UNIVERSITY OF SOUTHAMPTON

APPLICATION OF INDUCTIVELY-COUPLED PLASMA SOURCE MASS SPECTROMETRY TO THE ANALYSIS OF CLINICAL AND ENVIRONMENTAL SAMPLES.

BY

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ABSTRACT

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APPLICATION OF INDUCTIVELY-COUPLED PLASMA SOURCE MASS SPECTROMETRY TO THE ANALYSIS OF CLINICAL AND ENVIRONMENTAL SAMPLES.

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Methods were devised for the accurate and precise determination of total concentration and isotopic composition of lead in clinical and environmental samples, using Inductively Coupled Plasma-Source Mass Spectrometry (ICP-MS). Blood samples were analysed directly, following a simple aqueous dilution with a diluent containing ammonia solution, $(NH_4)_2H_2EDTA$ and Triton X-100. Diluted samples could be nebulised for periods up to 5 hours without blocking the Meinhard nebuliser. Deciduous teeth, dust, soils and other environmental samples were dissolved, or extracted, with nitric acid prior to analysis.

The described methods gave excellent agreement with atomic absorption spectroscopy for total lead concentration and with Thermal Ionisation Mass Spectrometry (TIMS) data from the University of Texas for lead isotopic ratios. The accuracy (<0.1% deviation from TIMS) and precision (cv=0.5%) of the ICP-MS method for isotopic analyses of tissue and environmental samples were sufficient to discriminate between different sources of lead ingested by humans.

The methods have been applied to studies of the source identification in childhood lead poisoning. Detailed studies were also made of environmental lead exposure of children living in Birmingham and to preliminary studies of children and adults in other UK towns. Significant differences in lead isotopic composition were observed and allowed some tentative identifications of source apportionment.

DECLARATION

I declare that all the work presented in this thesis was performed by me, Michael Jeremy Campbell, with the exception of the micro-sampling flame atomic spectrometry analyses of lead in whole blood which were performed by Mrs Sylvia Diaper, Trace Element Unit, Southampton General Hospital.

ACKNOWLEDGEMENT

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Contents.

CHAPTER 1

INDUCTIVELY COUPLED PLASMA-SOURCE MASS SPECTROMETRY

1.1 INTRODUCTION

Inductively coupled plasma-source mass spectrometry (ICP-MS) is a relatively new technique in which samples are ionised by an argon inductively coupled plasma (ICP) and the ions produced are analysed using a quadrupole mass spectrometer. The technique is capable of rapid, accurate and precise isotope ratio and multi-element analyses at the sub microgramme per litre level. Sample solution, in the form of an aerosol, is presented to a high temperature (≈10000K), atmospheric pressure, argon gas plasma. Ions are sampled from the plasma into a quadrupole mass spectrometer via a sampling interface. The necessary pressure reduction from atmospheric pressure (1×10^5Pa) to the pressure required for the successful operation of a quadrupole mass spectrometer (≈1×10^{-4}Pa), is achieved with a three stage differentially pumped vacuum system. The majority of the ions produced in the plasma have a single positive charge (>99% for most elements) so that the ion is detected at the isotopic mass of the analyte. The amplitude of the signal (current) produced, by ions falling onto an ion multiplier, is directly proportional to the analyte concentration in the sample. The spectra obtained by ICP-MS are easy to interpret, consisting of well resolved peaks at characteristic charge to mass ratios and are relatively free from interferences. The technique is most commonly used for the analysis of solutions, but samples may also be presented as slurries, or as vapours by electrothermal vaporisation or laser ablation, or as solids by direct probe insertion. The technique can also be readily interfaced to high performance liquid chromatography (HPLC) systems.

1.2 A BRIEF HISTORY OF ICP-MS

In 1973, Gray and his colleagues at Applied Research Laboratories (UK) Ltd, demonstrated the feasibility of using a gas plasma as an ion source for a mass spectrometer[1,2]. The mass spectrometer was based on the flame sampling quadrupole mass spectrometer developed by Hayhurst and his colleagues at Cambridge[3]. The plasma used for this initial study was a DC plasma, the capillary arc (DCP). The plasma plume was brought up against a fine (0.05mm diameter) orifice in a 2mm molybdenum disk, swaged into a copper sampling cone, allowing ions to be introduced directly into the spectrometer. Simple spectra with low backgrounds were obtained, enabling isotope ratios with a precision of ≈0.5% at the ppm level to be determined with counting times of 10-30 min.[4]

The capillary arc had a relatively low operational temperature of ≈5000K and was not sufficiently energetic to produce substantial levels of ionisation for elements with a first ionisation potential above 8ev. In addition, the degree of ionisation declined with increasing analyte concentration causing severe inter-element interferences, so work with the DCP was abandoned.

Two alternative plasma sources were available; the micro-wave induced plasma (MIP) and the inductively coupled plasma (ICP). The MIP, whilst producing a higher ionisation temperature (≈5900K) than the DCP was thought to have similar drawbacks because of its intolerence to sample loading and relatively low ionisation temperature, so Gray decided to work with an argon ICP which had a much higher core temperature of approximately 10000K (see section 1.3.1).

Gray's initial work stimulated work at other centres; Douglas et al at Toronto University in Canada adopted the MIP approach, whilst Houk et al at The Ames Laboratory, Iowa State University, USA, proceeded with an argon ICP.

The first successful extraction of ions from an RF ICP into a quadrupole mass spectrometer used a sampling system based on the earlier DCP work[5] and was achieved in 1975 by Houk and Gray during a short period of collaboration.

Sample introduction into an ICP is achieved through a torroidal space at the base of the plasma enabling the sample to be efficiently transported into the hot core of the plasma, unlike the DCP where most of the sample flows around the plasma which is at lower temperature than the core. The core temperature in an ICP of ≈10000K is much higher than that of in a DCP (≈5000K), so sample decomposition, volatilisation and ionisation processes should go much nearer completion in an ICP. However, the expected advantages of the ICP were not fully realised owing to the formation of a cooler, boundary layer of gas which formed over the sample aperture[5]. The residence time of a few micro seconds for ions in this boundary layer was long enough to allow significant recombination or cluster ion formation to occur, giving rise to a more complex spectrum than predicted, derived from hydroxide and other polyatomic species. The plasma temperature close to the sampling cone for an ICP of ≈6500K was considerably higher than that for a DCP of ≈3500K. This caused another unexpected difficulty. The aperture size(s) of 70μ (and below) for the molybdenum sampling disc that had been adequately cooled for DCP work could not be sufficiently cooled for the much hotter ICP and melted back to an aperture size of ≈120μ wherupon an intense "pinch" discharge would occur[6]. The discharge was due to the compression of the free electron population in the plasma as it passed through the sampling aperture due to the pressure differential across it[7].

Douglas and co-workers at Toronto had adopted an alternative philosophy for sampling ions from their MIP, based on molecular beam technology developed by Campargue[8]. A second cone was placed behind the sampling cone and the gas pressure in the region between the cones was reduced using a rotary vacuum pump. Ions from the plasma were extracted into the intermediate pressure zone and, as the pumping capacity of the system was improved considerably, larger apertures of 0.4mm could be used. This type of sampling interface was more rugged and could be adequately cooled to allow sample introduction from an ICP plume without any melting ocurring at the sampler tip. It was also tolerant of a much higher dissolved solids content in the sample solution, being much less susceptible to orifice plugging than the alternative interface. The establishment of this intermediate zone meant that the compression of the free electron population could be controlled by the selection of a suitable intermediate pressure and the pinch discharge eliminated. The results of Douglas and French's early experiments with MIP-MS[8] and later work with an improved MIP source[9] demonstrated that it was possible to obtain useful results from such a source.

The intermediate pressure stage technique was adopted by Gray and Houk and is used on all ICP-MS instruments today. The larger sampling apertures that could be used were broad enough to prevent the formation of the boundary layer across them and true bulk plasma sampling was achieved with the expected advantages of the ICP being observed for the first time[10].

In 1984, the first commercial instruments were launched. The Sciex Elan system was based on the instrument used by Douglas at Toronto[11], the ion source being an argon ICP (Douglas had abandoned work with the MIP which had the drawbacks forseen by Gray and Houk). The other commercial system, the VG plasmaquad, was developed in collaboration with Gray along the lines of Gray's prototype.

The development of ICP-MS and a review of the field up to 1987 has been compiled by Houk and Thompson[12].

All the work reported in this thesis was carried out using a VG PlasmaQuad which is described in the following sections.

1.3 PLASMA CHEMISTRY AND THE INTERFACE

1.3.1 THE PLASMA

A plasma can be defined as being a hot (≈10000K, in the case of the argon ICP), conducting gas with a high population of free ions and electrons, but is electrically neutral overall. The plasma ionisation source used in most ICP-MS systems is an argon ICP which is sustained by inductive coupling of radio frequency energy (RF) into the flowing gas. The oscillating high frequency current is produced by means of a positive feedback from the output to the input of a triode valve. Output is "frequency locked" to the resonant frequency of a piezoelectric crystal (13.56MHz). The frequency almost universally adopted for ICP-MS applications is 27.12MHz and this is achieved with a frequency doubling circuit.

A two or three turn load coil, earthed either centrally (Sciex Elan) or at the end nearest the interface (VG PlasmaQuad), is used to induce the RF energy into the plasma. The plasma torch, consisting of three concentric quartz tubes, is mounted on the central axis of the load coil, with the end of the central quartz tube (injector tip) located some 3 to 5mm before the first turn. Argon is passed down the outer tube at a flow rate of ≈12$\mathrm{l\,min^{-1}}$. This "coolant" flow is used to support the plasma and the plasma is lifted clear of the two internal quartz tubes by passing a lower flow rate of argon $\approx 0.5 l min^{-1}$ through the second tube. Sample solution in the form of an aerosol is swept through the injector and into the base of the plasma. In order for the RF energy to be induced in the argon gas stream, to form a plasma, the gas stream must first be "seeded" with ions, to make it conducting. This is accomplished using a Tesla coil with the output lead wrapped around part of the plasma torch. When the coil is energised, the electrical fields generated are sufficiently strong to cause local ionisation of the argon gas stream, allowing the RF energy to couple with the ionised gas, forming a plasma. The plasma is effectively the secondary coil of a step-down transformer, with the load coil acting as the primary. It is sustained by RF heating and any fluctuations in the impedence of the plasma are compensated for by impedence and phase matching circuitry on the torch box, which modify the RF supply from the generator accordingly.

The function of the plasma in ICP-MS systems is to provide an efficient ionisation source for samples introduced to it, for subsequent detection by the quadrupole mass spectrometer to which it is interfaced. In principle, the argon ICP is capable of ionising all elements with a first ionisation potential below that of argon (15.8ev). In fact, 54 elements are ionised to 90% or more. The theoretical extent of ionisation can be calculated from the Saha equation:

$$\frac{N_{ij}}{N_{aj}} = \frac{1}{N_e} \cdot \frac{(2\pi m_e KT)^{3/2}}{h^3} \cdot \frac{2Z_{ij}}{Z_{aj}} \cdot e^{-e_j/KT} \qquad (1.1)$$

where N_{ij} = ionic concentration of species j

N_{aj} = atomic concentration of species j

N_e = free electron population

m_e = mass of electron

K = Boltzman constant

h = Planck's constant

Z_{ij}/Z_{aj} = partition function between ionic and atomic states

e_j = ionisation energy of species j

T = ionisation temperature

Taking natural logarithms and rearranging, equation (1.1) can be re-written as follows:

$$\ln(N_{ij}/N_{aj}) = (-\ln(N_e . h^3) + 3/2 \ln(2\pi m_e KT) + \ln(2Z_{ij}/Z_{aj})) - e_j/KT \qquad (1.2)$$

Inspection of equation (1.2) shows that, for a given set of instrumental conditions, the only variable in the equation is e_{ij}, the ionisation potential for a given species. It is usually more convenient to evaluate the extent of ionisation from equation (1.1).

The number of electrons (N_e) present in the ICP is equal to the sum of ionised species in the plasma, since it is electrically neutral overall. Gray[13] suggested a value for N_e equal to 2.5×10^{21} electrons/M^3. Crain et al[14] measured the ionisation temperature of the plasma by determining the extent of ionisation for As and Sb over a range of instrumental conditions. They stated that the typical value for the ionisation temperature was ≈7500K (although their observations over a range of nebuliser gas flow rate and forward power settings suggested that this value was high for the Sciex Elan (range Sb 6400-8000K, As 6400-7100K)). Using these data, and assuming that the plasma is in local thermal equillibrium (LTE) and that Z_{ij}/Z_{aj} is ≈1, it is possible to calculate the extent of ionisation for a given element. The constant term in equation 1.1 is evaluated as 1.44×10^{51}, using these values. Table 1.1 presents the predicted percentage ionisation for a range of elements, calculated on the above basis:

Table 1.1. The extent of ionisation predicted from the Saha equation for selected elements.

Element	1^{st} ionisation energy (J)	% ionisation
Lithium	5.39	99.67
Fluorine	17.42	2.5×10^{-4}
Sodium	5.14	99.77
Magnesium	7.65	89.70
Argon	15.76	3.2×10^{-3}
Zinc	9.39	38.11
Indium	5.79	99.38
Barium	5.21	99.75
Lead	7.42	92.84

It can be seen that the ICP is an efficient ionisation source and provides a substantial reservoir of ions. It should be remembered that the Saha predictions are based on an assumption that the plasma is in LTE. Measurements of plasma temperatures have demonstrated that the plasma is not in LTE[15]. Species in a plasma, such as neutral atoms, ions, molecules and electrons are distributed over many energy states. The observed temperatures for various plasma processes (represented by gas kinetic temperature, T_{Gas}, excitation temperature, T_{Exc}, electron temperature, T_E and ionisation temperature, T_{Ion}) vary considerably from one another[15,16]. The range of values reported[16a] extends from 2300K (for T_{Exc}) to 52000K (for T_E). The consensus value for T_{Ion} is ≈7500K[14] and T_{Gas} at the core of the plasma is ≈10000K[17].

1.3.2 ALTERNATIVE PLASMA GASES.

The argon ICP is an efficient ionisation source for metal and non-metal elements with ionisation potentials below 9.5ev[18]. The extent of ionisation can be predicted from the Saha equation (section 1.3.1), if the condition of local thermal equilibrium can be assumed and the plasma ionisation temperature can be estimated. Houk[19] has performed such calculations for elements ionised in an argon ICP and reports that some 54 elements are ionised with an efficiency of 90% or more. However, for selenium, sulphur, phosphorus and the halogen group elements the extent of ionisation predicted declines from 33% for phosphorus and sulphur to 9×10^{-4}% for fluorine. Consequently, the detection limits for these analytes are relatively poor, in the μgml^{-1} range[20], approximately three to four orders of magnitude higher than typical detection limits observed in ICP-MS. If these elements are to be determined at the μgl^{-1} level or below, a more efficient ionisation source would be required.

Chan and Montaser reported the development and use of a helium ICP source for atomic emission spectrometry[21]. They observed that detection limits for chlorine and bromine were improved by factors of 63 and 34 respectively. Helium has a first ionisation potential of 24.6ev, considerably higher than that for argon, 15.8ev, and so a helium plasma should prove to be a superior ionisation source. However the density of helium is almost ten times lower ($0.179gl^{-1}$) than that of argon ($1.78gl^{-1}$) and therefore a vacuum system with a much greater pumping capacity is required to maintain the operational pressures required for quadrupole mass spectrometers, if the standard aperture sizes for the sampling and skimmer cones are to be used. Suitable pumping speeds can be obtained with turbo-molecular pumps (such pumps are now fitted as standard on the current production VG PlasmaQuads, to improve pumping efficiencies with an argon ICP).

As an ion source for ICP-MS, the helium plasma ought to exhibit fewer polyatomic species than an argon ICP, since it is essentially a monoisotopic gas (99.99987%). Such polyatomic species that did occur, due to the plasma gas, would be observed at lower mass than in an argon ICP, thereby removing some problematic interferences observed in the argon plasma. For example, the isobaric interferences on ^{75}As, ^{77}Se and ^{80}Se due to $^{35}Cl^{40}Ar$, $^{37}Cl^{40}Ar$ and $^{40}Ar_2$ would move to masses 39 ($^{35}CL^{4}He$) and 41 ($^{37}Cl^{4}He$) and if the purity of the helium was high enough no dimer would be seen at mass 80. Also, the possibility of determining ^{39}K and ^{40}Ca would exist since argon would not be present to cause interferences at these masses (currently these elements are in a skip scan region to protect the detector from damage due to the high flux of argon ions.)

Researchers at Texas University[22] and The University of Cincinatti[18] in the USA have performed some preliminary studies in which a helium plasma was interfaced to a quadrupole mass spectrometer. In the Texas study, Koppenal et al used a helium ICP with modifictions to the load coil and matching circuitry of the standard torch box[23], the instrument used was an otherwise standard VG PlasmaQuad. Caruso and co-workers at Cincinatti, used a helium MIP and their initial research was carried out with a plasma source mass spectrometer of their own design, although later research was done with a modified VG PlasmaQuad[18,24]. Both groups reported improved detection limits for the halogen elements by three orders of magnitude or more[18,22]. No data has so far been presented concerning matrix effects or the tolerance of sample loading for a helium source plasma mass spectrometer, so it has yet to be established if such a system is robust enough for routine analytical use.

1.3.3 SAMPLE INTRODUCTION TO THE PLASMA

Several different sample introduction techniques have been used in ICP-MS. These techniques fall into two categories, (i) dry sample introduction and (ii) liquid sample introduction. The first category includes: (i) electrothermal volatilisation (ETV)[25] from an electrically heated graphite furnace or rod, (ii) direct probe insertion, where sample solution has been allowed to dry onto the probe's surface (iii) laser ablation[26], in which material is volatilised from a solid using a finely focussed laser shot and (iv) volatilisation of an analyte by chemical means eg. formation of a volatile hydride or other volatile compound such as osmium tetroxide[27]. In all these cases the sample is first volatilised and then transported to the plasma on an argon carrier gas stream. The second group includes ultrasonic and pneumatic nebulisation techniques. Gregoire recently compared ETV, vapour generation and conventional solution nebulisation for the determination of total osmium concentration and isotopic composition[28].

The most widely used of these is pneumatic nebulisation. The solution presented to the nebuliser can be the eluent of an

HPLC system[29], a slurry[30] or a simple liquid sample. Dean et al[31] investigated the use of flow injection and reported that it could reduce the severity of problems associated with high salt content, viscosity and high acid concentration. When pneumatic nebulisation is used, the sample solution is normally pumped via a peristaltic pump to the nebuliser, although free-running uptake can be used. The solution stream is passed down a fine bore tube within the nebuliser (≈1mm) and encounters a high pressure (≈25psi) argon gas stream as it emerges from the tube. The gas pressure shears the liquid flow into droplets, forming an aerosol. The optimum droplet size for efficient volatilisation and ionisation within the plasma is $\leq 4\mu$[32]. The typical range of droplet size obtained from a commonly used type of nebuliser, a Meinhard TR-30-A, varies from 1.2 to 10.5μ[32]. Larger droplets are removed from the aerosol by a double pass spray chamber (which is usually water cooled to minimise solvent loading into the plasma) and pass to waste. Aerosol droplets of the desired size, representing 1-2% of the original aerosol, emerge from the spray chamber and pass via a link-piece into the torch and then into the annular space at the base of the plasma. As the aerosol passes further up into the plasma it encounters a strong temperature gradient which rapidly causes the aerosol to be desolvated, dissociated, volatilised and finally ionised. The ionised sample is passed through a sampling interface and into the quadrupole mass spectrometer.

1.4 THE VACUUM SYSTEM

Mass spectrometers separate ions or charged organic molecule fragments on the basis of their mass to charge ratio. A high vacuum is needed to minimise collisional losses of ions with neutral gaseous species within the spectrometer and consequent degradation of analytical performance.

An ICP-MS has three distinct vacuum stages; (i) the ICP which acts as an ion source; (ii) the sampling interface and (iii) the mass spectrometer itself. The mass spectrometer is separated from the interface by a slide valve which is opened for the operation of the instrument. Ions are extracted from the ICP, which operates at atmospheric pressure, into the quadrupole mass spectrometer through the sampling interface which consists of two co-axial nickel cones. The plasma plume is brought up against an aperture (1mm) in the outer (sampler) cone and a free jet of plasma gas is inhaled into the interface and through the inner (skimmer) cone aperture (0.75mm) into the main body of the spectrometer. The necessary pressure reduction is achieved using a three stage pumping system. The pressure between the sampling and skimmer cones is reduced from atmospheric pressure ($1x10^5$Pa) to ≈200Pa by a rotary vacuum pump, so approximately 99.9% of the sampled bulk plasma is immediately pumped away to waste.

In operation, the slide valve used to separate the high vacuum side of the instrument from the sampling interface is withdrawn, allowing ions to stream through the skimmer aperture and into an intermediate pressure zone. The pressure in this zone is reduced by a large capacity diffusion pump to $\approx 1\text{x}10^{-2}$Pa and finally, a smaller diffusion pump in the vicinity of the quadrupole further reduces the pressure to $\approx 5\text{x}10^{-5}$Pa, which is an acceptable pressure for the operation of a quadrupole mass filter.

The diffusion pump draws gas molecules into a hot oil (diphenyl ether) sprayed from a central tower. As the oil falls into the base of the pump it is cooled by means of an external cooling water circuit and the entrained gas molecules diffuse out to be removed by a backing rotary vacuum pump. In this type of instrument, a pressure reduction of $\approx 1\text{x}10^{10}$ is achieved, allowing the successful interfacing of two components with widely differing operational parameters.

1.5 ION OPTICS

The ion multiplier used in the VG PlasmaQuad is mounted on the optical axis of the instrument i.e. the same axis as the plasma. The ion multiplier is sensitive to photons and so must be shielded from the intense light source of the plasma to reduce detector noise. This is achieved by mounting a small metal disk known as a photon stop on the axis. The ion beam is focussed around the photon stop and into the first element of the quadrupole, the pre-filter, by means of an array of electrostatic lenses known as the lens stack. There are ten lenses in the array and the DC potential (±150v) applied to each element can be varied independently, using a potentiometer, to give maximum transmission for a given ion or optimum general sensitivity. The lenses are interactive and have to be tuned iteratively to provide maximum transmission of the desired species. It is possible to selectively tune the lens array to reduce levels of doubly charged or polyatomic species, but this usually results in a decrease in sensitivity for the analyte signal of interest.

Instrumental stability, and often sensitivity is adversly affected by capacitive build-up and discharge on a lens element which causes perturbation of the local electrostatic fields. This can arise from contamination of the lenses and will result in unusual potentiometer settings which can only be corrected by the removal and cleaning of the lens stack.

1.6 THE QUADRUPOLE MASS ANALYSER

The device used to analyse the ion beam extracted from the bulk plasma into the spectrometer is a quadrupole mass analyser[33,34]. A quadrupole consists of two pairs of rods (electrodes) mounted rigidly in a square array. Opposite electrodes are connected together and a radio frequency of opposite phase and a DC potential of opposite polarity is applied to both pairs. This gives rise to an electric field between the rods, perpendicular to the central (Z) axis of the array. Ideally, the rods should have a hyperbolic section, but in practice cylindrical section electrodes are used as they are easier to construct and provide a good approximation of the field generated using hyperbolic electrodes.

An ion entering the quadrupole along the Z axis will be caused to undergo a transverse motion under the influence of the electric field. The trajectory followed by the ion can be described by differential equations known as Mathieu equations:

$$\frac{d^2u}{d^2E} + (a-2q\cos 2e) = 0$$

where

$$a = a_x = -a_y = \frac{4eu}{mw^2r_o^2}$$

$$q = q_x = -q_y = \frac{2eV}{mw^2r_o^2}$$

$$E = \frac{1}{2}wt$$

and V = peak amplitude of RF voltage at frequency f

u = DC voltage

r_o = inscribed radius of quadrupole

m = mass of ion

e = charge on electron

w = angular frequency of RF

t = time

There are two types of behaviour described by these equations: (i) The ion adopts unstable motion, moving away from the Z axis and ultimately strikes an electrode or the body of the spectrometer and is neutralised or (ii) the ion adopts a steady oscillation about the Z axis and emerges at the exit of the quadrupole.

1.6.1 STABILITY DIAGRAM

If the Mathieu equations are solved for a given quadrupole (r_0 fixed), a plot of "a" against "q" depicts regions where the trajectory of a given ion is stable (Figure 1a), allowing the ion to emerge from the exit of the quadrupole and be detected. The amplitude of the RF voltage determines the mass to charge ratio for an ion to follow a stable trajectory. As the magnitudes of V and U are increased, successive stability zones are encountered which correspond to the transmission of progressively heavier ions through the quadrupole (Figure 1b). If the RF and DC voltages are selected such that the line of the slope passes through the tip of each successive stability zone in turn, the transmitted ions can be detected discretely. Hence, the range of stable masses transmitted is determined by the ratio of the RF to DC potential applied to the electrodes. For the quadrupole used in the VG PlasmaQuad, a ratio of 6:1 results in a stable mass range of 1amu. The magnitude of the DC potential therefore governs the resolution of the spectrometer.

A mass spectrum is obtained by increasing the RF and DC potentials up to their maximum values, whilst maintaining a constant ratio (in order to obtain uniform resolution across the mass range). Normally, the quadrupole is swept from low to high mass repeatedly (eg 200 sweeps), in order to accumulate sufficient data for a spectrum.

Fig. (1a)

Fig. (1b)

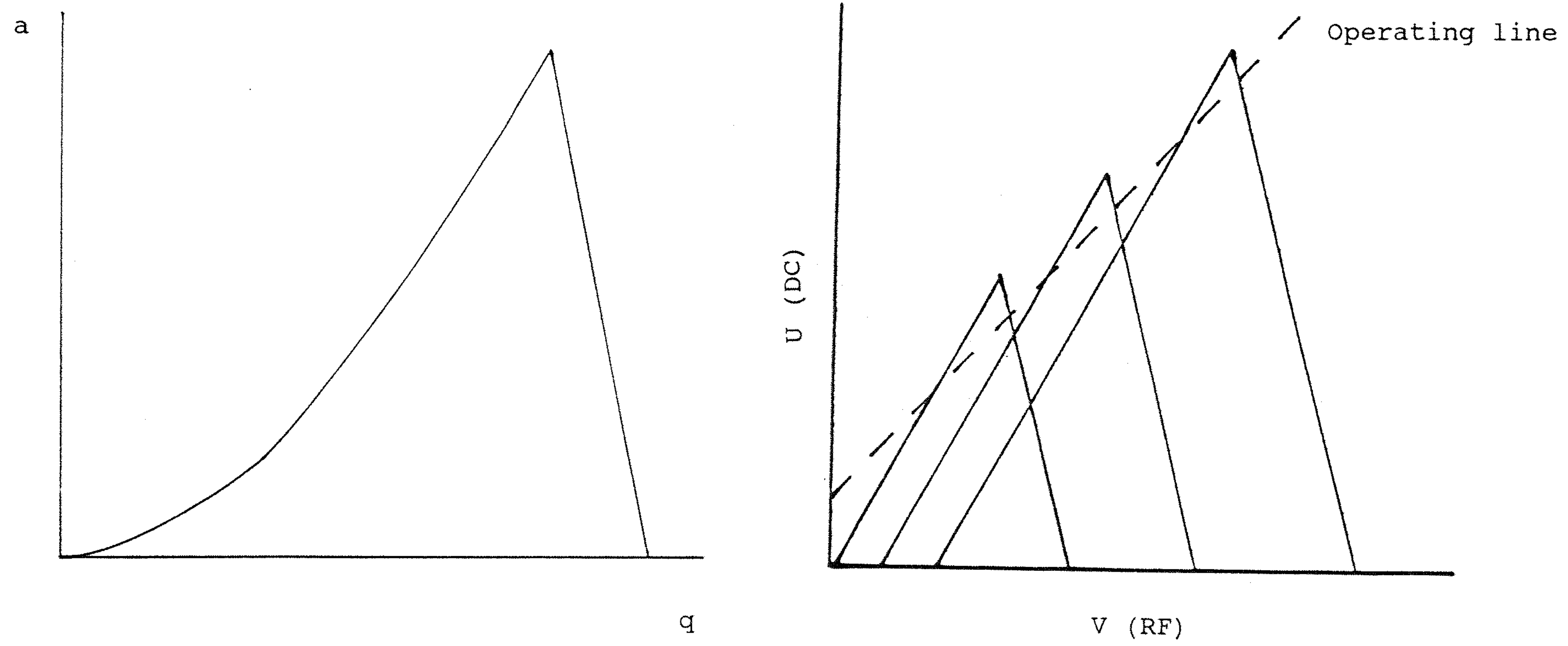

Figure 1 (a) Stability diagram depicting area where solution to Mathieu equation is stable. (b) Diagram showing succesive stability zones.

1.7. INTERFERENCES IN ICP-MS

In principle, ICP-MS should be virtually free from spectroscopic interferences, however interferences are observed[9,10]. These interferences can be classified into three groups: (i) isobaric, (ii) polyatomic and (iii) interferences due to formation of doubly charged ions.

1.7.1 ISOBARIC INTERFERENCES

Isobaric interferences occur when two elements present in an analyte have isotopes with the same nominal mass[13], eg ^{64}Ni and ^{64}Zn. It is possible to make a correction for the contribution to the signal at mass 64 from nickel and zinc, based on measurements at other, interference-free, nickel and zinc isotopes since the natural abundancies of both elements are known and fixed in nature[35] (^{64}Ni=1.16%, ^{64}Zn=48.89%). However, it is usually sufficient to make quantifications based on another isotope which is free from isobaric interference.

1.7.2 POLYATOMIC INTERFERENCES

Polyatomic interferences arise when charged species containing two or more atoms are formed within the plasma. Such species are detected at the mass to charge ratio corresponding to the sum of the masses of the constituent atoms eg $^{40}Ar^{16}O$ forms a polyatomic interference on ^{56}Fe, the major isotope of iron (91.52%). The mechanism for the formation of these polyatomic species is not yet understood. The extent of their formation is dependent upon the matrix concentration of their constituent ions but is not sufficiently stable to allow the use of correction routines similar to those used to compensate for isobaric interferences. An alternative isotope of the analyte of interest, free from interferences, should be used for quantitation. In cases where a polyatomic species overlaps with a monoisotopic element, for example $^{40}Ar^{35}Cl$ on ^{75}As, formation of the polyatomic species must be avoided by careful choice of the matrix and reagents or by a chemical separation of the interfering species eg by complexation or ion exchange chromatography. Plantz et al[36] reported the use of a complexing agent, bis(carboxymethyl)-dithiocarbamate to separate analyte metals from alkali and alkaline earth metals in urine and seawater samples. Beauchemin et al[37,38] obtained a 50-fold pre-concentration of trace elements in a sea water reference material (NASS-2) by adsorption of the sample onto silica-immobilised 8-hydroxyquinoline. This approach removed some polyatomic interferences and eliminated signal supression due to high sodium concentration in addition to reducing the detection limits as a result of the pre-concentration of the sample. The same group analysed trace elements in marine sediment[39] and two biological reference materials[40]. Polyatomic interferences were minimised by careful choice of reagents and dissolution procedure and by optimising instrumental parameters to give the lowest level of polyatomic ions possible. Good results were obtained by use of standard addition and isotope dilution techniques, but they noted that external calibration did not give good agreement with the accepted results in the absence of a complexation stage[37].

Lyon et al[41] evaluated ICP-MS for the simultaneous multi-element analysis of trace elements in the field of Clinical Chemistry by an inter-laboratory comparison of results obtained for four biological reference materials. The other techniques used in the study were ICP-AES, multi-element AAS and GFAAS. They reported broad agreement for the analytes investigated, but for analytes with masses below 82 mass units the results obtained by ICP-MS were severely degraded by polyatomic interferences. It was suggested that the polyatomic species containing chlorine (which has two isotopes at masses ^{35}Cl (75.4%) and ^{37}Cl (24.6%) and is present at high concentrations in biological matrices) could be eliminated by precipitation of the chlorine with silver nitrate. Although this approach would reduce such interferences it is of little practical use since the composition of the trace elements in the solution could be altered irreproducibly by co-precipitation. In a subsequent paper, Lyon et al[42] reported the use of gel filtration columns (Sephadex G-25M pre-packed PD-10) to overcome chloride interferences. They reported reasonable agreement for selenium analyses in a range of biological reference materials and stated that chloride interferences were completely eliminated.

Much of the early research in ICP-MS was focused on interferences and their avoidance[43-47]. It is possible to minimise polyatomic interferences by optimisation of instrumental parameters, as stated above, but this often results in considerable loss of sensitivity for the analyte. It is prudent to evaluate the severity, and indeed occurrence, of any potential polyatomic interference in a particular matrix before abandoning a major isotope for quantitation.

1.7.3 DOUBLY CHARGED SPECIES

The third classification of potential interferences in ICP-MS is that due to the formation of doubly charged analyte ions in the plasma. All elements with ionisation potentials below the first ionisation potential of argon (15.8ev) can, in principle, form doubly charged ions in an argon ICP. The doubly charged ions are detected at half the mass of the parent atom since the quadrupole mass analyser separates ions on the basis of their mass to charge ratio. In general, the levels of doubly charged ions observed on the commercial instruments are <2% of the parent ion signal. The extent of doubly charged ion formation for a given species is constant under a particular set of instrumental conditions.

It is possible to correct for such interferences in the same manner as that outlined for isobaric interferences. The extent of oxide and doubly charged ion formation observed under standard operating conditions with a PlasmaQuad was investigated by Gray and Williams[47]. The load coil geometry and the position of its connection to earth influence the ion energies of analytes entering the spectrometer. Gray[48] investigated the relationship between load coil design and the populations of oxide and doubly charged species, however, the responses observed were broadly similar for all the designs studied.

Jarvis et al[49] made novel use of the formation of doubly charged species to avoid polyatomic interferences on europium in high barium matricies by quantifying the element as Eu^{2+}. Barium can occur at high concentrations ($>100\mu g ml^{-1}$) as barytes (barium sulphate) in solutions of geological samples containing low levels of rare earth elements (typically 1-1000$ng l^{-1}$), making direct determination of europium impossible because of severe polyatomic interferences. Europium has two isotopes ^{151}Eu (47.77%) and ^{153}Eu (52.23%), both of which are interfered with by barium oxide and hydroxide polyatomic species: $^{135}Ba^{16}O$, $^{134}Ba^{16}O^{1}H$ on ^{151}Eu and $^{137}Ba^{16}O$, $^{136}Ba^{16}O^{1}H$ on ^{153}Eu. The instrument was re-tuned and the resolution adjusted to give maximum sensitivity for Eu^{2+} and the europium signal was measured at masses 75.5 and 76.5. Excellent agreement was obtained for analysis of a geological reference material using this technique ($1.14\mu g g^{-1}$ Eu found, $1.16\mu g g^{-1}$

Eu certified).

1.7.4 HIGH RESOLUTION ICP-MS

The resolving power of the quadrupole used in the PlasmaQuad is capable of resolving two peaks separated by one mass unit. This is adequate to separate at least one isotope of every element from all other isotopes, however interferences may occur, as discussed earlier. Bradshaw et al[50] recently published outline details of a high resolution ICP-MS instrument in which the ICP is interfaced to a double focusing, magnetic sector mass analyser. The resolution of the instrument is sufficient to resolve polyatomic interferences from analyte signals at the same nominal mass. For example, the polyatomic interferences on masses 51 and 56 due to $^{35}Cl^{16}O$ and $^{40}Ar^{16}O$ interfere with vanadium and iron on conventional ICP-MS instruments, however the accurate masses of these species are ^{51}V =50.944amu, $^{35}Cl^{16}O$ =50.964amu, ^{56}Fe =55.935amu and $^{40}Ar^{16}O$ =55.957amu, with the high resolution instrument these signals are completely separated. In addition, Bradshaw et al reported that photon noise had been reduced by two orders of magnitude and, since the sensitivities observed were comparable with those for conventional ICP-MS, they predict that detection limits could be reduced by 1-2 orders of magnitude.

1.7.5 ORIGIN OF POLYATOMIC SPECIES

The early work of Gray, Houk and Douglas involved sampling ions from a gas plamsa under the so called "boundary layer" conditions[7]. In this mode of operation, ions were extracted through a cooler layer of gas with residence times of the order of 1-2μs. It was assumed that recombination reactions to form polyatomic species were initiated in this region and that some reactions were completed within the interface itself. However, with the universal adoption of the type of intermediate pressure interface pioneered by Douglas et al[9], and the use of larger sampling apertures the boundary layer was punctured and true, bulk plasma was sampled. The spectra obtained in this second mode ("continuum sampling") still contained peaks due to polyatomic species, thereby implying that the recombination reactions occurred either within the plasma or within the interface. Douglas and French[51] recently applied a gas dynamic treatment to the plasma sampling process and they concluded that in the flow through the sampler and skimmer cones, the density and gas kinetic energy of the plasma drop so rapidly that the degree of ionisation is frozen at that of the source. This indicates that little or no recombination reactions take place. If the correct geometric relationship is maintained between the cones, a representative sample of ions from the ICP is obtained.

The influence of plasma parameters such as RF forward power, gas flow rates and water vapour loading, on the polyatomic and doubly charged ion populations have been studied by several groups[52-55]. The role of water vapour loading into the ICP, and its influence on the performance of ICP-MS instruments, has been reported by three groups[52-54]. The influence of water vapour loading was studied on three different commercially available instruments: Hutton and Eaton[53]: VG PlasmaQuad, Zhu and Browner[52]: VG PlasmaQuad and Sciex Elan and Tsukahara and Kubota[54]: Seiko SPQ-1600S (Seiko Instruments Inc. Tokyo, Japan). For the PlasmaQuad and SPQ-1600S instruments, these authors reported that the signal intensities for M^+ were increased as the water vapour loading was reduced. Similarly, doubly charged and polyatomic signals declined as water vapour loading was reduced. However Zhu and Browner reported divergent behaviour between the Sciex Elan and the VG PlasmaQuad with respect to the optimum response for a given analyte as a function of water vapour loading. They noted that wheras the Elan gave an essentially flat response for M^+ over a wide range of water vapour loadings (corresponding to spray chamber temperatures from 0 to 20°C), the intensities for M^+ increased at higher loadings (spray chamber temperature 25 to 35°C). This behaviour was mirrored by doubly charged and polyatomic species, although the increase was much less intense (ie lowest response for doubly charged and polyatomic species was obtained with spray chamber temperatures below 20°C).

One possible explanation of the divergent behaviour of the two instruments with respect to water vapour loading may be related to differences in plasma potential between the two systems. The Sciex Elan uses a "centre-tapped" load coil which is earthed at the centre, whereas the VG PlasmaQuad uses an assymetrically tapped load coil earthed at the end of the coil nearest the interface. The load coil geometry used for the Elan system is reported to eliminate the discharge between the plasma and the interface almost completely[56]. Houk et al[57] investigated the plasma potential observed for an Elan at various sampling depths, aerosol flow rates and foward power settings using a Langmuir probe. They found that under normal operating conditions, the central analyte channel was at a potential of ≈1.2v compared to the literature value of ≈20v for other load coil geometries[58]. The ion kinetic energies were also lower for systems using the centre tapped load coil.

1.7.6 SIGNIFICANCE OF INTERFERENCES IN ICP-MS

Signal suppressions due to the presence of high concentrations of easily ionised elements (eg sodium IP=5.14v) or high dissolved salt contents (>1%) in a matrix have been reported in ICP-MS[13,59,60]. The extent of these effects is dependent upon the concentrations of matrix elements, their mass and ionisation potentials and can vary from minimal levels to >90% signal attenuation[61]. Deposition of material on the cones from solutions with high dissolved salt contents, leads to a loss of signal due to partial blockage of the sampling orifice. The problem of deposition of material on the cones has been studied by Douglas and Kerr[62].

Polyatomic interferences are most serious below mass 80 (since the most abundant species in the plasma are derived from light elements with masses ≤40 amu). However, oxide and hydroxide species can present problems at higher masses eg MO^+ interference of light REE on heavier REE[63]. The severity of such interferences depend on the matrix concentrations of the parent species and the analyte: if the analyte is present at low concentration, any polyatomic interference may be severe on the small analyte signal. Polyatomic interferences can be minimised by careful choice of reagents and, where appropriate, by the use of separation techniques. In general, oxide and hydroxide ion intensities are low, 1-2%. Similarly, doubly charged ion intensities are rarely more than a few percent[47]. The oxide and doubly charged response has been improved by a factor of about 5 on the PQ2 series[49].

If care is taken to detect and identify interferences arising for a given analysis by ICP-MS, they rarely present an intractable problem, since acceptable alternative isotopes free from such interferences are often available or interferences may be overcome by chemical means, as outlined above.

CHAPTER 2

OPTIMISATION OF ICP-MS AND THE DEVELOPMENT OF SUITABLE METHODOLOGY FOR THE ANALYSIS OF LEAD IN WHOLE BLOOD.

2.1 INSTRUMENTAL OPTIMISATION PROCEDURES

2.1.1 INTRODUCTION

In order to be able to make precise and accurate determinations of lead concentration and isotopic compositions in clinical and environmental samples, suitable experimental procedures had to be developed and the analytical performance of the instrument established. The original VG PlasmaQuad suffered from a large number of component failures, resulting in considerable periods of instrumental "down time" (>70%), necessitating the replacement of various electronic units which were subsequently found to be interactive and should have been matched to one another. Instrumental drift also varied in severity from a few percent (2-5%) to over 50% during the course of an experiment, typically 2-3 hours. The drift and fluctuations in sensitivity could largely be overcome by the incorporation of an appropriate internal standard, but clearly was not acceptable in the longer term (as the magnitude of the corrections was often significantly greater than 10%). Against this background, it was difficult to characterise the performance of the instrument as there were a few short lived episodes where drift was negligible and precisions obtained for aqueous lead standards were encouraging (1-2% for $100\mu g l^{-1}$ Pb), suggesting that ultimate performance levels would be acceptable.

In March 1987, the instrument was exchanged for the then current production version, which differed from the original in several important aspects: (i) a more reliable free-standing RF generator replaced the original unit which had been mounted at the base of the cabinet that housed the gas contol panels. (ii) A mass flow controller was added to the nebuliser gas line which improved the stability of aerosol production. (iii) The vacuum system was upgraded, reducing the risk of oil vapour contamination of the quadrupole and lens stack and allowing larger sampler and skimmer aperture sizes to be used. (iv) A solid state RF generator was used to produce the RF supply for the quadrupole and (v) better cooling of the electronic units was achieved by altering the direction of air flow through the instrument, improving instrumental stability.

In addition to the instrumental improvements, the instrument was moved into a smaller, air-conditioned laboratory where the temperature could be maintained at 20±2°C. The heat associated with the ICP RF generator was ducted outside the room using a $100m^3$/hr fan, cooling port air flows and plasma exhaust gases were also ducted away. Finally, the instrument's power supply was fed through a constant voltage transformer which eliminated mains spikes and voltage fluctuations.

The reliability, stability and sensitivity of the new instrument have been excellent. Typical sensitivity for an equivalent $1\mu gml^{-1}$ aqueous lead solution of $\approx 3x10^{6}$ counts/sec on the ^{208}Pb isotope represents almost a fifteen fold improvement on the original, with a background noise signal of less than 25 counts/sec. Routinely, the stability exhibited over a 3-4 hr period of 5% is achieved.

Although much of the development work for total concentration and isotopic composition of lead analysis procedures was carried out on the original instrument, it has been repeated and refined using the current instrumentation. The optimisation procedures and preliminary experimentation presented in this chapter were performed with the replacement instrument. With the exception of the data obtained for the MSFAAS/ICP-MS comparison (see Section 3.3.2), all the data presented in this thesis was obtained with the second PlasmaQuad.

2.2 INSTRUMENTAL OPTIMISATION

Although ICP-MS is capable of accurate and precise multi-element determination across the full periodic classification table, the best performance for a limited mass range scan is obtained by optimising the instrument for an element of interest within the desired range.

This section deals with general principles of optimisation which were used to set up the instrument for routine analysis.

2.2.1 ICP FORWARD POWER

Above a threshold value of 900W, a broad optimum exists where increasing the RF forward power into the plasma has little apparent effect on the population of M^+ ions within the plasma. Above this threshold value, all material entering the ICP is ionised to its maximum extent, as predicted by the Saha equation. All the work reported in this thesis was carried out with a RF forward power of 1.35kW, unless stated otherwise. Typically, the reflected RF power was between zero and one watt, indicating efficient coupling of the RF energy with the plasma.

2.2.2 SAMPLE INTERFERENCE AND TORCH ALIGNMENT

The condition of the skimmer and sampling cones has been reported to have a marked effect on stability and sensitivity at all masses[64]. The condition of the cones was checked before experimentation and cones were usually cleaned on a daily basis with a proprietary stainless steel polish called "Polaris" to restore the high surface finish in the nickel cones. Great care was taken to ensure that all traces of the polish were removed after use, by firstly rinsing in water, then by cleaning in deionised water in an ultrasonic bath for 10 min. Finally, cones were rinsed in de-ionised water and allowed to dry before being re-mounted in the interface. The consequences of inefficient removal of the polish were presented in a paper by Dean <u>et al</u>[65].

It was noted that the small amount of play about the sampler cone mounting screws made it possible for the sampling and skimmer cones to fall on different axes with consequent deterioration in performance. Therefore care was taken to centralise the sampling cone to ensure that the plasma: interface: detector alignment was about a common axis.

The plasma torch is mounted centrally within a three turn load coil with the end of the inner quartz injector tip approximately 3-5mm from the first turn. All gas line unions were checked for leaks in order to prevent loss of stability in the analyte signal. The plasma was then struck and the instrument set into its operational mode and left to stabilise for approximately 30 min.

The position of the plasma with respect to the interface was adjusted to give optimum response for a convenient concentration (50-100 μgl^{-1}) of an aqueous standard, monitored at the appropriate mass. Crude adjustments of the torch box position in three dimensions were possible using the existing tripod nuts and studding arrangement and the lateral and axial threaded rod/spring adjusters. However, accurate re-positioning was impossible and a trial and error approach to the alignment problem had to be adopted. (In order to make spatial resolution measurements of the plasma a sophisticated, computer controlled, stepper motor driven torch box carriage was commisioned. This device which makes the alignment process a simple task is discussed in detail in Chapter 4).

2.2.3 OPTIMISATION OF ION TRANSMISSION FOR FULL MASS RANGE ANALYSIS

The electrostatic fields, generated by the potentials applied to the lens stack, required to give maximum sensitivity for a heavy ion, eg Pb^+, will not give optimum sensitivity for a light ion such as Li^+. The ion optics can be tuned by adjusting the potentiometer that governs the potential applied to one of the 10 electrostatic lenses. In the case of a specific analysis for a limited mass range, best sensitivities are obtained by optimising the ion optics for an element in the middle of the range of interest. As the major focus of interest of this thesis was the analysis of lead, the ion optics were tuned for maximum sensitivity on ^{208}Pb. Reasonable compromise sensitivity levels for all elements can be found by tuning the lens stack to optimise for indium, mass 115. The individual lens elements are interactive and care needs to be taken to avoid false optima. This is done by de-focusing the lenses and re-tuning to verify that a given set of conditions provided maximum sensitivity. (Operator experience rapidly leads to a knowledge of the likely maximum level of sensitivity for a given analyte in a particular matrix). It is preferable to select a tuning standard solution at a modest concentration in order to reduce the risk of memory effects.

In general, there was little day to day variation in the set of potentials required to optimise the lens stack for lead. Any significant variation was usually accompanied by a loss of sensitivity and was indicative of a fault with the instrument or a capacitive build-up on a lens element due to surface contamination.

Once the ion optics had been tuned, the auxillary, coolant and nebuliser gas flows to the plasma were adjusted to give maximum sensitivity, although these were found to be relatively constant for a given torch and nebuliser. The nebuliser gas flow rate was observed to have a marked effect on both sensitivity and stability, but once the mass flow controller had been correctly adjusted for a given nebuliser it normally required no further attention. The ion optics were re-optimised to compensate for any changes due to the optimisation of gas flows.

A short (30s) spectral accumulation over the mass range of interest was performed in order to check that the peak shapes, mass calibration and resolution were satisfactory. After any necessary adjustments in the light of the test spectrum had been made, the instrument was ready for operation.

2.3 ANALYSIS OF LEAD BY ICP-MS

2.3.1 INTRODUCTION

The VG PlasmaQuad was purchased to perform a specific study to investigate the source apportionment of lead in children. This project entailed the accurate and precise determination of lead isotope ratios in clinical and environmental samples. As the samples obtained for the project were irreplaceable, suitable methodologies for accurate and reliable determinations of lead isotope ratios had to be established on synthetic and less valuable authentic samples. Details of the project are given later (see Chapter 3).

The following sections deal with the optimisation of the instrument for the analysis of lead and the development of the appropriate methodology.

2.3.2 INSTRUMENTAL OPTIMISATION FOR THE ANALYSIS OF LEAD

The instrument was set up as described earlier (Section 2.2), but tuned to give maximum sensitivity for a standard lead solution ($100\mu g l^{-1}$ NBS 981, 1% HNO_3, 0.1% Triton X-100). The resolution controls were adjusted to give baseline separation of peaks; once set this parameter required no further adjustment in normal operation.

2.3.2.1 RF FORWARD POWER

The RF forward power is the energy supplied to the ICP for ionisation of analyte within the plasma. This parameter was set by aspirating the lead standard into the plasma with a fixed pumping rate and nebuliser gas flow and increasing the forward power from 800W to 1.5kW in 100W increments. The plasma was allowed to stabilise for 2 min at each power setting and the response was monitored by scanning the spectrometer from 200-210amu and summing the integrated lead data for all four isotopes. The experiment was repeated at five different pumping rates corresponding to peristaltic pump speed of 200, 400, 600, 800 and 1000 (0.2, 0.4, 0.6, 0.8, and 1.0 mlmin^{-1}) in order to investigate the effect of sample loading on the plasma. The ion optics were adjusted for optimum sensitivity at 1.3kW forward power at each flow rate before the experiment was begun. Each accumulation took 20s and three scans were performed for each set of parameters.

The results are presented on figure 2.1. In all cases, increasing the uptake rate from 0.2mlmin^{-1} resulted in an increase in signal. In general, maximum signal was obtained with an uptake rate of 0.8mlmin^{-1}, above this value the increase levelled off. As the RF forward power was increased from 0.9 to 1.5kW, the signal response also increased. However, at RF powers above 1.3kW the increase in signal again levelled off. With an RF power of 800W, the initial increase in signal with sample uptake rate, was followed by a decrease above 0.4mlmin^{-1}. At low sample uptake rates, there is more than sufficient energy in the plasma to fully ionise the analyte , so an increase in sample (analyte) loading results in an increase in signal (at all RF power settings studied). However, the maximum analyte signal with an RF power of 0.8kW, occurs at a sample loading of 0.4mlmin^{-1}, at higher loadings there is no longer a surplus of energy in the plasma and any additional loading serves to "cool" the plasma, resulting in a decrease in signal.

It was decided to conduct all subsequent analyses with an RF forward power of 1.35kW and a sample uptake rate of 0.8mlmin^{-1}. It was thought to be a sensible precaution to run the RF generator at the lowest acceptable output in order to enhance valve life-time and give the best output stability.

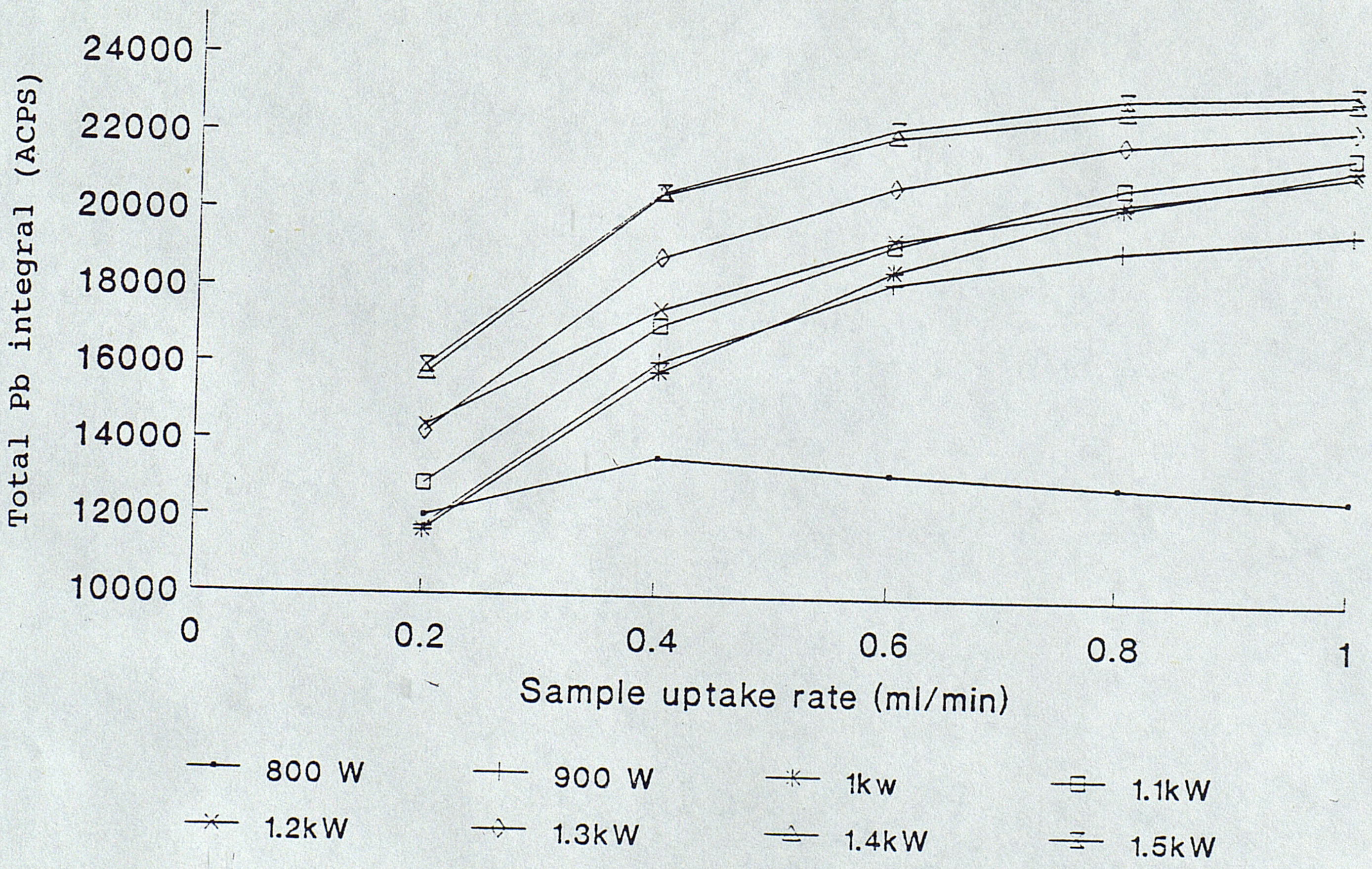

Figure 2.1. Variation of signal intensity as a function of plasma RF power and nebuliser uptake rate.

2.3.2.2 SAMPLE UPTAKE RATE

The effect of the sample uptake rate on sensitivity was confirmed for a forward power of 1.35kW by aspirating the lead standard solution into the plasma at various peristaltic pump speeds from 100-1000 units (0.1-1.0 $mlmin^{-1}$) in increments of 100 units with all other parameters held constant. Optimum sensitivity and stability was obtained with a pump speed of 800 units (0.8 $mlmin^{-1}$) and this was used for all subsequent work.

2.3.2.3 INVESTIGATION OF THE EFFECT OF DWELL TIME ON ISOTOPE RATIO PRECISION

The length of time that data acquired by the mass spectrometer is stored in a single memory channel in the MCA is called the dwell time. It governs the time that the quadrupole spends at a given mass and so controls the scan rate. Usually, several memory channels are allocated to a single mass unit - typically 20 channels for a limited mass range scan for lead. This parameter was of obvious importance in determining the precision attainable for an analysis. A series of standard lead solutions (NBS 981 from 1 to 50μgl^{-1}) was analysed over a range of dwell times using 512 memory channels on the MCA. The total scan time was held constant (at 30s) by varying the number of sweeps (repititions) performed at each dwell time. Five acquisitions were performed at each dwell time to enable a comparison of the relative precisions to be made. The conditions studied were as follows:

Number of sweeps	720	360	180	90	45
Dwell time (μs)	80	160	320	640	1280

The variation of the precision with dwell time and concentration is presented in figure 2.2. The precision improved as a function of concentration for all dwell times studied, as one would expect. The influence of dwell time itself did not have a large effect on the precision since similar precisions were obtained at concentrations above 5μgl^{-1} Pb. The mean values obtained for the ^{206}Pb:^{207}Pb ratio (n=5) and associated relative standard deviations for each dwell time studied were as follows:

Dwell time (μs)	80	160	320	640	1280
^{206}Pb:^{207}Pb	1.2258	1.2236	1.2262	1.2238	1.2306
RSD %	0.16	0.37	0.17	0.45	0.50

No trend was observed in the ratio data, implying that mass fractionation was not a function of dwell time. The dwell time chosen for future work was 320μs since it gave the best precision on both the averaged ratio and for the individual values.

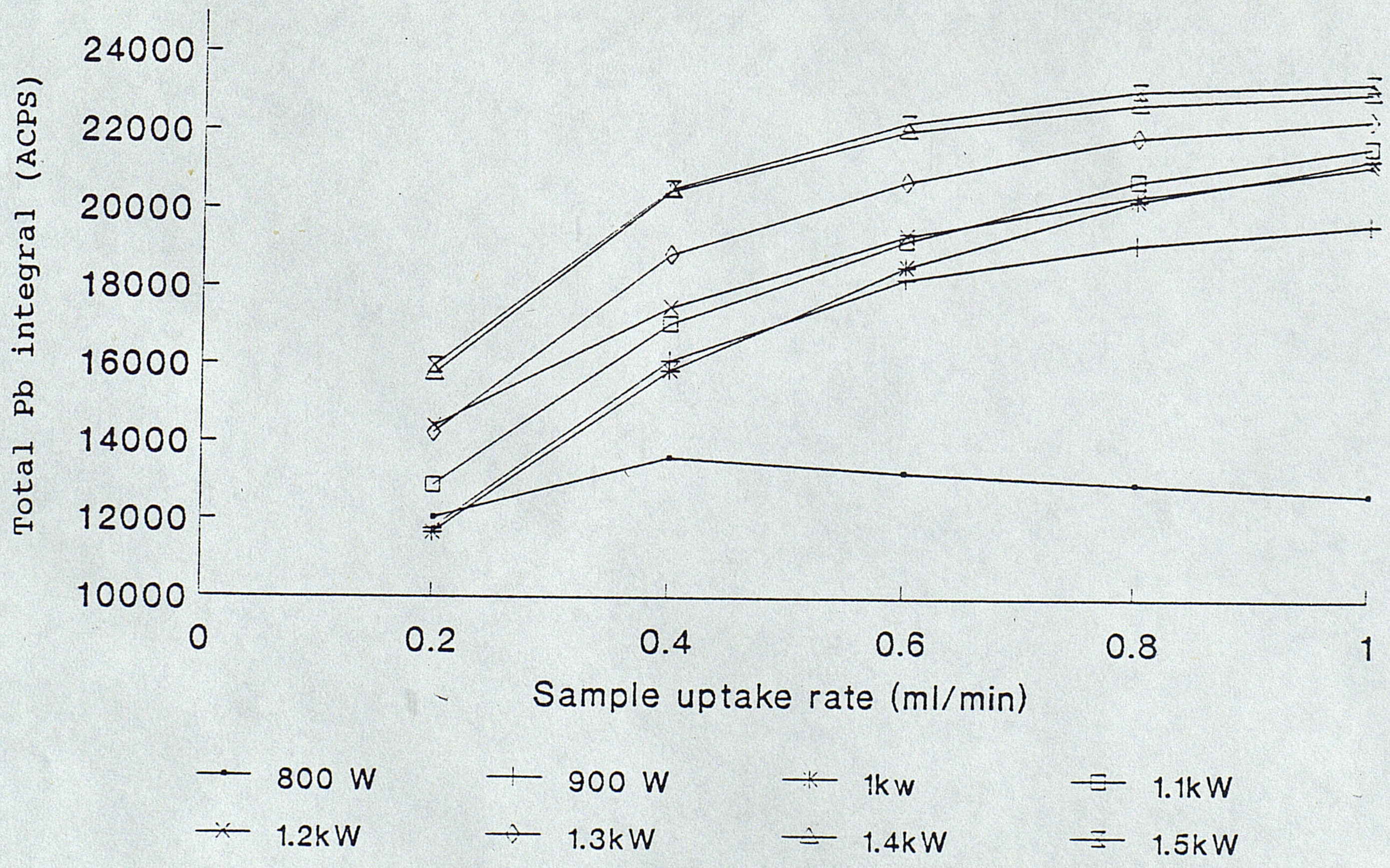

Figure 2.1. Variation of signal intensity as a function of plasma RF power and nebuliser uptake rate.

2.3.2.4 OPTIMISATION OF TOTAL ACQUISITION TIME

The duration of scan time was investigated to find the shortest time that would give an acceptable level of precision. The standard was analysed for a range of total acquisition times from 30s to 5min. Five runs were performed at each time to enable comparison of precision with acquisition time to be made.

The observed variation of the precision of the ^{206}Pb:^{207}Pb ratio is plotted on figure 2.3 as a function of run time. It can be seen that no significant improvement in the precision is obtained with counting times longer than 90s (≈0.1% RSD). It was decided to use a 90s counting time for all subsequent work since this gave acceptable precision, allowed a reasonable sample throughput and did not consume a large sample volume (≈12ml of solution was consumed per sample since five measurements were typically made and each spectrum had to be transferred to the computer, integrated and stored, before a subsequent accumulation could be started).

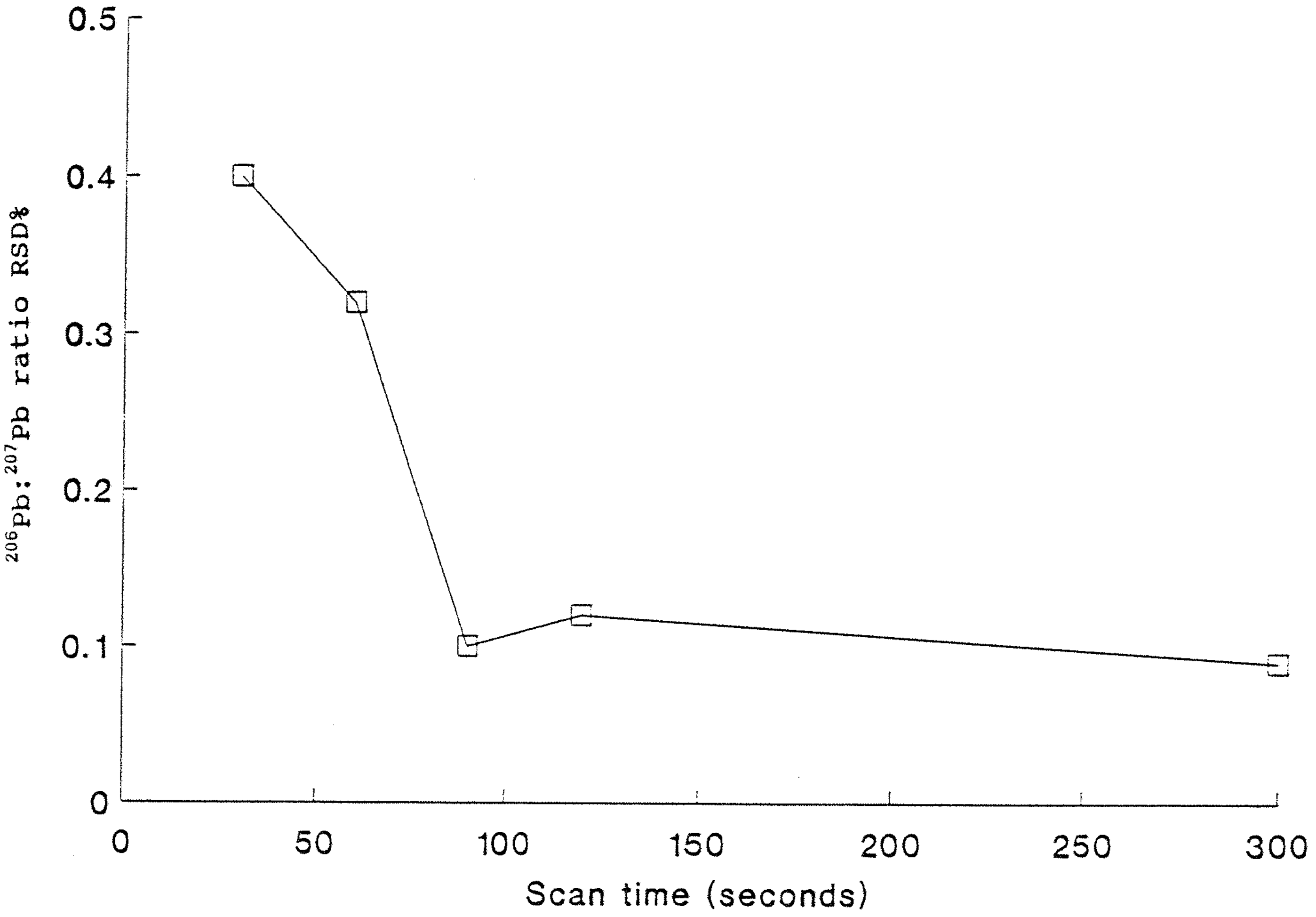

Figure 2.3. Variation of ^{206}Pb:^{207}Pb ratio precision with total acquisition time.

2.4 DEVELOPMENT OF METHODOLOGY FOR THE ANALYSIS OF LEAD IN WHOLE BLOOD

The possibility of analysing blood samples following a simple aqueous dilution was investigated. This approach had the benefits of ease of sample handling, reduced risk of contamination and greater throughput over the alternative method of wet oxidation followed by ashing of the samples in a muffle furnace. A 1+24 aqueous dilution of whole blood was found to be acceptable from the standpoint of ease of sample introduction into the ICP. However, it was observed that, on standing, the blood samples showed evidence of protein precipitation which could have lead to the blocking of the Meinhard concentric glass nebuliser and may have influenced the concentration of lead in solution by co-precipitation. The precipitate readily re-dissolved in the presence of a small quantity of ammonia solution, so it was decided to incorporate the ammonia, ammonium-dihydrogen orthophosphate, di-ammonium -EDTA matrix modifier developed by Shuttler and Delves[66] for the analysis of lead in blood by graphite furnace atomic absorption spectrometry. The addition of the modifier had three advantages (i) it promoted cell lysis; (ii) it prevented protein precipitation and (iii) it ensured that there would be no loss of trace elements from the solution to the walls of the sample container. Diluted whole blood samples were found to be stable for at least 24hr[66]. The surfactant Triton X-100 was included in the diluent/modifier.

The addition of a surfactant improved sample transport efficiency by wetting the walls of the delivery tubing and spray chamber, the latter having the benefit of reduced memory effects and improved draining of the link piece between the spray chamber and the plasma torch. The nebulisation efficiency was slightly enhanced, leading to improved sensitivity by the reduction of the surface tension of the sample solution. The optimum final concentration of Triton was found to be 1% v:v.

The sample preparation scheme used for all the blood lead analyses reported in this thesis is given below.

To a 25ml volumetric flask add:

(i) ≈4ml deionised water.

(ii) 1ml matrix modifier containing 0.14M ammonia, 0.003M ammonium dihydrogen EDTA and 0.029M ammonium dihydrogen phosphate.

(iii) 1ml blood sample.

(iv) Internal standard element (50μgl^{-1})

(v) Triton X-100 (5ml 5%v/v)

(vi) Make up to volume with deionised water.

The relative quantities of these additions were always maintained, but in cases where the volume of a given blood sample was limited all quantities were adjusted appropriately to the volume of blood taken. The internal standards used for normalisation purposes for quantitative blood lead analyses were bismuth, thallium or platinum. Quality control blood samples were analysed in all analytical runs and were prepared as for blood samples above, except that the quality control blood sample was added at step (iii) in place of the clinical sample. Bovine blood lead standards were prepared for quantitative analyses by substituting bovine blood in place of the clinical sample and making appropriate additions of an aqueous lead standard solution to produce the desired calibration range. All aqueous standard solutions were prepared by serial dilution from concentrated stock solutions that were stabilised in nitric acid.

CHAPTER 3

THE APPLICATION OF INDUCTIVELY COUPLED PLASMA-SOURCE MASS SPECTROMETRY TO THE ACCURATE AND PRECISE ANALYSIS OF LEAD ISOTOPE RATIOS IN CLINICAL AND ENVIRONMENTAL SAMPLES.

3.1. INTRODUCTION

Lead is a cumulative poison in man and animals and can be fatal if ingested in sufficient quantities. No evidence has yet been presented to suggest that lead is an essential trace element in human metabolism, but a report by Schwartz[67] indicated that low levels of lead were required for normal growth in rats.

The symptoms of lead intoxication in man include colic, nausea, anaemia (and lethargy) and encepalopathy (although acute encepalopathy is more commonly observed in paediatric cases). The anemia is as a result of the inhibition of enzymes associated with heme synthesis, notably δ-aminolaevulinic acid dehydratase and ferrochelatase. The former catalyses the formation of porphobilinogen from δ-aminolaevulinic acid and the latter is an enzyme controlling the incorporation of iron into the porphyrin ring of the heme molecule[68]. Lead also affects the fragilty of the erythrocytes resulting in a shorter life-span for the red blood cells. In children, prolonged exposure to elevated levels of lead can result in mental retardation and permanent physical damage to the brain. In recent years, attention has been focussed on the possibility that lead at low concentration might have a detrimental effect on the intelligence of children.

Most of the body burden of lead, about 90%, is stored in the bones, with the remainder distributed between the blood, soft tissues and a rapidly exchangeable bone fraction. The clearance half-life for lead in bone is ≈20 years whereas in the soft tissue and blood it is of the order of three weeks. Most of the lead in blood is associated with the red blood cells, ≥90%, so from an analytical viewpoint it is best to determine whole blood lead concentrations.

The most common source of ingestion of lead is from diet for the general public or by inhalation for occupationally exposed workers. The estimated daily exposure covers a wide range from 20-400μg Pb, although estimated daily uptake from all sources is probably 3-40μg Pb[69]. For children with pica (a compulsion to eat non-food materials) environmental sources such as soil and paint flakes may be important. Approximately 5% of lead ingested is absorbed through the gastrointestinal tract, however it is believed that all inhaled lead reaching the airway lumen is absorbed into the blood stream. Lead can cross the placental barrier between mother and fetus and the developing child will exhibit a blood lead ratio similar to the mothers' blood lead ratio.

The main mechanism for excretion of lead is via the renal system in the urine although smaller fractions are excreted in sweat, hair, nails and in the milk of lactating females. A general review of the usage of lead and its toxicological properties has been presented by Boeckx[70].

3.2 THE BIRMINGHAM PROJECT

Harvey _et al_[71] conducted a rigorous investigation of the relationship between paediatric blood lead concentration and intelligence for a carefully defined group of native Birmingham children of 2.5 years of age. In addition to the usual assessment of IQ rating, the behaviour, development and health background of the child, its family structure, the quality of the parents' relationship, their occupations and educational backgrounds, together with the age and quality of the housing and some other parameters were also investigated. Against this background, a full assessment of confounding variables (factors which could lead to the incorporation of erroneous data or mask a true effect) was made before blood lead concentration was correlated against IQ rating. They concluded that, once confounding variables had been taken into account, blood lead concentration was not a significant predictor of intelligence rating, over the range of concentration studied (0.3 to 1.45 $\mu moll^{-1}$). It was noted, however, that a relationship existed between the age of housing and blood lead concentration with a reduction in mean blood lead concentration from 0.79$\mu moll^{-1}$, for children living in houses built before 1939 to 0.66$\mu moll^{-1}$ for those living in properties built after 1939.

This relationship was further investigated as part of a second survey of Birmingham children, aged 5.5 years. The group of children investigated was selected in the same way as in the previous study, but blood samples were taken on two occasions (November 1984 and March 1985) and additional samples of household dust and garden soil were collected from the child's home. All these samples were sent to Southampton for lead isotope ratio analysis by ICP-MS. Further details and the results of these analyses are presented later in this chapter (Section 3.6).

3.3 LEAD ISOTOPE RATIO ANALYSIS IN WHOLE BLOOD SAMPLES

In order to ensure high standards of accuracy and precision, all experimental work incorporated appropriate, well characterised quality assurance samples. The internal quality control blood samples were made from bovine blood spiked with known quantities of lead. A batch of these samples was sent to Manton[72] at Texas State University, USA, for analysis by thermal ionisation isotope dilution mass spectroscopy (TI-IDMS). As a result, both the total lead concentration and the isotopic composition of these standards was accurately and precisely known.

The quadrupole mass filter sorts ions on the basis of their mass to charge ratio, hence determination of elemental isotope ratios is straightforward. However, lighter ions are not detected with the same efficiency as heavier ions, giving rise to the phenomenon known as mass discrimination[73]. Within an analytical run, the effect of mass discrimination is sufficiently constant that it can be adequately compensated for by the application of a correction factor derived from the analysis of a known isotopic standard. Alogorithms exist within the software suite to allow the computation and application of the factor to the data produced in an analytical run, making the correction for mass discrimination transparent to the user (see section 3.7.5).

The methodology for accurate determinations of whole blood lead concentration was directly applicable to the determination of isotope ratio data. It should be noted that the same data are obtained for both isotope ratio and concentration determinations ie an integrated count rate at each mass of interest. The only difference lies in the final manipulation of the data: for isotopic data, the relevant integrated count rates are ratioed, whereas for quantitative analysis they are summed for a set of isotopes and compared against an appropriate calibration.

3.3.1 PRECURSOR PEAKS

In order to be able to discriminate between different sources of lead with similar isotopic compositions, the highest possible precision and accuracy must be obtained for isotope ratio determinations. This capability was of paramount importance for the Birmingham study if subtle variations in lead isotopic ratios in clinical samples were to be attributed to relative exposure to various environmental sources.

One phenomenon that caused degradation of the precision attainable for isotope ratio determinations using the mark 1 VG PlasmaQuad was the presence of precursor peaks on the spectrum, particularly at high mass. Precursor peaks were thought to be due to the premature detection of a fraction of the ion flux at a given mass, typically <2% of the parent peak, and resulted in the formation of a distinct minor peak on the low mass side of the main peak. The presence of such peaks could lead to problems with the integration process due to the location of a false or variable valley position, thereby altering the total peak area in an irreproducible manner with obvious consequences for isotope ratio precisions. In order to avoid these problems the high mass resolution had to be increased to give baseline separation between the major peak and its precursor, with consequent loss of sensitivity.

The origin of precursor peaks was thought to be due to an assymmetry of the electrostatic fields generated by the quadrupole. Russ[74] found that precursor peaks could be virtually eliminated by placing permanent magnets at strategic points around the flight chamber, thereby modifying the shape of the quadrupole electrostatic field. When the PQ2 model was launched by VG precursor peaks were no longer apparent. Since the quadrupole RF generator, DC supply and the quadrupole were ostensibly identical to those on the Southampton instrument, I made enquiries to discover the change that had led to the elimination of the peaks. In fact the original supplier of the quadrupoles, VG Masslab, had altered the wiring on the quadrupole such that each pair of rods was supplied with RF and DC via a single copper wire with a "T"-piece junction connecting the two rods, rather than having a separate wire for each rod. The wires supplying each pair of electrodes were symmetrical and impedance matched, giving rise to a uniform field on the quadrupole. After the modification had been pointed out to VG, they agreed to modify the wiring on our instrument and the precursor peaks were eliminated. It was then possible to degrade the resolution and still maintain baseline separation between adjacent masses with a consequent increase in sensitivity. The performance of the instrument was improved and precisions of <0.5% were obtained for the ^{206}Pb:^{207}Pb ratio for a 25μgl^{-1} standard.

3.3.2 VALIDATION OF BLOOD LEAD ANALYSIS TECHNIQUE

It was essential that the accuracy and precision of the blood lead method and instrumental reliability were established as being satisfactory before irreplaceable paediatric blood samples were committed for analysis. In order to assess the technique, it was decided to analyse a large batch of blood samples (n=51) by ICP-MS[75] and the established, accurate flame atomic absorption spectrometry (MSFAAS)[76] technique for total lead concentration. The samples were selected from those received for routine clinical analysis by the Supraregional Assay Service and were chosen to give an approximately even distribution of low, medium and high exposure levels (ranging from 0.24 to 2.88 μmoll^{-1}). The sample preparation and scanning parameters used were as stated in Chapter two (Section 2.5). NB this data was obtained with the original instrument.

The concentration of lead in whole blood was determined by preparing a set of calibration standards by adding aqueous lead at 0 to 3.0 μmoll^{-1} to whole bovine blood. Test blood samples and internal quality control samples were then evaluated against these standards. The data used for the calibration were the sum of the blank subtracted integrated (area counts/s) data for ^{206}Pb, ^{207}Pb and ^{208}Pb for the standards, normalized against the bismuth internal standard count rate. The quality of the data was assured by the analysis of a set of three internal quality control standards

(IQC X, IQC Y and IQC Z) before and after each group of five samples until all the samples in a run had been analysed. Only if these QCs fell within a defined range was the data accepted (vide infra).

In order to establish the accuracy and precision of isotopic ratio analysis in whole blood, all available internal quality control samples (n=10) were analysed by ICP-MS and the results compared against those obtained by TI-IDMS. Compensation for mass discrimination was achieved by calibrating the instrument against a known isotopic certified reference material (CRM) (NBS 981 (25μgl^{-1})) and re-analysing this solution, declared to the system as a sample, immediately afterwards to verify that the correction routine satisfactorily compensated for the phenomenon.

3.3.3 RESULTS AND DISCUSSION

The 51 blood samples were analysed over a three week period with a typical experimental run lasting between 3-4 hr. Data were normalized against the count rate for the internal standard element (bismuth), to compensate for instrumental fluctuations and loss of analyte as dried blood on the wall of the injector tube at its constriction. A typical linear calibration curve is shown in Figure 3.1. The target values for the QC standards, together with the mean ICP-MS data obtained and the within run and between batch relative standard deviations are presented in Table 3.1.

The accuracy of the method can be seen from Figure 3.2 which compares the ICP-MS and MSFAAS data for the blood samples analysed. The accuracy of the ICP-MS data is comparable to that obtained for the routine technique. The regression equation for the data is:

$$ICPMS = 0.966MSFAAS - 0.0165\mu mol l^{-1}$$

with a correlation co-efficient of 0.994.

The results of the lead isotopic ratio determinations for the IQC standards are presented in Table 3.2 and compared with the TI-IDMS data obtained by Manton[72]. The initial calibration for mass discrimination was carried out using bovine IQC standard IQC Z, for reasons of simplicity. However, later corrections were made using an aqueous lead standard, NBS SRM 981 ($25\mu gl^{-1}$) without problem. It is obvious from Table 3.2 that either mode of calibration for mass discrimination provides an acceptable correction and gives excellent agreement with the TI-IDMS data. In all subsequent isotope ratio determinations, an aqueous NBS 981 calibrating standard was used to compensate for mass discrimination.

Further details of these and some other validation experiments (using the original instrument) are given elsewhere[75].

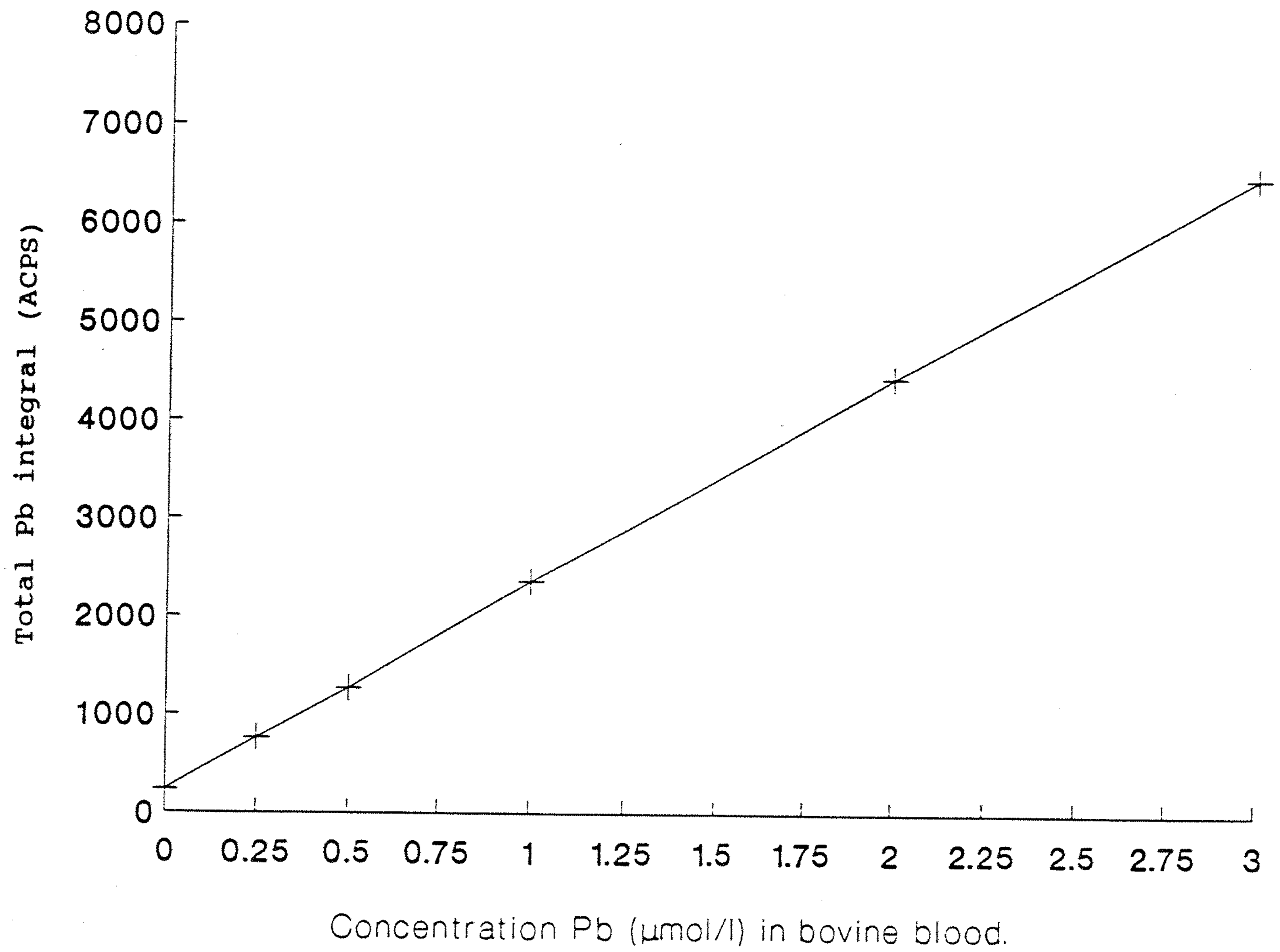

Figure 3.1. Calibration graph for lead in whole bovine blood.

Table 3.1. Comparison of IDMS and ICP-MS results for the analysis of lead in some bovine blood IQC standards, including the within run and between batch precision data for the ICP-MS measurements.

Sample	Mean concentration Pb (μmoll^{-1})			Relative standard deviation	
	Target range	ICP-MS	IDMS	Within-run (N)	Between-run (N^*)
O	0.08-0.13	0.10	0.085	3.42 (5)	23.60 (11)
X	0.49-0.62	0.54	0.55	1.40 (5)	5.52 (32)
Y	1.60-1.75	1.64	1.70	1.52 (5)	3.57 (31)
Z	3.15-3.40	3.35	3.27	2.64 (5)	3.44 (28)

N =Number of observations

N^*=Total number of measurements made over a three-week period.

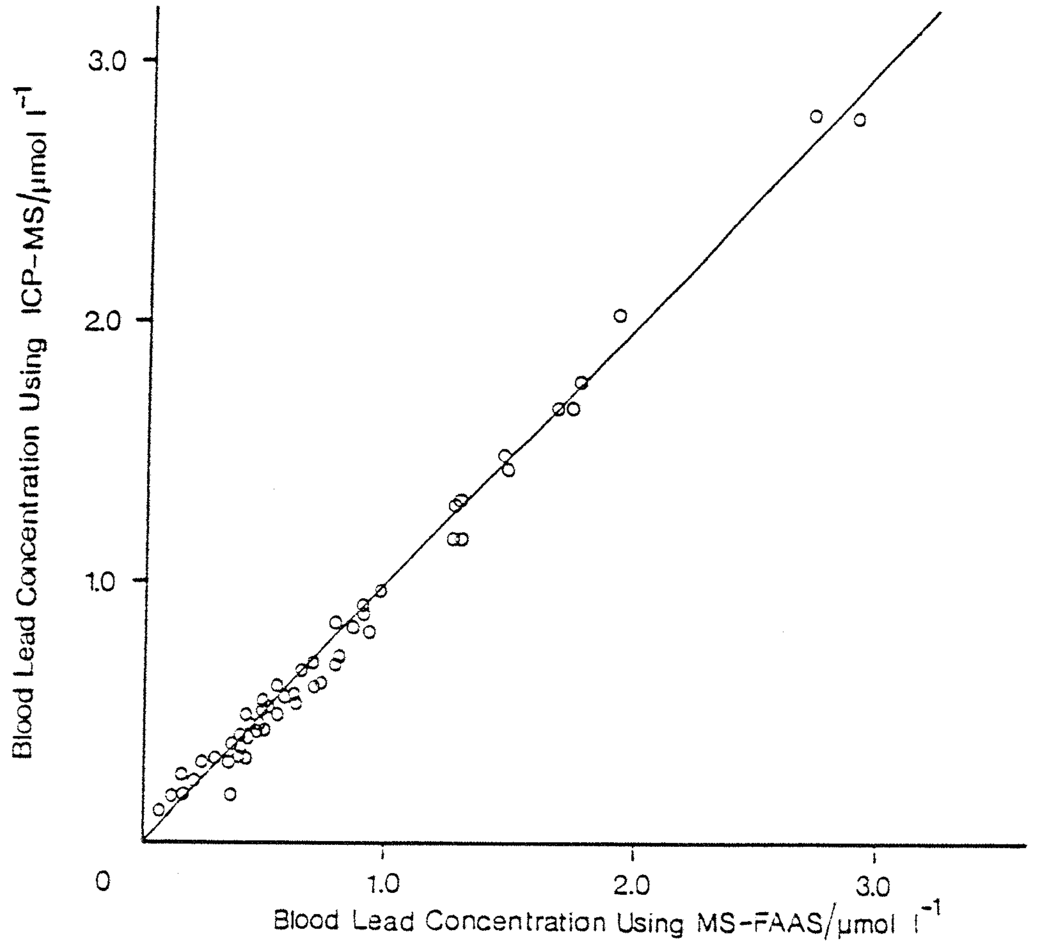

Figure 3.2. A comparison of ICP-MS with micro-sampling FAAS for determination of lead in blood.

Table 3.2. A comparison of ICP-MS and IDMS results for the analysis of the isotopic composition of lead in bovine blood IQC standards.

IQC Code	Concentration Pb (μmoll^{-1})	^{206}Pb:^{207}Pb			Error in ICP-MS Data %	
		IDMS	ICP-MS			
			A	B	A	B
O	0.085	1.127	1.180	1.122	4.70	-0.44
F	0.35	1.125	1.107	1.121	-1.60	-0.36
W	0.36	1.115	1.149	1.101	3.05	-1.26
X	0.56	1.153	1.171	1.143	1.56	-0.87
A	0.69	1.113	1.096	1.112	-1.53	-0.09
D	1.30	1.113	1.104	1.110	-0.81	-0.27
Y	1.68	1.157	1.146	1.151	-0.95	-0.52
B	1.77	1.109	1.111	1.102	0.18	-0.63
C	3.09	1.108	1.106	1.101	-0.18	-0.63
Z	3.27	1.159	1.151	1.170	-0.69	0.95

A = Using IQC Z as a calibrant.   B = Using NBS 981 as a calibrant.

3.4 SOURCE APPORTIONMENT STUDIES

Lead has four naturally occurring isotopes, ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb. The ^{204}Pb isotope is naturally occurring lead which was laid down in ore deposits as the planet formed. The other isotopes of lead are also formed as the daughter nuclides of radioactive decay, from thorium and uranium by alpha emission. The half-lives for the decay leading to these isotopes are given below:

$$^{238}U \xrightarrow{8\alpha} {}^{206}Pb \qquad T_{1/2}=4.5\mathrm{x}10^{9}Y$$

$$^{235}U \xrightarrow{7\alpha} {}^{207}Pb \qquad T_{1/2}=7.1\mathrm{x}10^{8}Y$$

$$^{232}Th \xrightarrow{6\alpha} {}^{208}Pb \qquad T_{1/2}=1.4\mathrm{x}10^{10}Y$$

The isotopic composition of a given lead ore deposit is therefore dependent upon the age of the deposit and the relative quantities of uranium and thorium in the parent strata. Lead ores from different geographical locations may be isotopically distinct. Such differences in isotopic composition will be reflected in any artefacts made from the ore. It is possible to differentiate between two lead ore samples on the basis of their isotopic composition, if the magnitude of the difference in isotope ratio is greater than the limit of accuracy of the instrumental technique (≈0.5% for ICP-MS). If an artefact is produced from two isotopically distinct sources of lead, its isotopic composition will be dependent upon the relative proportions of the sources used and their isotopic compositions. If equal quantities of lead from each source were used, the isotopic composition of the artefact would correspond to the average value of the two sources. In this way, it is possible to make apportionments of the relative contributions of a given source of lead to a mixture of sources if the isotopic compositon of the source and composite material are distinct and known. The isotopic ratio of an artefact containing n isotopically distinct lead sources is given by:

$$R_{obs} = \Sigma X_a R_a + X_b R_b + \ldots\ldots X_n R_n \qquad (3.1)$$

where R_a is the ratio of the artefact.

X_a, X_b, X_n are the fractional compositions of the sources a,b,n and $\Sigma\ X_a,\ X_b \ldots\ldots X_n = 1$ (3.2)

Equation 3.1 may be re-written as:

$$R_{obs} = X_a R_a + (1 - X_a) R_{av} \qquad (3.3)$$

where R_{av} is the average ratio for all constituents other than a.

The use of these equations is demonstrated in the following example:

The tetra-ethyl lead anti-knock agent which is added to petrol to promote smooth burning and lubricate the valves in internal combustion engines, consists of lead from two different sources: Broken Hill (Australia $^{206}Pb:^{207}Pb = 1.03$) and a Canadian source ($^{206}Pb:^{207}Pb = 1.16$). Petrol vehicle exhaust residue was determined to have a $^{206}Pb:^{207}Pb$ ratio of 1.064, due to the anti-knock additive, by analysis using ICP-MS (section 3.3.5).

Hence $R_{obs} = X_C(1.16)+(1-X_C)1.03$ (3.4)

Where X_C is the fractional composition of Canadian lead

$= 1.064= X_C(1.16)+(1-X_C)1.03$

and $X_C=0.266$ since $X_{BH}+X_C=1$ then $X_{BH}=0.74$

The manufacturers estimate that Broken Hill and Canadian lead are used in proportions of 70:30. Given the approximate nature of their estimate and that a small quantity of lead is re-cycled during manufacture, the values obtained, 74% Broken Hill and 26% Canadian lead, agree well and indicate the usefulness of this type of calculation.

3.5 EDINBURGH STUDY

3.5.1 INTRODUCTION

In 1987 Fulton et al[77] reported the results of a study in which childrens' blood lead concentrations were determined and correlated against a rating of intelligence. The study concluded that there was a significant 2.6 point reduction in IQ for children with an elevated blood lead concentration. In an earlier investigation, with a similar experimental design, a group of London children were studied by Smith et al[78]. The blood lead concentrations observed in the two studies were similar (ranges of 2.9-34.0μg/100ml Edinburgh and 7.0-43.0μg/100ml London) however, the reduction in IQ with increased lead exposure of 0.7 points observed in the London study was not significant. One major difference between these two studies was the much higher concentrations of lead observed in the deciduous teeth of the Edinburgh children which were nearly three times higher than their London counterparts (9.9μgg^{-1} for Edinburgh and 3.5μgg^{-1} for London). Blood lead concentration acts as an index of recent exposure, whereas deciduous tooth lead levels reflect cummulative exposure starting before birth as the teeth develop. It was decided to determine whether the sources of lead exposure were different at those periods of the childs development indicated by tooth lead and blood lead concentrations at the time of shedding the teeth, for both studies.

Samples of teeth and blood obtained from children who participated in either the Edinburgh or the London studies were kindly made available to us by the organisers. The isotopic composition of lead in these samples was investigated using ICP-MS.

3.5.2 BLOOD LEAD ANALYSIS

Twenty and twenty one venous blood samples (1-2ml) were supplied from the Edinburgh and London studies respectively. The samples had been stored in a freezer at -20°c since collection and were in a sufficiently good condition to allow representative portions to be taken for analysis for this study. As an additional control for this work, eight blood samples taken from a group of adults living outside London were also analysed. Matched maternal and placental cord blood samples from subjects living in Dundee (n=8) and blood samples from mothers of young children living in Ayr (n=20) were made available for the study (vide infra).

The normal blood lead sample preparation procedure, as discussed in section 2.5, using a platinum internal standard was followed for these samples. Internal quality control standard Z (IQC Z) was analysed before and after each group of five samples, as outlined previously. Aqueous lead isotope ratio standard NBS 981 was analysed at the outset of each analytical run to calibrate for mass discrimination and repeated immediately afterwards and before each analysis of IQC Z as a check of the calibration.

3.5.3 TOOTH LEAD ANALYSIS

Thirty acidifed solutions of ashed, deciduous teeth and ten whole deciduous tooth samples were obtained from the organisers of the Edinburgh and London studies respectively. Unfortunately, it was not possible to obtain matched blood and tooth samples from either survey. The whole, caries-free, deciduous teeth were cleaned by repeated washes in a hot "Decon" solution (10% v/v) with rinsing in deionised water in between washes. The teeth were dried, weighed, placed in acid washed quartz conical flasks and nitric acid (5ml 20% v/v) was added. The flasks were heated on a hot plate until the solution was near dryness and a further aliquot of nitric acid was added. The flasks were again warmed to ensure all the material was in solution before being transfered to volumetric flasks (20ml). The final solution contained platinum ($100\mu g l^{-1}$) as an internal standard and Triton X-100 (0.1%v/v). The tooth solutions provided from the Edinburgh study were diluted 1+9 for analysis and contained the same concentrations of platinum and Triton as the London samples. Appropriate blanks samples were prepared alongside the tooth samples as a check of lead contamination resulting from the preparation routine.

3.5.4 ENVIRONMENTAL SAMPLES

In order to be able to assess the likely source apportionment of the observed tooth and blood lead isotope ratios, a limited number of environmental samples relevant to each group of children, were analysed.

Duplicate samples (20-50mg) of paint flakes, playground dust, lead plumbing pipe work (taken from domestic water supply pipes in Edinburgh) and UK petrol vehicle exhaust residue were dissolved in nitric acid (10ml 50%v/v) in acid washed quartz conical flasks. The samples were covered, left to stand overnight and then heated to near dryness on a hot plate. A further aliquot of nitric acid was added and the samples were warmed, in order to ensure that all extractable lead was taken in to solution. The solutions were transferred to weighed, acid washed universal containers and diluted to approximately 25ml, by weight, with deionised water. The samples were diluted to give approximately $100\mu gl^{-1}$ of lead on the basis of the assumed lead content of the sample and the weight taken (NB all samples of a given type were diluted to the same extent). The final solution also contained a platinum internal standard ($100\mu gl^{-1}$) and Triton X-100 (0.1% v/v). Also samples of drinking water from Edinburgh, with a known low lead (1 to $4.6\mu gl^{-1}$) content, were also analysed.

Additionally, a small number of dietary samples were analysed to make a tentative assesment of the likely contribution of lead from foodstuffs.

3.5.5 RESULTS AND DISCUSSION

The internal quality control data for the $^{206}Pb:^{207}Pb$ ratio showed an an average bias relative to thermal ionisation mass spectrometry[72] of -0.172% and -0.177% for the aqueous lead standard (NBS CRM 981) and the quality control blood sample (IQC Z) respectively. The average within batch relative standard deviations associated with these data ranged from 0.26 to 0.49%. A similar level of accuracy for the isotope ratio measurements on the sample solutions to that observed for the concurrently analysed quality control materials was considered to be likely[75].

The clinical samples were assigned to groups on the basis of their total lead concentrations and geographical location from information received from the study organizers. The observed lead isotopic ratios for the clinical data is presented in table 3.3. The results are presented graphically in figure 3.3, the error bars represent the 95% confidence limits of mean values.

Table 3.3. Isotopic composition of lead in tissues of subjects participating in the Edinburgh study.

Code	A	B	C	D	E	F	G	H	J	K
Location	AYR		EDINBURGH				DUNDEE	LONDON		
Tissue	BLOOD		BLOOD		TEETH		BLOOD	BLOOD		TEETH
Mean total Pb*	7.2	21.3	6.0	20.9	3.7	24.5	6.5	11.2	15.5	3.8
$^{206}Pb:^{207}Pb$	1.120	1.150	1.122	1.147	1.129	1.151	1.123	1.125	1.111	1.108
SDx1000	7.0	14.8	6.4	10.6	19.6	8.1	5.2	3.7	9.1	5.9
No. samples	10	10	10	10	15	15	8	8	21	10

* μg/100ml blood; μgg^{-1} teeth, diet.

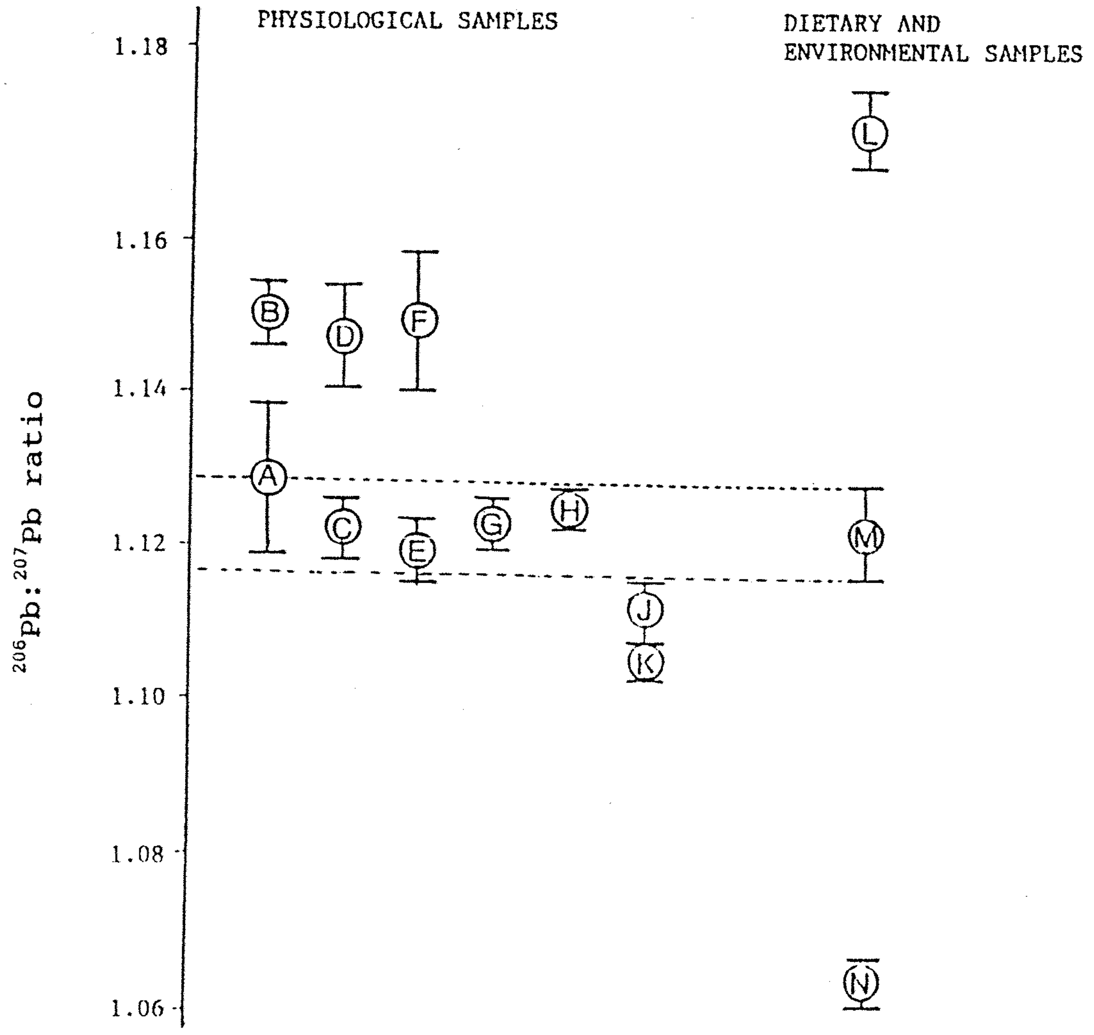

Figure 3.3. Observed $^{206}Pb:^{207}Pb$ ratios in body tissues, dietary and environmental samples.

Blood samples from Ayr and Dundee had been analysed in previous studies for their total lead concentrations by Sherlock et al[79] and Zarembski et al[80] respectively. The occupants of the city of Dundee are known to have one of the lowest blood lead concentrations observed in the UK. The matched placental cord and maternal blood samples analysed were used as a low level exposure control group (Group G). Two sets of blood samples taken from mothers of young children living in Ayr, were analysed since Sherlock et al[79] had demonstrated that water lead levels in Ayr had posed a problem prior to 1981 when the water supply was treated with lime to reduce its plumbosolvency by increasing its pH. Group A had low blood lead and water lead ($<10\mu gl^{-1}$) in 1980 and 1983; Group B had high blood lead in both years and also high water lead
($420-2570\mu gl^{-1}$ in 1980 and $60-288\mu gl^{-1}$ in 1983).

The observed ^{206}Pb:^{207}Pb ratio for the UK exhaust residue (N) was 1.064 and reflects the isotopic composition of lead used in the tetra-ethyl anti-knock agent added to petrol in the UK. The sample of Victorian lead pipe work (L), taken from the Edinburgh water supply system, had a ^{206}Pb:^{207}Pb ratio of 1.182.

Groups A, C, E, G and H represent low levels of lead exposure. Since the 95% confidence intervals for these groups overlap, the ratios determined for these samples are part of the same distribution (i.e. on a statistical basis it is not possible to distinguish between the sources of lead ingested by members of these groups.) These data also fall inside the 95% confidence interval about the mean ratio (^{206}Pb:^{207}Pb =1.123) for the low level lead environmental (playground dust) and dietary sources (drinking water, diet and bovine tissues) (M). It seems reasonable that this value represents an average background composition for low level lead sources in the UK (see also Chapter 4).

It is apparent fom figure 3.3 that the dominant source of lead for children in Edinburgh (groups C, D, E and F) is different from that for London children (groups J and K). The data suggest that lead from the water supply is a dominant source for the Edinburgh children (contributing 50 to 60% of the body burden), since the Scottish subjects with higher lead concentrations in their tissues (B, D and F) have ratios closer to that for water than their controls (A, C, E and G). Note that the Ayr subjects in Group B were found to have much higher lead concentrations in their water supply than their counterparts in Group A (Sherlock et al[79]). Lead from petrol vehicle exhaust emmisions appears to be a major source for London children (Groups J and K), since their tissue ratios were biased towards the petrol lead ratio in comparison to their control group (Group H) which reflected the UK low lead exposure composition. It seems likely that some 30 to 40% of the London children's body lead burden may be attributable to lead derived from the petrol additive.

Given that the lead concentration in the teeth of the Scottish high exposure group was approximately three times higher than that of their London counterparts, it is possible that the degree of ante-natal and neo-natal lead exposure had a greater effect on the developing nervous systems of the Edinburgh children. The importance of the role of water lead during pregnancy and the neonatal period in childhood lead exposure in Edinburgh, shown by the teeth data presented (Groups A, B and K), may help to explain, at least in part, the diffrence in the magnitude of the effects of lead on childhood intelligence reported by Fulton et al[77] and Smith et al[78].

3.6 THE BIRMINGHAM BLOOD LEAD ISOTOPE RATIO STUDY

In order to investigate the finding of Harvey et al [71] that a correlation existed in Birmingham between paediatric blood lead concentration and the age of the housing in which the child lived, samples of blood were taken from participating children on two occasions (November 1984 and March 1985) together with samples of garden soil and household dust and sent to Southampton University for isotopic analysis by ICP-MS.

3.6.1 TEMPORAL STABILITY OF THE BLOOD LEAD RATIO

Before embarking on the analysis of paediatric blood samples it was important to demonstrate that the blood lead ratio for an individual did not fluctuate significantly with time when not excessively exposed to a specific source of lead. In order to assess the temporal stabiltiy of the blood lead ratio, samples were taken on three occasions over a 220 day period from a group of six healthy adult volunteers (although there are obvious differences in the behaviour patterns, and therefore possible exposure risks of adults compared to children, it would not have been possible to obtain ethical approval for such a study in children). The samples were collected stored and prepared for analysis as described in the following section.

The results of the study are presented in figure 3.4. In all but one case, the subjects showed little variation of the $^{206}Pb:^{207}Pb$ ratio, indeed the inter-group relative standard deviation 0.48% (n=18) is less than the typical within batch rsd ≤0.8% which indicates that the variation on the ratio was less than the uncertainty on the measurement. The one outlying result (depicted by the square symbol) shows a 1.1% change in ratio from 1.139 to 1.127 between the first and second measurements. It is possible that this change was due to a contamination of the first sample at some stage. The only female subject in the study exhibited very little change in her blood lead ratio ($^{206}Pb:^{207}Pb$ =1.118 rsd=0.05% n=3) over the course of the experiment. On such a small sample size and with no details of the subject's age, it is impossible to draw any conclusions as to the influence of the menstrual cycle on the blood lead ratio.

On the basis of this study, the temporal stability of the blood lead ratio was assessed to be essentially constant over a period of 220 days. The blood samples collected for the Birmingham study were collected over a period not exceeding 150 days and could therefore be assumed to exhibit sufficient temporal stability such that any changes observed between the two samples from a child would signify a change in the source(s) of lead exposure for it.

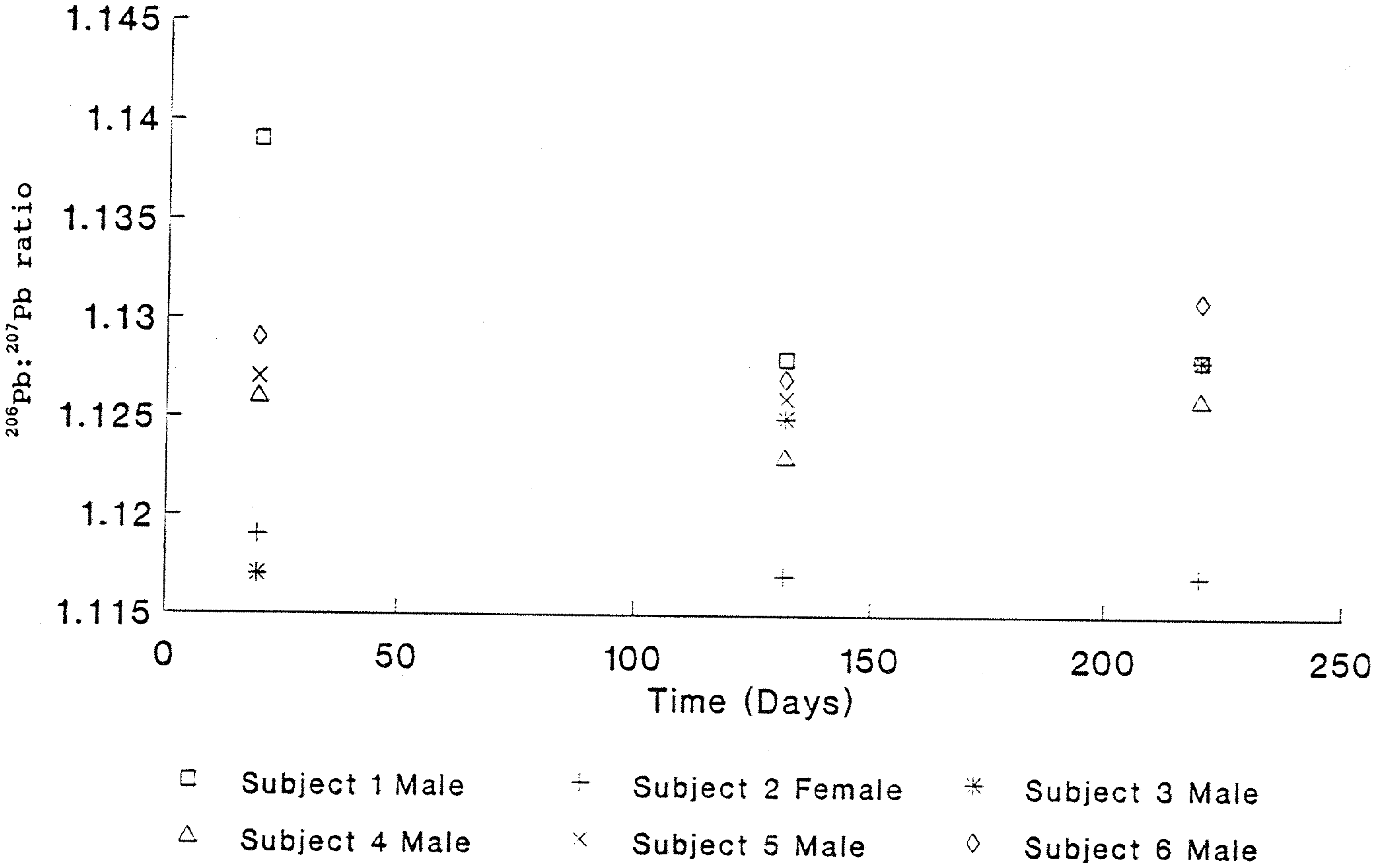

Figure 3.4. Temporal stability of blood lead isotopic composition.

3.6.2 BLOOD LEAD ISOTOPE RATIO ANALYSIS

The paediatric blood samples (2ml) for the Birmingham study were collected by venepuncture and transferred from the syringe into trace element-free polycarbonate tubes (Teklab, Birmingham), containing an EDTA anti-coagulant. The samples were mixed with the EDTA by gentle, repeated inversion to prevent clotting of the blood, and were stored in a freezer at -20°c until required.

When a batch of samples was taken for analysis it was transferred to a refrigerator at +4°c prior to the preparation of the sample for analysis. The samples were placed on a roller mixer and allowed to mix and attain ambient temperature for at least one hour prior to preparation for analysis. The usual blood lead solution preparation procedure was applied to the Birmingham paediatric blood samples (section 2.5). Typically, blood samples for six children were analysed in a given analytical run such that isotope ratio data from both blood samples for an individual child were analysed in the same run. The following analysis sequence was used for each batch of blood samples:

(i) Blank solution
(ii) NBS Calibrating standard (25μgl^{-1})
(iii) NBS Test standard (25μgl^{-1})

(iv) Internal quality control blood sample (IQC Z)
(v) Blood samples 1-6
(vi) NBS Test standard ($25\mu g l^{-1}$)
(vii) IQC Z
(viii) Blood samples 7-12
(ix) NBS Test standard ($25\mu g l^{-1}$)
(x) IQC Z

After analysis of a blood solution, an ammonia solution (5% v/v) was aspirated for approximately 2 minutes to clean the delivery tubing and prevent clogging of the sinter in the drain line and blockaging of the nebuliser, by deposition of proteinaceous material. This was followed by a wash with Triton X-100 (1% v/v) for a further two minutes, to reduce the lead signal on ^{208}Pb to the blank level, prior to the introduction of the next sample. A typical analysis procedure would last about four and a half hours.

Each solution was measured five times to allow the precision of the analysis to be assesed. If it was not possible to obtain a precision better than 1.5% the data was examined for outliers and these were rejected. In cases where it was not possible to achieve sufficient precision samples were re-analysed. The acceptance limit for the ^{206}Pb:^{207}Pb ratio of IQC Z were ±1% of its target value (1.159 ±0.01159). All experimental runs were acceptable by this criterion.

3.6.3 SCANNING PARAMETERS AND DATA STORAGE

All data was initially stored on the hard disk of the IBM XT Personal Computer. After data reduction and calculations had been performed, the raw spectral files were archived onto floppy disks. Each spectrum took approximately 90 seconds to accumulate, the scan conditions were as follows:

Mass range : 190-210 amu
Dwell time : 320μs
MCA Memory : 512 Channels
Integration: Valley
Dead time : 10ns

The concentration of lead in these samples had previously been determined by MSFAAS and no attempt was made to repeat this analysis using ICP-MS, since the purpose of this study was the determination of the lead isotope ratio of these samples. It was possible to get a very good indication of blood lead concentration by normalising the data against the internal standard and using the known concentration of IQC Z as a single calibrating standard (this approach was used to confirm some clinical lead analyses where the sample was subsequently analysed by ICP-MS to identify the likely source of a dangerously high blood lead concentration. See chapter 4).

3.7.3 BIRMINGHAM ENVIRONMENTAL LEAD ISOTOPE RATIO STUDY

The household dust and garden soil samples that were collected from the homes of children participating in the Birmingham study were initially sent to Dr Ian Thornton at Imperial College, London, for determination of the total lead concentration of the samples. The samples, which had been seived and milled to give a uniform particle size, were then sent to Southampton University for lead isotope analysis.

In order to minimise the risk of heterogeneity, relatively large portions of sample (≈1g) were analysed. Each environmental sample was analysed in duplicate and five measurements were made on both final solutions. This policy left sufficient of the original material to enable a repeat preparation to be made if required. Soil and household dust samples were weighed by difference in small (≈0.4g) plastic weighing boats and transferred to acid washed quartz conical flasks (50ml). Nitric acid (4ml 50% v/v) was added to each flask and left to stand overnight. The samples were placed on a hot plate and evapourated to near dryness then a further aliquot of nitric acid was added. The procedure was repeated and a third aliquot of nitric acid was added, the samples were allowed to warm to ensure that all the extractable lead was taken into solution then they were removed from the hot plate and allowed to cool. The samples were transferred to weighed, acid washed universal containers and then diluted to approximately 25ml with deionised water, by weight. The final (stock) solution contained approximately 2% nitric acid, which was sufficient to ensure the stability of the trace element content of the solution.

Although it is likely that the dissolution procedure would have extracted all the lead from the sample into solution, analytical recoveries were not investigated since interest was focussed on the isotopic composition of the samples rather than their concentration and appropriate certified reference materials were not available. Clearly the isotopic composition of the lead extracted into solution would not be a function of the efficiency of the dissolution procedure used, unless contributions from the reagent blank became excessive. As with all other work reported, blank samples were prepared alongside the environmental samples (blank levels were never observed to be higher than 0.1% of the environmental concentration).

The stock solutions were diluted 1+499 with deionised water for analysis. The final solution also contained a thallium internal standard ($50\mu gl^{-1}$) and Triton X-100 (0.1% v/v). This dilution gave a typical final lead concentration in the range $50\text{-}150\mu gl^{-1}$.

The following analysis protocol was used for all the environmental sample analyses:

(i) Blank solution
(ii) NBS 981 Calibrating standard (100μgl^{-1})
(iii) NBS 981 Test standard (100μgl^{-1})
(iv) NBS 982 Test standard (100μgl^{-1})
(v) Duplicate dust samples for child A
(vi) Duplicate soil samples for child A
(vii) Duplicate dust samples for child B
(viii) NBS 981 Test standard (100μgl^{-1})
(ix) NBS 982 Test standard (100μgl^{-1})
(x) Duplicate soil samples for child B
(xi) Duplicate dust samples for child C
(xii) Duplicate soil samples for child C
(xiii) NBS 981 Test standard (100μgl^{-1})
(xiv) NBS 982 Test standard (100μgl^{-1})

In this way, all the environmental samples provided for three individuals could be analysed in duplicate in a single analytical run. The scanning parameters and data storage were as described earlier (section 3.6.3).

The NBS 982 standard was used for quality control purposes. If a result for the NBS 982 ^{206}Pb:^{207}Pb ratio deviated from the certified value[81] by more than ±1% results bracketed by the standard would be rejected. The limit was 2.141±0.0241 and no data had to be rejected by this criterion.

3.7.4 BIRMINGHAM STUDY RESULTS

3.7.5. MASS FRACTIONATION

The quadrupole mass filter does not collect all ions with equal efficiency, heavier ions being collected with a greater efficiency. This effect would lead to erroneous determinations of isotopic ratios if not adequately compensated for. The extent of such fractionation is relatively constant and can be determined by the analysis of well characterised isotopic standards, to produce a correction factor which can subsequently be applied to all analyses. A software derived correction factor provided with VG PlasmaQuad makes these corrections transparent to the user, once a suitable standard and its "true" isotopic composition has been declared to the system.

It was observed that the instrumentally derived correction gave a slight systematic bias to the data[82] . To compensate for this effect, the results were re-corrected by multiplying the data by a factor equal to the certified NBS ratio value, divided by the average ratio value for the isotopic ratio standards run before and after a group of samples. This procedure compensated for the small bias introduced by the software routine and had the added advantage of reducing the influence of short term drifts.

The influence of the secondary correction routine can be observed on figure 3.5. This figure shows the variation in the observed ^{206}Pb:^{207}Pb ratio for NBS 981 (graph A) and IQC Z (graph B), corrected using the instrumental routine only. The dotted lines on the figure represent ±0.5% of the "true" ratio values for the standards, ten results for IQC Z, on graph B, fall outside this limit. The data presented in graph C was corrected using the secondary correction routine and only three results now fall outside the limit. Each point on the graph was the mean value for five analyses on a given sample and the data was collected over a three month period as the quality control data for the Birmingham blood lead survey (section 3.6.2 and 3.7.7).

The variation of mass fractionation with time was monitored during the analysis of the Birmingham environmental samples, over a period of fifty seven days. The instrumentally derived mass fractionation correction coefficient for the ^{206}Pb:^{207}Pb ratio was used as an index of variation. The average correction factor was 0.98184 (n=22) with a standard deviation of 5.92×10^{-3} (c.v=0.6%). This result indicates that mass fractionation is relatively constant in the long term, and is not greatly influenced by day to day alterations in instrumental parameters such as the electrostatic lens settings and gas flow rates. When the secondary correction routine was applied to the data, the mean correction factor

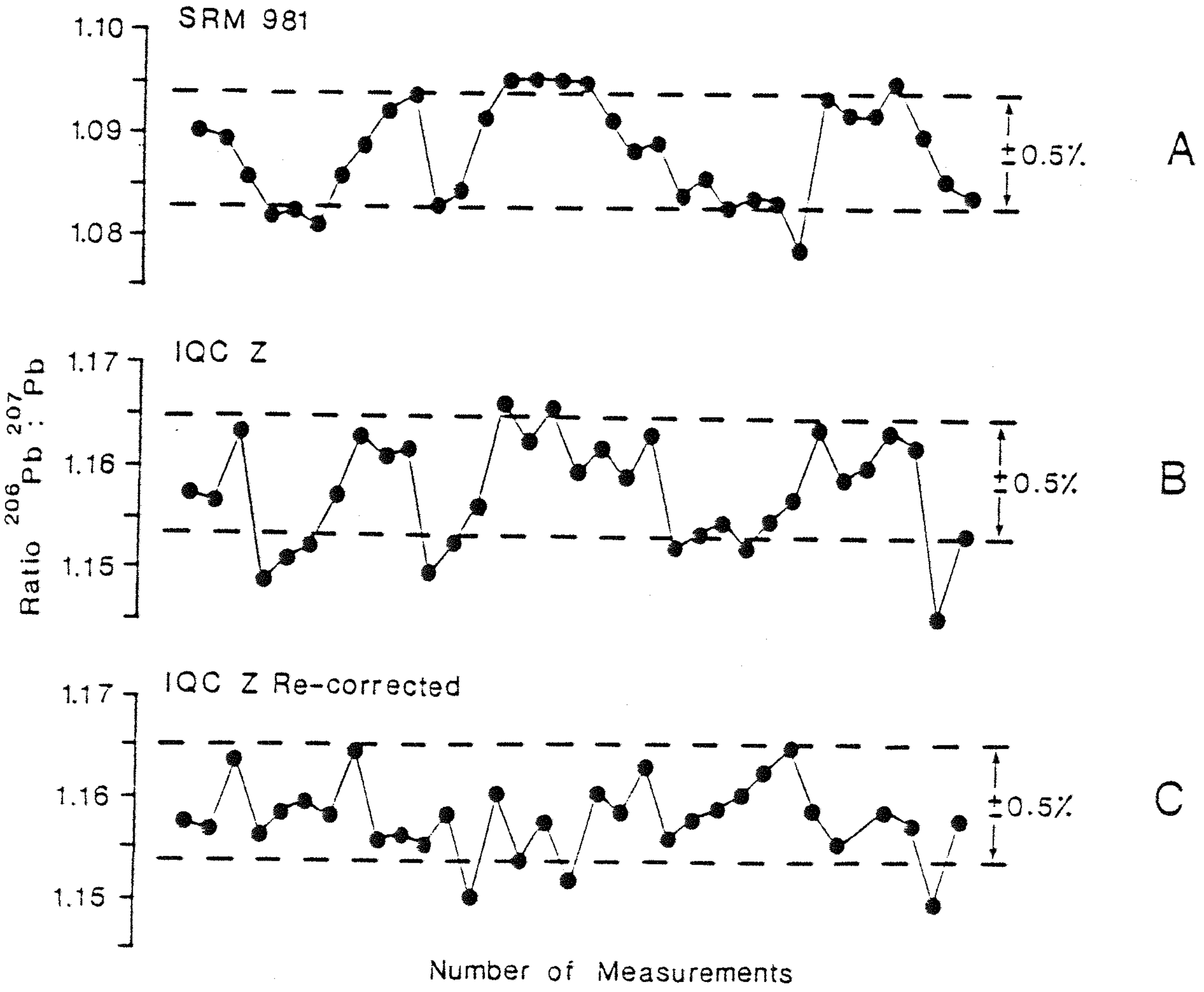

Figure 3.5. Variation of observed $^{206}Pb:^{207}Pb$ ratios for (a) NBS 981 and (b) IQC Z over a four month period using instrumental correction for mass fractionation (a and b) and a secondary correction for IQC Z (c).

was 0.99995 with a relative standard deviation of 0.2%, indicating the improvement of accuracy over the original correction routine. The observation that the secondary correction coefficient is so close to unity indicates that bias had been virtually eliminated by this approach.

3.7.6 QUALITY CONTROL DATA

In order to ensure the accuracy of analyses performed, internal quality control samples IQC Z and NBS 982 were analysed for each batch of blood or environmental samples as described earlier (section 3.6.2 and 3.7.3). The analysis of all the blood samples from the 55 children participating in the Birmingham study was achieved in twelve analytical runs, in each run IQC Z was analysed before and after each group of six blood samples; a total of thirty three measurements. After correction for mass fractionation, the mean observed $^{206}Pb:^{207}Pb$ ratio for IQC Z was 1.1580 with a between batch coefficient of 0.31%, the average within batch coefficient of variation was 0.47%. Once again, for this series of measurements the precision obtained for the isotope ratio measurements for the internal standard treated as a group was less than the uncertainty associated with a typical single determination. The accepted value obtained by Manton using TI-IDMS for the IQC $^{206}Pb:^{207}Pb$ ratio was 1.1590, hence the percentage error for the ICP-MS result relative to TI-IDMS was -0.09%.

Similarly, for the environmental samples, a standard solution of NBS 982 was analysed as an internal quality control sample. The NBS certificate[81] states that the $^{206}Pb:^{207}Pb$ ratio is 2.14101, the observed mean value for NBS 982 from eighteen runs, involving fifty four separate determinations, was 2.14364 with a between batch relative standard of 0.2%. The average within run relative standard deviation was 0.24%, similar to the between batch precision. The improved within run precision may be attributable to the higher lead concentration of the NBS 982 standard, $100\mu gl^{-1}$, compared to the diluted IQC Z concentration of $27.12\mu gl^{-1}$, resulting in improved counting statistics. The percentage error in the ICP-MS result for NBS 982 compared to its certified value was +0.123%, which was similar to the accuracy obtained for IQC Z from the clinical analyses.

The small deviations (≈0.1%) from the accepted ratio values for both quality control materials, implied that the correction protocol (discussed in section 3.7.5) was adequate to compensate for the effect of mass discrimination.

These results indicated that the analysis of the internal quality control samples, for lead isotope ratio, was both accurate and precise and suggested that the clinical and environmental samples interspersed between such quality control standards should be as reliable.

3.7.7 BIRMINGHAM BLOOD LEAD ISOTOPE RATIO ANALYSIS RESULTS

Blood samples were taken from fifty five children from the Birmingham area in November 1984 and March 1985 (section 3.2). The samples were analysed for their lead isotopic composition, as described in section 3.6.2. The observed ratio for each child in both years, is presented on figure 3.6.

The results for the $^{206}Pb:^{207}Pb$ ratio for the 1984 blood samples are plotted against the 1985 data on figure 3.7. If a child's blood lead ratio had not changed from the November value when sampled again the following March, the result would lie on the line $y=x$. The dotted lines on the figure represent a deviation of ±1% from $y=x$. A linear correlation was performed on the 1984 vs 1985 data. The correlation coefficient was 0.6651, the equation for the line being: $y=0.392 +0.709x$. There was a small change in the mean $^{206}Pb:^{207}Pb$ ratio over the course of the study from 1.1209 in November 1984 to 1.1244 in March 1985 . The 1984 and 1985 data were compared using a Willcoxon matched pairs signed ranks test and it was found that the change in ratio of +0.3%, was significant at the $p<0.01$ level. This finding means that the chance that the 1984 and 1985 data are part of a single distribution is less than 1% ie the change in ratio is statistically significant.

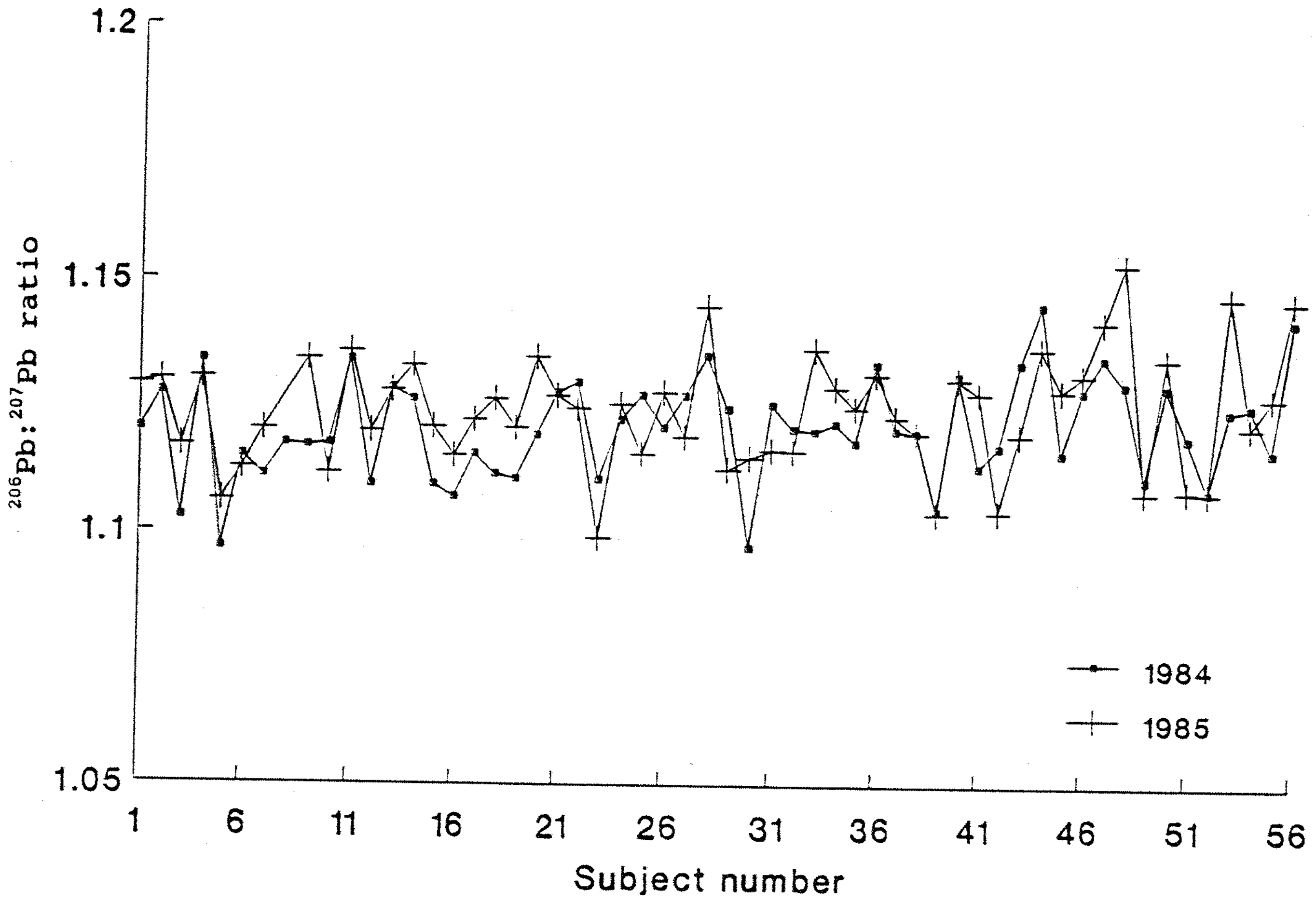

Figure 3.6. Observed blood lead ^{206}Pb:^{207}Pb ratio for the Birmingham study.

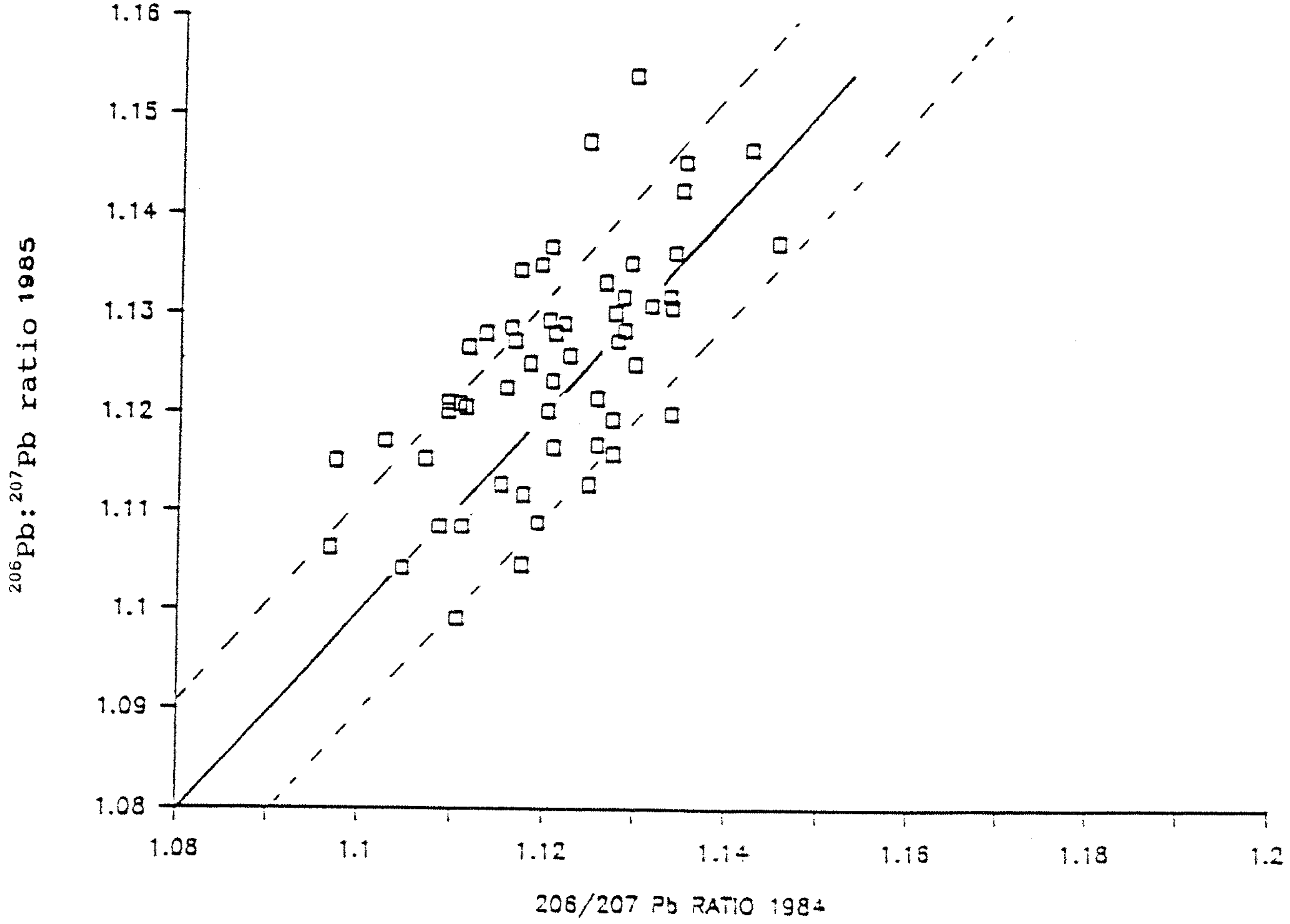

Figure 3.7. A comparison of the 1984 vs 1985 blood lead ^{206}Pb:^{207}Pb ratio for the Birmingham study.

The observed $^{206}Pb:^{207}Pb$ ratio in UK petrol vehicle exhaust residue was 1.064 (section 3.5.5) and the increase in the children's ratio would be consistent with a decrease in the contribution of lead from petrol to the childs body burden of lead. However, the blood lead concentration data (obtained by MSFAAS) does not provide any evidence for any reduction in the overall body burden of lead, the average concentration in 1984 being $0.628\mu moll^{-1}$ compared to $0.633\mu moll^{-1}$ in 1985. Since both data sets exhibited large relative standard deviations of 47 and 36% respectively, indicating that there was a wide distribution of blood lead concentrations in each year, it is possible that a small decrease in blood lead concentration would go unnoticed (each MSFAAS analysis was performed in triplicate and was only accepted if the results agreed within $\pm 0.3\mu moll^{-1}$). However, there is no reason to suggest a decline in the contribution to the lead body burden from petrol, since the reduction of the allowed concentration of lead in petrol was not begun until the end of 1985 and there is no reason to suspect a significant reduction in the local traffic density. Further investigation is required to explain the observed change in the blood lead isotopic composition.

3.7.8 BIRMINGHAM ENVIRONMENTAL SAMPLES ISOTOPE RATIO ANALYSIS

The samples of household dust and garden soil provided for each child taking part in the survey were analysed in duplicate as discussed earlier (section 3.7.3).

The average ^{206}Pb:^{207}Pb ratio observed in the soil samples was 1.1318 (n=100) with a relative standard deviation of 1.1%. The household dust samples had a ^{206}Pb:^{207}Pb ratio Of 1.1135 with a similar relative standard deviation of 1.4%. The average soil and dust ratios from each subject's home, are plotted on figure 3.8. The mean ratios for household dust, garden soil and the 1984-5 blood samples are plotted on figure 3.9. The error bars represent the 95% confidence intervals about the mean. Where two error bars do not overlap, as is the case for the soil and dust results, the difference between the means is significant (if there is an overlap then data should be tested to examine if a statistically significant difference exists or not). It is clear from the figure 3.9 that the soil and dust samples represent different, isotopically distinct sources of lead. The isotopic composition of the blood samples is intermediate between the soil and dust ratios, suggesting that both sources could contribute to the overall body lead burden.

3.7.9 FUTURE WORK

The work presented in this section has demonstrated that small variations in blood lead isotopic composition can be determined by ICP-MS. If the observed change in blood lead ratio between 1984 and 1985 is to be explained, further samples of other sources to which the children were exposed must be obtained. In a subsequent phase of this work, the organisers intend to make diet and water samples available to us that were collected at the same time as the blood and environmental samples. This should enable us to draw some conclusions about the apportionment of lead sources for the Birmingham children.

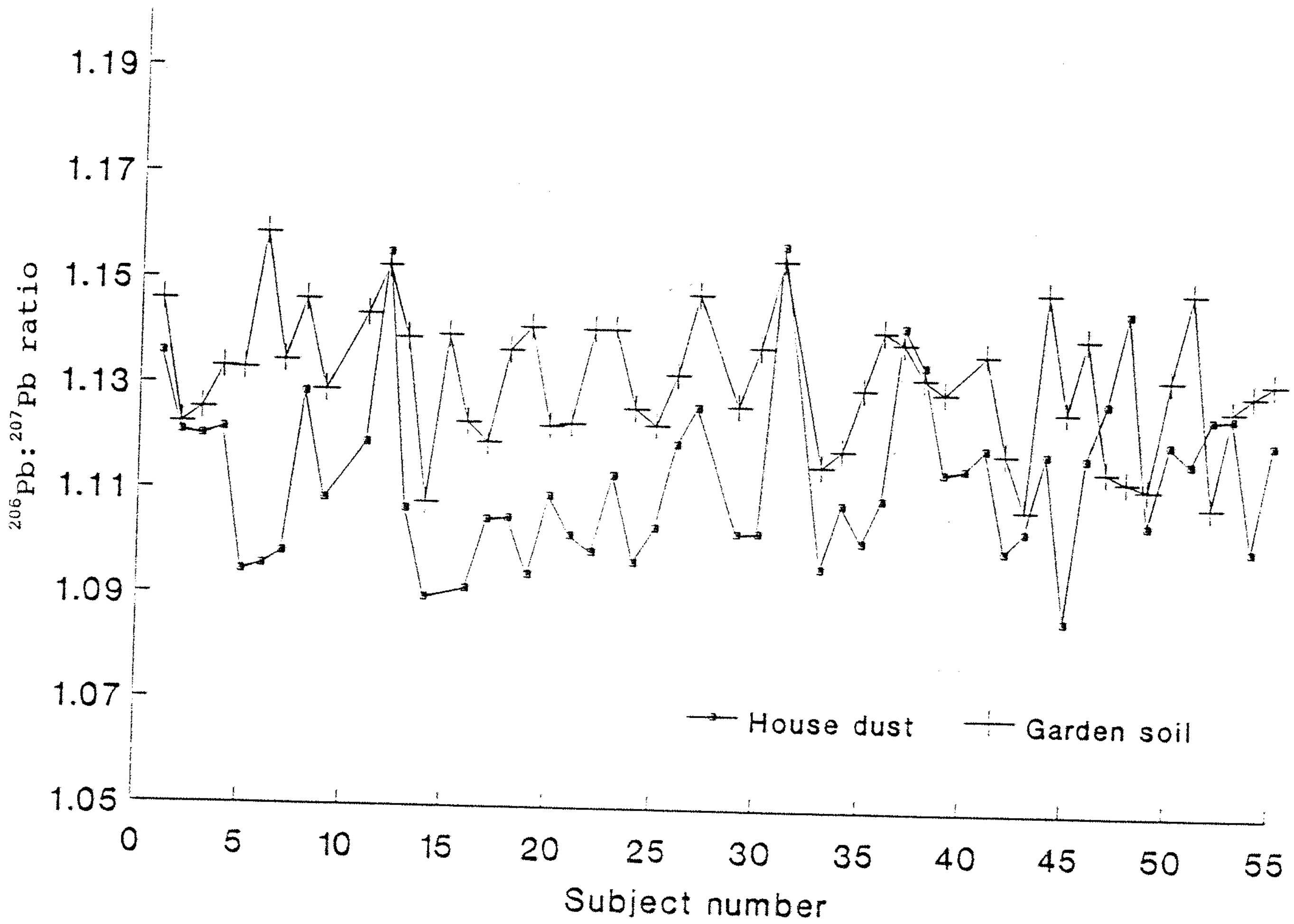

Figure 3.8. Observed ^{206}Pb:^{207}Pb ratio for soil and dust samples taken from the homes of subjects participating in the Birmingham study.

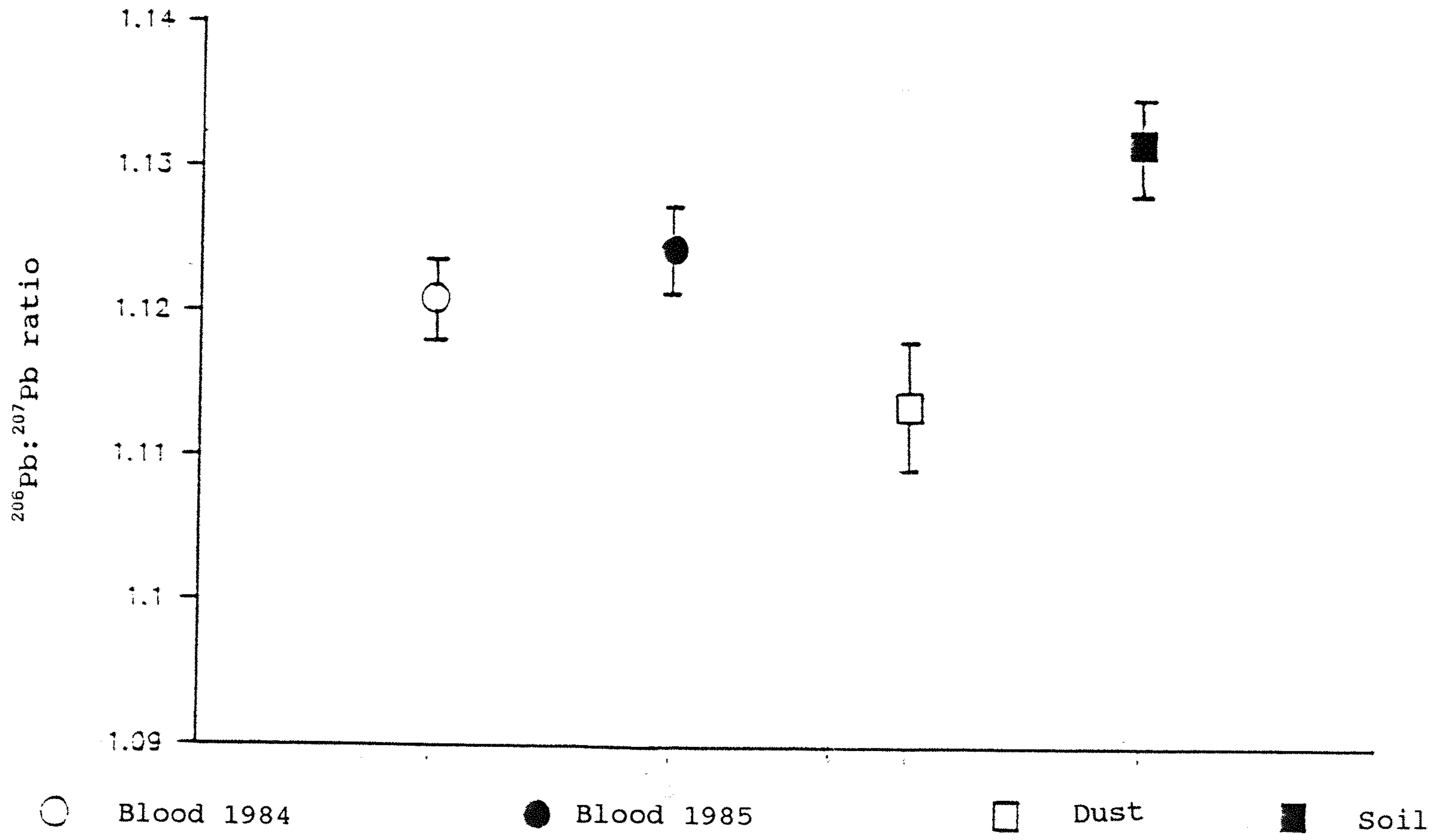

Figure 3.9 Summary of mean $^{206}Pb:^{207}Pb$ ratios for clinical and environmental samples taken for the Birmingham study.

CHAPTER 4

MISCELLANEOUS ANALYSES

4.1 SOURCE IDENTIFICATION IN PAEDIATRIC LEAD POISONING

As part of its activities for The Supra-regional Assay Service, our laboratory routinely analyses blood samples for lead concentration by MSFAAS. In some cases of excessive paediatric lead exposure the source of the poisoning has been identified from isotopic composition analysis using ICP-MS. In addition to the clinical sample, environmental samples (eg paint, soil etc) were obtained from the child's home to enable evaluation of the most likely source of the lead.

The environmental samples (10-500mg) were treated as discussed earlier (section 3.7.3) to ensure all available lead was in solution. The solutions were screened for lead concentration against an aqueous calibration, using flame atomic absorption spectrometry. Any solutions found to contain significant quantities of lead (ie $\geq 0.1\%$ Pb w/w) were subsequently analysed by ICP-MS for their lead isotopic composition.

In one such case, a blood sample was obtained from a female infant of asian origin. The child had a blood lead concentration of $3.1\mu moll^{-1}$, aproximately three times higher than the upper limit for normal paediatric exposure ($1.2\mu moll^{-1}$). This level of exposure was considered to be dangerously high and it was therefore important to identify the source of lead and remove it from the child's environment. The child's blood lead isotope ratio was determined as described earlier (section 3.6.2), and monitored periodically over a period of eight months.

Blood samples were obtained from the childs parents and her sibling in order to establish her likely basal blood lead ratio (this would normally be similar to that of her imediate family since they are likely to be exposed to the same sources of lead from a common environment and diet) and confirm that there were no other incidences of excessive lead exposure in the family. Additionally, samples of wood splinters from the childs cot (which the child had been observed to chew on), soil, paint and foodstuffs (herbs and spices) were provided and screened by FAAS for their lead content.

Twelve measurements were made on the child's blood lead ratio over the period of the investigation. The results of these measurements are presented on figure 4.1. The childs mean $^{206}Pb:^{207}Pb$ ratio was 1.0740 with a relative standard deviation of 0.55% whereas the ratios observed for the child's parents and sibling were 1.1210, 1.1241 and 1.1236 respectively. The blood lead concentrations of the child's parents and sibling were within the normal range for paediatric exposure (0.4, 0.4 and 0.2μmoll^{-1}) so the source of lead causing the poisoning was being ingested by the affected child alone. Obviously, the source of the poisoning had to have a $^{206}Pb:^{207}Pb$ ratio <1.0740 to have caused such a bias from the familial value of ≈1.12. However, none of the environmental samples provided was found to contain more than 0.1% lead by weight and so could not be responsible for the poisoning. The parents then provided us with samples of two patent Indian "medicine" samples which were occasionally administered to the child. Although these samples were found to contain 1.5 and 5.5μgl^{-1} of lead respectively the observed $^{206}Pb:^{207}Pb$ ratios (1.1046 and 1.0876 respectively) were too high to be the major source of lead involved, and although they would contribute to the overall lead body burden they would cause the child's ratio to approach her family's ratio. Eventually the parents provided a third "medicine" that was given to the child when she complained of colic, a symptom also associated with lead poisoning. This sample was also screened by FAAS and found to contain 85% lead by weight. Subsequent analysis by ICP-MS determined the $^{206}Pb:^{207}Pb$ ratio to be 1.0724 which confirmed that this was the source of the poisoning.

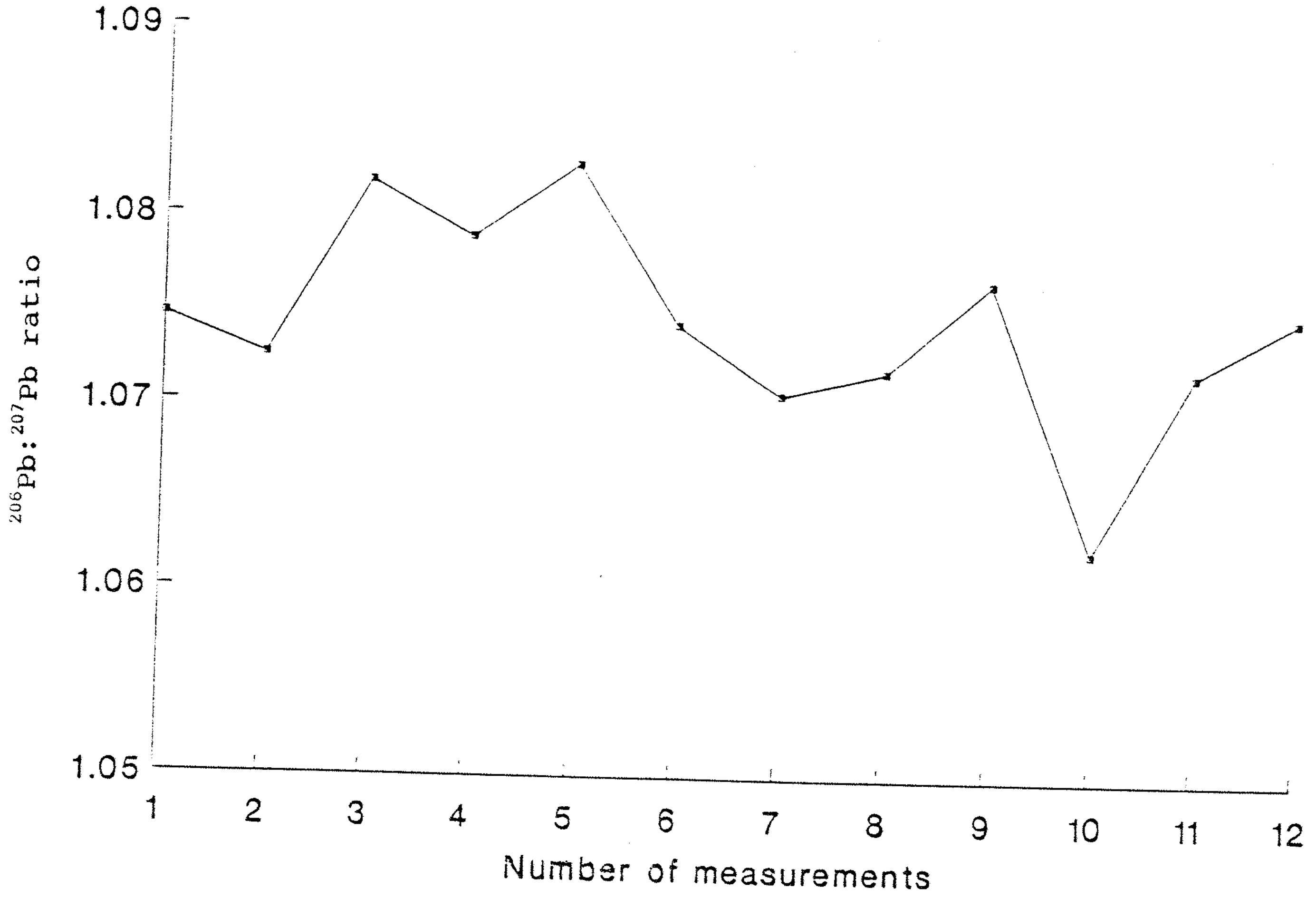

Figure 4.1. Temporal variation of blood lead ^{206}Pb:^{207}Pb ratio for a child with lead poisoning.

4.2 REGIONAL VARIATIONS OF BLOOD LEAD ISOTOPE RATIO

In the UK, excessive exposure to lead is rare, often arising in paediatric cases where the subject suffers from pica (a compulsion to eat non-food items eg paint flakes soil etc), or in adults as a consequence of industrial exposure, or very occasionally as a result of deliberate poisoning. However, the tissues of the public at large will contain some lead as a result of exposure to a number of environmental sources which may include components from diet, the water supply, petrol vehicle emmissions and atmospheric pollution as a result of industrial activity[70]. Our observations suggest that exposure to such low level sources may give rise to a particular, regional value for the blood lead ratio. Based on observations of the $^{206}Pb:^{207}Pb$ ratio for individuals, having low blood lead concentrations ($<1\mu moll^{-1}$), from five UK towns, the typical ratio was found to be ≈1.125.

Manton[83] noted that despite lengthy periods (3-5 years) living in their adoptive country, expatriates taking part in a survey of blood lead isotopic composition in Dallas, Texas, USA, showed blood lead ratios that were distinct from native Americans. The average $^{206}Pb:^{207}Pb$ ratio for native Americans was 1.1955 (n=6 c.v =0.37%) whereas the foreigners had ratios of 1.1600, 1.185, 1.1900 and 1.167 (Australian, S.African, Hungarian and S.African).

Each year we receive blood samples from a group of Zambian mine workers for blood lead concentration determinations as part of their industrial monitoring programme. Five of the samples were re-analysed for isotopic composition, using the normal procedure. The mean ^{206}Pb:^{207}Pb ratio observed was 1.1417 with a relative standard deviation of 0.29%.

A further, unusual example of the variation of lead isotopic composition in human tissues with geographical origin, was provided by the analysis of some Roman rib bones. The rib bones were taken from some Roman remains found in a West Country cemetery. The samples had been cleaned, and the lead extracted using ion exchange chromatography, by colleagues in The Geology Department at Southampton University. The mean ^{206}Pb:^{207}Pb ratio for the samples was 1.1791 (n=6 c.v. =0.1%).

The ratio data cited above is presented on figure 4.2. These results demonstrate that tissue lead compositions vary as a function of geographical origin. The tissue lead composition would not be expected to alter significantly for an adult expatriate in the absence of chronic exposure to a given source of lead, since most of the body burden of lead is stored in the skeleton and the rate of exchange of lead between the skeletal deposit and the soft tissues is very slow ($T_{1/2} \approx 20$ years).

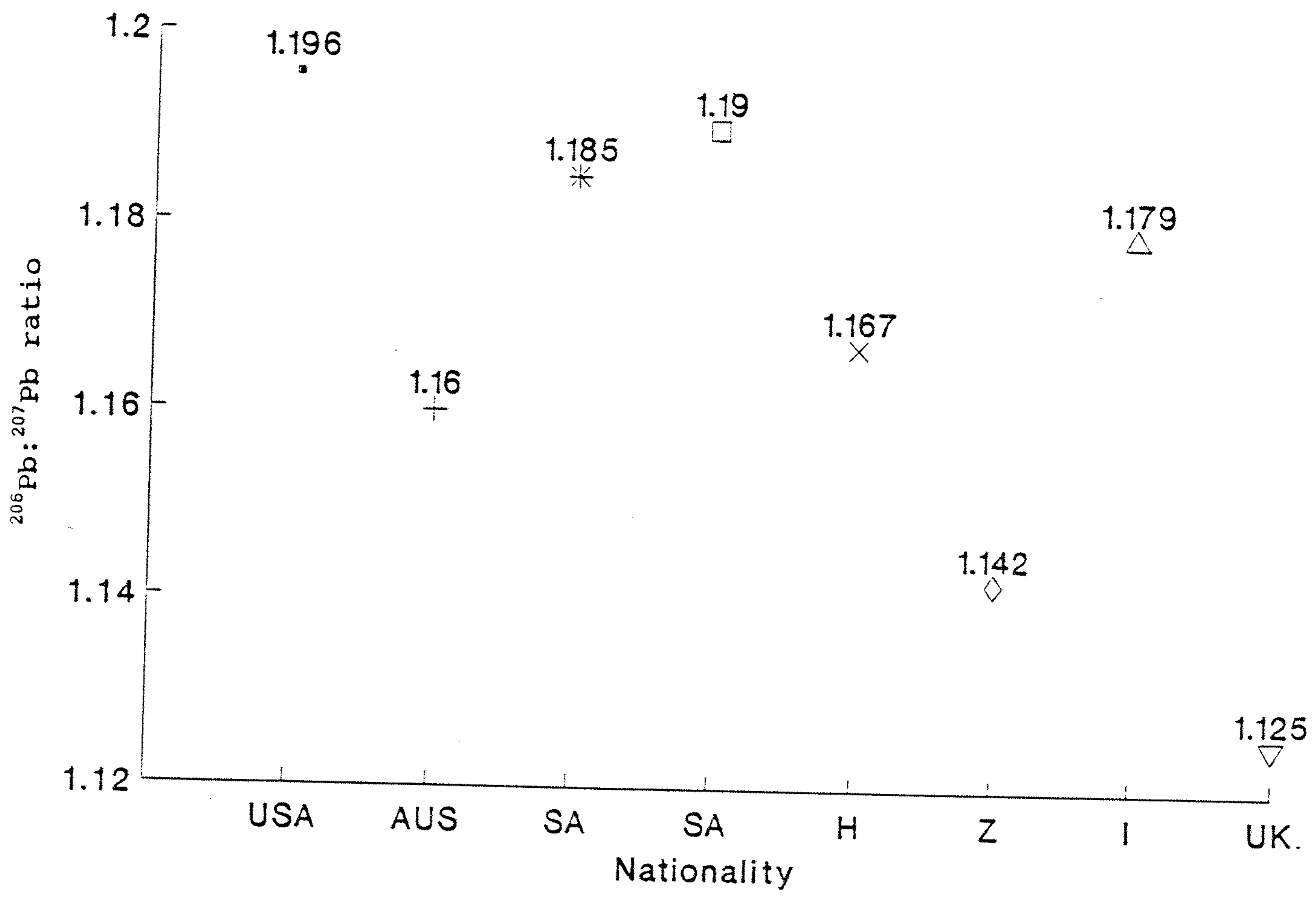

Figure 4.2. Variation of observed blood lead ^{206}Pb:^{207}Pb ratio with geographical origin of subject.

4.3 SPATIAL FEATURES OF ICP-MS

Commercial ICP-MS instrumentation uses the concentric quartz plasma torch developed by Fassel which has a two or three turn load coil. Once the plasma has been struck, a torroidal space is formed at the base of the plasma through which the sample aerosol can be passed into the hot core of the plasma, resulting in ionisation of analyte and matrix elements. Maximum sensitivity for ionic species is obtained if the central analyte channel within the plasma, is aligned with the optical axis of the spectrometer. In order to be able to study the spatial properties of the ICP and investigate the influence of instrumental parameters such as gas and nebuliser flow rates, RF power and aerosol loading on the intensities and distributions of species within the plasma a mechanism for the precise, reproducible positioning of the plasma with respect to the sampling interface is required.

The existing torch box adjustment facility on the mark I PlasmaQuad used a series of adjusting screws for lateral and longitudinal movement, and a triangular array of studs and nuts for vertical control. This system gave a crude adjustment in three dimensions (±1mm), but was not sufficient to allow any spatial profiling of the plasma to be undertaken.

Dr Ray Bacon of the department of Medical Engineering at Southampton University was approached and asked to devise a robust torch box carriage that could be accurately and precisely repositioned in three dimensions. The system he devised could be moved in the X, Y and Z planes by means of a series of precision slide rails which are driven by lead screws connected to three stepper motors; one for each axis. The original system for providing the longitudinal translation (X axis), an argon gas piston, was retained to move the torch from its standby position up towards the interface. Fine adjustments about the X axis were then controlled using a stepper motor (an additional three way gas solenoid valve was added to the argon cylinder to release the cylinder gas pressure when the X axis stepper motor was being driven). The stepper motors were all controlled via a dedicated computer and were driven sequentially (although simultaneous movement in three dimensions was feasible). The maximum and minimum extension in a given direction were controlled by the tripping of limiting switches and these were also used to provide a frame of reference for the torch box position. The minimum step size in a given direction was 5μ and corresponded to a single unit in the control software (devised by John Egan of Medical Engineering). The software provided three speeds for stepper motor control: slow, medium and fast representing 1, 10 and 100 unit movements.

The torch box movement system was used to demonstrate its profiling capabilities by monitoring barium species as the plasma was stepped over the sampling aperture. M^+, M^{2+} and MO^+ species intensities were followed as a function of the displacement of the plasma from its notional centre point in both directions along the Y axis (ie left to right across the sampling cone). The step size was 0.25mm over the first 1mm of the displacement and then 0.5mm for the second (the maximum spatial resolution obtainable was 0.005mm). In order to obtain reasonable signals for the oxide and doubly charged species a 10μgml^{-1} barium solution was used (the monopositive ion response was measured at ^{137}Ba (11.37% abundant) to avoid detector saturation, due to high ion flux at the major barium isotope, ^{138}Ba). The results of this preliminary study are presented on figure 4.3 (Y axis scale expansions are given in the legend). It appears that the true centre of the plasma, judged by the criterion of maximum signal, was displaced by ~-0.2mm from the nominal centre. The system is capable of much finer adjustments than were used for this demonstration and the device will be of great value for plasma profiling work and studies of the influence of plasma parameters on the population and distribution of ionic species within the plasma.

Unfortunately, the torch movement system was not available until near the end of my period at Southampton University and so it was not possible for me to embark upon the intended, detailed plasma profiling studies.

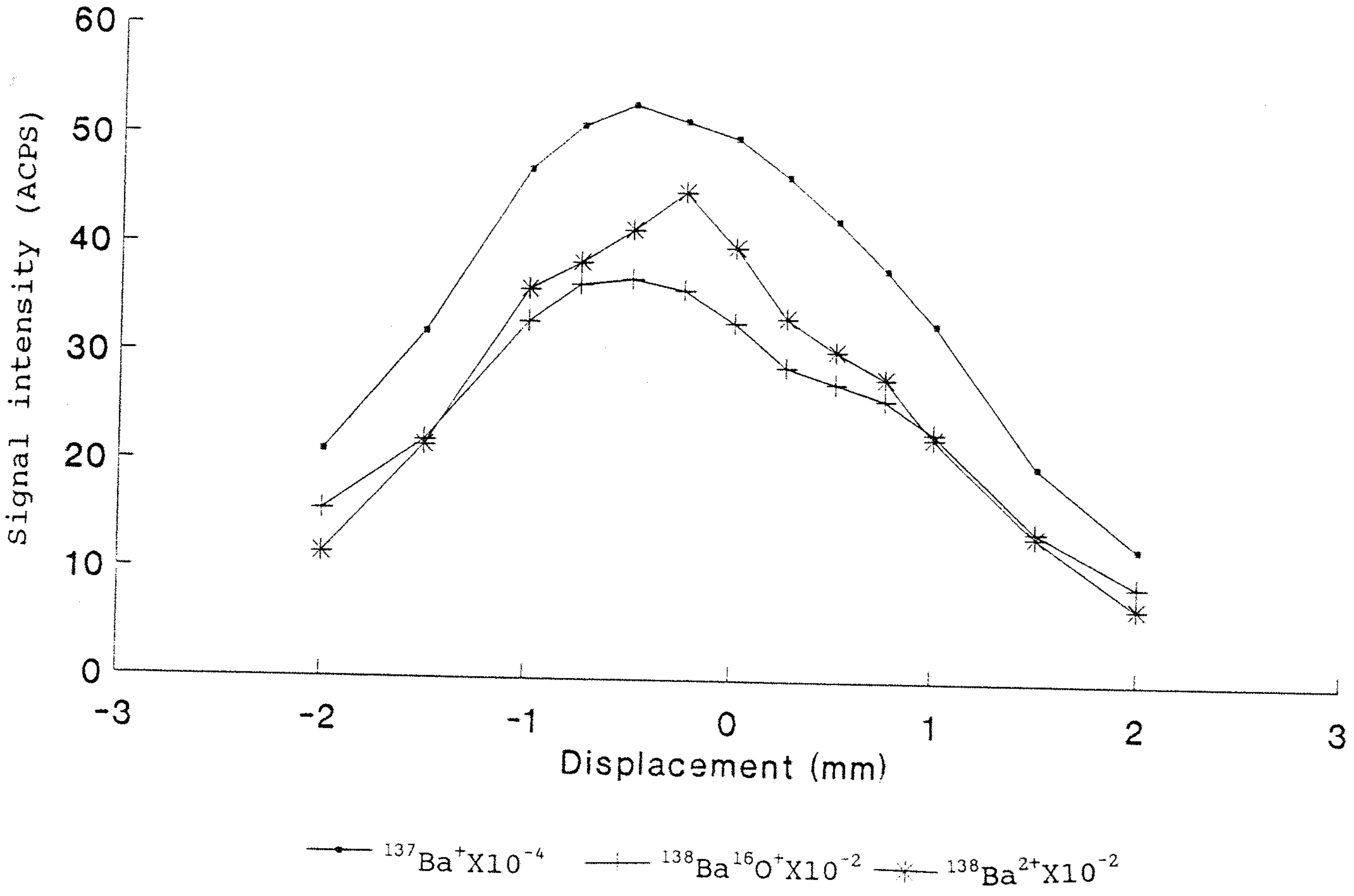

Figure 4.3 Plasma profile for Barium species.

4.4 GENERAL DISCUSSION AND FUTURE WORK

The work presented in this thesis has demonstrated that ICP-MS can be used to measure total lead concentrations in whole blood, with comparable accuracy to MSFAAS analyses (section 3.3), following a simple sample preparation involving a 1+24 aqueous dilution (section 2.5). The technique is capable of accurate (RSD ≈0.1%) and precise (RSD ≈0.5%) lead isotopic composition analysis in clinical and environmental samples (sections 3.5, 3.6). Blood samples can be analysed over a period of 5 hours without unacceptable loss of sensitivity. Mass fractionation effects can be readily compensated for and are almost constant for a given instrument under normal operating conditions over long periods of time. ICP-MS has also been used to determine lead isotopic ratios in clinical and environmental samples to identify a source of excessive lead exposure in a case of childhood lead poisoning (section 4.1). The technique shows promise for apportionment studies of lead in humans, if all relevant sources can be sampled and careful experimental design and control is exercised.

This thesis has dealt with the use of ICP-MS largely for isotopic analysis. However, it is capable of multi-element analyses at sub ngg^{-1} levels and so has found widespread use in many diverse fields of analytical chemistry eg nuclear[84,85], aerospace[86], food analysis[87] and clinical chemistry [88-91].

In addition to its role of determining concentrations of trace elements in clinical samples, perhaps one of the most interesting applications of the technique will be the study of the role of trace metals in metallo-enzymes and transport of trace elements across membranes, using stable isotope tracers (ethical approval would be easier to obtain for studies using stable isotopes rather than radio tracers). It could prove a very powerful tool if coupled with GC-MS (for separation and analysis of biochemicals) for investigations of metabolic pathways in normal subjects and those suffering from metabolic disorders eg Wilsons disease (associated with an inability to metabolise copper). With the body of data that the technique can provide from clinical analyses it will be possible to determine reference values for most elements in various tissues and extend this to studies of the different metal containing species in body fluids and tissues.

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