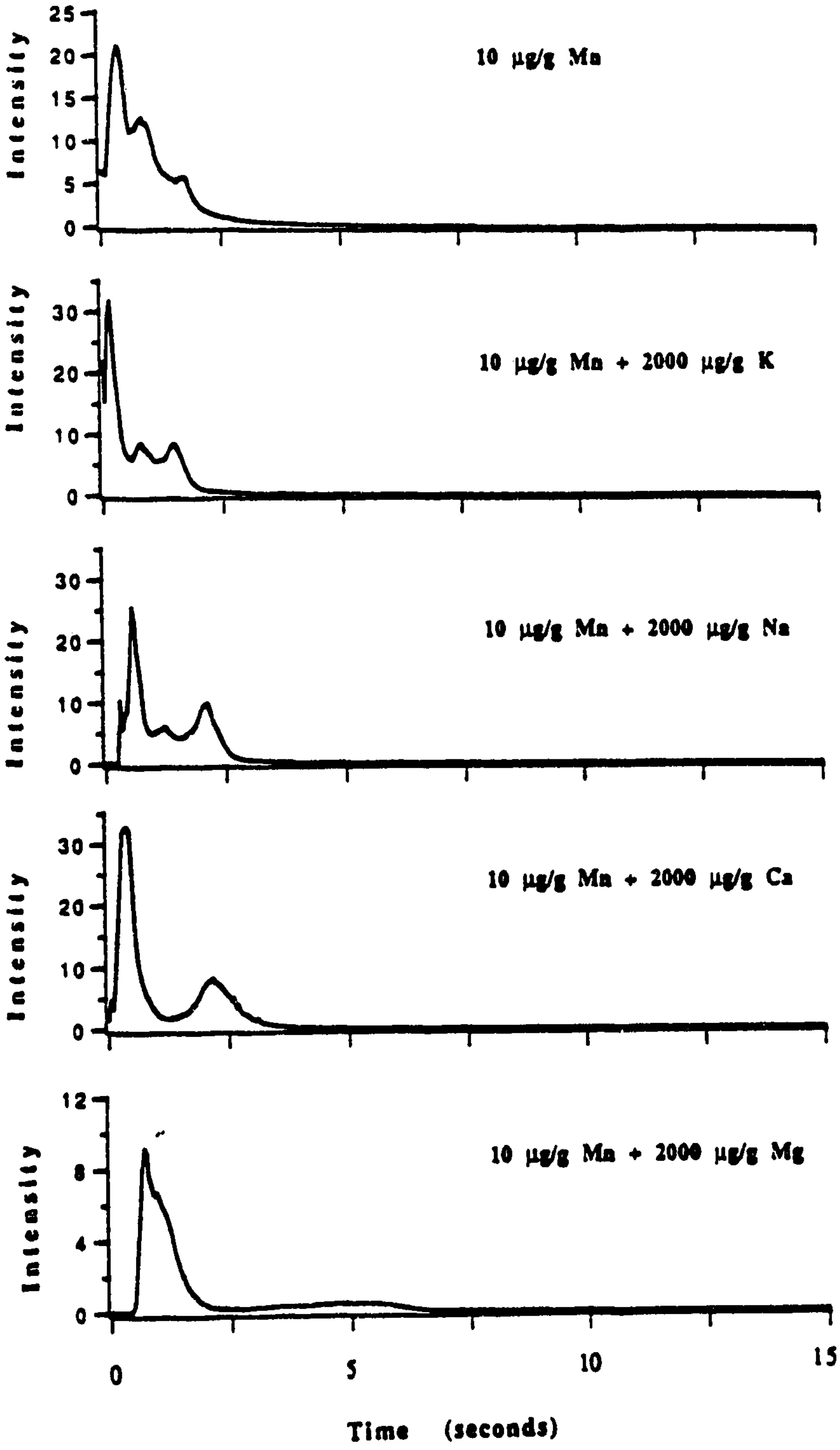

Figure 2.19 Effect of K, Na, Ca and Mg on the emission intensity-time profile of Mn

CHAPTER 3

ASSESSMENT OF HOMOGENEITY IN CERTIFIED REFERENCE MATERIALS

3.1 Abstract

Knowledge of the homogeneity of a material is a prerequisite for reliable determinations of concentration levels, otherwise results on test portions may not be representative for the material as a whole. Moreover, the homogeneity of reference materials used for assessment of analytical methods, apparatus, and personnel is of particular importance in trace and ultratrace analysis. This study shows that direct sample insertion ICP with its sensitivity, small test portion size, and multi-element capability is an invaluable tool for the study of sample homogeneity in subsamples of approximately 1 to 20 mg dry weight. In this investigation the variability in distribution of Al, Fe, Mn, Zn and Cu in Rice Flour (SRM 1568a) and Wheat Flour (SRM 1567a) from NIST was examined. The homogeneity of aluminum in the Rice Flour SRM is high at test portion sizes of 10 to 15 mg, but for the Wheat Flour SRM the variability among test portions is significant. The other elements showed a uniform distribution in both materials.

3.2 Introduction

Valid analytical information provides the backbone for conclusions reached and decisions made in an ever widening range of regulatory, legal and research activities. Accurate information is particularly needed in long term environmental studies of food, biological materials, and soils, where intercomparability of data over time and among laboratories is critical. Also, information on the degree of homogeneity of the population under investigation is a prerequisite for reliable interpretations. Moreover, the homogeneity of reference materials used for assessment of analytical methods, apparatus, and personnel is of particular importance in trace and ultratrace analysis. Inhomogeneity reflects both natural variation and adventitious contamination during preparation and handling. The latter if significant will elevate element concentration levels substantially above natural levels, impairing the validity of the measurements..

Kratochvil, Wallace and Taylor reviewed sampling for chemical analysis (1). They pointed out that heterogeneity of trace components can produce major sampling problems. Sampling errors cannot be controlled by use of blanks, standards, or reference samples and so are best treated independently. As analytical methodology improves and instrumental methods allow, or often require, the use of smaller and smaller analytical test portions, the error in the sampling operations becomes increasingly significant. Numerous researchers have written about sampling of plants for metal content. The topics discussed include both how to select appropriate leaves from a plant and variations within a single leaf. The following paragraphs summarize research in microsampling of reference materials for elemental analysis since that review (1).

Solid sampling GFAAS has been used for quick estimation of the distribution of elements in biological and environmental materials (2-6). Mohl and coworkers used this method to investigate the homogeneity of lead and cadmium in reference materials (7). These materials included NIST SRM1567 Wheat Flour, SRM1568 Rice Flour and SRM1577a Bovine Liver, and BCR #63 Milk Powder, #184 Bovine Muscle, #185 Bovine Liver, #186 Pig Kidney, #189 Wholemeal Flour and #191 Brown Bread. The sample size ranges were from 0.1 to 5 mg, with a weighing error of less than 0.05 mg. The homogeneities observed in this study for cadmium and lead were significantly different in the various materials.

The same group used solid sampling GFAAS to evaluate and estimate homogeneity in several plant reference and candidate reference materials from different sources (8). The distributions of cadmium, copper, lead, and nickel were determined in two spruce shoots from the environmental specimen bank (ESB) of Germany, and in NIST SRM1575 Pine Needles, SRM1570 Spinach, SRM1573a Tomato Leaves and NIES #7 Tea Leaves. A high level of homogeneity for these elements was found in all the materials.

Ihnat (9-11) prepared and characterized the homogeneity of ten new agricultural / food candidate reference materials. The materials include bovine muscle powder, whole egg powder, corn bran, microcrystalline cellulose, wheat gluten, whole milk powder, corn starch and three wheat flours representing a wide range of matrix types and trace element levels. Characterization was for some twenty five major, minor, trace and ultra trace elements of nutritional, toxicological and environmental significance. Solid sampling electrothermal atomic absorption spectrometry was applied to the determination of copper and lead in sub-milligram test portions. Good homogeneity was observed for virtually all materials and analytes with the exception of Cr and Pb in limited instances. The materials were thus deemed suitable for detailed characterization of elemental concentrations to yield recommended values and to bring these biological products to reference material status.

The NIST natural-product leaf standard reference materials have been widely used in developing reliable methods of analysis. A small amount of mineral matter present in these materials was separated by flotation and characterized qualitatively by microscopy and quantitatively by neutron activation analysis (12). Several elements are concentrated in the mineral fraction, a heterogeneity that can lead to analytical error though incomplete dissolution or sampling statistics. Since these materials are prepared from field-grown leaves, a small amount of siliceous or calcareous mineral matter (grit) is generally present, even though a washing step may have been used in the preparation. The presence of this grit leads to two consequences that must be taken into account in the use of these reference materials. First, some of the trace constituents are present in a phase that may not be solubilized by the method used to decompose the bulk cellulose matrix. If this possibility is not recognized, incomplete dissolution may lead to error in any analytical method which requires the sample to be in solution. The certificates accompanying botanical SRMs typically caution users about this problem; the first certificate for Orchard Leaves reported separate values for soluble and total iron and lead. Ihnat has found that several elements in leaf SRMs are only partially soluble in $HNO_3/HClO_4$ (13).

A second analytical consequence of the presence of a minor phase occurs when a trace constituent is present in high-concentration in a few grains. In this case, it is necessary to collect enough of the grains containing the trace component to allow a reasonable chance of estimating the overall concentration. The statistics of sampling may, if the degree of concentration into only a few grain is high enough, lead to heterogeneity which cannot be resolved by any analytical method. This issue has been studied extensively in the geochemical literature, and specifically as applied to the preparation of reference materials. Harris and Kratochvil (14) point out that " when the constituent being analyzed for is present as only a small fraction of the total, the number of units or particles required in the sample becomes enormous if sample error is to be kept small." Heydorn (15) et al. have reported a heterogeneous distribution of vanadium in SRM 1571 Orchard Leaves which appears to be due to the element being concentrated in a minor phase. They show by both experiment and calculation that the sample size required for a 1% sampling uncertainty for vanadium is 19 g.

The biological reference material TORT-1 lobster hepatopancreas has been analyzed for aluminum by instrumental neutron activation analysis (INAA) and graphite furnace absorption spectroscopy (GFAAS) (16). The methods gave similar results of 43 ± 3 μg/g and 42 ± 2 μg/g respectively for 200 to 300 mg test portions. Analysis of six portions from each of six bottles of TORT-1 showed no statistical difference at the 95% confidence level for between and within bottle variances. Therefore, the material can be considered homogeneous for aluminum if 200- to 300-mg test portions are taken. However, the variance was greater and the average lower when 30-mg test portions were analyzed for aluminum by GFAAS. The pattern of the results, together with the need for HF in the dissolution procedure, suggests the presence of aluminum-containing microparticle mineral matter in the material.

Thus the analysis of any botanical samples by solution-based analytical techniques must ensure that the mineral is dissolved as well as the bulk matrix. Commonly, a small amount of HF is added to complete dissolution. Whenever a new technique is applied to real samples, even if it has been shown to be reliable for the analysis of these SRMs, caution must be observed that any sampling inhomogeneity be considered and appropriately dealt with.

Homogeneity within certain limits is an important property of reference materials for quality control purposes in various analytical tasks. Therefore methods for reliably checking homogeneity are of great importance. Early studies have shown that direct sample insertion ICP is an invaluable tool for quick estimates of the content and distribution of elements in numerous materials and biological structures through its capability of analysing test portions as small as 1 to 20 mg dry weight. Due to the small test-portion size and multi-element capability, the method is particularly suited for homogeneity studies. Thus, this analytical approach has been used to investigate the distribution (homogeneity) of Al, Zn, Cu, Mn and Fe in two certified reference materials, Rice Flour (SRM 1568a) and Wheat Flour (SRM 1567a), from NIST.

3.3 Experimental

3.3.1 Instrumentation.

A schematic diagram of the DSI-ICP-AES system is shown in Figure 2.1. The torch, stepper motor, and the mechanical probe driving system have been described in references 13 and 15 in Chapter 2. The system was modified so that both the stepper motor and the data acquisition could be controlled by an IBM PC computer though a PCMotion TM stepper motor control board and a DT 2801-A data acquisition board housed in the IBM PC backplane. The stepper motor control board can control up to four motors simultaneously. Detailed hardware specifications are listed in Chapter 2, Table 2.1. The operating conditions are summarized in Chapter 2, Table 2.2.

The data acquisition program was written by X. Liu in quick-BASIC. The program is menu driven with screen edit functions providing convenient changes of operating parameters, such as sample weight, ADC gain, probe positions, and times for ashing and data acquisition.

The graphite electrodes were purchased from Bay Carbon Inc., USA and modified by drilling a 3 mm diameter hole about 6 mm deep into the base so as to allow the cup to be mounted on a support rod. The graphite contained considerable metallic impurities; to eliminate them, each cup was inserted for 30 s into an argon-oxygen gas plasma prior to use. The inner diameter and depth of the graphite cups were 3.17 and 4.78 mm respectively, giving a volume of 40 μL.

3.3.2 Chemicals

Standard stock solutions of 1000 ng/mL were prepared from commercial ICP standard 1000 ppm solutions (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085, USA) by dilution with distilled/ deionized water. The argon and oxygen gases were from Linde, Union Carbide Canada, Limited, Toronto. To minimize impurity levels, cellulose filter paper (Pall Ultrafine Filtration Corporation, Glen Cove, New York 11542 USA) was soaked in concentrated HNO_3 for 2 days, washed with deionized water, then dried. Nitric acid was double sub-boiling distilled in quartz (Seastar Chemical Inc., Sidney BC).

3.3.3 Sample Drying and Mixing

Bottles of Rice Flour (SRM1568a) and Wheat Flour (SRM 1567a) were purchased from the National Institute for Science and Technology, Gaithersburg, M.D. USA 20899. All bottles were tumbled end-over-end for at least 2 hours. After tumbling, about 5-g portions were transferred to clean, dry glass weighing bottles and vacuum-dried at room temperature for 24 hours at a pressure not greater than 70 Pa with a liquid nitrogen cold trap between the pump and samples.

3.3.4 Procedure

For solution standards, a small piece of wet cellulose filter paper was fitted into a graphite cup. After drying of the paper under an IR lamp, a 10 µL micropipet was used to add known concentrations of the standard solutions onto the filter paper, which was then dried again. The cup was inserted into the ICP to 18 mm below the load coil for 30 sec.

for ashing, then raised into the plasma for atomization. For instrument reproducibility studies 10 μL of a 10-ppm standard solution of each of Al, Cu, Zn, Fe and Mn were pipeted onto separate filter papers in individual graphite cups. After drying under an IR lamp, each cup was inserted into the plasma for analysis

The botanical powder samples were placed into preweighed graphite cups until half full, then the cups were reweighed to give the weights of the samples. The samples were ashed and analyzed as for the standards. The observation time usually was 30 seconds. The signals collected by the IBM PC were stored on floppy discs for integration and data processing.

3.4 Results and Discussion

Since the purpose of this study was to determine the distribution of five elements in the reference materials rice flour and wheat flour, we first determined the reproducibility of the instrument and procedure. The relative standard deviations (RSDs) for a set of 19 solutions are listed in Table 3.1. Most elements had RSD values around 5% except for zinc, which was 10%. Since zinc is a volatile element, it is sensitive to the temperature of the atomization step. Overall, all the elements were measured with good precision and reproducibility. The direct sample insertion ICP design was thereby shown to be quite satisfactory for the analysis of solution samples of at least four of the five elements.

Rice Flour and Wheat Flour reference materials were chosen for this sampling study because the aluminum content in rice flour showed a quite uniform distribution in the preliminary study, in contrast to wheat flour. The original homogeneity evaluations on these two materials were done on test portion weights of about 500 mg by instrumental neutron activation analysis at NIST in Gaithersburg, MD. Their work showed that all five elements were uniformly distributed in the two materials at this test portion size (17,18).

Table 3.1 Comparison of relative standard deviations of replicate measurements on rice and wheat flour and on a set of standard solutions

Element	Al	Cu	Zn	Fe	Mn
Standard solution (n=19)	4%	4%	10%	6%	4%
Rice Flour (n=26)	16%	17%	14%	14%	12%
Wheat Flour (n=33)	52%	18%	15%	20%	19%

n = the number of replicates

Table 3.2 Analytical Results for the Determination of Al, Cu, Zn, Fe and Mn in the NIST Standard Reference Materials Rice Flour(SRM1568a) and Wheat Flour(SRM 1567a)

Sample	element	certified value (µg/g)[a]	found value (µg/g)[b]
Rice Flour	Al	4.4 ± 1.0	4.6 ± 0.7
	Cu	2.4 ± 0.3	3.0 ± 0.5
	Zn	19.4 ± 0.5	21.9 ± 3.0
	Fe	7.4 ± 0.9	10.5 ± 1.5
	Mn	20.0 ± 1.6	26.2 ± 3.3
Wheat Flour	Al	5.7 ± 1.3	4.8 ± 2.5
	Cu	2.1 ± 0.2	1.5 ± 0.3
	Zn	11.6 ± 0.4	11.2 ± 1.7
	Fe	14.1 ± 0.5	16.4 ± 3.3
	Mn	9.4 ± 0.9	9.7 ± 1.8

[a] Certified concentrations computed as described in reference #19. Uncertainty is stated as a 95% confidence interval plus an additional allowance for systematic error among methods used in certification.

[b] Uncertainty is one standard deviation; n=26 for rice flour, 33 for wheat flour.

In this work samples of about the same size as used at NIST for their homogeneity studies, 513.8 mg for rice flour and 524.3 mg for wheat flour, were subdivided and transferred to 30 graphite cups. Each cup, containing about 16 mg of the sample, weighed to ±0.1 mg, was inserted into the plasma for ashing and determination.

The relative standard deviations for the resulting 31 analyses of rice flour and 34 analyses of wheat flour are listed in Table 3.1. The RSD values for the five elements for rice flour are uniform, around 15%, whereas those for wheat flour are somewhat higher except for aluminum which is extremely high. This suggests that there may be minerals in wheat flour which contain high aluminum concentrations. Figures 3.1, 3.2 and 3.3 show plots of individual analytical values for the standard solutions, rice flour and wheat flour. In Figure 3.1, Al, Cu, Fe, and Mn have much the same pattern, suggesting that the variation probably is caused by uncertainties in sample handling operations and in instrument stability. The volatility of zinc and its sensitivity are probably responsible for its greater variability and larger relative standard deviation.

In Figure 3.2, all five elements show about the same variation pattern, suggesting that the rice flour is quite uniform in composition. The observed variation may be due to weighing errors and to variability in element distribution in rice flour. The average RSD of 15% might be expected for such small test portion sizes in botanical materials.

The variation in results in wheat flour were greater than for rice flour, as can be seen from Figure 3.3. The aluminum content in test portions 2, 9, and 21 were extremely high, while the iron content in portions 11 and 28, and the manganese content in portions 25 and 28, were also high. This indicates the presence of some small particles that had higher concentrations of different elements. Such particles would cause heterogeneity problems in chemical analysis.

A detailed study of several "outlier" points for aluminum in wheat flour revealed the appearance of two peaks during the 60 seconds of data acquisition. This suggests that two different forms of aluminum might be present in the material. Figures 3.4 and 3.5 show typical situations for this matrix.

Figure 3.4 shows emission intensity as a function of time for the five elements in test portion 21 of wheat flour. Aluminum clearly shows a double peak. This profile suggests that one form of aluminum may be more volatile and produces the first peak. A second, less volatile form of the aluminum may vaporize later and give a second peak. Since the other four elements did not show the same pattern, it is unlikely that different portions of the sample atomized at different times . By excluding the three "outlier", the RSD decreased to 25% which means that the aluminum concentration in flour was much uniform than the whole sample.

Figure 3.5 shows emission intensity as a function of time for the five elements in test portion 28 of wheat flour. In this sample aluminum behaved normally, but iron showed marked tailing. Since the other four elements did not show an unusual pattern, some of the iron may have been present in a different form and vaporized later.

Upon completion of the set of experiments just discussed it was discovered that baseline drift had taken place over the several hours required to collect the data. Although this prevents use of the results for absolute quantitative purposes, these determinations are internally self-consistent and still useful for homogeneity assessment. The downward drift over about 4 hours of instrument operation may have been caused by thermal expansion in the optical system arising from heat generated by the plasma. This would cause the standard deviation to be bigger than it should. Because of this drift problem the experiment on rice flour and wheat flour was repeated, with some modifications. In order to reduce overall time, a 30 second data acquisition time was used. Also, calibration was done in the middle of the sample determinations in order to balance positive or negative errors caused by drift.

The new data are summarized in Tables 3.3 and 3.4, and in Figures 3.6 and 3.7. In this new set rice flour weighing 495.7 mg was subdivided into 32 individual portions, and wheat flour weighing 495.3 mg was subdivided into 35 individual portions.

Comparing Tables 3.1 and 3.3, we see that the average RSD was reduced overall for both rice flour and wheat flour. For Cu, Zn and Mn, the RSD values in rice flour and wheat flour were similar, but the value for Fe in rice flour was high, almost completely owing to an unusually high value for test portion 23 (see Figure 3.6). This point, if due to random error, can be rejected by the Q-test, thereby reducing the RSD to a value similar to the RSD found for wheat flour. Also, deletion of this portion brings the average value for iron to 7.7 ± 0.9 µg/g, close to the certified value of 7.4 ± 0.9 µg/g.

The results for aluminum in this case were different than for iron. Even with one outlier, the RSD value was reduced to 36% from 52% from the previous set. By deleting this outlier (test portion 16), the RSD value was further reduced to 19% and the average value of aluminum became 5.3 ± 1.0 µg/g. Plots of emission intensity as a function of time are shown in Figure 3.8 for all five elements in wheat flour for test portion 16, where again an unusual profile is seen for Al. It is clear that aluminum is not distributed uniformly in wheat flour at the 15-mg test portion level. Overall, one can conclude that any one element cannot be used to estimate distribution of another element in the same material, nor the same element in different materials.

Table 3.3 Comparison of relative standard deviations of replicate measurements on rice and wheat flour and on a set of standard solutions

Element	Al	Cu	Zn	Fe	Mn
Rice Flour (n=32)	14%	7%	9%	21%	8%
Wheat Flour (n=34)	36%	8%	7%	12%	7%

n = the number of replicates

Table 3.4 Analytical Results for the Determination of Al, Cu, Zn, Fe and Mn in the NIST Standard Reference Materials Rice Flour(SRM1568a) and Wheat Flour(SRM 1567a)

Sample	Element	Certified value (μg/g)[a]	Found value (μg/g)[b]
Rice Flour	Al	4.4 ± 1.0	4.9 ± 0.7
	Cu	2.4 ± 0.3	2.7 ± 0.2
	Zn	19.4 ± 0.5	21.7 ± 1.9
	Fe	7.4 ± 0.9	8.0 ± 1.7
	Mn	20.0 ± 1.6	16.8 ± 1.3
Wheat Flour	Al	5.7 ± 1.3	5.6 ± 2.0
	Cu	2.1 ± 0.2	2.5 ± 0.2
	Zn	11.6 ± 0.4	15.3 ± 1.0
	Fe	14.1 ± 0.5	14.2 ± 1.7
	Mn	9.4 ± 0.9	9.1 ± 0.7

[a] Certified concentration computed as described in reference #19. Uncertainty is stated as a 95% confidence interval plus an additional allowance for systematic error among methods used in certification.

[b] Uncertainty is one standard deviation.

Table 3.4 indicates that the agreement of the DSI results with the certified values is quite good considering the convenience and speed of the method. These encouraging results demonstrate that even for difficult sample matrices a standard solution calibration method can be used in the elemental analysis of solid powder samples by DSI-ICP-AES.

Finally, we can estamate the approximate RSD in 500 mg sample test portion by using RSD in 15 mg test portion. From Ingamell's sampling equation $K=WR^2$, where K is sampling constant, R is relative standard deviation, W is weight of sample analyzed, we see that $R^2(500)=R^2(15)*15/500$. Even if R(15) equals 50%, the R(500) only has 9%. The results in this study has proved this point.

3.5 Conclusions

These studies have shown that direct sample insertion ICP with its multi-element capability is a valuable tool for the study of sample homogeneity in botanical materials by allowing the direct analysis of test portions as small as 1 to 20 mg dry weight. The homogeneity of aluminum in NIST Rice Flour is high at test portion sizes of 10 to 15 mg, but for Wheat Flour variability among test portions is significant. On the other hand, copper, zinc, iron and manganese showed uniform distribution in both rice flour and wheat flour at these test portion sizes.

3.6 References

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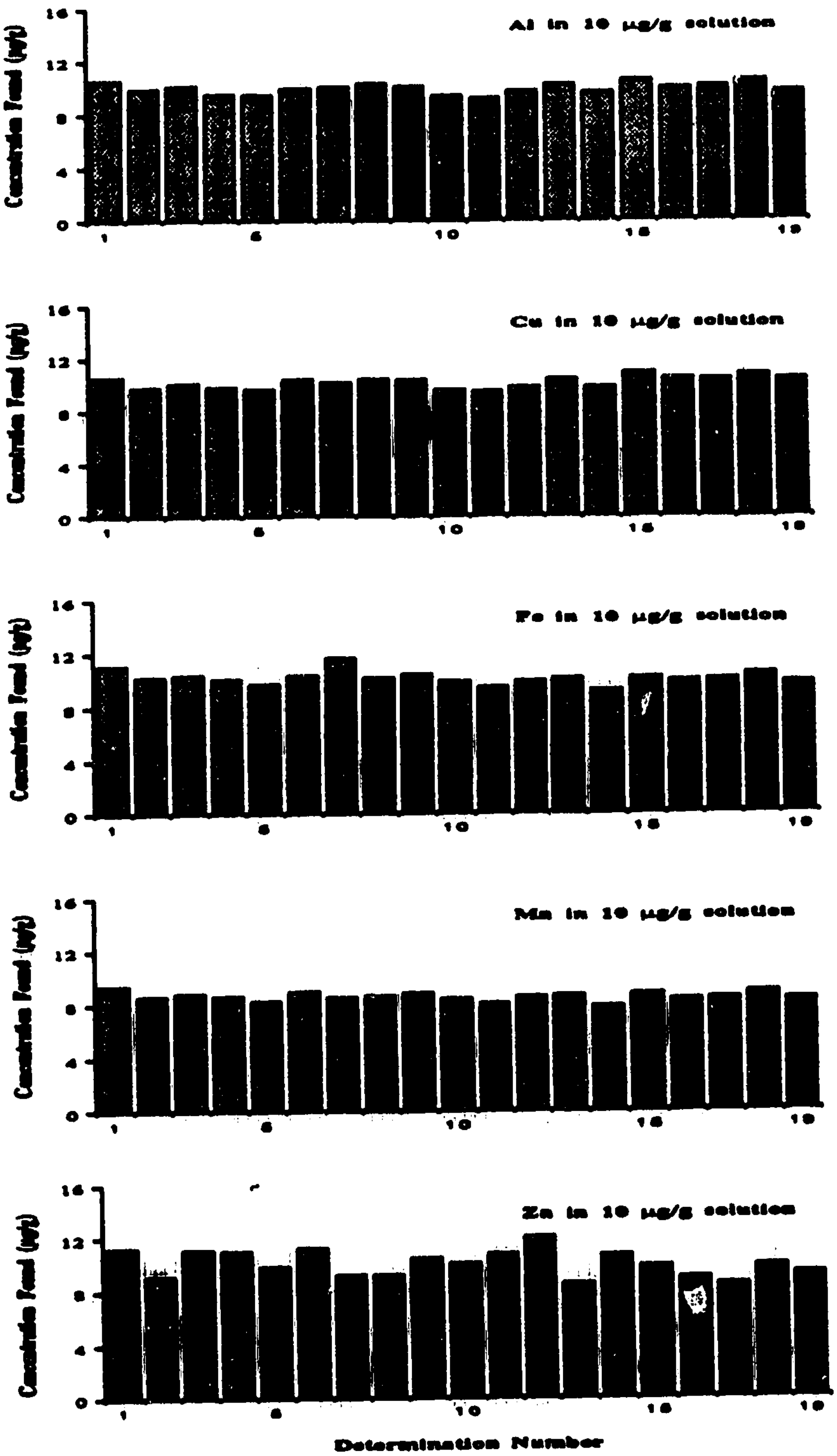

Figure 3.1 Variability of results for the determination of Al, Cu, Fe, Mn and Zn in successive 10 µg/g standard solutions pipetted into graphite cups for direct insertion into ICP

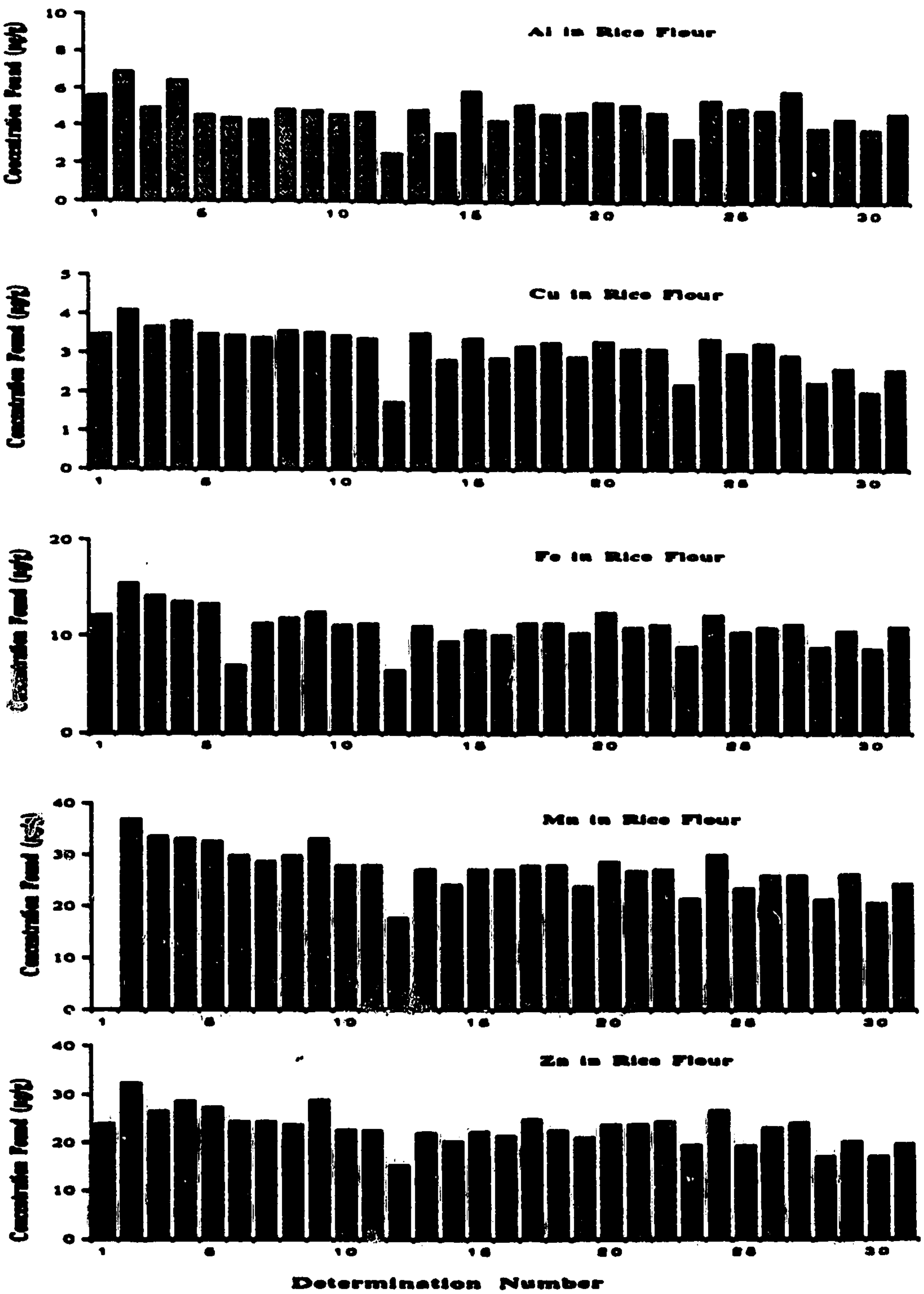

Figure 3.2 Variability of results for replicate determinations of Al, Cu, Fe, Mn and Zn in NIST Rice Flour (SRM1568a)

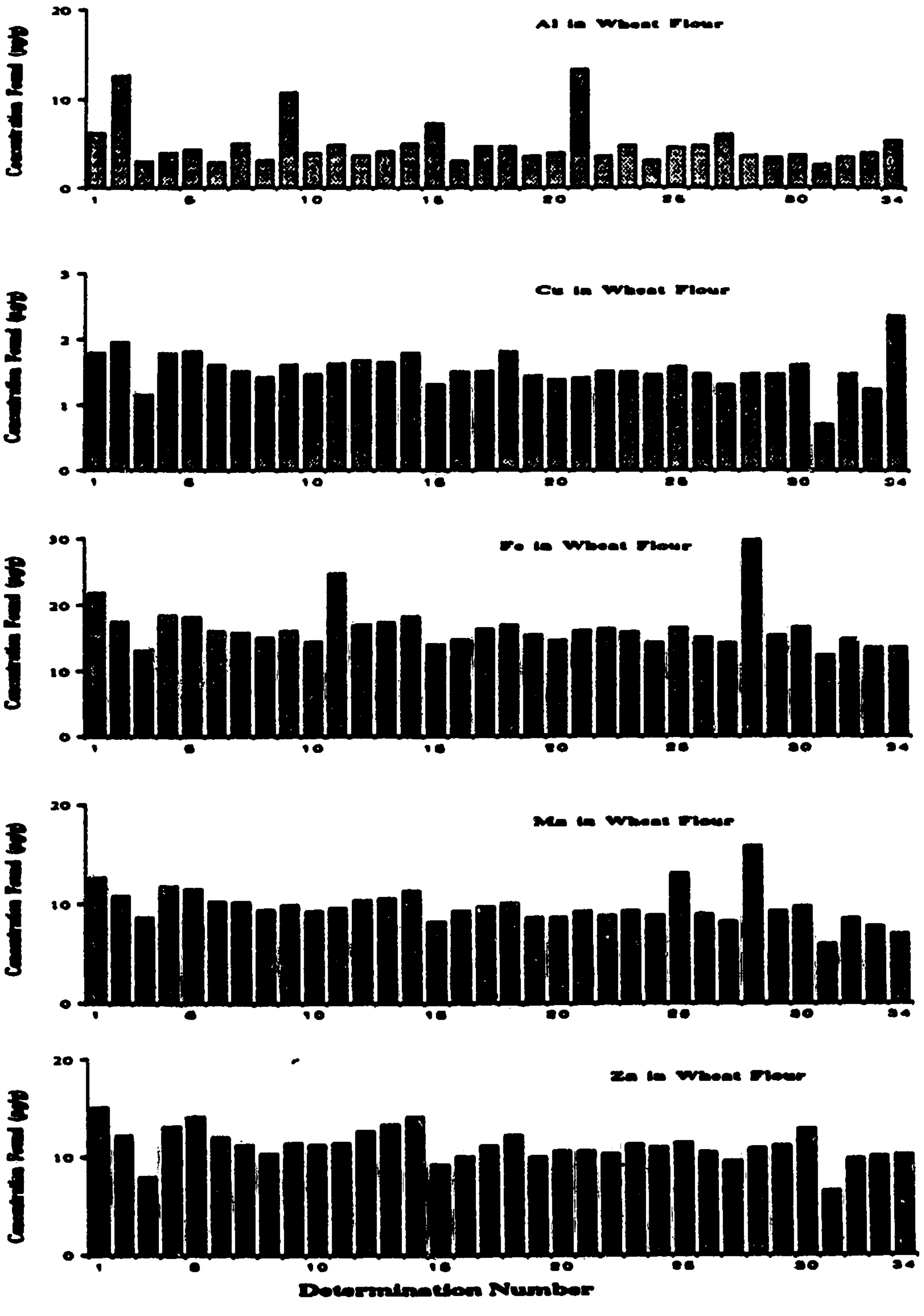

Figure 3.3 Variability of results for replicate determinations of Al, Cu, Fe, Mn and Zn in NIST Wheat Flour (SRM1567a)

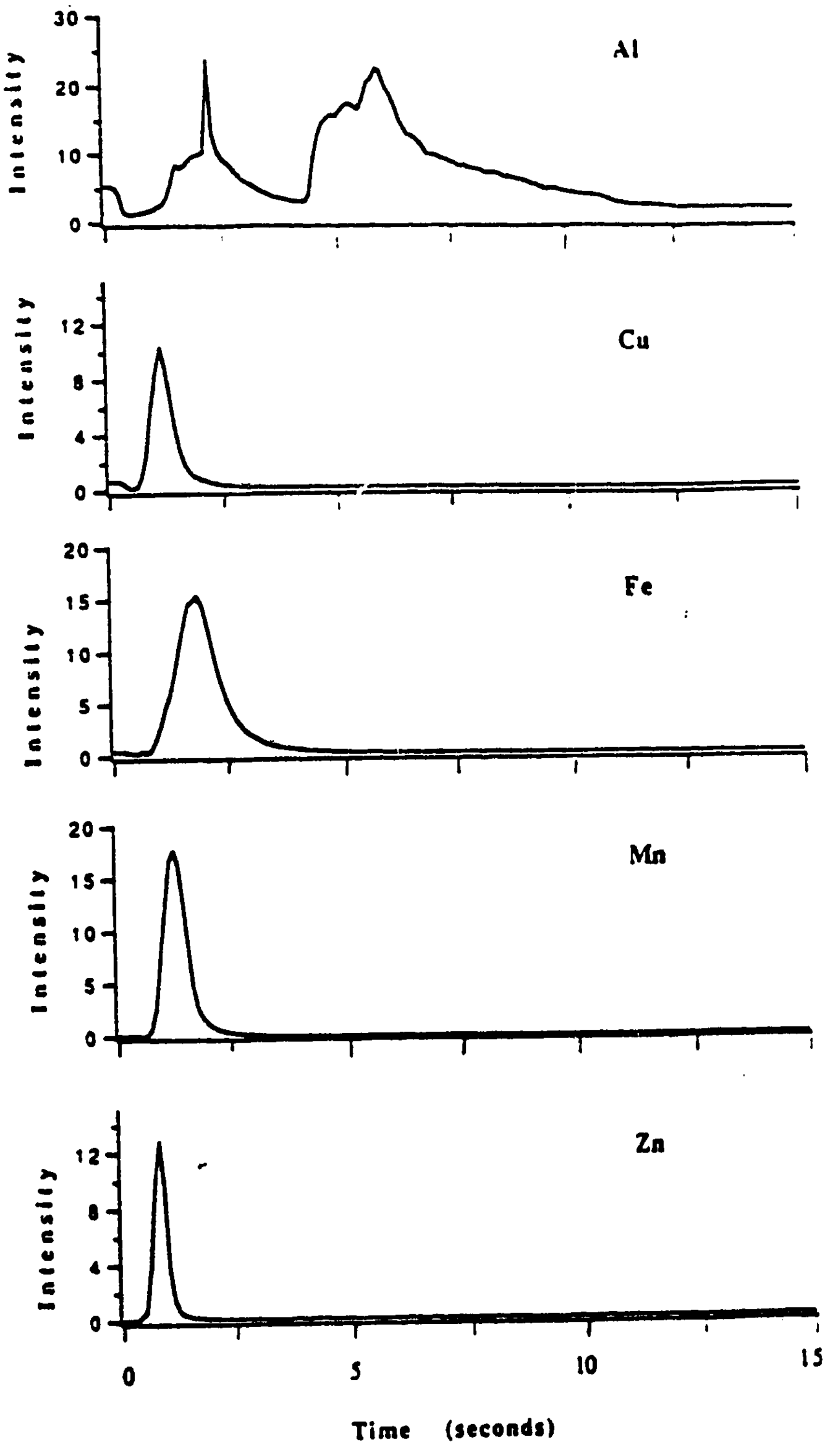

Figure 3.4 Emission intensity as a function of time for Al, Cu, Fe, Mn and Zn in test portion 21 of NIST Wheat Flour (SRM 1567a)

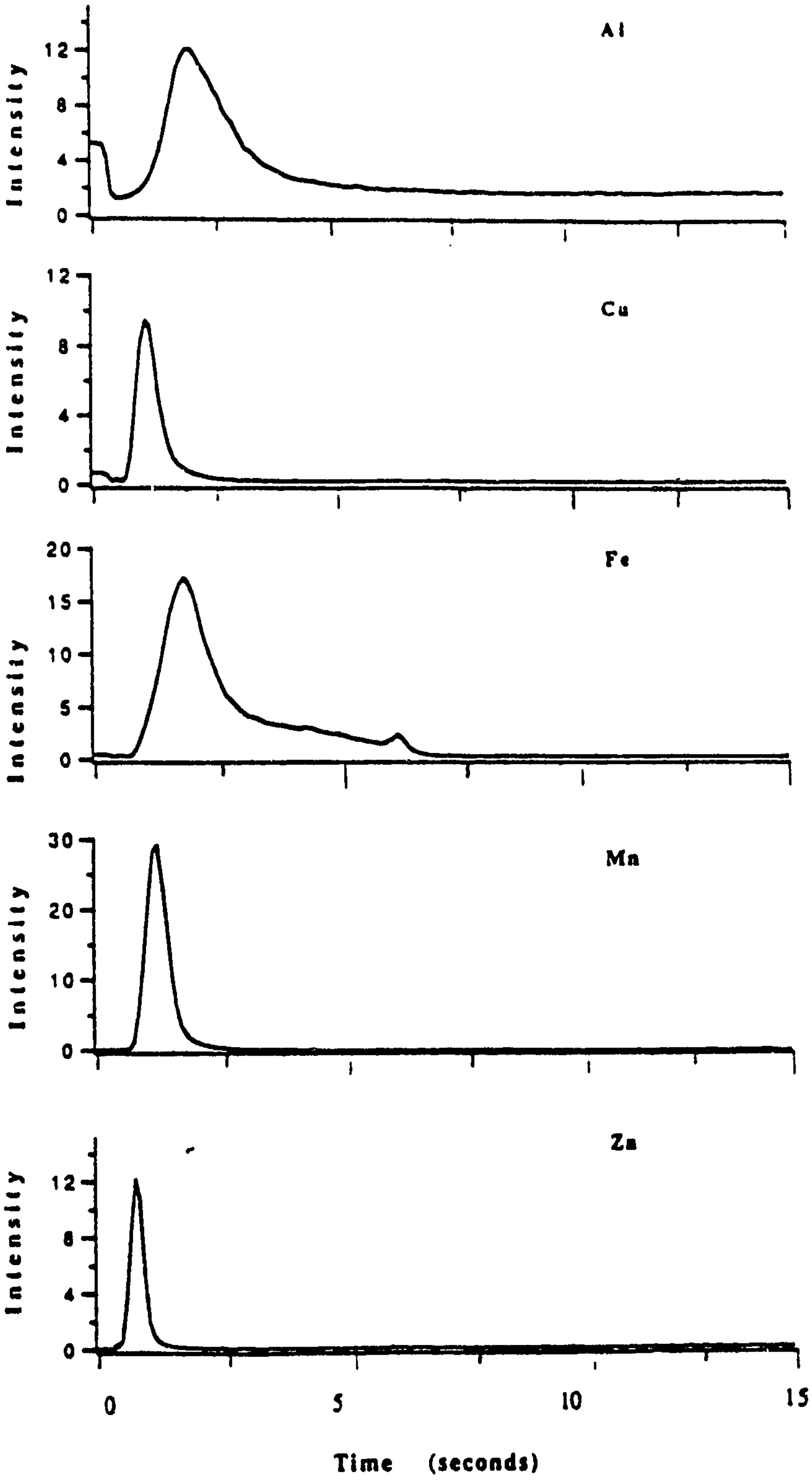

Figure 3.5 Emission intensity as a function of time for Al, Cu, Fe, Mn and Zn in test portion 28 of NIST Wheat Flour (SRM 1567a)

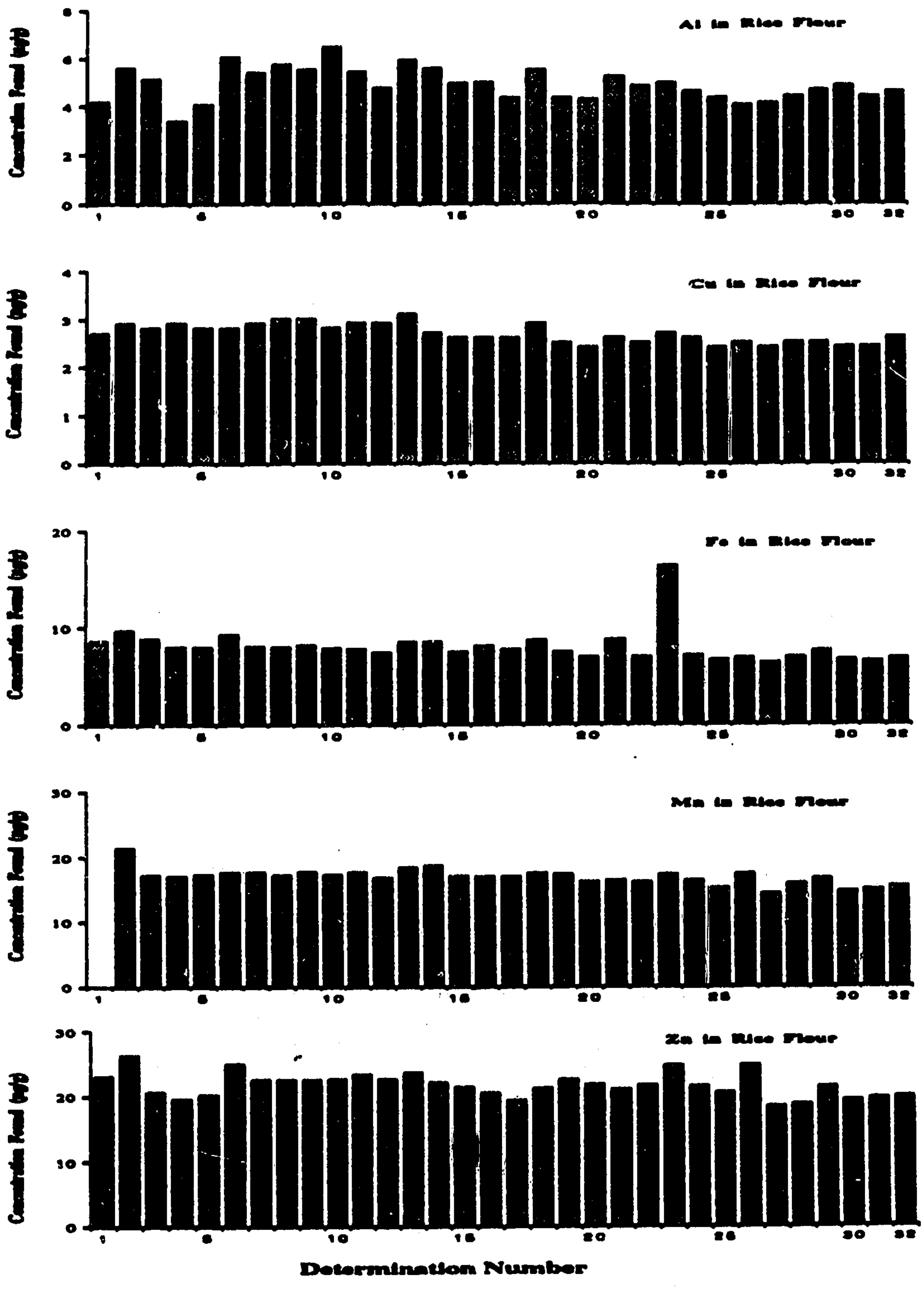

Figure 3.6 Variability of results for replicate determinations of Al, Cu, Fe, Mn and Zn in NIST Rice Flour (SRM1568a)

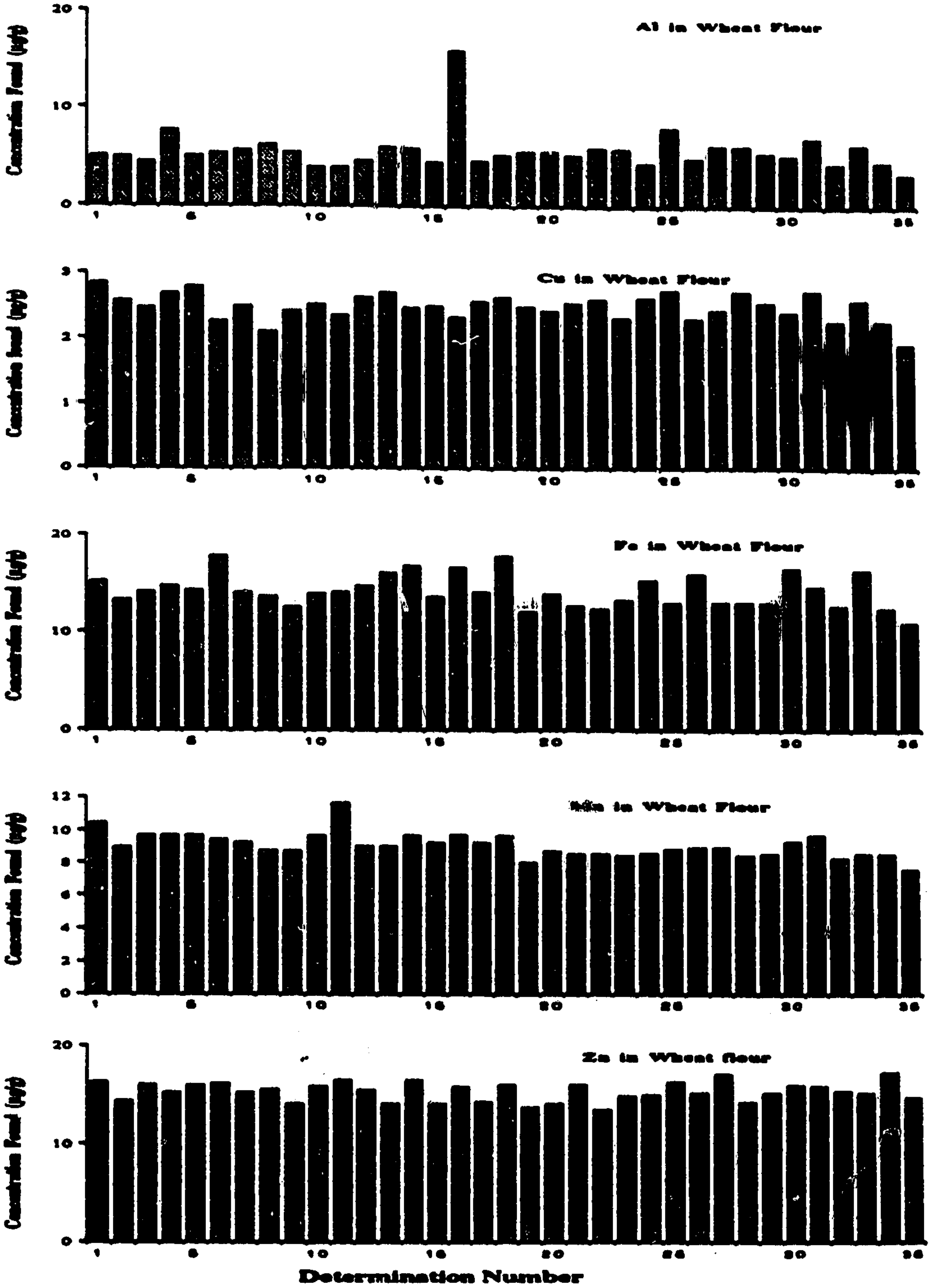

Figure 3.7 Variability of results for replicate determinations of Al, Cu, Fe, Mn and Zn in NIST Wheat Flour (SRM1567a)

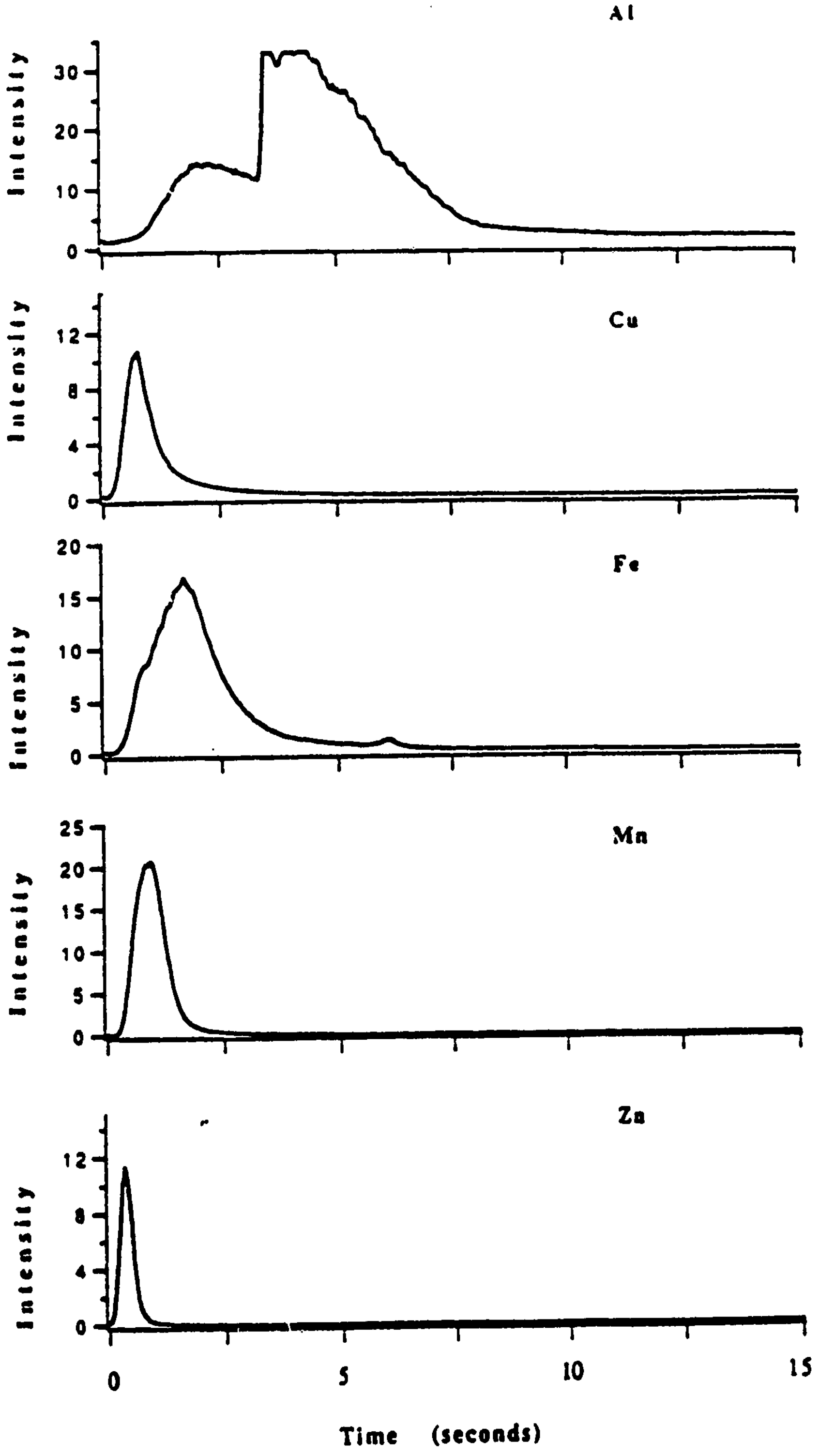

Figure 3.8 Emission intensity as a function of time for Al, Cu, Fe, Mn and Zn in test portion 16 of NIST Wheat Flour (SRM 1567a)

CHAPTER 4

DETERMINATION OF AL, FE, CU, MN and Zn IN POTENTIAL CANADIAN AGRICULTURE REFERENCE MATERIALS BY DSI-ICP

4.1 ABSTRACT

The concentrations of Al, Fe, Cu, Zn and Mn in eight candidate agricultural reference materials from Agriculture Canada were studied by mixed Ar-O_2 gas direct sample insertion ICP. The materials, which included bovine muscle powder, whole egg powder, corn bran, microcrystalline cellulose, wheat gluten, whole milk powder, corn starch and wheat flour, gave reproducible results that were comparable with published data. The materials were assessed for within vs. between bottle uniformity by one-way analysis of variance and were found to be homogeneous at 5 to 10 mg test portion levels for all five elements studied. Exceptions were aluminum in flour and iron in cellulose.

4.2 INTRODUCTION

Agricultural and food commodities represent an extremely wide range of composition that is not fully reflected in currently available biological reference materials (BRM's) for trace element concentrations. A cost effective approach to monitoring and maintaining the reliability of analytical procedures should include the incorporation of appropriate, compositionally similar, reference materials into the scheme of analysis. With a view to filling some of the gaps in the world repertoire of reference materials for use by analytical laboratories, Dr. Milan Ihnat of Agriculture Canada recently prepared twelve candidate agricultural reference materials (1-4). These products, representing a range of food types, include bovine muscle powder, hard red spring wheat flour, soft winter wheat flour, durum wheat flour, wheat gluten, corn bran, corn starch, potato starch, whole egg powder, whole milk powder, microstalline cellulose, and sugar.

Steps in the preparation of these materials included freeze drying, grinding or ball milling, X-ray sterilization, sieving, blending and packaging to yield sizeable quantities of each material in finely powdered form. The code numbers and full names, plus mean particle size, are listed in Table 4.1. Details of particle size distribution can be found in references 1 to 4.

Table 4.1 Candidate agriculture/food reference materials prepared by M. Ihnat, Agriculture Canada

Code	Name	mean particle diameter (μm)
136	Bovine muscle powder	50
188	Whole egg powder	150
186	Corn bran	150
189	Microcrystalline cellulose	90
187	Durum wheat flour	130
165	Hard red spring wheat flour	100
166	Soft winter wheat flour	100
184	Wheat gluten	130
183	Whole milk powder	170
162	Corn starch	70
185	Potato starch	70
182	White granulated sugar	290

It is anticipated that these materials will fulfill a number of roles. First, and most importantly, it is expected that a fair proportion of these materials will be important for quality control in agricultural and food analyses. Secondly, several materials with expected very low concentrations of inorganic analytes, such as sugar, cellulose and the starches, may serve as blank reference materials for many routine purposes. Incorporation of such "blank" materials into a procedure may provide better measures of sample ashing or digestion blank values than use of reagents alone.

Characterization has been done for up to some twenty five major, minor, trace and ultratrace elements of nutritional, toxicological and environmental significance. Solid sampling electrothermal atomic absorption spectrometry was applied to the determination of copper and lead in sub-milligram subsamples. A variety of other analytical techniques via an interlaboratory cooperative effort was used to determine many other elements in 100 to 2000 mg test portions. Good material homogeneity was observed for virtually all materials and analytes at these sizes with the exception of Cr and Pb in a few instances. A preliminary study of the concentration of nine elements, namely Ca, Cu, Hg, K, Mn, Na, P, Se and Zn, in bovine muscle, corn bran, whole milk powder, wheat flour, gluten and whole egg powder were published in reference 1.

As part of the analytical characterization of these materials, our group has focused on aluminum content, since the reliable determination of this element at ppm levels and below in biological materials is difficult. Eight agriculture materials, obtained from Agriculture Canada through Dr. M. Ihnat, were analyzed for their aluminum concentration by several analytical methods (5). Graphite furnace atomic absorption spectroscopy (GFAAS) gave reproducible results that showed all of the materials to have a homogeneous distribution of aluminum (5). Inductively coupled plasma atomic emission spectrometry (ICP-AES) was not sufficiently sensitive to be useful, due partly to the low aluminum concentrations in the materials and partly to the limited sample sizes that could be dissolved by microwave digestion. Instrumental Neutron Activation Analysis (INAA) gave reproducible results which were high compared to the GFAAS results owing to phosphorus and silicon interference (5). Correction of the INAA aluminum results for phosphorus interference gave values that agreed with the aluminum values by GFAAS. Phosphorus was determined by derivative activation analysis of a solvent-extracted phosphovanadamolybdate complex. Results for the phosphorus analyses were fairly reproducible. The detailed analytical results for aluminum by GFAAS and NAA are listed in Appendix D.

Since the GFAAS method gave difficulty at low concentration levels, the aluminum concentration in whole milk powder, which is low, could not be determined . In addition, the sample digestion procedures and standard addition methods used in this work are very time consuming and complicated. The present study used direct sample insertion ICP to eliminate these problems. With direct sample insertion there are no sample digestion and pretreatment steps, and the samples are not diluted. The capability of simultaneous multi-element determination with ICP-AES is another advantage. However, since DSI can handle only a few milligrams of material per analysis, sample heterogeneity is likely to introduce variability into results.

The aluminum content in the eight agriculture samples was determined by Ar-O_2 mixed gas DSI-ICP. In addition to aluminum, Cu, Zn, Fe and Mn were investigated. Assessment of the reference materials for within vs. between bottle homogeneity was done by one-way analysis of variance.

4.3 EXPERIMENTAL

4.3.1 Instrumentation

Figure 2.1 shows a schematic diagram of the DSI-ICP-AES system. The torch, stepper motor, and the mechanical probe driving system have been described in references 13 and 15 in Chapter 2. The system was modified so that both the stepper motor and the data acquisition can be controlled by an IBM PC computer though a PCMotion ^{TM} stepper motor control board and a DT 2801-A data acquisition board housed in the IBM PC backplane. The stepper motor control board can control up to four motors simultaneously. Detailed hardware specifications are listed in Chapter 2, Table 2.1. The optimized operation conditions are summarized in Chapter 2, Table 2.2.

The data acquisition program was written by X. Liu in quick-BASIC. The program is menu driven with screen edit functions providing convenient changes of operating parameters, such as sample weight, ADC gain, probe positions, and times for ashing and data acquisition.

The graphite electrodes were purchased from Bay Carbon Inc., USA and modified by drilling a 3 mm diameter hole about 6 mm deep into the base so as to allow the cup to be mounted on a support rod. The graphite contained considerable amounts of metallic impurities. In order to eliminate them, each cup was inserted for 30 seconds in the argon-oxygen gas plasma prior to use. The inner diameter and depth of the graphite cup were 3.17 and 4.78 mm respectively, giving a volume of 40 μL.

4.3.2 Chemicals

Standard stock solutions of 1000 ng/mL were prepared from commercial ICP standard 1000 ppm solutions (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085, USA) by dilution with distilled/ deionized water. The argon and oxygen gases were from Linde, Union Carbide Canada, Limited, Toronto. To minimize impurity levels, cellulose filter paper (Pall Ultrafine Filtration Corporation, Glen Cove, New York 11542 USA) was soaked in concentrated HNO_3 for 2 days, washed with deionized water, then dried. Nitric acid was double sub-boiling distilled in quartz (Seastar Chemical Inc., Sidney BC).

4.3.3 Sample Drying and Mixing

Four bottles each of Bran, Flour, Gluten, Whole Egg Powder, Whole Milk Powder, Bovine Muscle, Starch and Cellulose, obtained from Dr. Milan Ihnat, Agriculture Canada, Ottawa, were tumbled end-over-end for at least 2 hours. After tumbling, about 5-g portions were transferred to clean, dry glass weighing bottles and dried at 85^{o} C for 4 hours.

4.3.4 Procedure

For solution standards, a small piece of wet cellulose filter paper was fitted into a graphite cup. After drying of the paper under an IR lamp, a 10 µL micropipet was used to add known concentrations of the standard solutions onto the filter paper, which was then dried again. The cup was inserted into the ICP to 18 mm below the load coil for 30 seconds for ashing, then raised into the plasma for analysis.

The botanical powder samples were placed into preweighed graphite cups until half full, then the cups were reweighed to give the weights of the samples. The samples were ashed and analyzed as for the standards. The observation time usually was 30 seconds. The signals collected by the ADC were stored on floppy discs for integration and data processing.

4.4 RESULTS AND DISCUSSION

Since oxygen was added to the plasma gases, the cup part of the graphite electrode was burned off. This was done so that any elements present in nonvolatile form or soaked into the cup were bought into the plasma. This made calibration simple. However, calibration plots were usually only linear up 100 µg/g. This was adequate for most of the materials and elements studied except for aluminum in whole egg powder, where the levels were very high. It is suspected that either an aluminum compound was added to the egg powder during preparation, or the material was contaminated.

Results for the determination of copper, zinc, iron, manganese and aluminum in Gluten, Flour, Milk Powder, Egg Powder, Bran, Cellulose, Bovine Muscle and Starch are shown in Tables 4.1 to Table 4.8. Six test portions were taken from each of four bottles of each material. The aluminum reference values provided in the tables are from previous GFAAS analyses in our group (5). The copper, zinc, and manganese values are from a report by Ihnat (1). Generally, the ICP average were in good agreement with the reference values, although higher standard deviations were observed as expected because of the small test portions used.

The aluminum concentration found in bovine muscle in this work was 1.8 µg/g, which is about double that reported by GFAAS. This may have been caused by incomplete dissolution in the atomic absorption analysis. Since only nitric acid was used in the digestion of bovine muscle, it is likely some aluminum in mineral form was not dissolved. The neutron activation results by N. Motkosky (5) are more in line with the ICP results, giving an aluminum concentration of 2.5 ± 0.6 µg/g compared to 0.98 ± 0.28 µg/g for GFAAS.

The aluminum concentration in whole milk powder was the lowest among the eight agricultural materials studied. GFAAS was not able to provide useful values because the Al concentration was below the detection limit (5). However, since 0.5 µg/g is much higher than the detection limit by DSI-ICP-AES, it could be easily determined.

Figures 4.1, 4.2 and 4.3 show typical Al, Cu, Fe, Mn and Zn peak profiles during atomization. The atomization time was 15 seconds. The Y-axes in these figures are arbitrary emission intensity units at the selected wavelength. These peak profiles demonstrate that the peak shape is different for different materials, even for the same element. The peak rise time is very fast for zinc and quite slow for aluminum; aluminum usually has the largest peak width with iron next.

Figure 4.4 shows signal intensity vs. time for a test portion of Whole Egg Powder, which has the highest aluminum concentration in the materials studied. The peak shape for aluminum in this material is very tailing, with numerous spikes. Figure 4.5 shows the aluminum peak profile for Whole Milk Powder, which had the lowest aluminum concentration in the group, about 0.7 µg/g. It can be seen from the plot that the aluminum peak is still significantly larger than the background noise level. This concentration could not be measured by GFAAS in our group (5) because the concentration was too low.

Figures 4.6 and 4.7 present signal-time profiles for the five elements in Starch and Cellulose. Zinc was not detected in the Cellulose samples, while copper, zinc and maganese in Starch were among the lowest concentrations in the set of materials. However, for all three elements the peak heights were still much higher than the noise level, and quantitation was possible.

Figure 4.8 shows a profile for a test portion for Flour with a typical aluminum concentration for this material, while Figure 4.9 shows the profile for a test portion having a high concentration of aluminum. In this portion two aluminum peaks are seen, whereas the profiles for the other four elements do not show a similar pattern. This suggests that the double peak is not caused by uneven atomization of the material, but rather that the aluminum may be present in different forms in the test portion, one form atomizing less readily.

Table 4.2 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Gluten in µg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
1328	5.1 ± 1.1	32 ±4	53 ± 12	12 ± 3	8.9 ± 1.6
1288	4.7 ± 0.4	32 ± 3	49 ± 4	12 ± 1	7.8 ± 0.9
532	4.4 ± 0.3	30 ± 2	45 ± 4	11 ± 1	7.9 ± 0.5
639	4.9 ± 0.8	30 ± 7	51 ± 9	12 ± 2	8.5 ± 1.4
Average	4.8 ± 0.7	31 ± 4	49 ± 8	12 ± 2	8.3 ± 1.2
Literature	6[b]	54[b]	—	14[b]	10.9 ± 1.2[c]

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #1

[c] Literature value from reference #5

Table 4.3 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Flour 187in µg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
935	4.6 ± 0.4	25 ± 3	42 ± 3	16 ± 1	12 ± 1
640	4.9 ± 0.4	26 ± 2	41 ± 3	17 ± 1	15 ± 5
971	5.1 ± 0.2	25 ± 2	45 ± 4	17 ± 1	19 ± 4
489	5.0 ± 0.3	25 ± 2	42 ± 4	17 ± 2	15 ± 4
Average	4.9 ± 0.4	25 ± 2.0	43 ± 4	17 ± 1	15 ± 4
Literature	4.5[b]	22[b]	—	16[b]	13.2 ± 1.3[c]

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #1

[c] Literature value from reference #5

Table 4.4 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Bran in μg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
390	3.0 ± 0.2	20 ± 3	17 ± 2	3.3 ± 1.6	0.9 ± 0.2
1428	3.0 ± 0.1	20 ± 3	17 ± 1	3.2 ± 0.2	1.6 ± 0.9
312	3.0 ± 0.2	19 ± 3	17 ± 1	3.3 ± 0.3	1.0 ± 0.2
927	3.0 ± 0.1	22 ± 1	17 ± 1	3.3 ± 0.4	1.1 ± 0.3
Average	3.0 ± 0.2	20 ± 3	17 ± 1	3.3 ± 0.3	1.1 ± 0.5
Literature	2.5 [b]	18 [b]	—	2.5 [b]	1.4 ± 0.3 [c]

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #1

[c] Literature value from reference #5

Table 4.5 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Whole Milk Powder in μg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
295	0.55 ± 0.08	31 ± 3	1.7 ± 0.2	0.13 ± 0.02	0.8 ± 0.1
1235	0.49 ± 0.02	32 ± 3	1.7 ± 0.1	0.14 ± 0.01	0.7 ± 0.3
997	0.51 ± 0.05	32 ± 3	1.7 ± 0.2	0.13 ± 0.02	0.6 ± 0.1
1262	0.50 ± 0.05	31 ± 3	1.6 ± 0.1	0.13 ± 0.01	0.6 ± 0.1
Average	0.51 ± 0.06	35 ± 3	1.7 ± 0.2	0.13 ± 0.01	0.7 ± 0.2
Literature	0.5 [b]	30 [b]	—	0.2 [b]	—

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #1

[c] Literature value from reference #5

Table 4.6 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Bovine Muscle in µg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
1817	2.8 ± 0.2	106 ± 8	68 ± 6	0.31 ± 0.02	2.0 ± 0.3
57	2.9 ± 0.1	108 ± 9	64 ± 6	0.32 ± 0.05	1.6 ± 0.3
422	2.6 ± 0.5	96 ± 16	63 ± 13	0.29 ± 0.06	1.7 ± 0.5
3019	3.0 ± 0.2	107 ± 8	65 ± 5	0.32 ± 0.03	1.8 ± 0.1
Average	2.8 ± 0.3	104 ± 11	65 ± 8	0.31 ± 0.04	1.8 ± 0.3
Literature	2.9 [b]	140 [b]	—	0.4 [b]	0.98 ± 0.28

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #1

[c] Literature value from reference #5

Table 4.7 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Whole Egg Powder in µg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
751	3.7 ± 0.7	71 ± 14	140 ± 20	2.0 ± 0.4	490 ± 70
1930	3.3 ± 0.3	65 ± 5	120 ± 10	1.7 ± 0.1	470 ± 50
329	3.6 ± 0.4	72 ± 10	130 ± 10	1.8 ± 0.2	510 ± 60
512	3.0 ± 0.7	61 ± 10	110 ± 30	1.5 ± 0.3	440 ± 80
Average	3.5 ± 0.6	67 ± 10	120 ± 20	1.7 ± 0.3	480 ± 60
Literature	—	70 [b]	—	2.0 [b]	499 ± 39 [c]

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #1

[c] Literature value from reference #5

Table 4.8 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Cellulose in µg/g.[a]

Bottle No.	Cu	Fe	Mn	Al
479	0.15 ± 0.02	0.84 ±0.05	0.054 ± 0.006	4.1 ± 0.2
662	0.15 ± 0.02	0.72 ± 0.03	0.048 ± 0.009	5.3 ± 1.8
1791	0.14 ± 0.01	0.73 ± 0.05	0.048 ± 0.005	4.0 ± 0.4
497	0.14 ± 0.01	0.78 ± 0.07	0.048 ± 0.004	3.9 ± 0.2
Average	0.15 ± 0.02	0.77 ± 0.07	0.050 ± 0.006	4.3 ± 0.7
Literature	—	—	—	3.8 ± 0.7 [b]

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #5

Table 4.9 DSI-ICP-AES Results for the Determination of Cu, Zn, Fe, Mn, Al in Starch in µg/g.[a]

Bottle No.	Cu	Zn	Fe	Mn	Al
142	0.11 ± 0.01	0.11 ± 0.04	1.1 ± 0.3	0.056 ± 0.005	2.0 ± 0.3
869	0.12 ± 0.02	0.14 ± 0.05	1.0 ± 0.3	0.055 ± 0.008	1.9 ± 0.2
1591	0.14 ± 0.05	0.13 ± 0.04	0.9 ± 0.2	0.052 ± 0.009	2.0 ± 0.2
140	0.14 ± 0.02	0.13 ± 0.04	0.9 ± 0.1	0.053 ± 0.013	1.9 ± 0.2
Average	0.13 ± 0.02	0.13 ± 0.04	1.0 ± 0.3	0.054 ± 0.009	1.9 ±0.2
Literature	—	—	—	—	2.20 ± 0.55 [b]

[a] Uncertainties for analyses of each bottle are standard deviations based on n=6

[b] Literature value from reference #5

The results were tested for differences in within vs. between bottle homogeneity by a one-way analysis of variance. Prior to running the ANOVA program the data were tested by the Dean and Dixon Q test (6) and values falling outside the Dean and Dixon criteria at the 90% confidence level were deleted. The full data sets are listed in Appendix A and C. The values with an asterisk fall outside the 90% confidence interval. The statistical analyses were performed on an Apple Macintosh computer using the statistical package Statworks (Data Metrics Inc. and Heyden and Son Inc., Philadelphia, USA)

The statistical table F ratios, which express the relative magnitude of the between-bottle to within-bottle homogeneity, for 3 between-bottle and 15, 17, 19 and 20 within-bottle degrees of freedom (7) are 3.29, 3.20, 3.13, and 3.10 at the 95% confidence level. The F values found for the agricultural materials are given in Table 4.10. All of the F ratios fall below the table values at the 95% confidence level except for two. This indicates that the materials are homogeneous in almost all cases at the 10-mg test portion size.

The two F values exceeding the table values were aluminum in Flour 187 and iron in Cellulose 189. The conclusion we reach is that aluminum is not uniformly distributed in the Flour. In Chapter 3, we observed that there was evidence for some particulate matter in the Flour. The same situation is likely being encountered here. Additional support for the presence of localized high concentrations of aluminum is given by Figure 4.9, where double peaks were observed in the aluminum profile for one of the test portions. Almost all abnormally high results had a similar profile, suggesting that in these test portions the aluminum is present in two forms, one more resistant to atomization than the other. This resistant form is likely mineral in nature. The same situation held for Cellulose, where for one test portion the concentration of aluminum was very high and the peak shape was also double (Figure 4.10). However, the Q-test does not allow rejection of this result at the 90% confedence level. This single high value causes the average value for bottle #662 to be much higher than for the other three bottles, causing the calculated F value to exceed the table value.

Sample heterogeneity has always been a problem for micro sampling of materials because different sizes of test portions can lead to different results (8). Since these test materials were from field-grown agricultural materials, a small amount of mineral matter (grit) can be expected to be present, even though a washing step may have been used in the preparation. The presence of this grit leads to two consequences that must be taken into account in the use of these reference materials (9). First, some of the trace constituents may be present in a phase that may not be solubilized by the method used to decompose the bulk matrix. Second, the presence of a minor phase may occur in which a trace constituent is present mostly in a small number of high-concentration grains. Under these conditions the statistics of sampling may, if the degree of concentration is high enough, lead to heterogeneity which cannot be resolved by any analytical method. This issue has been studied extensively in the geochemical literature (10) and specifically as applied to the preparation of reference materials (11).

The statistical table F ratios, which express the relative magnitude of the between-bottle to within-bottle homogeneity, for 3 between-bottle and 15, 17, 19 and 20 within-bottle degrees of freedom (7) are 3.29, 3.20, 3.13, and 3.10 at the 95% confidence level.

Table 4.10 Calculated F ratios by Analysis of Variance for Cu, Zn, Fe, Mn and Al in Eight Agricultural Materials

Sample	Calculated F Ratio				
	Cu	Zn	Fe	Mn	Al
Gluten 184 (n=17)	0.86	0.36	0.92	0.61	1.00
Flour 187 (n=20)	2.23	0.06	1.54[b]	0.51	3.33[c]
Bran 186 (n=15)	0.01	1.05	0.37	0.02	1.88[a]
Milk Powder 188 (n=20)	1.29	0.32	0.50	0.25	1.83[b]
Bovine Muscle 136 (n=20)	1.97	1.82	0.41	0.43	1.43[a]
Egg Powder 183 (n=20)	2.85	1.61	2.03	2.31	1.21
Starch 162 (n=19)	2.05[a]	0.63	1.24	0.31	0.30
Cellulose 189 (n=19)	2.14	—	5.50	1.23	2.84[a]

[a] Number of freedom =n-1

[b] Number of freedom =n-2

[c] Number of freedom =n-3

The statistical table F ratios, which express the relative magnitude of the between-bottle to within-bottle homogeneity, for 3 between-bottle and 15, 17, 19 and 20 within-bottle degrees of freedom (7) are 3.29, 3.20, 3.13, and 3.10 at the 95% confidence level.

4.5 Conclusions

These results indicate that DSI-ICP-AES can used for the rapid and satisfactory determination of Al, Cu, Zn, Fe and Mn at the ng/g level in agriculture materials. Most of the values obtained in this work were comparable with other methods. An ANOVA study of within vs. between bottle homogeneity indicated that a majority of the five elements studied were distributed evenly in the sample materials. Therefore, results obtained from direct solid sample insertion at test sample portions of around 10 mg can be expected to be the same as for sample digestion methods at test portion sizes of around 500 mg. Finally, DSI-ICP-AES has been shown to be a good method for rapid analysis of several elements and for assessing their micro-distribution.

4.6 References

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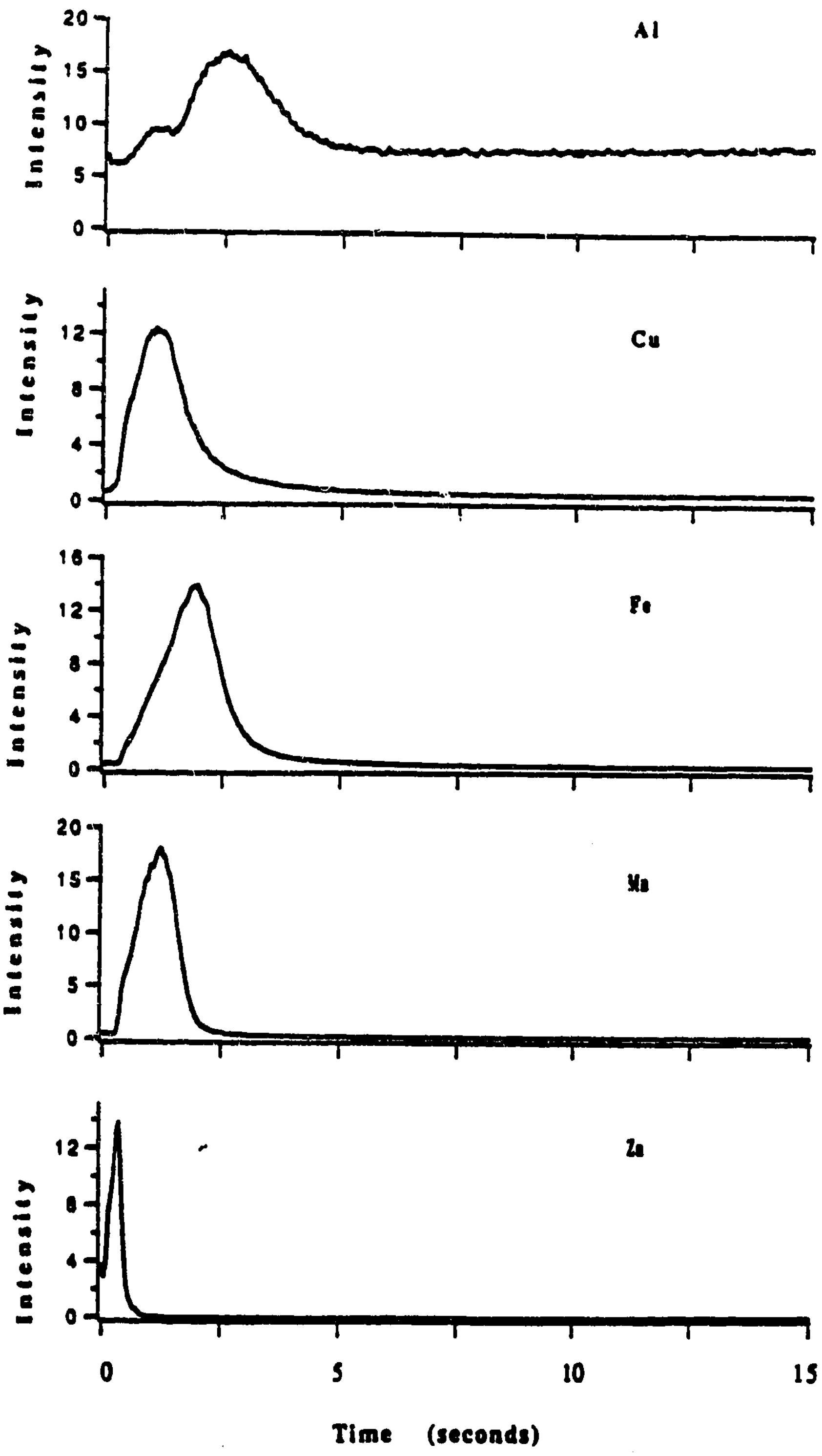

Figure 4.1 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Bran (186)

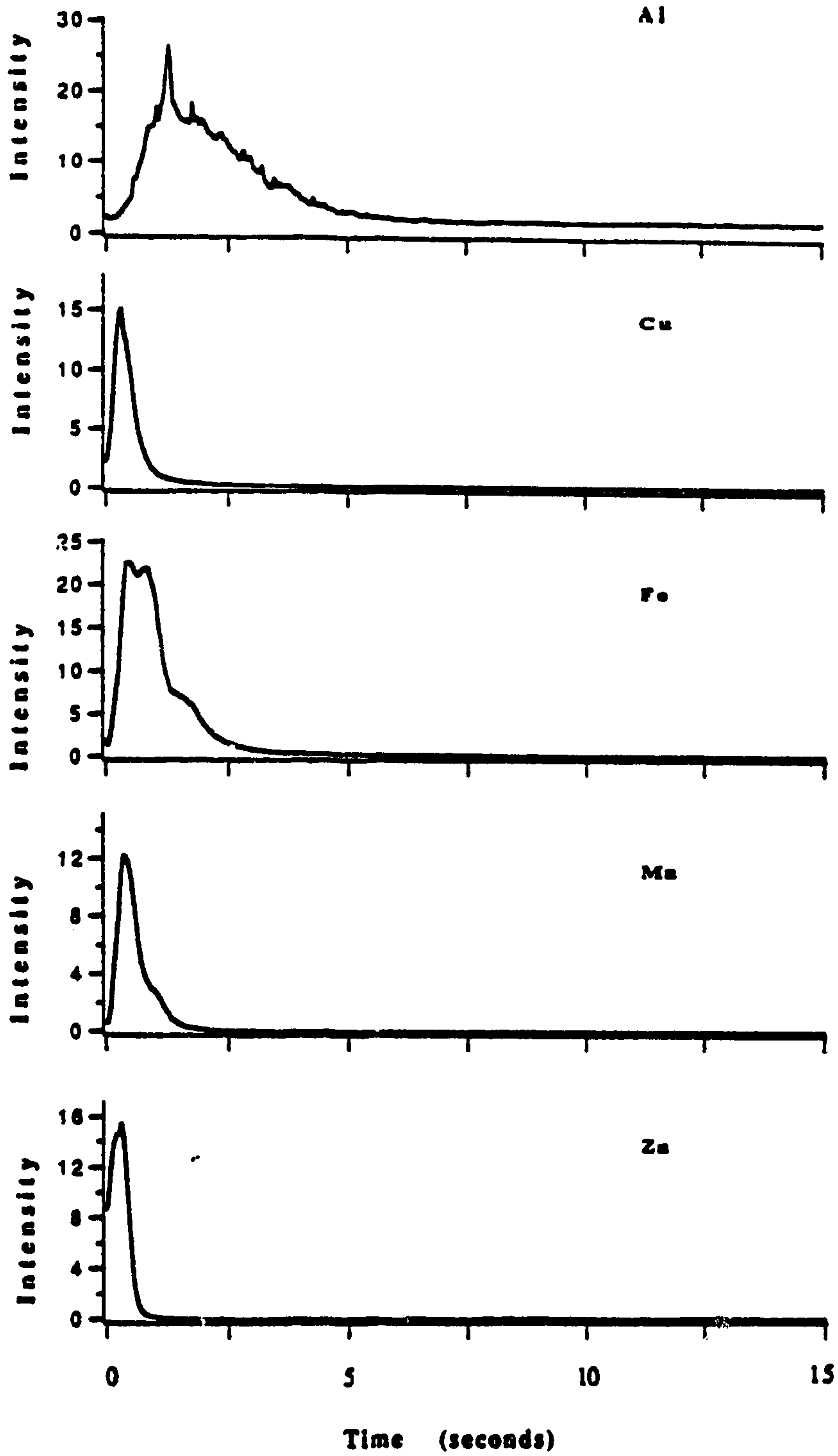

Figure 4.2 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Gluten (184)

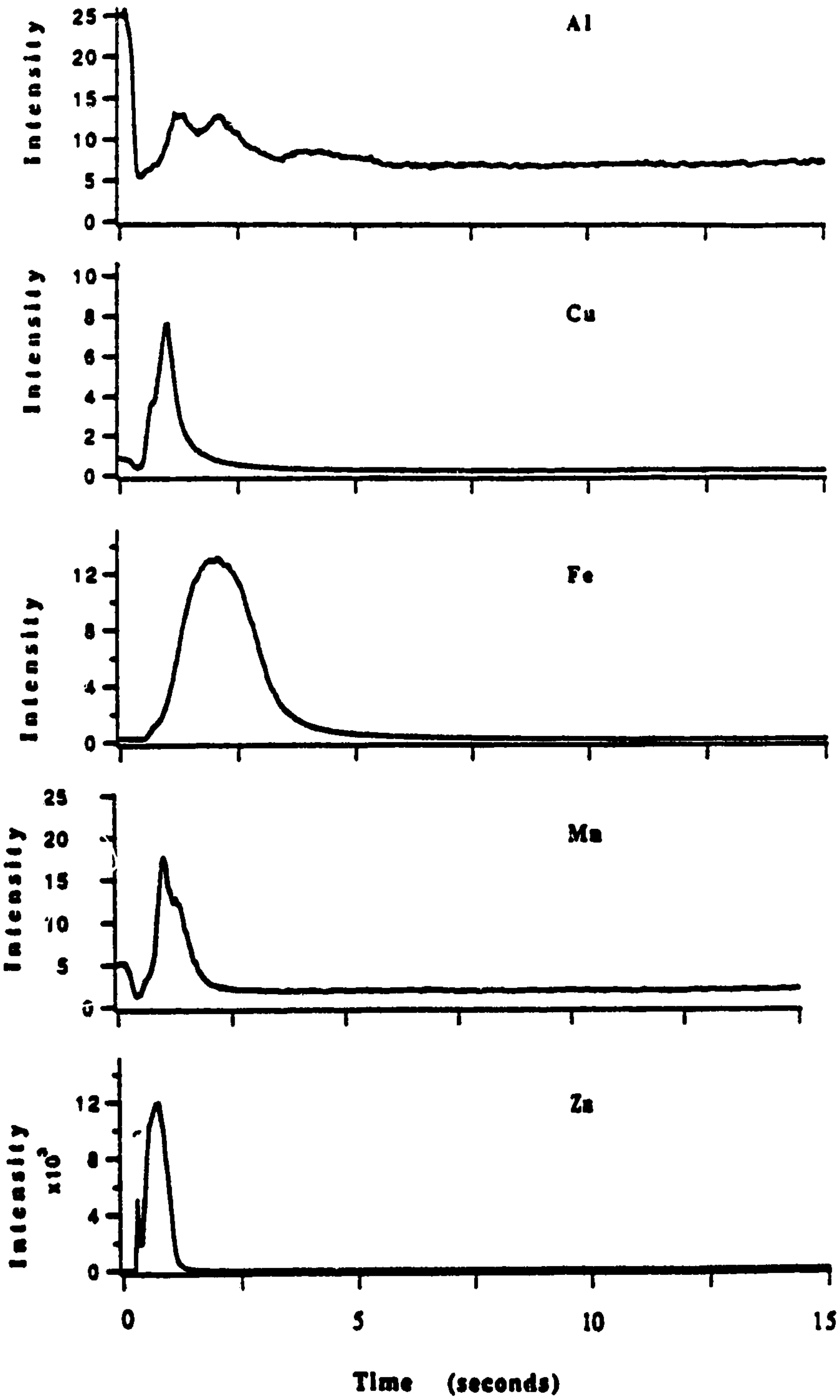

Figure 4.3 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Bovine Muscle (136)

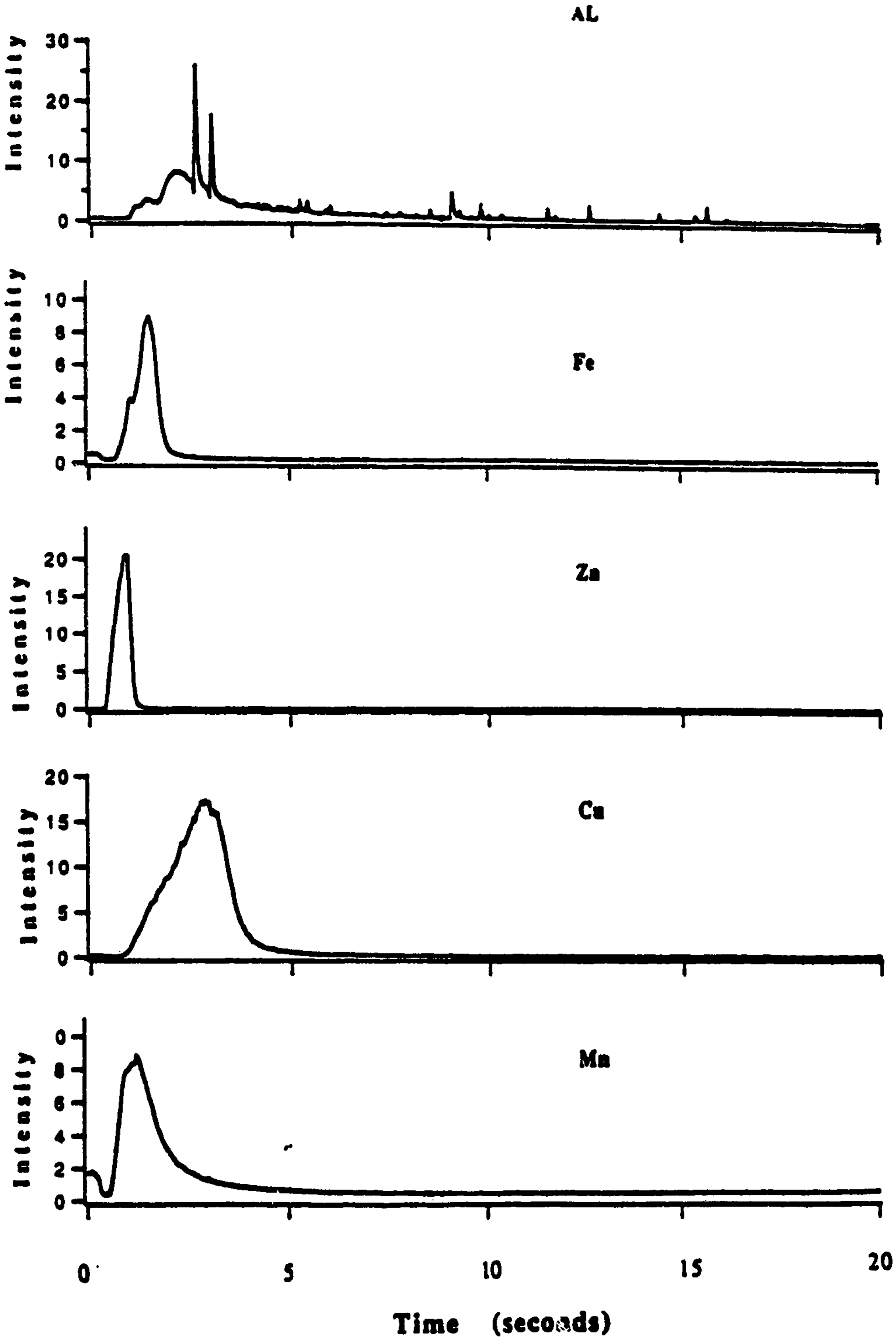

Figure 4.4 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Whole Egg Powder (183)

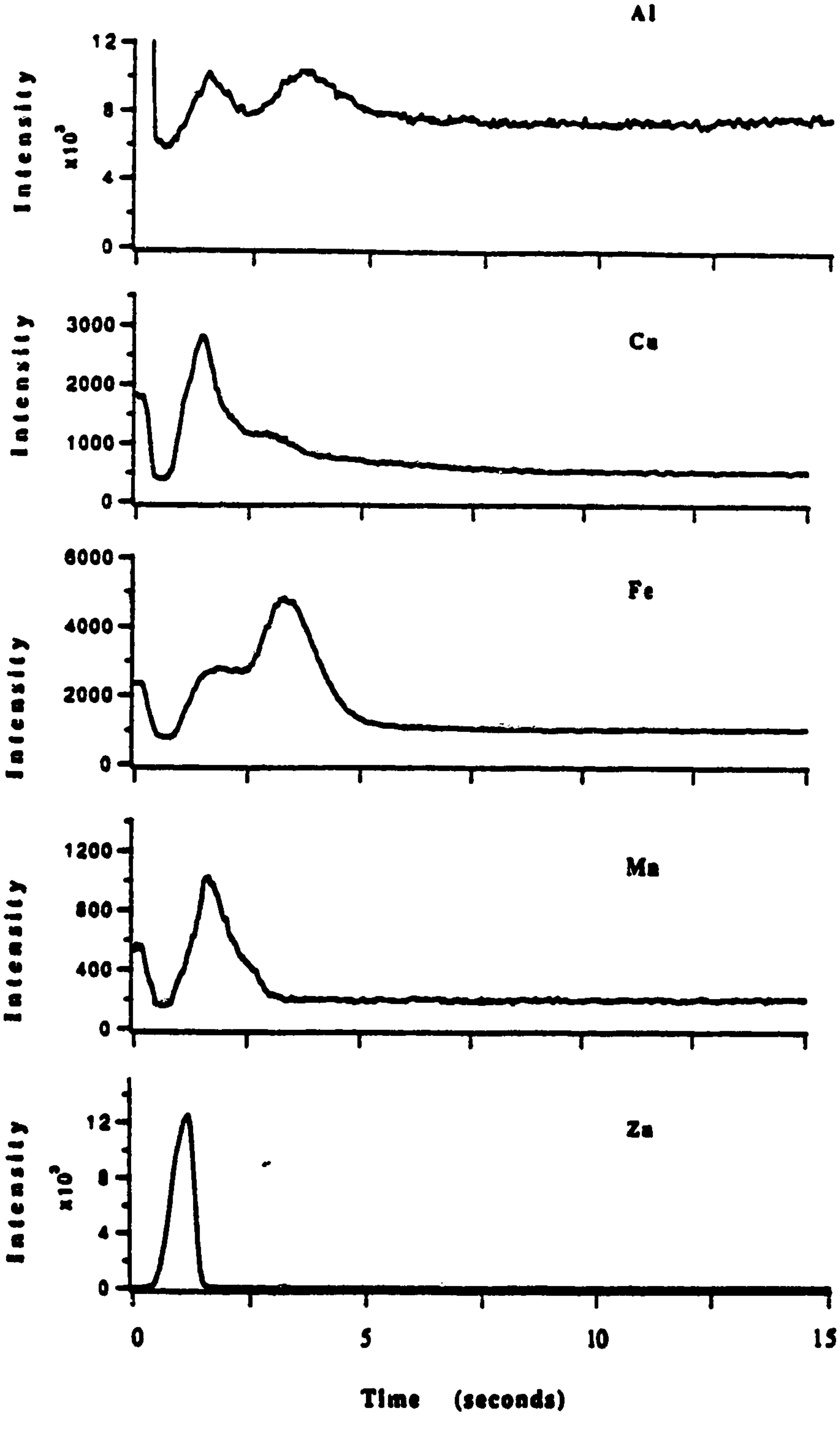

Figure 4.5 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Whole Milk Powder (188)

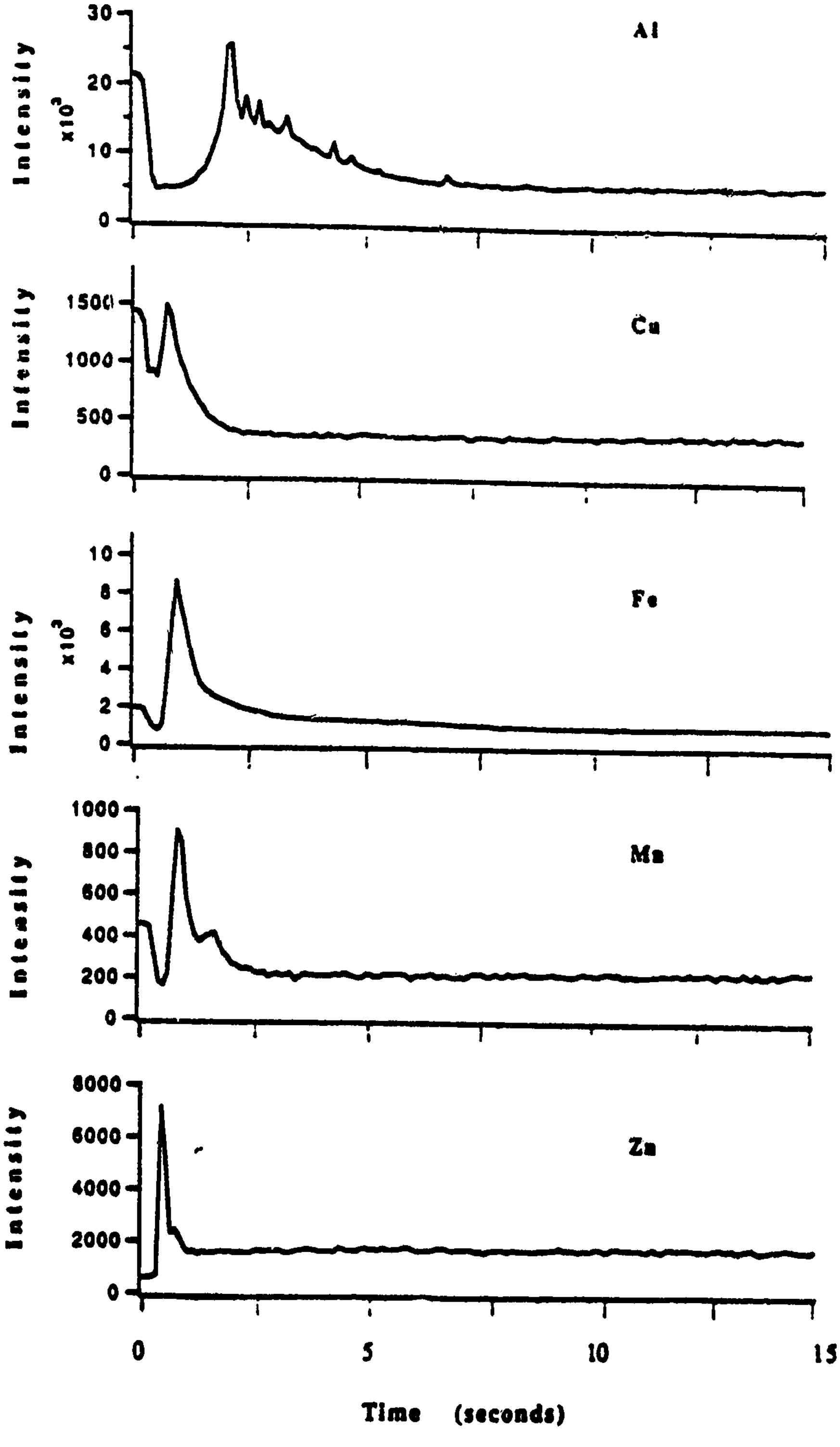

Figure 4.6 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Starch (162)

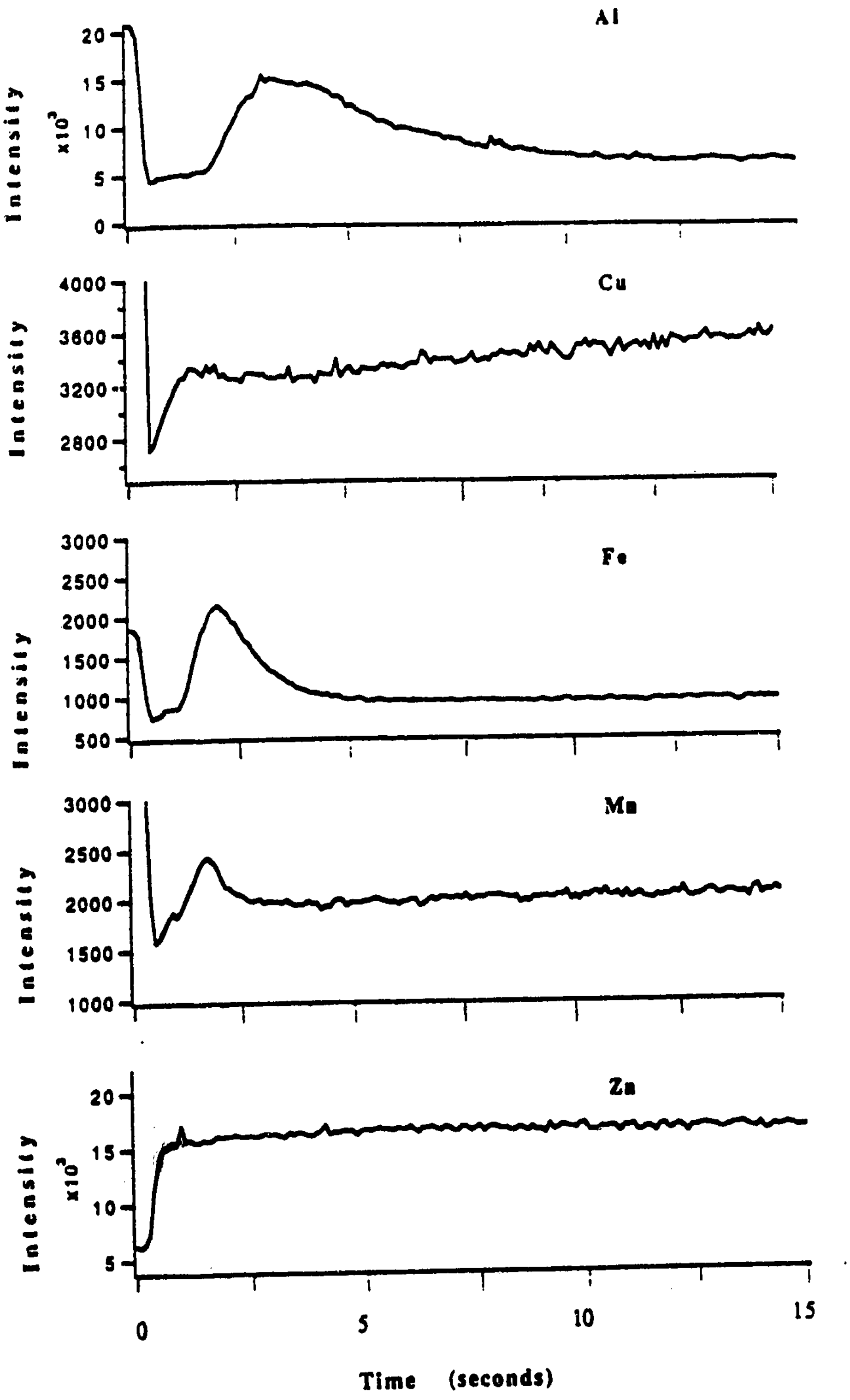

Figure 4.7 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Cellulose (189)

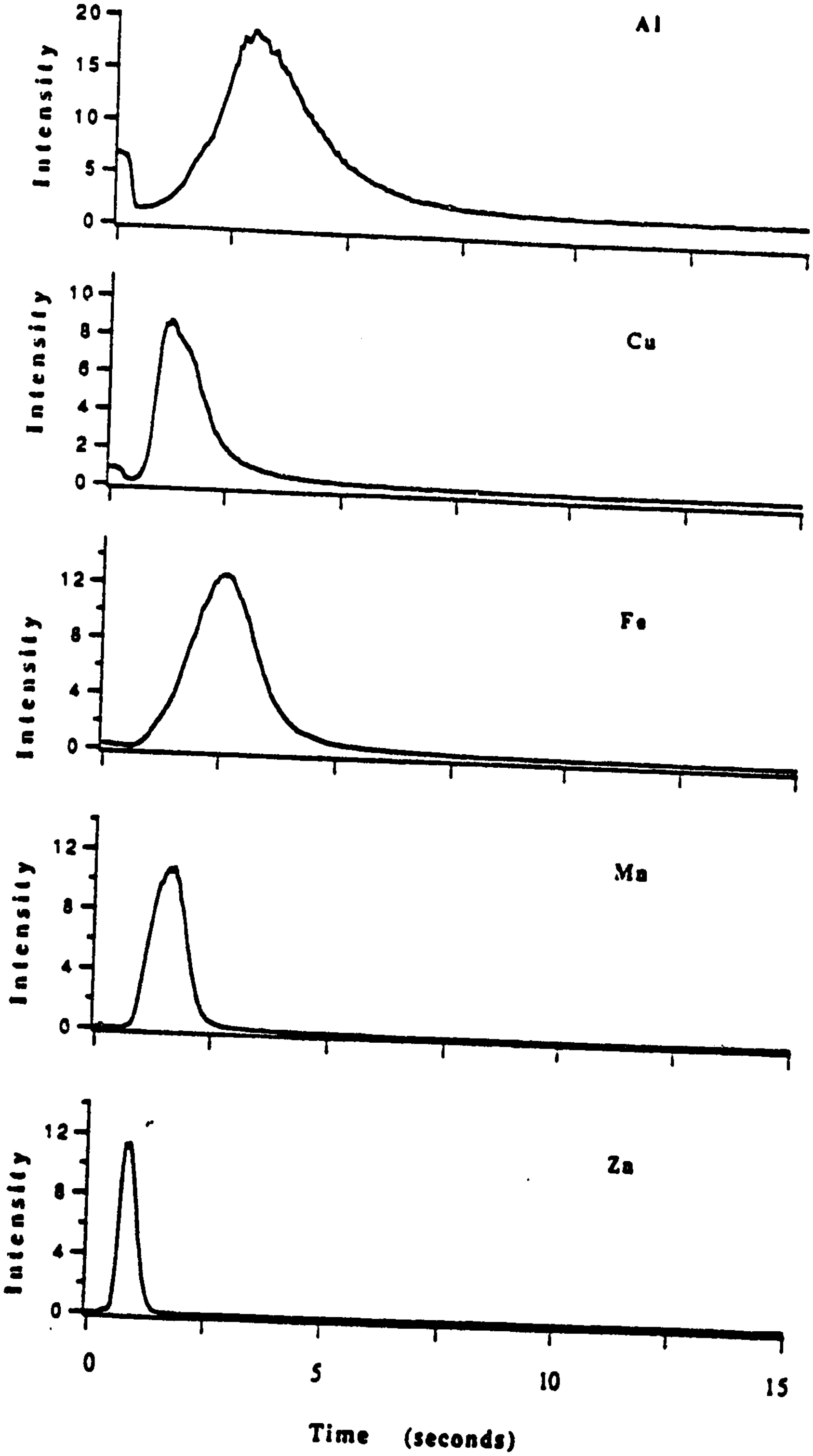

Figure 4.8 Plots of signal intensity (arbitrary units) for Al, Cu, Fe, Mn and Zn as a function of time (seconds) during atomization from graphite cup. Sample: Flour (187)

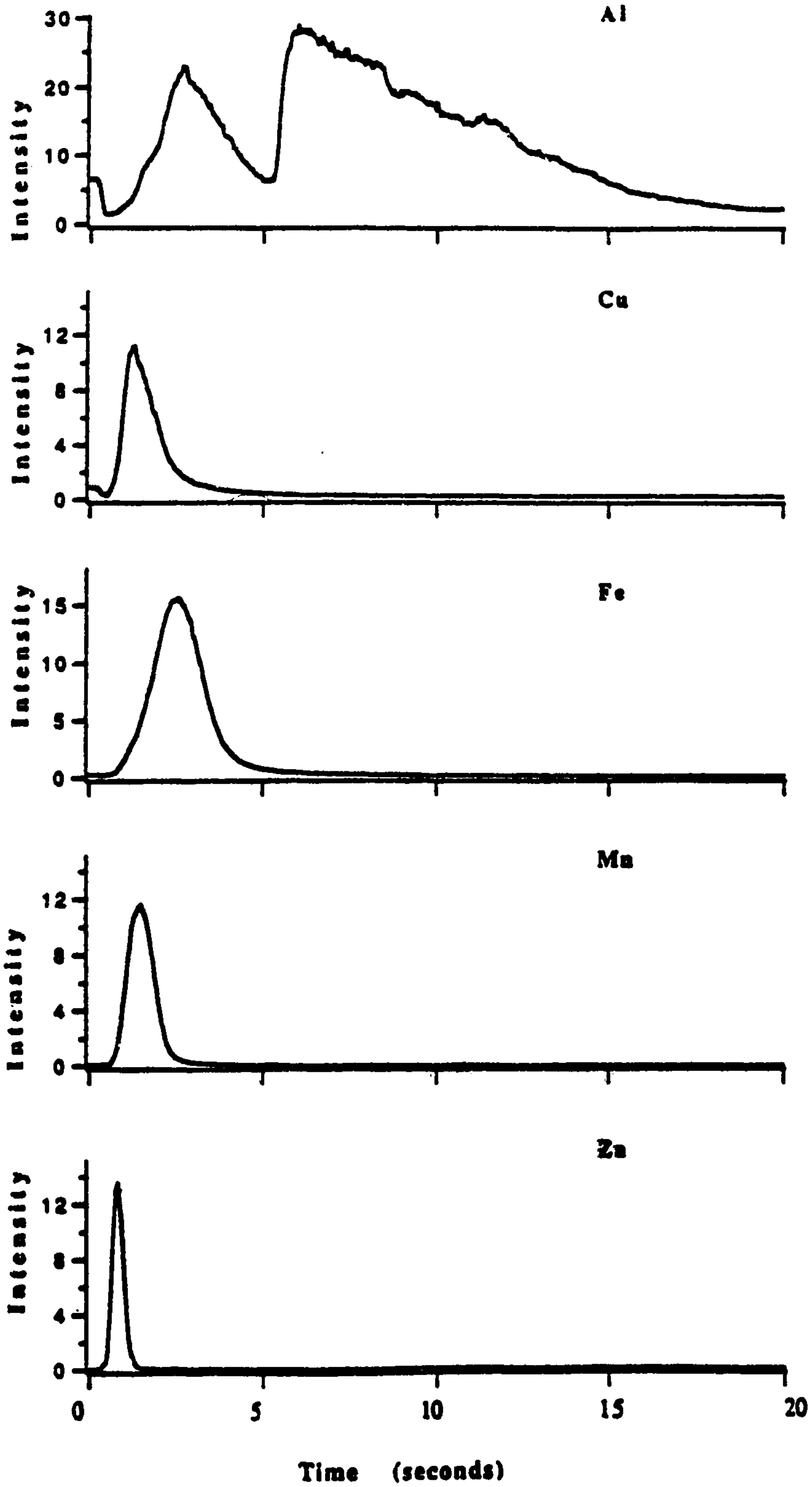

Figure 4.9 Emission intensity as a function of time for Al, Cu, Fe, Mn and Zn in a test portion of Flour (187)

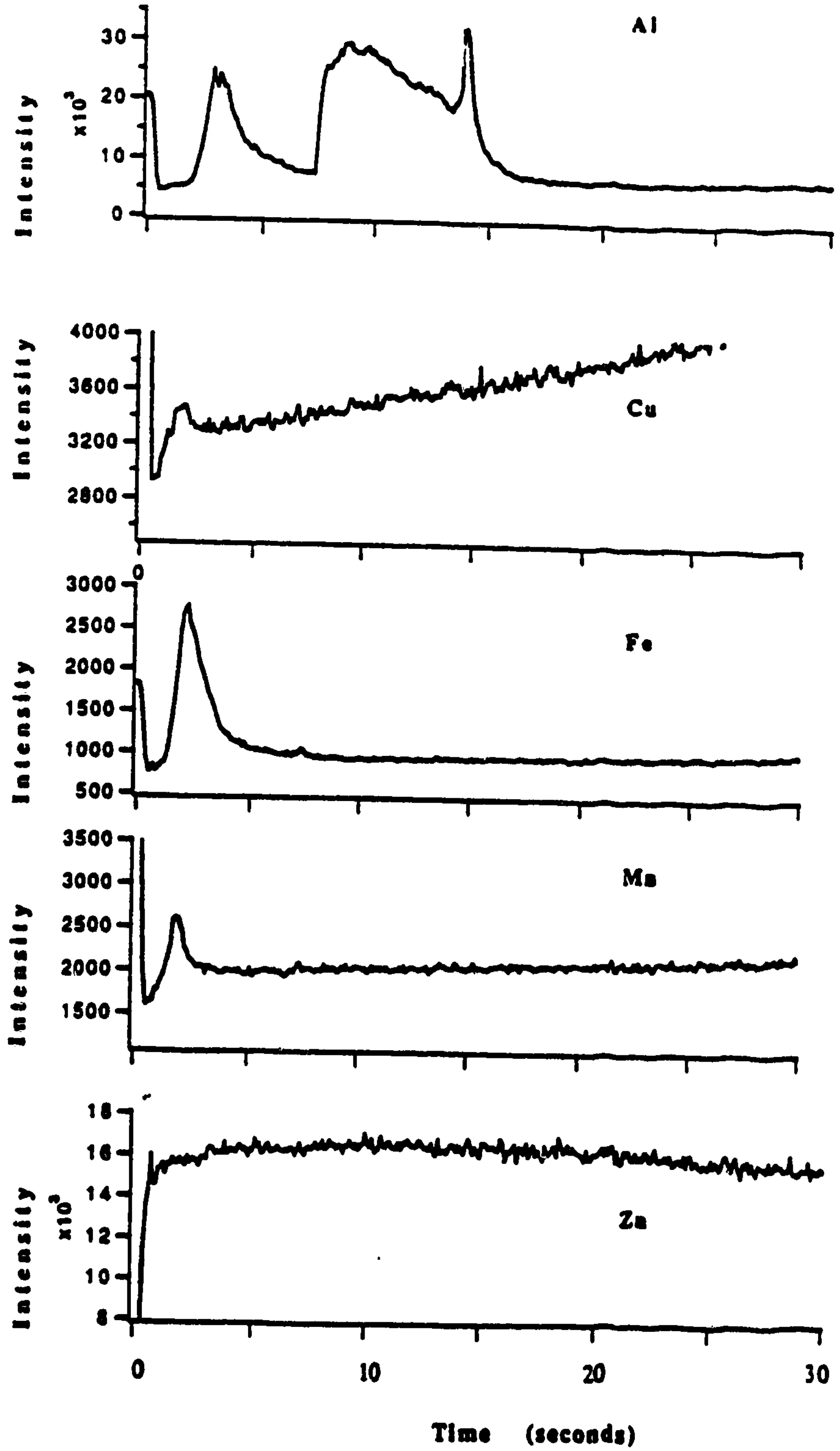

Figure 4.10 Emission intensity as a function of time for Al, Cu, Fe, Mn and Zn in a test portion of Cellulose (189)

Chapter 5

Summary and Suggestions for Future Work

5.1 Summary

During the course of this work, direct sample insertion ICP has shown promise for simultaneous multielement analysis. Working conditions have been optimized for the determination of Al, Zn, Fe, Cu and Mn in botanical samples. The method was applied to the determination of these elements in the NIST standard reference materials Rice Flour (SRM1568a), Wheat Flour (SRM1567a), Citrus Leaves (SRM1572), Corn Kernel (RM8413) and Corn Stalk (RM 8412). The results are generally in satisfactory agreement with NIST certified values.

The variability in distribution of five trace elements in Rice Flour (RM 1568a) and Wheat Flour (RM 1567a) from NIST was examined in depth. The distribution of aluminum in Rice Flour may be considered uniform at test portion sizes of 10 to 15 mg, but for Wheat Flour variability among test portions of this size is significant. The other four elements were found to be uniformly distributed in these two reference materials at test portion sizes of 10 to 15 mg. The variability in aluminum is speculated to be caused by the presence of small particles of mineral matter in the wheat flour.

The concentrations of Al, Fe, Cu, Zn and Mn in eight candidate agricultural reference materials from Agriculture Canada were also studied by mixed gas Ar-O_2 direct sample insertion ICP/AES. Most of the values found are comparable with published data where available. The assessment of these materials for within vs. between bottle homogeneity was done by one-way analysis of variance. All of the elements were evenly distributed in the samples at 10 mg test portion sizes, except for aluminum in flour and iron in cellulose, where hot spots were seen.

5.2 Future Work

Even though DSI-ICP-AES shows promise for the determination of trace metal concentrations in agricultural materials, there is still room for improvement. First, the small size of the test portions requires the analysis of a large number of portions if heterogeneity is present or suspected. An automatic sample insertion system would be a definite advantage for handling large numbers of samples. A sample tray holder and a computer controlled multi-purpose sample insertion system, along with automatic data collection and processing, are needed.

Second, the data acquisition system needs to be improved. Since the sample signal is transient, different elements and different sample materials have different peak profiles and rise times. Therefore the fixed channel data acquisition system should be changed to a more flexible arrangement to fit the data acquisition requirements of each channel. Another electronic system that needs to be improved is the amplifier, which should modified to adjust the gain automatically to match the sample signal requirements. In samples that contain high concentrations, the gain needs to be lowered in order to avoid saturation, whereas the gain needs to be raised to meet very low concentrations. Also, to improve background correction, the detector should be able to monitor the sample signal and background changes at the same time. It is likely a PDA combined with an Echelle optical system could do this job.

Third, the software controlling the system should be modified to be more user friendly. A menu driven, dialogue style program with on line help messages should be included in future software developments.

Finally, since ICP-MS has better detection limits than ICP-AES, using ICP-MS may give lower detection limits and allow more elements to be detected at the same time. Also, sometimes, information on isotope distribution is also useful, and ICP-MS provides this information. Since present ICP-MS sample introduction systems use solutions, aluminum cannot be determined by ICP-MS because aluminum has no isotope other than mass 27, a mass value that coincides with boron oxide. The boron arises from boric acid which is used to complex the excess HF used in the dissolution of aluminum-containing trace minerals in botanical materials. Therefore, DSI-ICP-MS might be used instead of solution nebulization ICP-MS. This approach would have all the ICP-AES advantages but in addition lower detection limits and isotope information on distributions. However, ICP-MS is not that compatable with transient signals, therefore some work must be done in this area.

Ultimately, the development of an automated ICP along with a self-training, self-optimized user friendly software package and incorporating the above suggestions would be a worthwhile goal.

APPENDIX A

This appendix contains the data files for the determination of aluminum, copper, zinc, iron and manganese in Cellulose, Starch, Bran, Gluten, Flour, Bovine Muscle, Whole Milk Powder and Whole Egg Powder. Four bottles of each material were analysed. The number of test portions from each bottle was six. The values with an asterisk fall outside the 90% confidence interval, therefore they were not used for calculation of the means or for statistical analysis.

Starch

	Bottle No.	Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)	Al (µg/g)
1	142	0.13	0.13	1.37	0.06[illegible]	2.41
2	142	0.12	0.11	0.92	0.055	1.93
3	142	0.11	0.09	1.54	[illegible]	2.22
4	142	0.11	0.13	0.81	[illegible]	1.73
5	142	0.11	0.05	0.87	0.049	1.50
6	142	0.09	0.16	1.10	0.057	2.12
7	869	0.09	0.10	0.99	0.054	1.91
8	869	0.12	0.09	0.94	0.061	1.67
9	869	0.11	0.12	1.05	0.053	1.78
10	869	0.16	0.19	0.98	0.058	1.8[illegible]
11	869	0.14	0.22	1.54	0.068	2.26
12	869	0.11	0.15	0.67	0.046	1.78
13	1591	0.12	0.16	0.84	0.056	2.18
14	1591	0.11	0.07	0.68	0.041	1.67
15	1591	0.23*	0.11	0.66	0.040	1.85
16	1591	0.12	0.15	1.13	0.058	2.04
17	1591	0.12	0.18	0.84	0.054	1.87
18	1591	0.12	0.13	1.21	0.061	2.18
19	140	0.15	0.15	0.83	0.053	1.90
20	140	0.18	0.15	0.90	0.036	1.70
21	140	0.12	0.07	0.72	0.049	1.83
22	140	0.13	0.16	0.86	0.050	2.13
23	140	0.14	0.15	0.97	0.055	2.10
24	140	0.11	0.07	2.3*	0.076	1.75

Bovine Muscle

	Bottle No.	Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Al (µg/g)	Mn (µg/g)
1	1817	3.09	112.0	71	1.95	0.35
2	1817	3.05	100.0	65	1.93	0.30
3	1817	2.63	99.0	77	2.49	0.32
4	1817	2.59	109.0	60	1.77	0.29
5	1817	3.00	98.0	69	2.11	0.32
6	1817	2.69	116.0	63	1.82	0.30
7	57	2.81	106.0	63	1.05	0.29
8	57	3.00	112.0	68	1.78	0.33
9	57	2.69	101.0	57	1.75	0.27
10	57	2.99	120.0	73	1.80	0.41
11	57	2.93	114.0	62	1.80	0.31
12	57	2.80	97.0	60	1.66	0.28
13	422	2.03	64.5	44	1.10	0.20
14	422	2.00	94.2	53	1.28	0.25
15	422	3.12	107.0	69	2.27	0.32
16	422	2.66	103.0	78	2.14	0.38
17	422	2.97	99.0	71	4.7*	0.32
18	422	2.69	105.0	61	1.93	0.28
19	3019	2.94	113.0	68	1.85	0.33
20	3019	3.18	105.0	66	1.74	0.34
21	3019	3.14	95.0	64	1.89	0.30
22	3019	2.98	104.0	68	1.74	0.33
23	3019	3.00	117.0	68	1.93	0.33
24	3019	2.56	105.0	56	1.66	0.27

Whole Egg Powder

	Bottle No.	Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)	Al (µg/g)
1	751	4.64	92.2	155	2.48	445
2	751	3.80	65.3	132	1.89	507
3	751	4.69	80.8	165	2.29	582
4	751	3.88	70.1	139	1.94	538
5	751	3.07	59.2	107	1.49	432
6	751	3.17	57.0	112	1.55	415
7	1930	3.04	65.9	124	1.68	488
8	1930	3.51	69.5	135	1.82	555
9	1930	3.75	69.5	129	1.85	463
10	1930	3.05	66.5	109	1.55	455
11	1930	3.02	57.8	108	1.53	418
12	1930	3.36	60.0	116	1.62	430
13	329	3.71	70.1	129	1.75	524
14	329	3.82	76.0	133	1.86	536
15	329	4.04	83.8	138	1.98	549
16	329	3.15	59.2	114	1.55	456
17	329	3.14	61.7	110	1.56	426
18	329	3.96	79.0	138	1.92	566
19	512	3.57	67.7	127	1.75	514
20	512	4.10	77.2	151	2.09	565
21	512	2.96	55.1	101	1.43	418
22	512	2.37	53.0	89	1.27	367
23	512	2.72	56.7	99	1.37	401
24	512	2.36	54.7	88	1.23	388

Whole Milk Powder

	Bottle No.	Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)	Al (µg/g)
1	295	0.64	32.0	1.93	0.16	0.80
2	295	0.55	32.5	1.67	0.14	0.77
3	295	0.53	31.5	1.59	0.13	0.76
4	295	0.57	32.3	1.74	0.14	0.81
5	295	0.57	30.7	1.73	0.12	0.99
6	295	0.41	25.9	1.33	0.11	0.65
7	1235	0.50	36.4	1.84	0.14	0.46
8	1235	0.51	32.9	1.66	0.14	0.77
9	1235	0.48	32.1	1.59	0.13	0.30
10	1235	0.51	30.4	1.65	0.14	0.87
11	1235	0.45	28.1	1.44	0.12	0.65
12	1235	0.49	32.0	1.72	0.14	1.04
13	997	0.50	34.2	1.69	0.13	0.04*
14	997	0.54	34.8	1.74	0.14	0.65
15	997	0.55	34.5	1.82	0.15	0.54
16	997	0.51	32.0	1.82	0.14	0.76
17	997	0.52	29.5	1.61	0.13	0.72
18	997	0.41	26.4	1.31	0.10	0.48
19	1262	0.57	32.5	1.64	0.14	0.63
20	1262	0.46	26.3	1.35	0.11	0.44
21	1262	0.42	33.2	1.64	0.13	0.02*
22	1262	0.48	30.2	1.62	0.13	0.72
23	1262	0.52	32.0	1.56	0.13	0.42
24	1262	0.52	30.8	1.59	0.13	0.58

Bran

	Bottle No.	Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)	Al (µg/g)
1	390	3.03	21.2	18.8	3.6	3.14*
2	390	3.25	21.8	18.3	3.6	1.19
3	390	3.06	19.0	17.8	3.2	0.84
4	390	3.07	22.1	17.2	3.2	0.95
5	390	2.72	14.0	14.6	2.7	0.64
6	1428	3.03	22.2	17.3	3.5	0.76
7	1428	2.89	15.8	16.6	3.2	2.79
8	1428	3.24	22.9	16.9	3.2	1.30
9	1428	3.01	20.3	15.7	3.0	1.44
10	312	3.22	17.6	17.9	3.6	1.04
11	312	3.10	19.2	17.8	3.5	0.89
12	312	3.09	15.8	16.4	3.3	1.17
13	312	3.14	23.2	16.7	3.3	1.10
14	312	2.94	20.2	16.0	3.0	0.76
15	312	2.75	18.9	14.9	2.9	0.85
16	927	3.22	23.5	19.1	3.8	0.66
17	927	2.97	21.1	16.6	3.3	1.17
18	927	3.05	21.7	16.3	3.1	1.10
19	927	2.93	22.2	16.1	2.9	1.45

Cellulose

	Bottle No.	Cu (µg/g)	Fe (µg/g)	Mn (µg/g)	Al (µg/g)
1	479	0.08*	0.85	0.045	3.05*
2	479	0.169	0.79	0.055	4.31
3	479	0.151	0.84	0.060	4.12
4	479	0.134	0.79	0.055	2.85
5	479	0.163	0.92	0.055	4.27
6	662	0.154	0.71	0.049	4.62
7	662	0.182	0.78	0.063	5.07
8	662	0.142	0.69	0.049	6.10
9	662	0.136	0.71	0.040	3.84
10	662	0.134	0.72	0.039	8.57
11	662	0.146	0.71	0.050	3.79
12	1791	0.15	0.77	0.054	4.39
13	1791	0.14	0.69	0.053	4.22
14	1791	0.13	0.81	0.044	3.76
15	1791	0.13	0.69	0.042	3.83
16	1791	0.16	0.69	0.049	4.11
17	1791	0.13	0.71	0.044	3.39
18	497	0.13	0.83	0.053	4.08
19	497	0.13	0.68	0.042	3.52
20	497	0.14	0.80	0.049	3.99
21	497	0.13	0.82	0.047	3.87
22	497	0.14	0.69	0.053	4.07
23	497	0.14	0.84	0.045	3.81

Flour

	Bottle No.	Cu (µg/g)	Zn (µg/g)	Fe (µg/g)	Mn (µg/g)	Al (µg/g)
1	935	4.03	28.2	43.0	16.2	13.3
2	935	4.70	27.7	42.1	17.6	11.9
3	935	4.77	25.4	42.9	16.5	12.6
4	935	4.18	20.5	36.0	13.9	10.8
5	935	4.92	25.6	40.2	16.9	12.6
6	935	5.03	24.7	46.1	16.6	20.1*
7	640	4.62	26.5	44.1	18.7	17.2
8	640	4.35	22.6	37.4	14.8	11.6
9	640	5.03	26.8	41.0	17.2	11.8
10	640	5.14	25.5	81.4*	17.7	23.3
11	640	4.95	25.4	39.8	16.2	12.6
12	640	5.31	26.2	41.8	17.2	13.3
13	971	4.76	22.2	46.1	16.5	17.7
14	971	4.95	27.2	46.3	16.9	46.9*
15	971	5.32	26.2	50.2	17.5	22.6
16	971	4.98	24.7	41.8	16.2	17.3
17	971	5.17	26.1	41.9	17.4	14.9
18	971	5.15	26.1	75.2*	18.1	23.1
19	489	4.72	28.0	44.4	18.4	12.8
20	489	5.02	27.1	41.5	17.2	34.5*
21	489	4.79	25.5	40.7	16.5	17.2
22	489	5.58	23.6	46.5	17.7	19.6
23	489	4.97	23.6	45.8	15.8	12.3
24	489	4.86	22.3	35.3	13.5	11.6

Gluten

	Bottle No.	Cu (ug/g)	Zn (ug/g)	Fe (ug/g)	Mn (ug/g)	Al (ug/g)
1	1328	6.60	31.3	68.2	15.0	11.5
2	1328	5.55	34.1	57.1	14.4	8.6
3	1328	4.76	34.1	52.9	12.1	8.4
4	1328	5.04	35.2	49.5	11.6	8.9
5	1328	3.58	26.6	35.2	8.5	7.0
6	1288	4.78	27.1	49.0	11.5	7.9
7	1288	4.47	28.8	46.8	11.9	6.8
8	1288	5.11	33.5	55.6	13.2	8.9
9	1288	4.27	32.4	45.7	11.1	7.4
10	1288	4.38	34.1	44.2	10.8	7.0
11	1288	5.16	36.0	52.6	12.5	8.7
12	532	4.58	29.6	49.1	11.8	7.6
13	532	4.85	29.9	48.4	12.1	7.8
14	532	4.23	26.6	41.5	10.0	8.8
15	532	4.30	32.0	43.1	10.5	7.5
16	532	4.10	30.1	41.4	9.8	7.8
17	639	5.34	23.7	55.7	13.0	9.4
18	639	5.45	34.5	59.4	14.4	9.5
19	639	5.45	42.1	56.4	13.4	9.3
20	639	3.95	24.1	38.8	9.3	6.2
21	639	4.05	28.8	43.7	9.7	8.0

APPENDIX B

This appendix contains the data files for aluminum, copper, zinc, iron and manganese in 10 µL of various standard solutions, and in NIST Rice Flour and Wheat Flour. The Rice Flour and Wheat Flour data include two sets. In the first set the total Rice flour and Wheat Flour comprised 513.8 mg and 524.3 mg respectively. In the second set the total Rice Flour and Wheat Flour comprised 495.7 mg and 495.3 mg respectively. Each total was weighed into 31 to 35 test portions in graphite cups.

10 µg/g Solution

	No.	Al (ug/g)	Cu (ug/g)	Fe (ug/g)	MN (ug/g)	Zn (ug/g)
1	1	10.62	10.56	11.13	9.34	11.29
2	2	9.93	9.84	10.24	8.64	9.14
3	3	10.06	10.03	10.38	8.78	11.12
4	4	9.49	9.82	10.10	8.56	11.03
5	5	9.41	9.67	9.76	8.25	9.92
6	6	9.93	10.45	10.32	8.92	11.29
7	7	9.95	10.13	11.60	8.48	9.23
8	8	10.24	10.39	10.15	8.62	9.20
9	9	10.00	10.28	10.43	8.76	10.46
10	10	9.31	9.52	9.93	8.34	10.03
11	11	9.08	9.50	9.45	7.97	10.72
12	12	9.67	9.75	9.87	8.48	12.04
13	13	10.13	10.34	10.07	8.62	8.51
14	14	9.57	9.73	9.17	7.75	10.69
15	15	10.49	10.81	10.18	8.73	9.83
16	16	9.93	10.45	9.87	8.23	8.97
17	17	9.95	10.32	9.96	8.31	8.42
18	18	10.44	10.70	10.37	8.81	9.80
19	19	9.52	10.30	9.73	8.23	9.23

Rice Flour (1)

	Al (ug/g)	Cu (ug/g)	Fe (ug/g)	Mn (ug/g)	Zn (ug/g)
1	5.62	3.45	12.16		23.73
2	6.87	4.09	15.35	36.93	32.16
3	4.95	3.66	14.09	33.58	26.37
4	6.44	3.79	13.50	33.02	28.43
5	4.59	3.47	13.25	32.46	27.26
6	4.38	3.43	6.94	29.67	24.13
7	4.28	3.39	11.35	28.55	24.19
8	4.85	3.56	11.83	29.67	23.50
9	4.77	3.50	12.41	33.02	28.72
10	4.56	3.43	11.13	27.71	22.53
11	4.72	3.35	11.21	27.71	22.41
12	2.49	1.74	6.38	17.77	15.13
13	4.82	3.52	10.99	27.16	21.98
14	3.59	2.82	9.43	24.31	20.26
15	5.85	3.39	10.60	27.16	22.04
16	4.31	2.88	10.04	27.16	21.29
17	5.10	3.18	11.35	27.71	24.62
18	4.62	3.28	11.32	27.99	22.33
19	4.74	2.90	10.26	23.94	20.89
20	5.28	3.31	12.33	28.55	23.50
21	5.13	3.12	10.77	26.88	23.59
22	4.72	3.12	11.05	27.16	24.13
23	3.31	2.21	8.92	21.54	19.29
24	5.36	3.37	12.16	29.95	26.48
25	4.92	3.01	10.40	23.47	19.23
26	4.82	3.26	10.91	26.01	22.90
27	5.85	2.97	11.19	26.09	23.99
28	3.92	2.25	8.95	21.32	17.14
29	4.41	2.61	10.57	26.29	19.90
30	3.85	1.99	8.73	20.68	16.97
31	4.67	2.59	11.02	24.93	19.49

Wheat Flour (1)

	No.	Al (ug/g)	Cu (ug/g)	Fe (ug/g)	MN (ug/g)	Zn (ug/g)
1	1	6.18	1.78	21.68	12.69	15.10
2	2	12.62	1.95	17.41	10.79	12.24
3	3	3.00	1.14	13.08	8.67	8.02
4	4	3.95	1.78	18.42	11.83	13.18
5	5	4.26	1.81	17.97	11.52	14.10
6	6	2.90	1.61	15.99	10.24	12.01
7	7	4.82	1.51	15.65	10.12	11.26
8	8	3.02	1.42	14.98	9.37	10.40
9	9	10.60	1.61	15.90	9.84	11.49
10	10	3.77	1.47	14.28	9.31	11.29
11	11	4.74	1.62	24.65	9.57	11.49
12	12	3.61	1.66	16.88	10.35	12.61
13	13	4.05	1.64	17.30	10.57	13.33
14	14	4.90	1.78	18.11	11.35	14.07
15	15	7.11	1.30	13.98	8.17	9.20
16	16	3.00	1.49	14.65	9.26	10.09
17	17	4.51	1.51	16.24	9.65	11.21
18	18	4.51	1.79	16.74	10.07	12.29
19	19	3.51	1.43	15.40	8.64	10.00
20	20	3.85	1.38	14.48	8.56	10.63
21	21	13.14	1.40	15.96	9.20	10.63
22	22	3.44	1.51	16.18	8.78	10.26
23	23	4.67	1.49	15.85	9.23	11.26
24	24	3.00	1.45	14.26	8.76	10.95
25	25	4.44	1.57	16.41	13.03	11.43
26	26	4.62	1.47	14.90	8.87	10.49
27	27	5.93	1.30	14.26	8.25	9.60
28	28	3.62	1.47	29.67	15.79	10.89
29	29	3.23	1.45	15.18	9.17	11.21
30	30	3.56	1.59	16.49	9.71	12.89
31	31	2.54	0.70	12.30	5.91	6.45
32	32	3.38	1.47	14.81	8.50	9.80
33	33	3.90	1.23	13.45	7.72	10.14
34	34	5.33	2.35	13.42	6.97	10.23

Rice Flour (2)

	Cu (μg/g)	Zn (μg/g)	Fe (μg/g)	Mn (μg/g)	Al (μg/g)
1	2.7	23.1	8.58	—	4.19
2	2.9	26.2	9.63	21.3	5.60
3	2.8	20.6	8.77	17.1	5.13
4	2.9	19.6	7.95	17.0	3.40
5	2.8	20.1	7.90	17.3	4.07
6	2.8	25.0	9.27	17.6	6.04
7	2.9	22.5	8.04	17.6	5.36
8	3.0	22.5	7.90	17.1	5.73
9	3.0	22.5	8.08	17.7	5.47
10	2.8	22.6	7.81	17.3	6.43
11	2.9	23.4	7.76	17.5	5.36
12	2.9	22.6	7.31	16.7	4.73
13	3.1	23.6	8.36	18.3	5.87
14	2.7	22.1	8.40	18.5	5.54
15	2.6	21.3	7.40	16.9	4.92
16	2.6	20.5	8.00	16.8	4.95
17	2.6	19.3	7.63	16.8	4.34
18	2.9	21.1	8.58	17.4	5.47
19	2.5	22.4	7.40	17.2	4.32
20	2.4	21.7	6.90	16.1	4.24
21	2.6	21.0	8.72	16.3	5.18
22	2.5	21.6	6.90	16.0	4.81
23	2.7	24.6	16.30	17.1	4.96
24	2.6	21.4	7.03	16.2	4.59
25	2.4	20.4	6.58	15.1	4.31
26	2.5	24.6	6.80	17.3	4.03
27	2.4	18.3	6.26	14.2	4.09
28	2.5	18.7	6.85	15.7	4.35
29	2.5	21.3	7.53	16.5	4.62
30	2.4	19.3	6.62	14.5	4.83
31	2.4	19.6	6.48	14.8	4.42
32	2.6	19.9	6.76	15.2	4.59

Wheat Flour (2)

	Cu (μg/g)	Zn (μg/g)	Fe (μg/g)	Mn (μg/g)	Al (μg/g)
1	2.84	16.3	15.2	10.4	5.04
2	2.56	14.4	13.2	8.9	4.96
3	2.47	16.0	14.1	9.6	4.49
4	2.68	15.2	14.7	9.6	7.68
5	2.78	15.9	14.3	9.6	4.99
6	2.26	16.1	17.8	9.4	5.29
7	2.48	15.2	14.0	9.2	5.61
8	2.09	15.5	13.6	8.7	6.21
9	2.41	14.1	12.6	8.7	5.41
10	2.51	15.8	13.9	9.6	3.87
11	2.35	16.5	14.1	11.6	3.85
12	2.62	15.4	14.7	9.0	4.58
13	2.69	14.1	16.0	9.0	5.88
14	2.47	16.5	16.8	9.6	5.75
15	2.50	14.1	13.6	9.2	4.44
16	2.32	15.7	16.6	9.7	15.80
17	2.56	14.3	14.1	9.2	4.54
18	2.62	16.0	17.8	9.6	5.15
19	2.48	13.7	12.1	8.0	5.49
20	2.42	14.1	13.9	8.7	5.47
21	2.54	16.0	12.7	8.5	5.10
22	2.60	13.5	12.4	8.5	5.86
23	2.32	14.9	13.2	8.4	5.81
24	2.63	15.0	15.3	8.6	4.37
25	2.74	16.3	12.9	8.8	7.97
26	2.30	15.1	15.9	8.9	4.83
27	2.43	17.1	13.0	8.9	6.19
28	2.72	14.2	13.0	8.4	6.13
29	2.55	15.1	13.0	8.5	5.41
30	2.40	15.9	16.5	9.3	5.15
31	2.74	15.8	14.7	9.7	6.93
32	2.28	15.3	12.8	8.3	4.38
33	2.60	15.1	16.4	8.5	6.28
34	2.27	17.3	12.5	8.5	4.56
35	1.93	14.7	11.0	7.6	3.28

APPENDIX C

This appendix contains the results for aluminum, copper, zinc, iron and manganese determinations in NIST Citrus Leaves, Corn Kernel and Corn Stalk. The values with an asterisk fall outside the 90% confidence interval, therefore they were not used for calculation of the means or for statistical analysis.

	Al (μg/g)	Cu (μg/g)	Zn (μg/g)	Fe (μg/g)	Mn (μg/g)
Citrus Leaves	77.6	16.0	23.2	72.0	13.2
	77.6	15.5	18.6	76.8	16.0
	88.8	16.7	37.7	70.8	13.8
	104.8	19.4	28.6	88.7	17.9
Corn Stalk	69.8	8.6	44.3	158	13.3
	64.2	7.8	41.1	126	12.9
	68.6	9.1	28.4	131	12.9
	72.2	8.9	40.4	135	14.5
	68.8	8.0	38.0	120	12.1
Corn Kernel	4.9	3.0	—	50.2*	3.9
	4.9	2.9	19.3	19.8	3.8
	2.0	2.8	18.1	18.8	3.7
	4.2	2.9	19.1	19.3	3.8
	2.9	3.2	18.6	20.6	3.6
	6.5	2.9	18.1	28.3	3.6
	8.1	3.1	19.0	19.0	3.6
	—	2.8	20.2	22.2	3.6

APPENDIX D

This appendix contains the aluminum values generated by GFAAS and INAA in Corn Kernel, Corn Stalk, Cellulose, Starch, Bran, Gluten, Flour, Bovine Muscle, Whole Milk Powder and Whole Egg Powder. Data is from Reference 14 in Chapter 1.

Sample	Al by INAA, μg/g	Al by GFAAS, μg/g
Flour	15 ± 1	13 ± 1
Gluten	11.6 ± 0.9	11 ± 1
Bran	0.6 ± 0.3	1.4 ± 0.3
Egg Powder	585 ± 12	499 ± 39
Bovine Muscle	2.5 ± 0.6	0.98 ± 0.28
Milk Powder	—	0.44 ± 0.25
Cellulose	4.4 ± 1.1	3.8 ± 0.7
Starch	1.8 ± 0.2	2.2 ± 0.6
Corn Kernel	4.8 ± 0.4	3.9 ± 0.3
Corn Stalk	105 ± 21	76 ± 5