ATOMIC ABSORPTION STUDIES

IN

CLINICAL AND FORENSIC ANALYSIS

by

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ABSTRACT

A review is presented of the role of trace metals in biological systems. Concentrations of trace metals to be found in human tissues are listed, and the analytical methods for determining these are discussed, with special reference to atomic absorption spectrometry and to the methodology of trace analysis of biological material. Graphite tube atomization atomic absorption spectrometry is applied to the determination of several trace metals in urine, with both direct injection of the sample into the graphite furnace and with preliminary solvent extraction. Liver is analysed for several trace metals by the same technique after digestion with either a nitric acid / sulphuric acid mixture or with tetramethyl ammonium hydroxide. A study has been made of the matrix modification of several elements by acids and hydrogen peroxide, with particular reference to the stabilization of mercury in the graphite tube with hydrogen peroxide and hydrochloric acid. A detailed examination is presented of the factors involved in producing the bending of analytical growth curves which was observed with the experimental system employed.

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PREFACE

The work described in this thesis was carried out in the Chemistry Department of Imperial College of Science and Technology between October 1973 and June 1976. It is entirely original except where due reference is made, and no part has been previously submitted for any other degree.

The units, symbols and abbreviations used in this thesis are a combination of those recommended by the Working Party of Medical Editors (608) and those recommended by Applied Spectroscopy (609). It is hoped that the nomenclature and units presented are consistent, if not strictly SI. In Chapter 7 many of the units and calculations are in cgs units. The majority of the equations used in this chapter originate in the monograph of Mitchell and Zemansky (361), which employs cgs units throughout. Changing from cgs units to SI units would necessitate the recalculation of many of the constants of proportionality in these equations.

Any eccentricities in the style of prose are the responsibility of the author. It may be relevant to recall the text of a letter from the late John Saltmarsh - a fellow of King's College, Cambridge - to the Editor of Victoria County History:

> ' I am very sorry to learn that the insertion of unauthorised commas is not, as I had supposed, the work of some officious proof-reader employed by the printers, but a deliberate interference with my prose style on your instructions. This is a gross impertinence, and I resent it as a personal affront. I cannot admit the principle that punctuation is part of the style of printing. It is an integral part of my own prose style, and as such under my absolute control. The insertion of unauthorised commas destroys the rhythm of my sentences, and in some instances the commas inserted by your sub-editor have perverted my sense. I must absolutely insist that all the commas inserted by the sub-editor shall be removed.'

Willie's Epitaph

Little Willie from his mirror
Licked the mercury right off,
Thinking in his childish error
It would cure the whooping cough.
At his funeral his mother
Smartly said to Mrs. Brown
" 'Twas a chilly day for Willie
When the mercury went down."

The Penguin Book of Comic
and Curious Verse

Chapter 1.

TRACE METALS IN BIOLOGICAL MATERIAL

1.1 Introduction

The developments in analytical methodology which in recent years have created the ability to analyse small samples for low-level constituents have come at an opportune moment in history. It is now, with intense interest in the fields of environmental pollution, in agriculture and in human health and disease, that the determination of trace amounts of many elements has become an important aspect of analytical chemistry.

The role of analytical methodology is particularly important in clinical chemistry where marginal deficiencies or excesses of elements may not be characterized by obvious signs of disease. and can only be diagnosed by timely, accurate analyses. In fact, the actual biological roles played by some elements in nutrition, health and disease have yet to be established. The short-term consequences of extreme deficiency or excess of many elements are well documented, but in the region of marginal imbalances, which may have important long-term consequences for man, there is a definite lack of reliable data.

In forensic chemistry, and particularly toxicology, the main purpose in analysing a biological sample for metallic elements will be to establish an unnatural excess.

The determination of traces of metals in biological systems has always created a challenge to the analyst. Many metals are present in concentrations at and below the parts per million level, and in the past the techniques of colorimetry, polarography and emission spectrography

have all been stretched to their limits of detection and often have been coupled with preconcentration steps to enhance their sensitivity. The relatively recent development of practical atomic absorption spectrometry has found a major application in the analysis of biological systems, for to a large extent this technique meets the general criteria of any analytical method, viz. sensitivity, specificity, precision and rapidity.

Atomic absorption spectrometry (A.A.S.) was first described as a viable analytical technique by Walsh in 1955 (562), and interestingly some of the first applications of the method were in the fields of medicine, biology and agriculture (571-573). At the present time atomic absorption spectrometry with conventional pneumatic nebulization - flame atomization has achieved the distinction of being the most widely used technique for determining traces of metals in biological fluids. More recently, since about 1969, various atomization devices have been employed as alternatives to the flame; the two main advantages of these 'non-flame' devices are the vast increases in sensitivity attainable, and the decreased sample volume requirements.

The purpose of the present investigation was to take one of the non-flame devices, viz. the graphite tube atomizer, and to evaluate its performance for determining a variety of elements in blood, urine and liver tissue. This work was the basis of a research contract between Imperial College and the Home Office Central Research Establishment (H.O.C.R.E.). The contract was originally intended to be a continuation of a previous one which had involved the design and construction of a multi-channel atomic absorption spectrometer incorporating graphite tube atomization, and which had been used for the simultaneous determination of up to nine elements in hair samples (451). It was envisaged that two of the three years of the contract period would

be spent in developing the methodology for analysing biological material, and the final year spent in applying this methodology to the multi-channel instrument.

A decision was made by H.O.C.R.E. at the beginning of the third year to recover the multi-channel instrument, and this necessitated a change in the direction of research: the final year was spent in developing methods for the precise determination of various elements in liver tissue. A project at H.O.C.R.E. involved the application of spark source mass spectrometry to the routine screening of liver samples for metals; if an unusual level of some element were noticed, then atomic absorption spectrometry, particularly with graphite furnace atomization, would be employed to provide an accurate value.

1.2 Metals Present in Human Tissues

The metallic constituents of biological tissues can be divided into two groups depending on their concentration. The first group, the macro elements, comprises those - sodium, potassium, calcium and magnesium - which are present in concentrations of milligrams per hundred grams. These elements have been studied for a number of years and many of their functions have been thoroughly investigated. Adequate methods for their determination by flame emission or flame atomic absorption spectrometry are available, and these elements will not be considered further in this study. The second group of elements, the micro or trace elements, comprises almost any other element which may be present in living tissue at low concentration (parts per million or less: for mammals, a trace element may be defined as one that makes up less than 0.01% of the body's mass). In total, there are

approximately fifty trace elements which have been found in the human body, and they make up less than 0.2% of the total weight of the body (473); only about twenty of these trace elements are consistently present in human tissues (Figure 1).

Underwood (549) has made a convenient division of the trace elements into three groups: (a) those essential for higher animals, (b) the possibly essential, and (c) the non-essential. An essential trace element must fulfil the following criteria, given by Cotzias (93): the element must be present in all healthy tissues of all organisms; the concentration of the element in these tissues must be relatively constant; and withdrawal of the element must produce similar structural or physiological abnormalities in different species - these effects must be prevented or reversed by addition of the element. Schroeder (461) also considers metals which are an integral part of purified enzymes to be essential.

Trace elements deemed essential at the present time are vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, molybdenum, tin, selenium, silicon, fluorine (as fluoride) and iodine. It is interesting to note that six of these, viz. vanadium, nickel, tin, selenium, silicon and fluoride have only been proven essential in the last six years. This is evidence of both the current intense interest in trace elements, and the constant evolution of the subject: less than half a century ago only iron and iodine were considered unequivocally as essential, and now it is thought that to the above list of fourteen essential elements may ultimately be added arsenic, barium, boron, bromine and strontium.

The remaining trace elements that occur in living tissue are believed to be acquired from the environment in amounts that depend on their ambient levels and on the extent of contact. This group includes aluminium, silver, gold, bismuth and gallium, and those metals which are

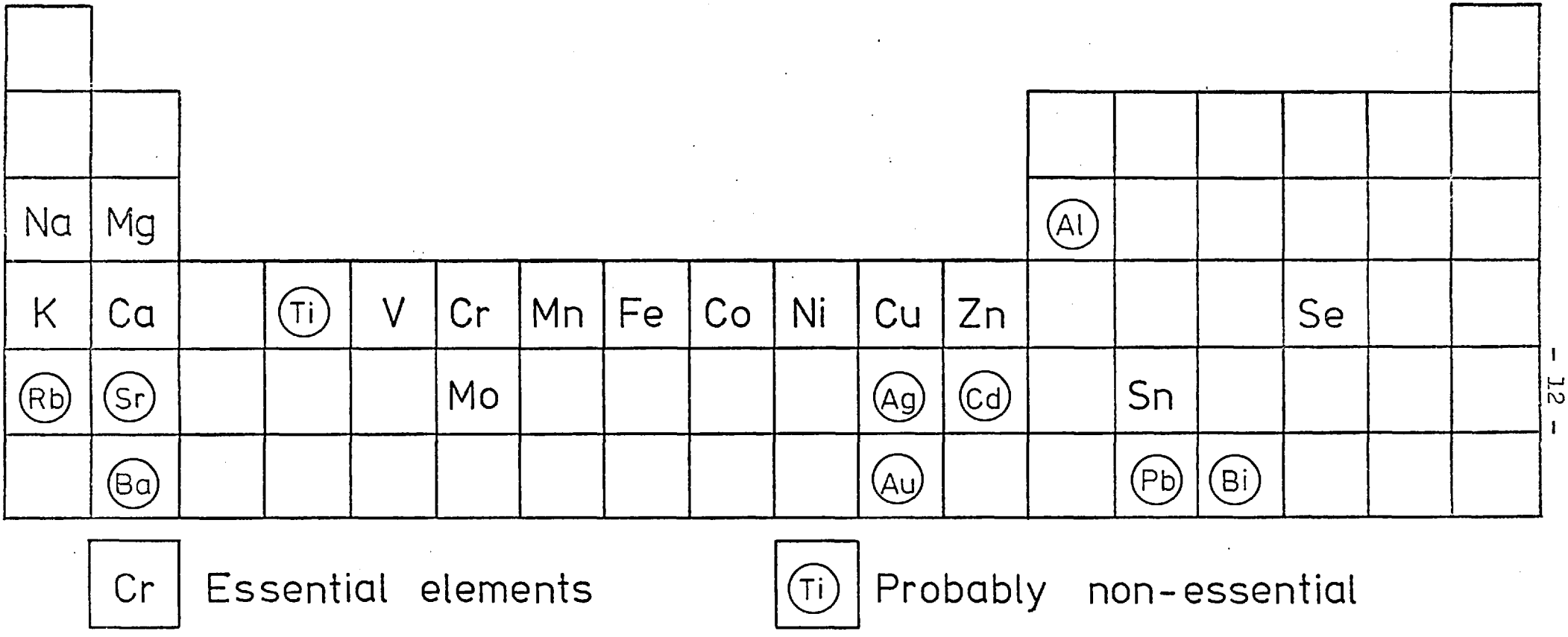


Fig. 1 Metals consistently present in human tissues

known to be toxic in relatively low concentrations: arsenic, cadmium, lead and mercury. Other toxic metals may be present as a result of occupational exposure or accidental or intentional ingestion: beryllium, tellurium, antimony and thallium. In this context, some elements cannot be clearly classified as toxic or non-toxic, since any essential trace element will become toxic when it enters an organism in large amounts. Underwood (549) has pointed out that each element will exhibit a spectrum of actions that depend on the dose and the nutritional state of the recipient with respect to the element. Starting with a state of deficiency, increasing amounts of the element will evoke an increasing biological response until a plateau is reached, the extent of which is determined by homeostatic capacity. Larger intakes may produce pharmacological actions, followed by toxic effects. This is illustrated in Figure 2, adapted from the work of Smith (501).

1.3 Analytical Methods for the Trace Metal Analysis of Biological Material

The choice of a particular technique for the determination of trace metals will depend primarily on its sensitivity, selectivity, precision and accuracy, and secondarily on the range of applicability of the technique, its convenience and cost. If an analysis is to give results which are meaningful to health-related studies, then it must meet the three criteria of selectivity, precision and sensitivity, in that order (352); these are discussed further in Chapter 2.

Methods which have been employed in trace metal determinations include X-ray spectrometry, emission spectrometry, spark source mass spectrometry, neutron

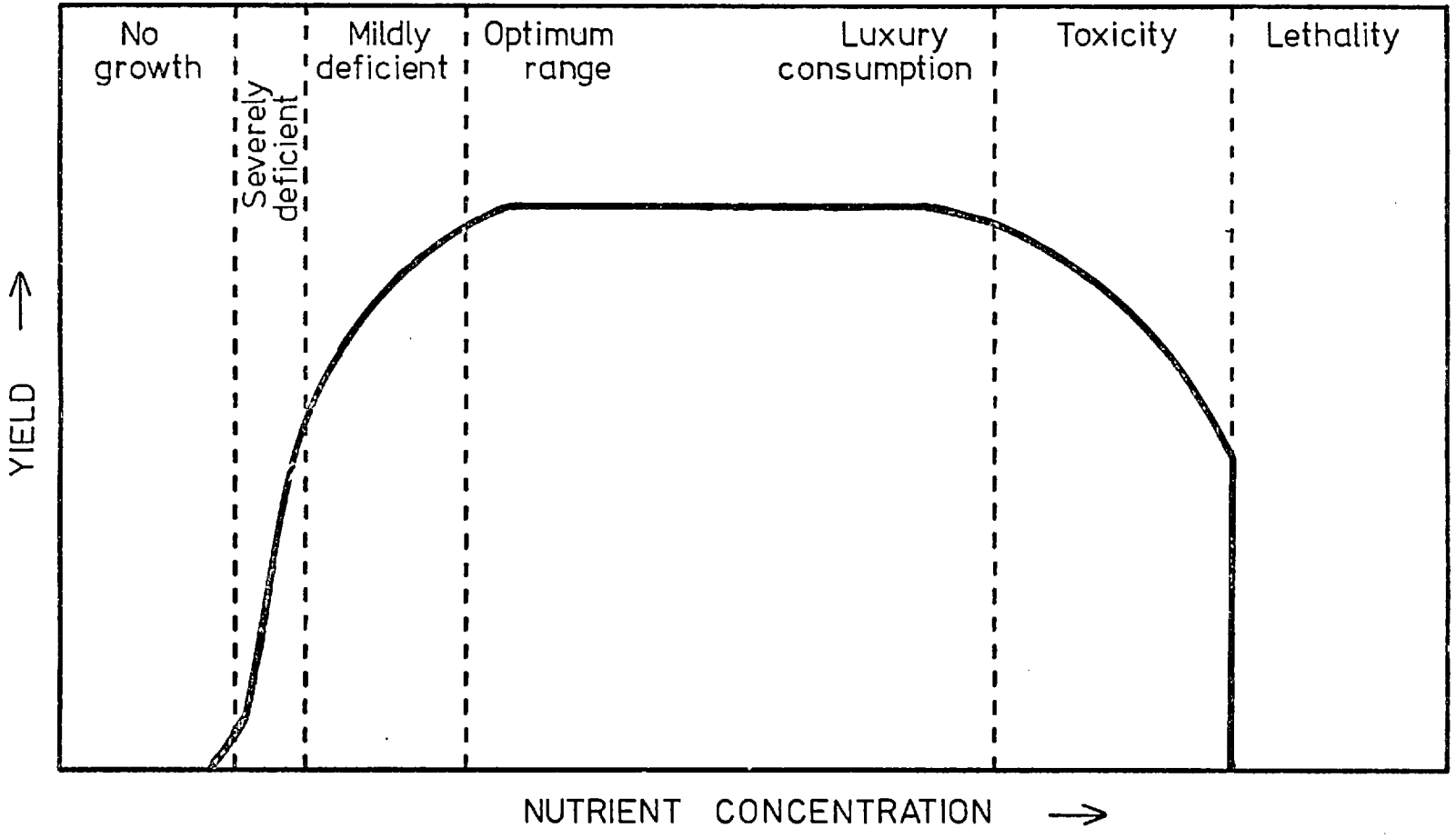


Fig. 2 Idealised growth vs. essential nutrient concentration diagram.

activation analysis, and atomic absorption spectrometry. In the past colorimetric methods have been applied but these, while convenient, are seldom of sufficient sensitivity for the determination of biological concentrations of trace elements. Recent years have shown renewed interest in inorganic polarography and the availability of improved instrumentation in this field points to a wider application in the future.

1.3.1 X-ray spectrometry

While X-ray spectrometry has not been considered primarily a tool for trace analysis, improvements in conventional X-ray equipment have enabled it to be employed usefully in this field. The development of electron probe X-ray microanalysis and X-ray microscopy has had a great impact on the analysis of small samples.

1.3.2 Spark Source Mass Spectrometry

This is a comprehensive technique of high sensitivity, but it suffers from many interferences and from relatively poor methods of detection; it is difficult to make quantitative. It has been widely used, however, in obtaining the data for "Reference Man" (section 1.4) and continues to be employed for the trace metal analysis of biological material (306, 533).

1.3.3 Neutron Activation Analysis

To date this technique is one of the most sensitive developed for the detection of a large number of elements, though for greatest sensitivity high neutron fluxes and long irradiation times are necessary, which add greatly to the cost of the technique. It is extremely valuable as an independent method, however, since it is free from matrix effects.

While the three techniques described above are of great value in the analysis of biological material, being suitable for multi-element determinations, they are sophisticated techniques and require expensive equipment and specialist operators; in addition they are difficult to apply to the routine analysis of large numbers of samples.

1.3.4 Emission Spectrometry

Flame emission spectrometry was developed to a high standard by Lundegårdh (312) over 40 years ago, and recently it has undergone a partial renaissance as a result of the improved instrumentation and high temperature flames developed for atomic absorption spectrometry (412). Flame emission methods do have limitations: a high resolution monochromator is required, and care must be taken to eliminate band emission superimposed on the line emission. At present the majority of applications reported for flame emission consists of determinations of sodium and potassium in serum, but possibly in the future flame emission will emerge as a competitive technique for some elements and a complementary technique for others, when compared to flame atomic absorption.

A technique complementary to flame atomic emission is flame atomic fluorescence (271). This may provide higher sensitivities for some elements but it has not been applied to any great extent to biological analyses.

Emission spectroscopy with an arc or spark source has been used for a number of years; its primary value lies in its ability to determine simultaneously, quickly and reliably the qualitative absence or presence of almost all elements. Photographic detection and microdensitometry can achieve a degree of quantitation, though the procedure does suffer matrix effects (378), and is time-consuming. The advent of direct reading spectrometers

has reduced many of these drawbacks, and improvements in the arc and spark sources have further enhanced the technique. Much of the early data in the trace element field was obtained by emission spectrometry (276) and it continues to be widely employed (377, 565). A new development in sources for emission spectrometry is the inductively coupled plasma (151). and the inductively coupled plasma - optical emission system promises to be an important simultaneous multi-element technique, having minimal inter-element effects and a large dynamic range. The technique is admirably suited for analyses in the trace element field, though as yet it has found few applications to biological problems (186, 274, 373).

1.3.5 Atomic Absorption Spectrometry

Since the development of commercial atomic absorption instrumentation in the early 1960's, this technique has proved its worth in many clinical and biological laboratories, and it is likely that at the present time more determinations of trace metals in biological materials are made by this technique than by any other. The advantages of atomic absorption spectrometry are well known (268) and the technique is considered in greater detail in Chapter 3.

1.4 Literature Values of Trace Metal Levels

Interest in environmental risks (as opposed to occupational risks) from such elements as lead. mercury and cadmium has illuminated the need for information on natural (or "normal") levels of these elements in both the environment and man, together with the natural variability of these levels. Once these natural levels are known, it is possible to measure unnatural perturbations against them. It is important to appreciate

that an "average" value may not correspond to the "normal" value.

Only a few whole human bodies have been analysed (570), but even these few analyses are sufficient to show the considerable variation in trace metal levels which may occur - due to sex, age, occupation, environment, past medical history, etc. More recent techniques such as the whole-body counting of radionuclides (591) or whole-body neutron activation (20) have been employed in determining the composition of man, but they are in general only applicable to a few elements. The procedure employed at present is to assemble a "Reference Man" from analysis of tissues from a wide range of samples; further analyses can establish inputs and outputs of the elements of interest.

Some twenty years ago the International Commission for Radiation Protection (I.C.R.P.) identified the need for a "Standard Man" and in the 1959 report "Permissible Dose for Internal Radiation" (Publication 2) was published a standard based on the available data. In 1963, recognising the deficiencies of these data, and the possibilities offered by new techniques, a "Standard Man Task Group" was set up to define the anatomy, composition and physiology of a standard man; the Task Group comprised Dr. W.S. Snyder and Mary Jane Cook, both from Oak Ridge National Laboratory, Dr. Lucien Karhausen, C.E.A., Paris, Dr. Gwyneth Parry Howells, M.R.C., London, and Dr. Isobel Tipton, University of Tennessee (532).

Table 1 gives the trace element content of the body of "Reference Man", based on a 70kg weight, and also the concentrations of these elements to be found in his blood (whole blood, serum and red blood cells). The principal storage areas are also indicated (53, 222, 533).

Table 1 Trace Metal Content of "Reference Man"

Element	Body /mg	Body /p.p.m	Blood	Plasma mg total	R.B.C.	Storage
Vanadium	18	0.3	0.088	0.031	0.057	>90% in fat
Chromium	1.5	0.02	0.14	0.074	0.044	37% in skin
Manganese	12	0.2	0.14	0.025	0.12	43.4% in bone
Iron	4200	60	2500	3.6	2400	70.5% in haemoglobin
Cobalt	1.5	0.02	0.0017	0.0014	0.00034	18.6% in bone marrow
Nickel	10	0.1	0.16	0.09	0.07	18% in skin
Copper	72	1.0	5.6	3.5	2.2	34.7% in muscle
Zinc	2300	33	34	5.6	2.8	65.2% in muscle
Molybdenum	9	0.1	0.083	..	..	19% in liver
Tin	6	0.1	0.68	0.1	0.55	25% in fat and skin
Selenium	13	0.2	1.1	..	..	38.3% in muscle
Fluorine	2600	37	0.95	0.87	0.17	98.9% in bone
Iodine	11	0.2	0.29	2.6	0.35	87.4% in thyroid
Arsenic	<18	0.3	2.5	<0.093	0.59	follows phosphorus
Barium	22	0.3	<1.0	<0.62	..	91% in bone
Boron	14	0.2	0.52	..	..	
Bromine	200	2.9	24	17	7.5	60% in muscle
Cadmium	<50	0.7	0.036	..	..	27.8% in kidney & liver
Lead	120	1.7	1.4	0.14	1.2	91.6% in bone
Mercury	13	0.2	0.026	0.009	0.017	69.2% in fat and muscle
Antimony	? 8	0.1	2.02	0.16	..	25% in bone
Aluminium	61	0.9	1.9	1.3	0.14	19.7% in lung 34.5% in bone
Gold	<10	0.1	0.0002	..	..	52% in bone
Lithium	2.2	0.03	0.10	0.093	0.061	50% in muscle
Uranium	0.09	0.001	0.0046	..	..	65.5% in bone
Beryllium	0.036	..	<0.0005	..	..	75% in bone
Rubidium	320	4.6	14	2.2	12	follows potassium
Caesium	1.5	0.02	0.015	..	..	follows potassium
Titanium	9	0.1	0.14	0.12	0.08	49.1% in lung and lymph
Zirconium	420	6.0	13	1.2	12	67% in fat
Niobium	? 110	1.6	13	<0.25	13	26% in fat
Tellurium	? 8.2	0.1	0.18	0.09	0.078	Probably in bone

It should be pointed out that spark source mass spectrometry was the technique employed for most of the analyses used in formulating "Reference Man", and hence only elemental levels were recorded, i.e. there was no indication of the forms in which the elements were bound. Spark source mass spectrometry is not without its disadvantages as an analytical technique, and it is possible that the values given in Table 1 may have to be modified in the light of new data from alternative analytical techniques. Problem areas associated with the analyses such as collection, separation and handling of the tissues would obviously need to be carefully controlled. Other factors are less easy to control: some tissue analyses may give only "less than limit of detection" information, whilst determinable quantities may be found in other tissues - leading to problems of data analysis. All the "Reference Man" tissues were obtained from accident cases, and it was hoped that these represented a typical population, although all the cases may not have been in optimum health (222).

Intake of trace elements is from the air and diet, and excretion routes include urine, faeces, sweat and hair. Air intake is closely related to metabolism and is well documented, but estimates of food and water intake present difficulties. Widely different values of the intake may result from the methods used; these methods include national consumption and population estimates, market surveys and controlled balance studies of a few individuals (188).

Average daily intakes of trace metals, and the corresponding excretions in urine, faeces and sweat are given in Table 2, together with the "normal" concentrations of these elements in hair (53, 218, 222, 451, 533). Air intakes were calculated as the upper limit due to intake of urban air on the basis of a total daily air intake of 2.3×10^4 dm^3 (222).

Table 2 Intakes and Excretions of Trace Elements

ELEMENT	INTAKE		EXCRETION			
	F.+ F. mg/day	Air mg/day	Urine mg/day	Faeces mg/day	Sweat mg/day	Hair mg/g
Vanadium	2.0	...	0.015	...	...	...
Chromium	0.15	0.1	0.07	0.08	0.059	0.69 - 0.96
Manganese	3.7	2	0.03	3.6	0.097	1.0
Iron	16.0	30	0.25	15	0.5	130
Cobalt	0.3	< 0.1	0.2	0.09	0.017	0.17 - 0.28
Nickel	0.4	0.6	0.011	0.37	0.05	0.0075
Copper	3.5	20	0.05	3.4	1.59	16 - 56
Zinc	13.0	< 100	0.5	11	5.08	167 - 172
Molybdenum	0.3	< 0.1	0.15	0.12	0.061	...
Tin	4.0	0.34	0.02	3.5	2.23	...
Selenium	0.068	...	0.04	...	0.34	0.3 - 13
Fluorine	1.8	...	1.0	0.15	0.65	...
Iodine	200	0.5 - 35	0.17	0.02	0.006	0.015
Arsenic	1.0	...	0.195	...	...	2
Barium	0.75	0.09	0.05	0.69	0.085	5
Boron	1.3	...	1.0	0.27	...	7
Bromine	7.5	...	7.0	0.07	0.2	12.5
Cadmium	0.15	< 1	0.1	0.05	...	2.8 - 1.8
Lead	0.44	10	0.045	0.3	0.1	18 - 19
Mercury	0.02	...	0.015	...	0.0009	6
Antimony	< 0.15	...	< 0.07	...	0.011	6.5
Aluminium	45	100	0.1	43	6.13	5
Lithium	2.0	...	0.8	...	(present)	...
Beryllium	0.012	< 0.01	0.001	0.01	...	...
Rubidium	2.2	...	1.9	0.3	0.05	...
Titanium	0.85	...	0.33	...	0.001	0.05
Zirconium	4.2	...	0.14	...	...	...
Niobium	0.62	...	0.36	...	0.003	2.2
Tellurium	0.112	...	0.53	...	...	...

N.B. F.+ F. = food and fluids

An analyst may be called upon to determine the metallic content of almost any biological sample, and data have been published on metallic levels in liver (144, 241, 383), kidney (101, 304, 419), brain (27, 193, 196, 495) and lung (252, 514), as well as blood (22, 421), urine (284), faeces (190), sweat (218), hair (208, 451) and fingernails (32, 197). Three of the most important biological samples are blood, urine and liver and published data on the concentrations of trace metals in these materials are collected in the following tables, Tables 3 - 5.

In order to calculate the normal concentrations in the blood of "Reference Man" the following blood parameters were employed (85):

	Male Adult
red blood cell volume	12-15 cm^3/lb
plasma volume	18-20 "
blood volume (total)	30-35 "

Thus the 5015 cm^3 of whole blood in "Reference Man" (70kg = 154 lb) is divided into 2932 cm^3 plasma and 2083 cm^3 red blood cells

1.5 Functions of Trace Elements; the Effects of Deficiency and Excess

Mammals developed and evolved in the presence of high environmental concentrations of some elements, and it is to be expected that these elements, viz. Si, Al, Ti, Ba, Zr, Nb, Li, La and Ge will have low orders of toxicity, whereas elements scarce on the earth and in seawater will be more toxic to living things. Appropriate body mechanisms for handling and excreting elements such as Sn, Ge, Be, As, Mo, Hg, Sb, Bi, Pb, Cd, Ag, Se, Au and Te,

Table 3 Normal Levels of Trace Metals in Blood

All values are in $\mu g/100cm^3$

Element	Whole Blood		Serum		Plasma		R.B.C.		Reference
	mean	range	mean	range	mean	range	mean	range	
Vanadium	1.75				1.06		2.74		"Ref. Man"
	1.7				1.0		2.6		53, 175
					1.0	0.1-			473
			0.46						206
Chromium	2.79				2.52		2.11		"Ref. Man"
	2.6				2.4		2.0		52, 53, 229
					2.8	2.0 -			473
	1.2								551
					2.9	1.1-6.4	2.6	2.1-3.6	154
					2.9		2.3		356
					2.4	1.6-3.9	2.2	1.1-4.1	396
			4.7						205
					2.2	0.7-5.2			276
			52.0,17.0						466
Manganese	2.79				0.85		5.76		"Ref. Man"
	2.6				2.9		2.2		53
					0.83	0.25 -			473
	2.4	1.6-3.2							51
					0.43				389
		0.6-1.1				0.12-0.16		2.2-2.4	94, 397
					1.3				251
Iron	49,850				123		115,000		"Ref. Man"
	47,500				114		111,000		53
					114	90 - 85-150			473 204
Cobalt	0.034				0.048		0.016		"Ref. Man"
	0.033				0.038		0.015		53
					0.018	0.007 -			473
		0.17-1.5							583
	0.85	0.28-1.7							207
	0.018	0.007 - 0.036							525
	4.3	0.35-0.63			1.2	0.4-7.3	20.8	3.0-50.8	276
Nickel	3.19				3.07		3.36		"Ref. Man"
	3.8				4.2		4.9		53, 229
					0.42	0.1 -			473
				2-10					381, 518
					2.5	1 - 8			88
	3.0	1 - 8.5							276
			2.2	0.1-7.7					519
							5		205

Table 3 (Continued)

Element	Whole Blood		Serum		Plasma		R.B.C.		Reference
	mean	range	mean	range	mean	range	mean	range	
Copper	112				120		106		"Ref. Man"
	107				112		98		53
					116	100 -			473
				85-150					204
Zinc	678				190		134		"Ref. Man"
	650				160		1230		53
					98	89 -			473
				80-110					192
	880				121				552
	810				124				559
	660				109				37
	710				120				276
	700				130				583
Molybdenum	1.66								"Ref. Man"
	0.41						<0.8		53, 65
					0.4				473
Tin	13.56				3.4		26.4		"Ref. Man"
	13				3.3		25		53
					3.3				473
Selenium	21.9								"Ref. Man"
	27				11		26		53, 54, 65
					1.1				473
	18.2				14.4		23.6		124
Arsenic	49.9				<3.17		28.3		"Ref. Man"
	49				4		110		53
					19.0				473
						0-40			66
	6 - 20								374
				3.5-7.2					234
	22.1								85
Barium	< 20				< 21				"Ref. Man"
	6.9				7.9		5.6		53, 70, 376
Cadmium	0.72								"Ref. Man"
	0.74				< 9		1.5		53, 229
					< 10				473
	< 5								229, 288
			0.33						88

Table 3 (Continued)

Element	Whole Blood mean	Whole Blood range	Serum mean	Serum range	Plasma mean	Plasma range	R.B.C. mean	R.B.C. range	Reference.
Lead	27.9				4.8		57.6		"Ref. Man"
	27				4.3		34		53
					4.6				473
	~ 20								256
	35	1 - 60							433
Mercury	0.52				0.31		0.82		"Ref. Man"
	0.65				0.30		0.67		53
	< 3								176
	8 - 16								540
Antimony	40.4				5.46				"Ref. Man"
	0.47				5.4		0.5		53, 584
Aluminium	37.9				44.3		6.7		"Ref. Man"
	32.0				44.0		16.0		53, 70
		2-94							582
	13								256
			17						484
Gold	0.004								"Ref. Man"
	0.004				< 0.25		0.004		53, 308, 584
		< 5							303, 411, 535
Lithium	1.99				3.17		2.93		"Ref. Man"
					< 0.5		< 0.5		53, 376
					3.1				473
		< 0.003							436
			1.1						88
Uranium	0.09								"Ref. Man"
	0.055				< 0.1		< 0.11		48, 53, 584
Beryllium	< 0.01								"Ref. Man"
	< 0.01				< 0.4		< 0.01		53, 584
		< 2							302
Rubidium	280				75		576		"Ref. Man"
	270				120		530		53
Caesium	0.30								"Ref. Man"
	0.28				0.31		0.28		53
Titanium	2.79				4.09		3.84		"Ref. Man"
	2.6				< 4.0		< 5.6		53, 584
Zirconium	260				41		580		"Ref. Man"
					< 0.6		< 0.6		53, 584
Niobium	260				< 8		624		"Ref. Man"
					< 1		< 1		53, 584
Tellurium	3.59				3.07		3.74		"Ref. Man"
					< 3		< 3		53, 584

Table 4. Normal Levels of Trace Metals in Urine

Element	Table 2 Values		Other Values		Reference
	mg/day	range/p.p.m.	mean/p.p.m.	range/p.p.m.	
Vanadium	0.015	0.01 - 0.03	0.016	- 0.03	53, 467
			0.029		536, 537
Chromium	0.07	0.047 - 0.14	0.010	- 0.040	53, 462,466,477
			0.087	- 0.133	536, 537
Manganese	0.03	0.02 - 0.06	0.30	- 0.98	53, 469
			0.033		536, 537
				0.02 - 0.14	88
Iron	0.25	0.17 - 0.50	0.18		53
			0.98		536, 537
				0.1 - 0.6	88
Cobalt	0.2	0.13 - 0.4	0.098	- 0.160	53, 476
			0.21		536, 537
				0.001 - 0.007	88
Nickel	0.011	0.007 - 0.022	0.085	- 0.100	53, 474
				0.005 - 0.08	88
Copper	0.05	0.03 - 0.10	0.059		53, 475
				- 0.05	204
			0.07	- 0.11	536, 537
				- 0.06	117
Zinc	0.5	0.33 - 1.0	0.90	- 1.92	53, 478
			1.80		536, 537
				0.2 - 1.2	88
Molybdenum	0.15	0.10 - 0.30	0.10	- 0.15	53, 470
			0.14		536, 537
				0.01 - 0.06	88
Tin	0.02	0.013 - 0.04	0.017×10^{-3}		468, 473
Selenium	0.04	0.027 - 0.08	0.03		53, 472
Arsenic	0.195	0.13 - 0.39	0.170		53, 465
				< 0.1	88
			0.17		473
Barium	0.05	0.03 - 0.10	0.017×10^{-3}		53, 473
Cadmium	0.1	0.07 - 0.2	0.044		53, 463
			0.012		473
				0.002 - 0.02	88
Lead	0.045	0.03 - 0.09	0.035		53,400, 464, 473
			0.04		479
			0.04		117
				0.01 - 0.14	88
Mercury	0.015	0.01 - 0.03	0.01		53, 473
				< 0.03	88
Antimony	< 0.07	< 0.14	$< 0.05 \times 10^{-3}$		53, 473
Aluminium	0.1	0.067 - 0.2		0.03 - 0.1	88
Lithium	0.8	0.5 - 1.6	0.57		53, 473
Beryllium	0.001	0.001 - 0.002	0.001		302
			0.0011		473
Rubidium	1.9	1.3 - 3.8			
Titanium	0.33	0.2 - 0.6			
Zirconium	0.14	0.09 - 0.28			
Niobium	0.36	0.24 - 0.72			
Tellurium	0.53	0.35 - 1.1			

Table 5 Normal Levels of Trace Metals in Liver

Element	Liver Ash		Dry Weight		Fresh Weight.	Reference
	mean/p.p.m.	range/p.p.m.	mean/p.p.m.	range/p.p.m.	/p.p.m.	
Vanadium	< 1	<1 - 1		< 0.04	< 0.01	162, 534, 535
Chromium	0.4	< 0,1 - 15	0.026		0.009	162, 534, 535
Manganese	100	39 - 500	3.7		1	162, 534, 535
Iron			520			535
Cobalt	< 4	< 4 - 80	0.23		0.06	162, 398. 534
Nickel	< 5	< 5 - 46	< 0.2		< 0.07	162, 534, 535
Copper	500	200 - 2500	19	10 - 50	7	162, 534, 535
Zinc	3200	1600 - 8600	130	50 - 140	50	162, 534, 535
Molybdenum	76	13 - 200	2.8		0.09	162, 534, 535
Tin	22	< 5 - 160	0.85		0.3	162, 534, 535
Selenium			2.1		0.43	135
Arsenic			0.5	0.01 - 1.0	0.1	494
Barium	< 0.3	< 0.3 - 0.6	0.07	0.05 - 0.8	< 0.003	162, 534, 535
Cadmium	180	50 - 1500	6.7	5 - 30		162, 534, 535
Lead	130	26 - 800	4.8	0.9 - 4.6		162, 534, 535
Mercury			(0.022)	1.5 - 20	0.3	(161), 534
Aluminium	44	10 - 300	1.7			162, 534, 535
Gold	< 10	< 10 - 10	< 0.0001			162, 398, 534
Lithium			< 0.02			53
Uranium			0.04			53
Beryllium			0.0009			161
Rubidium			30			594
Caesium			0.05			594
Titanium	< 5	<5 - 250	< 0.2			162, 534, 535
Zirconium			< 4			53

N.B. Water content of liver is approximately 70%

Liver ash is approximately 5.4% (range 5-9%) of the dry weight (162)

which are found in low concentrations on the earth's surface but are now mined and used industrially in large quantities, will not have developed in mammals. By comparing the industrial consumption of various elements with the amounts present in "Reference Man" and in the earth's crust and seawater, it is possible to estimate potential exposures (Table 6. adapted from references 222 and 471).

Elements present in biological samples may be divided into five categories: essential, possibly essential, toxic to living things, slightly toxic to living things, and probably inert to living things (471). As has been mentioned before, depending on the dose and the nutritional state of the recipient, any element will evoke a spectrum of actions - starting with deficiency and proceeding through normal and therapeutic states to toxicity.

All substances are toxic in large enough doses, but the toxicity of a particular substance is determined by the efficiency of the homeostatic mechanisms of excretion - the lower the efficiency of excretion then the smaller will be the difference between normal intake of a trace element by food, water and air and the toxic intake (a typical ratio of toxic level to requirement is about 50). If a situation arises where the human body burden of an element is considerably increased over that of primitive man - as a result of man's actions in smelting ores and refining metals, in canning foods, and in putting alkyl lead into petrol - then the normal intake of the element may approach the toxic intake and even normal intakes may give rise to sub-clinical effects. This situation is especially important for five elements, to which living things exhibit low tolerances: the toxic metals Cd, Pb, Hg, Sb and Be; these metals have been involved in many deaths and disabling diseases (99, 310, 420).

Table 6 A Comparison of the Industrial Consumption of Metals with the Amounts in "Reference Man", the Earth's Crust and Sea Water.

Element	Industrial Consumption (/1000 tons)	"Reference Man" /mg	Earth's Crust /p.p.m.	Sea Water /p.p. 10^9
(i) Essential Elements				
Vanadium	5	18	110	2
Chromium	459	1.5	200	0.05
Manganese	1,050	12	1,000	2
Iron	109,000	4,200	50,000	10
Cobalt	6	1.5	23	0.27
Nickel	170	10	80	5.4
Copper	1,400	72	45	3
Zinc	1,278	2,300	65	10
Molybdenum	25	9	1	10
Tin	59	6	3	3
Selenium	0.5	13	0.09	..
Fluorine	587	2,600	700	1,300
Calcium	86,273	1,000,000	36,300	400,000
Magnesium	89	19,000	20,900	1,350,000
Sodium	15,091	100,000	28,300	10,500,000
Potassium	3,230	140,000	25,900	380,000
(ii) Possibly Essential Elements				
Arsenic	22	< 18	2	3
Barium	700	22	400	30
Boron	70	14	3	4,600
Strontium	6	320	450	8,100
(iii) Toxic to Living Things				
Cadmium	7	< 50	0.2	0.11
Lead	816	120	10	0.03
Mercury	3	~3	0.5	0.03
Antimony	19	8	0.2	...
Beryllium	0.3	0.04	2	...
(iv) Slightly Toxic to Living Things				
Tungsten	7	(present)	1	0.1
Germanium	11	(present)	2	0.07
Uranium	2.7	0.09	2	3
Bismuth	1	0.2	0.2	0.017
Tellurium	0.1	8	0.002	...
Palladium	0.02	(present)	0.01	...
Rhodium	0.002	(present)	0.001	...
(v) Probably Inert to Living Things				
Aluminium	3,534	61	81,300	10
Titanium	413	9	4,400	1
Zirconium	61	420	160	0.022
Lithium	2.6	2	30	180
Silver	0.03	0.8	0.1	0.3
Niobium	2	110	24	0.01

N.B. Industrial Consumption is for the United States in 1968.

1.5.1 Essential Elements

Essential elements may be associated with a wide range of functions which can be divided into four groups: electrochemical (Ca, K, Mg, Na), catalytic (Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, Zn), structural (Ba, Ca, Fe?, Mg) and miscellaneous, including unknown functions (Cu, Fe, Se, V, Zn) (53). These functions can be related to groupings in the Periodic Table; the essential trace elements are mostly transition elements, with unfilled d-orbitals. Many interactions occur between the trace elements, and the Mn-Fe,Cu and Zn-Fe and As-Se interactions are of great nutritional importance.

Vanadium is now considered to be essential to man, and acts pharmacologically in inhibiting cholesterol synthesis (53, 431). It is slightly toxic, unless injected intravenously - when it becomes very toxic - and it is considered a contaminating, not a polluting, element (471). Workers with vanadium are likely to suffer green tongues (467).

Chromium was established as being present in all living things by 1948 (471). It is present in an enzyme concerned with sugar and phosphorus, and has been shown to act as an adjunct, perhaps a co-factor, to insulin, and thus is essential for sugar metabolism. Also, it is essential for cholesterol metabolism (53). Deficiency of chromium has been associated with atherosclerosis, and the depletion of chromium in tissues which occurs with age is probably due to insufficient intakes: refined foods contain little chromium. Chromium (III) is moderately toxic, and chromium (VI) is highly toxic, possibly even carcinogenic (431, 462, 466, 477).

Manganese was proven essential to all organisms by 1936 (471). It is a constituent of several enzymes and proteins, and activates numerous other enzymes (53). Unequivocal manganese deficiency has not been identified

in man, probably due to the high manganese content of cereal grains, green vegetables and tea. It is a moderately toxic element (431, 469).

Iron is essential to all organisms, and functions catalytically in activating a number of oxidases (471). It is a constituent of many oxidising metallo-enzymes, respiratory pigments and proteins of unknown function (53). Iron deficiency has long been recognised as the most common nutritional deficiency in man, but many aspects of iron metabolism remain undetermined. The low level of iron in cereal diets is a major cause of iron-deficiency anaemia in the less-developed countries, though losses of blood by various means are common contributory causes. Iron is only slightly toxic (431).

Cobalt was proven essential to all organisms by 1948 (471). It has a catalytic function, activating a number of enzymes, but it is believed to be physiologically active only in the form of vitamin B_{12}. Ruminant animals are important synthesisers of vitamin B_{12}, with the consequence that cobalt deficiency in man may occur in strict vegetarianism, giving symptoms of pernicious anaemia. True pernicious anaemia is a consequence of defective absorption of vitamin B_{12}. Cobalt is moderately toxic if injected intravenously (53, 431, 476).

Nickel has been shown to be present in all human tissues (520) and is now considered essential (379). It is associated with RNA and DNA, is present in proteins and activates certain enzyme systems. It is unlikely that nickel deficiency could be a problem in man, though some diets may contain minimal amounts. The toxicity of inorganic nickel compounds is not great unless nickel dusts are inhaled; this may result in lung cancer. Nickel carbonyl is also carcinogenic. It is found that patients with cirrhosis of the liver and uraemia have lowered serum nickel levels, while those suffering myocardial infarction, strokes and

burns have elevate serum nickel concentrations (53, 431, 471, 474).

Copper has been recognised as essential for all organisms since 1928 (471). It is a constituent of many metallo-enzymes, especially those connected with oxidation, and also of proteins. Although copper deficiency has not been described in human adults, it does occur in infancy, resulting in anaemia, chronic diarrhoea, and the sex-linked recessive disorder "kinky-hair syndrome" which involves retardation of growth and mind. Decreased plasma copper concentrations may be due to several diseases, such as celiac disease, or the prolonged use of penicillamine (53, 431, 475). It has recently been shown (272) that low intakes of copper are associated with increased cholesterol concentrations; this may have important implications for human cardiovascular disease. Copper is moderately toxic to mammals, and diseases suspected to be caused by excess copper are arthritis and scleroderma. Elevated plasma copper concentrations occur during pregnancy or as a result of taking oral contraceptives containing oestrogens. They may also occur in several illnesses linked to stress, as well as major surgery and myocardial infarction. Liver diseases will typically give increased serum copper levels, possibly due to interference in the biliary excretion of copper. Pellagra results in increased concentrations of copper in serum, urine and hair. Wilson's disease results in high levels of copper in urine, plasma, liver and brain; this disease is hereditary and is thought to be caused by a missing gene, one which governs the copper transport system. The result is the absorption of large amounts of copper (53, 431, 471).

Zinc was proven to be an essential element in 1934 (471). It is a constituent of many metallo-enzymes and of several proteins of unknown function. It has been established that zinc is involved in iron metabolism and in wound healing, and that it is necessary for reproduction, for

growth and for the formation of DNA and RNA. Conditions associated with zinc deficiency include pregnancy, taking of oral contraceptives, Iranian and Egyptian dwarfism, various infections, alcoholism, liver damage. sickle cell anaemia, heart attacks, cystic fibrosis, loss of taste and smell, Mongolism and idiocy. Most of these conditions are improved by the administration of zinc, and zinc has been found to stimulate wound healing. In fact, zinc is probably the most important micro-nutrient, though it is often neglected. It is only slightly toxic to man; up to 150mg a day has been given by mouth to patients for periods of several years (53, 431, 461, 471, 478).

Molybdenum was known to be essential by 1953 (471). It is a constituent of the xanthine oxidase system and several other enzymes. It is closely linked with copper nutrition, and high intakes of molybdenum will induce copper deficiency. No clinical manifestations in man have been traced to molybdenum deficiency; it is moderately toxic (53, 40, 471).

Tin was not recognised as an essential element until 1970 (480), possibly because its presence had not been detected consistently in tissue samples - it is easily lost due to its volatility. It is possible that tin (which readily forms co-ordination compounds) reacts with proteins or nucleic acids (431). Tin deficiency is not known to occur in man; the toxicity of inorganic compounds is low and at present exposures there are no hazards. Alkyl tins, however, arehighly toxic (53, 468, 471).

Selenium is essential and participates in three enzyme-catalysed reactions (508). A close relationship exists between selenium and vitamin E, and selenium occurs in a few amino acids and many proteins. No pathological disorders are known in man which can be attributed to selenium deficiency or excess; selenium is not carcinogenic

but excessive amounts can cause discoloured and decayed teeth, yellow skin, brittle nails and partial or total loss of hair (53, 471, 472).

1.5.2 Possibly Essential Elements

Arsenic has not yet been proven essential to man (465), but from antiquity it has enjoyed a dual reputation of benevolence and malevolence. Organic arsenic compounds have been employed in the treatment of syphilis, sleeping sickness and various parasitic diseases. Soluble arsenic compounds are highly toxic to man, and it has enjoyed a reputation as a simple, inexpensive and convenient poison for both suicide and homicide. The lethal dose to man is of the order of 125mg, but this may vary since some tolerance to arsenic may occur (99, 420, 471).

Barium has not as yet been confirmed as essential for life (471). It is present as a natural contamination of dusts, is added to Diesel oil to control smoke in exhausts, and shows little toxicity when swallowed or breathed, though accumulation in the lungs may give rise to pathological changes in the lung (53, 471). It is scarcely toxic when insoluble: barium sulphate is swallowed in large amounts prior to the X-raying of stomach and intestine.

Strontium has not yet been proven essential to man, although its biological role has been studied extensively in recent years because of concern over the biological implications of ingesting Sr^{90} (471).

1.5.3 Toxic Elements

Cadmium has long been known to be poisonous (99) but it is only recently that it has been mined in sufficient quantities to become an environmental contaminant (463). It is always associated with zinc, and has the ability to displace zinc in biological systems (285, 478).

Lead is the largest contaminant of the environment, locally in rivers near lead mines, but widely in air, water and soil from the combustion of petrol containing tetraethyl and tetramethyl lead (464, 471). Lead is a moderately toxic element, though overt lead poisoning in children eating lead paint is well known (117). Lead accumulates with age, and this has resulted in the body burden of "Reference Man" today being a hundred times that of primitive man (479). Sub-clinical lead toxicity has been suspected in urban dwellers, but has not yet been confirmed (53, 67, 285 471, 279).

Mercury is a ubiquitous element, and there appears to be little hazard to the general population from natural levels of inorganic mercury or aryl mercury compounds (471). It is, however, an accumulative poison (99) and is highly poisonous if injected intravenously or taken orally as $HgCl_2$. Alkyl mercury is highly poisonous, and the dumping of methyl mercury has led to two outbreaks of poisoning in Japan, in Minimata and Niigata, and ingestion of grain treated with alkyl mercury fungicide has resulted in many deaths (285, 471).

Antimony in the form of stibnite has been employed as a women's cosmetic for 6,000 years, but today potential exposures are low except in industrial situations (471). Potassium antimony tartrate has been used as a treatment for liver flukes, even though large doses may produce heart toxicity, liver damage and even death. Antimony has been the agent in both accidental poisoning - from dissolved enamel glazes - and homicidal poisoning; the lethal dose for man is 600 - 900mg (99, 420).

Beryllium is probably the most toxic metal and causes an incurable lung disease, but fewer than 900 cases of this have been reported, most being the result of occupational inhalation (471). Levels of beryllium in food and water

are low, and the main site of entry into the human body is the lung, where beryllium accumulates and may cause lung cancer. Beryllium particles entering the skin through a scratch or cut cause an ulcer to develop; this will not heal unless the particles are removed (471).

Thallium is not normally present in human tissues. Its poisonous character has been recognised for a long time, and although occupational thallium poisoning can occur in some industries, it is the use of thallium for criminal purposes that has received the most attention. The potency of thallium as a poison lies in its odourless and tasteless properties, and in its ability to produce a variety of clinical signs. The lethal dose is 1 - 2g (99, 102, 420, 425).

1.5.4 Other Elements

Aluminium is considered to have little biological significance. Titanium is poorly absorbed by the body and this results in tissue levels being much lower than those in the environment. Gallium has low toxicity, but it is found to concentrate in some tumours. Germanium has little biological significance (53, 471).

Two elements have found some therapeutic usage: lithium in the treatment of manic depression (92, 436, 452, 513), and gold for rheumatoid arthritis (4, 133, 193, 308).

Chapter 2.

METHODOLOGY OF TRACE ANALYSIS OF BIOLOGICAL SAMPLES

2.1 Trace Analysis

Although Sandell (453) has defined a trace constituent as being "below the threshold of quantitative determination", this definition, which conflicts with the title of his treatise "The Colorimetric Determination of Traces of Metals", is not particularly helpful. It is now generally agreed that "trace analysis" refers to concentrations lower than 100 p.p.m., although various workers have interpreted the term according to their own needs. Ultratrace analysis could refer to concentrations less than 1 p.p.m.(503), and further divisions have been proposed by Minczewski(358):

trace	microtrace	ultramicrotrace	submicrotrace
10^{-1} to 10^{-3}	to 10^{-6}	to 10^{-9}	$< 10^{-9}$ (%)

Kaiser (248) in an interesting and highly personal account on "Guiding Concepts Relating to Trace Analysis" has suggested that "trace" should be retained for qualitative descriptions, and a new term e.g. "Scope 5;14" implemented as a definition (where the numbers are the negative decadic logarithms of sample weight and component concentration).

Trace analysis currently occupies a very important position in many disciplines, but in an academic environment of analytical chemistry emphasis is often laid on developing instrumentation and insufficient attention may be paid to practical samples. Meinke (349) has pointed out that few analysts are capable of analysing at much below the p.p.m. level on practical samples, except under special circumstances. In fact, the analysis of real samples at the sub-p.p.m. level by most trace techniques requires great care, together with special facilities and special

reagents. In many cases the sophistication of the modern analytical methods has hidden the fundamental problems of analysis. It should always be borne in mind that the result in the analysis of real samples will be the product of several steps, each of which may increase the error associated with the determination; these steps include collection, handling and storage of the sample, preparation for analysis, and the final determination step (71).

2.2 Sample Collection

The first problem is to define the system being measured, and then to establish what constitutes a representative sample of it; the second problem is actually taking the sample. Good sampling is essential for a meaningful result in any analysis, but in many instances insufficient attention is given to this operation. Often the errors introduced by sampling or by the non-homogeneity of the sample will be greater than those associated with the determination (71); sampling errors will depend on the type of material to be analysed - biological materials being one of the more difficult, and especially so when analyses are to be undertaken at the trace level. A common assumption that the system under investigation should be homogeneous may be erroneous: variations in the analytical results from separate sample sites may reflect the true situation and not the competence of the analyst or the analytical technique.

In clinical cases it is often thought that a sample taken in a routine manner will reflect the state of the patient, but this assumption may not always be acceptable, especially if the analytical result is to be related to other results to indicate progress. The analysis can only reflect one moment in time in a dynamic system which is

constantly adjusting to many internal and external factors. A further problem, not often encountered in the sample types involved here, but which may occur in environmental systems with a finely balanced equilibrium, is whether the actual process of sampling affects the system under investigation.

In a situation involving a patient. the specimens of choice for trace metal determinations will be blood and urine. though sometimes special samples may be provided e.g. hair and nail clippings in chronic arsenic poisoning. Blood and urine will also be available from post-mortem cases, and samples of liver will usually be taken - liver is a very important specimen, being the major detoxication organ of the body.

Urine is a most valuable fluid for toxicological analyses, as from it indications of excessive excretion of Pb, As, Hg, Bi, Cu, Fe, Au, Ag and Zn can be obtained (98). Urine is normally freely available and since metal levels are often quoted in terms of excretion over 24 hours, a 24 hour sample is preferred. Urine excretion may vary between 500 and 1500 cm^3 per day (85), and "normal" levels expressed as concentrations will cover a wide range. Even at post-mortem when the bladder is apparently empty, traces of urine may still be available in sufficient quantities for sensitive micro-determinations (98). Urine is a single phase specimen and sub-sampling should present few problems. Urine commonly contains a high proportion of particulate matter and if it is necessary to establish the quantity of trace metal actually dissolved, then the biologist's maxim of regarding anything passing through a 0.45 micron Millipore filter as being dissolved, could be applied.

Blood can be regarded as a single phase specimen for purposes of sub-sampling, though for some purposes it may be preferable to perform an analysis on serum or

plasma rather than whole blood. Ten or twenty millilitres of whole blood can usually be taken from an adult patient though samples from children will necessarily be smaller, and for routine screening purposes often only finger-prick samples will be taken.

A sample of liver normally contains some blood i.e. the sample consists of two phases, and a decision must be taken whether to separate the phases or to take a representative proportion of each. Since a whole liver is rarely available for trace metal analysis, it is important to record the sample site, unless it can be shown that the analyte concentration is constant throughout the organ. Liver biopsies will provide smaller samples, of only a few milligrams.

2.3 Sample Handling and Storage

During each stage from sample collection to final analysis, the sample may come into contact with a variety of surfaces, be they implements or containers, made from a variety of materials. The twin evils of contamination of, or loss of trace metals from, the sample may result.

Contamination of the sample by containers. apparatus, reagents and the ambient air is all too common and is sometimes very difficult to avoid. Various workers have discussed the role of commonly available materials in the contamination process (226, 363, 435). In particular, Robertson (435) has shown that many of these materials contain concentrations of metallic impurities which are high compared to the levels of the same metals in a typical sample - sea water. Table 7 gives a small selection of his extensive data.

Table 7 Trace Element Content of Sea Water and Common Laboratory Materials (parts/10^9)

	Zn	Fe	Sb	Cr
Sea Water	10	10	0.3	0.5
Pyrex	730	2.8×10^5	2.9×10^3	-
Quartz	25	-	40	200
Polyethylene	25	1.1×10^4	0.8	19
Teflon	9	35	0.4	< 30
Rubber	4.1×10^7	<100	360	4.2×10^5
PVC	7.1×10^3	2.7×10^5	2.7×10^3	2
Perspex	< 10	<140	< 0.01	< 10
Kimwipe	4.9×10^4	1×10^3	16	500

The problems of contamination are best controlled by the use of apparatus made of inert material wherever possible, and polyethylene, Teflon and synthetic quartz are generally agreed as being the best materials. Stainless steel instruments are involved in the handling of liver samples, and though these may introduce some metallic contaminants, it is difficult to imagine pathologists using plastic implements in the interests of trace analysis - let alone the laser used by Hislop and Parker (215) for cutting bone samples.

Polyethylene is the principal material used because it is cheap, but it may contaminate some samples: it has been shown that the "Vacutainer" tubes used for collecting blood specimens may give rise to zinc and lead sample contamination, and therefore the use of siliconised tubes for lead determinations is recommended (202). Disposable pipette tips, indispensible for electrothermal atomization atomic absorption, have been cited as a source of Zn and Fe

contamination (504). In a study of the removal of trace metal impurities from polyethylene, Karin et al. (254) recommended the use of 8N nitric acid and a three-day leach period for optimum removal of the contamination.

Some workers recommend the provision of special facilities for trace analysis, with sealed rooms and positive-pressure filtered air (363, 602).

The purity of laboratory reagents is discussed later (section 2.4).

Loss of trace amounts of metals from samples in storage and handling may be as great a problem as that of contamination. Losses may take place by adsorption, precipitation, permeation, ion exchange, volatilization, liquid-liquid extraction, or sequestration by chemical or biological action (226).

Adsorption is likely to occur whenever samples come into contact with large surface areas - be they suspended particles or container walls. The particles may be already present in the sample e.g. calcium phosphate in urine, or the formation of a precipitate may result in adsorption or occlusion of the trace constituents. Glass is especially effective in holding trace metal ions, such as lead, which polymerise on hydrolysis.

An ion exchange process may occur with glass, or even with plastics if these have acquired ionic functional groups following treatment with strong acids.

Many metals may be lost from solutions as a consequence of the volatility of their compounds (180), and it has been shown (216) that conditions favouring the formation of covalent halides e.g. dehydrating agents in the presence of halide, will permit the quantitative distillation of

Cr, As, Sb, Sn, Se, Re, Ru and Os, and the vaporization of large proportions of Ge, Mo and Hg. The losses of metals from standard solutions are considered in section 2.6.6.

Most workers agree on the undesirability of glass as a storage vessel, and Minczewski (357) gives the following order of preference: "Teflon" and fluorinated plastics > polyethylene > quartz > platinum > glass. Dyck (134), however, working with silver ion and "Desicote" glassware coating, found that plastics were preferable to glass only over a period of a few days, and that plastics were more difficult to clean. For the storage of urine samples awaiting mercury determination, glass containers have been recommended in preference to plastic or paper containers (542).

Two factors associated with contamination and loss in sample handling and storage are the ratio of solution volume to surface area and the time that the solution and container are in contact. If these factors can be minimised then improvements in the accuracy and precision will result; it can be concluded that dilute solutions should not be stored for long periods.

If samples are submitted for analysis, and analysed promptly, then there will be no need to preserve them chemically. Urine and blood samples may be stored in a refrigerator at $< 10^{o}C$ provided that an anticoagulant is added to the blood samples. It is recommended that samples of urine to be analysed for mercury are preserved with potassium persulphate at the time of collection to prevent losses of mercury (542). Many biological samples now are kept deep-frozen prior to analysis, but blood samples will haemolyse under these conditions. Omang and Vellar (393, 394) have studied the concentration gradients of Ca, Cu and Na

arising in samples of sweat, urine and serum stored at room temperature, under refrigeration and deep-frozen. They found that calcium in serum or urine is precipitated during storage at room temperature or under refrigeration, and that concentration gradients do occur during thawing of frozen samples; in both cases they conclude, not surprisingly, that the specimens should be thoroughly shaken before analysis. If freeze drying is employed to dry biological specimens, then the problems of storage will be reduced (292).

2.4 Sample Preparation; Reagent Purity

It is unfortunate that many of the techniques of trace analysis do not allow the direct determination of analyte but necessitate some preliminary manipulation and chemical treatment. Electrothermal atomization atomic absorption spectrometry is perhaps better placed in this respect than many techniques: it is possible to analyse directly solid and various liquid samples (32, 158, 227, 257). Sample preparation steps may include grinding, mixing, ashing and dissolution. Separation steps may be utilised to separate the analyte from the matrix or other interfering species, and sometimes preconcentration steps are necessary. The reagents involved in any of these steps are important sources of contamination.

In the present work, although the direct analysis of urine was possible, sometimes it was desirable to employ solvent extraction procedures, and these are considered in greater detail in Chapter 4. For liver samples, destruction of the matrix was achieved by wet oxidation, and the methods employed for this are discussed in Chapter 5.

The need for pure reagents and solvents in trace analysis is obvious, but may be extremely difficult to achieve, especially since the content of impurity in the reagent should be lower by one order of magnitude than that to be determined in the sample. Interest in ultrapure substances intensified in the 1950's due to the demand for high purity materials for semiconductor devices, and now there is available a variety of methods for the preparation of high purity reagents - in fact the preparation of an ultrapure substance presents fewer problems than its storage.

Water, the most commonly used solvent, is usually purified using ion-exchange resins or distillation in glass or quartz apparatus (364), but this presents a dilemma: ion exchange removes most metallic impurities but introduces organic matter, while distillation reduces the level of organic contamination but tends to introduce metals. Alternative techniques for the purification of water are electrodialysis and sub-boiling distillation. A commercial unit from Millipore, which acts by passing water through a reverse osmosis membrane, granulated charcoal, two mixed bed ion exchange columns and a 0.22 μm membrane filter, has recently been evaluated by Knott (275) who compared the trace metal levels in the purified water with those from ordinary deionised water (Table 8).

Glass distilled water stored in a polyethylene container gave adequate blanks for the elements studied in the present work, with the exceptions of calcium, magnesium and zinc which gave varying sized atomic absorption peaks.

Distillations are widely employed for purifying volatile reagents although various other separation methods, including the use of chelating agents, can be used - for acids, bases and organic solvents; these have been listed by Mizuike (364).

Table 8 Comparison of Metal Levels in Millipore and Deionized Water

Metal	Millipore System	Deionized Water
	(concentrations in g/cm^3)	
Al	$< 5 \times 10^{-11}$	1×10^{-9}
Ca	$< 5 \times 10^{-11}$	$< 5 \times 10^{-11}$
Cd	$< 3 \times 10^{-12}$	$< 3 \times 10^{-12}$
Cr	$< 1 \times 10^{-10}$	$< 1 \times 10^{-10}$
Cu	$< 5 \times 10^{-11}$	1×10^{-10}
Fe	$< 2 \times 10^{-11}$	1×10^{-9}
Mg	3×10^{-11}	2×10^{-10}
Mn	$< 1 \times 10^{-11}$	$< 1 \times 10^{-11}$
Na	7×10^{-11}	1×10^{-9}
Pb	$< 5 \times 10^{-11}$	$< 5 \times 10^{-11}$
Si	1×10^{-9}	5×10^{-9}
Zn	3×10^{-11}	3×10^{-11}

The highest purity acids commercially available are those from B.D.H. (Aristar) and Hopkin and Williams (Ultrar); the impurity levels are of the order of 0.1 p.p.m., compared to 1 p.p.m. for Analar grades, and the maximum levels of metallic impurities (in p.p.m.) are listed in Table 9. These acids were employed throughout this study.

Sub-boiling distillation has recently received attention as an ultrapurification technique. It involves heating a liquid without bubble formation, either using a radiant lamp or a strip heater, and condensation of the vapour on a cooled surface. In some respects the method is similar to both isothermal and conventional distillation, and it combines the advantages of both i.e. minimal handling of the reagent and the avoidance of unrectified liquid in the vapour.

Table 9 Metal Levels in Purified Acids (in p.p.m.)

Metal	Nitric Acid		Sulphuric Acid	Hydrochloric Acid
	Ultrar	Aristar	Aristar	Aristar
As	0.005	0.005	0.005	0.005
Cd	0.005	0.005		0.005
Ca	0.05	0.5		0.05
Co	0.005	0.005	0.005	0.005
Cu	0.005	0.005	0.01	0.005
Fe	0.1	0.2	0.05	0.2
Pb	0.01	0.001	0.005	0.001
Mg	0.01	0.5		
Mn	0.005	0.005		0.005
Hg	0.005	0.001		0.001
Ni	0.01	0.005	0.005	0.005
K	0.05	0.1		0.05
Na	0.1	0.5		0.05
Zn	0.01	0.04		0.02
Cr		0.05		
Sr		0.5		
Al				0.05

A sub-boiling still was constructed as part of this project (Figure 3), and its performance was evaluated using water.

A flow-rate of 100 cm^3h^{-1} was achieved, and the purified water appeared to have a lower metallic content than normal glass-distilled water. The design was constructed from glass and, in addition to water, would be suitable for organic solvents. Sub-boiling stills for acids are normally constructed from quartz or Teflon, but if the condenser and drain tube in the design shown in Figure 3 were quartz, then this still could equally well be used for acids.

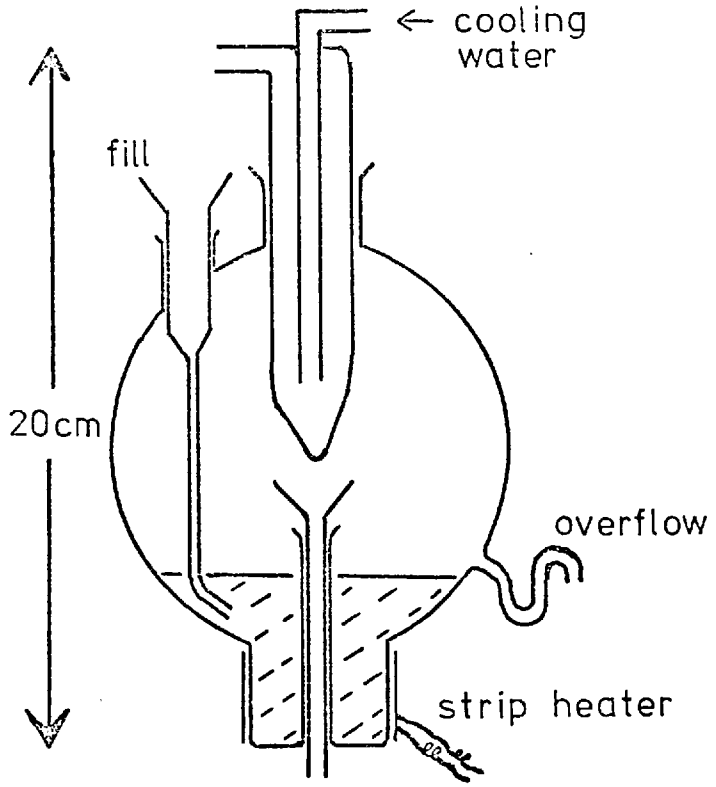


Fig. 3 Sub-boiling still

Kuehner et al. (289) have used sub-boiling distillation for the preparation of high purity acids and found that impurity levels were reduced by factors of ten to a hundred, and in the purified acids - nitric, hydrochloric, hydrofluoric, perchloric and sulphuric - no element was present in excess of 10 parts/10^9; most elements were well below the 1 part/10^9 level.

2.5 Sample Analysis; Selectivity and Accuracy, Precision and Sensitivity

In Chapter 1 the commonly employed techniques of trace metal determination were outlined, and it was pointed out that if an analysis is to give results meaningful in

health-related studies, then it must meet the three criteria of good selectivity (preferably specificity) and accuracy, precision and sensitivity, in that order (352).

2.5.1 Selectivity and Accuracy

Modern sophisticated and automated instrumentation often conceals the direct relationship which should exist between concentration of analyte and final analytical signal. The determination of one element in the presence of many others of differing concentration is not simple, and may be influenced by a range of factors; the continuous modification and improvements in analytical instrumentation may lead to anomalies in the literature, as shown in Table 10.

Table 10 Reported Blood Chromium Concentrations

Author	Year	Concentration $\mu g / dm^3$	Reference
Grushko, Y.M.	1948	35	Biokhimiya, 13, 124
Urone, P.F	1950	50	Anal.Chem. 22, 1317
Monacelli, R	1956	180	Clin.Chim.Acta. 1, 577
Volod'ko, L.V	1962	200	Vestn.Akad.Nauk., 1, 107
Schroeder, H.A.	1962	520; 170	J.Chron.Dis., 15, 941
Wolstenholme, N.A.	1964	1,000	Nature, 203, 1284
Feldman, F.J.	1967	29	Anal.Chim.Acta,38, 489
Hambidge, K.M.	1971	7	In Newer Trace Elements in Nutrition, N.Y.: Dekker.
Cary, E.E.	1971	7	J.Agr.Food Chem. 19, 398
Davidson, I.W.F.	1973	4.7	Amer.J.Obstet.Gynacol. 116, 601
Pekarek, R.S.	1974	0.72	Anal.Biochem. 59, 283

Obviously, these values for the concentration of chromium in blood do not represent a biological distribution. but rather improvements in techniques, more careful prevention

of contamination, or perhaps greater selectivity in the more recent methods - or alternatively the most modern methods may only be detecting a proportion of the chromium present in a blood sample.

Since the trace elements occurring in biological material will be in different forms which may respond in different ways to a given analytical technique, it is desirable to establish whether the technique is specific to one form, or responds to several, or gives a total elemental analysis. In inorganic trace analysis, specificity is generally taken as elemental specificity.

The accuracy of a method should be linked to its selectivity, taking due consideration of matrix effects, and the criteria which may be applied to establish the existence of selectivity and good accuracy in any given procedure will be considered in the section on calibration methods (section 2.6).

2.5.2 Precision

Precision is inversely proportional to the coefficient of variation (relative standard deviation) calculated from multiple determinations on a given sample. Although a high degree of precision is normally desirable, its importance will depend on the analytical situation. Since any biological parameter in a population will be scattered around a mean, it will have associated with it a natural standard deviation, often larger than that of the analytical procedure. Five per cent coefficients of variation in a procedure are often desired, but seldom achieved; since only relatively large differences in trace element concentration can be interpreted with confidence, a relative standard deviation of ten or twenty per cent will often be satisfactory. Improved interpretation of biological analytical data may result not only from better precision in the analysis but also from careful choice of meaningful samples.

From a forensic viewpoint it is sometimes said that "precision is desired, not accuracy". This is true in the type of forensic analysis which involves comparison of sample with control (100), but in toxicological work both precision and accuracy should be desired if the results are to be meaningfully related to literature values. The need for precision and accuracy in the determination of a metal in a biological sample for forensic purposes is not usually great, however, since it will normally be sufficient to establish either a normal level or a considerable excess.

It should always be borne in mind that a procedural precision will never be as good as that quoted in the original literature, and if inter-laboratory studies are attempted, even precisions of twenty to thirty per cent will be difficult to achieve (352).

2.5.3 Sensitivity

Sensitivity is the ability to detect differences in analyte concentration, and is closely related to the term "limit of detection". which is the ability to differentiate analyte signal from blank signal with a given degree of confidence (247). Greater sensitivity results from a larger signal change per unit concentration and a mathematical definition of sensitivity based on this concept has been proposed by Mandel and Stiehler (337) and Skogerboe *et al.* (491).

$$\text{sensitivity} \quad \gamma = \frac{dI}{dE \cdot s}$$

Where I = Intensity of signal measured
c = concentration of analyte
s = standard deviation of signal measurement

Skogerboe and Morrison (492) have shown how this approach may be employed to give an objective comparison of sensitivities of different methods.

In atomic absorption spectrometry, sensitivity is used in a special sense, and implies a fixed quantity, viz. the concentration or mass of analyte equivalent to 1% absorption (0.0044 absorbance).

Sensitivity in analysis is of course highly desirable, but even a non-detectable concentration of analyte will form a valid result if expressed as "less than the limit of detection". Analyses at or near the limit of detection of a given technique are obviously less precise, and it may be advantageous to choose an alternative technique of greater sensitivity, if one exists.

2.6 Calibration Methods

Most analytical procedures need to be calibrated, and the calibration methods commonly used, which must be applied to the total analytical procedure, will now be examined.

Since calibration methods are dependent on the use of standards, the concentrations of these must be reliably known. If chemical interferences occur, then both samples and standards must be treated similarly; the accuracy of an analysis depends ultimately on the accuracy of the standards.

2.6.1 Use of Synthetic Standards

Standard samples synthesised from pure substances are extensively used in analytical chemistry for relatively simple problems where the sample matrix is known, and contains relatively few components. This approach is very useful, especially when standards are prepared by simple dilution of analyte in a suitable solvent. A plot of analytical signal versus solution concentration is the analytical calibration graph; ideally this should be linear, should have zero intercept and should have a reproducible slope independent

of the matrix constituents (388). An intercept will be due to non-analyte signals (if the solvent is pure) and the slope will be influenced by matrix effects, as described in section 2.7. Solid standards, prepared by powder dilution, find some use in emission spectrography and X-ray methods and may find some application in electrothermal atomization atomic absorption spectrometry, but often they are impractical and subject to considerable error.

Standards may be prepared in an artificial matrix matching that of the sample, but in trace determinations of biological samples it will usually be difficult to obtain the matrix constituents in sufficient purity.

Whatever standards are prepared, they may be used either in constructing a calibration graph, or employed in the factor method or the bracketing method to obtain the analytical results (388).

2.6.2 Use of Certified Standards

Certified standards are useful for assessing the accuracy of an analytical procedure in any one laboratory, and become essential if several laboratories are engaged in a comparative study. As far as possible the standards used should match the composition of the samples. and for biological analyses there are available several certified standards. The United States National Bureau of Standards has prepared dried, powdered orchard leaves (Standard Reference Material 1571) and bovine liver (S.R.M. 1577), and biological standards are also available from other sources (605).

2.6.3 Use of Independently Analysed Samples

In the absence of available certified standards it is possible to calibrate by comparison with independent analytical methods. If an analysis is carried out with differing sample preparation, ashing or digestion, and

final determination steps, and agreement is reached between the results, then these results can be accepted as true indications of the composition of the sample. A useful independent method in trace element analysis is neutron activation analysis since this technique does not suffer matrix effects. and synthetic standards can be employed in its calibration. If a series of samples of similar nature are to be analysed, then it may be possible to choose one sample, analyse it by a variety of independent methods, and employ it as an "in-house" standard (388).

2.6.4 Use of Fundamental Relationships

In a limited number of situations it may be possible to achieve calibration by deriving a theoretical relationship between analytical signal and analyte concentration.

2.6.5 Standard Addition

The standard addition method (43) is a most important one in trace determinations, since it is the only calibration method that permits a quantitative determination when the analyte cannot be obtained completely free from the matrix. The method involves the use of the unknown and the unknown plus the addition of a known amount of standard; if a series of additions is made, a simple graphical technique can be employed to determine the unknown concentration. This is illustrated in Figure 4, where the signal produced by the sample is plotted on the ordinate and signals from the standard additions are plotted to the right of the ordinate. Extrapolation of the linear curve to the concentration axis to the left of the signal axis gives the concentration of analyte in the sample. It is possible to estimate the precision of the method without recourse to sample replication (294).

The standard addition method is only valid if the calibration graphs are linear and pass through the origin (a blank should always be run), and if the additions of

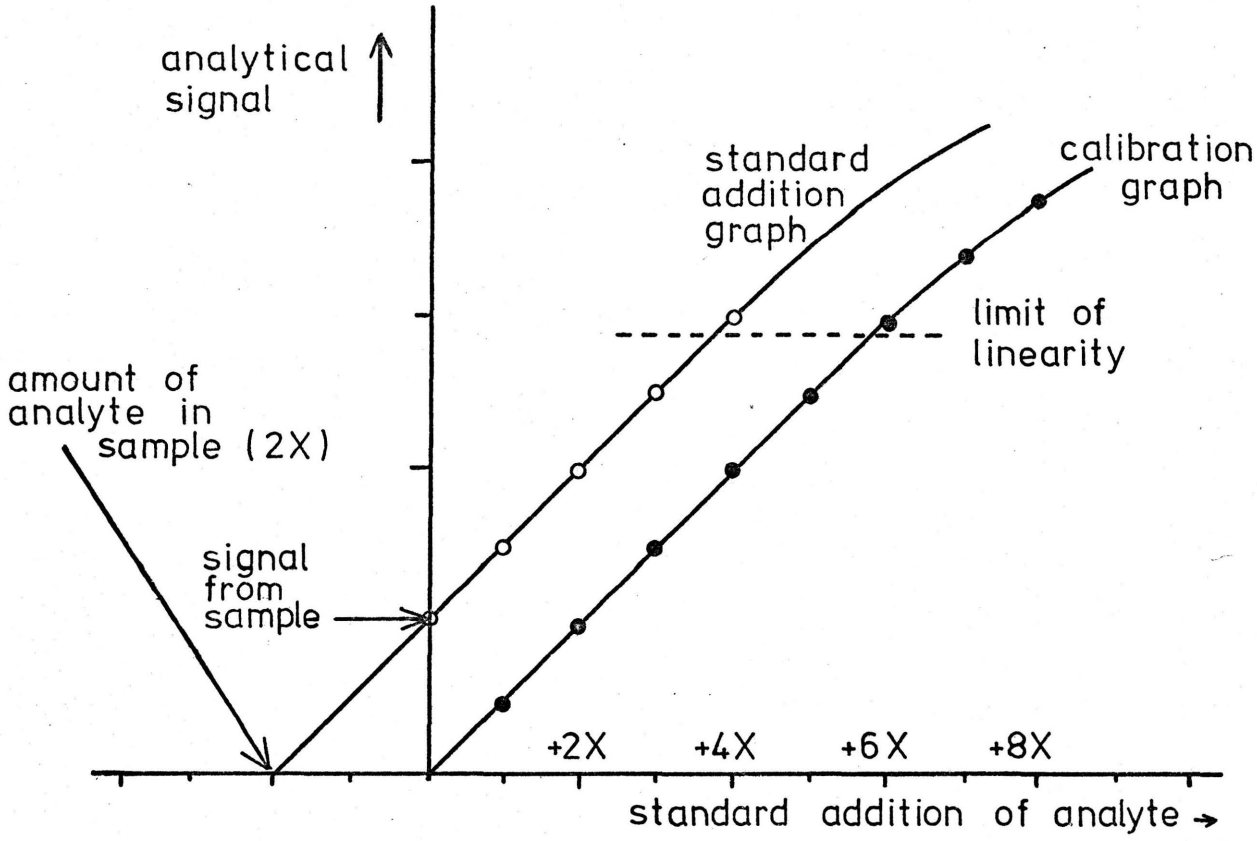


Fig. 4 The standard addition method

standard are in the same physical and chemical condition as the analyte. In flame atomic absorption spectrometry it is necessary to dilute the standard and additions to a constant volume to avoid corrections for differing volumes. This requirement does not arise in electrothermal atomization atomic absorption spectrometry since the signal is proportional to the mass of analyte, and is independent of sample volume; this allows the mechanics of the standard addition to be simplified: instead of pipetting a mixed solution of sample and standard into the atom cell, the sample can first be pipetted in, followed by an aliquot of standard. Japanese workers (544) have described the use of the standard addition method with carbon tube atomization atomic absorption spectrometry.

2.6.6 Standard Solutions

Most calibration methods require standard solutions and the preparation of stock solutions for flame spectrometry has been outlined by Dean and Rains (112). A more general, and perhaps more useful, listing has been given by Smith and Parsons (499) who made a critical selection of compounds suitable for preparing standard solutions, using the criteria of good stability, high purity, ease of preparation, good availability, high molecular weight, and low toxicity.

The 1000 p.p.m. stock solutions used in the present work were, where possible, prepared according to Smith and Parson's listing (499). The salts used in preparing the solutions are listed in Table 11. The final stock solutions were stored in polyethylene containers and were left slightly acid to prevent hydrolysis.

Table 11 Standard Solutions

Element	Salt Used	Weight Used (g/500 cm^3)
Copper	$CuSO_4.5H_2O$	1.9660
Lead	$Pb(NO_3)_2$	0.7993
Bismuth	Bi_2O_3	0.5574
Cadmium	$CdCl_2$	0.3066
Thallium	Tl_2SO_4	0.6177
Barium	$BaCl_2.2H_2O$	0.8896
Zinc	$ZnSO_4.7H_2O$	2.1990
Magnesium	$MgSO_4.7H_2O$	5.0706
Antimony	$SbCl_3$	0.9368
Nickel	$NiSO_4$	2.2388
Cobalt	$CoCl_2.6H_2O$	2.0188
Chromium	$K_2Cr_2O_7$	1.4145
Lithium	Li_2CO_3	2.6614
Manganese	$MnSO_4.H_2O$	0.8651
Arsenic	As_2O_3	1.3203
Mercury	$HgCl_2$	0.6768
Silver	$AgNO_3$	0.7874
Iron	$FeCl_3.6H_2O$	2.4201
Aluminium	$Al(NO_3)_3.9H_2O$	6.9520

A number of chemical suppliers produce standard solutions for use in atomic absorption analyses, but the use of these may be troublesome unless a complete recipe is available (see section 6.2.5). Standard solutions intended for use in flame atomic absorption usually contain high concentrations of chloride ion, but the use of these solutions in electrothermal atomization atomic absorption has been questioned by several workers. Campbell and Ottaway (72) state a preference for metal solutions of oxy-anion salts such as sulphate and nitrate rather than chloride solutions - since the latter would give a higher degree of molecular volatilization, decreasing the degree of atomization in the furnace. This has also been stated by Aggett and Sprott (5).

Adams, Kirkbright and Rienvatana (2) state that halide salts will vaporize without decomposition, and they obtained molecular absorption spectra from the halides of potassium and sodium in a heated graphite tube.

To test the hypothesis that chloride salts give rise to losses by molecular volatilization, several elemental standard solutions were made from both the chloride and an oxy-anion salt. Each standard was diluted to a given level with water, 2% (v/v) hydrochloric, 2% sulphuric and 2% nitric acid, and the atomic absorption signal for each solution was measured. The results for lead, cobalt, manganese and aluminium are given in Table 12.

The results for lead, cobalt, manganese were obtained by R.C. Henson, as part of his B.Sc. research project. The high lead blanks in the acids are ascribed to the use of G.P.R. or Analar acids instead of Aristar grade.

For the elements studied, there was no difference between the absorbances from the chloride salts and those from nitrate salts (within experimental error).

Table 12 Absorbance of Solutions Prepared from Chloride and Nitrate Salts (10 µl samples)

Lead (0.05 p.p.m.)

	Chloride	Nitrate	Blank
Aqueous	0.09	0.08	0.01
2% HCl	0.20	0.21	0.12
2% H_2SO_4	0.46	0.46	0.36
2% HNO_3	0.21	0.21	0.11

Cobalt (0.2 p.p.m.)

	Chloride	Nitrate	Blank
Aqueous	0.170	0.170	0.02
2% HCl	0.185	0.175	0.03
2% H_2SO_4	0.185	0.165	0.03
2% HNO_3	0.170	0.165	0.03

Manganese (0.02 p.p.m.)

	Chloride	Nitrate	Blank
Aqueous	0.26	0.26	0.02
2% HCl	0.27	0.28	0.03
2% H_2SO_4	0.28	0.28	0.03
2% HNO_3	0.26	0.27	0.03

Aluminium (0.1 p.p.m.)

	Chloride	Nitrate
Aqueous	0.06	0.07
2% HCl	0.075	0.075
2% H_2SO_4	0.075	0.075
2% HNO_3	0.075	0.07

When concentrated solutions are diluted to very low analyte levels, as is necessary for standardizing electrothermal atomization atomic absorption spectrometry, the stability of the solutions is important. At the nanogram-per-millilitre region it is not unusual to have standard and sample solutions change in concentration in a relatively short time - mostly decreasing in concentration by adsorption occurring on the container walls, though increases in concentration by contamination may occur. Where adsorption does take place, the extent and rate will depend on the element, its concentration, the pH of the solution and the type and history of the container (388).

Several workers have studied losses of metallic ions from aqueous solutions stored in various containers: West and co-workers, in a series of papers, studied the losses of silver (567), chromium (487) and selenium (488) in Pyrex, flint glass and polyethylene beakers, and investigated

the distribution of the metal ions on the container surfaces; Al-Sibaai and Fogg (17) examined the stability of dilute arsenic, iron and rhenium solutions; and losses of lead and cadmium during storage have been studied by Isaaq and Zielinski (231) and King et al. (262), respectively.

It can be concluded that serious losses of Ag, Mo, Mn, V and Ni occur in glass containers, and that losses of one to ten per cent in two weeks may occur for Sr, Zn, Cu, Fe, Pb and Al. Loss of cadmium will be minimised by the addition of nitric acid (262), while lead losses can be reduced by the addition of nitric acid or hydrogen peroxide (231). Anionic surfactants may be used for this purpose when acidic pH values are not tolerable (557).

Most elemental solutions are stable in polyethylene containers, especially when kept acidic ($pH < 2$) to minimise wall adsorption, though Dyck (134), working with silver, found plastics preferable to glass only over a short period. He also found plastics to be more difficult to clean, but perhaps silver is not a typical element: losses of fifty per cent or more may occur in only a few days (134).

Much attention has been focussed on the preparation and storage of mercury solution and samples for mercury determination (50, 152, 232, 305, 387, 443): losses of mercury at the 0.1 p.p.m. level may occur through volatilization or adsorption and it is recommended that mercury solutions at this level should be made up in either 5% (v/v) nitric acid and 0.01% dichromate (152) or in 1M hydrochloric acid (50). The use of hydrogen peroxide as a preservative (232)has been questioned (305). Glass containers are preferable to polyethylene (542), since use of the latter may cause high readings (50), due either to mercury leaching from the container surfaces or mercury vapour passing from the ambient air through the container wall.

2.6.7 The Blank

The blank is a solution containing everything in the sample solution except the analyte, and by subtracting the blank signal from the total analytical signal it is possible to correct for nonanalyte signals, assuming that there are no analyte-matrix interactions. In the analysis of real samples, however, it is seldom practical or possible to prepare an exact blank. Solvent and reagent blanks may be prepared and will correct for signals produced by these materials and their impurities. In some cases it is possible to prepare a matched-matrix blank, made up to reflect the composition of the sample e.g. blood serum, and it may even be possible to produce an internal blank by removing the analyte from the sample solution.

It is essential to carry a blank throughout the entire analytical procedure; it is then possible to recognise excessively high blank values.

2.7 Matrix Effects

The matrix is the major constituents of a sample, and it may or may not interfere with the determination of the trace constituents. It is well known that the same concentration of a trace element gives different sized signals in different matrices; this is known as the matrix effect, and may reveal itself as either an enhancement or a depression of the signal.

The difficulties caused by the matrix effect can be overcome by separation or isolation of the trace elements prior to determination, but this will defeat the primary advantage of electrothermal atomization atomic absorption i.e. the technique is a direct one that eliminates the need for (contaminating) reagents. The matrix effects in this technique may be caused by several factors: vaporization of analyte as molecules, molecular background absorption,

scattering of source radiation, or gas phase interaction of atoms with the matrix components.

In order to establish whether a determination suffers a matrix effect, it is necessary to effect a standard addition on the sample and to compare the plot with a calibration using aqueous solutions of the analyte: Figure 5.

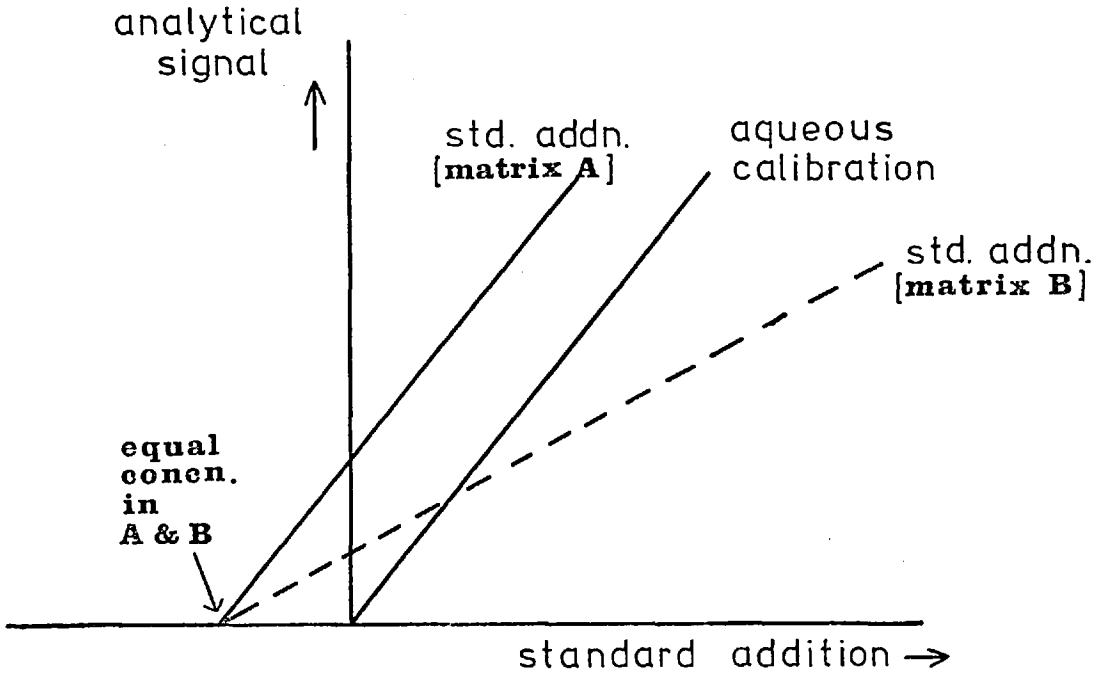


Fig. 5 Matrix effect

If no matrix effect is present in the sample, then the two graphs are parallel; a matrix effect causing signal depression gives a standard addition graph of lower slope than the aqueous calibration. This was the only type of matrix effect encountered in the present work, since those resulting in increased slopes compared to the aqueous calibration, caused by non-atomic absorption, would be eliminated automatically by the deuterium arc background corrector.

Since an accurate standard addition can only be performed over the straight line portion of the graph it is necessary to establish the extent of the linear region of the calibration graph for each element. If this is not done, the standard addition may result in large errors (Figure 6).

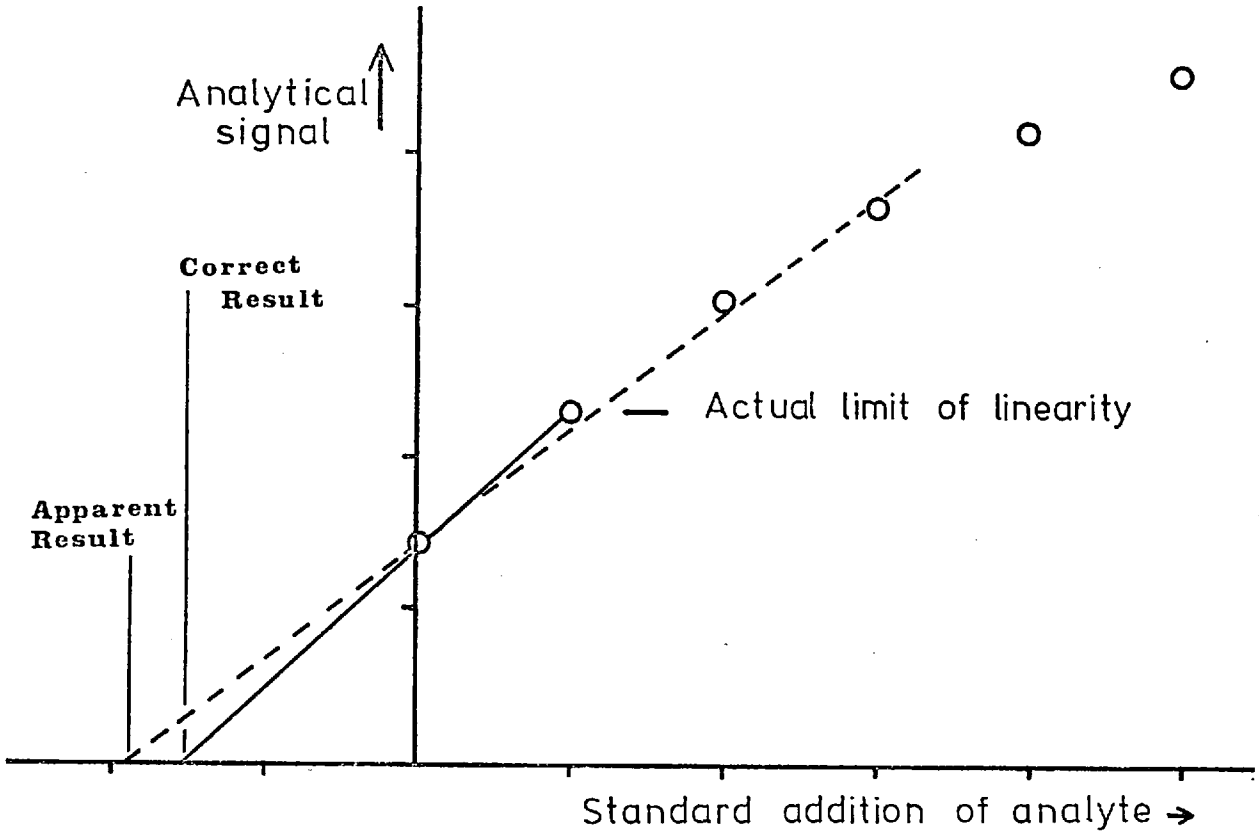
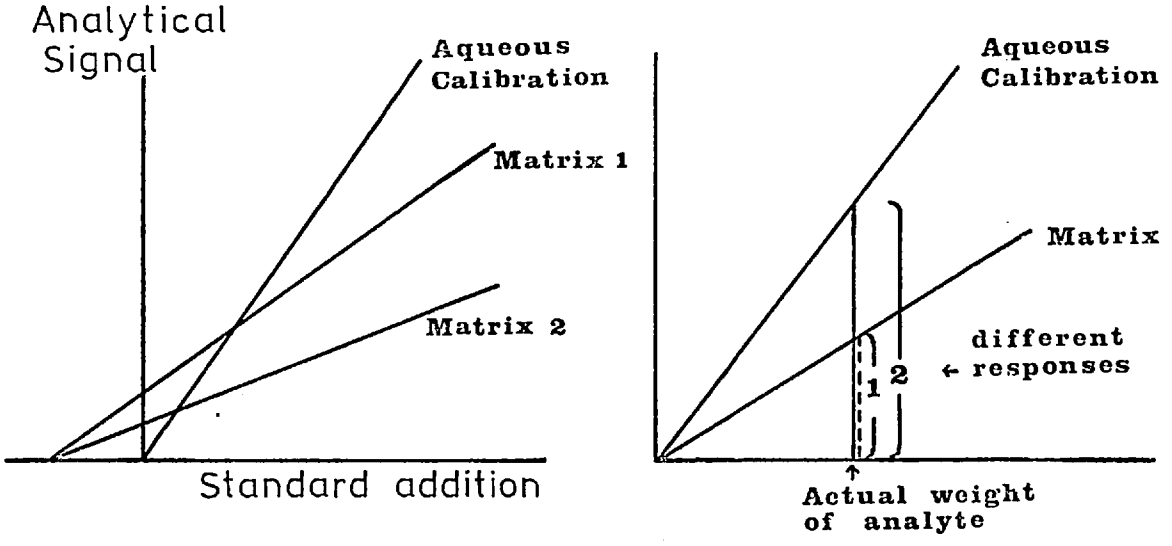


Fig.6 Possible error in standard addition analysis

This factor is especially important in electrothermal atomization atomic absorption where the calibration graphs are generally linear only up to 0.2 - 0.3 absorbance, compared to flame atomization atomic absorption where the linear region may extend to 0.6 - 0.8 absorbance. A discussion of the causes of the bending of the calibration graphs is given in Chapter 7, together with possible means of increasing the linear ranges.

For the purposes of comparing matrix effects between different samples and different sample preparation steps, the matrix effect may be defined as the ratio of the slope of the aqueous calibration to the slope of the standard addition (Figure 7).



definition:

$$\text{matrix effect} = \frac{\text{slope of aqueous calibration}}{\text{slope of standard addition}}$$

Fig. 7 Definition of matrix effect

It is obvious that standard additions of increasingly smaller slope compared to the aqueous calibration will be due to increasing matrix effects, up to a limiting case where the standard addition falls on the abscissa, and the matrix effect becomes infinite. The above definition allows for this, but the absence of a matrix effect will be defined as a matrix effect of unity (Figure 8).

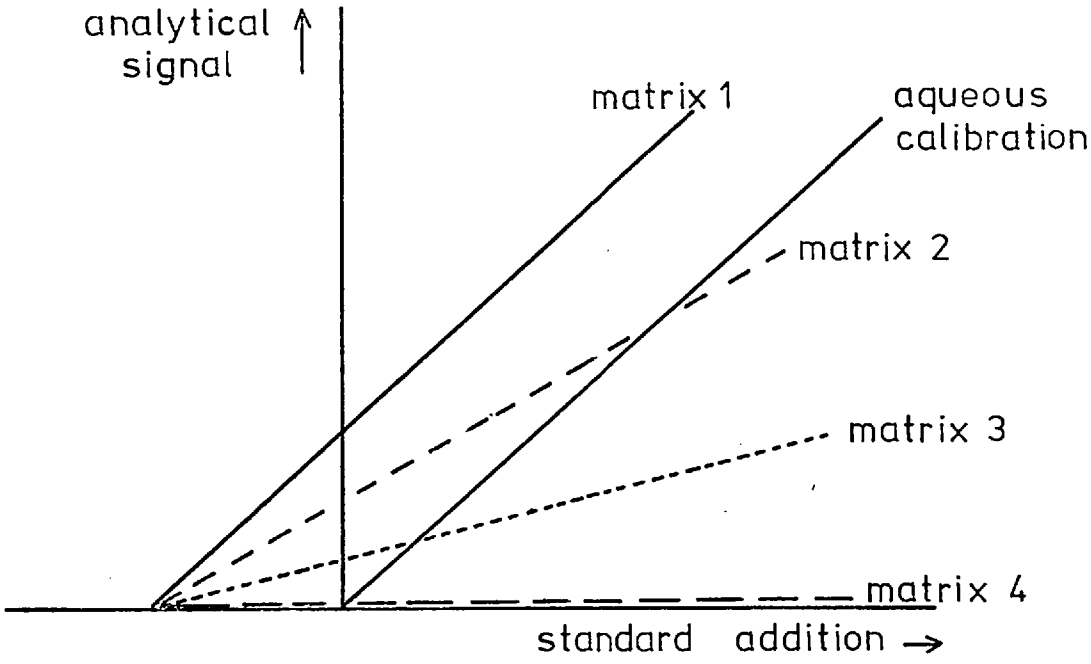


Fig. 8 Numerical values of matrix effects using the definition given in Fig. 7

matrix	1	-	matrix effect	= 1.0
"	2		"	1.73
"	3		"	3.73
"	4		"	∞

Chapter 3

INSTRUMENTATION

3.1 Atomic Absorption Spectrometry

Since the introduction of atomic absorption spectrometry (A.A.S.) as a viable analytical technique by Walsh (562) in 1955, it has gained wide acceptance for determining many metallic elements at trace levels. Having now truly "come of age" (568). atomic absorption is both well established and well documented. Countless reviews and several monographs have appeared: particularly useful are the books by Kirkbright and Sargent (268), by Price (424), by Christian and Feldman (89) - dealing with agricultural and medical applications of flame atomic absorption - and by L'vov (317) - which is notable for descriptions of graphite furnace atomization and detailed expositions of theory.

Reviews dealing with the biological applications of atomic absorption include the contributions by Willis (576, 577, 578), one of the first workers to apply the technique to biological problems, and reviews by Christian (88), Schroeder and Nason (473), Delves (117), Berman (42) and Matoušek and Stevens (348). From a forensic viewpoint, three rather trivial reviews have appeared: by Ramírez-Muñoz (427), by Kirk (265) and by Horncastle (221).

Atomic absorption spectrometry may be defined simply as the measurement of absorption of radiation by atoms. If the radiation is of a discrete frequency, ν, then the absorption coefficient k_ν may be defined by the equation:

$$I_\nu = I_\nu^o . e^{-k_\nu l} \qquad [1]$$

where I_ν^o and I_ν are the initial and final intensities of radiation passing through an absorption cell of

length l, containing the atomic population. A plot of k_ν vs. ν will give the profile of the absorption line. This profile is the consequence of a combination of various factors and the resultant half-intensity width for an absorption line is of the order of 0.002 nm. Those factors which contribute to the formation of an absorption line are considered in more detail in Chapter 7.

Two quantities related to k_ν are k_o, the peak absorption coefficient when Doppler broadening alone is present, and K the integrated absorption coefficient. a quantity independent of frequency. The difficulties involved in measuring k_ν and K have been examined by Kirkbright and Sargent (268), who also give an excellent account of the various methods of evaluating a more convenient quantity, the total absorption factor A_T. This is defined as:

$$A_T = \frac{\text{absorbed radiation intensity}}{\text{incident radiation intensity}} = \frac{I_o - I}{I_o} \qquad [2]$$

Where I = amount of radiation leaving the cell. Related terms are per cent absorption $\{(I_o - I)/I_o\} \times 100$ and absorbance $(\log I_o/I)$.

The atomic absorption method proposed by Walsh (562) involves measuring the absorption coefficient at the centre of an absorption line. This is achieved simply by measuring the total absorption of an emission line of much smaller half-width. Under these conditions the total absorption factor A_T can be evaluated by setting the limits of integration of:

$$A_T = \int (1 - e^{-k_\nu l})\, d\nu \qquad [3]$$

as zero and $\Delta\nu_s$, the source line width (in practice actually $\Delta\nu_s\sqrt{\pi}/2\sqrt{\ln 2}$), and it is possible to derive

an expression relating absorbance $A = \log(1-A_T)^{-1}$ to concentration of absorbing atoms (268):

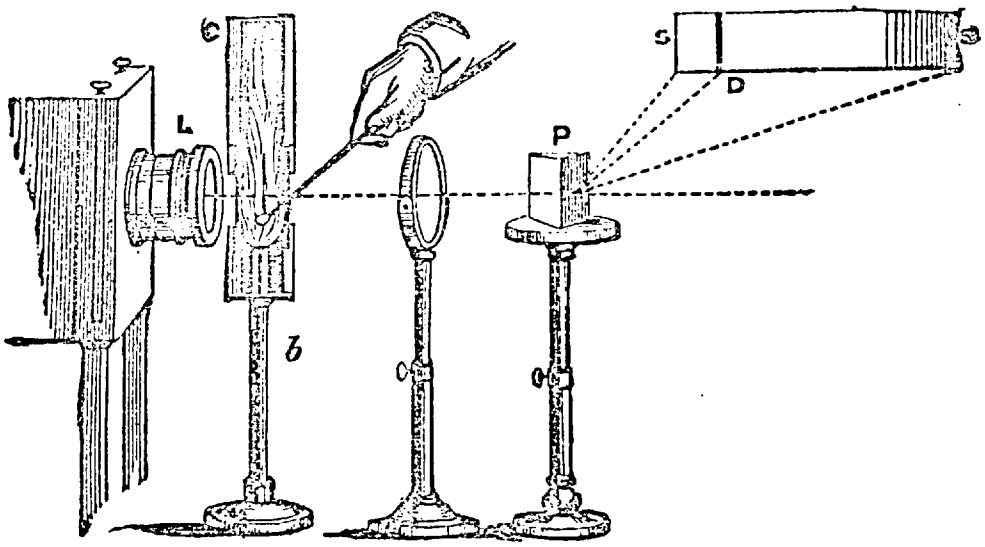
$$A = 0.434 \; b \; \frac{2\sqrt{\ln 2}}{\Delta\nu_D \sqrt{\pi}} \; \frac{\pi e^2}{m c} \; f_{ij} \; N_j \; l \qquad [4]$$

where:
- b = constant ($k_\nu = b k_o$)
- $\Delta\nu_D$ = Doppler half-width of the absorption line
- e, m = electronic charge and mass
- c = velocity of light
- f_{ij} = oscillator strength
- N_j = concentration of atoms capable of absorbing
- l = path length

Since the Doppler half-width is proportional to the square root of the temperature, the absorbance will be insensitive to small changes in temperature. A plot of absorbance against concentration should be rectilinear over a wide range.

The essential requirements of an atomic absorption system were illustrated by Tyndall in 1872 (Figure 9, reference 546) and comprise a primary source of radiation, a means of producing neutral atoms, a monochromator, a detector and some form of read-out. These components are found in all modern atomic absorption spectrometers, but other refinements have been added over the years to increase the sophistication (and cost) of the instrumentation.

Detailed descriptions of the components of atomic absorption instrumentation will not be given here, since much information is available in reviews and books and in manufacturers' literature. In the next three sections are given short reviews on electrothermal atomization and background correction - both of importance to the present study - and a brief outline of the instrumentation employed.



. — Optical arrangement used by TYNDALL to demonstrate the principle of atomic absorption. From left to right, emission source followed by the absorbing flame with its sample supplying system and the means to produce and observe the spectrum.

Fig. 9 Tyndall's Demonstration, 1872

3.2 Electrothermal Atomization in Atomic Absorption Spectrometry

A primary requirement in atomic absorption spectrometry is the efficient production of a stable atom population, and traditionally this has been achieved with flame atomization. To an extent, the use of flames descended from their application in flame emission spectrometry, but their intrinsic advantages have earned them a permanent place in atomic absorption methodology. Flames, however. do have limitations as atom cells (266), and the recognition of these limitations has led, in recent years, to much effort being devoted to alternative methods of atomization.

In general, the alternatives developed have widened the capabilities of atomic absorption as an analytical technique; it is now possible to analyse small samples of liquids or solids at sensitivities greater by one or two orders of magnitude than those attainable with flame cells (130, 266, 440. 526, 585).

A generic term "non-flame atom cell" has gained some acceptance in the literature; it covers any device used as an alternative to the traditional pneumatic nebulizer and flame atomizer. The term is perhaps unfortunate and a division of the various devices as to their mode of operation would be more informative, e.g. hydride generation, cold vapour, sampling boat, cathodic sputtering and electrothermal atomization.

The atomization devices which have aroused the greatest interest are the electrothermal atomizers, which rely on thermal energy transfer to the sample for atom formation. They may be further divided into tubes, filaments and strips, and loops.

3.2.1 Tube Furnaces

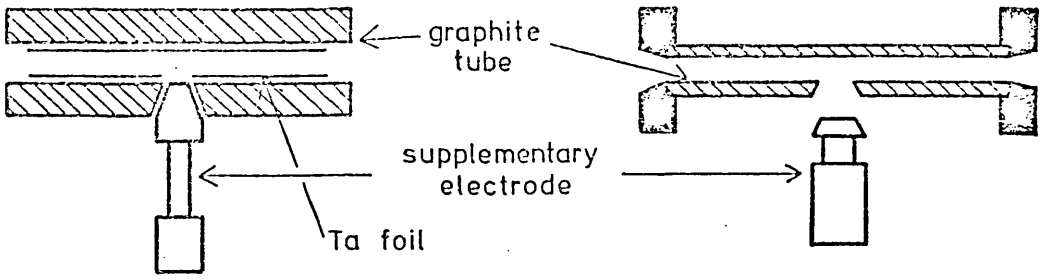
Although some workers have described atom cells based on heated quartz tubes (360), stainless steel tubes (224), tantalum tubes (432), and platinum tubes (46), it is the graphite tube atomizer which has received the most attention. The development of graphite furnaces in atomic spectroscopy may be traced to the work of King (260). King was interested in measuring the spectra from various elements under carefully controlled conditions, especially at different temperatures, as a means of understanding the absorption and emission processes taking place in stellar atmospheres. The furnace he designed cannot be strictly considered as an analytical device, since a dynamic equilibrium of atoms was maintained in the furnace, and thus it was impossible to determine the concentration of atoms in the cell.

L'vov's first furnace design (318-320) - based on the King furnace - was later improved (325); both furnace designs are shown in Figure 10. The later design employed resistive heating, and pressurization of the chamber with an inert gas gave increased atomic residence times. L'vov and co-workers have applied his furnace to several areas of analytical interest, but also to the determination of fundamental quantities - oscillator strengths (321, 327) and the Lorentz widths of atomic lines (326).

Massmann (344, 345) has described graphite furnaces for both atomic absorption and atomic fluorescence spectrometry. The absorption tube furnace is illustrated in Figure 10; it differed from the L'vov furnace in not being totally enclosed, nor was the sample introduced into a constant temperature tube. Sample solutions (5-200 µl) were introduced *via* the hole in the top of the tube, and solid samples (up to 1 mg) were introduced from the side; the aliquots of solutions were dried *in situ* by the application of current, prior to the atomization step. A commercial unit, the Perkin-Elmer HGA-70 - Heated Graphite Atomizer - (340) was based on Massmann's design of absorption tube furnace (Figure 10). Three heating cycles were used - for drying, ashing and atomization - and a "stop-gas" facility on the atomization step gave increased atomic residence times and improved sensitivities. An improved design, the HGA-74, was employed in this study and is described fully in section 3.4.

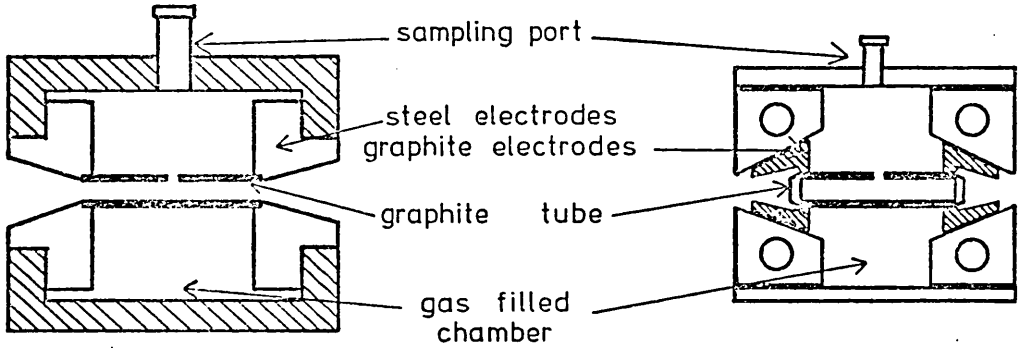
Woodriff's (588) design of furnace may be considered as intermediate to those of L'vov and Massmann; it is illustrated in Figure 10. The principal feature of the design is the capability for continuous sample introduction *via* the side arm.

The Varian carbon rod atomizer (C.R.A.) is illustrated in Figure 10; very small sample volumes (0.5 - 1 µl) can



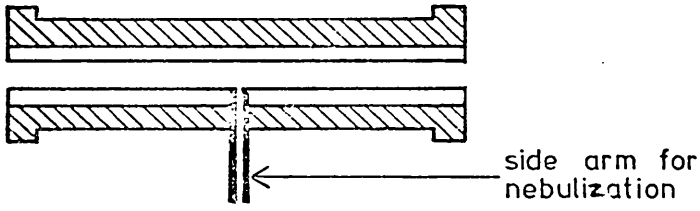
(a) 1959 version (b) 1967 version

L'vov's Graphite Furnaces

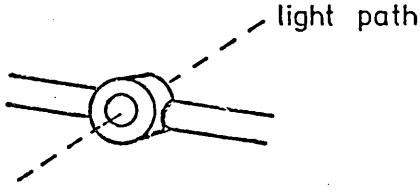


(a) Original 1963 version (b) Perkin-Elmer HGA (1970)

Massmann's Furnaces



Woodriff Furnace



Varian Furnace (Mini Massmann)

Fig. 10 Tube Electrothermal Atomizers

be used, and the volatile material passes completely out of the tube during the ashing step, rather than distilling to a cooler part of the tube.

Sometimes graphite tubes have been lined or coated with other substances for special purposes. L'vov's (318-320) original furnace was lined with tantalum to prevent the diffusion of vapour through the graphite, but the reason why Renshaw (432) employed a tantalum liner was to prevent the formation of barium carbide; an increase in sensitivity was also obtained. Baird and Gabrielian (30) lined a graphite tube with tantalum to prevent their samples from soaking into the carbon; they found improved analyte recoveries and precision in their analyses of arsenic and selenium in wastewaters. Woodriff and co-workers (296) plated the surfaces of graphite tubes with gold for use in mercury analyses; the mercury present in sample aliquots amalgamated with the gold, and was not lost during the drying stage.

Recently several workers have found that the use of pyrolytically coated graphite for electrothermal atomizers gives improved performance, since the coating prevents analyte samples from soaking into the surface of the tube. Though there have been descriptions of the treatment of carbon tubes prior to their installation in an atomic absorption set-up (490), it is generally agreed that the improvements are short-lived unless there is a continual renewal of the pyrolytic coating. Several workers (90, 339, 527) have described how methane may be introduced into the gas stream to effect this.

3.2.2 Filaments and Strips

The construction of atom cells may be simplified considerably by the use of carbon or metal filaments or strips; the "Alder-West"(8) cell design for a graphite

filament is shown in Figure 11.

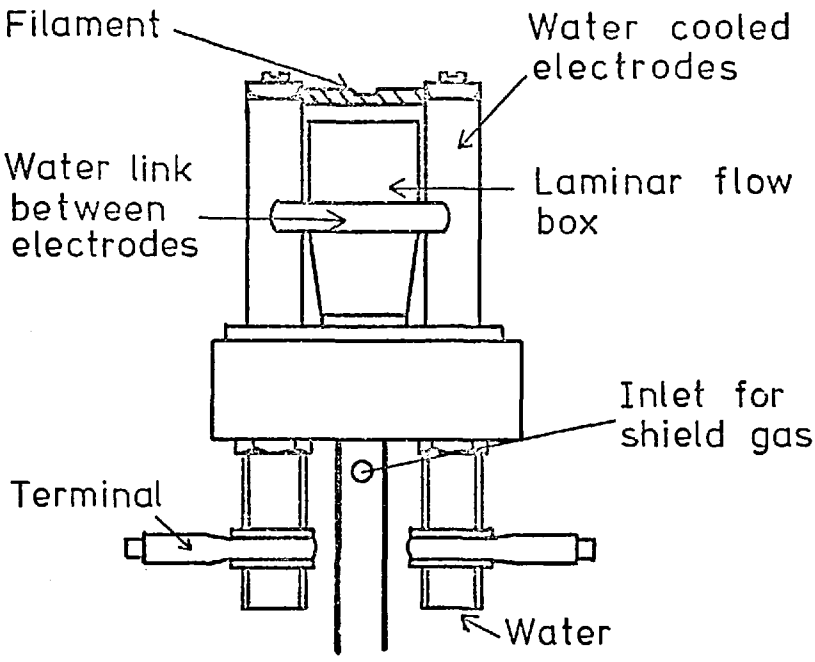


Fig.11 West's Carbon Filament (ref.(8))

During the atomization step the atoms are carried away from the filament surface by an inert gas stream; no further energy is supplied to the atoms once they have left the filament, and consequently the signals are of short duration (~ 0.5 sec.) Tantalum, tungsten and molybdenum have all been employed as alternatives to graphite.

3.2.3. Loops

The history of these simple devices may be traced back to 1859 when Bunsen (68) described the introduction of various metals and their compounds into a flame on a small platinum loop. Modern versions employ electrothermal atomization, and several kinds of wire besides platinum have been used (57, 83, 179). Sample size is 2 - 4 µl but

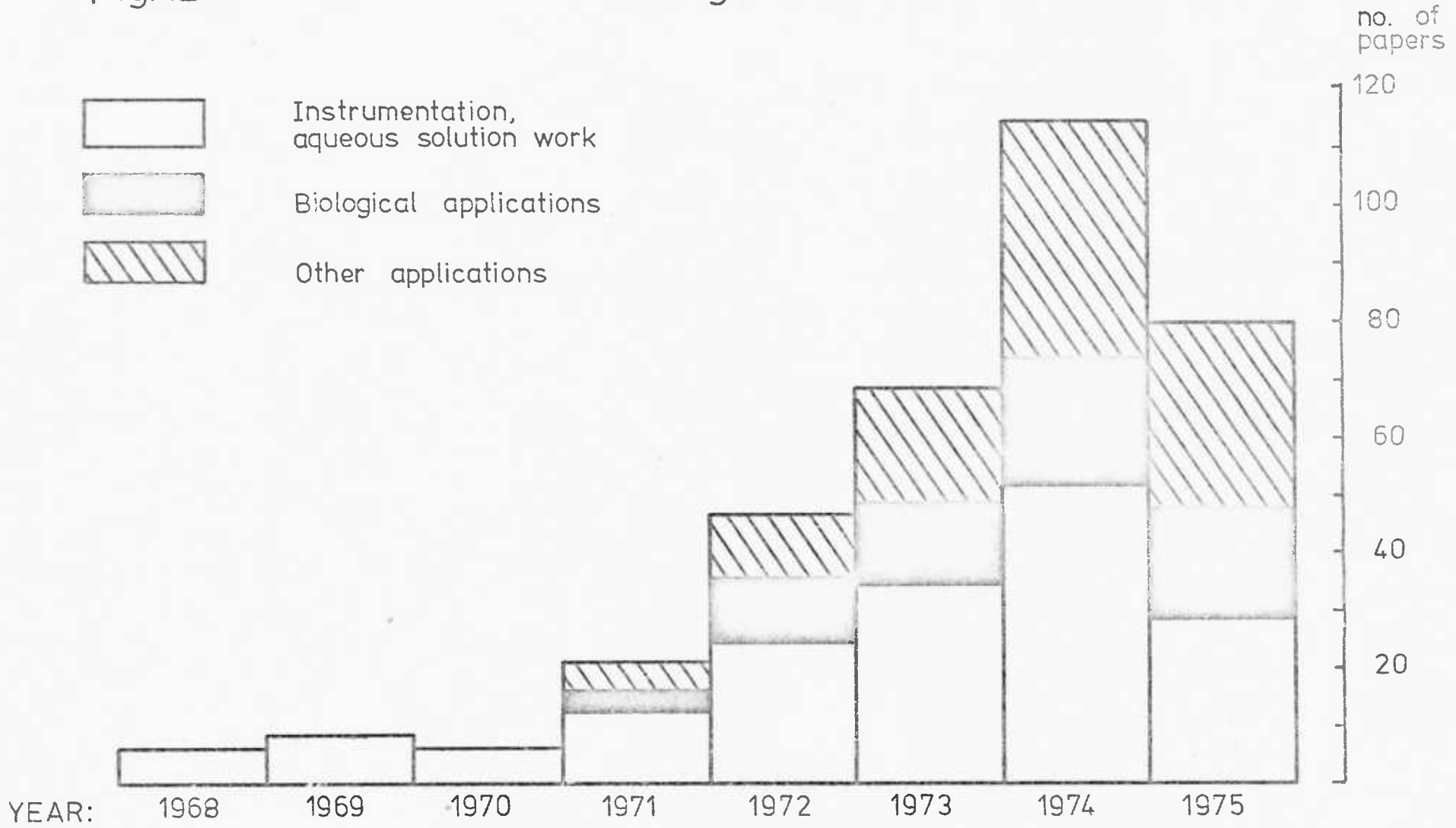
detection limits are not good.

In the period since Massmann (345) published his furnace design - in 1968 - and West (569) his filament design - in 1969 - many workers have produced their own varieties of electrothermal atomizer, but the advent of commercial instrumentation in this field has taken the edge off the race to produce modifications and improvements in design; the emphasis now is on applications of the technique. In this respect, electrothermal atomization atomic absorption is following the progress of any new technique: a few initial papers outling the technique, followed by a rush of interest and an upsurge in the number of papers published. As the method becomes well-established, the rate of increase of publications levels off, the content of the papers changes from instrumental variations to practical applications, and the rate of appearance of publications approaches that for all analytical papers (60, 61).

It is apparent that the "non-flame" methods of atomization have expanded the area of application of atomic absorption (and atomic fluorescence) spectrometry, but since about 90% of existing routine applications are satisfactorily covered by flame atomic absorption spectrometry, it is unlikely that flameless methods will ever replace the flame entirely (526).

Figure 12 indicates how, in the field of electrothermal atomization, emphasis has changed from papers outlining new instrumentation and data from pure aqueous solution work to an increasing proportion of papers detailing applications. It is interesting to see that electrothermal atomization was applied at an early stage to biological problems, and that biological applications still feature strongly; the other early application was to oil and petrol analysis. The number of publications used in Figure 12 was

Fig. 12 Publications involving electrothermal atomization



taken from the bibliographies which appear regularly in Atomic Absorption Newsletter; although these do not contain all publications in the field (60), they should at least be subject to a consistent editorial policy, and reflect the overall situation.

3.3 Background Correction in Atomic Absorption Spectrometry

In section 2.7 the causes of matrix effects were outlined, and it was pointed out that absorption by species other than the analyte atoms during an AA measurement might result in an enhancement of the analytical signal. Flames are notorious absorbers of radiation in certain spectral regions, particularly at short wavelengths, but scattering of radiation or molecular absorption by salts or other constituents of the sample can occur in both flames and electrothermal atomizers. In fact non-specific absorption can be several times greater in flameless atomic absorption or long tube atomic absorption (277) compared to the less sensitive flame technique, and the problem becomes increasingly apparent with biological matrices.

In electrothermal atomization A.A.S. it is sometimes possible to remove the species causing the background absorption by careful selection of ashing parameters, but usually there will be only a partial destruction of the interfering species before the analyte begins to atomize (439). It is claimed that if the inert gas is passed in through the ends of the tube and out through the dosing aperture, then the residual background absorption will be reduced - up to fifteen times (142). For precision and accuracy in most analyses with electrothermal atomization it is therefore necessary to employ some form of instrumental background correction, but before this is applied it will be desirable to minimise the background absorption either by instrumental or methodological modification e.g. correct sample dissolution, careful collimation of light beam, and judicious setting of ash and atomization times and temperatures.

Continuum source background correction (245, 277, 323) is available as an accessory from most instrument manufacturers. The principle is that absorption of the broad band of continuum radiation by sample atoms is negligible (since the spectral band width of the monochromator is typically two orders of magnitude greater than the atomic absorption line), but both the continuum radiation and that from the spectral source will be absorbed equally by non-specific effects. In practice it is necessary to match the two light paths as nearly as possible - usually by attaching mirrors to the conventional rotating chopper so as to alternate the two light beams. An alternative system is to use a semi-silvered mirror and a vibrating quartz plate placed in the exit slit of the monochromator to provide modulation, by short-range wavelength scanning, of the resonance line signal from the spectral source (362). A further variation - the use of polarization - of continuum source background correction has been described (586). An automatic background correcting system for use with transient (0.5 s duration) atomic vapour populations has been developed by Donelly et al. (128). In this system both the hollow cathode lamp and the hydrogen arc lamp are alternately pulsed.

The background correction system originally described by Koirtyohann and Pickett (277) employed a hydrogen lamp as continuum source, but deuterium lamps and arcs can be equally usefully employed (244); different instrument manufacturers promote slightly different sources. Epstein and Rains (143) have described the use of the very intense xenon-mercury arc lamp for background correction.

The alignment of the continuum source may be critical (338), and in flameless atomic absorption the geometry of the source is significant - an erroneous correction may be obtained unless the continuum image and that of the hollow cathode lamp are coincident. The intensities from continuum

and spectral sources must also be balanced, and in the wings of the continuum spectrum (Figure 13) this can often be achieved only by reducing the source operating current, restricting the area of the source beam, or using an extremely wide slit width.

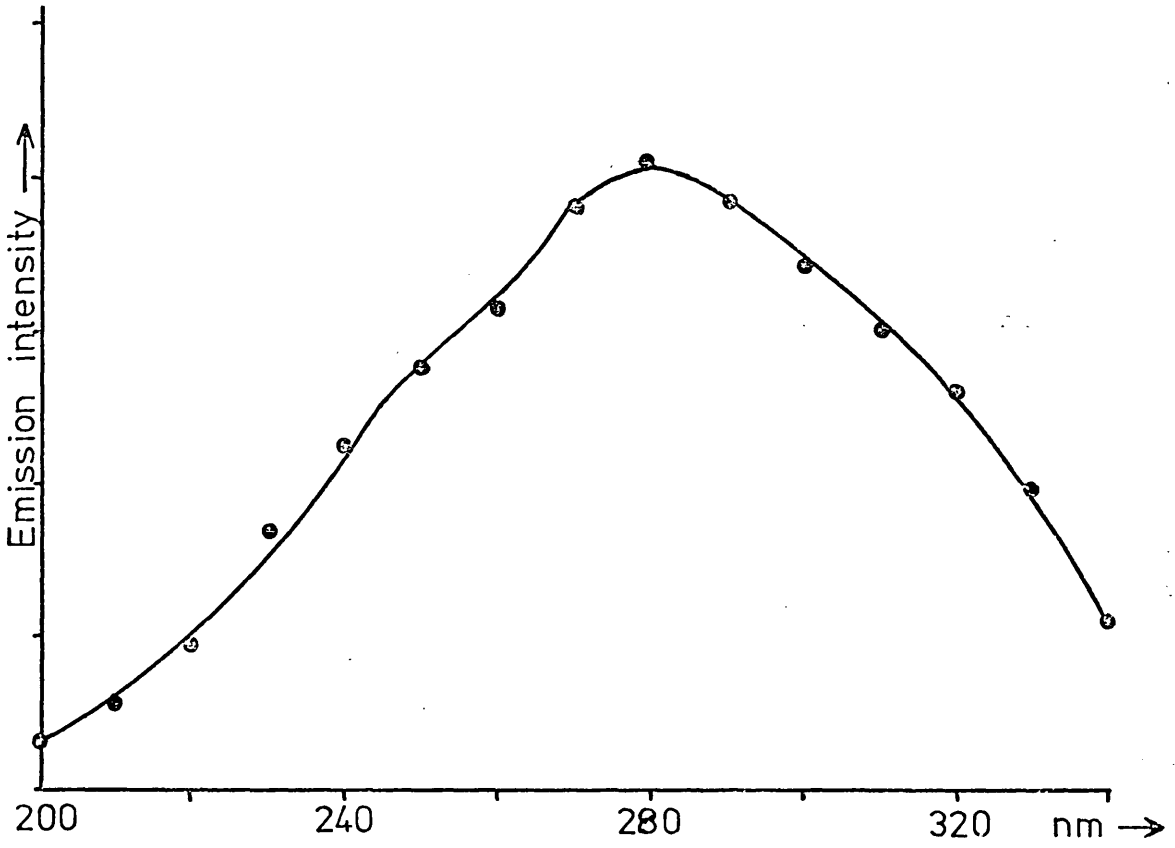


Fig. 13 Continuum spectrum from D_2 arc

A variety of factors - smoke from residual fragments of organic molecules, undissociated salt particles, even undissociated solvent vapour - will contribute to the background absorption in graphite furnace atomization. Several workers have plotted the molecular absorption spectra of species likely to occur in a sample matrix - species such as sodium and potassium halides. magnesium oxide, sulphur trioxide and sulphur dioxide - and have found that these consist mainly of broad absorption bands (2, 97, 189, 597).

Massmann (189) has also observed molecular absorption by CN and OH radicals and has pointed out that although molecular absorption bands can extend over 10 nm, complicated fine structure of the absorption spectra can also occur. This fine structure can prevent the correct functioning of a normal deuterium background correction system in the regions over which these species absorb; it is fortuitous that the upper limit to the working range of the D_2 arc ($\sim$ 360 nm) roughly corresponds to the limit of background absorption. Molecular absorption is not observed at the longer wavelengths e.g. Ca 422.6 nm (439)

A separate approach to background correction is to monitor the intensity of both the resonance line and a nearby non-resonance line (350, 438, 587), but since non-specific absorption is wavelength dependent, this method will only be successful if the two lines are close together. The method might be satisfactory for sequential correction, but in non-flame analyses it is essential to have simultaneous correction, as the size of the background signal may vary from one aliquot to the next.

One approach to background correction which is currently receiving interest is the use of the Zeeman effect to split resonance lines into pairs of non- absorbing lines at wavelengths outside the absorption profile of the atomic vapour. The effect may be produced - by the application of a magnetic field - in either the light source (279, 510), or the atom cell, whether flame (547) or electrothermal (110). The advantages of this approach to background correction are sensitivity, freedom from chemical pretreatment of the sample, speed and convenience of use, but unfortunately the range of applications is limited to the small number of chemical elements with practically useful Zeeman splittings.

3.4 The Experimental System

The atomic absorption spectrometer employed in this study was the Perkin-Elmer Model 300S. This is a single beam instrument incorporating integration (though not for transient signals), up to 40x scale expansion and automatic zero. Meter read-out is in either concentration or absorbance; absorbance signals resulting from atomization with the graphite furnace were recorded with an Oxford Instruments series 3000 recorder (response time for full scale deflection : 0.25 s). Background correction is effected by a deuterium arc, covering the range 190 to 325 nm. Since the spectrometer is single beam, a mirror-chopper system is employed - this allows radiation from the hollow cathode lamp and the deuterium arc to pass alternately through the atom cell and monochromator; the optical components of the complete spectrometer are shown in Figure 14. The monochromator is based on a Czerny-Turner mount, and incorporates two reversible gratings to cover the wavelength range 190 - 870 nm.

For flame atomization an air-acetylene flame was supported on a 10cm single slot titanium burner, but the atom cell used principally was the Perkin-Elmer HGA-74 graphite furnace.

The atomizer portion of the HGA-74 is shown in schematic form in Figure 15. The graphite tube (28mm long, 7mm o.d. 6mm i.d.) is held in position, and almost totally enclosed, by two graphite end cones which also carry the electrical current. Three holes in each of the end cones carry inert gas around the outside of the tube - the external gas flow - while an internal gas flow passes through the ends of the tube and out of the dosing aperture. This gas flow is the reverse of those in the HGA 70 and 72 atomizers, and is made possible by having quartz windows at each end of the atomizer unit. The advantage of the gas flow system in

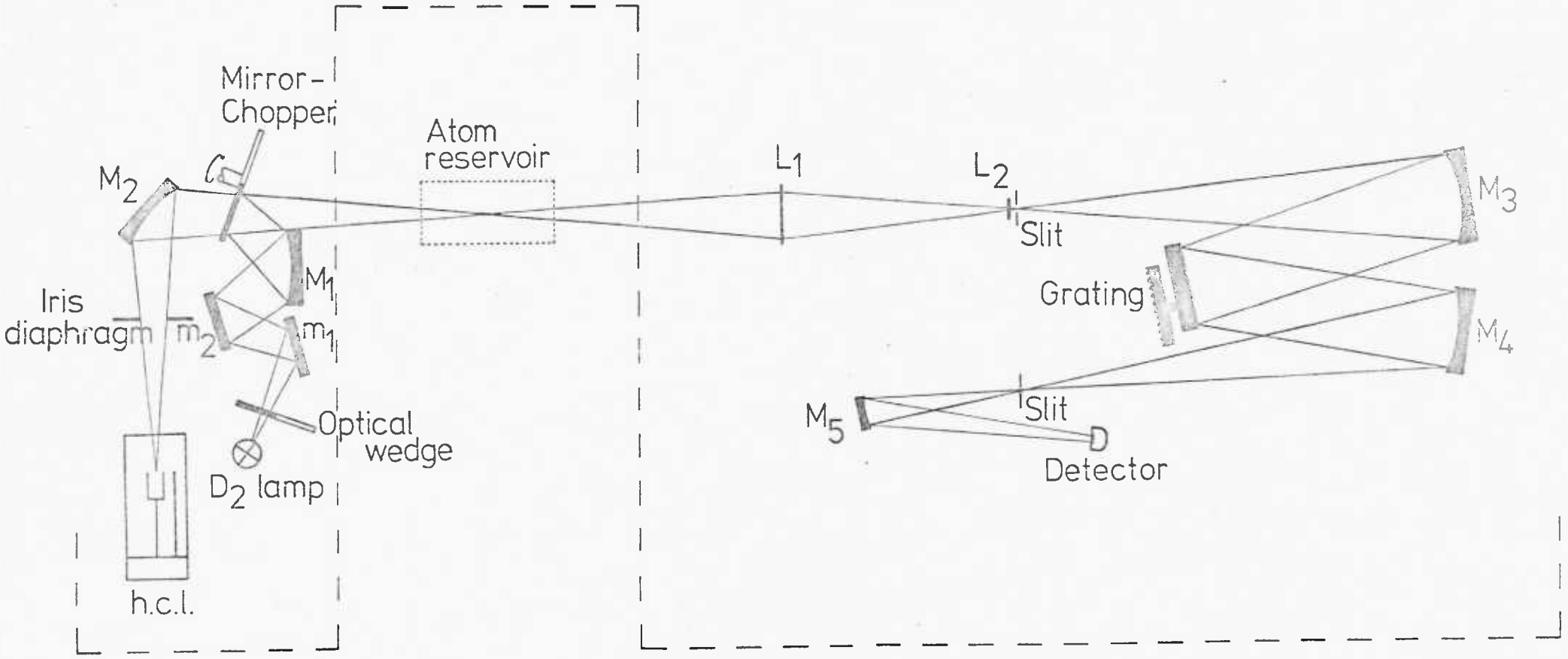


Fig. 14 Optical components of the Perkin-Elmer 300 AA Spectrometer with background correction

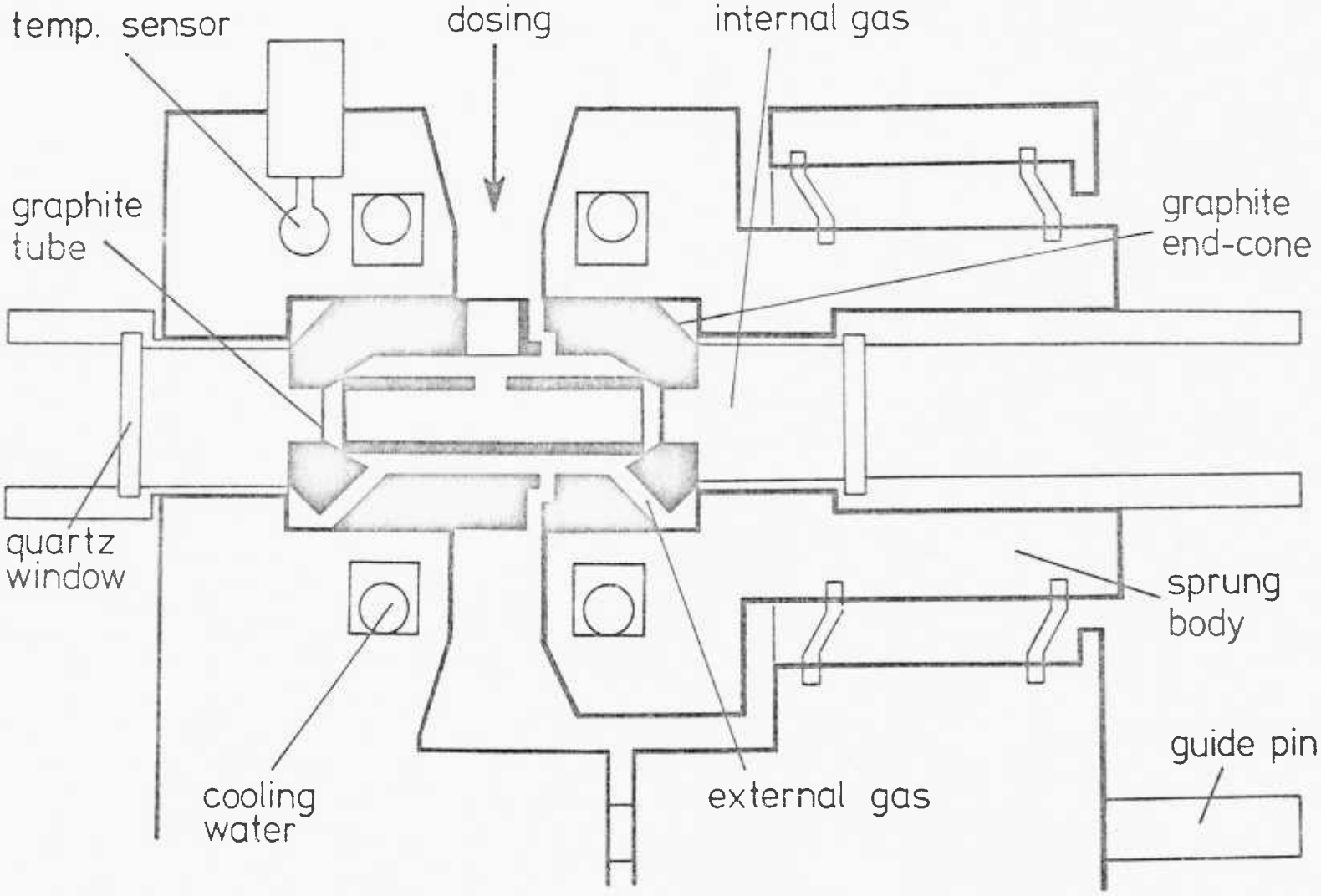


Fig. 15 Cross-sectional schematic of the HGA 74 atom reservoir

the HGA-74 is that the internal and external gas flows are independently adjustable; during the atomization step the external gas flow (900 cm^3/min.) is retained at its normal level, and thus will reduce incineration of the graphite tube, while the internal gas flow may be reduced from 300 or 350 cm^3/min. to zero ("Gas Stop") for increased atomic residence time, or to 50 cm^3 ("Mini Flow") for samples with a higher analyte concentration. The gas pattern of the HGA-74 also ensures the quick expulsion of any matrix components during the ashing cycle.

Either nitrogen or argon may be used as the inert gas. Nitrogen is cheaper, but this gas may react with the hot graphite to form cyanogen - which has an absorption band at the chromium wavelength (357.9 nm.). Nitrogen also gives decreased sensitivities compared to argon (Figure 16), and consequently argon was employed throughout this study.

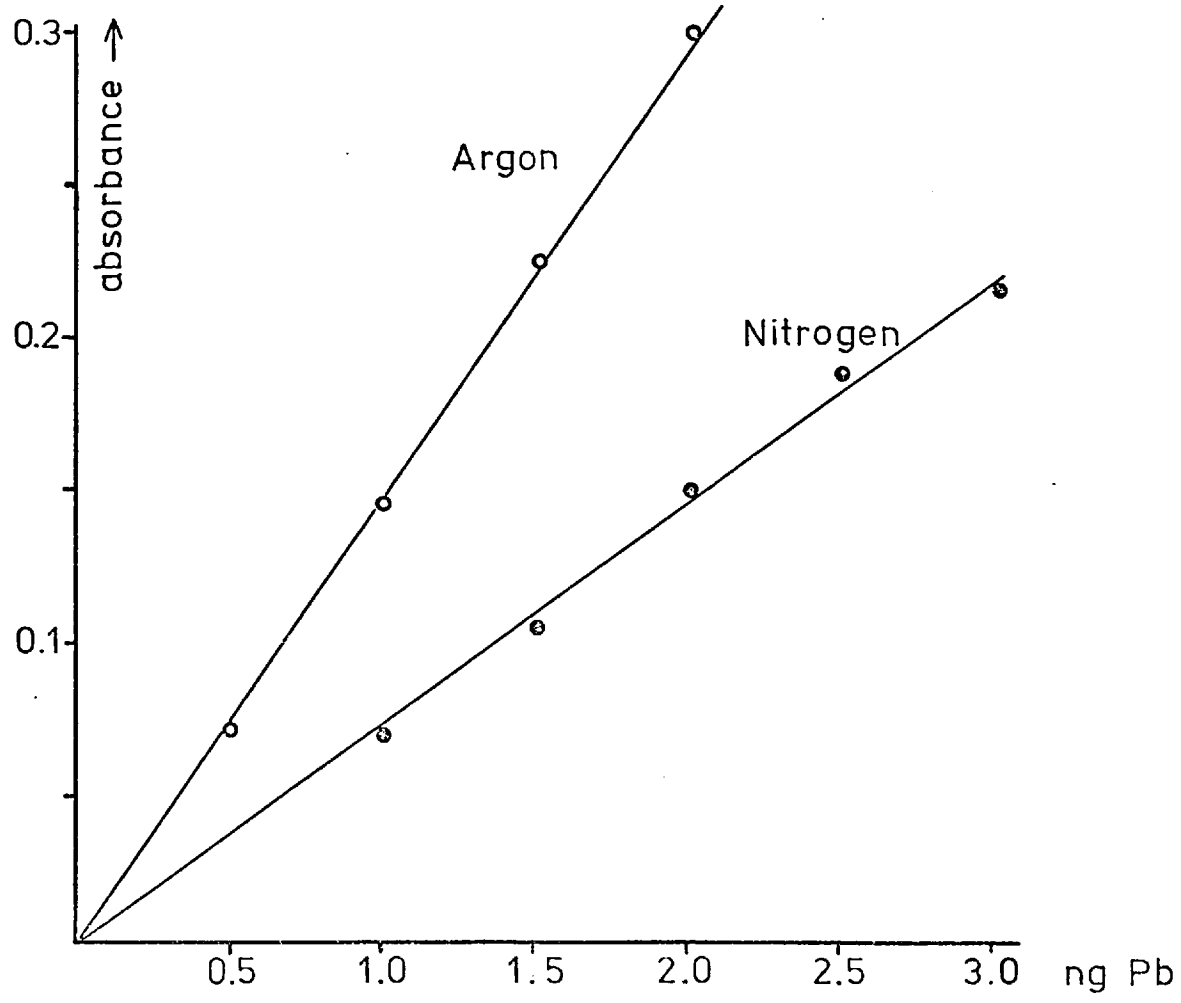


Fig. 16 Calibration graphs with argon and nitrogen

The control unit of the HGA-74 incorporates four variable time-temperature circuits which allow independent adjustment of the drying, ashing, atomization and heating-out steps. The digital switches 0-999 select temperatures 20°C - 2700°C; times may be set up to 30 h. A feature of the control unit is the ramp step, which allows selection of the initial and final temperatures of a temperature gradient, and varying rates of temperature increase. This ramp step produces steady ashing and, hopefully, the differential thermal destruction of a matrix. Since the ramp step can be used up to a maximum of 2700°C, it is possible to employ it for ramp atomization, and this is discussed in Chapter 7. Figure 17 shows how all the stages of the HGA-74 control unit may be used for a typical analysis.

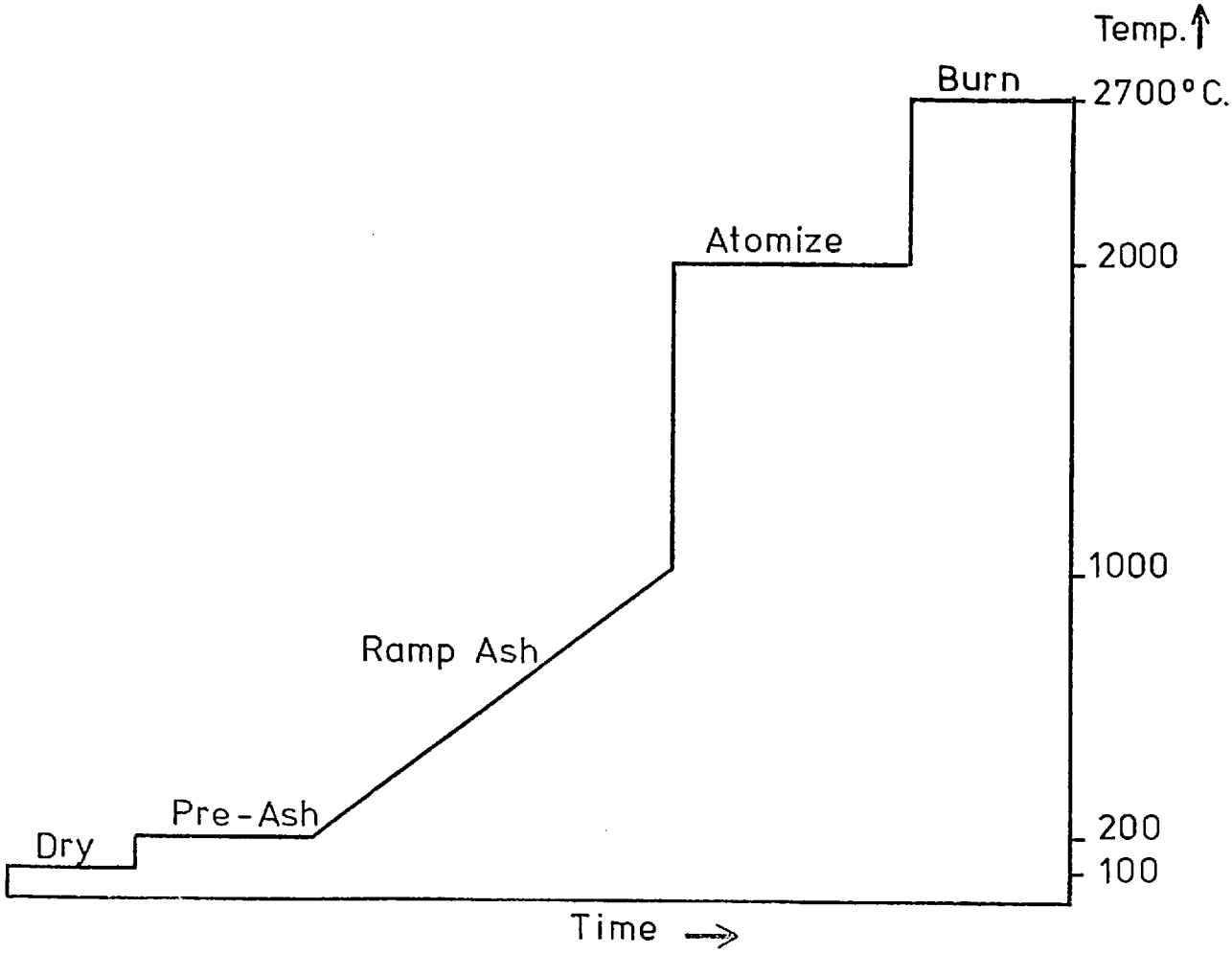
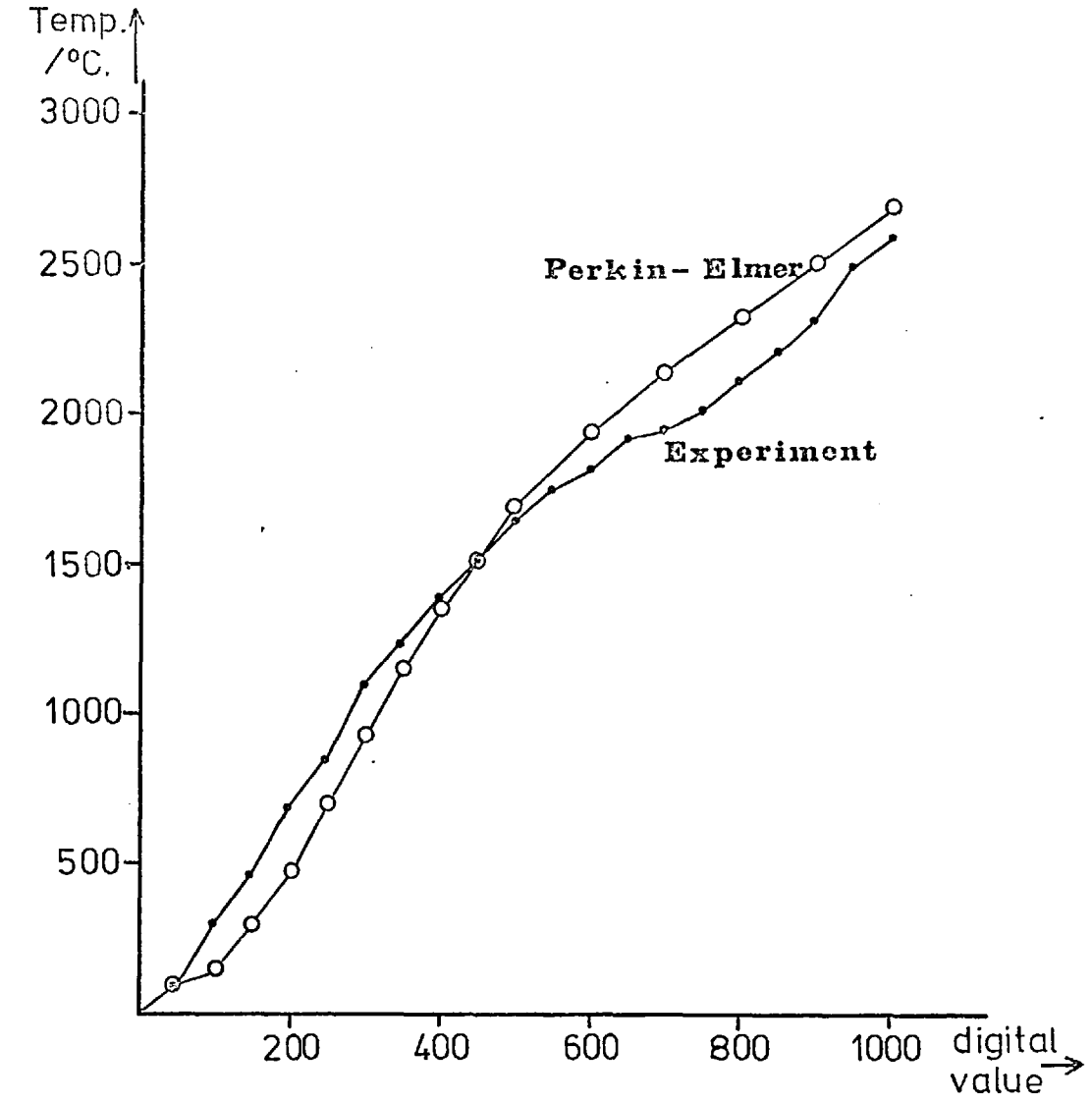


Fig. 17 Typical HGA-74 Programme

The HGA-74 control unit incorporates a thermometer, which indicates the temperature of the graphite tube. The thermometer is simply a calibrated resistance meter, only accurate to ± 50°C, and it may give varying readings for different tubes or for differing degrees of contact between the ends of the tubes and the end-cones. The temperatures indicated on the meter, corresponding to various digital values, were compared with the Perkin-Elmer calibration of temperature vs. digital value (409) and are shown graphically in Figure 18. The lower temperatures (up to 650°) were also checked with a thermocouple (Industrial Pyrometer Co. Ltd.) against the Perkin-Elmer data (409), and are also shown in Figure 18. A similar discrepancy was observed between experimental findings and the Perkin-Elmer data, and it was therefore felt that most confidence should be placed on digital values obtained experimentally for optimum ash and atomization temperatures, as opposed to relating these to the published temperature values (408, 409). A three-digit number would be of little value except to another HGA-74 user, however, and so these numbers were transposed to °C using the temperatures indicated on the meter; it was felt that these were the most accurate.

3.5 Optimization of Instrumental Parameters

The ashing and atomization steps were varied independently to determine optimum values for each element in aqueous solution; the drying and heating-out stages were kept constant, and the ramp ash stage was not used. The drying consisted of two stages: 70°C (digital 45) for 15-30s and 110°C (digital 60) for a further 15-30s (for 10-30 µl samples; longer time periods for samples > 30µl). The heating-out stage was at maximum current (≡ 2700°C) for 5s.



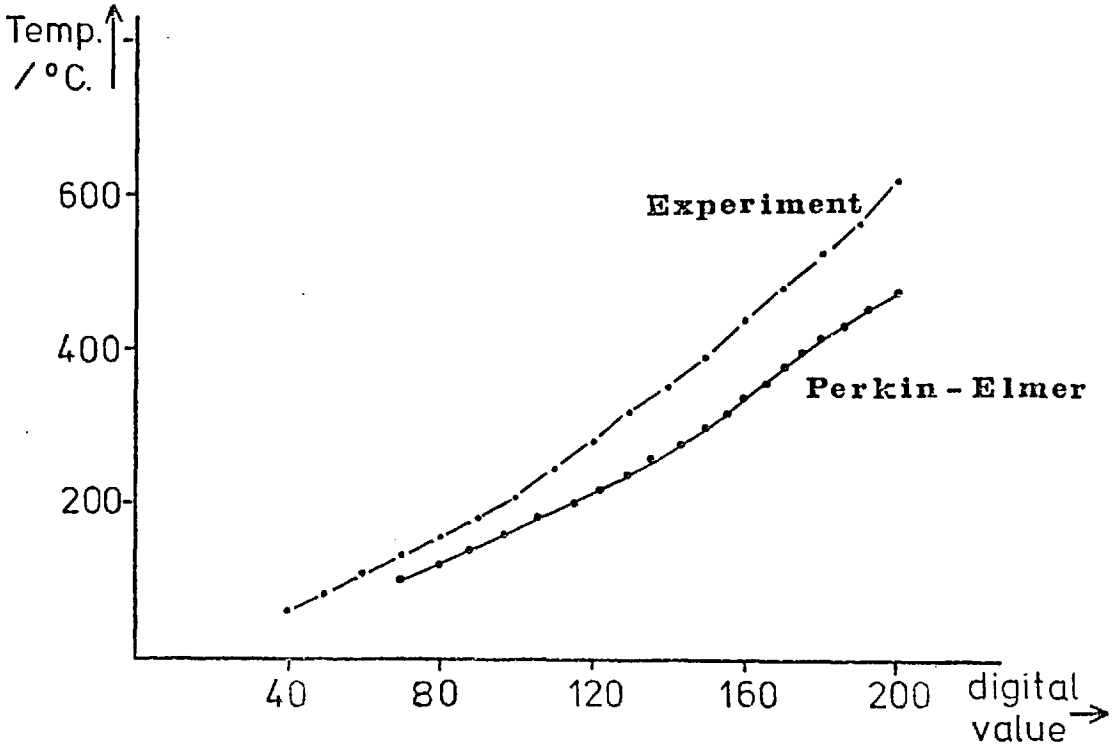


Fig. 18 Temperature calibrations for HGA-74

Typical graphs for optimizing ashing and atomization temperatures are given in Figure 19. These exhibit the maxima on a smooth curve obtained by other workers for aqueous solutions (340), though it should be noted that the presence of matrix may alter drastically both the shapes of the curves and the positions of the maxima.

The maximum ash temperature was taken as the highest temperature commensurate with minimal loss of analyte, whilst the optimum atomization temperature was selected as the lowest temperature on the plateau region - in order to increase tube lifetime. Table 12 gives the maximum ash and optimum atomization temperatures (in ^{o}C) of all the elements studied, and, for comparison, the values published by Perkin-Elmer (408, 409). Aggett and Sprott (5) and by Campbell and Ottaway (72) have published papers on the vaporization of elements from graphite surfaces; their data are given in Table 13.

3.6 Calibration Graphs, Sensitivities and Detection Limits: Analyte in Aqueous Solution

At the optimized furnace conditions, analytical growth curves were constructed to determine the range of the instrument. Argon was used exclusively as purge gas, and in most cases "Gas Stop" conditions of atomization were selected. The most sensitive resonance line was used for each element, except for zinc and bismuth.

The principal zinc resonance line is at 213.9 nm, and a very high sensitivity ($\sim$ 1 pg) is available at this wavelength, but initial experiments with distilled water gave erratic signals - either the distilled water was not sufficiently zinc-free, or atmospheric zinc was affecting the results. It is possible to overcome this problem by reducing the analytical sensitivity: by the use of a different spectral line, or the selection of "Mini Flow" conditions

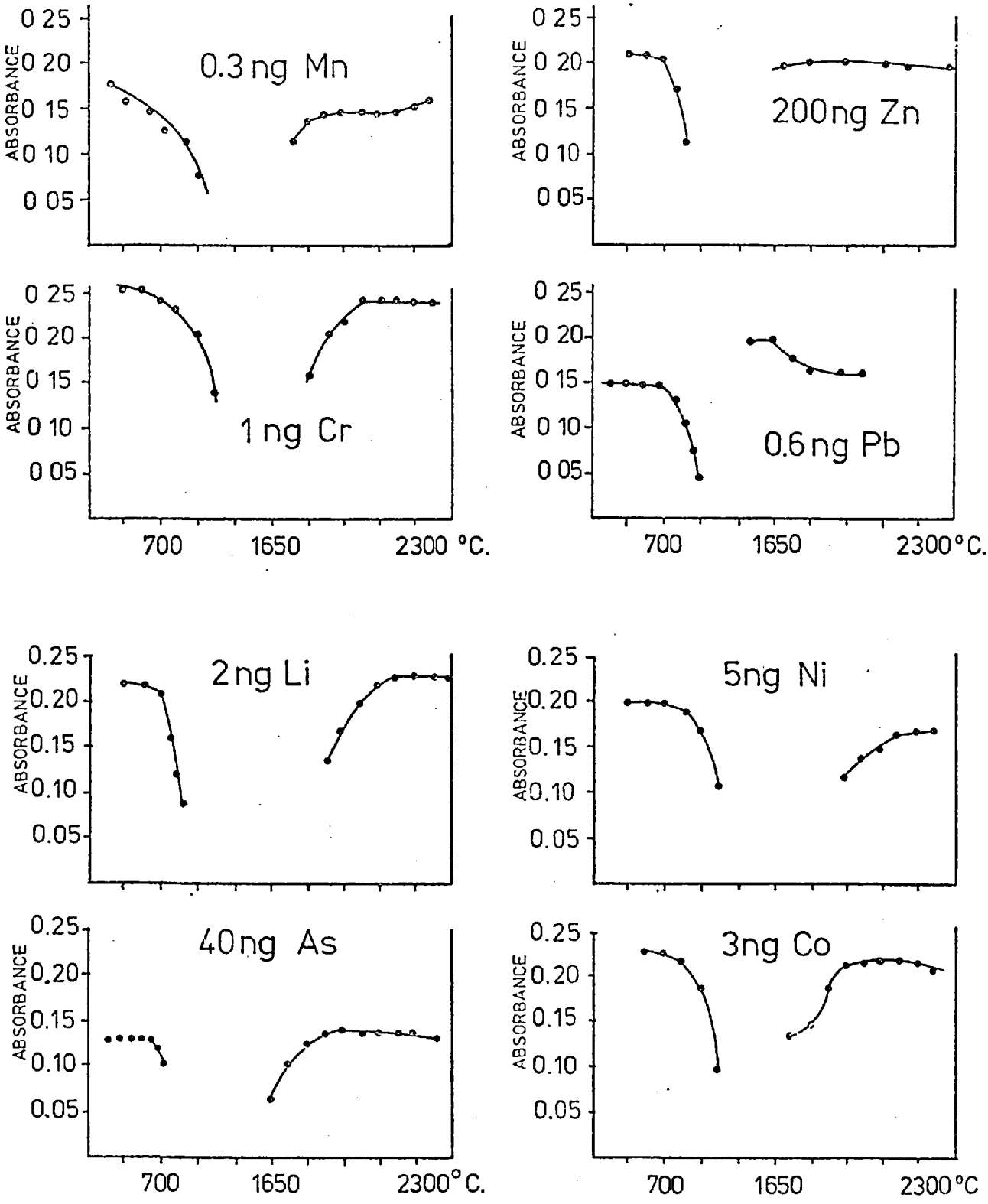


Fig. 19 Optimization of ashing and atomization temperatures

Table 12 Furnace Operating Parameters

Element	Maximum Ashing Temperature			
	P-E (409) °C	P-E (408) °C	This study °C	dig. value
Cu	1000	900	850	250
Zn	500		450	150
Cd	400	300	300	100
Ba	1400	1580	1350	400
Pb	550	700	700	200
Bi	400	350	300	100
Li	1000		700	200
Tl	400		300	100
As	250		700	200
Sb	450		350	120
Cr	1350	1200	700	200
Mn	1100	1000	450	150
Co	1000	1100	850	250
Ni	1200	1200	850	250
Element	**Optimum Atomization Temperature**			
Cu	2500	2550	2400	950
Zn	2000		2000	750
Cd	1500	1900	1650	500
Ba	2700	2700	2700	999
Pb	2000	2100	2000	750
Bi	2000	1900	2000	750
Li	2700		2400	950
Tl	2100		2200	800
As	2000		2000	750
Sb	2200		2200	800
Cr	2700		2400	900
Mn	2400		2200	800
Co	2600		2400	950
Ni	2500		2400	950

Table 13 Published Data on Vaporization of Elements

Element	M. pt. (84) /K	B. pt. (84) /K	min. vapn. temp. (5) /K	appearance temp. (72) /K
Al	930	2740	1820	2300
Sb	900	2020		1550
Ba	1000	1910		2200
Bi	540	1830	1130	
Cd	590	1040	543	850
Ca	1110	1760	1820	1800
Cr	2130	2950	1650	1800
Co	1770	3143	1570	1720
Cu	1360	2840	1430	1730
Au	1340	3080	1350	
Fe	1810	3020	1500	1750
Pb	600	2010	1130	1000
Li	450	1620		2100
Mg	920	1360	1570	1550
Mn	1517	2230	1480	1600
Hg	234	630		
Ni	1730	3000	1690	1800
Ag	1230	2485	1120	1150
Sn	500	2540	1410	1800
Ti	1930	3560		2420
V	2160	3650		2350
Zn	690	1180	1070	1100

during atomization, or the use of a slower rate of atomization (this point is discussed further in Chapter 7). For zinc it is unfortunate that the second most sensitive line, at 307.6 nm, is several thousand times less sensitive than the 213.9 nm line.

For bismuth, the hollow cathode lamp intensity at the bismuth 223.06 nm line was insufficient for the satisfactory measurement of absorption, and the alternative 306.77 nm line was used.

For lead, the 217.0 nm line was chosen, since this was the line selected for the multi-channel A.A. instrument (451). The 283.3 nm line, though in fact less sensitive than the 217.0 nm line, is the most popular lead line, due to an advantageous signal-to-background ratio.

The calibration graphs are given in Figure 20 and data obtained from these graphs are collected in Table 14. This lists: sensitivities, practical detection limits (weights needed to give a signal twice the noise level) - which are also the lower limits to the working range - and the upper limits to the working ranges, for both (a) the straight line portion of the growth curve, and (b) when some of the curved portion is included. It is always preferable to work over a straight line portion of a calibration graph, and if it is necessary to measure routinely values as high as (b), then curve correction would have to be employed, and this presents difficulties with discrete sampling on the Perkin-Elmer Model 300S. In the present study, however, most measurements were made at the lower end of the calibration graph.

Since many analysts, accustomed to colorimetry and flame atomic absorption, are unfamiliar with detection limits expressed as absolute weights, the practical detection limits in terms of concentration are given in Table 15, for 5 µl, 10 µl and 100 µl sample volumes.

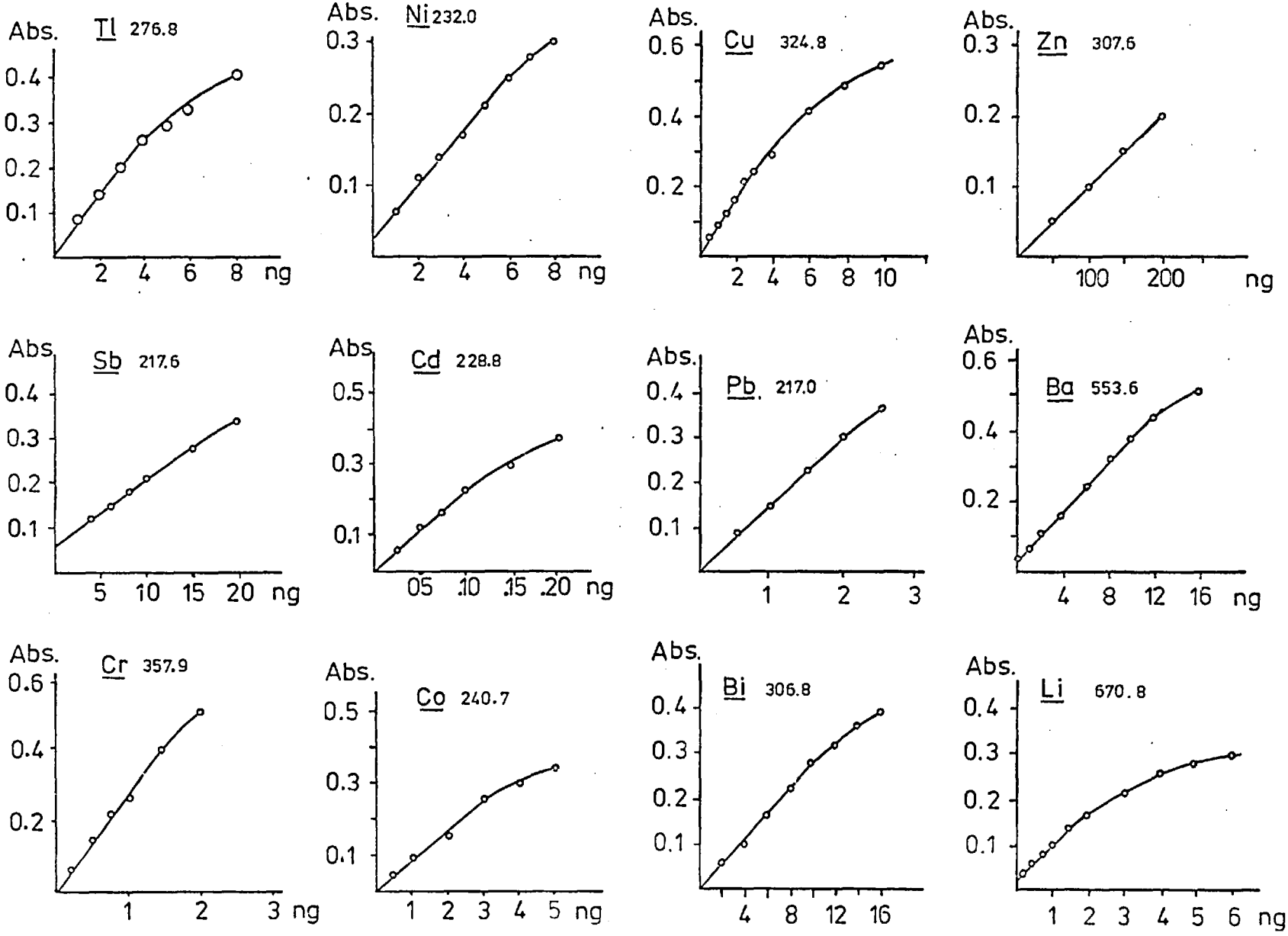


Fig. 20 HGA-74 calibration graphs

Table 14 Instrumental Capability

Element	P-E data (409)		This Study			
	opt. working range /ng	sens. /pg	detn. limit /ng	sens. /pg	upper limit to working range /ng (a)	(b)
Cu	1 - 8	50	0.12	54	5	8
Zn			10	4400	200	200
Cd	0.06-0.4	2	0.01	2	0.1	0.3
Pb	1 - 8	50	0.07	30	2.5	5
Ba	2 - 30	300	1.3	120	12	30
Bi	3 - 20	90	0.5	160	10	20
Li		60	0.3	40	2	6
Tl		130	0.15	70	4	8
Ni	3 - 20	140	0.98	110	6	10
Sb		290	0.7	290	20	20
Cr	0.5 - 4	10	0.02	17	1.5	3
Co	2 - 16	80	0.1	53	3	5

Table 15 Practical Detection Limits

Element	Absolute detn. limit /ng	Concentration detn. limit 5µl /p.p.m.	10µl /p.p.m.	100µl /p.p.10^9
Cu	0.12	0.024	0.012	1.2
Zn	10.0	2.0	1.0	100.0
Cd	0.01	0.002	0.001	0.1
Pb	0.07	0.014	0.007	0.7
Ba	1.3	0.26	0.13	13.0
Bi	0.5	0.10	0.05	5.0
Li	0.3	0.06	0.03	3.0
Tl	0.15	0.03	0.015	1.5
Ni	0.95	0.19	0.095	9.5
Sb	0.7	0.14	0.07	7.0
Cr	0.02	0.004	0.002	0.2
Co	0.1	0.02	0.01	1.0

Chapter 4.

TRACE METAL ANALYSIS OF URINE.

4.1 Introduction

In one of the earliest papers outlining the use of electrothermal atomizers for biological applications, Matousek and Stevens (348) wrote: "samples are injected into a (graphite furnace), and subsequently dried, ashed and atomized by the application of electric current. The resulting brief absorption signal is recorded and compared with standards. The procedure is particularly suitable for biological work. The samples require no pretreatment". Other authors have made similar claims regarding the usefulness of electrothermal atomizers - including the claim that selective volatilization of the matrix before the analyte or *vice versa* is a generally applicable method of reducing interference problems. If these claims are true, then the determination of trace metals in urine - or blood or digests of liver - should present few problems. Unfortunately, many of the early visions of the analytical possibilities offered by electrothermal atomization were extravagant, perhaps even naïve.

The determination of trace metals in urine - or other biological samples - is a severe test for electrothermal atomization atomic absorption. Urine represents a "heavy-matrix" sample and contains a variety of organic and inorganic substances including albumin, proteins, uric acid, calcium, potassium. chloride, sulphate and several other constituents (85). The total solids content of urine is 20-60 g/24h, and since the volume of a 24h urine sample may vary between 500 and 1500 cm^3 (according to fluid intake and the state of health or disease), there will be typically 4% solids present in a urine sample. The variability in the volume of urine excretion also means that the normal concentrations of trace elements will be spread over a wider range than those in other samples, *e.g.* blood.

4.2 Analysis of Urine Directly in the Furnace

4.2.1 Methodology

Since urine samples contain much dissolved and suspended matter, it can be forseen that background correction will be essential for meaningful results (section 3.3) and this is shown in the following traces (Figure 21) of the atomization peaks of a 10 µl urine sample at the lead 283.3 nm wavelength, without and with background correction.

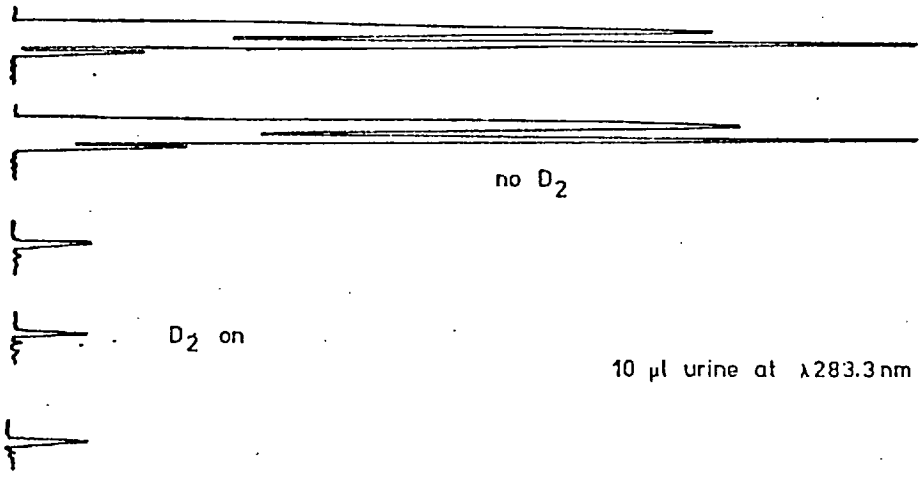


Fig. 21 Effect of background correction

It has been pointed out (338) that the background signal may show some variation in magnitude, though this may not always be so - as in the following example for zinc (Figure 22) where absence of background correction gave regular-sized peaks, and a standard addition effected on the sample gave a result much higher than the normal range.

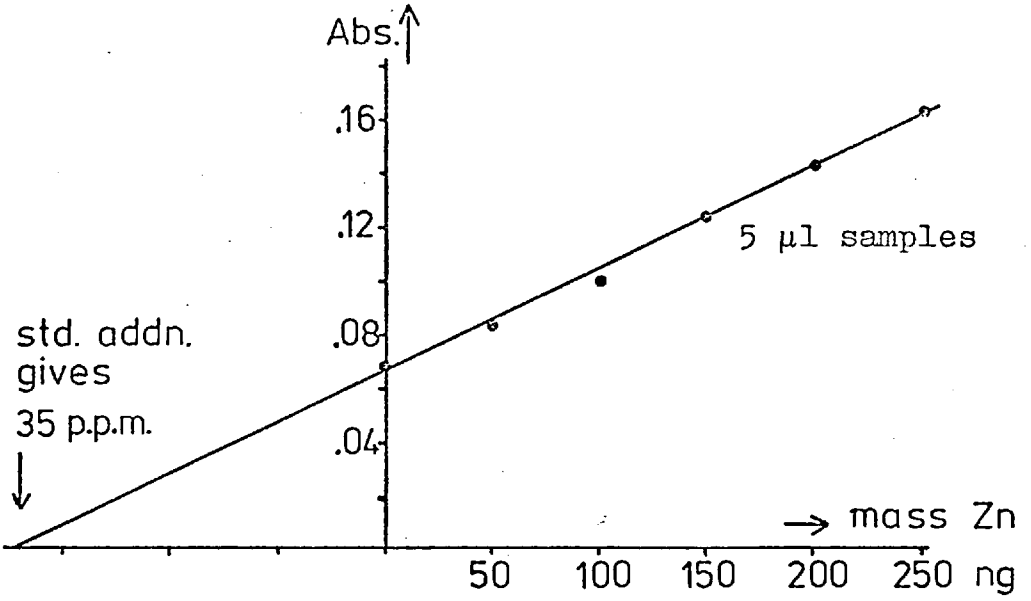


Fig. 22 Standard addition to urine (no background corrn.)

With background correction the result was reduced to the correct order of magnitude (Figure 23): normal range is 0.3 - 1 p.p.m.

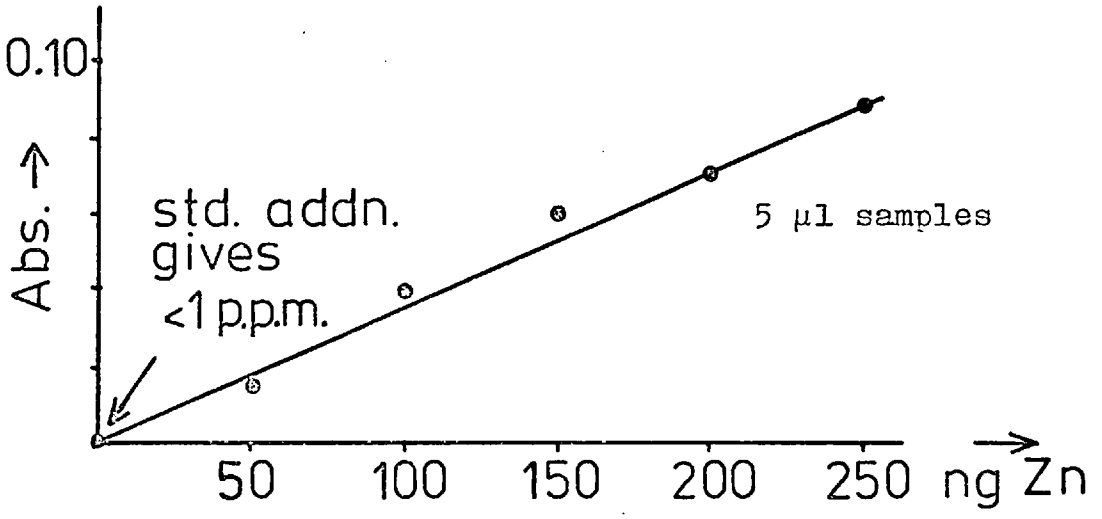


Fig. 23 Standard addition with background correction

The use of background correction will eliminate the effect of interferences giving signal enhancements, but interferences giving diminutions of signal may still be present i.e. matrix effects. The best way to establish if a matrix effect is present is to effect a standard addition on the sample; this has been outlined in section 2.7.

The requirements of the standard addition procedure will have to be met, and it is especially important that the additions do not exceed the linear range of the calibration graph; this linear range is often quite limited, as is shown in section 3.6. A further requirement is that the addition should be in the same chemical form as the analyte in the sample. In this work the standard additions were mostly simple aqueous solutions of the analyte element, but there was no guarantee that the analyte in the urine sample would not be bound in some way to the proteins or other constituents of the matrix. If they were, and were not dissociated during the ashing or atomization stages, then even a standard addition might give a low result.

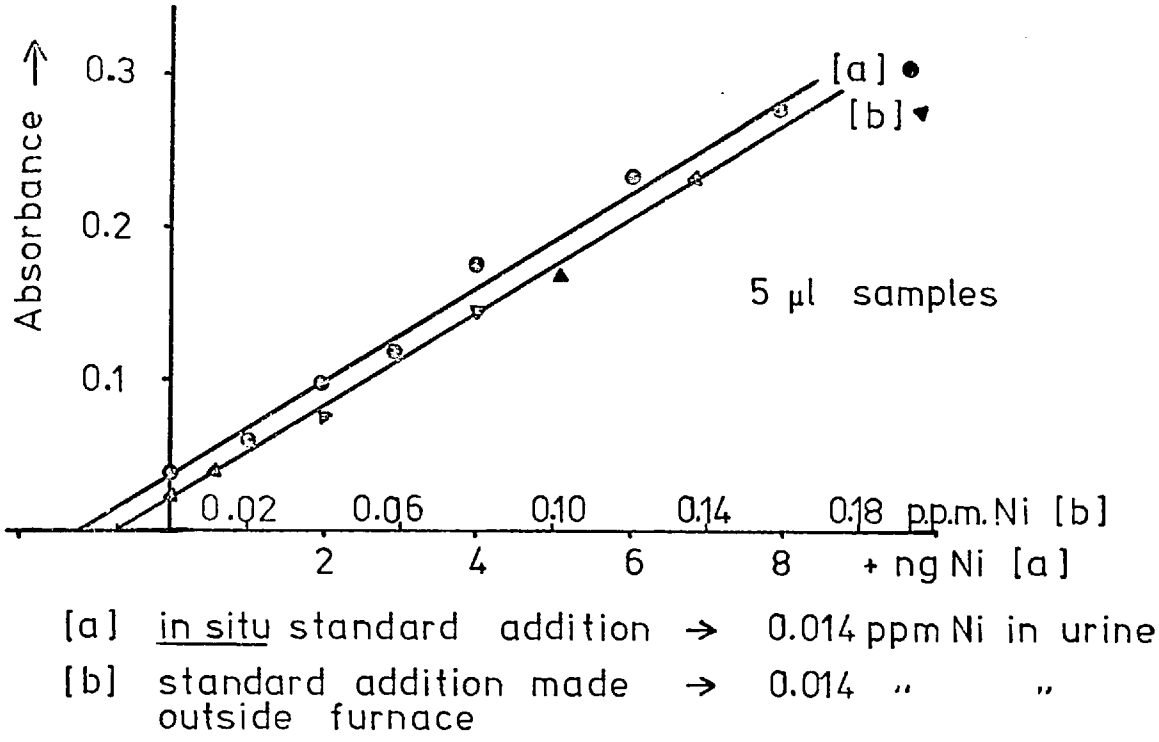
In flame atomic absorption it is necessary to make the standard addition to the sample prior to nebulization, but in electrothermal atomization this requirement does not apply, and additions to the sample can either be made externally (prior to an aliquot of the mixture being pipetted into the furnace), or in situ in the furnace. Obviously it is necessary to establish that these two methods give the same result - see Figure 24.

If reasonable care is taken, it is possible to pipette the standard addition on top of the sample aliquot, and to obtain meaningful results. Since this represents a convenient technique, it was employed throughout the present study. In each analysis, the initial stage of 80°C for 15s would ensure thorough mixing of the two aliquots prior to drying at 120°C for 15s.

4.2.2 Analysis of Urine

For each element studied, the following procedure was adopted:

(a) a urine sample was analysed by the method of standard addition; if the analyte level was below the limit of detection of the method (e.g. for thallium) a spiked urine sample was employed;



[a] in situ standard addition → 0.014 ppm Ni in urine

[b] standard addition made outside furnace → 0.014 " "

Fig. 24 Comparison of standard addition procedures

(b) an aqueous solution of similar or equal concentration to that of the analyte in the urine sample was analysed by the standard addition method;

(c) an analytical growth curve for the element in urine was constructed. by suitably spiking a urine sample.

Comparison of the slopes of the graphs obtained in (a) and (b) gave an estimate of the magnitude of the matrix effect present in the urine sample (section 2.7): absence of a matrix effect is defined as a matrix effect of unity. The standard addition in (b) also gave an indication of the accuracy of the method. From the calibration graphs

derived in (c), sensitivities and detection limits were calculated.

The instrumental conditions for each element were those listed in Table 12, though in an attempt to produce a generalized HGA-74 programme which would be suitable for multi-element determinations (451), a maximum ashing temperature of 400°C was employed in most cases. A ramp ashing step was incorporated into each analytical programme, and the maximum ashing temperatures used are listed in Table 16.

Standard addition graphs for Cd, Cu, Tl and Ni in urine are given in Figure 25; the sample sizes used and the measured analyte concentrations for Cr, Cu, Co, Ni, Sb, Zn, Ba, Cd, Pb, Li and Tl are listed in Table 16.

The calibration graphs for the analyte elements in urine are given in Figure 26, and the observed sensitivities and detection limits were found to be slightly poorer, in terms of mass of analyte, than the corresponding aqueous calibrations.

4.2.3 Correcting Ability of the Deuterium Arc

Although some workers (166, 482) using atomic absorption spectrometry have pointed out that deuterium arc background correction will only correct for limited amounts of matrix interferences, equipment manufacturers have not emphasised this limit to the usefulness of background correction.

It was noticed in this study that large scatter peaks appeared for some elements when the volume of the urine aliquot was increased. These peaks are in fact wavelength-dependent, as is to be expected for molecular scattering (581). At various analyte wavelengths, increasing volumes of unspiked urine samples were run to establish the sample size at which the large peaks appeared - this sample size indicated the limit to the correcting ability of the deuterium arc background corrector. The relationship between analytical wavelength and maximum volume corrected by the background corrector is shown in Figure 27.

Table 16 Analysis of Urine Directly in the HGA-74 Furnace

Element	Max. Ash Temp./°C	Sample size and type	Concn. found / p.p.m.	Matrix effect present
Cr	400	10 µl unspiked	0.003	1.23
		10 µl spiked 0.2 p.p.m.	0.18	
Cu	400	10 µl unspiked	0.025	1.30
Co	400	5 µl unspiked	0.06	1.34
Ni	400	5 µl unspiked	0.014	1.10
Zn	700	10 µl unspiked	< L.of D.	2.16
Ba	1400	10 µl unspiked	< L.of D.	
		10 µl spiked 0.2 p.p.m.	0.2	2.25
Cd	400	20 µl unspiked	0.0015	1.48
Pb	400	10 µl unspiked	0.08	1.81
Sb	280	10 µl unspiked	< L.of D.	
Li	400	10 µl unspiked	0.015	1.42
Tl	400	10 µl unspiked	< L.of D.	
		10 µl spiked, 3.5 p.p.m.	3.4	2.76

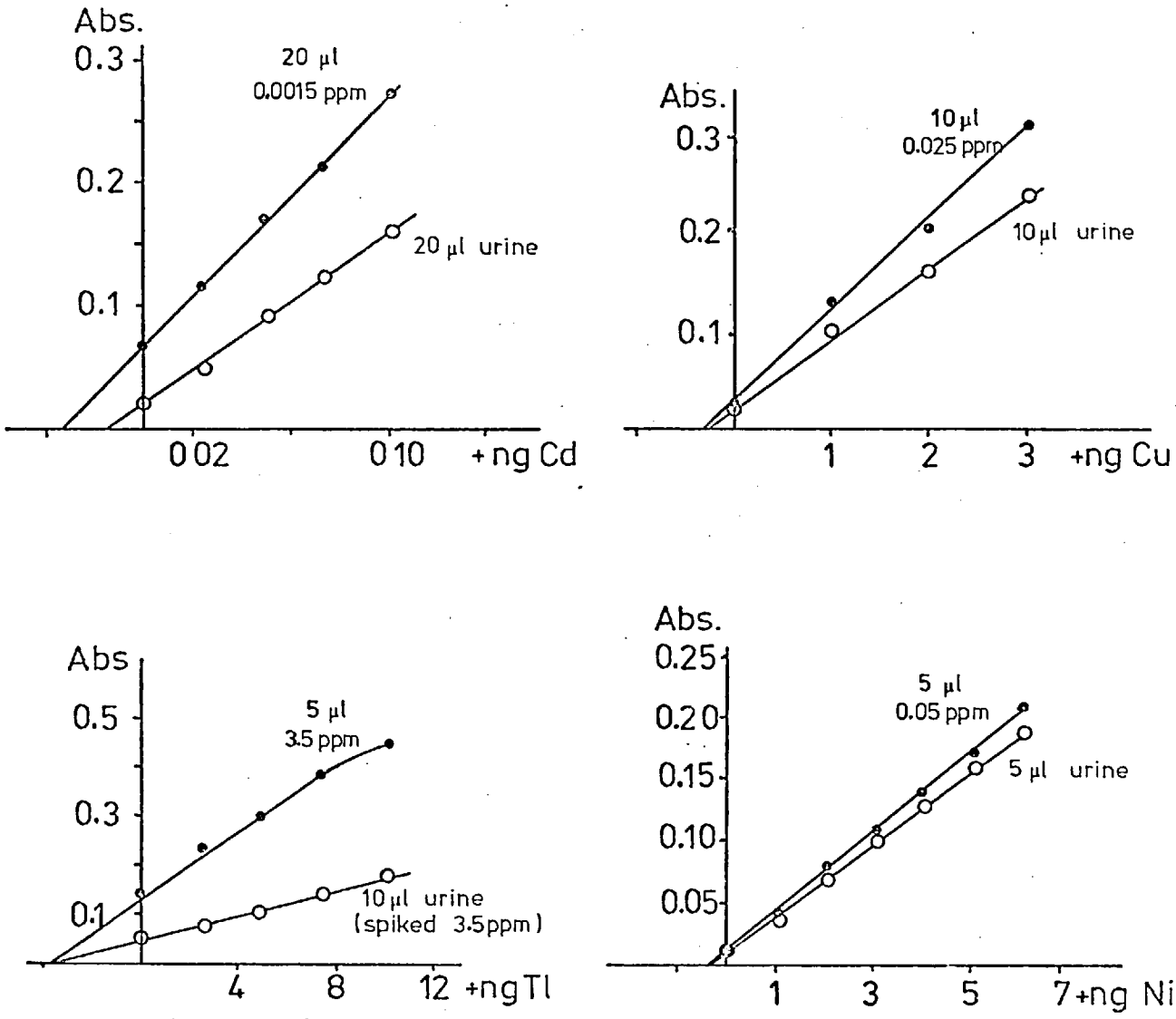


Fig. 25 Typical standard addition graphs for urine and aqueous solutions

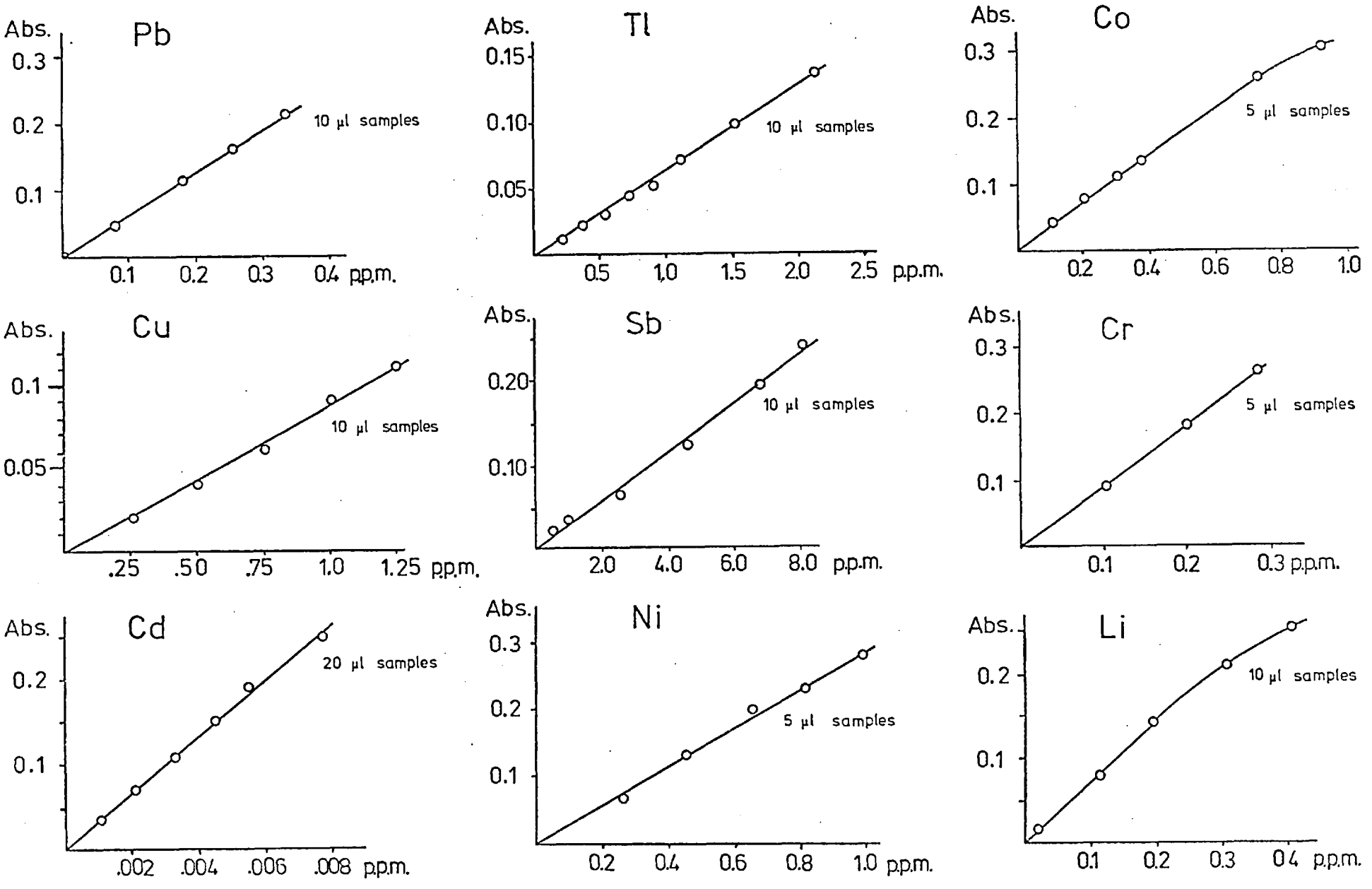


Fig. 26 Analyte in urine calibrations

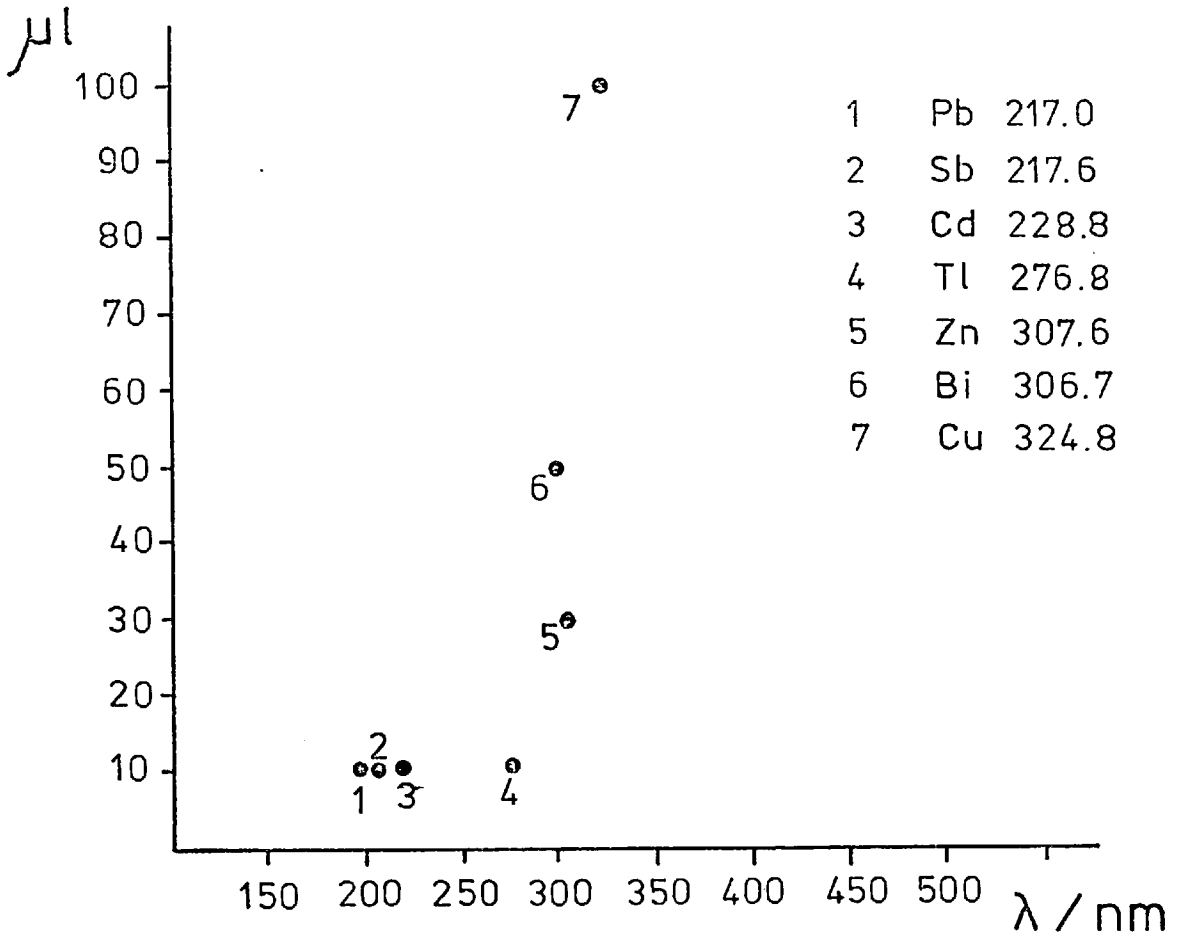


Fig. 27 Maximum volume corrected by D_2 arc (normal urine sample)

This figure shows that for wavelengths up to about 300 nm it is only possible to correct for the background interferences in 10 µl aliquots of urine; this is a severe restriction on the measurement of low concentrations of metals in urine by direct analysis in the furnace.

At wavelengths greater than 300 nm it is possible to correct for the background in volumes of urine larger than 10 µl- probably because molecular absorption from sodium or potassium halides is very low in this spectral region (2). This is a fortuitous situation since the upper limit to the working range of the deuterium arc is about 325 nm, though some workers (403) have reported background correction at the Cr 357.9 nm line. Using solid tissue samples, Lundgren and Johansson (315) found that the sample size was limited to about 200 µg, owing to the non-specific absorption from the salt content of the tissue.

4.2.4 Conclusions

The results given in Table 16 show that a matrix effect is present in all the urine / analyte combinations tested. The magnitude of the matrix effect ranges from 1.10 - for nickel - to 2.76 - for thallium, though whether the size of the matrix effect is dependent on the analyte element or on the actual urine sample would have to be established by further work. The magnitude of the matrix effect might be reduced by raising the maximum ashing temperature, but it is likely that the high solids content of urine would still produce a noticeable matrix effect. The variable solids content of urine samples precludes the use of calibration graphs based on pooled urine samples, a procedure which has been used for blood analyses (115), and for an accurate analysis by electrothermal atomization atomic absorption spectrometry a standard addition procedure will be essential. Using such a procedure, the concentrations of analyte elements in unspiked urine samples all lay within the normal range (Table 4), although the value found for lead was slightly high.

The calibration graphs for analyte element in urine (Figure 26) show the feasibility of determining any of these elements in urine, though a severe restriction on any method based on direct analysis of a urine sample in the graphite furnace is the volume limit of the correcting ability of the deuterium arc. This volume limit was found to be usually 10 µl, though with a new, bright deuterium lamp, it was possible to correct for the background in 20 µl samples (cadmium in Figure 26).

The method of standard addition on a urine sample in the graphite furnace was found to be satisfactory for Cr, Cu, Co, Ni, Cd, Pb and Li. Thallium could not be detected in an unspiked urine sample (concentration detection limit: 0.01 p.p.m.), and barium also could not be detected in an unspiked urine sample (concentration detection limit: 0.1 p.p.m.) At the 307.6 nm line, the zinc concentration detection limit

was 1 p.p.m., which is above the normal range of zinc in urine. A sensitivity enhancement of several thousand times is produced by the use of the zinc 213.9 nm line, but the sensitivity at this wavelength is so great that special precautions have to be taken to prevent zinc contamination. The concentration detection limit for antimony in urine was 0.4 p.p.m.; this was a consequence of a noisy output from the antimony hollow cathode lamp employed. Antimony, and also arsenic, hollow cathode lamps are well known to have low radiation outputs and short operating lives. The use of electrodeless discharge lamps for these two elements will lead to enhancements in brightness and stability, and thus to improvements in detection limits and precision.

In the study of bismuth, no problems were encountered in plotting a calibration graph with aqueous solutions of bismuth, but when bismuth standard additions were made on a urine sample the graph shown in Figure 28 resulted.

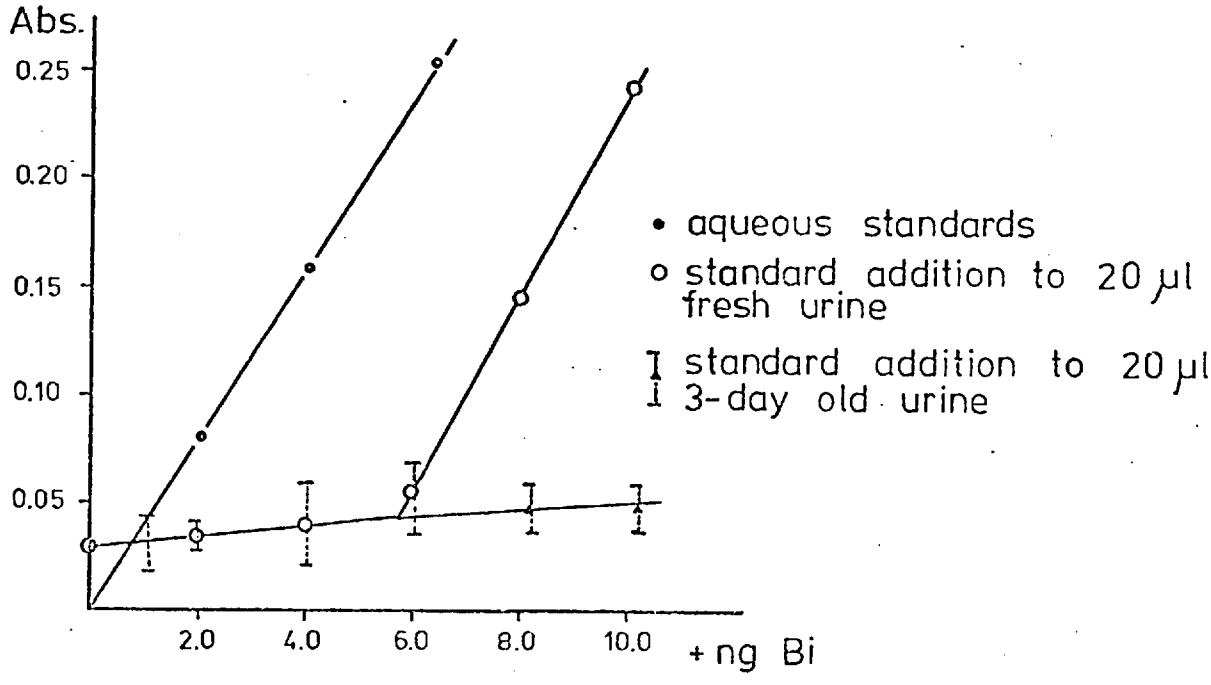


Fig. 28 Standard addition for bismuth in urine

The initial additions of bismuth to the urine did not result in significant increases in absorbance until a limit was reached, when the absorbance did increase. The limit appeared to be dependent on the age of the urine (Figure 28); in addition, the measurements on the 3-day old sample were less precise than those in the fresh sample.

A chemical solution to the problem was sought: the initial bismuth solutions used were simple aqueous dilutions of a 1000 p.p.m. stock solution, prepared by dissolving $Bi(NO_3)_2$ in 3% nitric acid. A new 1000 p.p.m. bismuth stock solution was made by dissolving bismuth metal in nitric acid, and dilution to give a final solution which was 2% in nitric acid. Improved standard addition graphs were produced when aqueous dilutions of this solution were used: Figure 29.

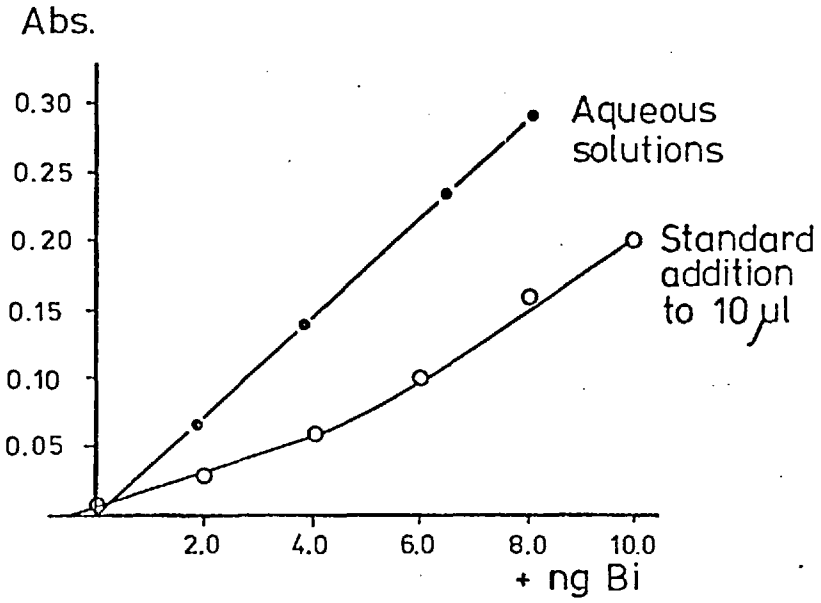


Fig. 29 Standards not acidified

When the diluted standards were made up in 2% nitric acid, and these solutions were used for the standard addition, the graph produced was further straightened: Figure 30. It was still advisable, however, to use a fresh urine sample to achieve an accurate standard addition.

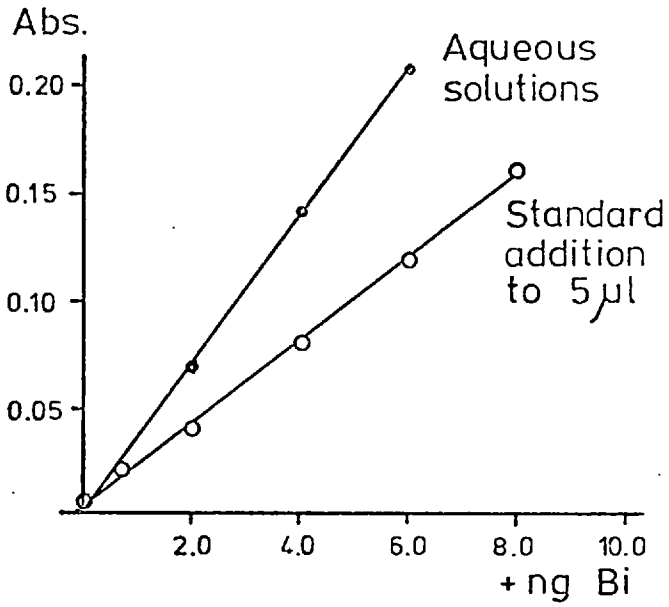


Fig. 30 Bismuth in urine - standards 2% nitric acid

4.3 Analysis of Urine with Preliminary Solvent Extraction

4.3.1 Introduction

It is sometimes found, in analyses employing flame atomization atomic absorption, that the amounts of analyte present are below those levels which can be easily determined, and it then becomes desirable to concentrate the analyte element. This is often achieved by solvent extraction, a procedure having the additional advantage of separating the analyte from possible interferences in the sample matrix. It is perhaps this factor which is the more important for electrothermal atomization atomic absorption where sensitivities (in terms of concentration) will often be one or two orders of magnitude better than those of flame atomization atomic absorption; even these high sensitivities, however, may be insufficient for some applications. e.g. thallium and barium in biological samples.

Solvent extraction will result in increased sensitivity for the analyte element if the volume of immiscible organic solvent is small relative to that of the aqueous sample, and a two- to ten-fold concentration enhancement is often achieved. The presence of organic solvent is an additional sensitivity-increasing factor if a flame is used as the atom cell (15, 16, 79): organic solvents will maintain or enrich a flame, raising the flame temperature and thus ensuring the rapid vaporization of the analyte. The lower viscosities and surface tensions of organic solvents (compared to water) lead to increased aspiration rates, and the lower surface tension ensures more efficient analyte vaporization (301, 437). A similar situation does not arise in electrothermal atomization atomic absorption where a given quantity of analyte element will result in a certain absorbance, independent of the solvent employed (6, 42). The behaviour of various metal chelates in organic extracts during ashing and atomization in a graphite tube atomizer has been examined by Gomišček et al. (177).

Methods other than solvent extraction may be employed to concentrate trace elements. Evaporation is possibly the simplest technique and has the advantage that large amounts of reagents or complicated glassware are not required. It is, however, a very slow procedure, and results in an increase in the total solids contents of the solution; it is best employed for water, mineral acids or organic liquid samples.

Ion exchange is currently gaining in popularity as a method of separation and preconcentration in trace element determinations - particularly in the analysis of water for ultratrace constituents. There are basically three different types of ion exchange resin: cation exchange, anion exchange and chelating, and all three have found wide use in trace element determinations. Many ion exchange resins have high capacities and large volumes of dilute solutions can be passed through the ion exchange column with nearly complete retention of the ionic species; the retained cations can then be eluted with a relatively small volume of dilute strong acid, e.g. 2M nitric acid. If a sample contains large amounts of Na, K, Ca and Mg ions then these may saturate the column, preventing the complete retention of trace cations. Several workers in the trace metal field have described the preconcentration of trace elements - with cation exchange (45), with precipitation ion exchange (522), with a liquid ion exchanger (531), and with an anion exchange resin to separate Sn, Cd and Zn from excess Cu (330).

Chelating resins contain functional groups similar to those in conventional chelating agents and thus are somewhat more selective than cation or anion exchange resins. Since most will not retain alkali or alkaline earth elements, these resins are extremely useful in the analysis of "real" samples.

Dithizone on a cellulose base was synthesised by Carritt (76) for concentrating metals from sea water, and

polyamine-polyurea resins have been studied by Dingman et al.(126 for the purpose of concentrating trace metals, but Chelex-100 ion exchange resin, commercially available from Bio-Rad Laboratories, appears to be the most widely employed chelating resin. It has been used by Blout et al. (47) for the trace determination of cobalt, nickel and bismuth, and by Agarwal et al. (3) for the concentrative extraction of Cu, Zn, Rb, Sr and Pb from urine. Ridley and Taylor (434) separated a large number of metals from sea water using Chelex-100 and found the optimum sample pH to be between five and nine. The recoveries for Tl(I), Sn and W(VI) were poor, but 90-100% recoveries for Ag, Bi, Cd, Ce(III), Co, Cu, In, Mo(VI) , Ni, Pb , Re(VII), Sc, Th, V(V), Y and Zn were obtained. Using chelating resins, Biechler (45) separated Cd, Cu, Fe, Pb, Ni and Zn from industrial waste water, and Sato et al. (454) separated heavy metals from estuarine water. Dingman et al. (125) have synthesised a resin containing dithiocarbamate groups which gave quantitative retention of Ag(I), Hg(II), Cu(II), Sb(III), Pb(II) and Cd(II), whilst Knapp et al. (273) used a reverse phase technique with diethyldithiocarbamates to concentrate Zn, Cu, Ni, Pb, Hg and Co - quantitatively at pH 4-7.

Descriptions of other preconcentrating techniques have appeared: Dawson et al. (108) used electrolytic extraction, whilst Newton et al. (375) simply soaked a tungsten alloy wire loop in the sample for a specified period of time; metal ions concentrated on the surface of the loop which could then be used as an electrically heated atomizer. The use of separation procedures for atomic absorption as applied to clinical chemistry have been reviewed by Delves (116), but it is solvent extraction which is by far the most commonly employed method.

In the present study, it was thought that two situations might arise when it would be desirable to employ a solvent extraction procedure:

(a) in heavy matrix samples where the size of the matrix varied between similar samples, and standard addition was necessary for an accurate result, the use of solvent-extracted samples and standards might provide a more satisfactory analysis throughput;

(b) for analyses with the multi-channel instrument (451), separation of the analyte elements from the biological matrices would be essential, since there was no facility on the multi-channel instrument for simultaneous background correction.

4.3.2 Requirements for Chelate and Organic Solvent in Solvent Extraction for Atomic Absorption Spectrometry

(a) Chelate A selective complexant is unnecessary in atomic absorption spectrometry since the method itself will impart a high degree of selectivity to an analysis; in addition a general chelating agent will allow several trace metals to be extracted simultaneously, and then to be determined in a single extract. The general chelating agents most commonly employed in atomic absorption spectrometry are listed in Table 17.

Several esoteric chelating systems have been employed in atomic absorption for a variety of purposes. Russian workers have used hexamethylene ammonium hexamethylene dithiocarbamate to extract heavy metals from a sea water matrix (69, 543), and another group of Russian workers has employed a diphenyl carbazene - pyridine - toluene system for the extraction of Zn, Cd, Co, Cu and Ni (62), whilst Japanese workers have favoured high molecular weight amines for various extractions, e.g. cadmium (539) and lead (182).

Table 17 General Chelating Agents used in A.A.S.

Compound	Metals Commonly Extracted	Reference
1,10-phenanthroline	Fe, Co, Cu, Zn, Cd, Pb, Mn, Ag	457
cupferron	V, Ti, Cu, Mn, Fe, Ni	114,368,509
8-hydroxyquinoline (oxine)	Al, alkaline earths + others	81,96,342,450
dithizone	Ag, Pb, Cd, Zn, Cr	442,449,453,595
acetylacetone (AcAc)	transition metals	388
sodium diethyldithiocarbamate (NaDDC)	Pb, Cu, Fe, Mn, Te	380,453
ammonium pyrrolidine dithiocarbamate (APDC)	Cu, Pb, Cd, Co, Mn, Fe, Ni, Bi, Zn, As, Ir, Pd, Pt, Se, Te, Tl, Mo, V, Cr	293,368

Not all extractions are based on chelate formation and a few metals can be extracted directly as their halide complexes from strongly acid solutions. These extractions may be very selective, since many common elements will not be extracted at all. Under appropriate conditions and with a suitable organic phase the following metals may be extracted as halides: Fe(II), Ga(III), In(III), Tl(III), U, Au, Pb, Hg, Cd, Cr(III), Sb(V), Ti(III) & Mo(VI) (168,253,388, 423). Halide extractions may be used either to concentrate the analyte metal or to remove a large excess of matrix element.

(b) Organic Solvent For atomic absorption determinations involving direct aspiration into a flame, the solvent

employed should support, or at least not extinguish, the flame. It should be of a suitable viscosity and not be too volatile, and it should not give unpleasant pyrolysis products [e.g. chlorinated solvents may give phosgene (297), though on occasions these solvents have been used (178)]. In addition it should not contribute adversely to the background radiation. 4-methylpentan-2-one (commonly called methyl isobutyl ketone, MIBK) is a popular choice of solvent in atomic absorption analyses, and is commercially available in a specially purified form of low metallic content. The solubility of MIBK in water (20 cm^3/dm^3 at 25°C) is too great to allow very large concentration factors, and heptan-2-one (methyl n-amyl ketone) would be a suitable alternative. A third choice, heptan-3-one is 4.5 times less soluble in water at 25°C than MIBK, and will extract metal chelates equally well, but the lower efficiency of nebulization of heptan-3-one may be a drawback (148). Acetone (347) and ethanol (311) have also been employed in some instances, and various solvents have been used for given determinations (96, 103, 153).

There are no special requirements for the solvent in electrothermal atomization atomic absorption spectrometry, and in addition to those solvents normally employed for flame work others such as chloroform, carbon tetrachloride and benzene have been used (6). The solvent may be selected on the basis of its extraction properties, but the requirement of solvent purity will be stringent. Insufficiently pure solvents could be purified by simple distillation or by sub-boiling distillation (section 2.4).

Since APDC and NaDDC, together with MIBK, have been extensively used for solvent extractions in atomic absorption analyses, and since they are all commercially available in purified form from a number of chemical suppliers, they were selected for study in the present work.

4.3.3 The APDC/MIBK and NaDDC/MIBK Extraction Systems

Descriptions of the chelating ability of APDC (336, 511) coincided temporally with the pioneering work of Walsh (562) on atomic absorption spectrometry (562), and the early workers with atomic absorption were quick to realise the usefulness of an APDC/MIBK extraction (155, 342, 575); this extraction system has also found application in emission spectroscopy (560).

APDC, the abbreviation of the commonly used term ammonium pyrrolidine dithiocarbamate (which should more correctly be called ammonium 1-pyrrolidinecarbodithioate), will react with over thirty metals, mainly in acid media (336). Applications of the extractions are numerous, but few systematic studies appear to have been made on the extraction variables, stability constants and efficiencies of APDC chelates. Standard works on solvent extraction (367, 509) usually have only a brief mention of APDC or NaDDC.

Slavin (493) and Sprague and Slavin (506) give some data on APDC, and a paper by Mansell (341) gives much useful information. Lakanen (293) performed a systematic study of the extraction of several metals over a wide pH range with APDC. Though he employed chloroform as the organic phase, much of his data should apply to MIBK extractions. Koirtyohann and Wen (278) reported the pH dependence of the extraction of Cu, Pb and Zn with the APDC/MIBK system. Wyttenbach and Bajo (593) studied the extraction of fourteen metals with NaDDC into chloroform, and Kinrade and Van Loon (263) have made a detailed study of the extractions by both APDC and NaDDC.

A summary of the pH levels at which several metals have been reported to be successfully extracted with APDC or NaDDC into MIBK or chloroform is given in Table 18. It is well known that the quantity of metal extracted by a chelating agent is strongly dependent on the pH of the

solution (367,509), but it is not widely realised that chelating agents will often alter the pH of the solution to which they are added. The use of a buffer solution is often advisable.

Chromium solutions can be extracted only if they are first heated to boiling point and then allowed to cool, before extraction is carried out (342). The extraction efficiency of manganese is dependent on the APDC concentration; none will be extracted if the APDC concentration is low (341).

Table 18 Extraction of APDC and NaDDC Chelates

	APDC		NaDDC	
Metal	pH Extracted	Reference	pH Extracted	Reference
Tl	3-10	81,293	8-11	453
Cu	2- 8.5	293,341,368	3.6,8-11	380,453
Pb	2- 4	293,341,368, 575	3.6,8-11	380,453
Cd	1- 5	293,341,368	8-11	380
Co	2- 4	293,341,342, 368	3.6,8-11	380,453
Mn	3- 4	293,341,342, 368	3.6	380
Fe	2- 5	293,368	3.6	380
Ni	2- 4	293,341,342, 368	3.6,8-11	380,453
Mo	4	293,342,368	-	-
V	4	81,293,342, 368	6	453
Bi	1- 6	293,341	8-11	453
Zn	2- 6	293,341,368	3.6,8-11	380,453
Cr	3- 9	342,368	3.6	380
Ag	1-10	293	8-11	453
As	2- 6	368	6	453
Hg	2- 4	353	8-11	453

4.3.4 Extraction of Trace Metals from Urine

Though numerous papers have detailed the application of APDC and NaDDC extractions to various analytical problems, and several workers have studied the pH dependence of these extractions from simple matrix solutions, there are few published studies on the equivalent extractions from urine or blood. In practical analyses it is also important to establish that extraction efficiencies are the same for standards as for samples (369).

Kubasik and Volosin (284) adjusted aliquots of a pooled urine sample to pH values between 1 and 12 and then measured the extraction of added amounts of lead, cadmium and thallium with NaDDC. Their experimental results are shown in Figure 31; they recommended a pH range of 3-7 for the simultaneous extraction of the three elements.

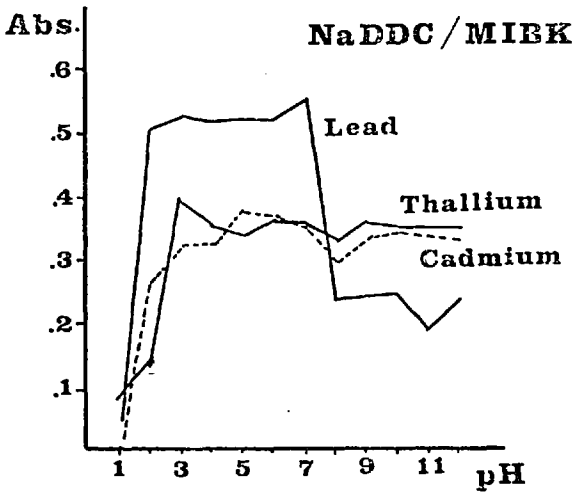


Fig. 31 Extraction of metals from urine

An investigation of the extraction - pH relationship for several metals in urine was undertaken, using both the APDC/MIBK and NaDDC/MIBK extraction systems. An initial study using lead served to establish an optimum operating procedure.

The extraction of lead from urine with APDC/MIBK over the pH range 1-12, carried out with adjustment of pH prior to addition of APDC, and vice versa, is shown in Figure 32.

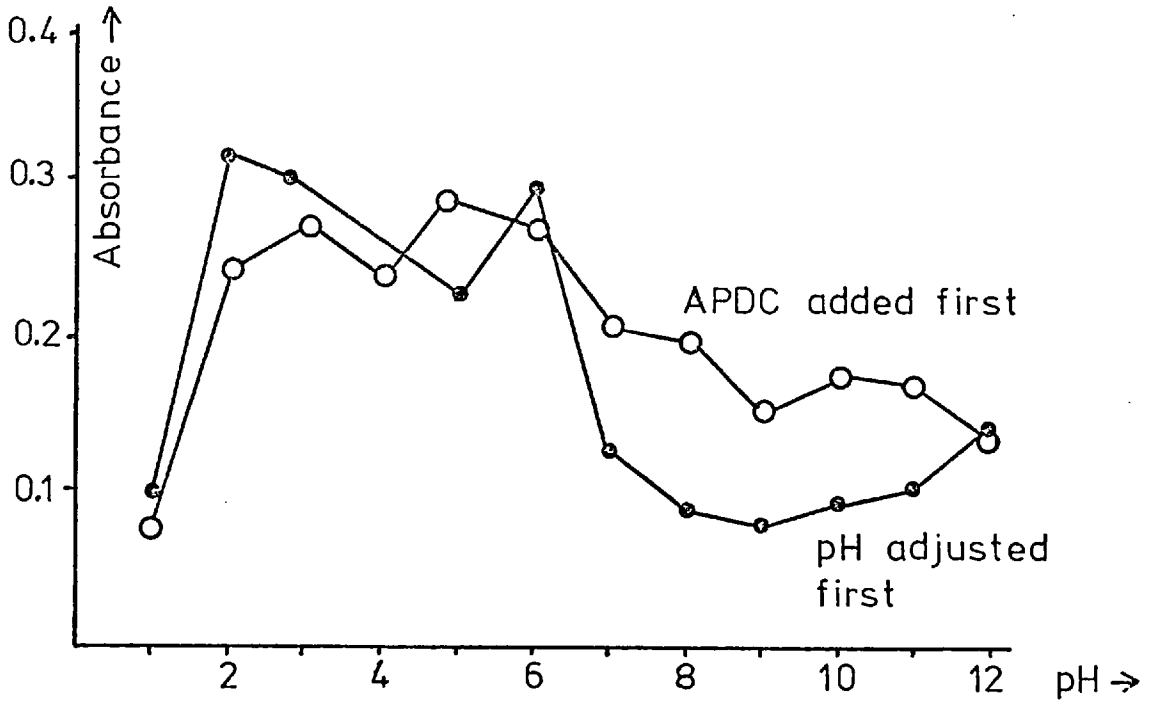


Fig. 32 Extraction of Pb from urine with APDC/MIBK

Urine is a strongly self-buffering medium, and standard buffer solutions were inadequate in adjusting its pH outside the range 6.0 - 8.0; consequently nitric acid was added to achieve low pHs. and sodium hydroxide solution was added to give high pHs. The effect of different acids (nitric, formic and acetic) was also investigated, and is shown in Figure 33.

Figures 32 and 33 were obtained from analyses of the MIBK layer by graphite furnace atomization atomic absorption, though flame atomization could have been used equally successfully. In view of the considerable gains in ease of operation and speed of analysis, flame atomization atomic absorption was employed for the remaining work in this section.

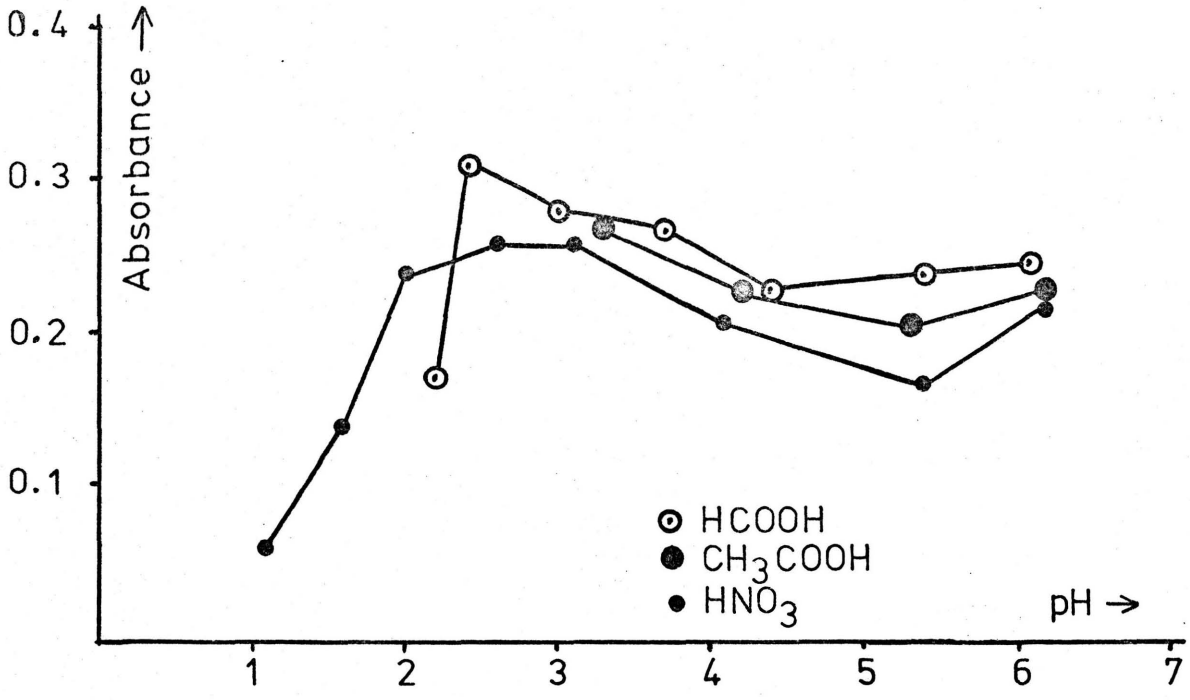


Fig. 33 Effect of different acids on extraction

The extraction vs. pH curves for lead with APDC and NaDDC in the pH range 2-12 are given in Figure 34.

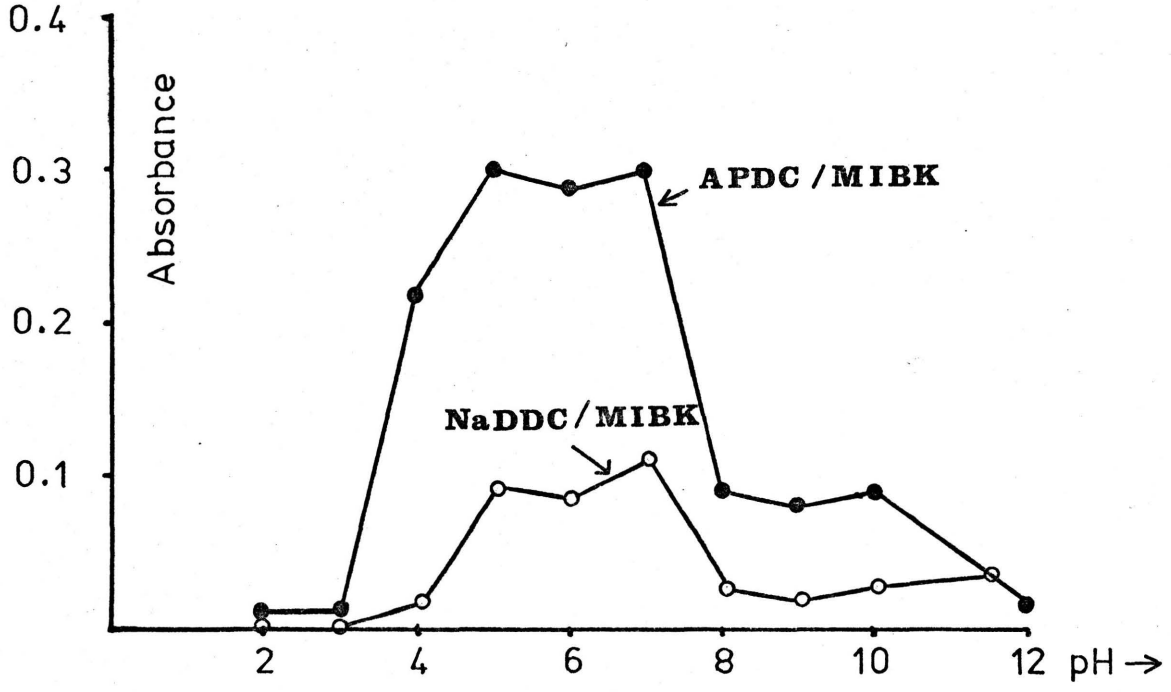


Fig. 34 Extraction of lead from urine

Since APDC extracts metals mainly from acidic media, for the other metals studied the only pH adjustments employed were those giving lower pHs.

The procedure adopted for Pb, Cu, Bi, Ni, Co, Cr, Fe, Mn, Cd, Tl, Zn, Li, Hg, As and Sb was to take a 20 cm^3 aliquot of suitably spiked urine, adjust its pH with nitric acid, add 1.0 cm^3 1% APDC or 1% NaDDC solution and 5.0 cm^3 MIBK, shake the mixture and centrifuge (both for fixed periods of time). Using 5 cm^3 MIBK this procedure gave sufficient clear extract for nebulization, but the use of 2 cm^3 MIBK gave a viscous extract, though this problem could be overcome by an initial extraction of the urine sample with MIBK. The solutions of APDC and NaDDC were prepared fresh daily, because of the instability of APDC solutions. Jenne and Ball (240) have recommended an initial extraction of APDC solution with MIBK to remove water-insoluble components and to reduce the content of trace metal impurities. They found that APDC solutions treated in this way could be kept for several weeks under refrigeration provided that a sufficient excess of APDC was used to compensate for the slow decrease in its chelating capacity.

The extraction vs. pH curves for Cu, Pb, Fe, Cd, Tl, Zn, and Hg are given in Figure 35, and from these the optimum pH range for the extraction of each element may be selected.

The extraction of several elements from a single sample has been considered by a number of workers. A few descriptions of the sequential separation of trace metals have appeared: Delves et al. (118) used a six stage extraction procedure for the determination of eleven metals in small samples of blood, whilst Korkisch and Sorio (281) used an initial preconcentration step followed by adsorption on an ion exchange column and selective elution to give separation of Cd, Co, Cu, Mn, Pb, U and Zn. A simultaneous separation of trace elements has more obvious advantages, and solvent extraction systems suitable for this have been studied in some detail.

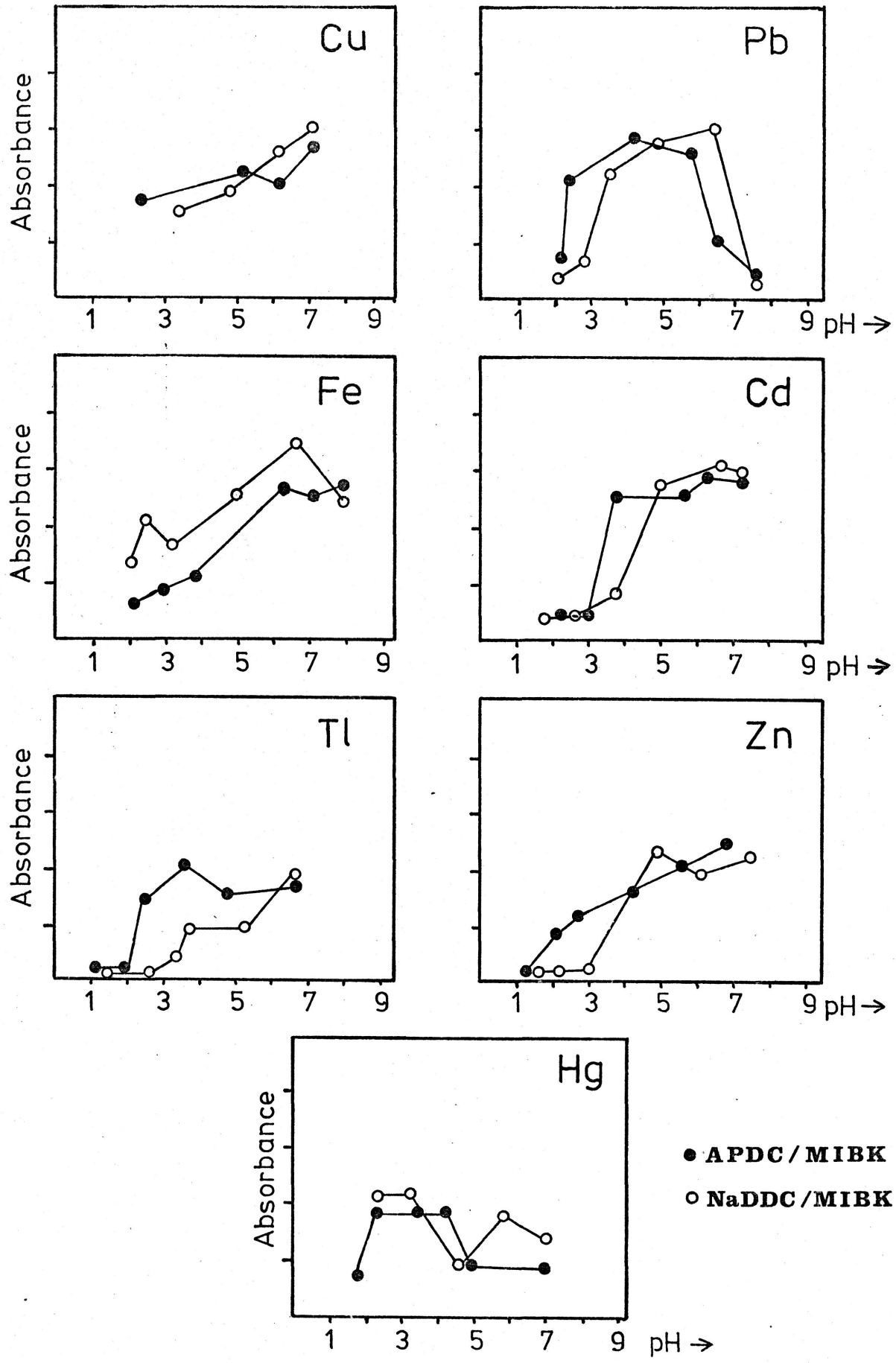


Fig. 35 Extractions from urine

Montford (366) compared six chelating agents and six organic solvents for extracting metals, and recommended that samples at pH 2 be extracted with NaDDC into MIBK, for the determination of Fe, Co, Cu, Cr, Mn and Ni. Montford found that dithizone formed an extractable complex only with copper, although Sachdev and West (449, 450) have reported the extraction of Ag, Cd, Co, Cu, Ni, Pb and Zn with dithizone at pH 7.5 into ethyl propionate. A more recent study by Appleby and Savory (23) involved the comparison of four chelating systems for the extraction of Cd, Se, Ag, Rb, Zn and Fe from serum; the APDC/MIBK system was chosen as being the most efficient. A further study from the same laboratory (300) involved the ashing of serum in a low temperature asher, dissolution of the serum ash in pH 4 buffer, and extraction with APDC into MIBK; this procedure was suitable for the simultaneous extraction of Se, Cr, Fe and Zn.

Most workers have in fact employed extractions based on APDC or NaDDC for multi-element separations. Willis (575) was an early advocate of the extraction of heavy metals from urine, and employed an APDC/methyl n-amyl ketone system for the simultaneous extraction of Pb, Hg, Bi and Ni at pH 3-4; he pointed out that urine samples preserved with acetic acid are at this pH. Goulden *et al.* (183) combined an APDC/MIBK extraction at pH 3 for the extraction of Cu, Zn, Fe and Cr from natural waters with an automated extraction system; this instrumental system was later improved by Pierce *et al.* (417). The APDC/MIBK system has been applied to sea water samples by Brooks *et al.* (59) - at pH 4 , and by Kremling and Petersen (283) - at pH 3-4 - for multi-element extractions.

Nix and Goodwin (380) extracted Fe, Mn, Cu, Co, Ni, Cr, Pb and Zn simultaneously from natural waters with NaDDC/MIBK at pH 3.6 using a phthalate buffer. An extraction system based on NaDDC and a mixture of butan-1-ol and 4 methylpentan-2-one has been employed by Jones *et al.* (243) for the simultaneous extraction of Co, Cr, Cu, Fe, Mn and Zn from sea water adjusted to pH 5.5. NaDDC and MIBK were

used by Olsen and Sommerfeld (392) to extract Mn, Cd and Pb from water samples at pH 3.5, but for storage of the samples the chelates were then back extracted into acetone - hydrochloric acid solution. Tweeten and Knoeck (545) employed a NaDDC/isoamyl alcohol extraction system to preconcentrate Zn, Cu, Fe, Pb, Cd and Mn from water samples adjusted to pH 2.3 - 4.0 with hydrochloric acid, but found that only copper was extracted quantitatively; the other metals were extracted with an efficiency greater than 90%, except manganese for which the recovery was only 59.6%.

Kinrade and Van Loon (263) have made a detailed study of solvent extraction systems for flame atomic absorption spectrometry. For all the nine chelating agents they examined satisfactory extraction of Ag, Co, Fe, Mn, Ni, Pb, Zn, Cd, Cr, Hg and Mo could not be obtained at any one pH value, though APDC showed the best extraction characteristics. They found a combination of APDC with NaDDC for extraction into MIBK gave the best range of pHs over which most of the elements examined could be extracted. At pH 3-6 Cd, Co, Cu, Fe, Ni, Pb and Zn could be extracted simultaneously; silver was also extracted if the narrower pH range 4.5 - 6.0 was used. The use of citrate buffer was recommended for its stability and non-interference in the extraction process. The addition of NaDDC was found to have a stabilizing effect on all of the metal complexes. At pH 5.0 the distribution ratios for the metal complexes of APDC with NaDDC were: Ag 1800, Cd 2500, Co 8000, Cu 5000, Fe 2800, Ni 4200, Pb 2300 and Zn 160.

From the results of the present work, and with consideration of the data of Kinrade and Van Loon (263), it was possible to formulate an optimized multi-element extraction suitable for urine (and possibly other matrices):

> take 10.00 cm^3 urine, adjust pH to 4.0 with nitric acid, add 0.5 cm^3 (1% APDC + 1% NaDDC), and 2.0 cm^3 MIBK, shake the mixture and centrifuge. Carry out analyses on the clear upper layer.

This extraction system was found to be suitable for the simultaneous extraction of Cu, Fe, Pb, Cd, Tl, Zn, Hg, Ni and Co. Chromium, manganese and lithium were not extracted by this system at pH 2.0 - 8.0. Chromium forms the very stable hexahydrate complex $Cr(H_2O)_6^{3+}$, and if chromium were to be determined, the sample solution would have to be first heated to boiling and then allowed to cool (342). The reason for manganese not being extracted was probably an insufficient concentration of APDC (341). The analytical sensitivities for Bi, Sb and As were insufficient to assess the extraction system for these elements, though it is probable that antimony is extracted (342).

4.3.5 Solvent Extraction and Electrothermal Atomization

Few studies have appeared on the behaviour of extracts of metal chelates in electrothermal atomization atomic absorption. Aggett and West (6) applied a carbon filament atomizer to the solvent extracts of five trace metals from aqueous solutions - using four organic solvents and five chelating agents. They envisaged that the organometallic species might sublime from the filament in molecular form rather than undergo atomization, but in fact the behaviour of the organometallic systems was virtually identical to that of aqueous solutions of the metals. Gomiscek et al. (177) studied the phenomena occurring during the atomization of metal tetramethylenedithiocarbamates in a graphite tube furnace, and found that a temperature of 100°C would vaporize the solvent, whilst temperatures of up to 330°C resulted in decomposition of the chelates - to metal sulphides in the presence of argon. Most metal sulphides would be stable up to 1100°C, allowing ashing up to this temperature to be employed, but since lead and cadmium sulphides are volatile, these elements would be lost at temperatures much lower than 1100°C.

A paper by Dudas (132) outlined the effect of drying parameters on the atomic absorption signal from APDC/MIBK extracts of cobalt and nickel. He found that drying at temperatures above 50°C immediately after sample injection resulted in reductions in the absorption of cobalt and nickel, and recommended that a time lapse of two minutes or more should precede the drying stage, in order to achieve maximum absorption signals. The slow drying by evaporation apparently allowed the organic extract to spread in the graphite tube to a greater extent. Various delay times were tried with the present equipment, using an MIBK extract of lead APDC (Table 19), but no significant difference in absorption was found.

Table 19 Effect of Time Delay on Atomic Absorption Signal of Lead APDC

Delay	Absorbance
0 s	0.203
30 s	0.200
1 min	0.205
2 min	0.210

The disparity between these results and those of Dudas may be due to the use of pyrolytically treated graphite tubes and untreated tubes, respectively.

Chapter 5.

TRACE METAL ANALYSIS OF LIVER

5.1 Introduction

Electrothermal atomization atomic absorption offers the possibility of direct analysis of liver tissue. Lundgren and Johansson (315) have described a graphite furnace design similar to that of L'vov (section 3.2.1) in which samples of heart and pancreatic tissue were placed on a carbon cup electrode in the side of a carbon tube furnace, and then dried for 1 minute, ashed (sometimes in the presence of air) for 3 minutes, and atomized. The sample size was limited to about 200 μg; larger samples gave excessive non-specific absorption from the salts present in the tissue. Pickford and Rossi (415) used solid sampling combined with graphite tube atomic absorption for the determination of Ag, Cu, Pb and Mn in N.B.S. Bovine Liver - a freeze-dried sample. Four or five milligram samples were used, but it was found necessary to employ a standard addition technique.

The analysis of solid (wet and dry) tissue samples in electrothermal atomization atomic absorption will be subject to interference from non-specific absorption - hence background correction is essential - and also matrix effects - with the need for a standard addition procedure. It was thought that a preliminary destruction of the organic matrix of tissue samples might reduce these problems, and have the additional advantage that the sample would be in liquid form and hence easier to handle.

Dry ashing and wet ashing are the most widely used methods for the destruction of organic matter.

Dry oxidation at low temperatures ($<100^{o}C$) involves activated oxygen radicals produced in a radio-frequency discharge. First introduced by Gleit and Holland (172), the

technique has been described by Gleit (171) and by Backhaus (28). Commercial instrumentation is now available and the technique is likely to find wider application in the future, especially for determinations of As, Se, Cd, Ge and Sn. A seventy-fold concentration enhancement for wet tissue is achieved, but the method is slow and may involve ashing times of up to 2 days for a 5g sample. Its use in the determination of beryllium, cadmium and tellurium in animal tissue has been documented (307).

In dry oxidation at high temperatures (400 - 700°C) the organic matter is decomposed in the presence of atmospheric or introduced oxygen. This is a relatively simple technique which can accommodate large samples and does not require the addition of (contaminating) reagents. It has the disadvantage that loss of trace elements can occur through volatilization, or through retention on the walls of the ashing dish or in the acid-insoluble fraction of the ash (555). Ashing aids may be employed to combat these sources of error.

Wet oxidation is a relatively low temperature oxidation carried out by oxidising agents in solution. Oxidising acids (nitric, sulphuric and perchloric in different combinations, and with modifications for special purposes) or alkalis (e.g. quaternary ammonium hydroxides) may be used. It is a rapid technique, and although volatilization losses are not entirely absent, they are lower than in dry ashing at high temperatures. The major disadvantage is the possibility of contamination from the large excess of reagent employed. The use of acids in wet oxidation is well documented (181). Hydrogen peroxide may be used as an oxidising agent. either in conjunction with acids (129), or on its own (119). The use of quaternary ammonium hydroxides as tissue solubilisers was introduced relatively recently (235).

Middleton and Stuckey (354), Gorsuch (181), Thiers (524) and O'Haver (388) have produced comprehensive comparisons of the recoveries of elements by different sample preparation techniques. The selection of a particular procedure for a given analytical problem may be restricted by the conflicting

recommendations which are often found in the original literature. Comparative studies have been carried out to find the optimum ashing procedure (169, 170, 230, 316). The usefulness of any particular technique must be gauged by actually applying it to a given problem (316).

5.2 Digestion with Nitric Acid and Sulphuric Acid

5.2.1 Introduction

The three most popular acid digestion mixtures, viz. $HNO_3 + HClO_4$, $HNO_3 + HClO_4 + H_2SO_4$ and $HNO_3 + H_2SO_4$, were applied to the digestion of liver tissue, but the nitric acid/sulphuric acid mixture was selected on considerations of safety and ease of operation. This is probably the most widely used mixture of oxidising agents, and many elements are said to be recovered satisfactorily by the use of this mixture - including Zn, As, Cu, Co, Ag, Cd, Sb, Cr, Mo, Sr and Fe (181).

5.2.2 Digestion Apparatus and Procedure

Although open flasks are often employed for wet digestions, it is sometimes preferable to use a closed system to prevent the loss of volatile elements. Such a system has been described by Gorsuch (181), and a very similar set-up was used in the present work. Other systems have been described (528, 529), and a novel approach by Koirtyohann and co-workers (1) was to place an encapsulated mixture of sample and digestant inside a microwave oven - the internal heating of the mixture gave rapid, safe and efficient ashing of the sample.

The apparatus used in this study is illustrated in Figure 36. It is similar to the system proposed by the "Petroleum and Related Compounds" Subworking Group of the Community Bureau of Reference (91) for the acid digestion of single cell protein. The apparatus employs commercially

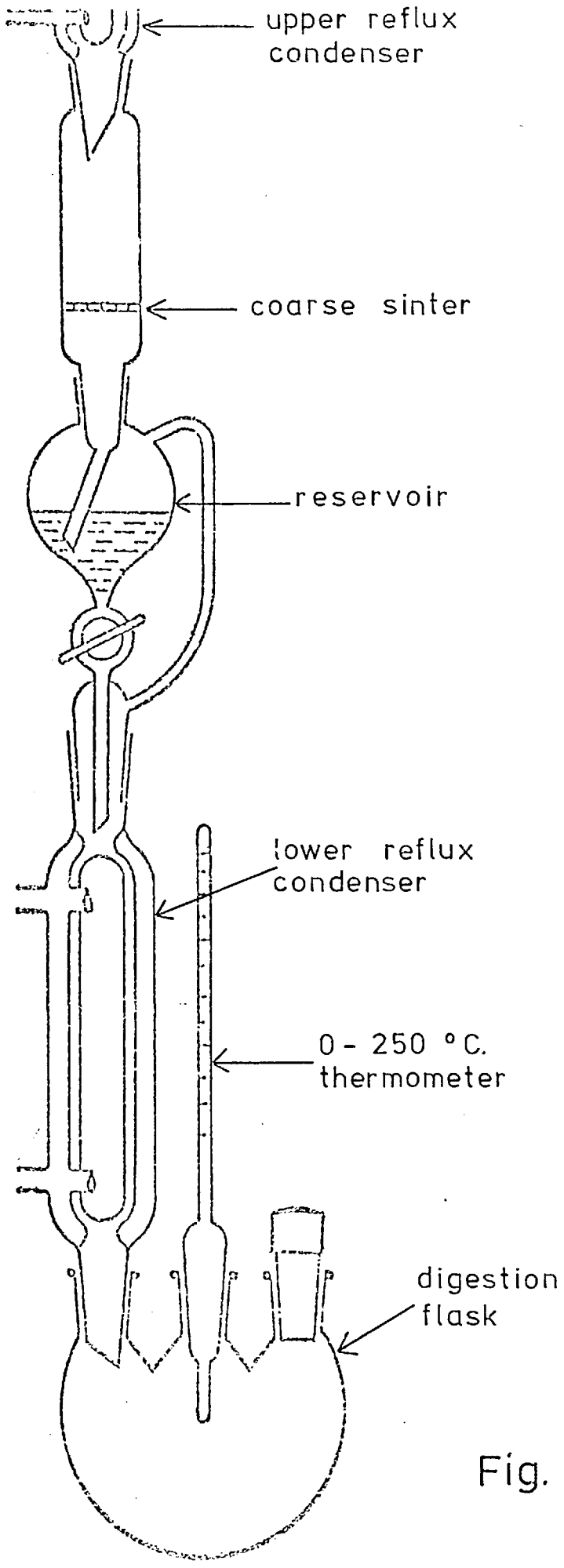


Fig. 36 Digestion apparatus

available components, and is designed to allow refluxing or distillation of the digestion mixture. Distillation will separate the acid mixtures, the components of which can then be recombined in the digestion flask. A single-necked flask was actually employed, but if a two- or three-necked flask is used it is possible to control the lengths of the distillation stages by monitoring, with a thermometer positioned in one of the necks, the temperature attained by the reaction solution. This can be especially useful with mixtures containing perchloric acid. Most elements will be retained during a digestion in this apparatus, and even the very volatile elements such as selenium should be retained by the scrubbing action of water placed on the coarse sinter.

When the digestion apparatus was first assembled, it was cleaned by boiling water in the flask until steam rose through the apparatus and issued from the top. After each digestion the apparatus was cleaned by boiling nitric acid in the flask.

The procedure adopted for the digestion of liver was to weigh a 2g sample of the wet homogenized tissue into the digestion flask. The digestion mixture of 10 cm^3 Aristar nitric acid and 3 cm^3 Aristar sulphuric acid was then added to the liver. The mixture was refluxed for one hour, with 10 cm^3 distilled water placed on top of the sinter and with coolant water flowing through both condensers. After one hour the lower condenser was drained and further heating of the mixture distilled the nitric acid into the reservoir. Heating was continued until white fumes of sulphuric acid appeared, when the heating was stopped and the nitric acid was drained into the digestion flask. The complete procedure was repeated until the sulphuric acid digest reached a pale straw colour which indicated complete digestion. The liver digest could be collected in two ways: either the nitric acid and sulphuric acid fractions could be separated and transferred to separate volumetric flasks, or the two fractions could be combined in the digestion flask and then transferred to one volumetric flask, before making up to volume. The latter procedure was adopted.

The digestion procedure worked reasonably well, though the hot plate used was not sufficiently powerful to effect complete separation of the nitric and sulphuric acids. During the course of a digestion the water placed above the sinter gradually drained down the apparatus.

The digestion method was examined by taking standard copper solutions through the entire procedure: recoveries of 100 $\pm$ 4% were obtained.

5.2.3 Analysis of Acid Digest of Liver

A single acid digest of a "normal" liver sample was used to establish working methods for the determination of Cu, Pb, Mn, Cd, Ag, Cr, Al, Ni, Li, Co and Ba. The digest of approximately 2 g liver with 10 cm^3 nitric acid and 3 cm^3 sulphuric acid was diluted to 25 cm^3 with distilled water. This solution is referred to as "the digest", and was further diluted for the determination of some elements. For each element the instrumental conditions listed in Table 12 were employed, and a ramp ashing step was incorporated into each analytical programme - the maximum ashing temperature for this step is given in Table 20.

Standard additions were carried out on suitable dilutions of the digest and acid blank for each element listed in Table 20; this table lists the volumes and dilutions used, and the matrix effects observed. Some standard additions were carried out using standards made up in an acid matrix equivalent to that in the sample.

A very noisy signal was given by the cobalt hollow cathode lamp employed, and it was difficult to distinguish the cobalt signal from the noise. Using ashing up to 650°C., no cobalt signal could be detected from up to 50 µl of 10x diluted digest. 20 µl samples of the digest gave detectable signals, but the results were not reliable. Barium could not be detected in either the digest or the acid blank.

Table 20 Analysis of Acid Digests of Liver

Element	Max. Ash Temp./°C	Standards	Sample Vol.	Sample Diln.	Sample Matrix	Blank Vol.	Blank Diln.	Blank Matrix
Cu	1100	aqueous	5 µl	10x	1.0	5 µl	10x	1.0
	1100	acid	5 µl	10x	1.0			
Pb	400	aqueous	5 µl	10x	2.3	5 µl	10x	2.3
	630	aqueous	5 µl	10x	1.17	5 µl	20x	1.07
	630	acid	5 µl	10x	1.31			
	630	acid	5 µl	20x	1.19			
Mn	650	aqueous	5 µl	10x	1.11	5 µl	10x	1.11
Cd	440	aqueous	5 µl	20x	1.79	5 µl	10x	1.79
	440	acid	5 µl	20x	1.1			
Ag	440	aqueous	10 µl	10x	1.15	5 µl	undil	1.44
	440	acid	10 µl	10x	1.1	10 µl	10x	1.2
Cr	400	aqueous	10 µl	20x	1.0			
Al	1100	aqueous	5 µl	20x	1.06	10 µl	10x	1.0
Ni	625	aqueous	10 µl	undil	1.74	10 µl	undil	1.0
Li	625	aqueous	10 µl	undil	1.19	10 µl	undil	1.28

Acid digestions were carried out on four human liver samples and duplicate samples of N.B.S. Bovine Liver with the help of R.C. Henson, a third year undergraduate student. The digests were analysed for Cu, Fe, Mn, Cr, Pb and Cd, using the methods outlined above. Since the matrix effects present in samples suitably diluted for the determinations of Cu, Fe, Mn and Cr were virtually unity, the concentrations of these elements were determined from aqueous calibration graphs. For lead and cadmium determinations the samples could not be diluted sufficiently to reduce the matrix effect present to unity, due to the low levels of these elements in the samples. Standard addition procedures would be necessary for accurate analyses of lead and cadmium, but there was insufficient time to carry these out. A blank correction was made to all the analyses, and the results obtained are given in Table 21.

Table 21 Analysis of Human Liver for Cu, Fe, Mn and Cr

Sample	Concentration / p.p.m. (fresh weight)			
	Cu	Fe	Mn	Cr
296/75 - (1)	10.5	211	1.6	1.6
" - (2)	10.6	200	1.6	1.5
297/75	6.0	237	0.8	4.1
298/75 - (1)	7.1	189	0.7	1.9
" - (2)	7.2	189	0.6	2.0
299/75 - (1)	10.5	137	1.1	1.9
" - (2)	8.1	158	0.7	1.8

The results in Table 21 are in satisfactory agreement with literature values of the concentrations of these metals in liver (fresh weight basis): Cu 3-15 p.p.m., Fe 90-300 p.p.m., Mn ~ 1 p.p.m. and Cr ~ 0.2 p.p.m.

The concentrations determined in duplicate analyses of N.B.S. Bovine Liver were: Cu 201, 197 p.p.m., Mn 7.2, 7.0 p.p.m. and Cr 1.9, 1.8 p.p.m., which are in fair agreement with the certificate values: Cu 193 ± 10 p.p.m., and Mn 10.3 ± 1.0 p.p.m.

5.2.4 Conclusions

Digestion of liver with a mixture of nitric acid and sulphuric acid, followed by analysis of the digest by electrothermal atomization atomic absorption spectrometry, is a satisfactory procedure for the determination of many trace metals in liver. If the matrix effect present in the sample is unity, or close to unity, then elemental concentrations can be obtained by comparison of the analytical signals with an aqueous calibration graph. For larger matrix effects, the use of standard addition procedures will be essential if accurate analyses are required. In general, matrix effects are reduced by raising the ashing temperature and by increasing the dilution of the sample. For ashing temperatures of 625 - 1100°C, e.g. Ni, Mn, Cu and Al, the observed matrix effects are close to unity, whilst for lead increasing the ashing temperature from 400 to 630°C reduced the matrix effect from 2.3 to 1.17. A two-fold increase in dilution - from 10x to 20x - reduced the lead matrix effect from 1.17 to 1.07.

The use of standards made up in an acid matrix matching that in the samples gave no benefits in the form of reduced matrix effects or ease of calibration. The procedure was actually complicated by the level of analyte present in the acid blank. For cadmium, although the matrix effect was reduced when acid solutions were used (Table 20), the precision of the measurements made with acidified standards was not as great as the corresponding measurements with aqueous solutions.

The levels of analyte elements in the acid blanks were high. For equivalent dilutions of the liver digest and the acid blank, the signal ratios observed were: Cu 76.9, Pb 1.65, Mn 2.09, Cd 5.54, Ag 3.25, Al 7.28, Ni 2.68 and Li 3.60. These ratios reveal that - except for copper - any error in measuring the blank signal will have a large effect on the accuracy of the analysis. A sample : blank signal ratio of 10 : 1 would be satis-

factory, but to achieve this further purification of the reagents would be necessary. Sub-boiling distillation (section 2.4) would be a satisfactory method for purifying acids. An alternative approach would be to use a simpler procedure, as has been described by Segar and Gilio (482). In order to reduce contamination during wet ashing these workers used nitric acid alone in pure fused silica flasks. Each digestion, however, took several days to complete.

5.3 Digestion with Tetramethyl Ammonium Hydroxide

5.3.1 Introduction

Alkaline digestants such as tetramethyl and tetraethyl ammonium hydroxides were originally employed in liquid scintillation counting (194), and a commercial product - Soluene-100 - was specifically formulated for use with toluene and xylene based scintillation counting solutions. These digestants have also found application in the gas chromatographic analysis of tissue samples and more recently have been applied to sample preparation prior to atomic absorption spectrometry.

Jackson et al. (235) employed Soluene-100 for the determination of Zn, Cu, Fe and Mn in animal tissues by flame atomization atomic absorption, and found that the preparation of the tissue samples was fast, with minimal handling, sample loss and contamination. Luyten et al. (316) evaluated Jackson's (235) method, and found that it gave good results for the determination of copper and zinc in human brain, but they were unable to dissolve fish meal with the Soluene-100. Soluene-100, which is tetramethyl ammonium hydroxide (TMAH) in toluene, was also employed by Kaplan (252) for the digestion of rat lungs prior to the determination of cadmium, nickel and zinc. Murthy et al. (371) used an aqueous solution of TMAH to digest rat liver, rat kidney and rat hair samples, and analysed the resulting homogenates for Zn, Cu, Cd and Pb by flame atomic absorption.

More recently graphite furnace atomic absorption has been employed in the analysis of TMAH digests. Gross and Parkinson (187) analysed liver and other tissue digests for Cu, Pb, Zn, Cd and Mn, and with N.B.S. Bovine Liver achieved good agreement with the certified results (except for copper). Barlow and Khera (31) employed the commercial product Soluene-350 for the digestion of placental and liver tissue prior to the determination of lead.

5.3.2 Digestion Procedure

Samples of liver (approx. 10g) were homogenized in a rotary homogenizer for five minutes. No water was added to the samples, and if not required immediately they were preserved by freezing. Two grams of freshly homogenized sample or mixed thawed sample (394) was weighed into a beaker and 5 cm^3 tetramethyl ammonium hydroxide solution (25% w/w, Hopkin & Williams Ltd. or B.D.H. Chemicals Ltd.) was added. The mixture was left for several hours at room temperature to effect dissolution; higher temperatures increase the dissolving power of the TMAH (31). The resultant solution was diluted to 25 cm^3 with distilled water in a volumetric flask. The final solutions varied in colour from dark brown to yellow.

5.3.3 Analysis of TMAH Digest of Liver

The 25 cm^3 of solution obtained from the digestion procedure is referred to as "the digest"; this digest was further diluted for the determination of some elements. The instrumental conditions for the elements - Mn, Cd, Ag, Cr, Al, Ni and Li - determined in the digest were those in Table 12. A ramp ashing step was incorporated into each analytical programme, and the maximum ashing temperatures used are listed in Table 22.

Standard additions were carried out on suitable dilutions of the digest and TMAH blank, for each element. The volumes and dilutions used are listed in Table 22, together with the size of the matrix effects observed.

5.3.4 Conclusions

Solubilization of liver tissue with TMAH is a simple, rapid and convenient method for the preparation of this material for analysis by electrothermal atomization atomic absorption. For elements which can be ashed to 600°C or above e.g. Mn, Al, Ni and Li, the residual matrix effect is low - even for an undiluted digest - and probably could be ignored in routine analysis. The more volatile elements

Table 22 Analysis of TMAH Digests of Liver

Element	Max. Ash Temp. /°C.	Sample			Blank		
		Volume	Dilution	Matrix	Volume	Dilution	Matrix
Mn	650	5 µl	10x	1.02	10 µl	10x	1.02
Cd	440	5 µl	100x	1.41	10 µl	10x	1.41
Ag	440	5 µl	100x	1.21	5 µl	100x	1.21
Cr	400	10 µl	100x	1.64	10 µl	10x	1.19
Al	1100	10 µl	1000x	1.11	10 µl	500x	1.11
Ni	625	10 µl	undil	1.07	10 µl	undil	1.07
Li	625	10 µl	undil	1.21	10 µl	undil	1.04

e.g. Cd, Ag and Cr are subject to more severe matrix effects; possibly higher ashing temperatures could be employed, but accurate analyses for these elements will only be achieved by employing standard addition procedures.

The analysis of TMAH by standard addition for each element showed that there were significant amounts of each element present (Table 23).

Table 23 Trace Metal Content of TMAH

Element	Concentration/p.p.m.
Cu	0.6
Pb	0.6
Mn	0.015
Cd	0.03
Ag	1.90
Cr	0.19
Al	50
Ni	0.05
Li	0.1

Murthy et al. (371) do not mention TMAH blanks in their study, while Kaplan (252) ran reagent blanks. but did not report any values. Jackson et al. (235) analysed reagent blanks and found that only zinc gave a measurable signal. These three workers employed flame atomic absorption, but the workers with the more sensitive electrothermal atomization techniques - Gross and Parkinson (187) and Barlow and Khera (31) - make no mention of reagent blanks.

From the present study it is apparent that the levels of metallic contaminants in commercial TMAH can be considerable. For equivalent dilutions of the liver digest and the TMAH blank, the signal ratios observed were: Mn 2.7:1, Cd 12.5:1, Ag 1.35:1, Cr 2.22:1, Al 4.03:1, Ni 1.30:1 and Li 1.75:1. For results of the greatest accuracy it will be necessary to purify the TMAH. Probably the most effective way of achieving this will be to solvent extract the metallic contaminants, e.g. with cupferron into chloroform.

Chapter 6

MATRIX MODIFICATION

6.1 Introduction

An important feature of electrothermal atomization atomic absorption is that pretreatment of the sample is unnecessary in many instances. Prior to the atomization stage it is often sufficient to employ drying and ashing steps to remove solvent and matrix constituents. Occasionally, however, analyses will be met where the relative volatilities of analyte and matrix are such that the matrix cannot be completely removed before a temperature is reached which is sufficient to effect partial atomization of the analyte element. This situation is especially likely to occur in the analysis of biological materials or in the determination of very volatile elements such as Se, Te, Cd, Hg or As. Modification of the matrix by chemical agents, either to the sample solution or directly to the sample aliquot in the furnace, is a means of overcoming the problem (141), and will be effective either by increasing the volatility of the matrix constituent(s) or by decreasing the volatility of the analyte element.

6.1.1 Matrix Modification to Increase Volatility of Matrix

This technique is well illustrated by the determination of copper in sea water by Ediger *et al.* (142) where the addition of ammonium nitrate solution produced a reaction with the sodium chloride present in the sea water.

$$NaCl + NH_4NO_3 \longrightarrow NaNO_3 + NH_4Cl$$

and ashing temperatures of less than 500°C resulted in the products volatilizing out of the furnace. Sodium chloride has been shown by Adams *et al.* (2) to exhibit considerable molecular absorption in the hot graphite tube over the wavelength range 190-360 nm, whilst sodium nitrate produces no molecular absorption over the same wavelength range.

Thus any residual sodium nitrate should not give a (molecular absorption) matrix interference. If the matrix constituents of a sample are known, then it may be possible to predict chemical reactions which would give products showing low molecular absorption at the analyte wavelength.

6.1.2 Matrix Modification to Decrease Volatility of Analyte Element.

The addition of nickel solutions to samples containing selenium, tellurium or arsenic will result in the formation of relatively involatile compounds in the furnace, and ashing temperatures higher than would be normal for aqueous solutions of these elements may be employed (141). Copper may be used instead of nickel, since it will form similar stable compounds.

The addition of ammonium fluoride, sulphate or phosphate to cadmium solutions allows ashing temperatures of up to 900°C to be employed, compared to a limit of 500°C for simple cadmium solutions. This effect is due to the formation of stable cadmium compounds in the furnace; ammonium salts are preferred since they are more easily decomposed by heat (141).

Other examples of this matrix modification technique have appeared in the literature, and will be discussed in the next two sections.

6.2 Matrix Modification to Decrease Volatility of Mercury

6.2.1 Introduction

Mercury is very difficult to determine by electrothermal atomization atomic absorption because even the drying of a mercury solution results in a significant loss of mercury - due to its high volatility. It is this high volatility of mercury - even at room temperature - and the ability to produce a monatomic vapour, which made possible Woodson's (589)

flameless atomic absorption determination of mercury in 1939 - a determination which predated the classical work by Walsh (562) on atomic absorption with flame atomization. Even the determination of mercury by atomic absorption in an air-acetylene flame is not very sensitive [sensitivity for 1% absorption = 7.5 $\mu g/cm^3$(407)], and although the toxicity of mercury has been recognised for many years, it is only within the past seven that methods have become available which are capable of determining mercury reliably at the low levels encountered in biological and environmental samples. These methods can be traced to the work of Brandenberger and Bader (55, 56) and Hatch and Ott (199); variations of the reduction - aeration continuous recirculation technique for the cold vapour determination of mercury, introduced by Hatch and Ott, are now employed in many laboratories. These highly sensitive techniques have led to a large volume of data on mercury levels. A recent review by Ure (550) on the determination of mercury by non-flame atomic absorption and fluorescence spectrometry includes the cold vapour method; this method is the subject of a detailed study by Hawley and Ingle (200) who optimized the experimental parameters and achieved, with their recommended method, a concentration detection limit of 1 part per 10^{12} Hg(II) for a 1 cm^3 sample.

Ediger (141) has described the addition of ammonium sulphide to mercury in nitric acid solution in a graphite tube furnace. The resulting stabilization allowed ashing temperatures of up to 300°C to be employed, and a detection limit of 0.2 ng was achieved.

Issaq and Zielinski (232) studied the determination of mercury with a graphite tube atomizer, and reported a 50x signal enhancement by the addition of hydrogen peroxide to aqueous mercury solutions. They found ashing temperatures up to at least 200°C could be used, and postulated the formation of a stable mercury-peroxy complex.

It was thought desirable to include mercury in this study of the analysis of urine for trace metals by ashing and atomizing directly in the graphite tube, and since the high volatility of mercury would preclude any ashing of an untreated urine matrix, an investigation of the chemical stabilization of mercury was undertaken.

6.2.2 Experimental Procedure

All mercury determinations were made at the 253.65 nm line using a spectral band width of 0.7 nm. The optimized programme for the HGA-74 comprised: initial drying at 105°C (digital value 60) for 30s, and ashing at 155°C (digital value 80) for a further 30s; a 10s atomization at 2000°C (digital value 720) under "gas stop" conditions, and a final heating at 2700°C for 5s. Argon was used as the purge gas. Mercury working standards were prepared by serial dilution of a 1000 p.p.m. stock solution (0.6768 g mercuric chloride dissolved in 500 ml distilled water). Hydrochloric (34-37 % w/w) and nitric (69-71% w/w) acids were of "Aristar" or "Ultrar" quality, and the hydrogen peroxide employed was 20 volume (6% w/v), general purpose reagent grade (Hopkin and Williams Ltd.)

6.2.3 Experimental Results

Initial experiments failed to reproduce the results of Issaq and Zielinski (232). The addition of hydrogen peroxide (4% v/v 20 vol.) to a 5.0 p.p.m. mercury solution gave no enhancement of the mercury atomic absorption signal, neither did the addition of nitric acid (4% v/v conc. HNO_3), nor a combination of nitric acid and hydrogen peroxide (4% v/v + 4% v/v). Comparison of the present experimental parameters with those of Isaaq and Zielinski showed the only real difference to be the mercury stock solution used. It was thought that their, commercially prepared, mercury solution might contain hydrochloric acid, and consequently test solutions were made up in hydrochloric acid. The results are given in Table 24.

Table 24. Absorbance from 50 ng Mercury in Solutions Containing Various Chemical Additions.

5.0 p.p.m. Hg solution	Absorbance from 50 ng
aqueous	0.007
4% HNO_3	0.007
4% H_2O_2	0.007
4% HNO_3 + 4% H_2O_2	0.007
4% HCl	0.045
4% HCl + 4% H_2O_2	0.12
4% HCl + 4% HNO_3 + 4% H_2O_2	0.12

The addition of hydrochloric acid gave an eight-fold enhancement of the mercury signal, and a combination of hydrochloric acid and hydrogen peroxide gave approximately 20x signal improvement, but hydrogen peroxide on its own appeared to have no effect. In order to clarify the situation, further experiments were undertaken.

(a) Effect of hydrochloric acid concentration

5.0 p.p.m. mercury solutions in 0-18% (v/v) hydrochloric acid were prepared and 10 µl aliquots analysed. The experiment was repeated after the addition of 2 cm^3 20 volume hydrogen peroxide to the 100 cm^3 of each solution. The resulting graphs are given in Figure 37. The possibility that the hydrochloric acid might contain appreciable amounts of mercury was considered (453), but mercury could not be detected in water blanks containing 0-12% hydrochloric acid.

(b) Effect of hydrogen peroxide concentration

For 5.0 p.p.m. mercury solutions in 8% (v/v) hydrochloric acid, the hydrogen peroxide content was varied between 0 and 4% (v/v 20 volume solution), and 10 µl aliquots analysed. The results are shown in Figure 38.

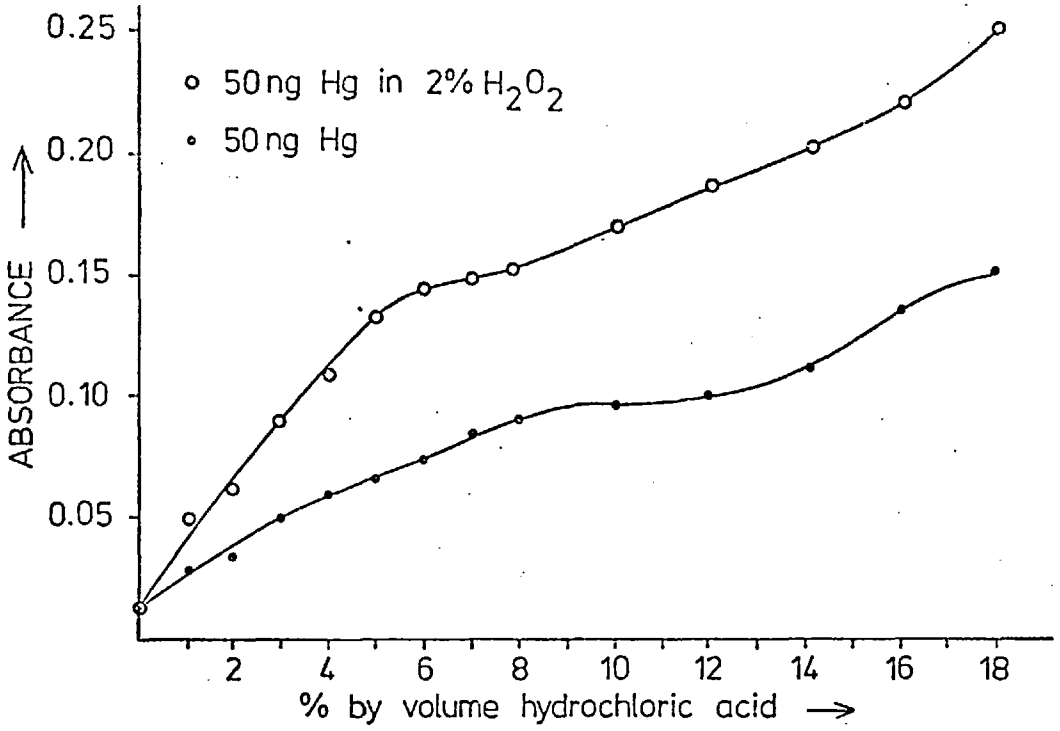


Fig. 37 Effect of hydrochloric acid concentration on mercury atomic absorption signal in the absence and presence of hydrogen peroxide

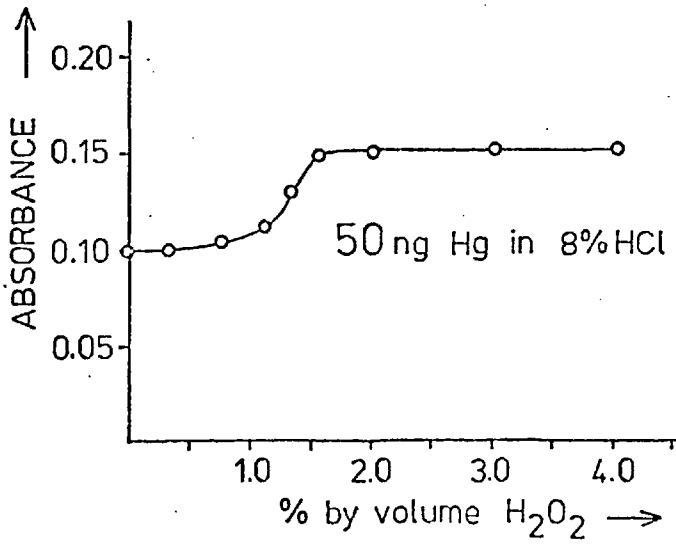


Fig. 38 Effect of hydrogen peroxide concentration on mercury atomic absorption signal

(c) Effect of chloride ion concentration

The chloride ion concentration in solutions of: (i) 5.0 p.p.m. Hg. (ii) 5.0 p.p.m. Hg containing 2% (v/v 20 volume) hydrogen peroxide, (iii) 5.0 p.p.m. Hg containing 6% (v/v) nitric acid, and (iv) 5.0 p.p.m. Hg containing 2% hydrogen peroxide and 6% nitric acid. was varied between 0 and 0.6 M by the addition of 0-40 ml 8% (w/v) ammonium chloride solution. 10 µl aliquots of each solution were analysed, and the resulting graphs are given in Figure 39.

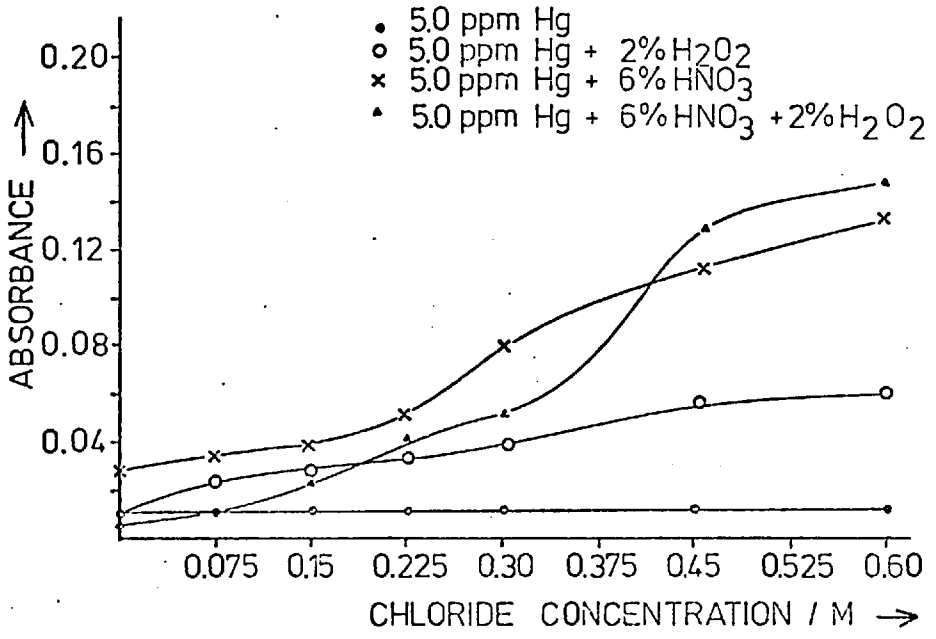


Fig. 39 Effect of chloride ion concentration on mercury atomic absorption signal

(d) Effect of ashing temperature and atomization temperature

Solutions of mercury containing 4, 8 and 12% hydrochloric acid, each with 2% hydrogen peroxide, were ashed at temperatures varying from 60 to 440°C. For temperatures over 105°C, a dry/ash programme was used: drying at 105°C for 30s, and ashing at the second temperature for a further 30s. Atomization at 2000°C gave plots of absorbance vs. ashing temperature (Figure 40). The temperatures over the

range 0-500°C were determined separately by the use of a thermocouple, and were found to be in general agreement (± 50°C) with the data supplied by the manufacturer (409).

Varying the atomization temperature for a two stage ash of 105°C/30s, 155°C/30s, gave the plot shown in Figure 40, for the absorbance from 50 ng mercury.

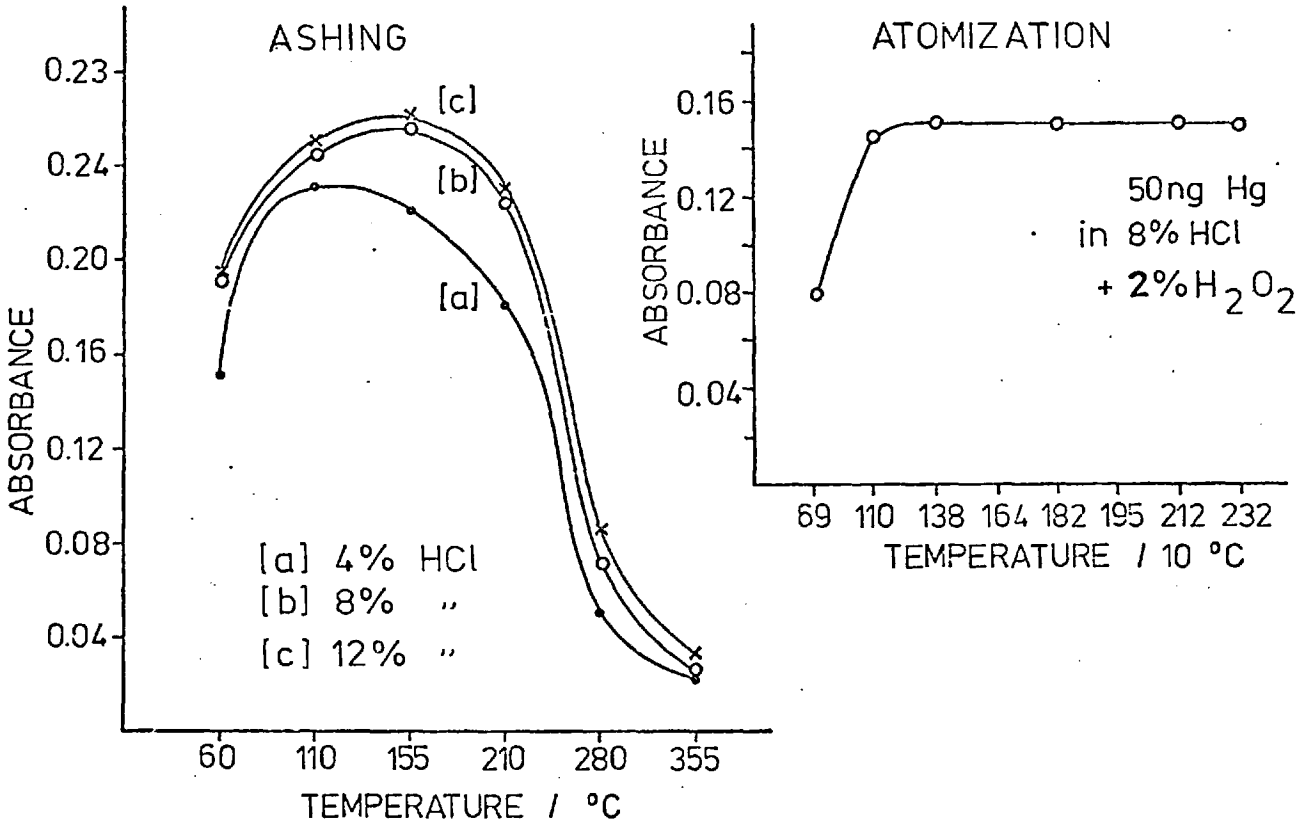


Fig. 40 Effects of furnace ashing and atomization temperatures on mercury signal

(e) Calibration

Using the optimized experimental conditions it was possible to construct a calibration graph for mercury in a 10% (v/v) hydrochloric acid and 2% (v/v) 20 volume hydrogen peroxide solution. The calibration graph is shown in Figure 41; it is linear up to 120 ng mercury, with a sensitivity of 1.65 ng and a detection limit of 3.8 ng.

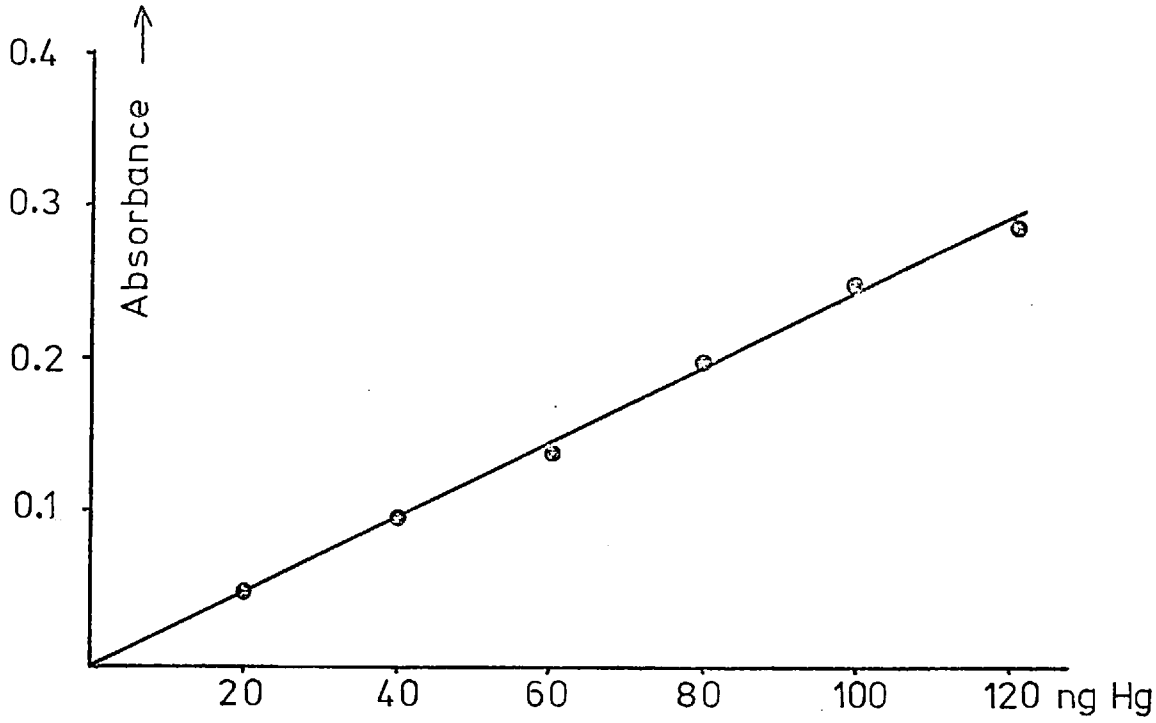


Fig. 41 Calibration graph for mercury

(f) Flame Atomization

Solutions of: (i) 100 p.p.m. mercury (aqueous), (ii) 100 p.p.m. mercury in 2% (v/v) hydrogen peroxide, (iii) 100 p.p.m. mercury in 8% (v/v) hydrochloric acid, and (iv) 100 p.p.m. mercury in 2% hydrogen peroxide and 8% hydrochloric acid, were examined by atomic absorption with atomization in an air-acetylene flame. Each solution gave the same absorbance value.

6.2.4 Mechanism of the Stabilization of Mercury.

It is difficult to envisage the formation of a mercury peroxy complex, as suggested by Issaq and Zielinski (232), which would be stable in the strongly reducing atmosphere present in a hot graphite tube. It was at first thought that the hydrogen peroxide might, by reducing the Hg(II) to Hg(I), be enhancing the measured absorbance, as has been observed in the atomic absorption determination of mercury with flame atomization (212). The fact that the observed enhancement occurred only in the graphite tube atomizer and not in the flame appeared to negate this argument.

Additionally, when ascorbic acid was added to dilute solutions of $HgCl_2$, no signal enhancement was observed for graphite tube atomization.

The facts which emerge from this study are that the mercury stabilization is effected by a combination of acid and chloride ion e.g. hydrochloric acid or hydrogen chloride, and the presence of hydrogen peroxide causes a further enhancement, though hydrogen peroxide on its own will not effect the stabilization. The hydrogen peroxide enhancement was not a simple pH effect; this is shown in Table 25.

Table 25. Effect of Varying pH with H_2O_2 and HNO_3 on the Hg Signal.

Sample	pH	Absorbance
120 ng Hg, 6% HCl	0.90	0.18
120 ng Hg, 6% HCl, 2% H_2O_2	0.60	0.23
120 ng Hg, 6% HCl, HNO_3	0.60	0.14
120 ng Hg, 10% HCl	0.40	0.21
120 ng Hg, 10% HCl, 2% H_2O_2	0.30	0.24
120 ng Hg, 10% HCl, HNO_3	0.30	0.19

The amounts of hydrochloric acid and hydrogen peroxide required to effect the enhancement of the mercury signal are too large to postulate the formation of any kind of stoichiometric complex. and it seems likely that the enhancement/stabilization is due to the conditions present in the vapour phase in the furnace. The conditions are that HCl gas is present in excess, and it is possible that mercury atoms will be held in the gas phase by the formation of $HgCl_2$. It is interesting to note that the maxima of the ashing profiles (Figure 40) are near to the constant boiling

temperature of hydrochloric acid: 110°C (84). By passing HCl gas into the furnace, via the argon inlet, during the ashing stage, the results given in Table 26 were obtained.

Table 26 Mercury Determinations in Presence or Absence of HCl gas at the Ashing Stage.

Sample	Absorbance (HCl gas)	Absorbance (No HCl gas)
10 µl H_2O	0.01	0.005
10 µl 5.0 p.p.m. Hg	0.15	0.005
10 µl 5.0 p.p.m. Hg, 2% H_2O_2	0.15	0.005
10 µl 5.0 p.p.m. 2% H_2O_2, 8% HCl	0.26	0.14

The data in Table 26 tend to support the gas-phase theory vs. the formation of a complex in solution, but the role of the peroxide is not clear. If the peroxide is acting in its capacity as an oxidising agent, then other oxidising agents would be expected to effect the enhancement. The effect of ammonium persulphate was tested, in both the absence and presence of silver nitrate catalyst; the results are given in Table 27.

Table 27 Mercury Determinations with Two Different Oxidising Agents.

Sample	Absorbance
10 µl 5.0 p.p.m. Hg	0.005
10 µl 5.0 p.p.m. Hg, 6% HCl	0.06
10 µl 5.0 p.p.m. Hg, 6% HCl, 2% H_2O_2	0.16
10 µl 5.0 p.p.m. Hg, 6% HCl, 2% ammonium persulphate	0.05
10 µl 5.0 p.p.m. Hg, 6% HCl, 2% ammonium persulphate + $AgNO_3$	0.05

The variation in the absorption maximum with change in the hydrochloric acid concentration, shown in Figure 40, is a possible cause of the discontinuities in the enhancements shown in Figure 37.

6.2.5 Conclusions

In the determination of mercury by atomic absorption spectrometry with graphite furnace atomization, it is possible to reduce the loss of mercury during the drying and ashing steps by the presence of hydrochloric acid. The formation of $HgCl_2$ by the high concentration of HCl gas in the hot graphite tube reduces the loss of mercury atoms, and results in a considerable enhancement of the mercury signal. Hydrogen peroxide effects a further signal enhancement, though this is neither a reducing, oxidising nor a pH effect of the hydrogen peroxide. It is possible, however, that an $HgCl_2 . H_2O_2$ adduct, of higher thermal stability than $HgCl_2$, is formed.

After the completion of this work a note by Owens and Gladney appeared (395). These authors also found that the presence of HCl was essential to achieve any enhancement of the mercury signal by hydrogen peroxide. The results of Issaq and Zielinski (232) are probably due to their failure to recognise the presence of HCl in commercially prepared standard solutions.

6.3 Matrix Modification by Acids and Hydrogen Peroxide

6.3.1 Introduction

There are few published data on the effect of the acid matrix on elemental sensitivity in graphite furnace atomization A.A.S., though it has been stated that chloride salts are unsuitable due to their tendency to volatilize as molecules (73) - see section 2.6.6. This point has yet to be proven satisfactorily, though Fuller (166) in

investigating the effect of acids on the determination of thallium found sulphuric and nitric acids to have negligible effect in the 0.001 - 1% v/v concentration range, whilst hydrochloric and perchloric acids gave severe depression of the thallium signals. Fuller attributed these results to the formation of volatile chlorides during the ashing step. Addition of sulphuric acid reduced the interferences both from the hydrochloric and perchloric acids and also from up to 100 $\mu g/cm^3$ sodium chloride.

Ediger (141) has pointed out the effects of perchloric acid and hydrogen peroxide on atomic absorption signals for gallium and germanium, and in view of the role of hydrogen peroxide in the mercury stabilization, and the lack of data on the effect of the acid matrix on elemental sensitivies, a study of the effects of hydrochloric acid, nitric acid, hydrogen peroxide, hydrochloric acid plus hydrogen peroxide and nitric acid plus hydrogen peroxide on analyte signals was undertaken.

6.3.2 Experimental Results

(a) Cu, Ni, Cr, Cd, Li, Ag, Fe, Ba, Mn and Au.

For each of these metals, the stock solution was diluted to give solutions which would result in atomic absorption signals of approximately 0.05 absorbance (for 10 µl aliquots): (i) in distilled water, (ii) in 2% (v/v) nitric acid, and (iii) in 2% (v/v) hydrochloric acid. After measuring the absorbance for each solution under the appropriate instrumental conditions (section 3.5), further measurements were carried out after the addition of hydrogen peroxide - at both 1% (v/v 20 volume H_2O_2) and 2% (v/v) levels. It was found that for the ten metals listed above the atomic absorption signals were not significantly enhanced or reduced by the presence of nitric acid, hydrochloric acid or hydrogen peroxide - except for manganese, where slight increases in signal with increasing peroxide concentration were observed. This was found to

be due to contamination of the hydrogen peroxide by manganese: at the manganese wavelength a 2% (v/v) hydrogen peroxide solution gave an absorbance of 0.01, and a 4% (v/v) solution one of 0.02.

(b) Al, Ga, In, Tl, Pb, Sb, Bi and Hg

Nitric acid, hydrochloric acid and hydrogen peroxide resulted in some definite interferences on the atomic absorption signals for these elements, which, with the exception of mercury, are all metallic p-block elements. The presence of hydrochloric acid and hydrogen peroxide gave enhanced mercury atomic absorption signals; a detailed study of this effect has been given in section 6.2. The results for the other elements are listed in Table 28.

Aluminium and lead gave larger signals with increasing hydrogen peroxide concentration for each of the aqueous and acid solutions; this was found to be due to contamination of the hydrogen peroxide by these metals. Ga, In, Tl, Sb and Bi could not, however, be detected in the hydrogen peroxide. The addition of increasing quantities of hydrogen peroxide to the gallium, indium and thallium solutions resulted in diminishing signal sizes; for antimony a signal enhancement was observed, whilst for bismuth the signal remained fairly constant.

A comparison of signal size from aqueous standards with those in acid matrices showed that the change from aqueous to nitric acid gave slightly enhanced signals for most elements, except for gallium where the enhancement was dramatic. The change from aqueous to hydrochloric acid gave slight enhancements for aluminium, gallium and lead, a large enhancement for bismuth, and a slight reduction for antimony. Indium and thallium gave larger reductions in the signal.

Table 28 Effect of Nitric and Hydrochloric Acids on the Atomic Absorption Signals of Al, Ga, In, Tl, Sb and Bi.

Sample	Absorbance								
	Aqueous	+ 1% H_2O_2	+ 2% H_2O_2	2% HNO_3	+ 1% H_2O_2	+ 2% H_2O_2	2% HCl	+ 1% H_2O_2	+ 2% H_2O_2
10µl 0.1 p.p.m. Al	0.06	0.10	0.11	0.07	0.10	0.11	0.08	0.09	0.10
10µl 4 p.p.m. Ga	0.10	0.09	0.08	0.24	0.19	0.18	0.11	0.08	0.07
10µl 0.1 p.p.m. In	0.06	0.05	0.045	0.075	0.07	0.068	0.035	0.033	0.03
10µl 0.1 p.p.m. Tl	0.14	0.10	0.10	0.15	0.14	0.13	0.06	0.07	0.075
10µl 0.03 p.p.m. Pb	0.05	0.08	0.08	0.08	0.09	0.095	0.075	0.08	0.09
10µl 0.5 p.p.m. Sb	0.06	0.065	0.07	0.04	0.048	0.05	0.05	0.06	0.09
10µl 0.2 p.p.m. Bi	0.06	0.06	0.06	0.10	0.09	0.09	0.14	0.145	0.145

6.3.3 Conclusions

The depression of the thallium signal by hydrochloric acid agrees with the work of Fuller (166), and a similar effect was observed for indium, though not for any of the other elements investigated. This questions the theory (5, 73) that chlorides will volatilize during the ashing step, with resultant loss of analyte. Presumably, if atomization takes place via the oxide state (73), then hydrolysis of the analyte element must occur in chloride solutions, and loss of the element as molecular chloride will be minimal for most elements (except indium and thallium).

Since the signal enhancements observed will most probably be due to chemical stabilization of the analyte, it should be possible to interpret the results in terms of compound formation in the furnace. Oxidising agents, such as nitric acid, will help the formation of metal oxide during the ashing step (73), and hence may give a slight signal enhancement. The dramatic enhancement of the gallium signal by nitric acid has also been observed by Fernandez (156) who recommended the use of nitric acid plus hydrogen peroxide because this gave more reproducible results than nitric acid alone, while still enhancing the sensitivity over that of aqueous solutions. Germanium also gives increased sensitivities in oxidising matrices such as nitric and perchloric acids (242). For gallium and germanium, the lower oxides (Ga_2O and GeO) are more volatile than the higher oxides (Ga_2O_3 and GeO_2), and thus enhancement of gallium or germanium signals will occur in conditions favouring the formation of the higher oxides - such as the presence of oxidising acids (242).

Chapter 7

BENDING OF ANALYTICAL CURVES

7.1 Introduction

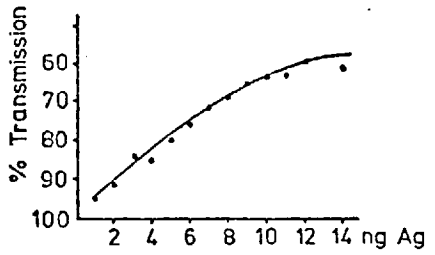
It is noticeable in many of the papers which have appeared on the subject of atomic absorption with electrothermal atomization that the calibration graphs obtained are either curved with an initial straight line portion - Figure 42 - or apparently curved right from the origin - Figure 43. In this work it was observed that the electrothermal atomization graphs were linear up to a limit which occurred at relatively low absorbance values, i.e. 0.3-0.4 - Figure 20 - compared to flame atomization where calibrations are linear up to 0.6-0.7 absorbance (407). It was also apparent that the extent of the linearity was very important when standard additions had to be carried out, as discussed in section 2.7, and an investigation into the causes of the increased curvature and smaller linear range was undertaken. Other workers (137, 258) have commented on the shapes of the calibration graphs, but have not attempted to identify the causes.

Several authors (320, 448, 458, 599) have considered the shapes of analytical growth curves in atomic absorption spectrometry, and have outlined the probable causes of bending of these curves - though almost exclusively for flame atomization systems. The factors involved in causing the greater curvature in calibration graphs from electrothermal atomizers may be divided into two groups, taking the basic classification of Rubeška and Svoboda (448):

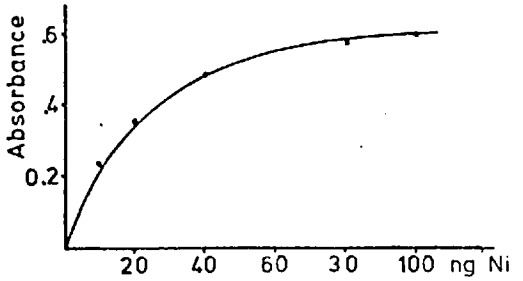
I Factors arising in the instrumental system

For measurements involving transient signals it is necessary to consider:

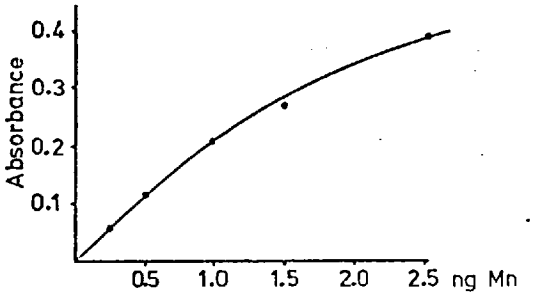
(i) the method of signal measurement (whether peak height or peak area);



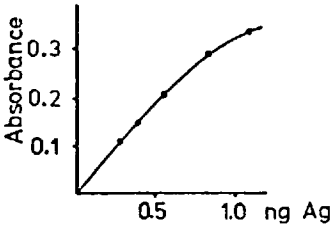
[a] West & Williams (569)



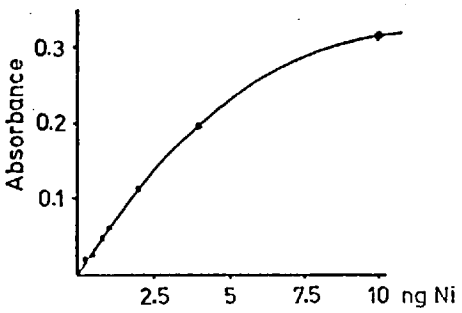
[b] Anderson, Johnson & West (21)



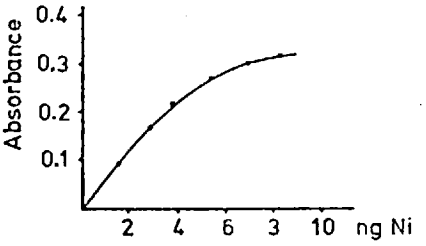
[c] Ebdon, Kirkbright & West (137)



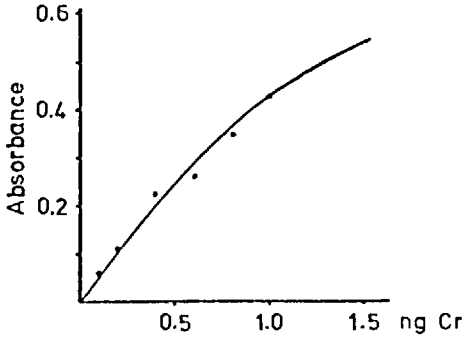
[d] Alder & West (9)



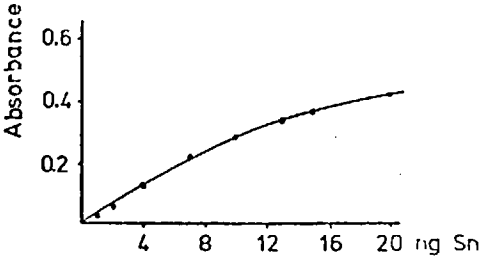
[e] Jackson & West (237)



[f] Alder & West (10)

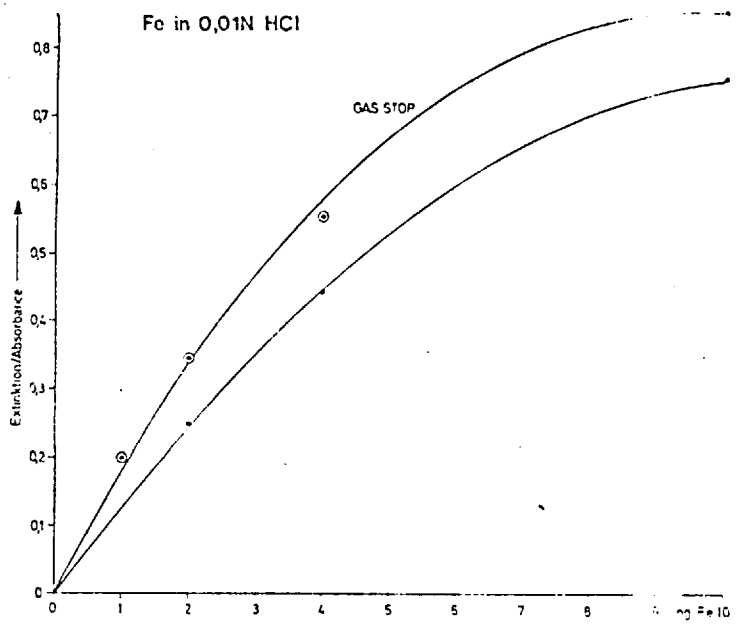


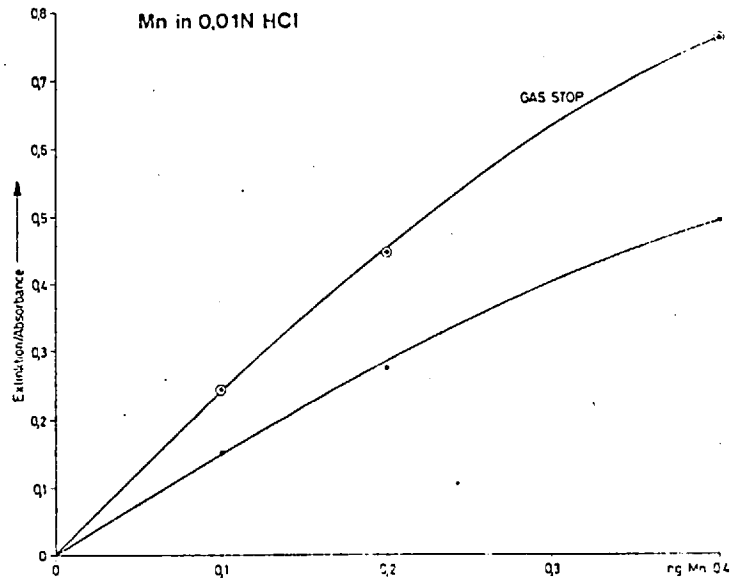
[g] Jackson & West (238)

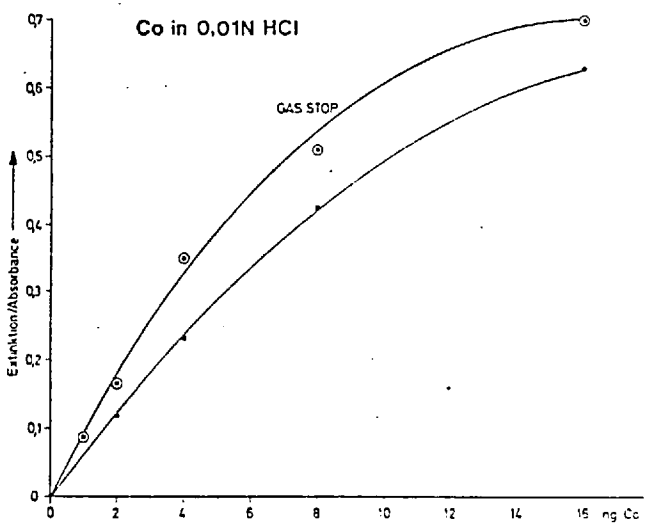


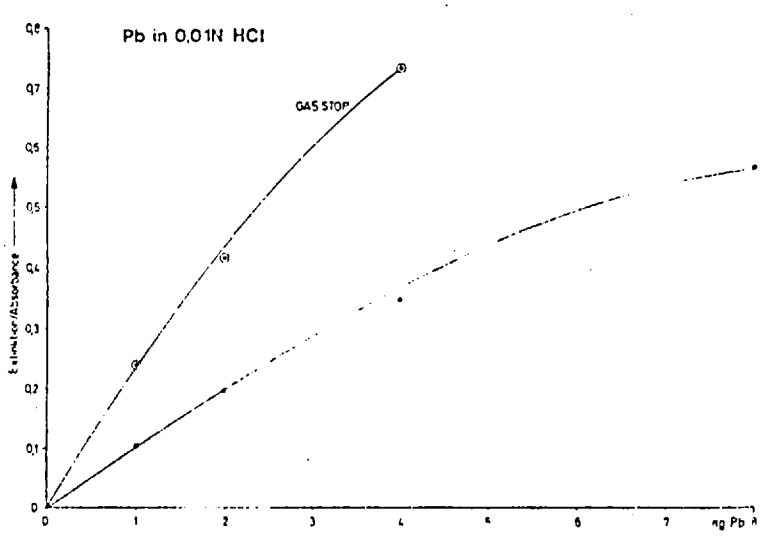
[h] Everett & West (147)

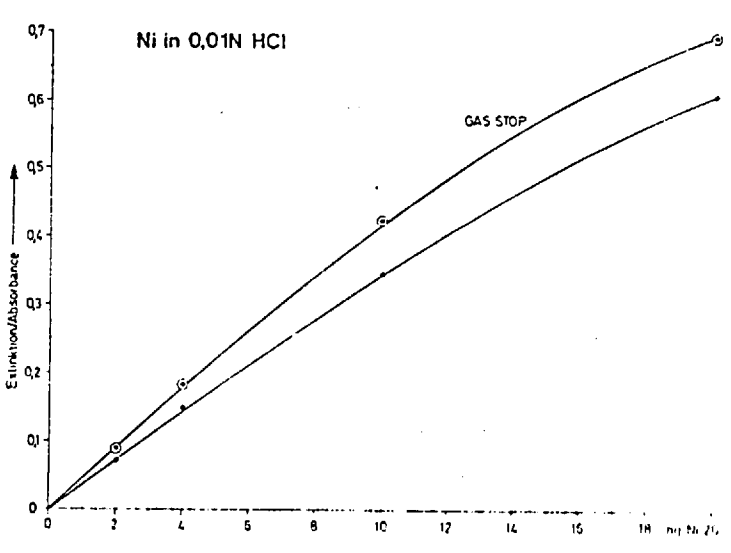
Fig. 42 Graphite filament calibration graphs











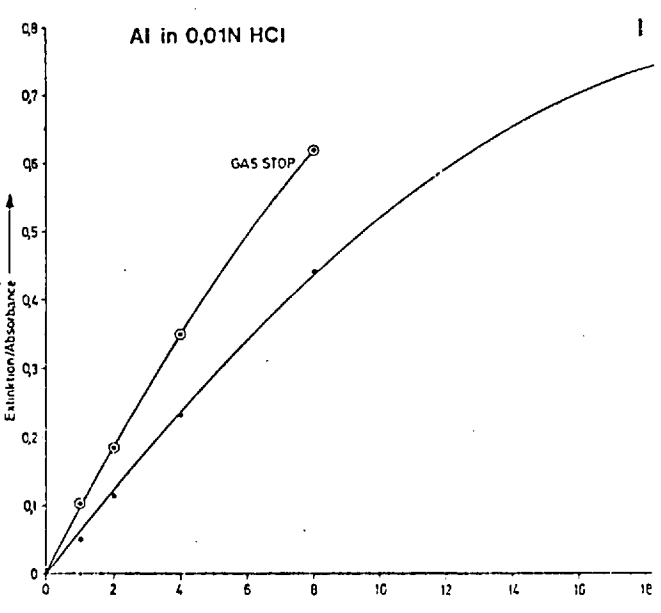


Fig. 43 Perkin-Elmer calibrations [ref. 409]

(ii) the chopping frequency (if modulation of the spectral source is employed);
(iii) the response time of the electronics (including the read-out device).

More general factors are:
(iv) light which is emitted by the source and falls on the detector but is not absorbed by the absorbing medium;
(v) inhomogeneities of the temperature and spatial distribution of the absorbing atoms in the absorbing medium;
(vi) emission by the absorbing medium affecting the measured signal.

II Factors related to the properties of the particular spectral line itself

(i) hyperfine structure of the line;
(ii) ratio of absorption and emission line widths;
(iii) resonance line broadening and shift in the absorbing medium.

Neglected in these factors is any process which disturbs the proportionality between the concentration of analyte in the sample volume and its concentration in the absorbing medium. In relation to this, Manning and Fernandez (340) have pointed out that different sampling procedures will give rise to different analytical curves, due to the porosity of the carbon surface. Analyte solution will soak into the graphite, the amount soaking in depending on the mode of sampling; the use of pyrolytic graphite (section 3.2.1) overcomes this problem.

These possible causes of the observed curved calibration graphs will now be considered in relation to the instrumental system employed in this work.

7.2 Method of Signal Measurement

It may be advantageous to employ integrated absorbance rather than peak absorbance when plotting growth curves in electrothermal atomization atomic absorption spectrometry. The theoretical approach (322, 324, 516), which is mainly due to L'vov, assumes that the element to be determined is completely atomized, that all of the atoms of the element enter the analysis volume, and that the removal of atomic vapour from the analysis volume is determined solely by diffusion (and hence is exponential). If N_0 is the number of atoms of an element in the sample, N is the total number of atoms entering the analysis volume at any instant t, T_1 is the atomization time, T_2 is the mean length of time spent by an atom in the analysis volume (the residence time, defined as the time taken for the absorbance to decrease from its peak value to a value e times smaller), and T_3 is the length of time during which the signal is recorded (Figure 44), then:

in the peak method. at time T_1, when the sample is completely atomized, N reaches its peak value N_{peak} and:

$$N_{peak} = N_0^2 \left[\frac{T_2}{T_1}\right]^2 \left[\frac{T_1}{T_2} - 1 + e^{-T_1/T_2}\right] \quad [5]$$

If $T_1/T_2 \ll 1$ then to a first approximation $N_{peak} = N_0$. Therefore, if the sample is completely atomized in a time $t \ll T_2$, the peak value N_{peak} corresponds to the total number of atoms in the sample, N_0. If $T_1 \geqslant T_2$ the value N_{peak} depends upon T_1 and T_2, and a simple relationship between N_{peak} and N_0 is possible only if T_1 / T_2 is constant.

The integration method depends upon measuring the integral value Q_N of atoms during the entire period for which atoms are produced and remain in the analysis volume. The integrated absorbance is represented by the area under an absorption peak, and it bears a simple relationship to the total number of atoms in a sample:

$$Q_N = \int_0^{T_3} N(t)\,dt \quad [6]$$

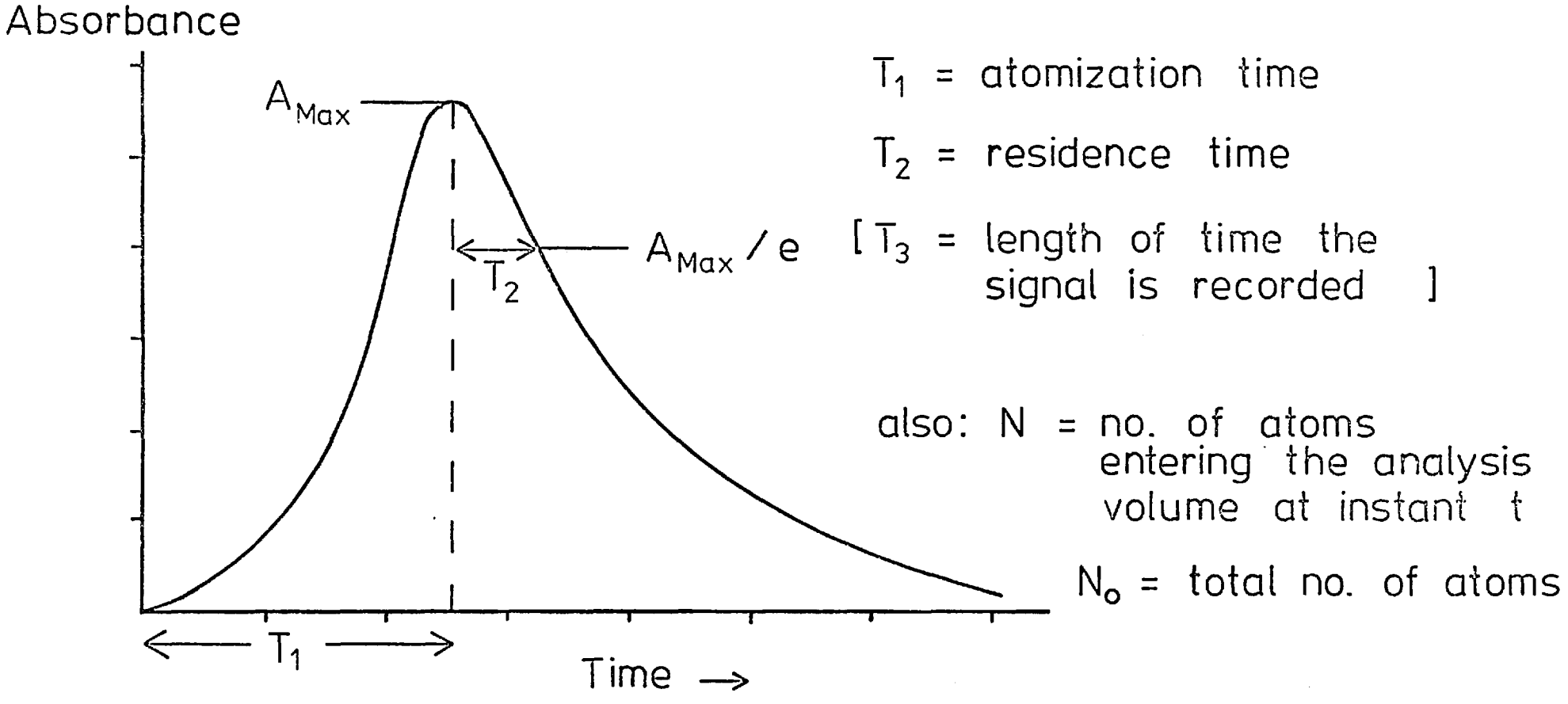


Fig. 44 Absorption profile for pulse atomization

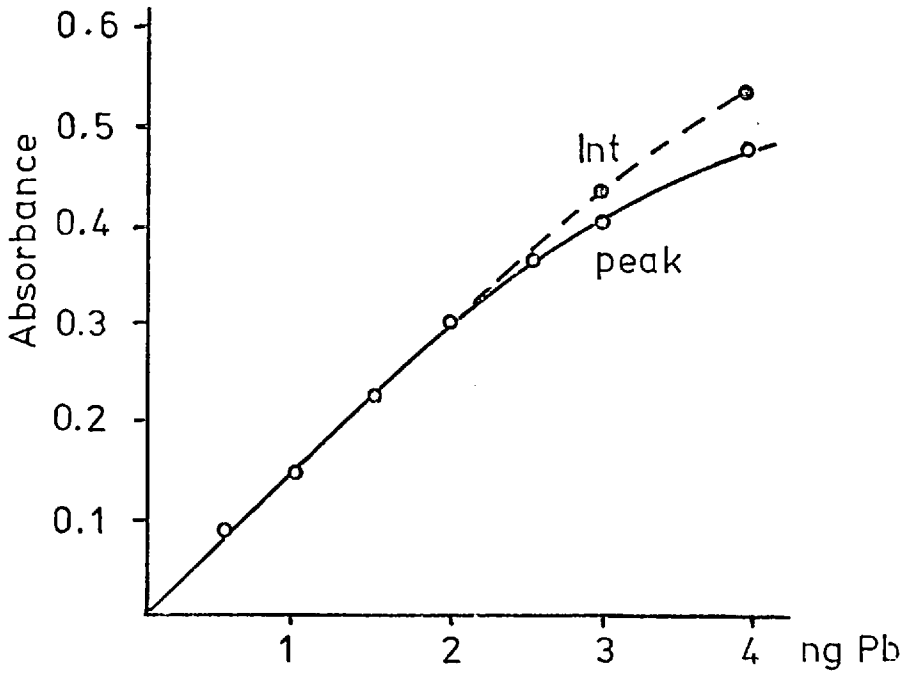
This integral reduces to: $Q_N = N_o . T_2$, with the proviso that $T_3 \geqslant T_1 + 4T_2$. Thus Q_N is independent of T_1 , the rate and duration of sample transfer to the analysis volume.

Katskov and L'vov (255) have shown that the integral is also independent of the time constant of the recording system. Thus measurements of Q_N will be less affected by both physical interferences which cause changes in the rate of atomization (i.e. matrix effects), and by changes in the signal half-width resulting from changes in the analyte concentration. By its nature a transient signal is liable to variation in its absorption profile and measurement of peak-area should be more precise than that of peak-height - though it is necessary for T_2 to remain constant, and for complete vaporization of the analyte to occur.

Interferences causing a change in the total number of atoms will obviously affect both the peak-area and peak-height measurements. The sensitivity of the peak-height method is highest when $T_2/T_1 \gg 1$, and in the peak-area method the magnitude of T_2 will govern the sensitivity.

Many workers in the field of electrothermal atomization applied to atomic absorption have been content to construct their calibration graphs by plotting peak absorbance vs. weight of analyte, due no doubt to the extreme difficulty of determining peak-area with an oscilloscope, or the tediousness of measuring peak-area on the paper output from a chart recorder. In fact, when a filament atomizer is used for the pulse vaporization of samples, the peak method of signal measurement is preferable for many elements. This is a consequence of the theory outlined above, and is due to the properties of a filament atomizer: open nature, rapid temperature rise and short residence time. The filament actually functions as a furnace and not as an isothermal cuvette (516). In the determination on the filament of the more refractory elements, such as molybdenum and vanadium, it will become advantageous to use the integration method (516).

It can be predicted that greater advantages will ensue with the use of signal integration when an isothermal cuvette is employed, such as the L'vov furnace and variations of the Massmann furnace. Mechanical methods of signal integration involve manipulation of the peak once it has been recorded on the chart paper; these methods include triangulation, cutting-out and weighing and measuring the product of peak height and width at half the height (although this will only be strictly valid for symmetrical peaks). Electronic integration of the signal is the method of choice, however, and various integration modules are available; the latest atomic absorption instrumentation incorporates peak integration facilities (74). In this study, the only feasible method for determining integrated absorbance was to cut-out and weigh the chart paper peaks. Integrated absorbance calibration graphs were plotted in this fashion, and compared with the corresponding peak absorbance calibrations (Figure 45). Integration gave some increase in the linear range.



N.B. Integrated absorbance plotted in arbitrary units to give initial slope equal to peak abs. plot

Fig. 45 Comparison of calibration graphs using peak and integrated absorbance

Several workers have recently employed signal integration in conjunction with graphite tube atomization atomic absorption spectrometry, and have reported both increased linearity of the calibration graphs for some elements (460, 516) and improved precision (332, 414). Improvements in precision and freedom from signal shape by the use of integrated absorbance have also been reported for the flame microboat technique (11). With the exceptions of the papers by Chakrabarti and co-workers (515, 516) and by L'vov and co-workers (49, 328), the data reported on integrated absorbance are, unfortunately, in arbitrary units, and not readily amenable to quantitative comparisons. It may be concluded, however, that the use of integrated absorbance increases the linear portion of the calibration, the increase being most noticeable for the more volatile elements, i.e. zinc and cadmium as compared to molybdenum and vanadium.

The use of peak absorbance rather than integrated absorbance is thus a contributory factor to the observed bending of the electrothermal atomization calibration graphs, but since even the integrated absorbance calibrations curve at lower absorbance values than corresponding flame calibrations, and the increased curvature is observed with filament atomizers (Figure 42) where theoretical considerations show that peak absorbance will give a true representation of atom concentration, it is possible that some other factor is involved.

7.3 Chopping Frequency

The spectral source in most commercial atomic absorption spectrometers is modulated at 50 Hz, the frequency of the British electrical supply. In the Perkin-Elmer Model 300S, a typical single beam instrument employing background correction, modulation is achieved by mechanical chopping of the source signal. The chopper has three sectors: one

blank which allows the light beam from the spectral source to pass through, one mirrored to reflect the light from the deuterium arc (Figure 14), and one blackened as a zero reference. Consequently, the actual absorption signal in the graphite furnace is viewed for 6.67 ms every 20 ms, and five sampling intervals occur in every 100 ms; Figure 46 shows these sampling intervals superimposed on the relatively fast lead signal obtained at 2700°C (adapted from reference 33).

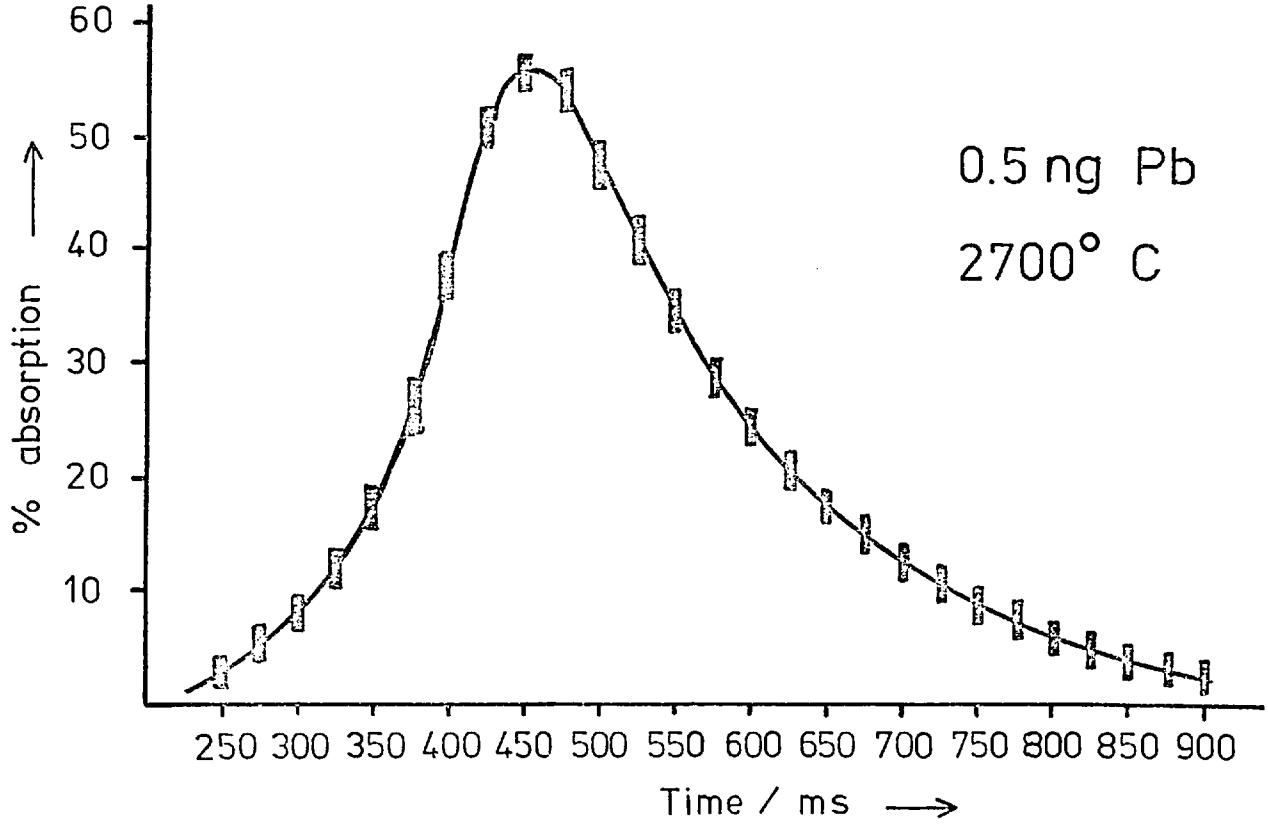


Fig. 46 Sampling of pulse atomization absorption signal

Alder (7) has discussed the measurement of short duration signals with a modulated system. If the gate time is **t** and the duration of the light signal is **T** , he calculated that a value of **T/t** of approximately 40 would give acceptable reproducibility of measurement. If this calculation is applied to the present case, then signals of duration greater than ~ 270 ms will be reproduced to an acceptable degree of accuracy. Table 29 gives signal

durations for the HGA-74 (33). Since even a mercury signal is longer than 270 ms, a chopping frequency of 50 Hz will be adequate for processing signals from the HGA-74.

Table 29 Typical Signal Durations for the HGA-74

Element	Time
Hg	600 ms
Pb	800 ms
Cd	1.40 s
Cu	2.20 s
As	2.20 s
V	8.0 s

For other flameless systems, however, where the signal duration is much shorter (such as the graphite filament), it will be advantageous to increase the chopping frequency in proportion to the decreased signal time, though a consequence of short signal duration will be shorter times available for averaging out the various sources of error.

7.4. Response Time of Electronics

The Perkin-Elmer Model 300S is a conventional atomic absorption spectrometer, designed to follow the relatively steady signals which arise from the use of flame atomization. Considerable electronic manipulation of the signal takes place before it is finally displayed. It was considered that some delay to the signal might be taking place in the electronics, resulting in a change in the shape of the calibration graph. A calibration graph obtained in the usual manner, by measuring peak heights on a chart recorder display of the absorption signal (the ~ 0.25 s response time of the recorder was considered adequate for this purpose) was compared with a calibration graph obtained by measuring the signal size at the photomultiplier tube. For this

purpose an oscilloscope was employed to monitor the output across the ballast resistor to the photomultiplier tube.

The calibration graphs are given in Figure 47, and it can be concluded that the internal response time of the instrument is adequate. In fact any dependence of the absorption signal on the response time could be eliminated by measuring integrated absorbance.

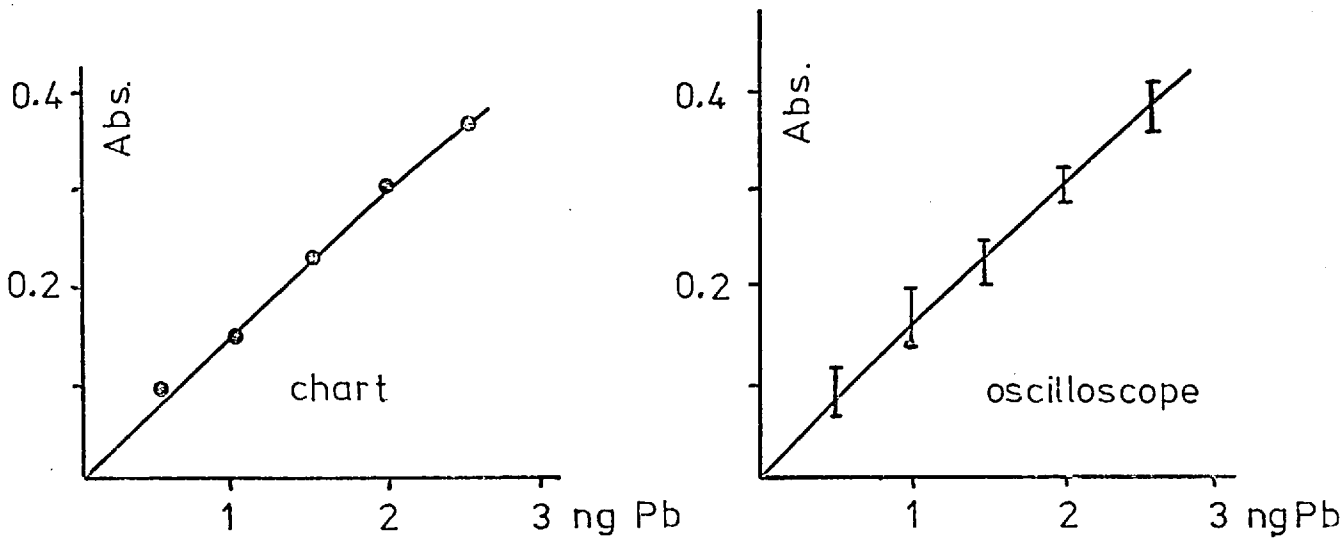


Fig. 47 Comparison of calibration graphs using chart recorder and oscilloscope signal presentation

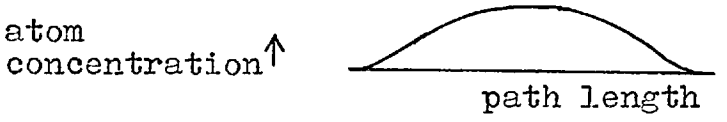
7.5 Inhomogeneities of the Temperature and Spatial Distributions of the Absorbing Atoms

Kilroe-Smith (258) has stated that non-linear calibrations from electrothermal atomizers are caused by a breakdown of the Beer-Lambert law, due to an unequal distribution of analyte atoms in the atom cell. He pointed out that the Beer-Lambert law:

$$A = k \cdot l \cdot c \quad [7]$$

where k = constant
l = path length
c = concentration of absorbing species

assumes that $\frac{dc}{dl} = 0$, i.e. a constant concentration of atoms along the length of the atom cell is required for the Beer-Lambert law to be valid, whereas the actual situation in an electrothermal atomizer might be:



A strict derivation of the Beer-Lambert law shows that the absorbance is proportional to the total number of absorbing species, and should be independent of their distribution in a fixed volume. In any case, at the time of peak absorbance in atomic absorption with electrothermal atomization, it is likely that the sample atoms are distributed evenly throughout the volume of the graphite tube.

7.6 Other Factors Arising in the Instrumental System

Various spectral interferences may occur in atomic absorption spectrometry: more than one absorbing line may be viewed by a given spectral band pass, a non-absorbed line may be emitted by the excitation source, spectral overlap may occur (384), and various other factors such as molecular absorption and scattering of radiation may be present. Most of these interferences will have equal effect on the measured atomic absorption whether a flame or a non-flame device is employed for atomization, unless the width of the absorption profile differs markedly between these two atom cells. An estimate of the size of the absorption line widths for a graphite tube atomizer is made in section 7.8.2: the line widths are of the same order of magnitude as absorption line widths in a flame.

Modulation of the spectral source will eliminate the possibility of emission by the absorbing medium affecting the measured signal.

7.7 Hyperfine Structure of the Spectral Line

The effect of hyperfine structure of the source emission line has been examined by Rubeška and Svoboda (448), but it can be predicted that this factor would affect equally the results from flame or electrothermal atomization atomic absorption.

7.8 Ratio of Absorption and Emission Line Widths

7.8.1 Introduction

Winefordner (579) has pointed out that in atomic absorption spectrometry a linear relationship between the observed absorbance and the concentration of analyte will

only result if the source line width is very narrow compared to the absorption line width. This point has been discussed in greater detail by Shimazu and Hashimoto (458) and by Rubeška and Svoboda (448). The latter have computed the shapes of analytical curves for different ratios of emission and absorption line widths: Figure 48.

It has been shown by L'vov (317) and Cantle and Mullins (74) - Figure 49 - that the linearity of calibration graphs in electrothermal atomization atomic absorption may be improved by pressurization of the inert gas in the cuvette. Increased pressure causes increased Lorentzian half-width of the absorption line, and a consequent higher ratio of absorption half-width to emission half-width.

7.8.2 The Absorption Line Width

If the absorption line from an electrothermal atomizer is narrower than that from a flame cell, it is possible that this is a contributory factor to the curvature of calibration graphs. Although direct measurements of absorption half-intensity widths have been made interferometrically in flames (64, 269), to date there have been no direct measurements by this method of the absorption line widths in a furnace atomizer. In 1940 King and Stockbarger (261) reported the measurement of absorption **f**-values using the King furnace (260). In this device a static population of atoms is maintained (as opposed to the transient populations produced in the modern electrothermal atomizers), and King and Stockbarger found that the equivalent widths of the absorption lines lay in the range 0.0002-0.0006 nm.

In order to estimate the size of absorption half-widths produced in electrothermal atomizers, it is necessary to resort to calculations from first principles and to consider the relative importance of those processes which contribute to the formation of an absorption profile.

It is well known (64, 361, 530) that several processes may contribute to the formation of an absorption line:

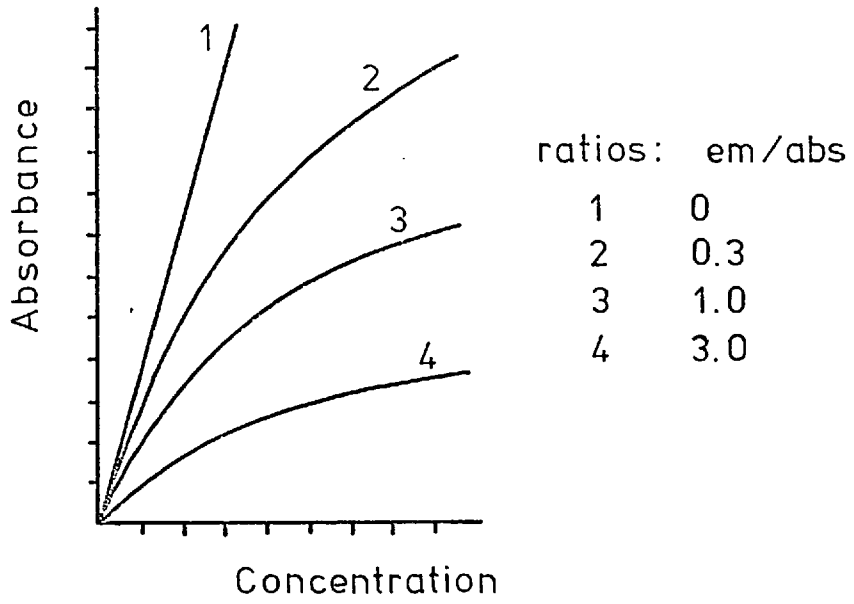
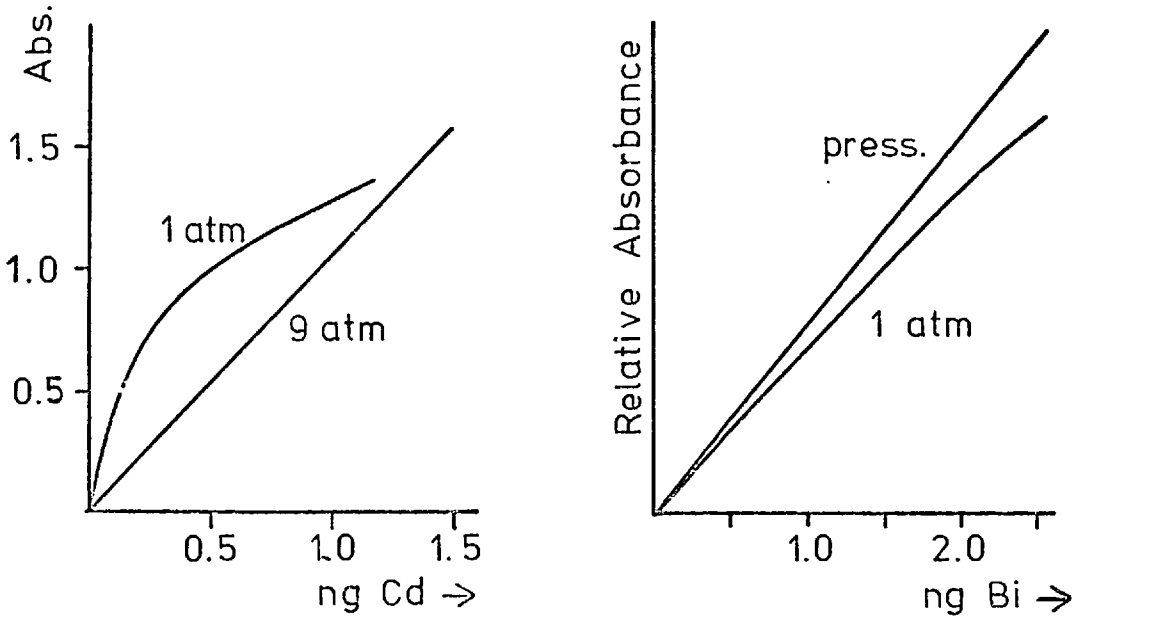


Fig. 48 Calculated analytical curves for different half-width ratios



[a] L'vov (317) [b] Cantle and Mullins (74)

Fig. 49 Calibration graphs at different inert gas pressures

(a) Natural Broadening

A spectral line has a finite half-intensity width which may be calculated by the Heisenberg Uncertainty Principle from the mean lifetime (τ) of the excited state. The line-broadening has a Lorentzian frequency distribution of the form:

$$I(\nu) = I_0 / \left[1 + \left[2(\nu-\nu_0)/\Delta\nu_N\right]^2\right] \quad [8]$$

where the half-intensity width, $\Delta\nu_N$, is given by:

$$\Delta\nu_N = 1 / 2\pi\tau \quad [9]$$

It is possible to calculate τ if the oscillator strength, f , is known, since:

$$\tau f = \frac{m c}{8\pi^2 e^2} \frac{g_2}{g_1} \lambda_0^2 \quad [10]$$

Where g_1 & g_2 are the statistical weights of the normal and excited states. respectively, and λ_0 is the wavelength corresponding to ν_0

Table 31 gives the values of $\Delta\nu_N$ calculated for some elements using the data in Table 30, and the following constants (84):

$$m = 9.1091 \times 10^{-28} g$$
$$c = 2.997925 \times 10^{10} \text{ cm s}^{-2}$$
$$e = 4.803 \times 10^{-10} \text{ e.s.u.}$$
$$\pi = 3.141593$$

(b) Doppler Broadening

Doppler broadening of a spectral line is the consequence of the emitting or absorbing atoms having a range of component velocities in the direction of observation. The distribution is Gaussian and is of the form:

$$I(\nu) = I_0 \cdot e^{-\left[(2(\nu-\nu_0)/\Delta\nu_D)\sqrt{\ln 2}\right]^2} \quad [11]$$

where the half-intensity width, $\Delta\nu_D$, is given by:

$$\Delta\nu_D = \left[2\sqrt{\ln 2} / c\right] \cdot \nu_0 \cdot \sqrt{2RT/M} \quad [12]$$

It is therefore straightforward to calculate Doppler half-intensity widths at any desired temperature - Table 31. The half-intensity widths in this table were calculated using the data from Table 30, and a selected temperature of 2800 K.

Table 30 Atomic and Spectral Data for Some Elements

Element	λ / nm	Transition	g_2/g_1	f	A
Al	309.27			0.23	26.98
Sb	217.59			0.04	121.75
As	193.70			0.095	74.92
Ba	553.56	$6\,^1S_0 - 6\,^1P_1$	3	1.4	137.34
Be	234.86	$2\,^1S_0 - 2\,^1P_1$	3	0.24	9.012
Bi	223.06			0.012	208.98
Cd	228.80	$5\,^1S_0 - 5\,^1P_1$	3	1.2	112.40
Ca	422.67	$4\,^1S_0 - 4\,^1P_1$	3	1.5	40.08
Cr	357.87			0.34	51.996
Co	240.72			0.22	58.93
Cu	324.75	$4^2S_{1/2} - 4^2P_{3/2}$	2	0.74	63.54
Ga	287.42	$4^2P_{1/2} - 4^2D_{3/2}$	2	0.32	69.72
Ge	265.16			0.84	72.59
Au	242.79	$6^2S_{1/2} - 6^2P_{3/2}$	2	0.3	196.966
In	303.97	$5^2P_{1/2} - 5^2D_{3/2}$	2	0.36	114.82
Fe	248.33			0.34	55.847
Pb	218.00			0.39	207.2
	283.31	$6^3P_0 - 7^3P_1$	3	0.21	207.2
Li	670.78	$2^2S_{1/2} - 2^2P_{3/2}$	2	0.7	6.94
Mg	285.21	$3\,^1S_0 - 3\,^1P_1$	3	1.2	24.305
Mn	279.48			0.58	54.938
Hg	253.65	$6\,^1S_0 - 6\,^3P_1$	3	0.03	200.59
Mo	313.26			0.20	95.94
Ni	232.00			0.095	58.71
Pt	265.94			0.12	195.09
Se	196.03			0.12	78.96
Ag	328.07	$5^2S_{1/2} - 5^2P_{3/2}$	2	0.51	107.87
Sr	460.73	$5\,^1S_0 - 5\,^1P_1$		1.54	87.62
Te	214.28			0.08	127.60
Tl	276.78	$6^2P_{1/2} - 6^2D_{3/2}$	2	0.27	204.37
Sn	286.33	$5^3P_0 - 6^3P_1$	3	0.23	118.69
Ti	365.35			0.22	47.90
U	358.49			2.1	238.03
Zn	213.86	$4\,^1S_0 - 4\,^1P_1$	3	1.2	65.38

Table 31 Natural and Doppler Half-Intensity Widths

Element	λ /nm	$\Delta\nu_N$/Hz	$\Delta\nu_D$/Hz
Ba	553.56	1.617×10^7	1.751×10^9
Be	234.86	1.540×10^7	1.612×10^{10}
Cd	228.8	6.357×10^7	4.684×10^9
Ca	422.67	2.971×10^7	4.246×10^9
Cu	324.75	3.724×10^7	4.389×10^9
Ga	287.42	3.374×10^7	3.374×10^9
In	303.97	2.823×10^7	2.350×10^9
Pb	283.31	9.258×10^6	2.786×10^9
Li	670.78	8.258×10^6	6.430×10^9
Mg	285.21	5.012×10^7	8.081×10^9
Ag	328.01	2.515×10^7	3.335×10^9
Sr	460.73	4.519×10^6	2.634×10^9
Tl	276.78	8.314×10^7	2.872×10^9
Zn	213.86	1.009×10^8	6.571×10^9

(c) Lorentz (Pressure) Broadening

Collision of an absorbing atom with other atoms or molecules of a foreign gas results in broadening, shift and asymmetry of the line profile. The earliest theory, due to Lorentz, gives an equation for evaluating the resultant half-intensity width, which is now known as the Lorentz half-intensity width $\Delta\nu_L$ (361):

$$\Delta\nu_L = \frac{1}{\pi} \text{(no. of broadening collisions per second per absorbing atom)}$$

$$= \frac{2}{\pi} \sigma_L^2 \; N \sqrt{2\pi RT\,(1/M_1 + 1/M_2)} \qquad [13]$$

where σ_L^2 = collisional cross section for Lorentz broadening of the atom concerned

N = no. of foreign gas species per unit volume

M_1, M_2 = atomic or molecular weights of the colliding species.

In calculating $\Delta\nu_L$ from equation [13] the limiting factor is finding a suitable value for the collisional cross section, which is usually an experimentally determined quantity. In their calculations of collisional half-widths

of some atomic spectral lines in common analytical flames, Parsons et al. (399) took two values to represent maximum and minimum collisional cross sections. For absorption lines originating from electrothermal atomizers, however, only element-argon or element-nitrogen interactions are relevant. Much experimental work has been carried out on the collisional broadening of spectral lines by neutral atoms, but this experimental work has mainly involved the alkali metals. In their review of the literature up to 1937, Ch'en and Takeo (86) found that, except for silver and mercury, all observations had been made on the absorption lines of alkali metal atoms. The review by Hindmarsh and Farr (211) of the post-1937 literature again shows that much of the experimental work involved the alkali metals - due to the more straightforward theoretical interpretation of the data from these elements. Atomic absorption spectrometry is not generally applied to the alkali metals, and data on those elements commonly determined by this technique are scarce.

Table 32 gives the result of an extensive literature search for collisional cross sections of elements of interest in atomic absorption spectrometry.

Table 32 Collisional Cross-Sections

Element	Temp/K	Line/nm	$\sigma^2 \times 10^{-14}$ cm^2				Ref.
			He	Ne	Ar	Kr	
Ga	1500	403.3	1.11	1.01	2.77		(404)
In	1300	410.2	1.20	1.26	3.21		(404)
Ca	850	422.673	0.68	0.74	1.79	2.45	(405)
Ca		422.673	0.78				(209)
Ca		422.673			1.90		(502)
Ca		657.3	0.76	0.86	1.20	1.77	(500)
Ca		657.3	0.65				(210)
Sr	750	460.733	0.75	0.85	2.21	2.69	(405)
Ba	940	553.548	0.77	0.76	2.00	2.68	(405)
Mg		285.213			1.90		(502)

If the data in this table are applied to the model situation of atomization at 2800K in a graphite tube, under "gas-stop" conditions of argon, the collisional half-intensity widths may be calculated - Table 33. The concentration of argon atoms is calculated by assuming the tube to be full of argon at one atmosphere pressure:

$$N = \frac{6.02252 \times 10^{23} \times 273.16}{22.415 \times 1000 \times 2800} \ cm^{-3} \quad [14]$$

Table 33 Collisional Half-Intensity Widths (Element + Argon)

Element	Line/nm	$\Delta\nu_L$/Hz
Ba	553.548	6.33×10^9
Ca	422.673	8.25×10^9
Ga	403.3	1.10×10^{10}
In	410.2	1.18×10^{10}
Mg	285.213	9.78×10^9
Sr	460.733	8.45×10^9

The more modern collision theory, due to Weisskopf and Lindholm, gives a more satisfactory explanation of the broadening process since it actually predicts line shift and asymmetry as well as giving estimates of the half-width (36, 58, 211). The theory assumes a general attractive interaction denoted as $-C_p / r^p$ where p is an integer. For a radiating particle in a gaseous medium the most important interaction is the van der Waals interaction between uncharged particles, and the earlier workers took this interaction viz. $-C_6/r^6$ as the basis for their theories. The theory predicts that:

$$\Delta\nu_L = 1.30 \ C_6^{0.4} \ v^{0.6} \ N \quad [15]$$

or $$\Delta\nu_L = 8.16 \ (C_6/\hbar)^{0.4} \ v^{0.6} \ N \quad [16]$$

and shift $$\beta = -2.96 \ (C_6/\hbar)^{0.4} \ v^{0.6} \ N \quad [17]$$

where: C_6 is the van der Waals constant for the interaction between particles

N is the concentration of perturbing particles

and v is the relative velocity of the particles, and equals $\sqrt{\frac{8RT}{\pi} \ 1/M_1 + 1/M_2}$ [18]

Dimensional analysis shows the units of C_6 to be $cm^6\ Hz^{-1}$.

It is possible to estimate C_{max} and C_{min} for a given interaction in terms of a_1, the polarizability of an excited atom, and a_2, the polarizability of a normal foreign molecule (113). Alternatively, in considering only the polarizability of the perturber (530):

$$C_6 \sim a.p^2/\varepsilon^2 \qquad [19]$$

where p = electron co-ordinate,

it is possible to estimate the magnitude of C_6. Atomic polarizabilities in the visible region are of the order of 2×10^{-24} (530), and taking p to be $\sim \varepsilon.3a_o$ - where a_o is a typical atomic radius - gives a value of C_6 of $10^{-58}\ cm^6\ Hz^{-1}$. Direct calculation of van der Waals interactions from quantum theory is possible (104).

In fact, experimental C_6 values exceed the theoretical ones by factors of about two or three (211), which has led some workers to examine (12, 6) potentials or even (12, 8, 6) potentials: use of these gives an improvement in the theoretical values of C_6, though the calculations are necessarily more complex.

(d) Broadening Due to Quenching Collisions

Collisional de-excitation and a consequent reduction in the lifetime of the excited state of a resonance line may occur if foreign gas molecules have vibrational levels very close to the resonance line excited state. The resulting line broadening has a Lorentzian frequency distribution, and a half-intensity width given by an expression similar to that for Lorentz pressure broadening (equation [13]). In quenching collisions transfer of energy must occur between the valence electron of the excited atom and the rare gas atom, and it can be expected that these collisions will not contribute much to the absorption line width if argon is present in the graphite furnace, though the effect will be larger for nitrogen. Calculated half-intensity widths

are given in Table 34, together with the experimentally determined values of the quenching cross-section (268). Conditions were taken to be 2800K and with the graphite tube full of inert gas.

Table 34 Quenching Half-Intensity Widths

Element	Temp/K	Line/nm	Argon		Nitrogen	
			σ_Q^2/cm^2	$\Delta\nu_Q$/Hz	σ_Q^2/cm^2	$\Delta\nu_Q$/Hz
Li	1400	670.78	$\leq 0.3 \times 10^{-16}$	$\leq 4.71 \times 10^5$	6.8×10^{-16}	1.10×10^7
Tl	1400	204.37	$\leq 0.1 \times 10^{-16}$	$\leq 6.60 \times 10^4$	6.4×10^{-16}	4.92×10^5
Pb	1400	207.2	$\simeq 0.0$	-	5.7×10^{-16}	4.38×10^6

(e) Holtzmark (Resonance) Broadening

This is due to collisions (or strong exchange-type interactions) of the absorbing atoms with other absorbing atoms of the same kind. The frequency distribution is Lorentzian, and the half-intensity width is given by:

$$\Delta\nu_H = \frac{4}{\pi}\ \sigma_H^2\ N \sqrt{\frac{\pi R T}{M}} \qquad [20]$$

Again, the effective cross-section, σ_H^2, has to be determined experimentally. Table 35 gives two typical values, and the calculated half-intensity width for 1 ng of the analyte element in a graphite tube at 2800K.

Table 35 Holtzmark Half-Intensity Widths

Element	Line/nm	σ_H^2/cm^2	$\Delta\nu_H$/Hz	Reference
In	410.2	5.80×10^{-13}	1.09×10^6	(404)
Ca	422.673	2.91×10^{-12}	2.66×10^7	(406)

(f) Stark Broadening

This is caused by the effect of an electric field on an absorbing atom, but it may be neglected in considerations of broadening processes occurring in a graphite tube atomizer.

(g) Self-absorption Broadening

This is an important factor under conditions of high optical density; it is considered in greater detail in section 7.9.

A compilation of calculated half-intensity widths for natural, Doppler, Lorentz, quenching and Holtzmark broadening for some elements is given in Table 36. Where necessary, "gas stop" conditions with argon as inert gas and a temperature of 2800K in the graphite tube are assumed. It can be seen that the Doppler and Lorentz effects will give the greatest contributions to the resultant line width, and it is possible to estimate this total width by convoluting the Dopper and Lorentz half-intensity widths (606):

$$\Delta\nu_T \simeq \left(\frac{\Delta\nu_L}{2}\right) + \left[\left(\frac{\Delta\nu_L}{2}\right)^2 + (\Delta\nu_D)^2\right]^{1/2} \quad [21]$$

The simpler equation:

$$\Delta\nu_T \simeq \left[\Delta\nu_L^2 + \Delta\nu_D^2\right]^{1/2} \quad [22]$$

gives values for $\Delta\nu_T$ which are approximately 4% lower than the values obtained by applying equation [21] .

Table 37 lists the calculated values of $\Delta\nu_T$ for various elements in the furnace and, for comparison, values of $\Delta\nu_T$ for flame atomization. Values of **a** (the natural damping ratio = $\Delta\nu_N\sqrt{\ln 2}\,/\Delta\nu_D$) are also listed.

King and Stockbarger (261) found that the equivalent widths of absorption lines in the King furnace (260) lay in the range 0.0002 - 0.0006 nm. If these values are converted to frequencies, they give the range 4×10^8 - 5×10^9 Hz [for wavelengths 200 - 400 nm, using the expression $\Delta\nu_D = (c/\lambda_o^2)\,\Delta\lambda_D$]. Recently, Koizumi and Yasuda (607), in calculating the magnetic fields necessary to effect Zeeman splitting of spectral lines, took an average value of 60×10^{-16} cm^2 for the effective cross section for Lorentz broadening, and calculated total half-intensity widths. They found a value of 3.5×10^9 Hz for the cadmium 228.8 nm

Table 36 Calculated Half-Intensity Widths

Element	Line/nm	$\Delta\nu_N$/Hz	$\Delta\nu_D$/Hz	$\Delta\nu_L$/Hz	$\Delta\nu_H$/Hz	$\Delta\nu_Q$/Hz
Ba	553.548	1.62×10^7	1.751×10^9	6.33×10^9		
Ca	422.673	2.97×10^7	4.246×10^9	8.25×10^9	2.66×10^7	
Cu	324.75	3.72×10^7	4.389×10^9			
Ga	403.3	3.37×10^7	3.374×10^9	1.10×10^{10}		
In	410.2	2.82×10^7	2.350×10^9	1.18×10^{10}	1.09×10^6	
Li	670.78	8.26×10^6	6.430×10^9			4.71×10^5
Mg	285.21	5.01×10^7	8.081×10^9	9.78×10^9		
Ag	328.07	2.52×10^7	3.335×10^9			
Sr	460.733	4.52×10^6	2.634×10^9	8.45×10^9		
Zn	213.86	1.01×10^8	6.571×10^9			
Tl	276.78	8.314×10^7	2.872×10^9			6.60×10^4

Table 37 Total Absorption Line Widths in Furnace and Flame Atomizers

Element	Line/nm	Furnace			Flame			
		$\Delta\nu_T$/Hz	a	Temp/K	$\Delta\nu_T$/Hz	a	Temp./K	Reference
Ba	553.548	6.78×10^9	3.01	2800	4.07×10^9	2.0	2270	556
					2.35×10^9	0.83	2500	217
					2.47×10^9	0.84	2760	217
Ca	422.673	1.00×10^{10}	1.62	2800	5.00×10^9	0.62	2500	217
					5.10×10^9	0.57	2760	217
					5.14×10^9	0.59	2730	331
Cu	324.75				4.55×10^9	0.46	2500	214
					5.14×10^9	0.56	2730	331
Ga	403.3	1.19×10^{10}	0.27	2800				
In	410.2	1.23×10^{10}	0.42	2800				
Li	670.78				7.40×10^9	0.58	2500	217
Mg	285.21	1.43×10^{10}	1.01	2800	8.69×10^9	0.32	2730	331
Ag	328.07				5.07×10^9	0.95	2730	331
Sr	460.733	9.20×10^9	2.67	2800				
Zn	213.86				6.92×10^9	0.28	2730	331

line, and 3.9×10^9 Hz for the lead 283.3 nm line. Damping constants were calculated to be 0.54 for the cadmium line and 0.84 for the lead line. The results of King and Stockbarger (261) and Koizumi and Yasuda (607) are in satisfactory agreement with the calculated values given in Table 37.

Table 37 shows that the half-intensity widths of absorption lines in a furnace are of the same order of magnitude as those in a flame, and the postulate that calibration graph bending is due to narrow absorption lines in the furnace must be invalid.

7.8.3 The Source Emission Line Widths

If hollow cathode lamps are run at relatively high operating currents, then self-absorption of the emission line by the atoms in front of the hollow cathode may take place, with consequent broadening of the line profile (44). Commercial atomic absorption instruments, especially those with a large number of mirrors and reflecting surfaces, need fairly high operating currents of the hollow cathode lamp to achieve a satisfactory through-put of light. None of the lamps used in this study was run at a current greater than the manufacturer's recommended maximum, but the possibility that self-absorption in the source was taking place, with resultant effect on the calibration graphs (561), was examined: Figure 50. Although some decrease in sensitivity was observed with higher operating currents, the linear range of the calibrations remained approximately constant.

7.8.4 Contribution by the Deuterium Arc

In plotting the calibration graphs in Figure 50 no background correction was employed, and it was observed that the graphs were less curved than when background correction was employed - Figure 51. These graphs show the typical shapes of growth curves for atomic absorption with line and continuum sources (599): Figure 52.

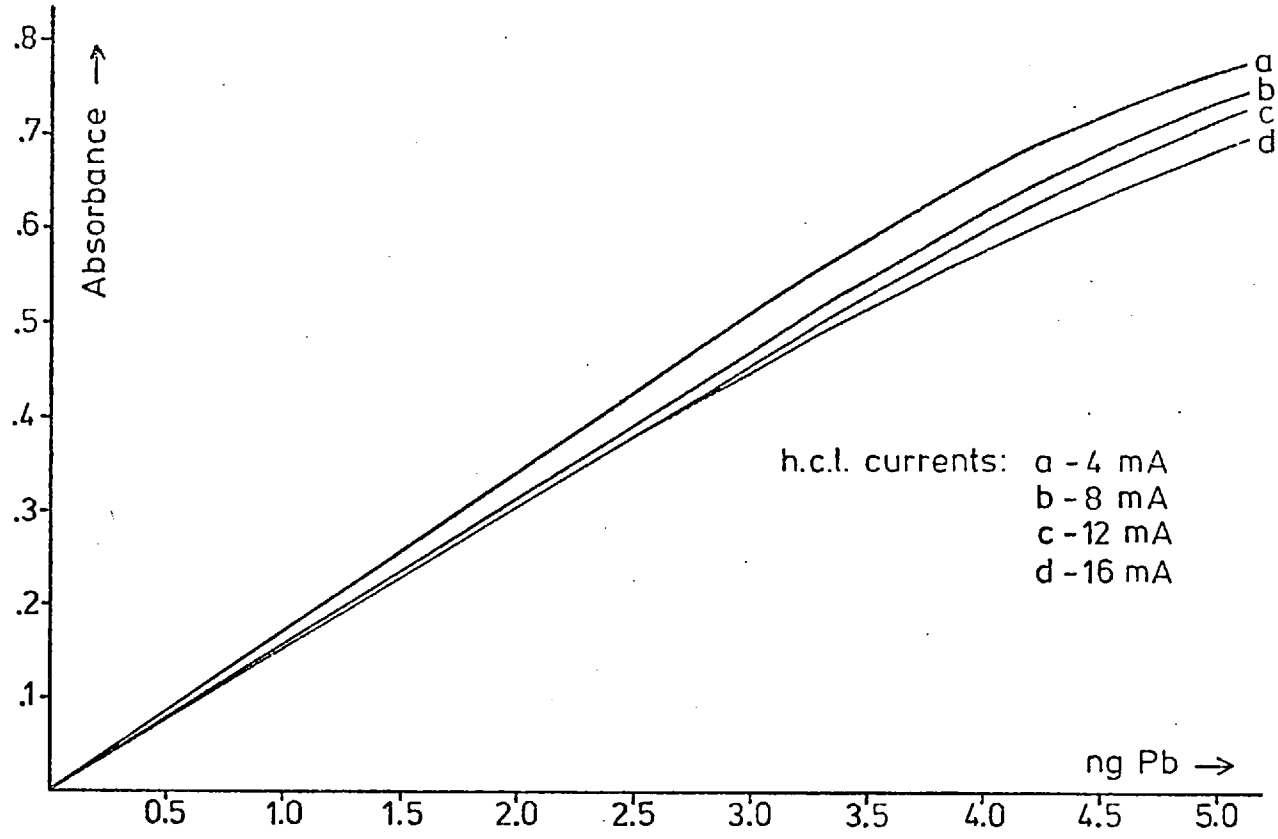


Fig. 50 Calibration graphs at different hollow cathode lamp currents

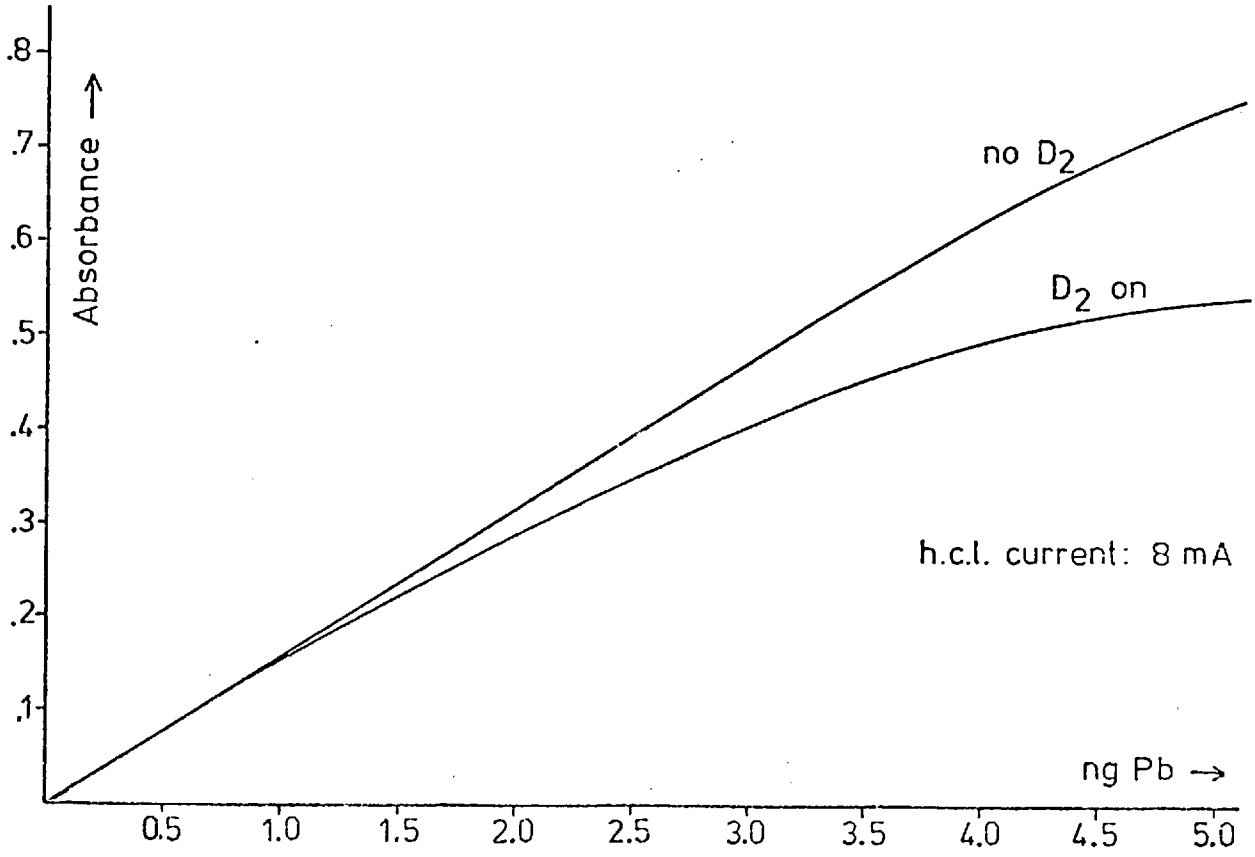


Fig. 51 Calibration graphs with and without deuterium arc background correction

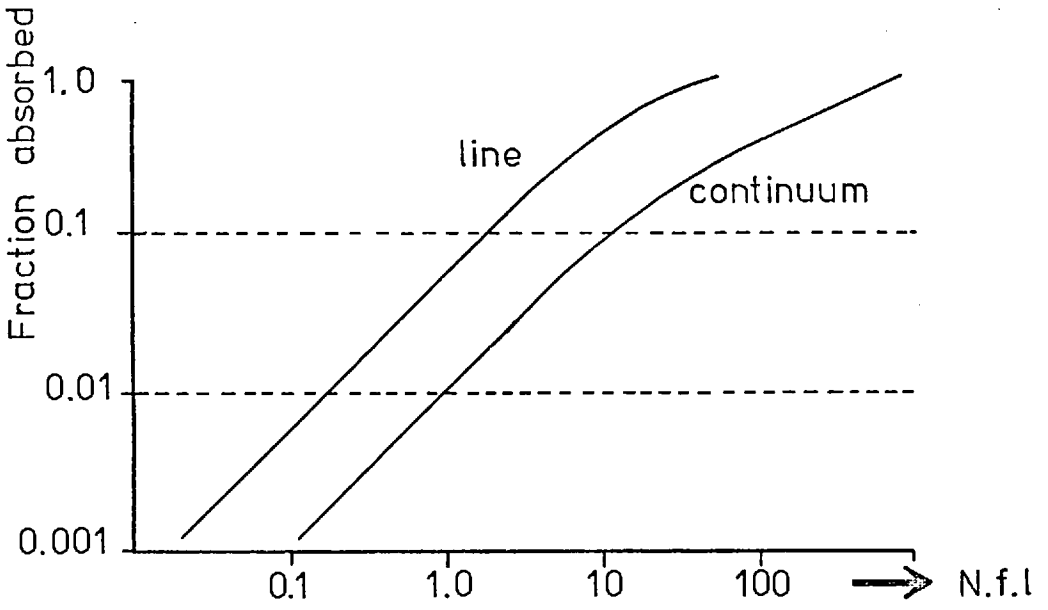


Figure 52 Representative growth curves for atomic absorption with line and continuum sources

In effect, when background correction was employed, the detector was monitoring the atomic absorption not with the desired narrow line from the hollow cathode lamp, but with a hollow cathode lamp spectral line broadened by continuum radiation from the deuterium arc. If a wide source line is employed for evaluating $\mathbf{A_T}$, the total absorption factor, then it can be calculated (268) that at low optical densities $\mathbf{A_T}$ is proportioned to the concentration of absorbing atoms, while at high optical densities $\mathbf{A_T}$ is proportional to the square root of the concentration of absorbing atoms. Figure 53 gives calibration graphs for copper and lead, plotted with both absorbance vs. mass of analyte and absorbance vs. $(\text{mass of analyte})^{\frac{1}{2}}$. The square root

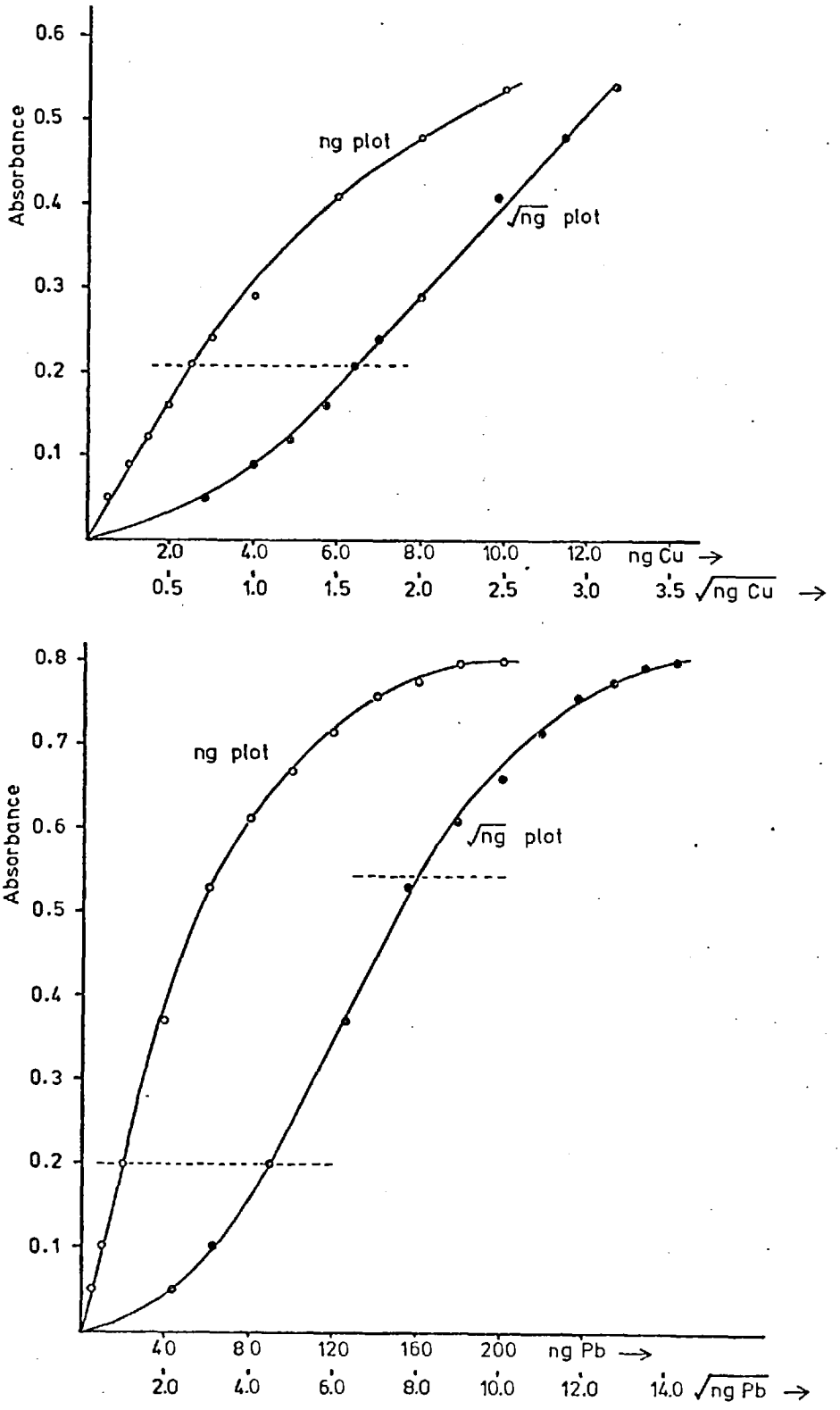


Fig. 53 Calibration graphs for copper and lead

relationship at higher analyte masses i.e. higher optical densities is clear, and is further illustrated in Figure 54 which is a log/log plot of absorbance and mass of analyte for copper.

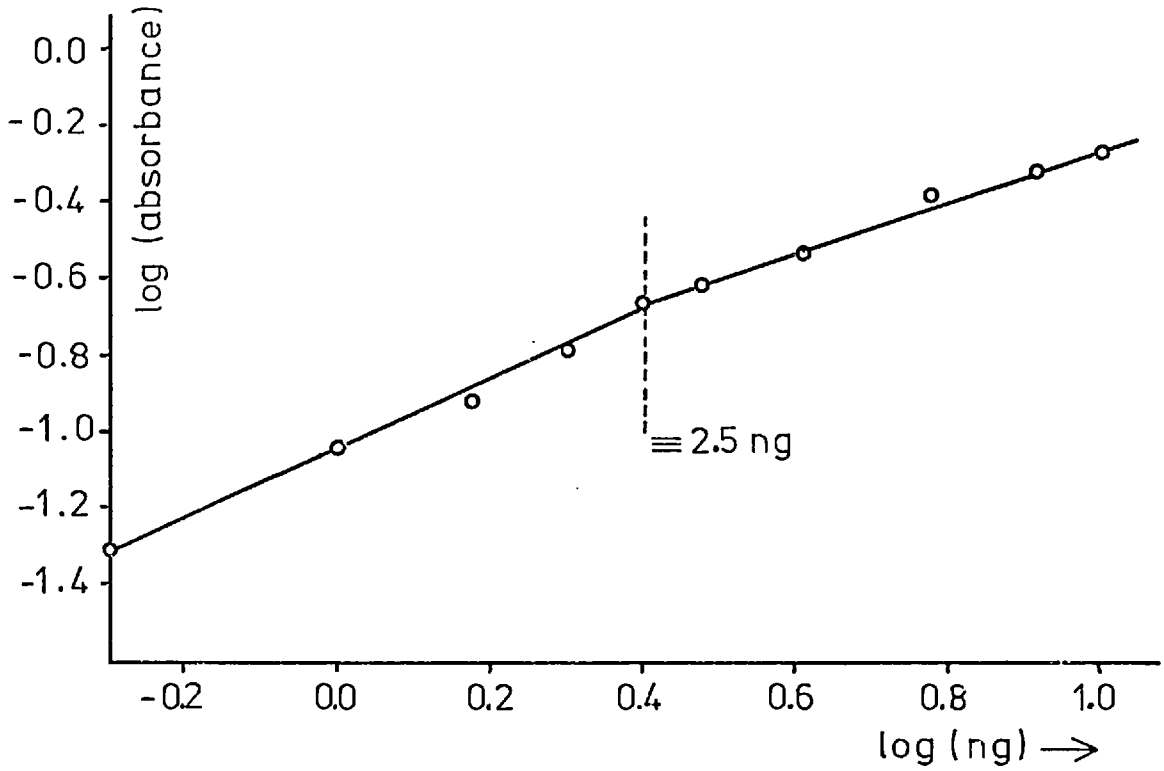


Fig. 54 Log / log plot of absorbance and mass of analyte for copper

7.9 Resonance Line Broadening and Shift in the Absorbing Medium

7.9.1. Introduction

Radiation of a given frequency passing through an absorbing medium is absorbed according to the equation:

$$I_\nu = I_\nu^o \; e^{-k_\nu l} \qquad [1]$$

where I_ν^o & I_ν are initial and final intensities of the radiation of frequency ν

k_ν = absorption coefficient

l = effective path length

k_ν is related to k_o , the peak absorption coefficient when Doppler broadening alone is present, by the equation:

$$k_\nu = k_o \; e^{-\left[\frac{2(\nu-\nu_o)}{\Delta\nu_D} \sqrt{\ln 2} \right]^2} \qquad [23]$$

When both Lorentzian and Doppler broadening contribute to a line profile, they may be combined by means of a Voigt integral:

$$k_\nu = k_o \; \frac{a}{\pi} \int_{-\infty}^{\infty} \frac{e^{-y^2}}{a + (\omega - y)^2} \, dy \qquad [24]$$

where $\omega = \dfrac{(\nu-\nu_o)\sqrt{\ln 2}}{\Delta\nu_D}$ $(\nu-\nu_o)$ is the distance of a frequency band from the line centre

$y = \dfrac{2\delta}{\Delta\nu_D} \sqrt{\ln 2}$ δ is the frequency displacement from $(\nu-\nu_o)$

and $a = \dfrac{\Delta\nu_N + \Delta\nu_L}{\Delta\nu_D} \sqrt{\ln 2}$ a is the natural damping ratio.

The optical density of a medium is defined as:

optical density = $k_o . l$ [25]

It is an important quantity since the value of k_o is directly related to the concentration of absorbing atoms.

The effect of increasing optical density on I_ν / I_ν^o (i.e. $e^{- k_\nu l}$) curves for a given spectral line is shown in Figure 55; in calculating the data for this figure the simplifying assumption of minimal Lorentzian contribution was made. Since the quantity ω has been used - a dimensionless quantity relating the width and shape of a line to the Doppler half-width - the curves in Figure 55 will be typical of most elements. For increasing optical density the transmitted intensity decreases but the line half-intensity width remains approximately constant, up to a limiting value at which the absorption saturates, first at the centre of the line and then outwards towards the wings.

The shape of the spectral profile will be different when the Lorentzian contribution cannot be neglected, since a Lorentzian profile has much more prominent wings than a Doppler profile, and consequently a lower peak height for the same total area - Figure 56; in this figure the two curves have equal total areas and equal half-widths.

Figure 57 shows how increasing optical density affects the absorption line width for a pure collisional broadened line (220). The dashed curves describe how an equivalent emission line will be distorted at high atomic concentrations, due to radiative disequilibrium near the border of the atom cell (in this example, a flame) (13).

Most absorption line profiles will contain contributions from both Lorentzian and Doppler components; the relative importance of these contributions is measured by the ratio of their half-widths: a . Consideration of Figures 55 and 57

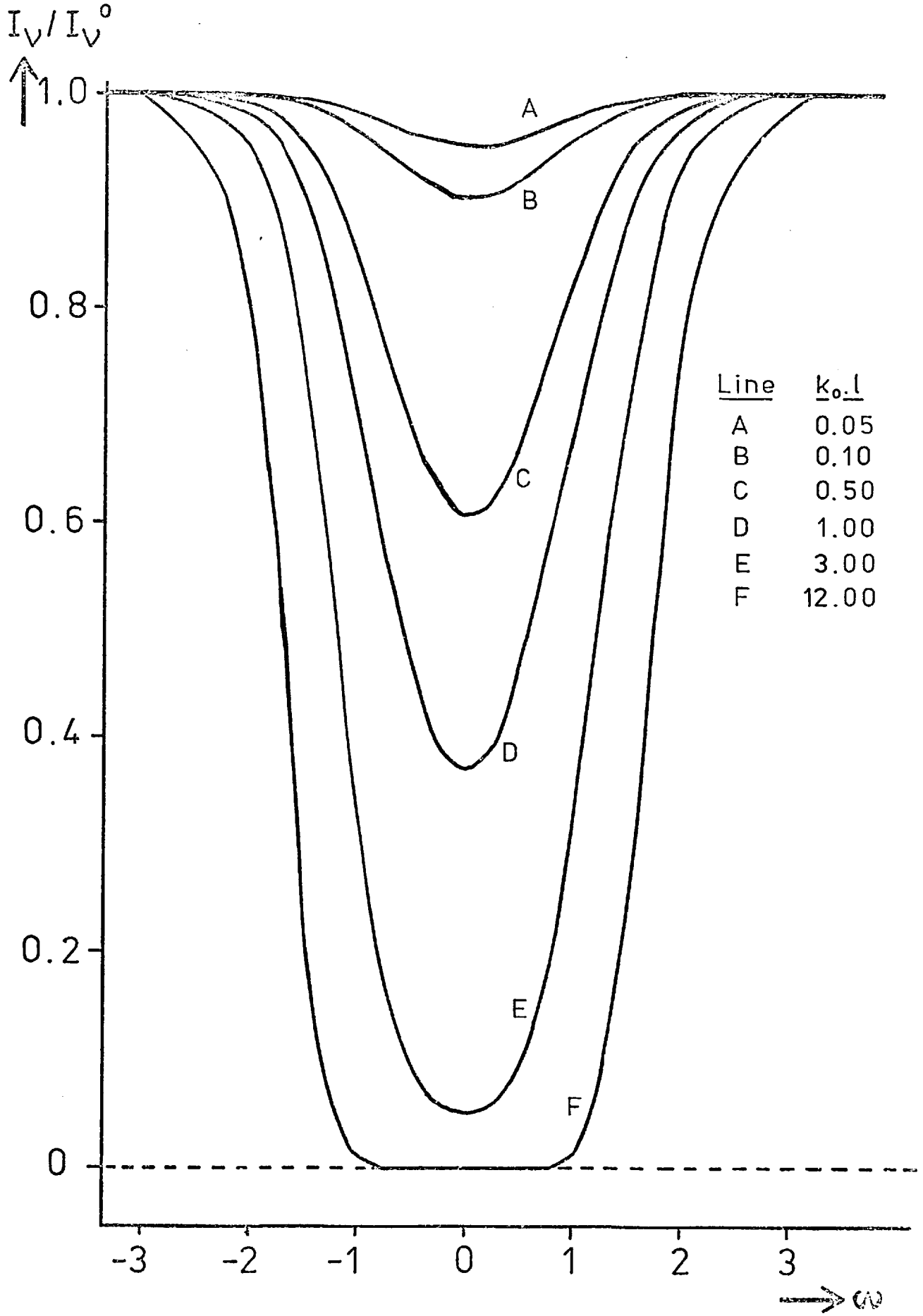


Figure 55 Variation of absorption line width with increasing optical density ($k_o.l$)

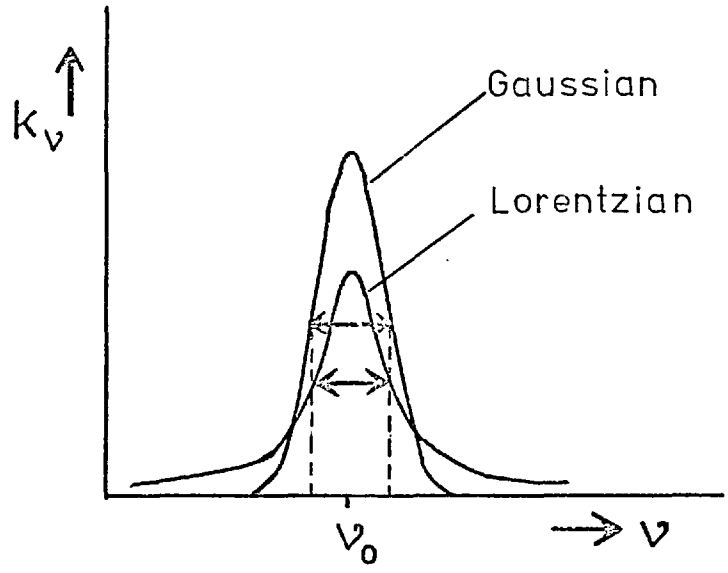


Figure 56 Comparison of Lorentzian and Doppler line profiles

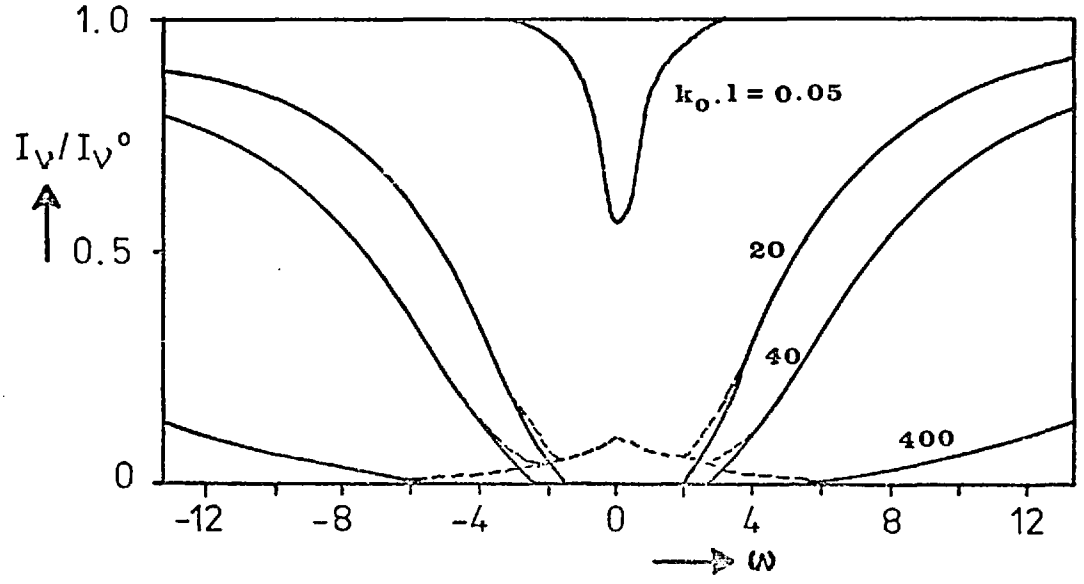


Figure 57 Variation of absorption line width with increasing optical density ($k_0.l$)

reveals that at low optical densities the absorption half-width is independent of atomic concentration, but increasing optical densities produce saturation of the absorption at the line centre followed by line broadening - due to the absorption factor increasing at the wings of the line. Large optical densities will thus increase the equivalent width of the line i.e. self-absorption broadening.

The equivalent width of a line is defined as:

$$W_\nu = \int (1 - e^{-k_\nu l})\, d\nu \qquad [26]$$

and since k_ν is proportional to $N.f$, W_ν is some function of $N.f.l$ and a plot of $\log W_\nu$ vs. $\log N.f.l$ is the curve of growth for the spectral line. Figure 58 shows a plot of $\log(W_\nu/\Delta\nu_D)$ - a quantity independent of line width - vs. $\log[(N.f.l/\pi\Delta\nu_D)\sqrt{\ln 2}]$ - N.B. this expression is equal to: $\log[10.6\, k_o l]$.

The three regions of Figure 58 are related to the optical thickness of the absorbing medium. Region A corresponds to optical thinness ($k_o l \ll 1$) and the curve of growth is a straight line. When the optically thin criterion is no longer applicable, W increases less rapidly

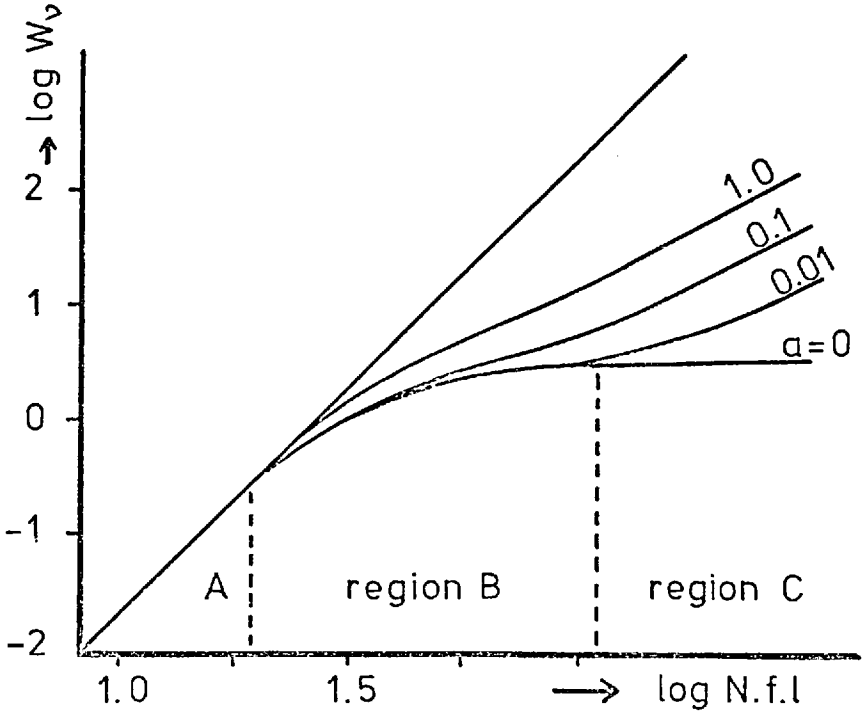


Fig. 58 Curves of growth for a spectral line at different damping ratios

than **N.f.1** and the curve flattens out: region B. Increasing **N.f.1** further results in the absorption occurring in the wings of the profile: region C. The absorption profiles corresponding to regions A-C are given in Figure 59.

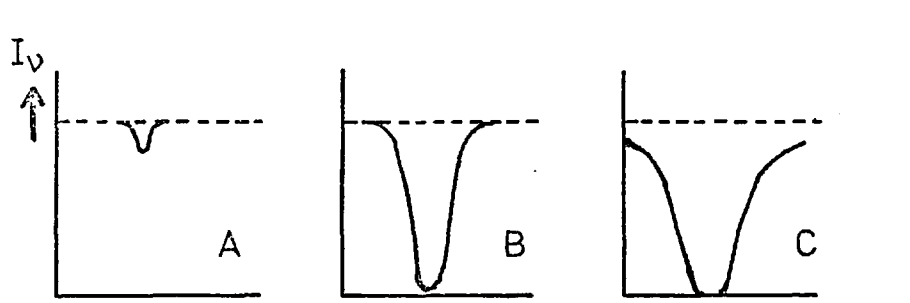


Figure 59 Absorption line profiles corresponding to regions A, B and C of the curve of growth (Fig. 58)

Figure 58 shows the relationship between total absorption and analyte concentration in the atom cell. In the Walsh (562) method of measuring atomic absorption, a narrow emission line is employed to monitor the absorption coefficient at the centre of the absorption line. The consequence of this is a linear relationship between absorbance and analyte concentration until a concentration limit is reached, above which there is progressive deviation from linearity. At very high optical density strong absorption occurs and the measured absorbance reaches a constant value independent of the analyte concentration (599) - Figure 52.

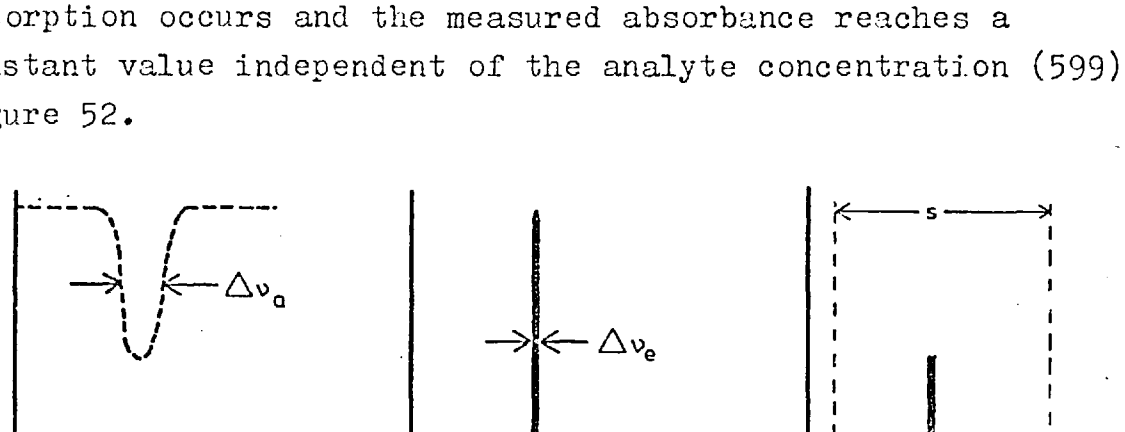


Fig. 60 The Walsh method of measuring Atomic Absorption

Typical values of optical density in flame and plasma atom cells are given in Table 38, taken from the work of Kirkbright and Ward (270) and recalculated using a plasma temperature of 5500 K. The values of $k_o \cdot l$ are all less than unity i.e. approximating to the optically thin condition.

Table 38 Typical Optical Densities

Element	N_2O-C_2H_2 flame 2800 K	Plasma 5500 K
Ca(I)	0.050	0.210
Ca(II)	0.0057	0.0509
Cu(I)	0.000562	0.0197
Zn(I)	0.00520	0.148

If conditions of high optical density are produced during atomization in a heated graphite tube (267) then it is possible that these conditions are a contributory cause of the typically small linear ranges and large curvatures of the growth curves from electrothermal atomizers.

7.9.2 Calculation of Optical Densities Occurring during Atomization in a Graphite Tube

The expression for k_o , the peak absorption coefficient for pure Doppler broadening, is well known (317, 361):

$$k_o = \frac{2}{\Delta\nu_D}\cdot\sqrt{\frac{\ln 2}{\pi}}\cdot\frac{\pi e^2}{mc}\cdot N.f \qquad [27]$$

and an expression for $\Delta\nu_D$, the Doppler half-width has been given previously:

$$\Delta\nu_D = \frac{2\sqrt{\ln 2}}{c}\cdot\nu_o\cdot\sqrt{\frac{2RT}{M}} \qquad [12]$$

Combination of these two equations gives:

$$k_o = \frac{\sqrt{\pi}\cdot e^2\cdot N\cdot f\cdot\sqrt{A}}{m\cdot\nu_o\cdot\sqrt{2RT}} \qquad [28]$$

and since $\nu_o = c / \lambda_o$, an expression for optical density, $k_o l$ is obtained:

$$k_o l = \frac{e^2}{mc}\cdot\sqrt{\frac{\pi}{2R}}\cdot f\cdot\lambda_o\cdot\sqrt{\frac{A}{T}}\cdot N\cdot l \qquad [29]$$

where: e = electronic charge (= 4.803×10^{-10} e.s.u)
m = mass of electron (= 9.1091×10^{-28} g)
c = velocity of light (= 2.997925×10^{10} cm s^{-1})
R = gas constant (= 8.3143×10^{7} erg.K^{-1} mol^{-1})

f = oscillator strength
λ_o = wavelength of line (cm)
A = atomic mass (g mol^{-1})
T = absolute temperature (K)
N = concentration of absorbing species (cm^{-3})
l = effective path length (cm)

Using constants expressed in the c.g.s. system, equation [29] reduces to:

$$k_o l = 1.1611 \times 10^{-6} \cdot f \cdot \lambda_o \sqrt{A/T} \cdot N \cdot l \quad [30]$$

In order to calculate optical densities occurring in graphite tube atomization it is necessary to estimate the effective path length and the volume of the atom cloud at atomization (in order to calculate the concentration of atoms present).

Assuming that the atom cloud fills the tube under "Gas Stop" conditions, then the volume of the atom cloud is given by $\pi . r^2 . l$ (r = radius and l = length of tube), and the concentration of atoms, N, by:

$$N = \frac{\text{mass analyte (ng)} \times 6.02252 \times 10^{23} \times 10^{-9}}{\text{atomic mass of analyte (A)} \times \pi . r^2 . l} \text{ cm}^{-3} \quad [31]$$

and the product $N.l$ is therefore:

$$N.l = \frac{\text{mass analyte (ng)} \times 6.02252 \times 10^{14}}{A \times \pi . r^2} \quad [32]$$

The value of $k_o l$ will be independent of the path length and depends only on the cross-sectional area of the tube (if the atom cloud fills the tube). This has been pointed out by L'vov (317).

Using equations [30] and [32], values of $k_o l$ were calculated for 0.1 ng quantities of 32 elements in the graphite tube (0.3 cm internal radius, 2.0 cm long) at

Table 39 Calculated Optical Densities for 0.1 ng Amounts of Various Elements in a Graphite Tube at 2800K

Element	λ/nm	$k_o l$
Aluminium	309.27	6.40
Antimony	217.59	0.37
Arsenic	193.70	74.46
Barium	553.56	30.91
Beryllium	234.86	8.78
Bismuth	223.06	0.09
Cadmium	228.80	12.11
Calcium	422.67	46.81
Chromium	357.87	7.89
Cobalt	240.72	3.22
Copper	324.75	14.09
Gallium	287.42	5.15
Germanium	265.16	12.22
Gold	242.79	2.43
Indium	303.97	4.77
Iron	248.33	5.28
Lead	217.00	2.75
	283.31	1.93
Lithium	670.78	83.32
Magnesium	285.21	32.45
Manganese	279.48	10.22
Mercury	253.65	0.25
Molybdenum	313.26	2.99
Nickel	232.00	1.34
Platinum	265.94	1.07
Selenium	196.03	1.24
Silver	328.07	7.53
Tellurium	214.28	0.71
Thallium	276.78	2.44
Tin	286.33	2.83
Titanium	365.35	5.43
Uranium	358.49	22.81
Zinc	213.86	14.84

2800 K, using the data in Table 30. The calculated values are listed in Table 39; for the calculations the efficiency of producing atoms from the sample was taken as unity. This assumption was also made by Winefordner (580) who compared the atom-producing ability of a graphite furnace with that of a flame, and found the graphite cell to be more efficient by a factor of 600. A comparison of the values of $k_0 l$ for Ca, Cu and Zn in a flame (Table 38) with those calculated for 0.1 ng amounts of the same elements in the furnace gives the ratios: Ca(I) 220, Cu(I) 280 and Zn(I) 100, i.e. of the same order of magnitude as Winefordner's ratios. It is possible that the gas expansion factor between the heated gas temperature and room temperature will affect the peak atomic concentration of analyte (581).

It would thus appear that the optical densities occurring during atomization in a graphite tube (and probably other electrothermal atomizers) are several orders of magnitude greater than typical optical densities in flame or plasma atom cells, and are thus contributory factors to the observed short linear ranges and high curvatures of growth curves from electrothermal atomizers. This conclusion was tested by performing atomic absorption measurements under differing conditions of optical density.

Two methods were used to vary the optical density during the atomization step. The first method utilized the ramp ash facility on the HGA-74 - in effect a ramp atomization was performed, giving a steady release of atoms from the sample into the analysis volume and thus maintaining conditions of lower optical density than a simple pulse vaporization of sample. Different rates of temperature rise were selected with the HGA-74, and the resultant calibration curves are given in Figure 61. Peak absorbance was plotted in this figure, and consequently there was a decrease in sensitivity for the slower atomization rates, since these produced smaller, but wider peaks - Figure 62. Integration of the signal restored the sensitivity, but the linear extent of each curve was not affected.
The second method of varying the optical density was to

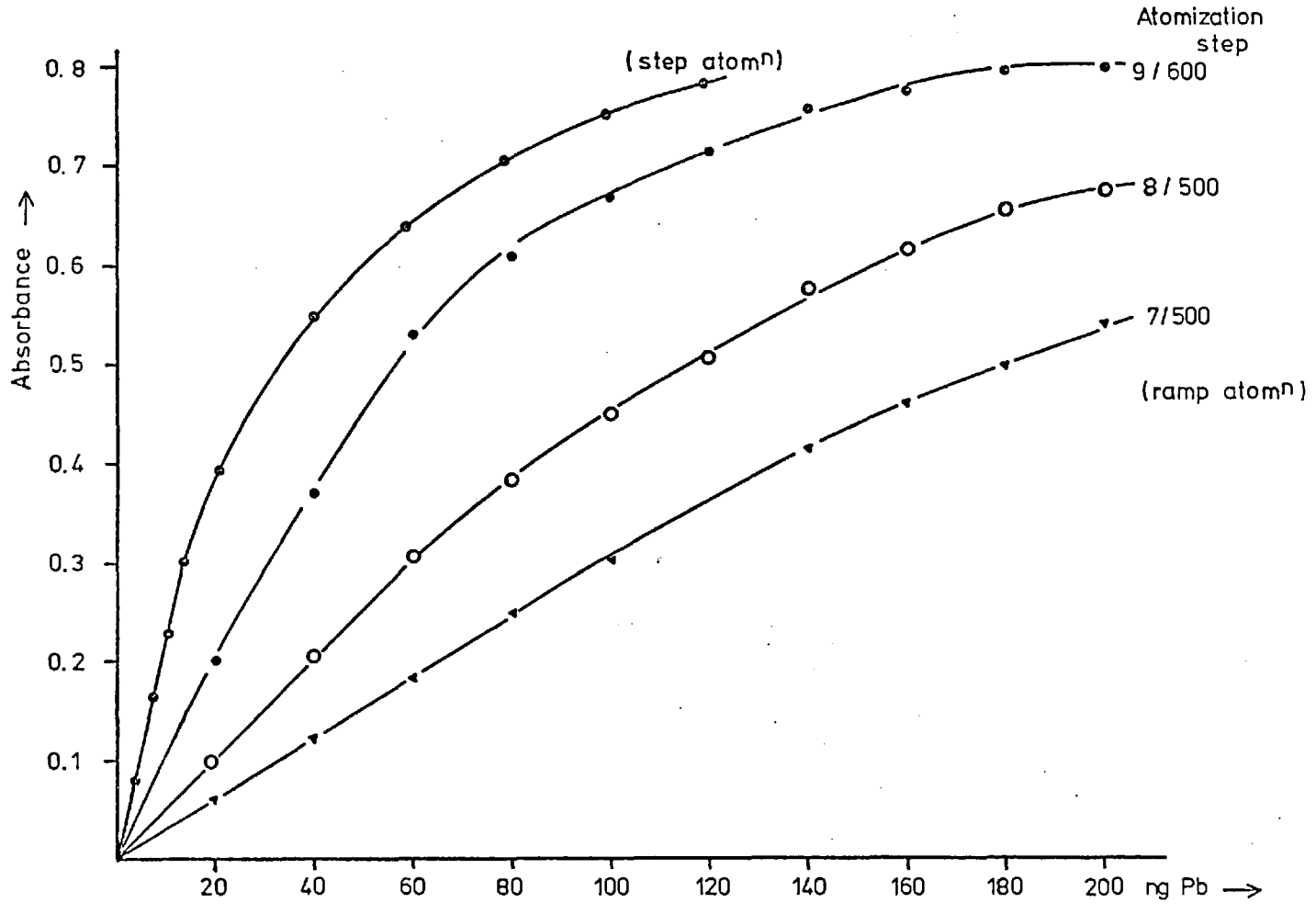


Fig. 61 Calibrations at varying atomization rates

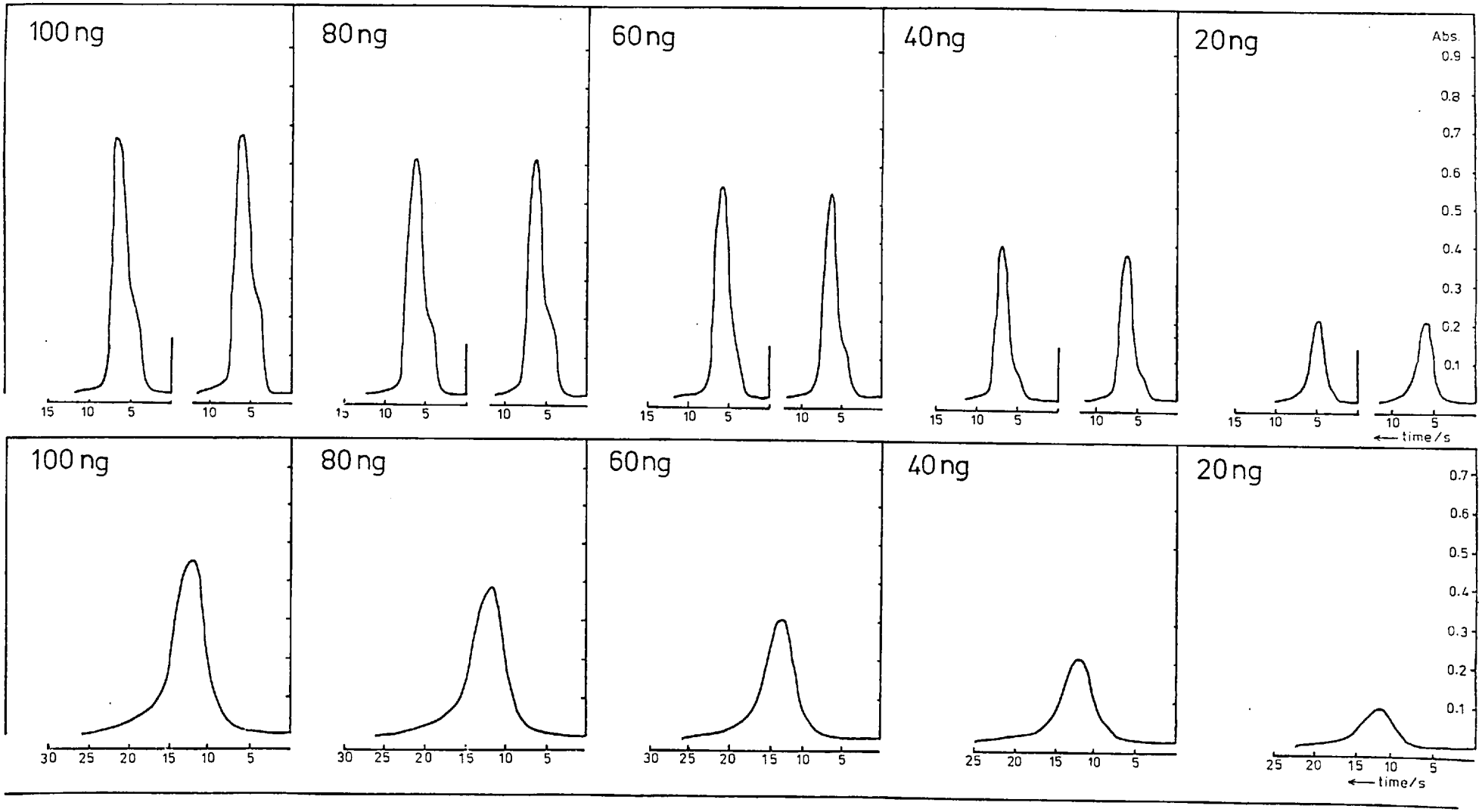


Fig. 62 Atomization of lead at two different rates (faster at top)

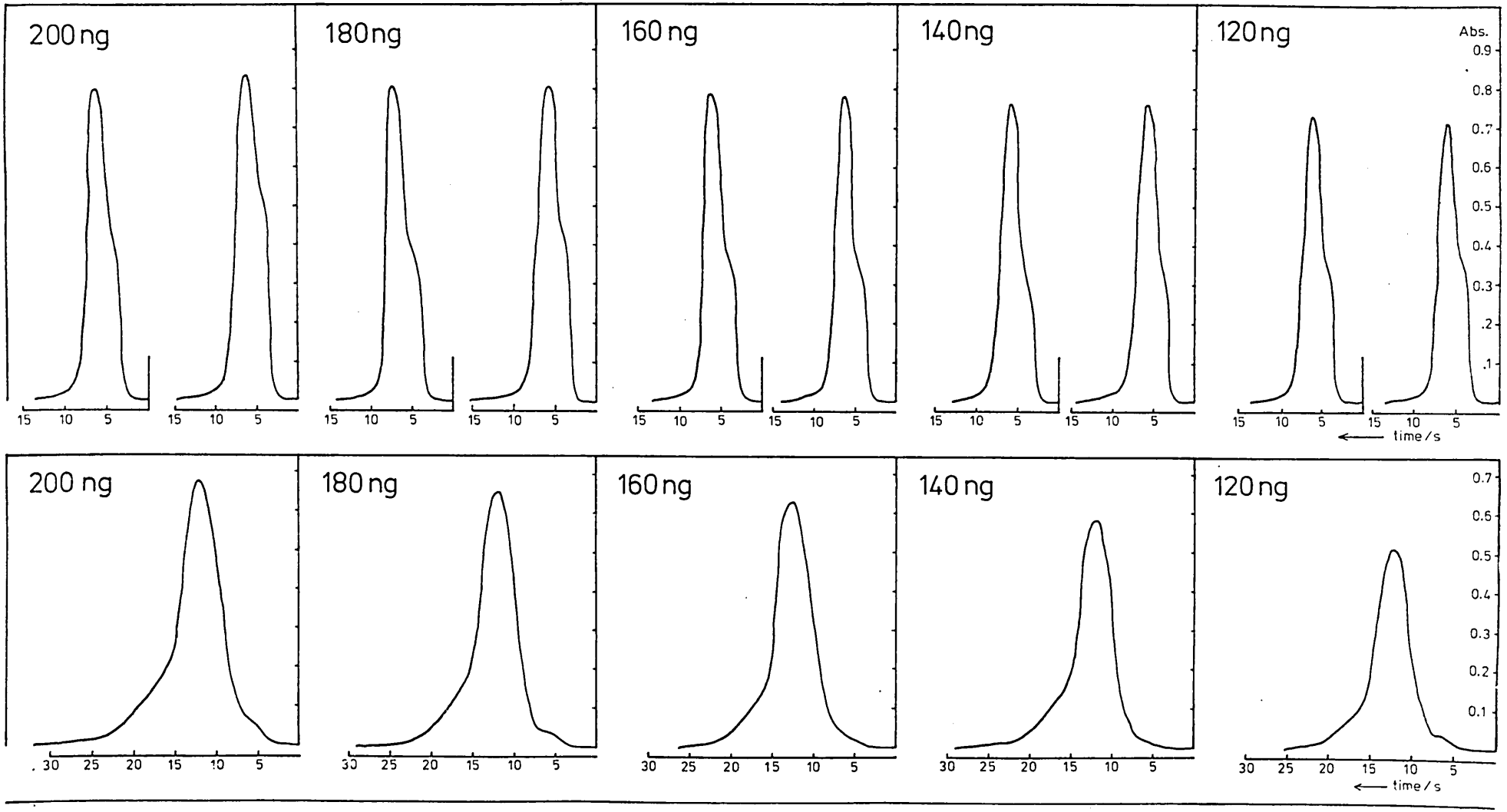

200 ng
180 ng
160 ng
140 ng
120 ng
Abs.
0.9
0.8
0.7
0.6
0.5
0.4
0.3
.2
.1
15 10 5
time / s
200 ng
180 ng
160 ng
140 ng
120 ng
0.7
0.6
0.5
0.4
0.3
0.2
0.1
30 25 20 15 10 5
time / s

Fig. 62 (cont.)

use different gas flow rates during the atomization step; since the two stages on the HGA-74 which are normally used for atomization are subject to automatic control of gas flow, the atomizations were carried out using the ramp step and with varying gas flow rates - Figure 63. As might be predicted, higher gas flow rates (which produced lower optical densities by faster removal of atoms from the analysis volume) gave lower peak absorbances, but greater linearity in the calibration graphs.

7.10 Methods to Increase the Linear Ranges of Calibration Graphs from Electrothermal Atomizers.

A large linear calibration range is desirable for any analytical technique, and it is one of the drawbacks of electrothermal atomization atomic absorption that the typical linear range is not large. The consequence of this is that only samples which have analyte concentrations falling within a small range can be analysed without recourse to sample dilution. When it is necessary to perform standard additions (section 2.7) care must be taken to ensure all the measurements lie on the linear region of the calibration.

Obvious advantages will be produced by extending the linear region of electrothermal atomization calibration graphs. Two methods of achieving this have been given in the previous section, viz. by a slow atomization step or by reducing the inert gas flow during atomization. The logical conclusion to reducing the gas flow rate - the use of "Gas Stop" conditions - is already widely employed because it gives maximum sensitivity. To retain the analytical sensitivity during a slow atomization it is necessary to employ signal integration, but occasionally it may be desirable to reduce the analytical sensitivity e.g. for zinc determinations at the 213.9 nm line (section 3.6.) It will then be sufficient to plot peak height.

A different approach to linearising calibration graphs has been proposed by Kilroe-Smith (258, 259). Instead of

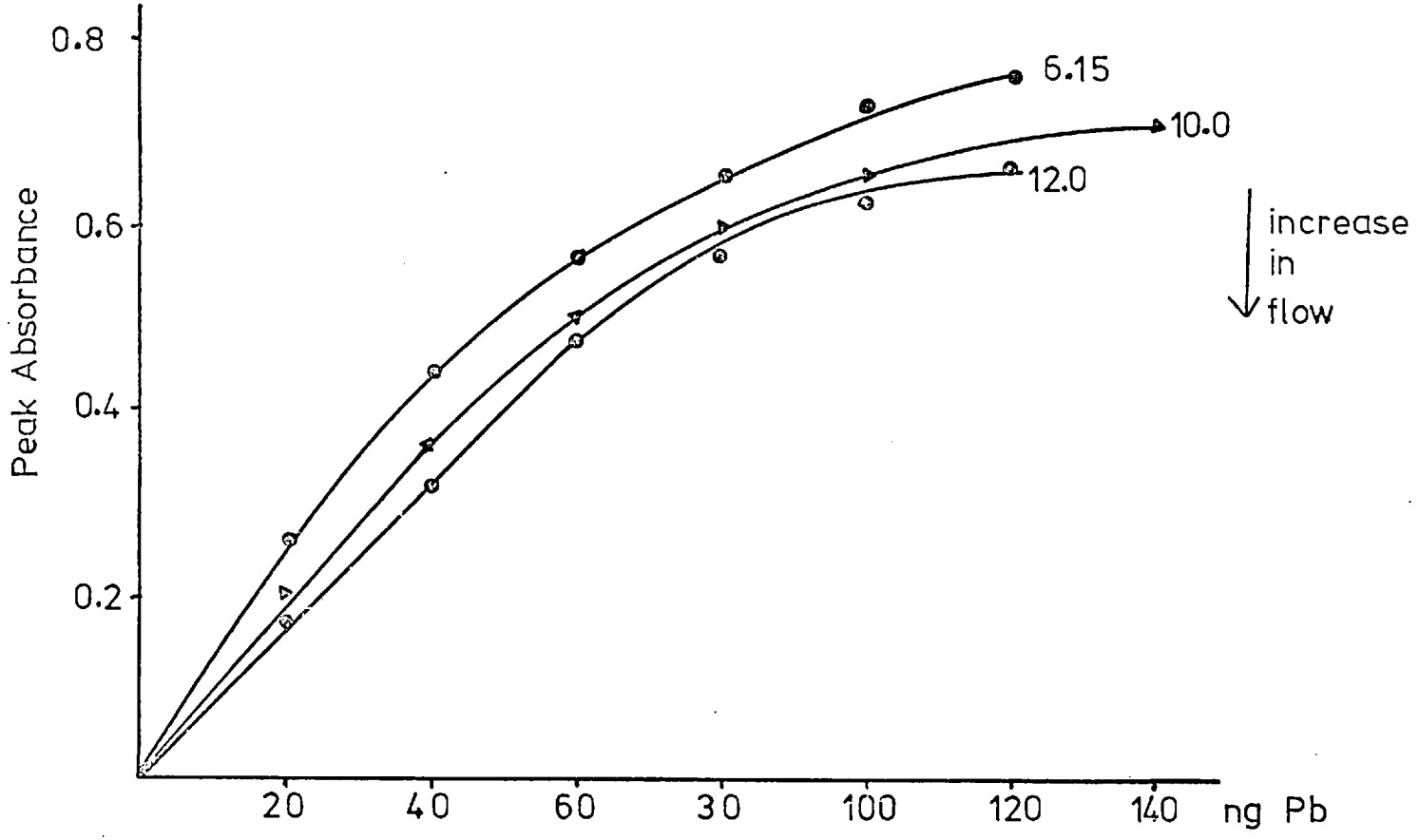


Fig. 63 Atomization at different inert gas flow rates

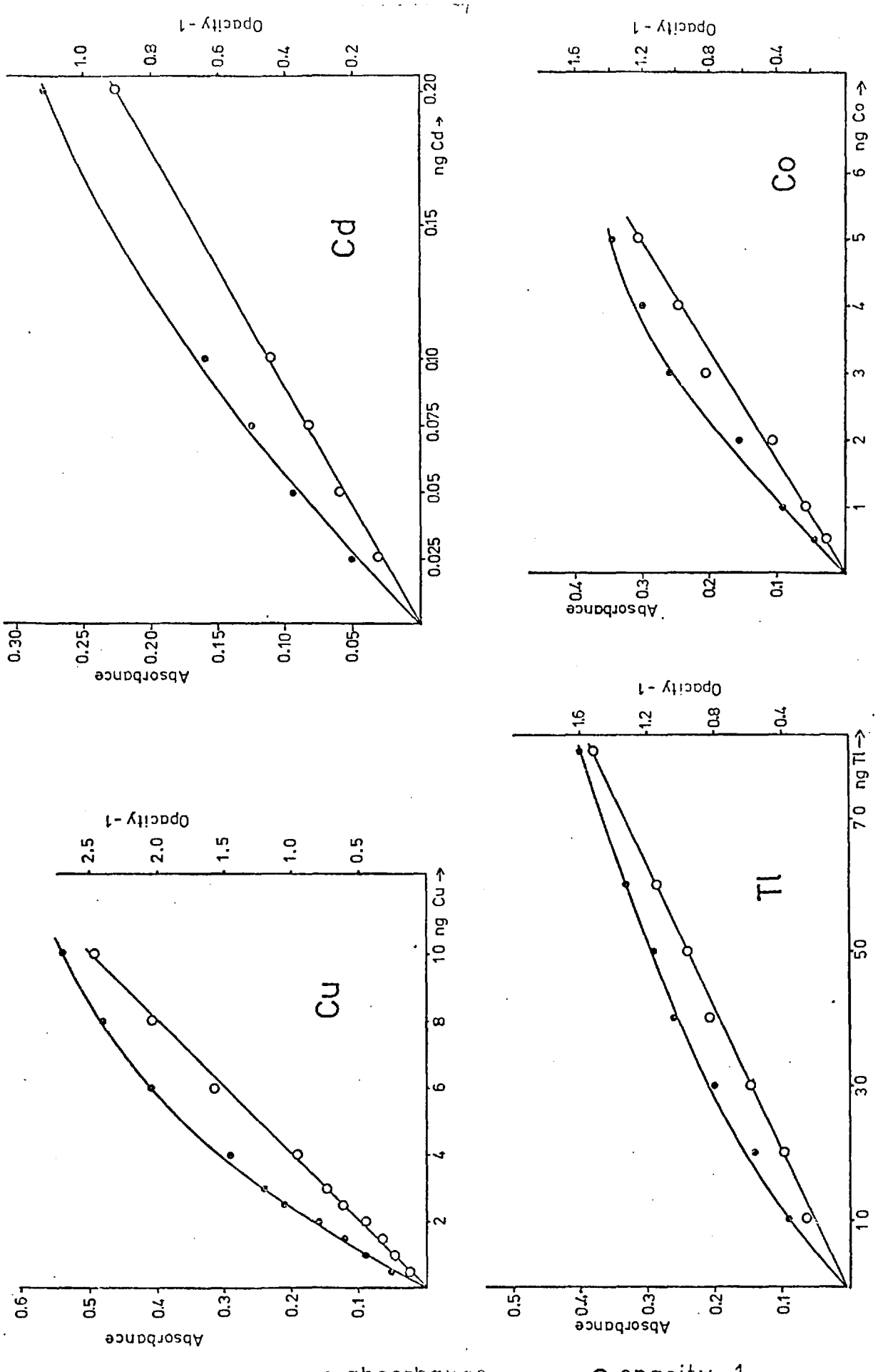


Fig. 64 Absorbance and opacity calibrations

plotting absorbance vs. mass of analyte, Kilroe-Smith plotted (opacity-1) vs. mass of analyte, and found that the linear region of the graph was increased considerably. Calibration graphs previously plotted for copper, cadmium, thallium and cobalt (Figure 20) were replotted using (opacity-1), and indeed showed increases in the linearity - Figure 64. A linear absorbance vs. mass of analyte plot produced a concave graph when replotted using (opacity-1) - Figure 65.

The reason why (opacity-1) vs. mass of analyte should produce a linear graph is unclear. Normally absorbance vs. mass of analyte is plotted since a linear relationship exists between these two quantities (268) but % absorption is sometimes used instead of absorbance. At low absorbances, absorbance ($\log I_0/I$) is proportional to % absorption $((I_0 - I)/I_0) \times 100$. (Opacity-1), which is $((I_0 - I)/I)$ is only equal to % absorption at very low absorbances - at higher absorbances there is no obvious relationship between the two terms. Kilroe-Smith (258, 259) makes no suggestion why (opacity-1) should produce a linear relationship to mass of analyte; it is possibly a mathematical quirk.

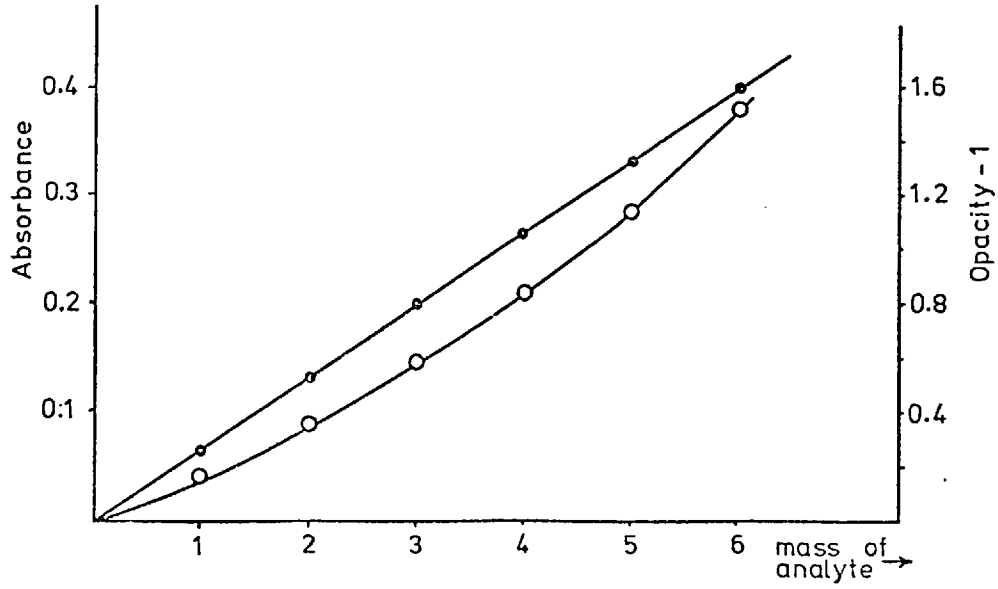


Fig. 65 Absorbance and opacity plots for a calibration linear in absorbance

Chapter 8

CONCLUSIONS AND SUGGESTIONS FOR FUTURE STUDIES

Atomic absorption spectrometry is well established amongst the techniques available to the modern analyst (80) and it has become the method of choice for the determination of many metallic elements at trace levels. The use of electrothermal atomization further extends the range of applicability of atomic absorption spectrometry. The work presented in this thesis shows that the technique can be successfully applied to the analysis of urine and liver.

Methods are given for the analysis of urine samples by direct injection into the furnace. Cr, Cu, Co, Ni, Cd, Pb and Bi can be determined in unspiked urine samples, but the method is restricted by the necessity of employing standard addition procedures, and by the volume limit to the correcting ability of the deuterium arc background corrector. Solvent extraction of urine improves the detection limits attainable, and an extraction procedure is presented which is suitable for the single-element or simultaneous extraction of Cu, Fe, Pb, Cd, Tl, Zn, Hg, Ni, Co and Sb.

Liver tissue may be digested with either a nitric acid / sulphuric acid mixture or tetramethyl ammonium hydroxide, and it is possible to determine Mn, Cd, Ag, Cr, Al, Ni, Li, Cu and Pb in the digests - in many cases without the need for standard addition. Levels of analyte elements in the acid and TMAH blanks were high, and for results of good accuracy purification of these reagents will be necessary. Sub-boiling distillation is a suitable method for acids, whilst a solvent extraction would be preferable for the TMAH.

Research on trace metals in man and in the environment is an area of importance to the overall understanding of the potential hazards and benefits to man's health. Losee and Adkins (610) have correlated geographical location to differing levels of dental caries. The correlation showed

dependence between type of food consumed and metal content of the soil where the food was grown, and potable water. Webb et al.(566) applied a cluster analysis technique to the multi-element analysis of cardiovascular tissues and showed that the technique could distinguish between types of tissue. Application of a pattern recognition technique (282) might provide a more satisfactory evaluation of the data, and thus provide a method for the diagnosis of disease. Klevay (272) has recently shown that low intakes of copper are associated with increased cholesterol levels. The determination of trace metals in biological systems, and the relation of the levels of these metals to states of health and disease - for both short-term and long-term exposures - is likely to increase in importance in the near future, but accurate analyses of these trace metals and their statistical interpretation will be more important than the development of new instrumentation, though further work on multi-element systems will be extremely valuable.

The use of a graphite tube atomizer means that it is possible to perform chemical operations actually in the furnace, and matrix modification is likely to provide a useful means of altering the volatility of an analyte element; this should reduce the difficulty of some determinations. Solvent extraction is a form of matrix modification; some chelates decompose to sulphides during the charring step (177), and higher ashing temperatures can be used. Matrix effects due to the high solids content of urine and liver digests were a recurring problem during this study, and these should be reduced or eliminated by the use of solvent extraction procedures.

The study on the causes of bending of the analytical curves showed that two main factors were involved - the contribution of the deuterium arc and the high optical densities formed during atomization. The latter could be reduced by a slow atomization step or by altering the inert gas flow rate - which led to increases in the linear ranges of the calibration graphs. Slow atomization also

gave reduced analytical sensitivities when peak absorbance was plotted; this could be useful in some instances when samples of high analyte concentration need to be analysed.

The theoretical work involved in calculating half-widths showed that much basic data does not exist, and since the graphite tube offers a very simple, well defined analytical system (analyte + graphite + inert gas in a fixed volume), the system could be usefully employed in more fundamental studies, e.g. measurements of oscillator strengths, collisional cross-sections, etc. - which was the purpose for which King originally designed his furnace.

REFERENCES

The list of 610 references presented covers the various topics studied in the thesis. The literature of the trace metal analysis of biological material is extensive, and the author's collection of relevant references - which is by no means exhaustive - contains over 3000 references; this excludes determinations of Ca, Mg, Na and K.

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ERRATA

p 63	line 5	for standard addition read standard addition line
p 67	line 12	for temperature read absolute temperature
p 74	line 10	for outling read outlining
p 95	line 4	for forseen read foreseen
p 97	line 9	for analyte read analyte atoms
p 141	line 35	for mercury-peroxy read peroxy-mercury
p 163	line 6	for signal shape read signal shape effects