Determination of Trace Elements in Hair for Monitoring Environmental and Occupational Exposure

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Contents

Hair has a number of advantages over more conventional sample types, such as serum, whole blood and urine, as an indicator of the intake and exposure to trace elements. While the analysis of these conventional sample types can only be used as a measure of recent exposure, hair, in contrast, can provide an historical record of exposure episodes. Furthermore the levels of trace elements in hair are higher than in blood and as a result small changes in intake are magnified. In addition, the collection of hair samples is non-invasive and the samples are easy to store. However, the difficulty in differentiating surface bound contamination from trace elements incorporated into the hair matrix complicates the interpretation of the hair analysis results. A review of the literature showed that a variety of washing procedures to remove external contamination have been proposed, but as yet no standardised procedures are available. In this study, methods for the pretreatment and determination of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in human hair by ICP-MS were developed. Chemical speciation of mercury and methyl mercury in hair was also achieved using LC-ICP-MS.

For the digestion of hair samples the optimum method for these elements was found to be a digestion mixture of nitric acid and hydrogen peroxide. Certified reference materials CRM 397 and CRM 07601 were digested using this method and the values obtained were seen to be in good agreement with the certified values. Investigations of various washing procedures to remove external contaminants showed that in unexposed hair samples cadmium, lead and mercury were significantly removed from hair using a 0.1M HCl wash, with 87%, 73% and 5% respectively being washed off. Whilst the removal of antimony, arsenic and chromium from unexposed hair was more efficient with 1% v/v sodium lauryl sulphate, with 43%, 40% and 13% of each element respectively being washed off. Selenium could not be removed from the hair by any of the washing methods studied. Experiments with simulated sweat spiked with each of the elements showed that exogenously bound chromium, cadmium and lead could be removed after washing with 0.1M HCl. In contrast, antimony, arsenic, selenium and mercury were irreversibly bound and could not be removed with any of the washing solutions investigated.

Using a LC-ICP-MS system it was possible to separate inorganic mercury and methyl mercury in hair, without any modifications to the existing instrumentation. The results showed that in order to determine methyl mercury the sample had to be cold digested in 2:1 HNO_3:H_2O_2 and that a minimum of 0.1g of hair is required. Using this method of analysis it was seen that with spiked simulated sweat solutions exogenously bound methyl mercury could be removed from hair after washing with 0.1M HCl, whereas the inorganic mercury was irreversibly bound to the hair. It was also found that inorganic mercury was the major mercury species in hair from dental subjects.

The results of a six week selenium supplementation study of 29 subjects showed an increase in hair selenium levels of 19%, confirming that hair concentrations of selenium can give a good reflection of selenium status in the body.

A control group of 40 unexposed samples were analysed to determine normal levels of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in human hair. Occupational studies were then carried out and the data obtained from these studies showed a significant increase in the elements of exposure. Hair samples from dental workers (n = 43) showed a mean mercury level of 813 ng g^{-1} compared to a control value of 287 ng g^{-1}; hair samples from semiconductor workers (n = 24) showed a mean arsenic level of 208 ng g^{-1} compared to a control value of 34 ng g^{-1}; hair samples from chromium platers (n = 7) showed a mean chromium level of 2201 ng g^{-1} compared to a control value of 985 ng g^{-1}; and hair samples from lead foundry workers (n = 13) showed a mean lead level of 124337 ng g^{-1} compared to a control value of 1030 ng g^{-1}. The lead levels in the foundry workers' hair samples were reduced by 94% after washing with 0.1M HCl from 124 000 to 7440 ng g^{-1}, which was still above the normal group mean hair lead level of 1030 ng g^{-1}.

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Chapter 1: Introduction

1.1. General Background

Human hair has received much attention through the history of mankind[1]. In early Japanese history a woman's hair was regarded second only in importance to her life and there are stories linking a woman's spirit to her hair. The Japanese tradition of respecting hair can be traced to Buddhist influences. It is believed that when Buddha died he was cremated and objects of worship such as his bones, teeth and hair were placed in separate pagodas and sent to different countries. The shaving of head hair in Buddhism represents devotion to a strict and religious life and because hair is seen to signify sexual desire and potency, shaving the head therefore denies such feelings[1].

In other cultures the concern about hair centred mostly on style rather than its spiritual importance. The Romans cropped their hair short but considered it undignified for a man to be bald. In contrast, the Syrians wore their hair hanging to the shoulders. Under the Manchus, Chinese men wore their hair shaved away from the forehead and hanging in a long plaited pigtail. From ancient times, Muslims have shaved their heads leaving only one long lock on the crown of the head (covered by a fez or tarbush) with which, it was thought, they could be drawn up to heaven after death. In Europe, hair has also been deemed an important asset through the ages, particularly in the production of wigs and clothing. Horse and oxen hair have been used in the manufacture of brushes, blankets, insulation, jewellery, plaster for houses and wigs[2].

Hair has protective, sensory and heat regulatory functions and it occurs in animals in a wide range of textures, from a 'downlike' fur to an armour of coarse heavy spines. The word hair is also applied to outgrowths in other animals e.g. insects, the hairy frog and even plants, though none of these have similar structures. Human beings are relatively hairless when compared to other mammals, whose hair is of great importance as it provides insulation, which can alter depending on the time of year and may also fulfil other important functions such as camouflage, mechanical protection and for sexual display.

Human hairs project above the surface of the skin almost everywhere, except over the sides and soles of the feet, the palms of the hands, the sides of the fingers and toes, the lips and portions of the external genitalia. There are about 5 million hairs on the human body and 98% of them are on the general body surface, not the head[6]. There are roughly 100 000 hairs on the head which can protect the scalp from ultraviolet light,

cushion a blow to the head and provide insulating benefits to the skull. The main function of the eyebrows is to protect the eyes from sweat and particles, eyelashes protect the eyes from sunlight, and people who lose them have constantly swollen and bloodshot eyes. Nasal hairs cool or warm inhaled air before it reaches the respiratory tract, prevent mucous from flowing over the upper lip and trap particles of dust[1,6]. The base of each hair follicle is surrounded by a plexus of sensory nerves. As a result of this, the movement of the shaft of single hair can be felt at a conscious level. This sensitivity serves as an early warning system that helps to prevent injury[2].

Hair is a unique biological material whose growth reflects the biomedical and environmental history of the subject[3,4,5,11]. In 1945, Flesch proposed that hair could function as a minor excretory organ for arsenic and possibly other toxic elements[5,6]. Early investigations included the determination of arsenic in Napoleon's (d.1821) hair[7,8]. The results showed that there were periods of arsenic exposure between 1816 and 1821, prompting suggestions that he was poisoned[9].

1.2. Human Hair[1]

There are three types of hair on the human body: 'overhairs' which are long and coarse; 'underhairs' which are soft and thin, of uniform length, often wavy and easily bent; and 'vellus' hairs which are short, very fine and soft, usually unpigmented and found on many 'hairless' areas of the body such as the forehead, eyelids and bald scalp. There are two types of hair classifications, morphological and hormonal. Morphological classification consists of six hair categories based on location, pattern and density. These are head, eyebrow and eyelash, beard and moustache, body, pubic and axillary hair. Hormonal classifications are:

i. Hair not dependent upon steroid hormones, but influenced by changes in growth or hormone output, e.g. the eyelashes, eyebrows and some body hair;
ii. Hair dependent upon female amounts of steroid hormones e.g. pubic and axillary hair;
iii. Hair dependent upon male amounts of steroid hormones e.g. beard and moustache hair, nasal tip hair, ear hair, and body hair.

Axillary hair is a 'secondary sex' hair characteristic and is generally absent until puberty.

Sensory hair is similar to other hair but differs in the degree of development of the individual components and in the presence of the connective tissue capsule. Leaflike

nerve endings, commonly called whiskers, are found at the hair follicle and its base. Sensory hairs are not found in humans but in all lower animals and on the eyebrows of the gorilla, chimpanzee and other primates.

Hair follicles appear first on the eyebrows, upper lip, and chin in the second month of fetal life. General developments appear about the fourth month. No new follicles appear after birth, and the follicles become more wide spread as the surface area increases. Hair direction is believed to follow the course of the arteries. The total number of follicles in an adult male is around 5 million, of which about 1 million are found on the head and around 100 000 constitute scalp hair.

Hair in different areas of the body differs not only in length, texture and colour, but also in diameter and shape and they all have different growth rates. The diameter of human scalp hair increases rapidly and uniformly during the first 3 or 4 years after birth, less rapidly during the next 6 years and scarcely at all from 12 years on. Beard and other body hair do not attain full growth until middle age. Ear hair is normally present in the new-born, absent in the child and young adult, and limited to the older male. Scalp hair begins to grow before birth, at the time of birth the average hair length is 2.5 cm. The growth rate in the new-born is about 0.2 mm per day but later increases to 0.3-0.5 mm per day. The total life span of a hair differs according to the region of the body and the type of hair. For example eyebrows, eyelashes and axillary hair have a life span of 3 to 4 months, in contrast to 4 years for scalp hair.

1.3. Growth and Structure of Hair[5,10]

Hair growth begins in the follicles which cover the body. There are roughly 100 000 follicles on the scalp, between 10 000 and 20 000 on the bearded area of the face and between 500 000 and 1 000 000 on the rest of the body. Hair is formed in the follicles, which lie just below the skin, from materials in the blood and other body fluids. As growth takes place the hair is extruded from the follicle as a fibre and rises above the skin as shown in figure 1.1.

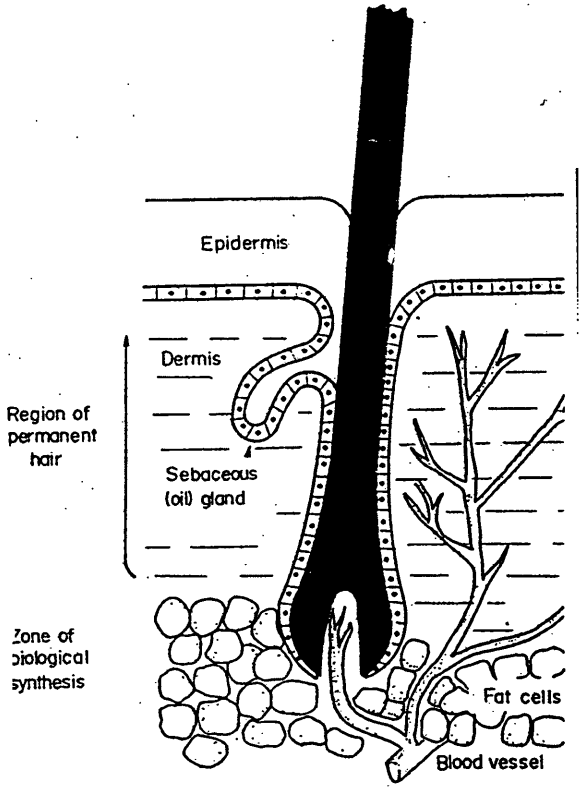


Figure 1.1. Hair follicles formed beneath the skin surface[9].

The visible hair is not a living tissue, the increase in length results from the continual extrusion of freshly formed material from the follicle. Hair follicles extend deep into the dermis, often projecting into the underlying subcutaneous layer. The epithelium at the base of a hair follicle surrounds a small hair papilla, which is a peg of connective tissue containing capillaries and nerves. The hair bulb consists of epithelial cells that surround the papilla. Each follicle is a miniature organ that contains both muscular and glandular components. The cells of the follicle walls are organised into several concentric layers. Beginning at the hair cuticle, these layers include:

i. The internal root sheath: this layer surrounds the hair root and the deeper portion of the shaft. It is produced by the cells at the periphery of the hair matrix. The cells of the internal root sheath disintegrate relatively quickly and this layer does not extend the full length of the follicle.
ii. The external root sheath: this layer extends from the skin surface to the hair matrix. Over most of that distance it has all of the cell layers found in the superficial epidermis.
iii. The glassy membrane: this is a thickened basement membrane, wrapped in a dense connective tissue sheath[6].

The hair filament can be separated into three zones along its axis. Figure 1.1 shows two of the zones and outlines the role of the blood vessels and the sebaceous sweat gland. Figure 1.2 shows the three zones, where the keratin zone occurs between the two zones in figure 1.1. The area around the bulb of the hair is the innermost zone and the site of biological synthesis and organisation. Keratinisation occurs in the next zone, where the hair fibre undergoes hardening through cystine cross linking. The final zone is the region of permanent hair, consisting of dehydrated and cornified cells and the intracellular binding material[6].

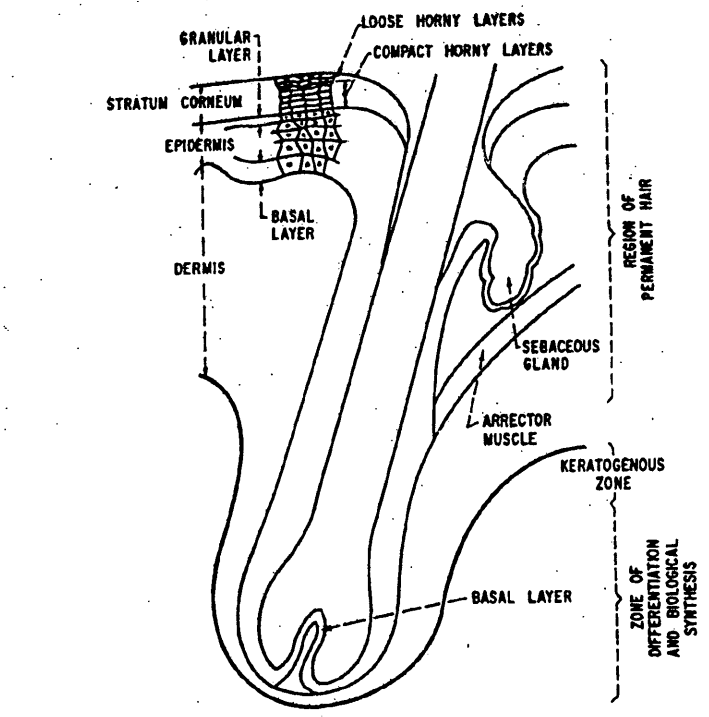


Figure 1.2. The three zones in a hair filament[6].

The hair shaft is the keratinised filament that is formed from the matrix of cells at the bottom of the follicle deep in the epidermal epithelium. The shaft is made up of the cuticle (a thin outer layer of scales), which encases and protects the cortex (a hollow cylinder of keratin- a name applied to a group of stable fibrous proteins) and the medulla, the central cavity in the cortex (which can be continuous or discontinuous and is composed of large cornified cells, loosely linked together). Figure 1.3 shows the main components of human hair.

The medulla is filled, to a greater or lesser extent, by cells of irregular shape and by tiny air bubbles. In old age the cells shrivel and the air bubbles become more numerous, the hair then reflects light more effectively. For this reason and because of the loss of pigment (melanin) from the cortex, old people usually have white hair. The melanins are usually attached to the protein matrix of the hair[13].

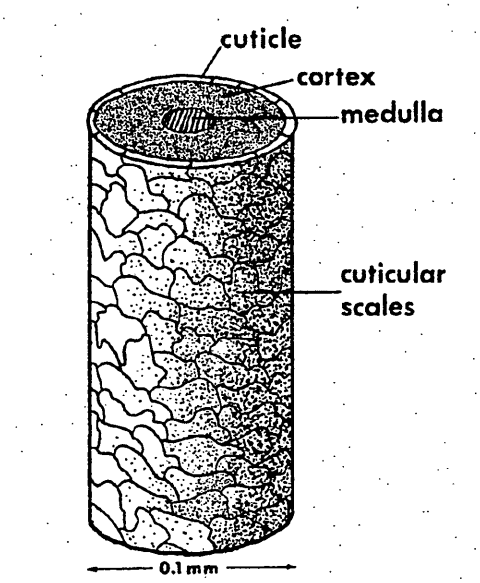


Figure 1.3. Schematic view of mature hair[11].

Keritisation is the hardening process for hair and nails. Hopps[11] states that keratin is not a specific chemical substance but a category of substances. Keratin, which is derived from the Greek word "keras" meaning horn[1], has been described as a highly stable, fibrous protein (or group of proteins) which contains disulphide bonds and which is remarkably resistant to enzymatic digestion and all but the strongest chemicals. This sulphur containing protein, gives hair its heat retaining property[1]. A typical keratin molecule in hair is a two (or three) stranded cable of highly orientated polypeptide chains wound into a helix, with secondary folds or distortions associated with a relatively unorganised matrix. The sulphur to sulphur bond, which is formed by two cystine residues contained in adjacent polypeptide chains, gives the keratin molecule its stability and resistance to enzyme digestion and chemical hydrolysis. The association of lipids with hair is important in that it affects the incorporation into hair of constituents from sebum, sweat and exogenous materials[11]. Hair phospholipids are thought to be chemically linked to keratin through their fatty acid side chains[11]. Many complex lipids are excreted by glands in the skin, primarily by sebaceous glands which

are associated with hair follicles. It has been shown that lipids provide an excretory pathway for chlorinated hydrocarbons in hair[12].

Human hair consists of approximately 80% protein and 15% water, with smaller amounts of lipids, pigments, polysaccharides and inorganic materials. Genetic makeup, diet, illness, weathering, cosmetic treatment and the environment are all factors which affect hair amino acid composition[1]. The water content of hair varies directly with the ambient relative humidity. The melanin levels range from 0.1-5%[13]. The lipids derived from sebum consist of free fatty acids, mono-, di- and triglycerides, wax esters, hydrocarbons and alcohols and account for 1% to 9% of the content. The mineral content of hair is between 0.25% to 0.95% on a dry weight ash basis[1]. The macroelemental composition of hair in weight percent is carbon, 46, oxygen, 28.2, nitrogen, 13.6, hydrogen, 7.5, sulphur, 4.1 and calcium, 0.3[26].

1.4. Hair Growth Cycle

The diameter of a typical scalp hair is about 0.1 mm and the mass of a 1cm length of a single hair is about 100 micrograms[5]. Scalp hair is believed to grow at a rate of 0.4 mm per day, while beard and pubic hair grow at approximately 0.2 mm per day[6]. It has been reported that on average head hair and chest hair grow at a little over 1 cm a month.

The hair follicle does not produce hair continuously, as periods of growth alternate with periods of rest. At any one time, about 90% of the hairs on the human head are in the growth phase, while the remainder are in the resting phase, in preparation for falling out. The hair cycle is divided into three stages:[1,6,11]

i. the *anagen* stage, during which active growth occurs
ii. the *catagen* stage, which is the transitional phase, and
iii. the *telogen* phase which is the resting stage.

The onset of anagen is accompanied by increased metabolic activity of the matrix cells just above the papilla and the bottom of the follicle is pushed more deeply into the dermis by continual cell division. The new hair begins as a thin filament pushing its way upward into the follicular canal, where the cells undergo differentiation to cuticle, cortex and medulla and the keratisation process is initiated. It is at this stage that endogenous trace elements are incorporated into the hair from the metabolic milieu of the matrix cells, together with the contributions from the connective tissue of the

papilla, circulating blood and lymph and other intra- and extracellular fluids. The developing hair becomes fully keratinised and is then extruded through the skin[6].

During the growth phase, the follicles of the scalp produce hair at a rate of between 0.2 to 0.5 mm per day. These growth rates are variable and dependant on age, race, gender and anatomical location. The growth phase lasts about 900 days and the resting phase about 100 days, so that the average life of a head hair (if not cut or combed out) is approximately 1000 days.

1.5. How Metals are Incorporated in the Hair Structure

Hair accumulates trace metals to a greater extent than many body tissues[1,6,11,14,15]. Table 1.1. taken from Lenihan[5], shows the higher concentrations of many metals in hair compared with other body tissues.

Element	Whole Body ($\mu g\ g^{-1}$)	Blood ($\mu g\ g^{-1}$)	Hair ($\mu g\ g^{-1}$)
Al	0.9	0.14	5
As	0.1	0.004	0.2
Br	3	4	30
Cd	5	0.01	1
Co	0.03	0.0004	0.1
Cr	0.1	0.03	1
Hg	0.2	0.01	2
Mn	0.3	0.01	0.3
Ni	0.1	0.04	3
Pb	1.5	0.2	20
Sb	0.04	0.1	0.2
Se	0.2	0.1	1
Ti	0.2	0.03	4

Table 1.1. Comparison of the levels of elements in the whole body, blood and hair.

The high affinity for metals is due mainly to the presence of cystine, which makes up approximately 14% human hair. The cystine cross-linkages are largely responsible for the strength and elasticity of hair. Many metals found in hair are bound to sulphur atoms in cystine or to sulphydryl (SH) groups present in other amino acids[5,6,16]. Hair is

essentially a cross linked, partially crystalline, oriented, polymeric network which carries various functional groups (e.g. acidic, basic and peptide bonds) which are capable of binding small molecules[1]. Metals can also bind to the hair structure through melanin, which determines the hair colour depending on the quantity and type of melanin incorporated into the hair shaft. Melanins are polyanion polymers containing negatively charged carboxyl groups and semiquinones at physiological pH allowing the binding of cations by ionic interaction[17]. The exact structures of the melanin polymers are not known because they can be complex copolymers of both eumelanin and phaeomelanin[13]. The two classes of melanin are based on their different colour contents. Eumelanin is a dark brown to black pigment that is insoluble in acid and alkali and contains 6-9% nitrogen, but an insignificant amount of sulphur. Phaeomelanin is a yellow to reddish-brown pigment that is soluble in alkali and possesses both nitrogen (8-11%) and sulphur (9-12%). The two classes of melanin are also chemically distinct: eumelanin is mainly a polymer of monomer units of 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid, whereas phaeomelanin contains benzothiazine units derived from cysteinyldopa[13]. Organic amines and metal ions have a high melanin affinity, because they are positively charged at physiological pH and interact with the melanin polymer by electrostatic forces between their cationic groups and the negative charges in the melanin polymer. The ionic binding can also be enhanced by other forces such as van der Waal's attraction[17]. Uncharged metals e.g. elemental mercury, may also bind to the hydrophobic core of the melanin polymer in the hair structure[13].

Proposed sources of trace elements in the hair[6,26] include:

i. Those taken up by the matrix during its continuous formation.
ii. Those deposited from sebum, the sebaceous gland being a source of trace elements derived from body tissues. The sebaceous excretion is quite significant, with the total lipid component from 10 cm^2 areas of the forehead skin amounting to 0.7-2.4 mg during a 24 h period.
iii. Those sorbed or otherwise transferred to the hair from eccrine sweat. Eccrine sweat is a source of entry and also a factor in the removal of trace elements. Its quantity and concentration vary enormously, depending on the individual and environmental conditions. Eccrine sweat is a water solution containing principally sodium, potassium and chlorine as well as urea, organic acids and trace elements.

iv. Those simultaneously incorporated into the permanent hair from apocrine sweat. This sweat is influenced by hormones and has limited distribution.
v. Those deposited from the external environment after the hair has been extruded from the skin.
vi. Those from cosmetic and/or pharmaceutical preparations applied to the scalp or other body surfaces.

Incorporation of trace elements via the hair root matrix and through the hair sheath is most likely, but the contribution from sebum, sweat and desquamated epidermis are also significant[18]. The contamination from impurities in air and water, soaps, dyes and other cosmetic preparations must also be considered.

It is important to distinguish the processes in which the metals bind to the hair whether it be via endogenous or exogenous means. The binding of metals to hair protein can give useful information about the medium from which the hair was formed i.e. the circulating blood. However, the binding of elements from external sources complicates the interpretation of hair trace element concentrations.

Endogenous trace elements are those incorporated into the hair at the root during anagen, i.e. the growing stage[1,6,19], either through the blood or at the later stage via sweat and sebum. Eccrine sweat is a quantitatively important source of trace elements that may be incorporated in hair after its formation. The lipids and waxes of sebaceous secretion and desquamated epithelium provide the physical/chemical means to incorporate trace elements into the hair substance in such a way that they cannot be washed off or extracted without also removing the truly endogenous trace elements[11].

The incorporation of exogenous trace elements can take place from a wide variety of sources and whilst their presence certainly reflects environmental exposure, it also leads to confusion in the interpretation of hair analysis results and so this is an area that has caused much discussion[1,5,6,9,11,26,38]. Exogenous sources of trace elements include particulates in air, solutes present in water and cosmetics. When hair is impregnated with water, its weight increases by 12-18%. The process of absorption is very rapid with 75% of the maximum possible amount being absorbed in 4 minutes. Thus, along with water and lipids (from sweat, shampoos and cosmetics), various soluble substances, including trace element pollutants can enter hair. The impact of exogenous contamination is illustrated by the case of a man whose grey hair turned green as a result of exposure to copper salts in the water he showered in[1]. It is noteworthy that hair can take up elements from aqueous solutions and that this takes place via a

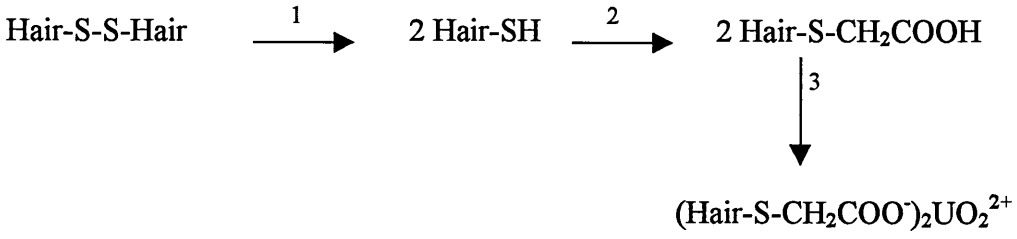
mechanical swelling process as well as by diffusion and chemical bonding through active groups (SH and NH_2) of the proteins. Rates of penetration into and desorption from the hair strongly depend on the type of element and physical properties such as the pH and the length of contact.

Work carried out to investigate the role played by cystine and cysteine in hair in the binding of exogenous metals suggest various reactions for the incorporation of silver, uranium and mercury in hair[20,21]. Studies[20] have been carried out to investigate the rapid uptake of silver in hair by cysteine to form a silver mercaptide (see reaction 1) to allow transmission electron microscope (TEM) studies of hair to identify the position of the amino acid group.

$$\text{Hair-SH} + Ag^{+} \longrightarrow \text{Hair-SAg} + H^{+} \qquad \text{(Reaction 1)}$$

It has been shown that by 'staining' the hair in this way the cysteine groups in the hair have been identified. Cystine has been identified in a similar way, by first reducing the hair fibre with benzene thiol and then alkylating with iodoacetic acid and finally treated with uranyl acetate and this is shown in reaction 2 .

(Reaction 2)



1 C_6H_5SH

2 ICH_2COOH

3 $UO_2(Ac)_2$

Staining the cystine groups in this way was further enhanced with the addition of a lead citrate solution. This technique is commonly used in biological electron microscopy but the chemical nature of the process remains obscure[20]. The study of cystine (reaction 3) and cysteine (reaction 4) residues in hair[21] have also explained how methyl mercury binds to hair and it has been shown that this is enhanced in the presence of sulphite and urea. The amounts of cystine and cysteine residues are calculated from the amount of methyl mercury consumed[21].

(reaction 3)

$$\text{Hair-S-S-Hair} + CH_3HgI + SO_3^{2-} \longrightarrow \text{Hair-S-Hg-}CH_3 + \text{Hair-S-}SO_3^- + I^-$$

or (reaction 4)

$$\text{Hair-SH} + CH_3HgI \longrightarrow \text{Hair-S-Hg-}CH_3 + HI$$

A study of the possible ion exchange properties of human hair was carried out by Steinnes in 1975[22]. The study involved soaking hair samples for 20 h, in 50 μg ml^{-1} radiotracer solutions of ^{65}Zn, ^{59}Fe, ^{75}Ga, ^{115}Cd, ^{64}Cu and ^{140}La. The ion exchange properties of human hair were investigated by comparing the adsorption of elements on hair samples with the adsorption of the same elements on a strongly basic anion exchanger and a strongly acidic cation exchanger. Chloride solutions of the cations cadmium, zinc, iron, gallium and copper, were seen to exhibit anion exchange properties. This was thought to be due to the formation of negatively charged chloride complexes[22]. It has been suggested that the anion exchange capability of hair is associated with the amino groups present in the hair matrix[1,22]. The study used ^{140}La, which strongly adsorbed to a cation exchange resin, to investigate the cation exchange properties of hair. However, results showed that hair had a very limited cation exchange capacity[22]. This therefore suggests that metals bind to hair when they are present in a negatively charged species.

It is also known that the pH of the solution in contact with the hair, e.g. the sweat solution, affects the binding of ions to hair[1,6,22,23]. The relationship with pH and the amount of element adsorbed has been investigated where the hair samples were in contact with simulated perspiration solutions containing radiotracers of Au^{3+}, Hg^{2+}, Zn^{2+}, Ag^+, Cu^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Cr^{6+}, Mn^{4+}, Se^{4+}, Ba^{2+}, Sr^{2+}, Ca^{2+}, Sb^{5+}, As^{3+}, As^{5+}, Na^+, K^+, Cs^+, Cl^-, Br^-, I^- and PO_4^{3-} for 18h[23]. Only Na^+, K^+ and Cs^+ were found not to adsorb. For all of the elements tested the cation adsorption increased as pH 7 was approached, while anion adsorption decreased. The behaviour of Au^{3+}, Se^{4+}, Sb^{5+} and As^{5+}, were similar to that shown by anions[23] which is to be expected since the elements will be in anionic species such as SeO_3^{2-} and AsO_4^{3-}.

It has also been suggested, that any binding of cations and anions to the hair may involve free radicals on the surface with amongst others, carboxyl groups playing an important role here[24]. However there has been no work carried out to confirm this

claim. It has been shown that at low pH calcium is released as unbound carbonyl groups are protonated and fewer carbonyl anions are available for binding with calcium cations[24]. Melanin as mentioned previously is also capable of binding metal cations and in doing so they can inhibit other molecules binding, e.g. drugs, to the melanin[25]. Melanin possesses a free radical and it has been proposed that drugs that are good electron donors would undergo charge-transfer reactions. Metal ions such as sodium, nickel and lead can inhibit the binding of the drugs to melanin. Larsson et al[25] found that by soaking melanin solutions in metal salt solutions for 45 minutes, centrifuging and analysing by spectrophotometry methods, the binding of inorganic cations to melanin showed characteristics similar to an ion exchange mechanism[25]. The binding of nickel was thought to occur at the free carboxyl groups and that Ni^{2+} may be able to attain a close contact with the anionic sites. It was also suggested that reduced binding observed at low concentrations of nickel may have been caused by a competition with 'endogenous' cations present in the melanin polymer[25].

It is apparent from studies carried out that the composition of hair is determined by the entry of substances not only from the blood stream, but also from the external substance of the hair[26].

1.6. Trace Elements in Human Hair

Unlike other tissues, hair is a metabolic end product that incorporates trace elements into its structure during growth and functions as a minor excretory route[9]. It is known that hair serves as a site for accumlation and a pathway for the excretion of some thirty elements, which are mostly metals[32].

A major advantage of hair as a tissue for analysis is that it can act as an integrating dosemeter over a period of months. Other advantages include:

i. The ease of collection, storage and transportation without deterioration.
ii. Many heavy metals found at relatively high concentrations.
iii. Hair provides a record of contamination from inside the body (since it is a route of excretion for many metals) and from outside, since it traps metallic vapours and dust.
iv. Hair is very durable: e.g. 1900 year old samples have been analysed.
v. Extremely sensitive analytical techniques are now available for the determination of metals in hair[10].

1.6.1. Hair Trace Elements and Disease

Hair has been used to assess nutritional status in Egyptian dwarfs[9] who were found to have deficient zinc levels, with low hair zinc concentrations of 54 $\mu g\ g^{-1}$. After taking zinc supplements the hair levels increased to normal values, 150-190 $\mu g\ g^{-1}$. Depleted zinc levels in hair have also been associated with malnourished children[27]. Diabetic children have been shown to have below normal hair chromium levels[9]. This is due to the fact that chromium is involved in glucose tolerance in the body. It has also been shown that adult diabetics have both lower chromium (94 $\mu g\ g^{-1}$) concentrations in their hair compared with non-diabetics (240 $\mu g\ g^{-1}$) as well as lower zinc levels[9]. Results such as these have contributed to the recognition that adult-onset diabetes is a different disease from juvenile-onset diabetes. Other diseases that have been diagnosed through hair and urine analysis, include Wilson's disease, a genetic abnormality of copper metabolism and Hodgkins disease, a cancer of the lymph node that is associated with chronic lead poisoning[9]. However, other studies have shown that the hair copper level is not significantly different in those with Wilson's disease compared to normal[28,29]. Others have found that hair copper levels in those with Wilson's disease were normal, instead it was the zinc levels that were low[30].

It has also been suggested that hair calcium levels may be related to cystic fibrosis and myocardial infarction[6,9]. Depressed magnesium levels may also be a factor in cystic fibrosis, whereas elevated potassium and sodium levels may be a factor in cystic fibrosis and Celiac disease[6]. Depressed selenium levels have been linked with Keshan disease and multiple sclerosis[6,31]. Hypoglycemia sufferers have been shown to have elevated zinc levels and depressed copper levels in their hair, while mongolism seems to show depressed calcium, copper and manganese levels[9]. Depressed magnesium levels in hair have been linked with phenylketonuria[32] and rheumatoid arthritis, while schizophrenia sufferers have been shown to have elevated iron and lead and depressed cadmium and manganese levels in their hair[9]. Hair iron content has also been studied as a monitor of iron deficiency in patients with chronic inflammatory bowel diseases[53]. Recently, hair analysis has been used as an aid in the screening for breast cancer[33]. Pubic and scalp hair were studied for differences in intermolecular structure, because it has been seen that breast cancer patients have extra scattering in the X-ray diffraction patterns in their hair structure not seen in healthy hair samples. This is believed to be related to a mutation of a gene called BRCA1, which is associated with increased risk

of breast cancer. If further studies prove successful, hair analysis by X-ray diffraction studies could act as a simple method for screening for breast cancer. Using this approach 23 breast cancer patients out of a group of 51 were correctly identified. Other studies looking at trace element status in breast cancer sufferers have shown a decrease in hair selenium levels in women affected by the disease[34].

Elevated hair and nail arsenic levels have been linked with the incidence of Blackfoot disease in Taiwan[35]. A study of emotionally disturbed and violence prone male children, found elevated hair cadmium, lead, copper, manganese, iron, nickel and calcium levels and depressed phosphorus levels[36].

MacPherson et al[37], used beard calcium levels as a marker for coronary heart disease. Their study found that about 90% of people who had suffered an acute myocardial infarction had low beard calcium levels which increased after vitamin D supplements.

From the work reviewed it is apparent that during the past three decades, the determination of trace element concentrations in human scalp hair has become increasingly popular for monitoring environmental exposure, evaluating systematic intoxication, assessing nutritional status and disease diagnosis[38,39]. However, a number of factors complicate the interpretation of the results.

1.6.2. Hair and Exposure to Drugs

From the early 1980s hair has been used to study exposure to drugs. Drugs and drug metabolites remain in the hair indefinitely and this provides a 'much larger time window of detection'[40]. In contrast serum or urine drug levels decrease rapidly over a short period of time after exposure. Evidence for the long-term stability of drugs in hair is provided by the reported finding of cocaine metabolites in the hair of 4000 year old South American mummies. The fact that sampling is non invasive, storage is simple and further samples can be taken at a later date has made hair analysis popular in the solving of drug crimes in the USA[40].

The availability of sensitive analytical techniques such as Radio Immuno Assay (RIA) and Gas Chromatography coupled with Mass Spectrometry (GC-MS), allow the determination of drug metabolites in hair for forensic science purposes[78,85]. Hair analysis has been successfully used in the detection of cannabis, cocaine, heroin and opiates[41,42,43,44,45,46]. Hair analysis has also been applied in detecting the use of illegal anabolic steroids in athletes and cattle[47].

Monitoring individual drug dosage history and patient compliance can also be carried out using hair samples[48].

It has been suggested that hair analysis is particularly useful for the screening of personnel in highly sensitive positions, such as the army, administration or aviation, for illegal substances abuse[49].

Studies[1,50] had previously detected nicotine in hair and one such study in Austria also looked at metals in the hair of smokers and non-smokers. The results showed a significant increase in the levels of cadmium, lead, arsenic, cobalt, chromium and nickel in smokers' hair[50].

1.7. Problems Encountered with the Determination of Trace Elements in Hair

It has been considered that hair may be a useful medium for the determination of occupational and environmental exposure to trace elements[3,9,51]. However, before trace element levels in scalp hair becomes an acceptable indicator of exposure normal values have to be established, along with the standardisation of sample collection and sample preparation. In addition trace elements incorporated into hair from within the body (endogenous) must be differentiated from contamination by external (exogenous) sources[9]. Other factors to be considered include variations with hair colour, location, diameter, variations with sex or season, rate of hair growth and correlation between hair and other body tissues or fluids trace element levels.

Hair, because of growth, reflects previous elemental concentrations in serum and the body and any environmental influences[1]. External contamination from the environment and cosmetics such as shampoos, hair sprays, soaps and perfumes can occur. More permanent hair beauty treatments will also alter the hair trace element content. The location of the hair sample is also important and hence meticulous attention must be paid to the site from which the hair is sampled. Assuming that the growth of hair is approximately constant and continuous during the lifetime of the subject, it is expected that the concentrations of the trace elements will differ with increasing distance from the scalp, particularly during times of increased exposure. Variability in the concentration of elements depend on the distance the hair is from the scalp, generally the further from the scalp the higher the concentration of elements[14]. The longer the hair is exposed to external contamination the higher the levels of contaminants such as lead, arsenic, nickel, zinc and copper[18]. An example of this has been reported where

the levels of lead in hair were recorded as higher at the distal end of the hair, with almost 20 μg g^{-1} compared to 3 μg g^{-1} near the root.

One of the factors determining the concentrations of trace elements in hair is the intrinsic rate of growth of hair itself. For example the rate of hair growth is slowed down during periods of malnutrition. Hair of normal, healthy individuals generally should contain each essential trace element within a well-defined range of concentration. However, a number of factors can complicate the interpretation of the results such as changes in health and diet, exposure to external contaminants and cosmetic treatments[9].

The lack of established methods for hair analysis including the sampling of the hair and the pre-treatment methods used to remove exogenous contaminants, as well as the different analytical techniques used also make it difficult to compare the concentrations of elements found in hair.

1.7.1 Sample Collection

It has been suggested that hair is best collected from the area at the back of the head, called the vertex posterior (around the occipital bone seen in figure 1.4). Hair from this area has less variability in growth rate, where the number of hairs in the growing phase is relatively constant and the hair is less subject to age and sex-related influences[52]. It has also been shown that beard, pubic and axillae hair have no advantage over head hair as they can also be contaminated[52]. In the majority of studies hair has been collected from the occipital region (see figure 1.4) [3,15,16,67,68,97,100,110,116,131,148,154].

In a sampling protocol developed by the IAEA (International Atomic Energy Agency) it was stated that to assess internal body burdens of environmental mineral pollutants a single cutting from the occipital region is adequate[6]. Previous protocols had involved the collection of at least 100 individual hairs from 10-20 sites on the scalp. The IAEA also gave an outline of procedures that could be applied to other body hairs, such as beard or pubic that could be analysed if there were no scalp hair. They recommended that sample size should range from 500 mg to 1 g and the sample should be obtained from at least five and preferably ten or more separate locations along the nape of the neck. Hair specimens should be cut as close to the scalp as possible and should be limited to the first 5 cm of recent growth. It was accepted that hair samples would be cut from the nape of the neck with stainless steel scissors and that several hundred

milligrams of sample is normally sufficient for analysis. However, Kintz and Mangin reviewed the literature and showed that the sample size varies considerably among laboratories, but that samples sizes of 30-50mg are currently used[52].

In practice the sampling of hair from the head has been carried out in several ways. The routine method uses a pair of stainless steel scissors to take the sample. One method takes 2-3 cm specimens cut with stainless steel scissors from the part closest to the scalp in the sub-occipital region of the head[53,54]. It has also been suggested that plucking the hair from the scalp would provide more accurate information. However, it has been stated that the plucking of hair requires that the anagen hairs are selected (actively growing fibres). Classical plucking with the aid of a pair of rubber-coated tweezers seems to be the preferred method, because the hair can be removed with the root mostly intact and without further contamination from metals such as nickel found in stainless steel tweezers[55,56]. Hair samples can be conveniently stored in plastic vials, bags or envelopes.

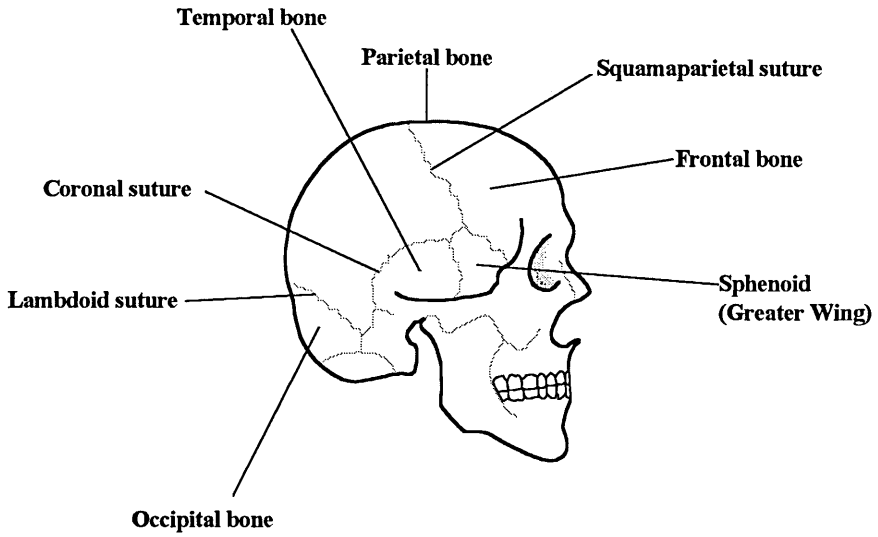


Figure 1.4. The regions of the skull[57].

1.7.2. Hair Sample Washing Procedures

Another of the problems encountered in the use of scalp hair for trace element analysis has been external contamination. This may result from the application of shampoo, water used for hair washing, cosmetic preparations and treatments, hand contamination of hair and from environmental and occupational sources such as atmospheric fallout[38]. The issue of hair washing to remove exogenous contamination must be addressed in order to ensure that the results of analyses reflect the amount incorporated into hair via metabolic processes and thus be of endogenous origin[58].

Factors which determine the extent of external contamination on hair include the type of shampoo used, hair treatments and brushing techniques, wash frequency and environmental and occupational exposure[59]. The levels of metals in hair also vary depending on the time of year the sample is taken[38]. Exogenous contamination can occur during the hair's growing stage and also after sampling.

Various washing techniques have been reported and these range from the use of only distilled water, to the use of ethylene-diaminetetraacetic acid (EDTA) solutions, various detergents and solvents[38]. One of the procedures adopted for hair analysis by X-Ray Fluorescence, involved the use of a mild detergent, isopropanol and distilled water. Prior to analysis the hair was washed in an anionic detergent (1% sodium lauryl sulphate), distilled water, isopropanol, distilled water and then air-dried in a laminar flow hood[38]. The results of this washing method are shown in the table 1.2.

The data show that mild hair washing had no statistically significant effect on the level of trace metals found in the hair. The study also reviewed commercially available shampoos, which contain chelating agents for the removal of trace metals and found that these cause significant amounts of calcium and iron to be released.

Trace Elements	Unwashed ($\mu g\ g^{-1}$)	Washed ($\mu g\ g^{-1}$)
Sulphur	40.5 ± 7.3	42.3 ± 8.0
Calcium	550 ± 348	402 ± 296
Iron	19.3 ± 5.9	18.8 ± 5.2
Nickel	1.15 ± 0.82	1.22 ± 0.51
Copper	21.8 ± 5.1	21.3 ± 6.3
Zinc	171.7 ± 59.2	150 ± 58.5
Selenium	0.61 ± 0.34	0.62 ± 0.27
Bromine	4.90 ± 4.10	3.30 ± 2.20
Strontium	18.9 ± 23.0	17.1 ± 22.6
Chromium	1.56 ± 0.74	1.60 ± 0.49
Manganese	1.55 ± 1.19	1.41 ± 1.02
Arsenic	0.55 ± 0.54	0.55 ± 0.50
Mercury	1.17 ± 0.74	1.20 ± 0.94
Lead	6.41 ± 4.40	5.96 ± 4.92
Rubidium	0.98 ± 0.58	0.78 ± 0.47

Table 1.2. Comparison of results for washed and unwashed hair samples taken from Yukawa et al[38].

The main washing procedures that have been investigated have included the use of organic solvents, detergents, water and commercial shampoo. In an investigation of washing procedures using water, hexane, ethanol, shampoo and detergent, there was no significant reduction in the hair chromium levels with the water wash but found 24% and 38% reduction with the organic and detergent wash respectively. It is interesting to note that the use of shampoo did not lead to a reduction in hair chromium levels. In an experiment in which hair was soaked in a $CrCl_3$ solution and then washed it was shown that the chromium remained partially bound to hair[58].

As mentioned in Section 1.5, the adsorption and elution of trace elements on human hair was studied in the US, by placing hair in a simulated sweat solution of each element studied. The hair was then washed with three solutions, EDTA, nitric acid and ammonium hydroxide and it was found that EDTA removed most of the bound species. All elements, except potassium and sodium, were found to adsorb and the extent of adsorption was pH dependent. Antimony, barium, cobalt, iron and manganese were removed with EDTA, while mercury and zinc were only partially removed after washing for 8 h with EDTA. In contrast to this no solvent could remove gold, selenium and silver[22,23].

In another investigation, in which hair was exposed to lead from a car exhaust, it was shown that the externally bound lead could not be removed by surfactants but only with EDTA solutions[1].

Investigations into the interaction of hair with surfactants, such as sodium lauryl sulphate (SLS), have been carried out[60,61,62]. The studies concluded that the extent to which the surfactant is bound to the keratin depends on the pH of the solution.

The IAEA method for washing hair involves sequential washing with acetone, water and acetone. This is probably adequate for the removal of dirt and dust, but there is no confirmation that it will efficiently remove exogenous trace elements from the hair and leave the endogenous elements completely undisturbed[1,6,38]. The protocol suggests that the hair (tied in a lock) is washed successively in acetone, three times in water and once more with acetone. Acetone should be of reagent grade and water of the highest purity. The solvents should be added in sufficient amounts to cover the sample entirely. The sample should be allowed to stand at room temperature for 10 minutes, in contact with the solvent with constant stirring and the washing should be carried out in a dust free enclosure.

A number of studies have used the IAEA method as matter of routine[16,88,112,130,136,163,175], however, others have departed from this protocol and used different organic solvents[109], such as isopropanol[3,154]. Also reported is the use of ionic surfactants, such as sodium lauryl sulphate[48], non-ionic surfactants[15,97,100,110,148,159,167] such as Triton X-100[15,100,110], a mixture of an organic wash followed by a surfactant wash[38,68,148,174] and a further wash with an acid such as hydrochloric acid[78].

One investigation of hair washing found that 50% of cadmium was removed by a SLS wash, compared with 15% removed in an acetone wash. Less impressive results were reported for copper, iron, manganese and zinc, which hardly washed off. The authors concluded that the IAEA methods could not be used as a wash method for head hair. They also investigated the number of washes and found that after the first two the amounts extracted had reached a constant level, except with EDTA where constant levels were obtained after four washes[63]. It has since been stated that EDTA should not be used for washing hair because it is believed that it removes a considerable amount of 'true hair minerals', i.e. endogenous species[1].

Hambidge reported, in 1982, that washing hair with either a non-ionic detergent or an organic solvent yields similar results for some trace elements, e.g. zinc, copper and chromium. However, a non-ionic detergent removes much more iron and magnesium

than an organic wash[76]. The study concluded that as a standard washing procedure has not been agreed upon hair analysis may be unreliable.

1.7.3. Hair Digestion

The low levels of lipid material in hair makes it easier to dissolve than most other biological samples. Acids such as nitric, perchloric, hydrochloric[3] and sulphuric have been used to digest hair samples, either singly, in combination[68,78,109,131,157,167] or with an oxidising agent such as hydrogen peroxide. Nitric acid is commonly used, either on its own[50,88,100,158,159,160,161], with perchloric acid[97,130,148], or with hydrogen peroxide[64]. Foo et al[110] used a sodium hydroxide solution to digest the hair. After a survey of methods, Kratzer et al[175] found that, sodium or potassium hydroxide digestions gave the best results in the digestion of hair for the analysis of mercury and methyl mercury. Also under scrutiny is whether or not a microwave oven should be used to digest the samples as opposed to open digestion methods. The consensus of opinion is to use microwave digestion methods properly to avoid losing any volatile analytes[1]. Open digestion methods are difficult to standardise and often depend on the skill of the operator[163].

In 1982, the Hair Analysis Standardisation Board made recommendations on the digestion of hair and the subsequent trace element determinations, but these provided little guidance on the actual digestion procedures[6] and just reiterated information about the sampling of the hair.

1.8.Trends of Metals in Hair

Over the years, several studies have investigated the influence of sex, age, race, hair colour and treatment on the levels of trace elements in hair.

1.8.1.Sex and Age

Taylor found that, with the exception of lead and cadmium, trace element concentrations of copper, nickel, selenium and zinc were greater in female hair than male hair[86]. In direct contrast to this, Gordon found that the levels of zinc in men and women did not differ, although in both sexes hair zinc levels decline with advancing age[65]. Other trends were outlined in Sky-Peck's study[39], which reported that the sulphur, iron and selenium contents were lower in females' hair while calcium, nickel, copper and zinc levels were significantly elevated. It was suggested that the differences

in sulphur, calcium, nickel and zinc levels may in part actually reflect hair treatment by women.

Copper hair levels have been seen to increase during the first 3 months after birth and then decline between 3-6 months, while zinc levels have been seen to decrease in infants who were not breast fed[86]. Hair chromium levels appear to decrease following birth and by the age of 2-3 years the levels are less than half of the value at birth. Apart from the neonatal period, increasing hair metal concentrations during childhood have been reported for zinc, mercury, selenium and copper, where the increased copper were observed only in males[86]. From studies that have examined trends in trace element concentrations in hair through adult life, it is evident that the gradual decrease in copper seen in females is maintained until middle life. There may also be a slow increase in hair zinc concentration during later life and an increase in barium is apparent in females from the age of fifteen which reaches a peak at 30-35 years and then gradually declines over the next decade or so. It has been suggested that the lead content of children's hair is twice that of adults but this has not been confirmed[6]. Most reports seem to indicate no change in lead concentrations with age but a peak at 35-40 years old in women has been noted[86].

It is generally seen that older people have less sulphur, calcium, zinc, selenium[66] and strontium in their hair[38]. This could be as a result of the levels of melanin in the hair decreasing with age and thus there are less sites for the metals to bind to in the hair structure. The scalp hair concentrations of copper, lead and cadmium for those under 30 years of age are significantly higher than those over 40 years of age[6].

The zinc, copper, cadmium and lead content in hair of males and females has been shown to be age dependent and varies between the sexes[67,68]. In both sexes the hair zinc and copper levels decrease from 12-80 years. In males the cadmium hair levels have been shown to increase until the 20th year after which they either remained at the elevated level or decreased slightly, whereas with females the concentration of cadmium increased from the 14th year to a peak between 40-50 years of age, and remained high. Lead hair levels gradually declined in males from 2 to 85 years, whereas the lead in female hair was similar to cadmium levels where it increased from 14 to 35 years, but then differed from the cadmium levels by decreasing sharply to 84 years. As cadmium and lead are nonessential elements it is expected that levels in hair are determined by the extent of exposure to both elements.

1.8.2. Race

It has been found that mean concentrations of lead, calcium, manganese, strontium, iron, copper and nickel were significantly higher in Caucasians than in Orientals, while arsenic, calcium, chromium, iron, lead, manganese and nickel were higher still in Blacks[86]. Mercury, rubidium and sulphur were highest in hair samples from Caucasian subjects and the greatest concentrations of selenium were found in hair from Oriental subjects. The most striking results were apparent for lead where the mean concentration in hair samples from Black subjects was 5 and 18 fold greater than the values for Caucasian and Oriental subjects respectively. A study carried out in Poland, in which Vietnamese and Polish students were compared, showed that the Poles had almost twice as much iron and half as much calcium in their hair[6]. A study of over 900 people from Japan, India, Canada, U.S.A. and Poland[69], showed that aluminium, calcium, copper, iron, lead, magnesium, manganese, phosphorus, potassium, selenium, sodium and zinc all occur at significantly higher concentrations than other elements in hair. Among the metals, zinc, copper, iron, aluminium and lead occur at higher concentrations in decreasing order. This order is to be expected since the aluminium and lead are nonessential elements. The high aluminium and iron levels in Indians were attributed to the use of aluminium and iron cookware. Canadians and Americans show, in general, higher concentrations of cadmium and copper, but in addition the Canadians also have higher antimony and zinc, while Americans have higher manganese and selenium levels. In the Polish samples, the cadmium and copper levels are higher in females than males, and in general the potassium and sodium levels are lower in males. Furthermore, the levels of mercury and tin in males, and antimony, cobalt, mercury and nickel in females of Japanese origin, were higher than in any other population. Similarly, the levels of aluminium, chromium, iron and potassium for males and arsenic, lead, magnesium, selenium and vanadium for females were highest in those of Indian origin.

1.8.3. Hair Colour

Hair colour is determined by the amount of melanin pigments in the cortex. Dark hair has higher amounts of these pigments than does blond or white hair, while red hair contains, in addition, siderin pigments[6] (the siderin pigments contain iron). Brown and black hair have high eumelanin concentrations, whereas blond hair has a higher concentration of phaeomelanin and red hair has a mixture of both melanin polymers[13].

These findings suggest the need to develop the means to consider the link between melanin and trace element levels in hair.

Taylor found that the zinc levels in blond hair were lower than those in brown and black hair[86]. Black hair was seen to contain more magnesium and cadmium, but less lead than brown hair. Zinc (especially in females), cadmium and nickel were high in red hair compared with brown, whereas lead was reduced. Colour was seen to be unrelated to copper and chromium[86]. It has also been seen that blonds have significantly less iron and manganese in their hair than brunettes, while red-heads have more chromium, copper and zinc in their hair[39].

Pigmented and natural grey hair have also been compared. Natural pigmented hair from women contained very high concentrations of copper compared with hair from men and grey hair from other women. Grey hair from women of any age contained less cadmium and lead than natural pigmented hair from men and women[39,86].

1.8.4. Hair Treatments

In 1990, Sky-Peck carried out a study on the distribution of trace elements in human hair and trends in the different hair types taking into account the treatment that the hair had undergone[39]. It was found that both peroxide bleaches and permanent-wave mixtures markedly altered the contents of sulphur, calcium, iron and nickel in hair. In addition, peroxide affected zinc levels and permanent waving increased copper and arsenic levels.

It was also found that commercially available shampoos contain chelating agents can cause the release of significant amounts of calcium and iron[39]. Furthermore, the copper and zinc levels are known to increase after showering, shampooing and dyeing.

1.8.5. Profile Along Hair Length

In a study of the variation of trace elements along the hair length using neutron activation analysis[38], it was shown that the concentrations of copper, calcium, iodine and magnesium increased to the tip of the hair, while chlorine and bromine concentrations decreased. No trends along the hair sample were seen for mercury, manganese, zinc or selenium. The trace element concentrations in adult hair demonstrated lower and less fluctuating values compared with those from children[39].

1.8.6. Anatomical Location

Assuming that the processes involved with the endogenous incorporation of trace elements into the hair are uniform over the entire body, samples collected from covered anatomical sites, such as the pubes, should be less vulnerable to exogenous contamination than samples collected from the scalp. However, Hopps reported that pubic hair is less suitable than scalp hair for analysis because it grows slower and is more prone to contamination from sweat and sebum[11]. The advantage of using pubic hair could be as a check against misinterpretation of elevated levels in scalp hair[6]. Lenihan suggests that beard hair is a more useful material as it can be sampled everyday which would minimise the exposure time to environmental contamination[5].

1.8.7. External Contamination and the Ranges of Metals in Hair

Studies carried out by Airey[15] in 1983, showed that mercury has a high affinity for hair samples. A large variation in hair mercury levels was found in one subject, ranging from 1.83 $\mu g\ g^{-1}$ on the top of the head to 13.8 $\mu g\ g^{-1}$ at the front. The large variation was caused by the donor habitually pushing hair out of his eyes with mercury contaminated hands. It was suggested that when sampling it is necessary to be aware of the donor's treatment of their hair. For example, was the hair treated with thiol-containing solutions, was the hair washed with mercurial soap which can raise the hair levels to 100 $\mu g\ g^{-1}$, or was the hair damaged by the use of curling devices. The donor's occupation and habits need to be documented so that details such as their use of contraceptive spermicides or cosmetics containing mercurials, such as skin lightening creams, are known. Skin lightening creams have been shown to produce hair mercury levels as high as 9220 $\mu g\ g^{-1}$ in Kenyan women[15].

1.8.8. Geographical Influences on Trace Elements in Hair.

Epidemiological studies have shown good correlation between the total heavy metal levels in hair and environmental exposure[19]. In India[97], as well as Spain[100], hair lead levels have shown good correlation with the amount of air borne lead in the atmosphere.

Genetic and dietary factors differ from area to area and this contributes partly to the variation of elemental compositions found in hair samples. One of the most important factors influencing zinc and copper concentrations in hair is diet[16]. The relationship

between soil and hair selenium levels is a clear illustration of the influence of geographical factors[70]. Higher mercury levels are found in people who eat lots of fish, for example Eskimos[16]. Dietary supplements also need to be considered when analysing hair in highly industrialised countries, where the taking of mineral supplements is common. Oral contraceptives are among the medications reported to have an effect on the concentration of trace elements in the hair, although the reports are contradictory[1,6]. One review showed that women who take oral contraceptives have decreased copper and increased zinc hair levels when compared to non-users[71]. Other reports have shown that while the zinc levels increased, silicon and chromium decreased and no changes in the levels of copper, manganese, gold or caesium were seen in the hair of those who took contraceptives[6]. Both of these studies were contradicted by another report which found that oral contraceptives had no effect on copper or zinc levels[72].

In an Indonesian study, high levels of calcium in hair were attributed to high calcium levels in sago grubs, which is a local delicacy consumed in large quantities on ceremonial occasions[73].

1.9. Trace Elements in Human Health

Ten trace metals are essential to warm blooded mammals: iron, copper, manganese, zinc, cobalt, molybdenum, chromium, selenium, vanadium and nickel, along with four bulk metals: sodium, potassium, magnesium and calcium. In addition to these, it has been suggested that cadmium and arsenic may be required in the body at very low levels[74]. Although these elements are essential, they can be toxic at high concentrations. There are also a number of elements which are non-essential and are known for their toxic effects. Metals such as cadmium, lead, chromium and mercury and metalloids such as arsenic, antimony and thallium, are extremely harmful as they can be easily incorporated into biological systems. Mercury and lead have long been recognised as occupational hazards, while compounds of arsenic and thallium have been used as commercial poisons. These elements have no known biological functions and their presence in the human body often interferes with normal metabolic processes.

The type of compound or oxidation state is crucial in determining the extent of the toxicity of the element. This can mean that different forms of the same element may have varying degrees of toxicity, for example, transition metals which exist in different oxidation states exhibit different toxicities.

Blood and urine analysis are the more traditional approaches to evaluating trace element levels in the human body. Trace elements are, for the most part, transported from organ to organ by the blood. However, the residence time for a given trace element in the blood is relatively short. Additionally elemental concentrations in blood and urine fluctuate in response to changing physiological and/or environmental conditions. Therefore, blood and urine are not reliable for evaluating trace element levels in body organs resulting from transient metabolic or environmental events[9]. Obtaining samples of blood and urine involve invasive procedures, whereas the collection of hair is non-invasive and the samples remain stable after collection for much longer[3]

A collection of some published values of the normal levels of metals in blood, hair and urine is given in table 1.3. The higher levels found for copper, iron and zinc compared with cadmium, lead and mercury is a reflection of the essential nature of the former. It should be noted that, in general, the data for trace metals in hair show much wider ranges of normal values than similar tabulations for normal values in more conventional samples such as blood[10]. Reasons for this could be that the hair types and thus contents vary with donor, and that on the same donor different areas of sampling can lead to different values of metals being determined[15].

Metal	Blood Serum ($\mu g\ ml^{-1}$)	Urine ($\mu g\ ml^{-1}$)	Hair ($\mu g\ g^{-1}$)
Copper	0.8-1.8	0.03-0.06	7-40
Iron	0.7-1.5	0.10-0.15	15-175
Zinc	0.8-1.1	0.40-0.60	150-250
Cadmium	0.001-0.007	0.001-0.005	0.4-2.4
Mercury	0.002-0.006	0.001-0.02	0.5-10
Lead	0.002-0.2	0.006-0.12	5-50

Table 1.3. Range of normal values for cadmium, copper, iron, lead, mercury and zinc in blood, hair and urine[10].

It should also be noted that the concentrations of the metals in hair are higher than the concentrations in blood or urine. This is as a result of the proteins that are present in

hair having a high affinity for metals and also, possibly, due to the fact that the metals are bound both endogenously and exogenously[75]. Many reports have shown a good correlation between those who have been exposed to heavy metals and the increased levels in their hair[76].

1.10. Sources, Health Effects and Hair Levels of Trace Elements

The elements of interest in this study are antimony, arsenic, cadmium, chromium, lead, mercury and selenium.

1.10.1 Mercury

In the ionic form, Hg^{2+}, mercury is toxic because it is easily soluble at pH 7. Organomercury (RHg^{+}) species are especially toxic since their lipophilic character allows them to penetrate the blood-brain barrier. Methyl mercury is the most toxic form of mercury. Mercury poisoning causes neurological defects, dark lining of teeth, diminished blood circulation, impaired concentration and co-ordination and tremors. At a very high level of exposure the symptoms can include loss of hearing, blindness and death.

The acute toxicity of mercury was identified in the workplace long before its chronic toxicity to the general public was suspected. It has been suggested that the Mad Hatter from *Alice in Wonderland* suffered from mercury poisoning[10]. Mercuric acid was used to prepare felt for hats from animal fur and the hat makers frequently developed tremors characteristic of mercury poisoning. Charles II, King of England from 1660-1685 and the founder of the alchemists, is believed to have died of mercury poisoning. His hair was analysed in 1966 by neutron activation analysis (NAA) and was found to contain 53 $\mu g\ g^{-1}$ mercury[5]. It is believed that the King had a laboratory at Whitehall where he experimented with the distillation of mercury which was a favourite pastime of the alchemists.

Robert Burns, Scotland's national poet, is also suspected of dying of mercury poisoning. It was believed that the poet died of rheumatic fever, but a sample of Burns' hair was examined by NAA in 1971 and found to contain 8 $\mu g\ g^{-1}$ of mercury, which is much higher than the normal low $ng\ g^{-1}$ levels. It is thought that Burns suffered from a diseased liver and his physician at the time gave him a course of mercury to cure the illness[5].

Mercury compounds have been used for the treatment of syphilis, in disinfecting preparations and as a component of fungicides. During the winter of 1971/1972 several thousand people in Iraq developed mercury poisoning after consuming grain which had been treated with fungicides containing methyl mercury. The grain was intended for use as seed, but considerable amounts were used to prepare food. There were many severe symptoms such as tremors, blurring of vision, partial paralysis, difficulty in hearing, blindness, deafness, loss of speech and damage to foetal membranes[5].

Between 1953 and 1956, a disaster involving mercury occurred in Minamata Bay in Japan, where increasing numbers of adults and children residing near Minamata Bay showed loss of co-ordination, numbness to limbs, partial blindness and loss of hearing. Convulsions, coma and death followed in 46 of 125 cases. By 1956, a congenital Minamata disease was observed in the offspring of symptom-free parents. The disease was subsequently linked to acute methyl mercury poisoning and traced to the consumption of fish that had 'bio-magnified' mercury from industrial wastes discharged into Minamata Bay[10]. It is believed that inorganic mercury used as a catalyst in the acetaldehyde process had methylated inside the factory and then had been discharged into Minamata Bay. The waste from the acetaldehyde process continued to pour into the Bay until 1968[77].

Recently, environmental mercury pollution due to gold mining in the Amazon region has caused worldwide concern[78]. It is estimated that around 100 tonnes of mercury are released annually, of which 45% is discharged into the river systems and 55% into the atmosphere. To determine the level of human exposure caused by the goldmining industry in Brazil, Akagi et al collected human hair, blood and urine samples from people living in the fishing villages and gold mining areas along the Tapajos river basin[78]. The results showed good correlation between blood and hair for the levels of total and inorganic mercury but no correlation between blood and urine for total and inorganic mercury levels was found. The survey concluded that in the fishing villages, the hair and blood of the inhabitants contained mercury at particularly high levels and that the mercury appeared mostly in the methylated form. Abnormally high levels of mercury in the inorganic form was found in the urine of goldshop workers, and the hair of the goldshop workers contained both inorganic and methylated forms of mercury, although the methylmercury levels were much lower.

Recently the US Environmental Protection Agency (EPA)[79] have discussed the introduction of stricter regulation of mercury in industrial emissions and in seafood. It

recommends a maximum daily dose of 0.1 μg kg^{-1} of body weight, which the EPA has calculated will result in a concentration of mercury in hair of 1 μg g^{-1}. This is compared with the World Health Organisation limit of 10 mg kg^{-1} of body weight[79]. The annual total world mercury production is 3660 tonnes, with 60% of this from coal, oil and wood burning, 36% from waste incineration and 4% from the non-ferrous metal industry[79]. Workers at risk of mercury exposure include dentists, fishermen, thermometer workers, molybdenum refinery workers and chemical industrialists. Occupational exposure to mercury has also been reported for workers in a fluorescent lamp plant in Japan, where the workers' urine samples were shown to contain more mercury than a control group[80]. Past occupations in which workers were exposed to mercury, include the fingerprint police in the 1940s, mercury miners and processors, pesticide and thermometer workers, chlorine manufacturers and polarography students[15].

Usually about 95% of ingested methyl mercury is absorbed into the body, but only 15% of inorganic mercury. When a meal which contains methyl mercury is consumed, peak blood levels are reported 4-14 h later. It then takes several hours for the mercury to be distributed in the body and months for it to be eliminated. The mercury remaining in the blood is in equilibrium with the levels in the rest of the body and similar concentrations can then be seen in hair[15]. Between 61-82% of inhaled inorganic mercury vapour is retained. The mechanisms by which the two forms of mercury enter the hair are still unknown, although it is known that inorganic mercury is more concentrated in hair than blood. A suggested mechanism therefore, is that of selective removal of mercury species from the blood in the hair follicle during hair formation. An alternative mechanism is that inorganic mercury in excreted sweat is adsorbed onto hair as it emerges from the scalp[15].

The prevalence of mercury poisoning (usually mild, though occasionally fatal) in dentists and their surgery assistants provides an interesting example of a hazard which, though widespread and clearly undesirable, is not yet adequately controlled[5]. The use of amalgam fillings presents no hazard to the patient as metallic mercury passes through the digestive tract with little or no absorption. Additionally amalgam is almost insoluble in saliva and gastric juice. Increased excretion of mercury has been found for a few days after the insertion of a filling, however, there in no evidence to suggest that this small amount of mercury is harmful to the patient. Mercury levels in the patient

have also been seen to increase following removal and restoration of a single amalgam filling, although even this increase is believed to be small[81].

A Glasgow study reviewed by Lenihan[5] indicated that head and body hair and nails, of dentists and dental assistants showed elevated mercury levels when compared with those who were not exposed. In most cases, the levels were two or three times higher than the unexposed workers. Other studies have also documented elevated mercury levels in dentists' hair[1,6,82].

1.10.2. Arsenic

Arsenic exists in two forms, inorganic and organic, both of which are naturally occurring in the environment and may be the product of industrial processes. Arsenic exists principally in two valence states As^{3+} (AsO_3^{3-}) and As^{5+} (AsO_4^{3-}). Although both are highly toxic, the former arsenite is considerably more toxic than the latter arsenate[83]. Other arsenic species found in the environment are dimethylarsinate (DMA), monomethylarsonate (MMA), arsenobetaine (AB) and arsenocholine (AC). DMA and MMA are moderately toxic whereas AC and AB are virtually non-toxic. Arsenobetaine, $[(CH_3)_3As^+CH_2COOH]^-$, commonly found in seafood, is essentially non-toxic and is excreted unchanged in urine[84]. The inorganic forms of arsenic are about 100 times more toxic than the organic forms. Based on epidemiological data, inorganic arsenic is currently classified as a human carcinogen[85].

Arsenic is a naturally occurring metalloid known for its toxicity. It is believed that Napoleon died of arsenic poisoning. His hair was found to contain 56 $\mu g\ g^{-1}$ arsenic and it is thought that volatile arsenic species formed by microbial conversion of the arsenic compounds on the wallpaper in his cell might have been responsible[5,86]. As early as 1775, Percival Pott linked the appearance of cancerous tumours in chimney sweeps and sheep dip workers to occupational exposure to arsenic compounds[5].

Arsenic compounds occur naturally but industrial processes such as nonferrous ore smelting and fuel combustion increase the levels released to the environment. Detergents made from sulphuric acid manufactured by the lead chamber process, which uses pyrites, usually contain arsenic as an impurity[5]. The world production of arsenic in 1995 was 50000 tonnes and around 70% of all the arsenic produced was used in the pesticide industry[87].

In 1995, Sen et al[97] linked Blackfoot disease with arsenic exposure and looked specifically at an arsenic contaminated artesian well in China They found that all the sufferers of this disease had consumed water from the well and exhibited high levels of As^{3+}, As^{5+}, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA).

In West Bengal, seven districts (with a population of 34 million) were found to exhibit high concentrations of arsenic in the water supply and it was claimed as the "biggest arsenic calamity in the world"[88,89,90,91]. The exposed people were found to have high levels of arsenic in their hair, nail and urine samples and many cases of arsenical skin lesions such as melanosis, leucomelanosis, keratosis and gangrene, along with lung and bladder cancers were reported[90].

Studies of occupational exposure to arsenic in copper smelters and community exposure to significant arsenic levels in drinking water have indicated an increased risk of lung and skin cancer, respectively[88]. Follow-up studies in Taiwan have linked increased incidence of bladder and kidney cancers with exposure to arsenic in drinking water[83]. Epidemiology studies have indicated a synergistic interaction between arsenic exposure and cigarette smoking in smelter workers in the induction of lung cancer and in the production of chromosomal aberrations. Arsenite has been shown to enhance the mutagenicity of UV treatment in bacterial cells and to inhibit DNA ligase activity in mammalian cells. The induction of chromosomal aberrations in human cells by DNA crosslinking agents combined with UV light was observed to be synergistically enhanced by arsenite[83].

Arsenic exposure also occurs in the semiconductor and timber treatment industries[92]. A study carried out in Glasgow in 1990 looked at the arsenic present in several groups of workers and showed that occupational exposure to arsenic greatly increases the concentration in urine. The average level of arsenic in urine samples of the control was found to be 4.4 $\mu g\ g^{-1}$, compared to 10 $\mu g\ g^{-1}$ in the electronics industry, 47.9 $\mu g\ g^{-1}$ for timber treatment workers, 79.4 $\mu g\ g^{-1}$ for glass workers using arsenic trioxide and 245 $\mu g\ g^{-1}$ for chemical workers manufacturing and handling inorganic arsenicals[93].

Arsenic is one of the most suitable elements for hair analysis because the keratin in hair has a greater affinity for arsenic than any other tissue[11].

In 1977 the Home Office in England carried out some experiments regarding arsenic in hair. They exposed a 49 year old male to arsenic by giving him a solution, known as Fowler's solution, consisting of 6 mg of arsenious oxide (containing the equivalent of

1% w/w arsenic trioxide in neutral solution) in a hot drink. Over a period of time the man's sweat, urine, head and beard hair were sampled and analysed for arsenic. The hair showed increased arsenic levels after only hours and over a period of months progressed down the hair length. In contrast to this the arsenic level in the urine peaked a day after ingestion and the levels in sweat did not change significantly throughout[94].

Chronic exposure to inorganic arsenic is a potential occupational hazard for unprotected employees working in the semiconductor manufacturing industry. The processes involved in doping silicon wafers to produce conducting circuits using arsine gas or solid sources containing elemental arsenic, leave arsenic residues on equipment such as ion implanters, ion source housings, epitaxial reactors and on nearby work surfaces. A study carried out in 1995 in San Diego, analysed the hair of workers where arsine gas (15% arsine, 85% hydrogen) was being used as a dopant. Despite proper ventilation in the workplace, and the elimination of smokers from the study, the workers showed considerably increased hair arsenic levels when compared to normal levels[95].

Hair levels were also monitored in workers at a gallium arsenide plant and a copper smelter. When compared with controls, the hair of the gallium arsenide workers showed slight inorganic arsenic contamination, whereas the hair of the workers in the copper smelter showed higher amounts of inorganic arsenic in their hair. This study also investigated the presence of other arsenic species in hair and found that in the copper smelter workers' hair there were no methyl arsonic acid (MMA) or trimethyl arsenic compounds (TMA), only DMAA, while MMA and TMA were seen in the normal group. As DMAA was not seen in the ambient air at the works it was assumed that the DMAA detected in the hair samples had been eliminated from the body and accumulated in the hair[96].

1.10.3. Lead

Lead is the oldest recognised toxic metal and is the sixth most produced metal after iron, aluminium, copper, manganese and zinc. Lead toxicity affects several organ systems, including the nervous, hematopoietic, renal, endocrine, skeletal and reproductive[9,113]. Alkyl lead compounds are the most toxic lead species. Nerve and brain damage, increased risk of premature births, adverse neuro-psychological effects among young children and endocrinal disturbances in adults are all documented effects

of lead exposure[97]. Exposure to lead has also been associated with hypertension in adults[98]. In addition, lead interferes with calcium metabolism in the body, by competing for calcium-binding protein sites, blocking voltage-dependant calcium membrane channels and inhibiting neurotransmitter kinetics. Other metabolic routes affected include those of zinc, copper, iron and vitamin D. However, of major concern is the impairment of cognitive and behavioural development in infants and young children caused by exposure to lead and its compounds. Children living in the urban environment are exposed to lead via the air they breathe, the water they drink and the food they ingest. The major route of the absorption of lead is through the gastrointestinal tract and approximately 50% of dietary lead is absorbed by children[99]. Lead is generally considered a ubiquitous health hazard for humans[100].

The ancient Greeks were the first to write about lead poisoning, but it is believed that the Roman ruling class was the first to experience the metal's adverse health effects on a large scale. The Romans used lead extensively for vessels of all kinds, including cooking utensils, water pipes and wine storage casks[99].

The fuel additive tetraethyl lead, batteries, bearing alloys, solder and radiation protection materials all contain lead[101]. The main source of lead in the environment today is from the fuel additives tetraethyl and tetramethyl lead. The lead contamination from petrol makes up about 70% of the total lead in the environment, natural sources such as volcanic dust make up 1% and the rest is made up from industrial sources (iron smelting 12%, lead smelting 5%, zinc and copper smelting 9% and coal burning 4%)[98].

In recent decades the lead levels in the environment have decreased with the introduction of lead free petrol, the banning of lead paint and the replacement of lead piping. A study carried out in Spain, evaluated the outcome of reducing the lead content in fuel from 0.4 to 0.15 g L^{-1} (the European Union maximum limit)[100]. The survey was carried out between 1990-1995 and it was found that all the levels in the hair and blood of adults and children were approximately half the original value after the five year period. Blood lead levels in adults declined by 47% (from 12.0 to 6.3 μg dL^{-1}) and in children's hair by 53% (8.8 to 4.1 μg g^{-1}). In many countries occupational exposure to lead continues to be a widespread problem, particularly in the manufacture of batteries, paints and pigments, plastics, ceramics and in secondary foundries and welding[102]. A 1986 study of lead workers in the mineral extraction industry in the Peak District, Derbyshire found that the miners who extracted the ore from the mines,

quarries and spoil tips did not have high blood levels of lead. However, the workers who worked in the dry grinding department had several cases of lead poisoning, symptoms being excessive tiredness, muscle cramps and arthralgia[103].

A 1998 US study showed that children who lived in a lead mining area showed considerably higher lead levels in their blood than children who did not live in the vicinity of a lead mine. This was further confirmed from analysis of the soil and dust levels in the areas[104]. Over 200 organolead workers had their blood levels analysed in a 1995 study in the US[105]. The study showed that recent exposure to organic lead and recent combined exposure to organic and inorganic lead were significantly and positively associated with elevated blood lead levels[105]. The study further showed that age and cigarette smoking were positively associated with blood lead levels, however, alcohol use was associated with lower blood lead levels. In 1998 a study of refinery workers, mechanics and petrol service technicians was carried out in Turkey. The workers all showed high levels of lead in urine, with the refinery workers having higher levels. Some of the mechanics who showed higher levels within their group were found to have been cleaning their hands in petrol, which had further increased their exposure to lead[106].

Lead is believed to enter the hair bulb during the short intense period of mitosis. It may also be absorbed into the metabolically inactive keratinous structure during relatively long exposure to dirt, dust, sweat, sebum, environmental sources and chemical agents applied to the hair. It is difficult to determine between exogenous and endogenous lead in hair, but it is reasonable to assume that a fraction of the lead in the hair close to the scalp is of endogenous origin and that the additional accumulation of lead in the more distal hair segments is most likely due to external sources[1]. A study in the US in 1972 analysed lead in human hair from 1871-1971. The results suggested a decrease in lead levels over the 100 years despite an increase in atmospheric lead[107]. Mean levels of 164.24 $\mu g\ g^{-1}$ and 16.23 $\mu g\ g^{-1}$in children's hair and 93.36 $\mu g\ g^{-1}$and 6.55 $\mu g\ g^{-1}$ in adult hair were determined in samples from the period between 1871-1923 and 1971 respectively.

Epidemiological studies of lead in hair to assess exposure were carried out as early as 1970, when it was found that children's hair accurately reflected community exposures. Hair from children in five districts in the US was compared and showed that arsenic, cadmium and lead increased when the local industries consisted of metal smelting

processes[108]. A Glasgow study on the effect of lead plumbing found that children who lived in houses with lead pipes had five times more lead in their hair compared to unexposed controls[5].

Elevated levels of lead have been found to correlate to exposure in battery factory workers and petrol station workers[6,109]. A 1993 study examined the relationship between hair and blood lead content in battery workers. It showed that the hair and blood levels were higher compared to the normal population and there was good correlation with the amount of occupational exposure[110]. Hair lead levels, as well as aluminium, antimony, magnesium, manganese and potassium, in professional drivers were found to be higher than in office workers in a study carried out in Hong Kong[111]. Other industrially exposed lead workers also showed higher lead levels than a normal group in Pakistan in 1994[112].

1.10.4. Cadmium

Cadmium is a ubiquitous environmental pollutant and is nutritionally nonessential and toxic. It interferes with the metabolism of three essential metals: calcium, zinc and iron[113]. Cadmium's pathway to man is from food, particularly leafy vegetables, grains and cereals. The tobacco leaf contains a substantial amount of cadmium and cigarette smokers often have much higher levels of cadmium in their bodies. Cadmium accumulates in the liver and kidneys and has a long biological half life, e.g. from 17-30 years in man. Toxicity involves two organ systems, the renal and skeletal systems and is largely the consequence of the interactions between cadmium and essential metals, in particular calcium[113]. Cadmium poisoning produces weight loss, bleeding, rhinopharyngitis, perivascular and peribronchial fibrosis, pulmonary emphysema and damage to the liver and kidneys[10]. It has also been linked with bone diseases such as the debilitating bone disease called itai-itai, first observed among Japanese fishing villagers, osteoporosis[113] and is believed to be implicated in the development of hypertension. Evidence has been found to link cadmium toxicity to emphysema and cancerous tissues[114].

Cadmium is considered to be far more toxic than lead with an ionic radius (95 pico metres) similar to that of as Zn^{2+} and Ca^{2+} and can therefore substitute zinc from its enzymes and calcium in bone tissue. Chronic cadmium poisoning can cause embrittlement of bones and extremely painful deformations of the skeleton.

Cadmium is an important industrial metal. It is used in anti-corrosion electroplating and as a component of nickel/cadmium batteries, colour pigments, in stabilisers in plastics and for metal surface treatment. Cadmium is also a side product of zinc smelting[101].

In 1975, a study was carried out to investigate the correlation of hair cadmium levels with the levels in critical organs and then to relate these to occupational exposure. The study used samples from the New York City Medical Examiners' Office and found that the elevated cadmium levels in the kidney and liver could be linked with occupational exposure[114]. Hair cadmium levels that correlated well with whole body levels were seen in a study where mice were fed cadmium chloride intravenously[115].

Hair cadmium levels from workers in a nickel-cadmium battery factory were found to be significantly elevated compared to normal values and the levels found correlated well with the length of exposure[116]. Elevated hair and urine cadmium levels were also found in inhabitants of a mining area in Japan, where a local mine had released the element into a river and polluted the drinking water system. The results showed that younger people showed higher levels of cadmium than older people. Furthermore, the inhabitants showing symptoms of severe bone pain had higher levels of cadmium in their hair and urine[117]. In 1998 a study looked at the cadmium and lead levels in children who lived near a zinc plant in one of the most polluted areas in Poland. The results showed that the levels of both the heavy metals were some of highest seen in the hair of children in Poland and that boys' hair showed higher levels than girls' hair and that a correlation could be seen between hair and blood levels[118].

1.10.5. Chromium

Trivalent chromium (Cr^{3+}) acts primarily by regulating insulin action[119]. However, chromates (CrO_4^{2-} and $Cr_2O_7^{2-}$) have long been recognised as skin irritants and are now classified as a potential carcinogen. Chromate can break membrane barriers and reach the cell nucleus because its size is similar to the sulphate ion, SO_4^{2-}. In the cell nucleus, chromate can oxidatively damage genetically important components.

Chromium is used in the manufacture of ferrous and non-ferrous alloys, pigments, electroplating, leather tanning, fungicides, corrosion inhibitors in cooling water, wallpaper, photographic films, magnetic tapes and printing inks.

The development of asthma in workers has been linked to the use of chromium containing materials and processes[120]. There have been reports of occupational asthma

associated with the use of chromium salts among metal-plating factory workers and stainless steel welders. Chromium exposure is also associated with respiratory diseases such as chemical pneumonitis, bronchitis, emphysema and lung carcinoma among workers employed in metal-plating, tanning and stainless steel welding[120]. Chromium is a potent skin sensitiser and it is one of the common contact allergens which cause contact dermatitis[120].

1.10.6. Selenium

The metalloid selenium is both an essential nutrient and a potential toxicant. Excess doses of selenium cause liver atrophy, necrosis and haemorrhages in animals. In general, selenites are more toxic than selenates or selenium salts. Selenium is essential at $\mu g\ kg^{-1}$ levels and toxic at higher concentrations. Selenium is an important metalloid with industrial, environmental, biological and toxicological significance[121].

The average selenium content of the earth's crust is 0.05-0.09 mg kg^{-1} which is about the same as cadmium and antimony, but higher than levels of molybdenum, silver, mercury and uranium. World production of selenium was approximately 1470 tonnes per year in 1995. Selenium is widely used in semiconductors, thermoelements and photocopy materials. Approximately 14% of selenium is used in the manufacture of inorganic pigments (principally as cadmium sulphoselenide which is used in plastics, paints, enamels, inks, rubber and ceramics). An additional 10-15% selenium is used in other applications including : accelerators and vulcanising agents in rubber production, in stainless steel, and as selenides of refractory metals for use in lubricants. Selenium is also used in the manufacture of glass and paints to neutralise the undesirable green colour caused by the impurities of iron. The sulphoselenide pigment is used to produce colours from yellow to maroon in the plastic and ceramic industry. Workers who are at the highest risk of selenium exposure in the workplace are heavy equipment mechanics, painters and mechanics in service stations. Selenium monosulphide and disulphides have been used in dandruff treatments and [^{75}Se]-selenomethionine is used as a diagnostic scanning and labelling agent for various malignant tumours.

Described symptoms and signs of poisoning due to inhalation of selenium fumes or Se-containing dust are non-specific and include: irritation of eyes, nose and throat, nausea, vomiting, increased body temperature, and fatigue. More serious exposures have caused inflamed mucous membranes, irregular heartbeats and heartburn[121].

Selenium has been found at μg g^{-1} in hair and it is thought that selenium has a high affinity for the keratin in hair and nail[1,6]. Measurement of the sulphur rich protein, keratin, has been found to offer a long term marker of selenium in biological processes[64]. The accurate determination of selenium in hair requires the total destruction of all organoselenium compounds. These compounds are the acid resistant sulphur and selenium containing amino acids, methionine, cysteine and cystine which are present in keratin[64].

In 1987 the World Health Organisation recommended hair as a potential biopsy material for the determination of selenium status in human beings. In 1997 such a study[122], on the low level exposure to selenium in the workplace, was carried out. The workers studied manufactured the drums for photocopy machines and were exposed to selenium during handling the raw materials and during the processes of coating, drum engineering, grit blasting, mask and heat stripping, spray washing and packaging the finished material. The hair of the exposed workers showed considerably higher selenium levels than the unexposed. Interestingly, the vegetarian exposed workers showed less selenium in their hair than the exposed non-vegetarian workers[122]. This finding indicates that there is a relationship between the diet and selenium levels in hair. Therefore it is clear that a history of the donor is required before any comparison between exposed and unexposed workers can be made.

1.10.7. Antimony

Antimony in nature is an element of low abundance, however the use and production of antimony have increased continually through the past decade. Antimony, like arsenic and selenium, is toxic at high concentrations[123]. In the US, the recommended permissible level in water is 5 μg L^{-1}. It is now commonly accepted that antimony is a carcinogen[124].

Arsenic and antimony have been used as 'pathfinders' for gold. As early as 3500BC, in ancient cities such as Athens, antimony was mined and smelted. Today the soil in those areas contain levels of between 3-650 mg kg^{-1} of antimony[125]. Inhabitants of these areas have been found to suffer from neurological conditions and slower response time in children have been reported, while lung and kidney cancers have also been linked to antimony exposure.

Antimony has recently been postulated to be linked to the Sudden Infant Death Syndrome (SIDS). It is thought that antimony used as a fire retardant material in PVC cot mattresses is converted by micro-organisms to toxic volatile derivatives such as trimethylstibine and stibine. However, the evidence linking antimony and SIDS is inconclusive[126].

In 1995, the US consumption of antimony was about 12 000 tonnes per year and that the annual world production was around 62 900 tonnes[87]. Antimony is a common trace constituent in ores such as zinc sulphide. It is used in alloys with tin and lead and for electroplating steel to prevent rusting. Antimony also has uses in fireworks, makeup, paints, contact lenses and car batteries (typically lead car batteries include 2.5-3% antimony).

Antimony levels in hair, urine and blood did not show any increase when a study was carried out on people who lived in an exposed area with high soil antimony in Germany[124]. Home grown produce in the area did not seem to accumulate antimony and the water levels were below the legal limit. Interestingly, the reference group in the study exhibited higher antimony levels in blood, urine and hair than the exposed group: 1.23 compared to 0.60 μg/24h for urine, 0.045 compared to 0.026 $\mu g\ g^{-1}$ in hair and 0.57 compared to 0.48 $\mu g\ L^{-1}$ for blood.

1.10.8. Other Elements in Hair

In 1997, workers in a Taiwan paint factory were shown to have significantly higher levels of lead and manganese, but lower levels of iron in their hair, than those in the normal group, whereas the chromium levels did not differ significantly from the normal group[127]. The process of paint manufacture involved making an alkyl enamel paint for anticorrosion primers, which meant adding red lead pigment (Pb_3O_4) and the drying agents lead naphthalate and manganese naphthalate.

Determination of zinc and copper concentrations in the hair of workers from zinc based industries in India showed that the levels of the metals were higher than normal in the smelters, but the levels were similar to those measured in miners who lived in the local city[128].

A comparison of hair levels of twenty four elements in non-ferrous smelter workers and a normal group found that the exposed group had significantly elevated levels of antimony, arsenic, selenium and silver compared to the normal group[129].

1.11. Ranges of Trace Elements in Hair and Correlation with Other Body Levels.

Studies have shown that zinc and copper hair concentrations do not correlate with tissue concentrations[86], however, cadmium does show a significant correlation between hair and tissue samples[114]. Animal experiments have shown that exposure to cadmium and mercury is reflected in elevated levels of these elements in both the internal organs and hair, furthermore there appears to be a dose related response. It has been stated that blood is not a suitable material for monitoring cadmium because the levels are always low and reflect recent exposure[1].

Chronic arsenic poisoning cases have shown that the levels of arsenic in hair correlate well with levels in the blood and urine[6]. Hair arsenic levels have been reported to occur within the range 0.3-0.74 $\mu g\ g^{-1}$ and it has been shown that hair arsenic levels reflect environmental exposure[1,19].

Hair chromium levels have been studied and it is generally accepted that serum levels do not reflect body chromium storage[1], however it should be remembered that the chromium levels in hair decrease with age.

Selenium levels in hair have been reported from 0.3 to 6.4 $\mu g\ g^{-1}$. It has been found that selenium hair concentrations show no apparent correlation with internal organs[130].

The highest antimony concentration in human hair reported was 10.8 $\mu g\ g^{-1}$ for persons living in South Africa and the lowest values reported are in the range 0.049-0.058 $\mu g\ g^{-1}$.

The highest mercury levels in hair found to have no symptomatic effects in adults is reported to be 96-185 $\mu g\ g^{-1}$. The known fatal level is 500 $\mu g\ g^{-1}$. The highest levels seen in people from the Iraqi village where contaminated grain was accidentally eaten were 535-649 $\mu g\ g^{-1}$. It has been shown that hair mercury levels are around 300 times higher in hair than in blood.

It has been stated that external exposure adds mercury to hair and as a result the correlation between hair and blood levels is affected[131]. Various equations have been used in an attempt to link hair to blood mercury levels[10,15]. An equation derived from a study of over 900 people in two coastal UK towns[6,10] was:

$$HAIR\ MERCURY = 0.367 \times BLOOD\ MERCURY + 0.694$$

(where the level in hair was in $\mu g\ g^{-1}$ and the level in blood was in $\mu g\ L^{-1}$).

A study in Greenland found that hair and blood mercury in people who had consumed mercury-contaminated seal could be described by the following equation:

$$HAIR\ MERCURY = 0.289 \times BLOOD\ MERCURY + 63.4$$

(where hair mercury concentration was expressed in $\mu g\ kg^{-1}$ and blood concentration in $\mu g\ L^{-1}$)[1].

In healthy persons it is believed that the concentration of lead in scalp hair may be 2 to 5 times greater than in bone, about 10 to 50 times greater than in blood and from 100 to 500 times greater than in urine[1,6,86]. It is suggested that normal hair lead levels[18] range between 3 to 51 $\mu g\ g^{-1}$. The concentration of lead in blood has been seen to correlate well with the hair lead levels[1,6,18]. Hair lead levels have also been shown to correlate well with bone lead levels[107].

The correlation calculated for lead in hair in children who had been environmentally exposed to lead is given by the following equation:

$$WHOLE\ BLOOD\ LEAD = 39.79 + 0.02757 \times HAIR\ LEAD$$

(where blood concentration was in $\mu g/100ml$ and hair concentration in $\mu g\ g^{-1}$)[6].

Normal ranges of all the metals of interest are outlined in Table 1.4.

Element	Range ($\mu g\ g^{-1}$) Minimum Maximum
Antimony	0.064 - 3.0
Arsenic	0.06 - 3.71
Cadmium	0.24 - 2.7
Chromium	0.13 - 3.65
Lead	3 - 52
Mercury	1.25 - 7.6
Selenium	0.48 - 2.53

Table 1.4. Ranges of elements in hair[18,132]

1.12. Analysis of Trace Elements in Hair

Neutron activation analysis, X-ray fluorescence spectrometry, atomic absorption spectrometry and inductively coupled plasma spectrometry have all been used for the determination of trace elements in hair. The choice of technique has been determined primarily by availability and cost considerations. Other significant factors in considering analytical techniques are sensitivity, selectivity, speed, dynamic range and multielemental capabilities[6].

1.12.1. Neutron Activation Analysis (NAA)

For many years, NAA enjoyed a favoured position among the techniques used to determine the trace element composition of hair[6]. This technique requires minimal sample preparation and can determine several elements simultaneously. NAA is based upon the quantitative detection of the radiation produced during the decay reactions induced after neutron bombardment of the samples. The instrumental form of NAA is based on the detection of induced γ-ray radiation using γ-detectors (NaI(Tl) crystals or Ge(Li) semiconductor detectors)[1]. In order to remove potential interferences, hair samples are first digested with acids and then the elements are complexed with suitable reagents e.g. zinc-diethyldithiocarbamate and methylisobutyl ketone iodide[136]. The complexes are then extracted into a solvent and after solvent removal, the samples are irradiated. Many elements of environmental significance, including copper, manganese, mercury, cadmium and arsenic, are readily activated by neutron bombardment, however, lead cannot be estimated accurately by activation analysis[5].

The International Atomic Energy Agency recommend the use of 10 cm of hair measured from the scalp surface for the determination of the average concentration of elements in one year, as hair grows at a rate of 10-12 cm per year[38]. Concentrations of low $\mu g\ g^{-1}$ are attainable using NAA, for elements such as mercury, manganese, cobalt, selenium, iron, copper, zinc, chromium, antimony and gold[16].

NAA has been used in many studies of hair analysis[16,111,117,129,133,134,135,136,137,138]. Using NAA it has been shown that the concentration of elements in hair have a wide range and vary greatly within different samples from the same donor. The variations were greater in adult hair compared to children's hair. Using NAA it was possible to see trends along the length of the hair[38]. Chlorine and bromine levels decreased as the distance from the scalp increased, whereas iodine levels increased towards the tip of the

hair. Calcium, copper, magnesium and manganese were found to increase towards the tip of the hair, while zinc appeared not to change. The mercury patterns along the hair lengths were seen to change with every sample.

1.12.2. X-Ray Techniques

X-Ray Fluorescence (XRF) has been used to analyse elements in human hair, where elements such as sulphur, iron, nickel, zinc, selenium, bromine, chromium, manganese, arsenic, mercury and lead have been determined at $\mu g\ g^{-1}$ levels. XRF is a non-destructive technique and allows the analysis of a single strand of hair. A 2 mm segment of hair is bombarded with X-ray photons of the appropriate energies, which induce fluorescence of the elements. The fluorescent X-ray emissions are detected and counted in a highly sensitive silicon-lithium detector which provides a measure of the total amount of each element in the hair segment[139]. Large variations (of up to 10 $\mu g\ g^{-1}$ [143]) in distribution along the same hair, even for essential elements such as sulphur, iron, copper, zinc and selenium, have been reported[38].

Proton induced X-ray emission (PIXE) spectrometry has been used considerably more than XRF for the determination of trace elements in hair[24,54,140,141,142,143,144]. PIXE is also a non destructive technique that can analyse very thin strands of hair as proton beams can be focussed to 10 μm diameter. PIXE was used for hair analysis as early as 1973[141]. Sample preparation is very simple but, when compared to NAA, PIXE has been shown to be somewhat limited in terms of the number of elements that can be determined[6].

1.12.3. Atomic Absorption Spectrometry (AAS)

Selectivity and sensitivity, coupled with simplicity and low cost have made AAS the most widely used technique for the determination of trace elements in hair[1,6]. Extensive sample preparation and single element analysis have not prevented many hair studies being carried out using this technique[3,19,35,67,68,70,97,110,95,96,118,127,128,145,146,147,148,149,150]. AAS has been used since the early 1970s for the determination of trace elements in hair and it involves digesting the hair. The liquid sample is aspirated into a flame at a temperature of around 2000-3000 K and the elements are atomised. Subsequently the absorption of radiation, from a hollow cathode lamp, of the element is measured. The relatively poor detection limits offered by AAS have meant that the technique is only useful for the determination of elements that are present at levels of

1 μg g^{-1} and above [151]. However, it has been possible to couple other systems to AAS instruments in order to improve the detection limits for some elements. For example, hydride generation has been used to determine selenium levels in hair samples[152], showing detection limits of 0.3 ng ml^{-1} for selenium. In an investigation of selenium in hair by HG-AAS an ultrasonic leaching method was used which had been shown to keep Se^{4+} and Se^{6+} intact[152]. Mercury hair levels have been determined by cold vapour AAS (CV-AAS). Results from a study[153] in 1982 showed that it was possible to determine occupational exposure in a group of dentists using CV-AAS and reported limits of 0.1μg g^{-1}.

Instead of a flame a graphite furnace can be used as the source of atoms and this is called electrothermal AAS (ET-AAS). ET-AAS has unique properties such as the long residence time in the furnace, high sensitivity and the ability to handle small samples of hair (1 mg) by direct solid analysis[154]. Hair analysis for cadmium, chromium, lead and manganese showed levels in the ng g^{-1} region and results from the work have provided useful information with respect to elemental distribution the length of the hair. Detection limits of 9 ng g^{-1} were determined for cadmium in hair by ET-AAS, from very low masses[155,156].

1.12.4. Inductively Coupled Plasma (ICP)

Recently, inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have been used for hair analysis. ICP analysis allows rapid multi-elemental analysis at very low concentrations in a range of environmental samples, such as water and soil, as well as biological samples[157]. Concentrations of ng g^{-1} and lower have been achieved for elements such as copper, zinc, arsenic, cadmium, antimony, thallium and lead[158] with both ICP-MS and ICP-AES [158,159].

Hair samples from occupationally exposed workers have been analysed at the μg g^{-1} and ng g^{-1} levels, by ICP-AES[112] and ICP-MS[160] respectively. ICP-MS studies have shown that multi-elemental analysis of both mercury and selenium in hair samples has been achieved[160]. There have also been studies of mercury in hair carried out using cold-vapour ICP-AES and ICP-MS including one such study determining mercury and selenium levels in neonatal hair samples[161] which used cold-vapour atomic absorption (CV-AA). Other multi-elemental studies, in hair samples and hair certified reference

materials[162], have also been carried out using ICP-MS[50,158,163]. Selenium has also been determined in the hair and blood of Turkish children using HG-ICP-MS[164].

1.12.5. Other Techniques for the Analysis of Hair Samples

A cheaper alternative analytical technique for analysing metals in hair is differential pulse anodic stripping voltammetry (ASV). Hair samples, collected from people who worked in lead acid and nickel cadmium battery factories and petrol stations, were analysed by ASV. In all cases of occupational exposure there were increased levels of lead and cadmium in hair yet at the same time depressed zinc hair levels[116]. A much older technique was spark source mass spectrometry, which was used to analyse hair in 1969. Unfortunately only a few hair samples were analysed for trace elements, but the levels were in the $\mu g\ g^{-1}$ range and so, for its time, this technique showed considerable promise[165].

Scanning electron microscopy (SEM) has been used to examine hairs since the early 1970s. SEM is a powerful microscope that can examine the topography of the hair surface. It allows surface analysis and determination of elements that are present at levels as high as a percentage of weight of the hair. Different types of hair have been investigated in detail using SEM[55,166]. Morphological changes in the hair follicle and the hair shaft have been shown to occur in various dermatology patients. Examples of these are 'Bamboo hair', where there is a softness in the hair cortex, which has been seen in those with Netherton's syndrome, 'hair casts' have been described in scalp disorders and 'uncombable hairs' have been shown to appear in localised areas of neurofibromas[55].

A group in Northern Ireland, in 1998, looked at capillary electrophoresis (CE) as a method for the determination of trace metals in hair samples[167] and compared this to high performance liquid chromatography (HPLC) and AAS techniques. The method investigated used large volume sample stacking CE of cobalt, copper, iron, lead and zinc, using precapillary online chelation. The study found that HPLC by complexation with a chelating agent only worked for cobalt and copper in hair samples with levels of 57.6 $ng\ g^{-1}$ and 17.31 $\mu g\ g^{-1}$ found respectively, which compared well to the results obtained with the CE method.

Determination of trace elements in human hair using ion chromatography to separate bivalent ions of copper, lead, zinc, nickel, cobalt and manganese has been reported[168].

The separation was carried out on a reverse phase column, followed by ultra violet detection. The separation of copper from lead was poor and the separation of nickel from zinc was very poor, but the method was said to give comparable results when the same samples were analysed by AAS and XRF. The system showed detection limits of 1 $\mu g\ g^{-1}$ for copper, zinc and nickel, and for lead and manganese 2 and 0.15 $\mu g\ g^{-1}$ respectively[169].

1.13. Chemical Speciation in Biological Samples

Speciation may be defined as either the process of identifying and quantifying the different, defined species, forms or phases present in a material, or the description of the amounts and kinds of these species, forms or phases[170].

Elemental speciation involves the separation and quantification of the different oxidation states (e.g. As^{3+} or As^{5+}) or chemical forms (e.g. monomethylarsonate and dimethylarsinate) of a particular trace element. Elemental speciation information is crucial because the toxicity and biological activity of many trace elements depend on their oxidation states or chemical forms as well as their quantities[188]. The toxicity of elements can vary considerably depending on the species, e.g. metallic mercury is associated with damage to the central nervous system, while compounds of mercury can cause mild irritant symptoms[172].

The absorption, distribution, reactivity to binding sites, bioavailability, toxicity and excretion of elements can be better understood if the information about the chemical species are available[172]. Although the speciation of trace elements in biological fluids and tissues has been approached in many different ways during the last decades, it is extremely difficult because of the complexity of the biological systems[171]. Approximately 15 elements, 20 oxidation states and 25 organic compounds have been involved in the speciation of biological samples[172]. Several biological matrices have been considered but blood, serum and urine are the most analysed, with tissue being found to be a relevant matrix for copper, zinc and cadmium. Organic compounds, such as ethyl, methyl and aryl derivatives, have been demonstrated for arsenic, mercury, lead and selenium, and other organic compounds (e.g. complexes with amino acids, proteins and macromolecules) have been demonstrated for silver, cadmium, copper, chromium, lead, selenium and zinc. Among the procedures for the separation and identification of species are various chromatographic techniques, as well as chemical chelation,

extraction, acid modified separation, heating and distillation. The analytical techniques employed have been AAS and ICP-AES or ICP-MS[172].

1.14. Chemical Speciation of Trace Metals in Hair

The aim of hair analysis is to estimate the amount of exposure from the environment and so it is imperative that each species is determined to give an assessment of toxicity. There has not yet been very much work done in the area of speciation of hair and what has been done mostly involves the determination of methylmercury and inorganic mercury in hair samples.

In one of the earlier studies of mercury speciation, methyl mercury was extracted from the hair with benzene. 50% of the methyl mercury broke down to inorganic mercury during alkaline digestion, however the addition of cysteine prevented the degradation[173]. Analysis of hair taken from a woman who attempted suicide by ingesting mercuric chloride showed that the levels of inorganic mercury peaked after 41 days in contrast to organic mercury that remained constant throughout[174].

Work by Kratzer et al using solvent extraction for the separation of inorganic and methyl mercury showed that neutral halides of the type HgX_2 and CH_3HgX can be extracted into non-polar organic solvents such as benzene, toluene or xylene, whereas the inorganic mercury present forms non-extractable anionic complexes[175,176].

Total mercury and methyl mercury were determined in hair from people living in the Amazon basin[78] and abnormally high levels of total mercury were observed in hair and blood from inhabitants of all the fishing villages investigated and more than 90% was methyl mercury. In the people from the gold-mining area however, the total mercury value was much lower and the level of methyl mercury varied widely in both blood and hair samples. Similar studies to Akagi et al[78] have been carried out on the scalp hair of fishing families in Chile[177]. In this study the total mercury was determined by CV-AAS and NAA, whilst the methyl mercury was determined by GC-ECD. The results from this study also showed significantly higher total mercury and methyl mercury levels in hair and blood compared to the normal group.

Methyl mercury and inorganic mercury in hair have also been separated on a HPLC column using a chelation extraction method with sodium pyrrolidinedithiocarbamate and detection with UV and CV-AAS. This method reported detection limits of 4 ng g^{-1} and so shows promise as a suitable technique for the analysis of mercury in hair[178].

1.15. Techniques used in this study

To determine the concentrations of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in hair for this study an ICP-MS was used. For speciation of the elements LC coupled with the ICP-MS, to allow the separation and detection of the different species, was used. Hydride generation coupled to ICP-MS was used in initial work (see Chapter 2).

1.15.1. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

In this investigation, ICP-MS was used in the analysis of trace elements in hair because it has excellent sensitivity at the levels required i.e. below 10 ng g^{-1}. It also allows multi-elemental analysis and provides rapid solution analysis.

For rapid qualitative analysis of aqueous samples the ICP-MS is unequalled[179]. The four most important attributes of ICP-MS that make it attractive to analytical chemists are:

i. ability to accommodate solution samples easily,
ii. low detection limits,
iii. broad elemental coverage, and
iv. isotopic analysis capability.

Over the past decade there has been a significant increase in the use of ICP-MS for elemental analysis[179].

The technique combines an inductively coupled plasma (ICP), as a high temperature ion source with a quadrupole mass spectrometer (MS), as a high sensitivity detector. It measures both elemental and isotopic composition and provides good sensitivity at low levels. The ICP-MS has the ability to yield extremely high analytical sensitivities and wide dynamic ranges allow multi-elemental analyses at low concentrations. ICP-MS detection limits are often up to three orders of magnitude superior to those in ICP-AES[180].

The argon plasma of the ICP-MS is initiated by an electrical discharge from a tesla coil and is maintained by the inductive coupling of 1.2 kW of radio frequency energy from the induction coil. Most of the power of the plasma is dissipated to the outer layers leaving a relatively cool inner layer. Ionisation temperatures in the plasma can be in excess of 7000 K and residence times of the sample of 2 ms provide enough energy to ionise most elements. The degree of ionisation is over 90% for the elements with first ionisation energies less than 10 eV and over 1% for the remaining elements except

nitrogen, oxygen, fluorine, chlorine, neon and krypton. Some of the elements, for example barium, can form doubly charged ions.

Samples are introduced into the plasma via pneumatic nebulisation (effectively spraying) of solutions to produce a fine aerosol. This aerosol then comes in contact with the argon plasma (around 1% of the sample). The input flux of aerosol water can influence the degree of formation of oxides and other species both within the plasma and in the ion sampling interface. This is limited by using a water-cooled spray chamber.

The next stage of the ICP-MS is the ion extraction, which takes place in the interface. The interface consists of a shallow, water-cooled sampling cone with a central 1 mm aperture, which is located on the axis of the plasma torch 5-10 mm from the end of the induction coil. A second, sharp-angled skimmer cone, with a 0.7 mm aperture is located coaxially 5-10 mm behind the sampling cone. The pressure in the intervening space is maintained at 100 Pa by a rotary vacuum pump and the pressure behind the skimmer cone is further reduced to 0.1 Pa. This allows continuous sampling from a central core (1.5 - 3 mm) of the bulk plasma without entering the cooler boundary layer. The gas coming into the first low pressure stage expands as a directed supersonic jet along the axis of the equipment, reaching terminal Mach numbers (ratio of speed of jet to speed of sound) depending upon the aperture size and sampler-skimmer cones distance. The bulk of the gas is pumped away by the rotary vacuum pump and a small fraction passes through the final aperture into the quadrupole mass spectrometer. A small disc located on the axis of the ion lens system prevents the intense photon emission from the plasma from reaching the detector of the mass analyser. The schematic in figure 1.5 shows all of the ICP-MS characteristics.

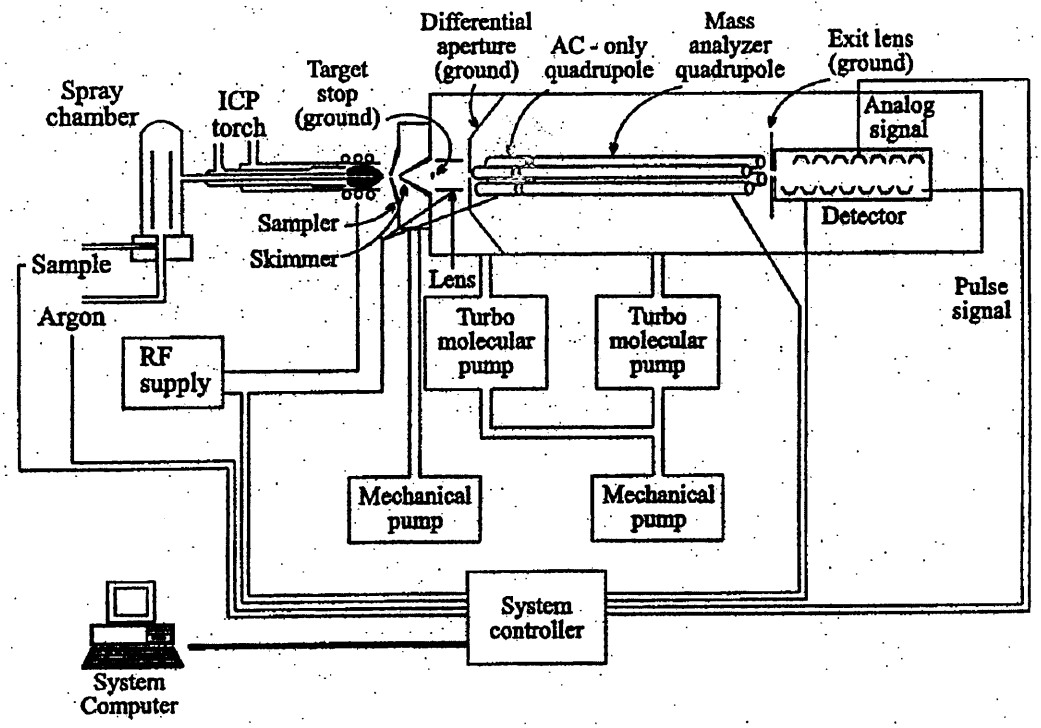


Figure 1.5. The ICP-MS[189]

The final stage involves the quadrupole MS. The operational vacuum within the mass analyser is maintained at 1-2 $x10^{-4}$ Pa by oil diffusion pumps backed by rotary vacuum pumps. The accelerating voltage of the ion beam is directed along the central axis of the quadrupole, which consists of four diagonally-paired cylindrical rods mounted in a square. A positive voltage with superimposed Rf voltage is applied to one pair, while the other receives an equal but negative dc voltage and an Rf voltage that is 180° out of phase with the first. This creates a two dimensional oscillating electric field within the quadrupole, which is zero along the axis of the rods and which imposes on the incoming ions' oscillatory trajectories that are defined by

i. the equations of motion relating to the mass to charge (m/z) ratio of the ions
ii. the magnitudes of the dc and Rf voltages
iii. the dimensional constants of the quadrupole.

At any given set of voltages only ions of a certain charge to mass ratio will have stable trajectories and reach the detector. Other ions will adopt unstable trajectories and either strike the rods or pass out of the quadrupole field. The complete mass spectrum (0-250 amu) can be scanned rapidly (e.g. in as little as 30 ms) by varying the magnitudes of the dc and Rf voltages whilst keeping their ratios constant.

Ions which pass through the quadrupole are detected by an electron multiplier. Output pulses are fed to a multi-channel scaler in which memory channels are swept synchronously with the mass scan. Depending on the number of elements to be determined either single ion monitoring for one or a selected number of elements with rapid switching between them, or a scanning mode over the whole mass range or pre-selected parts of it, can be used[179].

ICP-MS has been proven to be a versatile, sensitive analytical technique which offers a simple approach to the analysis of a wide variety of metals in a variety of biological materials[181,182,183,184,185]. Spectral effects can be minimised by using a different isotope of the element for the analysis and matrix effects can be corrected for by the use of internal standardisation, standard addition or isotope dilution, with the latter giving the most accurate and precise results. It is also possible that some biological samples, e.g. serum, blood and urine, can be analysed directly after a simple dilution with an appropriate solvent[186]. Mostly though, the samples analysed by ICP-MS are aspirated from sample digests and it has been possible to have microwave systems digesting samples on-line to decrease the total analysis time[187].

As already outlined in Section 1.12.4, ICP-MS has been used to analyse hair samples[50,130-135]. It is an ideal technique for hair analysis, as it has the ability to provide multi-elemental analysis and detection limits at trace levels[160]. A further advantage is that it allows the simultaneous determination of essential elements, such as chromium and selenium, which may appear at high $\mu g\ g^{-1}$ levels in hair, and trace elements which occur at much lower $ng\ g^{-1}$ levels [163]. As a result of the high sensitivity of ICP-MS it is possible to analyse small masses of hair, indeed single strands of hair have been analysed by ICP-MS[162], as have neonatal hair samples weighing approximately 25 mg.

The use of ICP-MS coupled with separation techniques for the purpose of speciation has gained a lot of attention in recent years[163-188,189,190.191,192,193,194,195]. Liquid chromatography (LC) is one of the most versatile chromatographic techniques that has been coupled to ICP-MS[196]. However, to date there have been no reports of speciation of hair samples by LC-ICP-MS.

1.15.2 Liquid Chromatography Coupled to Inductively Coupled Plasma Mass Spectrometry (LC-ICP-MS)

Improvements in instrumentation performance and design, with the addition of continually developing methods, have responded to the demand for more sensitive and reliable techniques in trace element speciation[197]. The ICP-MS is a trace metal detector with unique capabilities, including high sensitivity and when coupled with LC it provides a powerful separating tool[198,199,200,201,202]. The coupling of LC to ICP-MS is relatively simple[197,202,203]. The compatible flow rates mean that the LC column can be connected to the concentric nebuliser of the ICP by the use of tubing, which must be kept as short and narrow as possible to minimise extra column band broadening. The spray chamber is used to sort the aerosol droplets according to their size and generally, only 2% of the solution which passes through the concentric nebuliser actually reaches the plasma. A typical LC-ICP-MS set-up is shown in figure 1.6.

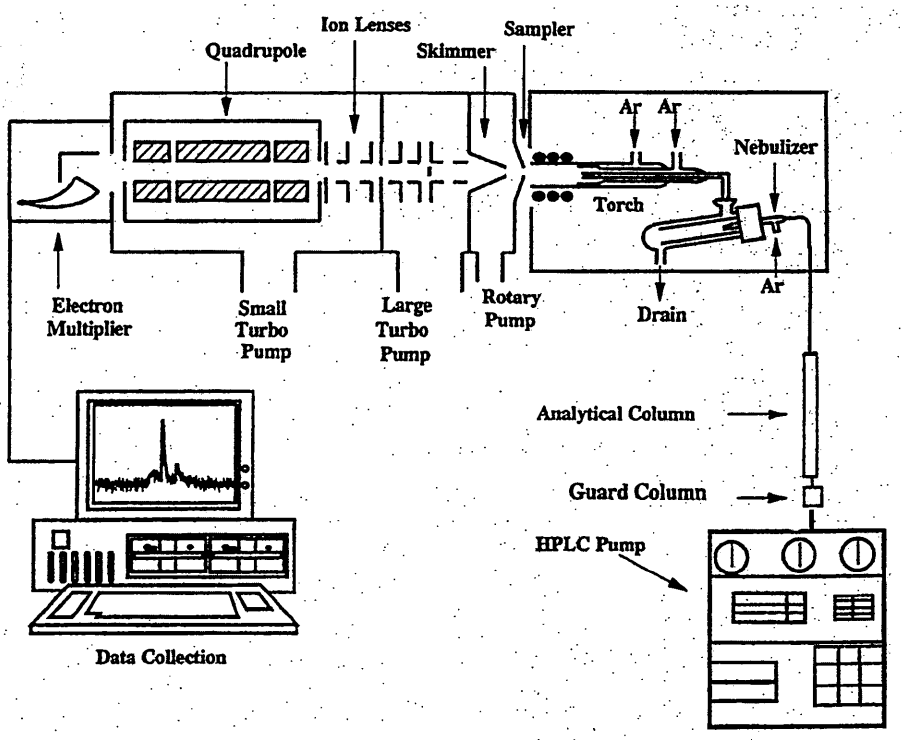


Figure 1.6. Schematic diagram of a HPLC system coupled to ICP-MS[203]

Other nebulisers, such as the glass frit, the ultrasonic and the direct injection nebulisers, increase the efficiency, but require lower flow rates.

When coupling LC to ICP-MS several precautions need to be addressed. The salt content of the mobile phase must be kept to a minimum (<2%), to prevent clogging of the nebuliser and erosion of the sampler and skimmer cones, which often forces a

change in the ion input aperture. This effect regulates the ion beam and thereby the sensitivity. Mobile phases that contain a large amount of organic solvent may cause plasma instability and may ultimately quench it. Another effect of using high organic content mobile phases is the build up of carbon as soot on the sampler cone which will cause a decrease in the analyte signal. A possible solution is to cool the spray chamber in order to prevent large amounts of organic solvent vapour from entering the plasma. Desolvation and the addition of oxygen gas to the nebuliser provide better solutions. The forward power of the plasma can be increased to aid the decomposition of the organic components, however, this may be accompanied by an increase in reflected power, which could harm the generator[189,198,200,201,202,239,239].

1.15.3. Chemical Speciation by LC-ICP-MS.

There has now been a considerable amount of work done in the area of LC-ICP-MS[204,205]. The successful speciation of arsenic[206,207,208,209] in urine[210,211] (with the separation of up to eight arsenic compounds), in fish[212,213,214] and in water[215] has been carried out by LC-ICP-MS. Also, the speciation of selenium[216,217,218] in urine[219] and other biological samples[220,221,222], as well as yeast[223] has been reported. Chromium compounds have been separated[224,225,226] in dyes[227] and cadmium speciation has been achieved in pig kidney samples[228]. Other elements separated and determined by LC-ICP-MS include gold drug metabolites in blood[229], lead in wine[230], blood[231] and rainwater[232], butyltin compounds[233], antimony compounds[234,235], and rare earth elements[236] in geological samples[237].

There does however, seem to be a lack of multi-elemental speciation by LC-ICP-MS in the literature. The separation of selenium and arsenic under the same conditions has been reported, but there were no retention times or chromatograms in the paper and so it was not clear whether they had been separated at the same time[238]. Lead and mercury species have been determined by the same method but not in the same samples[239], nickel and chromium were separated on the same column but not at the same time and vanadium could not be separated under the same conditions as nickel and chromium[240]. Metal ions in pond water were analysed after being complexed using a chelating resin and although arsenic, antimony, iodine, lead and some rare earth metals could be determined at the same time it was not possible to quantify the data, although two peaks were detected for arsenic[241]. It has been shown that arsenic and tin require different conditions for their respective species to be separated by LC-ICP-

MS[242]. Multi-elemental speciation by LC-ICP-MS has been carried out in France for arsenic, selenium, antimony and tellurium, using an anion exchange column, with 12.5 mmol L^{-1} ammonium hydrogen phosphate in water, with 3% methanol, at pH 8.5. The separation of the various species of arsenic and selenium were shown in different chromatograms and so it was not clear from the paper whether they could actually be separated in the same run[243]. Other workers have reported that it was possible to separate chromium and arsenic species in water samples by ion chromatography ICP-MS[244]. However, the Cr^{3+} had to be determined separately and although the chromium (Cr^{6+}) was at a different retention time than the five arsenic compounds separated, the sensitivity of the chromium peak was considerably lower than for the arsenic species.

1.16. Aims of the PhD

There is evidence to suggest that hair analysis provides useful information about occupational and environmental exposure to toxic elements. It also acts as a useful aid in the diagnosis of certain diseases relating to trace elements and can provide information about the use of certain drugs. What is also clear from the literature is that in order to make hair analysis a reliable and reproducible technique, certain aspects need to be investigated[1,5,6,9,11,15,19,38,76]. Most importantly, exogenous contamination and washing procedures need to be addressed in order to put the final results into perspective. Additionally, the hair values of elements need to be correlated to values in other body tissues to set non-toxic and toxic limits. Also of prime importance is the development of analytical methods which allow the accurate determination of trace elements in human hair[245].

It was stated in 1986 that because of inconsistencies in hair results and the fact that exposure can only be seen in extreme cases that, with the exception of exposure to methyl mercury, trace element analysis of hair is not a useful procedure[86]. However, the World Health Organisation has indicated benefits of hair testing in some cases and the International Atomic Energy Agency uses hair to monitor global trends of elements[246]. Suzuki[18] and Lenihan[5] have commented that there is a need for more standardised methodology, reference ranges and a correlation between hair ranges and other matrices. If an appropriate washing procedure was developed it seems that, by using new and sensitive analytical instrumentation, it would be possible to validate hair analysis for trace elements[18].

The aim of this study is to investigate the determination of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in human hair, so that routine analysis may be performed using a reproducible and accurate method. The main objectives of this are to establish a method to determine normal control values for these elements in hair and to apply the method to occupational samples to confirm occupational exposure. An investigation into washing procedures will be carried out to see if it is possible to remove exogenously bound species from the hair to allow the endogenous species to be determined. Speciation studies will also be performed using LC-ICP-MS, to investigate whether it is possible to differentiate between the various species, in washed and unwashed hair.

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Chapter 2: Hydride Generation with Inductively Coupled Plasma Mass Spectrometry

2.1. Introduction

ICP-MS has been applied in the determination of trace elements in various types of samples. However, several trace elements such as arsenic, selenium and antimony can be difficult to determine because of isobaric interferences and low ionisation ratios[1]. These interferences are eliminated and improved sensitivities are obtained when arsenic, selenium and antimony and other elements such as lead, germanium, bismuth, tellurium and tin are converted to the hydrides before determination by ICP-MS[2,3].

2.1.1. Hydride Generation

Elements that form hydrides are listed in table 2.1.

Analyte	Hydride Formula	Hydride Name	Melting Point (°C)	Boiling Point (°C)	Solubility in water (μg ml^{-1})
As	AsH_3	Arsine	-116.3	-62.4	696
Bi	BiH_3	Bismuthine	-67	16.8	-
Ge	GeH_4	Germane	-164.8	-88.1	Insoluble
Pb	PbH_4	Plumbane	-135	-13	-
Sb	SbH_3	Stibine	-88	-18.4	4100
Se	H_2Se	Hydrogen Selenide	-65.7	-41.3	37770-68000
Sn	SnH_4	Stannane	-146	-52.5	-
Te	TeH_2	Hydrogen Telluride	-51	-4	very soluble

Table 2.1. The properties of hydride forming elements[3].

Arsenic, selenium, tellurium, antimony, bismuth and tin (and to a lesser extent germanium) are volatilised by the addition of a reducing agent like sodium borohydride to an acidified solution. The principle reaction of the hydride generation method is[3]:

$$\text{Analyte } (l) + BH_4^- \rightarrow \text{Hydride } (g)$$

It is important for the elements to be in the correct oxidation state to allow the hydride formation i.e. As^{3+}, Sb^{3+}, Se^{4+}, Pb^{2+} and in the case of mercury, Hg^{2+} is reduced to Hg^0, elemental mercury[3].

Sodium borohydride is a particularly versatile reagent that is of particular value in the conversion of species in aqueous solution into volatile hydrides. In this role the sodium borohydride reagent is thought to act both as a reductant and a hydride source. The first step in the reaction with the hydride forming elements is thought to be reduction of the species to the hydride forming oxidation state, e.g. from As^{5+} to As^{3+}. Subsequent reaction with sodium borohydride converts the reduced species to the hydride compound. The borane generated by these reactions hydrolyses to give boric acid and gaseous hydrogen as shown here[4].

$$BH_3 + 3H_2O \rightarrow H_3BO_3 + 3H_2$$

2.1.2. Coupling of Hydride Generation (HG) to ICP-MS

The entire process of hydride generation ICP-MS consists of:

i. hydride release from the sample solution, i.e. conversion of an analyte in an acidified sample to the hydride form and its transfer to the gaseous phase, followed by
ii. the transport of the released hydride by a flow of purge gas to the plasma source[5].

Powell[6] stated that hydride generation coupled to ICP-MS should give two orders of magnitude greater sensitivity than the ICP-MS alone. The potential of the continuous flow HG system coupled to an ICP was recognised in 1978 when Thompson first published a series of papers on the technique[7]. Since then the main objective has been to obtain a method which allows the simultaneous determination of the hydride forming elements[8].

Hydride generation combined with ICP-MS has the following advantages over conventional aqueous solution pneumatic nebulisation sample introduction:

i. the transport efficiency of gaseous hydrides approaches 100% compared with less than 5% when pneumatic nebulisation is used,
ii. the detection limits are improved significantly,
iii. the spectral interferences from the sample matrix are reduced because the hydrides are separated from the sample matrix and

iv. hydrides can be easily decomposed and ionised in the plasma which enhances the signal intensity[9].

Table 2.2 compares results from previous studies of the sensitivity and limits of detection achieved by ICP-MS alone and by ICP-MS coupled with HG. It can be seen that the sensitivity and limits of detection of hydride forming elements are greatly improved with the introduction of HG to the ICP-MS.

Element	Sensitivity using direct nebulisation ICP-MS[10] (counts/ng ml^{-1})	Sensitivity using hydride generation ICP-MS[10] (counts/ng ml^{-1})	Limit of detection using direct nebulisation ICP-MS[5] (ng ml^{-1})	Limit of detection using hydride generation ICP-MS[5] (ng ml^{-1})
As	230	9080	0.14	0.017
Bi	955	13080	0.045	0.005
Se	75	2410	1.5	0.17
Te	137	2000	0.16	0.034
Sn	880	26065		
Sb	680	21660	0.019	0.031

Table 2.2. Comparison of detection limits and sensitivities for volatile hydride generation and direct nebulisation ICP-MS[5,10].

Hydride generation has been used with atomic absorption spectrometry[3,11,12] (AAS) and inductively coupled plasma atomic emission spectrometry[7,9,13] (ICP-AES) for many years. HG-ICP-MS was first reported in 1983 for the determination of arsenic and since then there has been a lot of interest in this area[1,2,14,15,16,17,18,19,20,21]. HG-ICP-MS has been used to determine the hydride forming elements of interest, antimony, arsenic and selenium, in various environmental samples[8,10,13,18,21,22,23]. Arsenic and lead speciation have also been studied in environmental samples using liquid chromatography (LC)-HG-ICP-MS[19,24].

HG-ICP-MS is achieved by connecting the gaseous flow of hydrides from a gas liquid separator to the spray chamber of the ICP-MS. There have been several publications on the coupling of a HG to an ICP-MS, whereby a modified spray chamber or nebuliser has been required. In 1996, Zhang et al[1] used the installed spray chamber with minor modifications as a gas-liquid separator for a HG-ICP-MS system. This was said to reduce problems associated with the coupling between HG and ICP-MS such as plasma

instability, severe memory effects and high blanks found with a conventional pneumatic nebuliser.

2.1.3. The Determination of Hydride Forming Elements in Hair

Selenium has been studied extensively using hydride generating techniques[2,12,25,26,27,28,29,30,31]. In an investigation of selenium in hair by HG-AAS an ultrasonic leaching method was used which was shown to keep Se^{4+} and Se^{6+} intact[32]. Selenium has also been determined in the hair and blood of Turkish children by HG-ICP-MS[33]. The study found a correlation between the hair and blood levels, and although the selenium status of the children was considered to be low, the girls showed higher levels of selenium in their hair than boys (317 compared to 292 ng g^{-1}). It was shown in a Polish study that the use of hydride generation improved the sensitivity of the analysis of selenium in hair when coupled to an ICP-AES[34]. In 1997, a study carried out in Japan, looked at the speciation of selenium in hair by HG-AAS[32]. The digestion procedure in this study was time consuming, involving refluxing with perchloric acid and preconcentrating the sample before selenium reduction with 6 mol dm^{-3} HCl.

Since elemental mercury is volatile, the hydride generation method is adapted so mercury is reduced to its elemental state. There have been studies of mercury in hair carried out using cold-vapour (CV) ICP-AES and CV-ICP-MS. In one such study, sodium borohydride was used with 3.5% dimethylformamide to generate elemental mercury in the gas liquid separator[35]. Cold-vapour atomic absorption (CV-AA)-ICP-MS has been used to determine mercury in hair in a recent study in Hull, England[36]. It was found that direct determination of mercury by ICP-MS yielded poor recoveries and chemical modifiers needed to be added to the analyte. CV-AA was coupled to ICP-MS to generate both quantitative and stable isotopic ratio analysis of mercury in hair samples, however a major problem of this hybrid system was that as the sample stream was very dry there was accumulation of mercury in the ICP-MS. However, the hair mercury values did show improved recoveries with the CV-AA-ICP-MS.

2.2. Metals of Interest and Hydride Generation

2.2.1. Antimony

The determination of antimony in environmental samples can be difficult as a result of interferences in the different matrices, from polyatomic ions with masses similar to that of antimony[21]. Such spectral interferences are reduced with hydride generation, as the hydrides are separated from the sample matrix and solution reagents. With nebulisation of the sample solution, molecular ions of the same nominal mass/charge as the analyte ions may be derived from the sample matrix, reagents, water or carrier gas and interfere with the determination of the analyte. The formation of most interfering molecular ions is precluded with hydride generation because the precursors do not reach the plasma torch and as a result of this the detection limits of the hydride forming elements are improved[2].

Antimony is one of the least problematic hydride-forming analytes, as stibine can be formed within a very broad interval of acidity/pH and sodium borohydride concentration. With continuous flow HG-AAS limits of detection within 0.08-1 $\mu g\ L^{-1}$ have been reported[3]. Potassium iodide, with concentrations of 0.1-5% m/v potassium iodide in dilute hydrochloric acid, or mixtures of potassium iodide, ascorbic acid and dilute hydrochloric acid can all be used to reduce Sb^{5+} to Sb^{3+}. Hydroxylammonium chloride with potassium iodide has also been used as a reductant and has proved useful for wet-digested samples because it has a good tolerance of oxidants. Other reductants include a hydroxylammonium chloride / potassium iodide / ascorbic acid / dilute hydrochloric acid mixture with L-cysteine at pH 2.1-2.15 and hydrazine sulphate heated followed by the addition of sodium iodide and thiourea (1% m/v in hydrochloric acid) / thiourea / potassium iodide / dilute hydrochloric acid mixture and of thiourea / ascorbic acid / dilute hydrochloric acid and oxalic acid.

Optimum conditions for the formation of the antimony hydride have been found to include the use of sodium or potassium iodide (10% m/v) to firstly reduce the antimony from the pentavalent to the trivalent oxidation state[18]. This was followed by the reaction of 0.6% (m/v) sodium borohydride, in 0.1M sodium hydroxide and 1.5M hydrochloric acid[18].

2.2.2. Arsenic

Arsenic is a mono-isotopic element and its isotope ^{75}As is susceptible to interference from $^{40}Ar^{35}Cl$, when chloride is present in the matrix. In addition to this, the sensitivity and detection limits of arsenic are poor in ICP techniques because arsenic, like selenium, has a relatively high ionisation potential and so is not easily ionised in the argon plasma[5].

The formation of arsenic hydrides is not affected by the concentration of sodium borohydride solution. Arsine is generated from solutions of As^{3+} within a rather broad acidity range; from 9-10M HCl to buffered solutions with near neutral pH. Hydride generation from As^{5+} cannot be performed above pH 0.3-0.5. The reduction of As^{5+} to As^{3+} is similar to that of antimony but may require longer reaction times and/or higher concentrations of reductant reagents[3]. The successful use of L-cysteine to reduce As^{5+} to As^{3+} to determine arsenic in steel by hydride generation ICP-AES has been reported[9].

2.2.3. Lead

The chemical yield of plumbane (PbH_4) is rather low, presumably because of the very low redox potential of the Pb^{2+}/PbH_4 couple and the possible alternative pathways of reduction producing e.g. elemental lead (Pb^0) instead of the volatile hydride. To generate the lead hydride a vigorous reaction is required between the acidified sample with added strong oxidant and large amounts of sodium borohydride (5-10%). Plumbane is generated within a narrow pH range of 1-2. Hydrogen peroxide is a common addition to the acid medium to improve the oxidation of Pb^0 to Pb^{2+} to form the hydride[3]. Peroxodisulphate has also been used as an oxidising agent to transform the lead into the metastable Pb^{4+}, before reduction with HCl to the hydride[18].

In 1995, a study looked at lead speciation by HG-ICP-MS using liquid chromatography to separate the species of lead[19]. A 6% v/v hydrogen peroxide solution was used on-line in the hydride system to oxidise the lead species, followed by reaction with 10% m/v sodium borohydride and 0.08 mol l^{-1} of hydrochloric acid. The study showed that it was necessary to use glass beads in the gas liquid separator and mixing coils in the hydride generator to maximise the surface area available for hydride evolution[19].

2.2.4. Selenium

In the determination of selenium by ICP-MS, the use of ^{80}Se, the major isotope (abundance 49.96%), is precluded by the presence of the argon dimer $^{40}Ar_2^+$, therefore a minor isotope such as ^{77}Se (isotopic abundance 7.50%) or ^{82}Se (isotopic abundance 8.84%) must be used[18]. It is possible to use ^{78}Se (isotopic abundance 23.61%) in some cases, but this too is interfered with by an argon dimer species $^{40}Ar^{38}Ar^+$. The use of a minor isotope results in lower sensitivity and a higher detection limit for selenium. It is thus necessary to use hydride generation to achieve higher sensitivity and lower detection limits.

Selenium easily forms the hydride, H_2Se. The reaction is fast, feasible in a broad range of hydrochloric acid molarity, has a relatively low sodium borohydride consumption and the detection limits are low in pg ml^{-1}.

Sources of error with this method include:

i. Volatilisation, adsorption and uncontrolled changes in the oxidation of the analyte can take place during sample preparation.
ii. Incomplete pre-reduction to Se^{2+}.
iii. Hydrogen selenide is very reactive and relatively soluble in water, therefore partial losses due to incomplete stripping of selenium from solutions and adsorption onto surfaces is possible[3].

The presence of noble and transition metals, strong oxidants, organic matter and other hydride forming elements has been known to interfere with the H_2Se formation[2]. Both higher hydrochloric acid and lower sodium borohydride concentrations have been shown to help such interferences. Catalysts of the HG reaction such as the halides (KI>KBr>NaCl) have been seen to improve the rate and efficiency of H_2Se generation and thus provide better tolerance to acids[3].

2.3. Problems with HG-ICP-MS

The major disadvantage of most hydride generation techniques, including those used with ICP-MS, is the inability to simultaneously determine volatile-hydride-forming elements and other elements[5]. Other problems commonly associated with the coupling between HG and ICP-MS are high blank values, long memory effects and plasma instability. The design of the gas-liquid separator is a key factor affecting all three problems[1]. It has been

especially noted that the variations in hydride generator design can have a profound effect on the analytical sensitivity of selenium[2]. In 1995, Jamoussi et al, showed that it was not possible to generate all hydrides under the same conditions[18]. The study showed that the lead and tin hydrides could be generated together using the same oxidising agents. It was also shown that arsenic and antimony would produce hydrides under the same conditions using potassium iodide and that selenium would form a hydride under the same conditions as those required to reduce mercury to elemental mercury, without any pre-reductants. A study[15] by the Laboratory of the Government Chemist (UK) in 1997, disagreed with Jamoussi et al[18]. The study showed that although it was possible to simultaneously determine arsenic, antimony and selenium in water by HG-ICP-MS[15], the selenium required a further off-line pre-reduction stage of heating with hydrochloric acid to reduce Se^{6+} to Se^{4+}. Thiourea was then added on-line to reduce pentavalent arsenic and antimony to the trivalent species, after which the sodium borohydride was added to form the hydrides.

This chapter reports the investigation of hydride generation when coupled to an inductively coupled plasma mass spectrometer, to see if it is a suitable method for the simultaneous analysis of the hydride forming elements of interest in hair samples.

2.4. Experimental

2.4.1. Reagents Used

Aristar grade hydrochloric acid (1.18gr.) (37%), nitric acid (69%), sodium hydroxide pellets, Plasma Emission Standards (1000 mg l^{-1} of chromium (III), lead, antimony, mercury and arsenic in 2% v/v in HNO_3) and hydrogen peroxide 30% v/v all supplied by BDH (Poole, Dorset, UK), were used.

Analar grade potassium iodide, sodium borohydride 99%, Spectrosol selenium 1000 mg l^{-1} standard, hydroxylammonium chloride (Spectrosol low in Hg), tin (II) chloride 2-hydrate and thiourea supplied by BDH (Poole, Dorset, UK), were used.

2.4.2. Preparation of HG-ICP-MS Solutions

A 1% m/v sodium borohydride solution was prepared fresh daily, by firstly dissolving 2 g of NaOH pellets in 500 ml of deionised water and then adding 5 g of $NaBH_4$.

The 8% m/v potassium iodide solution was also prepared fresh daily by dissolving 8 g of KI per 100 ml of 3M HCl.

All solutions were made in distilled and deionised water (MilliQ plus 185 ultrapure water system), and all the standards were made from stock solutions of 10 mg L^{-1} in 2M HCl and 10% HNO_3 and stored in plastic bottles (Nalgene).

All the glassware and bottles were cleaned in 5% v/v nitric acid.

2.4.3. Coupling the Hydride Generator to the ICP-MS

The ICP-MS was a HP 4500 (Hewlett Packard, Yokogawa Corporation, Japan) and the hydride generator was constructed from a PSA Analytical automatic Hydride Generator (PSA Analytical Ltd, Kent, UK).

The hydride system consisted of a gas liquid separator into which the reagents were pumped after having been mixed in a T-junction. The pumps used were the peristaltic pumps on the ICP-MS. The hydrides formed in the gas liquid separator were transported to the blend gas port of the spray chamber on the ICP-MS. This was set up as shown in figure 2.1. It was found that the addition of argon to the gas liquid separator, to promote the transport of the hydrides to the ICP-MS, was not required. Furthermore, when additional gas was used the plasma was very unstable.

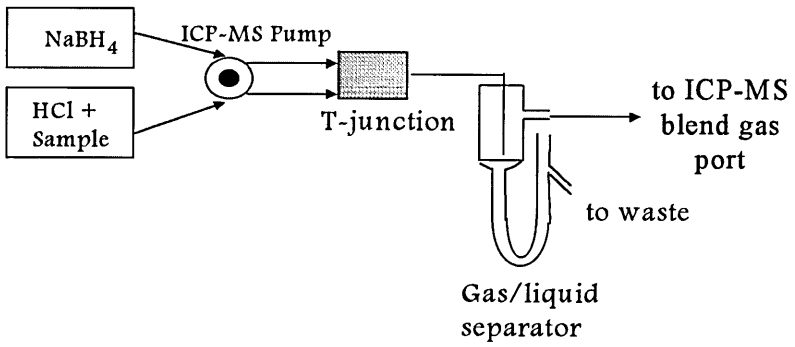

Figure 2.1. Schematic of HG-ICP-MS system.

2.4.4. Instrumental Conditions

Tables 2.3 and 2.4 show the instrumental conditions and the isotopes chosen for measurement on the ICP-MS respectively. The ICP-MS was tuned daily with the recommended tuning solution of 10 ng ml^{-1} Y, Ce, Tl and Li. The experimental conditions of the HG-ICP-MS system are stated in table 2.5.

Parameter	Setting
Rf Power	1200 W
Carrier Gas Flow Rate	1.15 L min^{-1}
Sample Depth	6.4 mm
Pump Speed	0.12 rps

Table 2.3. Normal Operating Conditions for Hewlett Packard ICP-MS 4500

Metal	Atomic Masses of Isotopes available	Chosen Isotope for Hydride Generation
Selenium	76,78,82	78
Arsenic	75	75
Mercury	196,198,199,200,201,202	202
Antimony	121,123	121
Lead	206,207,208	208

Table 2.4. The isotopes of each element available for HG-ICP-MS analysis. The most abundant isotope of each metal was chosen for the measurements.

Parameter	Setting/Concentration
Sodium Borohydride Concentration	1% m/v in 0.1M NaOH
Hydrochloric Acid Concentration	2M (18 ml in 100 ml)
Rf Power	950 W
Carrier Gas Flow Rate	1.35 L min^{-1}
Sample Depth	4.5 mm
Pump Speed	0.3 rps

Table 2.5. Experimental conditions for HG-ICP-MS

2.5. Results and Discussion

2.5.1. Optimisation of the HG-ICP-MS Parameters

Each of the variable instrumental settings were optimised in the system. Parameters including the Rf power, carrier gas flow rate, sample depth and pump speed were optimised individually using the ICP-MS software. The sodium borohydride and the acid concentrations were also optimised for each element. The initial optimisations were carried out using separate elemental standards.

2.5.1.1. Optimisation of Sodium Borohydride Concentration

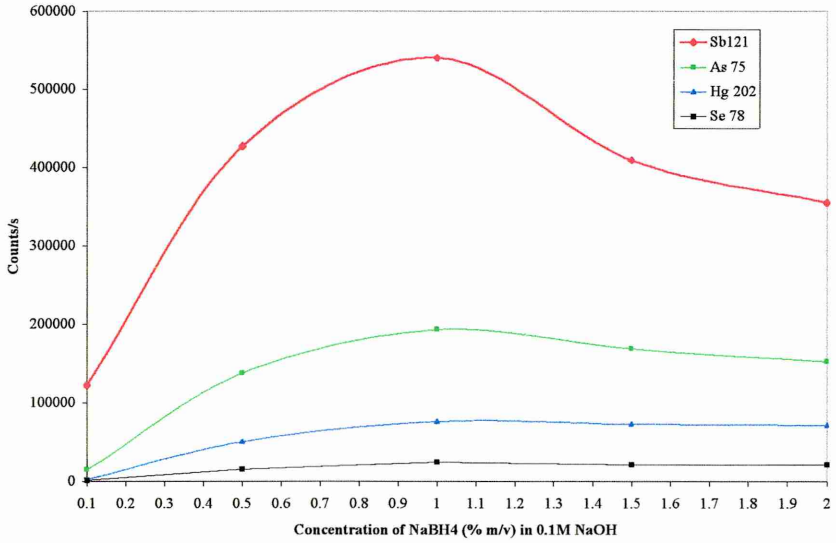

Figure 2.2. Optimisation of sodium borohydride concentration for the determination of 10 ng ml^{-1} individual solutions of antimony, arsenic, mercury and selenium in 1M HCl by HG-ICP-MS.

It can be seen from figure 2.2, 1% $NaBH_4$ was the optimum concentration for all of the elements.

2.5.1.2. Optimisation of the HCl Molarity of the Standards

Figures 2.3 and 2.4 show the results of varying the HCl concentration in which the single standards were made. Figure 2.4 displays the mercury and selenium results shown in figure 2.3 on an expanded scale.

Similar trends are seen for all elements studied, in that the signal obtained increased up to a HCl concentration of 1M, then remained generally constant. Consequently an optimum HCl concentration of 2M was chosen as the plasma seemed more stable with this concentration and because the calibrations of the elements were better in 2M HCl.

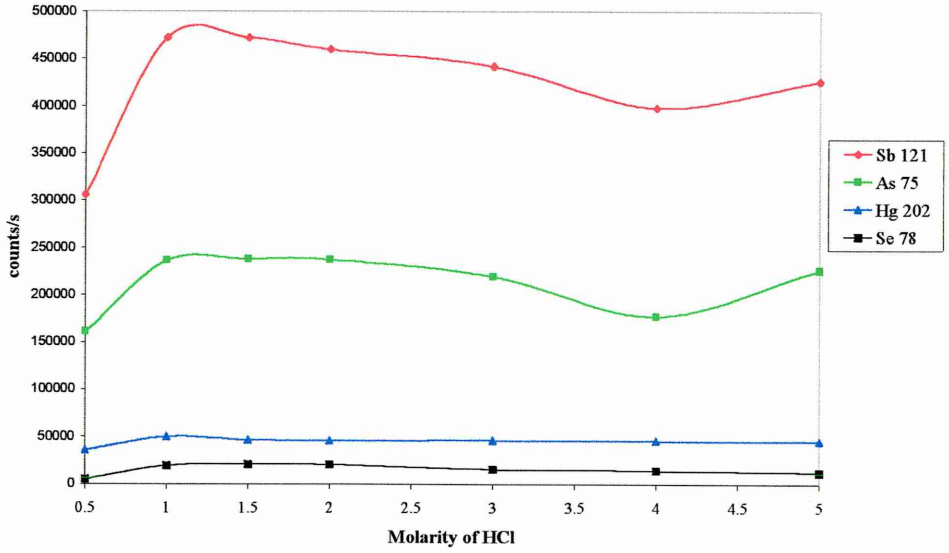

Figure 2.3. Optimisation of HCl concentration for the determination of 10 ng ml^{-1} individual solutions of antimony, arsenic, mercury and selenium in 1M HCl with 1% $NaBH_4$ in 0.1M NaOH by HG-ICP-MS

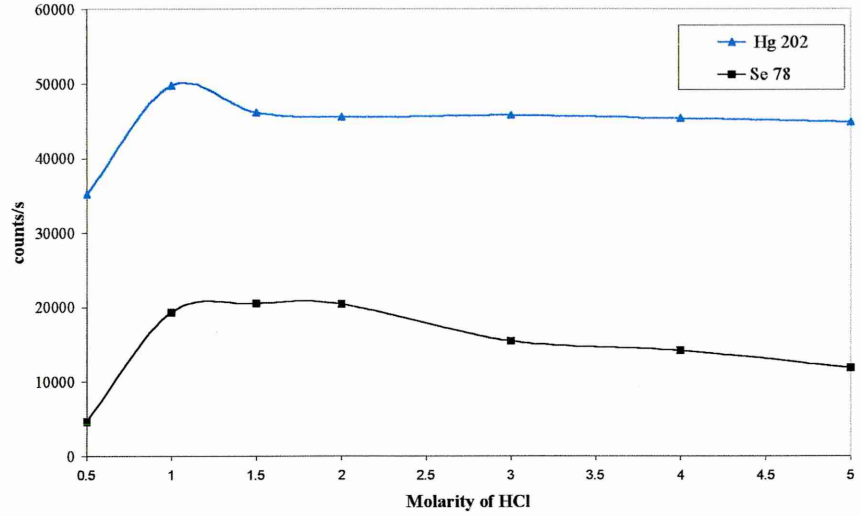

Figure 2.4. Optimisation of HCl concentration for the determination of 10 ng ml^{-1} individual standards of mercury and selenium with 1% $NaBH_4$ in 0.1M NaOH, by HG-ICP-MS

2.5.1.3. Optimisation of the Rf Power on the ICP-MS for Hydride Generation

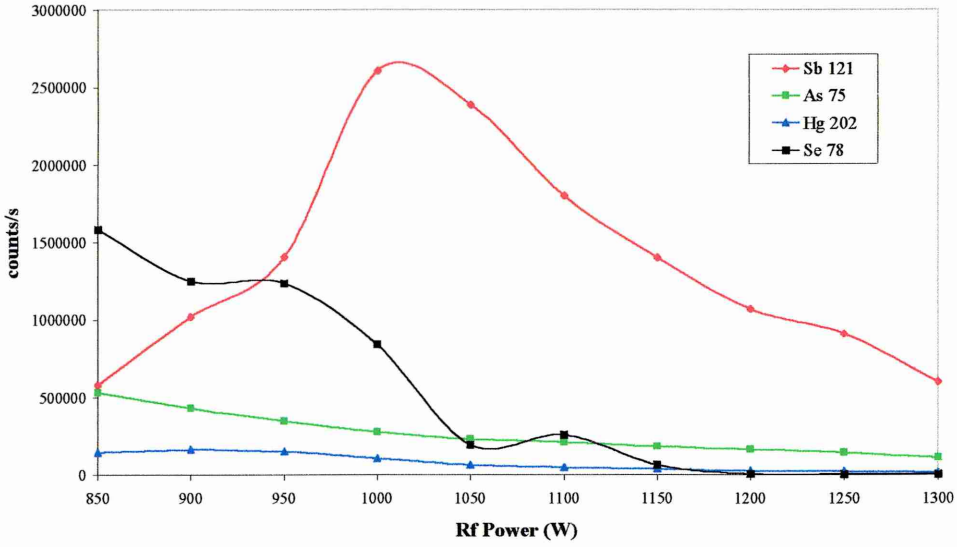

Figure 2.5. Optimisation of the ICP-MS Rf Power for the determination of individual 10 ng ml^{-1} standards of antimony, arsenic, mercury and selenium in 2M HCl with 1% $NaBH_4$ in 0.1M NaOH

The optimum Rf power was determined as 950W because at this power all of the elements had suitably increased counts from those at the normal value of 1200W. It was crucial that the Rf power was lowered to maximise the hydride signals for all the elements, with the exception of antimony, which gave a higher count rate generally. The ICP-MS manufacturers recommended that the Rf power not be below 900W, so 950W appeared to be the optimum setting.

2.5.1.4. Optimisation of ICP-MS Carrier Gas

It has previously been shown that the carrier gas flow affects the precision of the HG method in ICP analysis[18]. When the flow rate is low the gas reaches the ICP slowly and the intensity of the hydride or Hg^0 increases. Whereas, high carrier gas flow rates greatly dilute the hydride, hence reducing the intensity of the hydride or Hg^0.

The count rate for all of the elements in this study greatly increased around the flow rate range of 1.25-1.50 L min^{-1}. Below this range of carrier gas flow rates the flow rate was too low to be effective and above this range the dilution of the hydrides with the higher flow rate reduced the counts. The normal flow rate for the instrument at the time was around 1.12 L min^{-1}. An optimum of 1.35 L min^{-1} was chosen because at this flow rate the less sensitive elements, mercury and selenium, were at their highest counts and it was within the optimised range for arsenic and antimony. Figures 2.6 and 2.7 show the effect of carrier gas flow rate on the intensity of the signals. The antimony and arsenic values are shown in figure 2.6 and the lower selenium and mercury values are shown in figure 2.7.

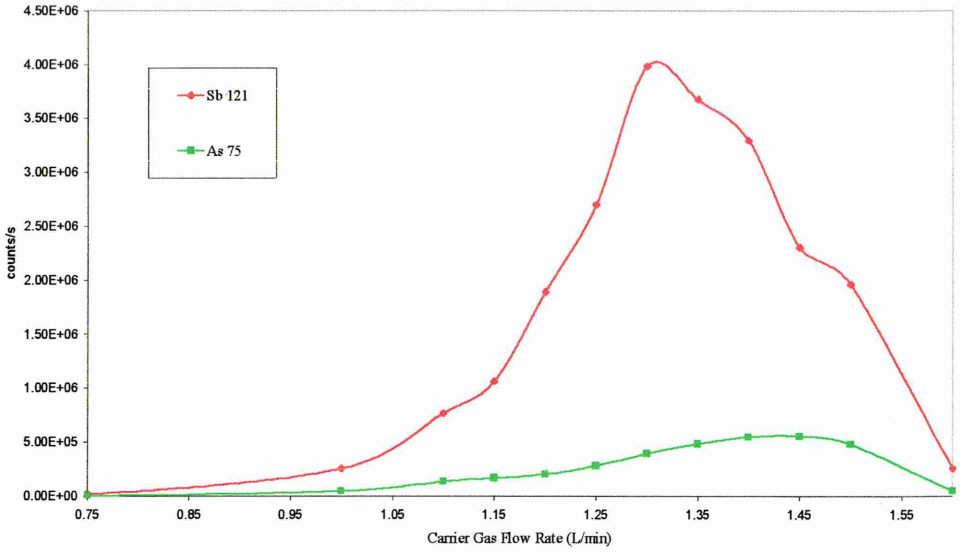

Figure 2.6. Optimisation of the carrier gas flow rate of the ICP-MS for the determination of 10 ng ml^{-1} individual solutions of arsenic and antimony with 1% $NaBH_4$ in 0.1M NaOH.

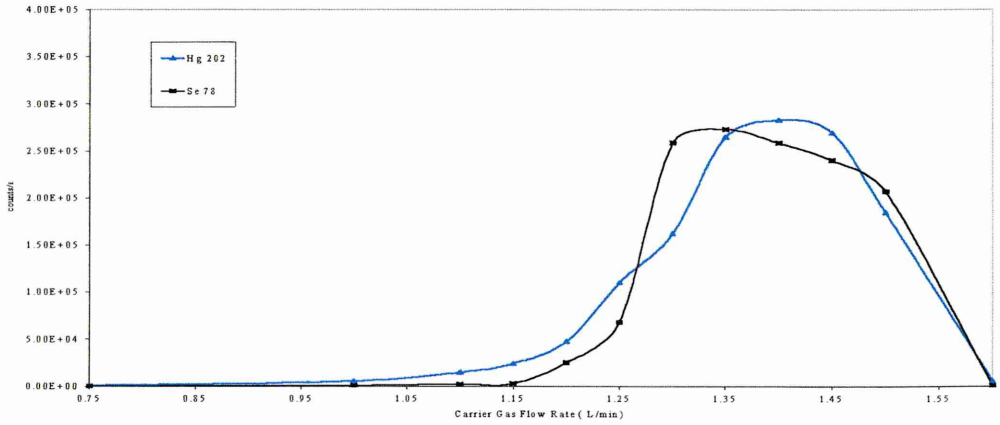

Figure 2.7. Optimisation of the carrier gas flow rate of the ICP-MS for the determination of 10 ng ml^{-1} individual solutions of Se and Hg with 1% $NaBH_4$ in 0.1M NaOH.

2.5.1.5. Optimisation of the Sampling Depth of the ICP-MS for Hydride Generation

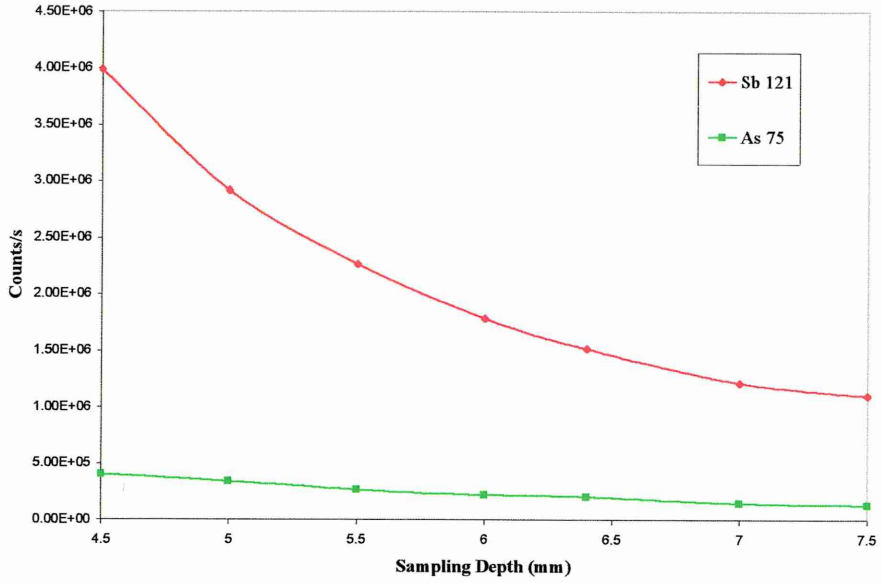

Figure 2.8. Optimisation of the sampling depth of the ICP-MS for the determination of 10 ng ml^{-1} individual solutions of arsenic and antimony with 1% $NaBH_4$ in 0.1M NaOH.

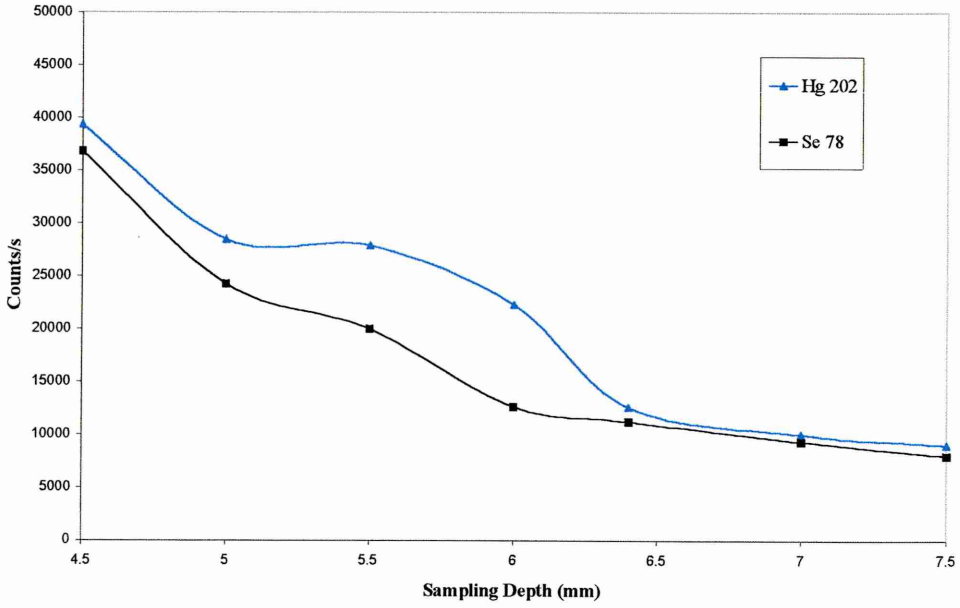

Figure 2.9. Optimisation of the sampling depth of the ICP-MS for the determination of 10 ng ml^{-1} individual solutions of mercury and selenium with 1% $NaBH_4$ in 0.1M NaOH.

The sampling depth optimisation for antimony and arsenic can be seen in figure 2.8 and the optimisation for selenium and mercury is shown in figure 2.9. The general trend is that

the smaller the sampling depth, i.e. the closer the sampling cone is to the plasma, the higher the count rate. The sampling depth of 4.5 mm was chosen as the optimum depth, because it provided a compromise between high count rate and limiting the damage to the sampling cone.

2.5.1.6. Optimisation of the Pump Speed of the ICP-MS

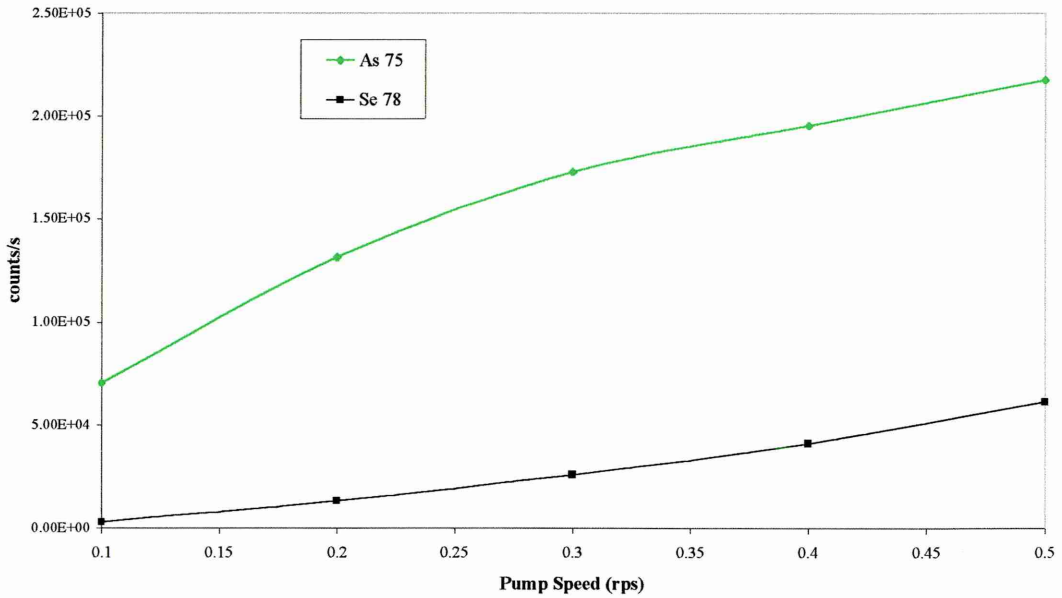

Figure 2.10. Optimisation of the pump speed of peristaltic pump on the ICP-MS for the determination of 10 ng ml^{-1} individual solutions of arsenic and selenium with 1% $NaBH_4$ in 0.1M NaOH.

Figure 2.10 shows that at the maximum pump speed of 0.5 rps the count rates for both arsenic and selenium were at their highest. Mercury and antimony showed the same trend. However, the pump speed used for the hydride generation was 0.3 rps because at this speed the observed increases in the count rates from the normal pump speed of 0.12 rps were considerable. It was important not to run the pumps at maximum speed as this would shorten the life of the tubing and can lead to plasma instability.

2.5.2. Comparisons of All the Sensitivities of the Elements Using ICP-MS and HG-ICP-MS.

Each element was prepared in a separate standard and run by normal ICP-MS (i.e. the set conditions outlined in table 2.3 in section 2.4.3), HG-ICP-MS with normal ICP-MS conditions and optimised HG-ICP-MS conditions (as in table 2.5 in section 2.4.3.). The results can be seen in table 2.6.

Element	ICP-MS normal conditions, response in counts/s	HG-ICP-MS normal conditions, response in counts/s	HG-ICP-MS optimised conditions, response in counts/s
Se	13544	737749	1659378
As	174222	3972873	5627002
Hg	76789	95504	155815
Sb	7551664	19015290	17355010

Table 2.6. Comparison of the count rates seen with a 20 ng ml^{-1} standard of each element using the ICP-MS with and without the hydride generator, with normal and optimised conditions.

It can be seen that the HG-ICP-MS optimised parameters give much higher count rates than the normal conditions of the HG-ICP-MS and the ICP-MS, with the exception of antimony, where higher counts are seen with the HG-ICP-MS using the normal ICP-MS conditions.

The calibration graphs obtained for mercury using the ICP-MS were better than the other hydride forming elements, but still the counts were considerably improved using the optimised conditions for the HG-ICP-MS.

Calibration graphs for arsenic showed that there was no significant interference from $^{40}Ar^{35}Cl$ (see figure 2.12). This was further investigated with HCl blanks and the HG-ICP-MS. It was seen that 1/1000 of the counts for arsenic would be due to interference from $^{40}Ar^{35}Cl$.

The response of the ICP-MS to the increasing concentrations of antimony is non-linear.

2.5.3. Comparison of the Reaction of Mercury and Stannous Chloride with that of Mercury and Sodium Borohydride.

It is known that the reaction whereby inorganic mercury (Hg^{2+}) is reduced to atomic mercury (Hg^0) is aided by stannous chloride[3] and so it was necessary to see if mercury was converted to the atomic state more efficiently using $SnCl_2$.

The average count rate for a 10 ng ml^{-1} mercury standard reduced using $NaBH_4$ was in the range of 34000 counts/s, whereas the counts attained after reduction with the $SnCl_2$ solution were never greater than the background counts, i.e. around 50 counts/s.

The results show that the count rates obtained from mercury reduced with the stannous chloride were low with relatively poor precision and that the sodium borohydride is a better reducing agent for mercury using the HG-ICP-MS.

2.5.4. The Analysis of Lead using the Hydride Generator

The counts obtained for lead using HG-ICP-MS were very low, at less than 100 counts per second, which is equivalent to background counts on the ICP-MS.

It is likely that the lead had been reduced to Pb^0 and so oxidation of the lead samples with hydrogen peroxide was necessary[3]. However, the addition of 1 ml of 1%, 3% and 30% H_2O_2 to solutions of Pb^{2+} did not improve the signal count rate. Using hydrogen peroxide, the sodium borohydride concentration was also increased. The highest count rates for lead were observed with a solution of 1M HCl, 1ml 3% H_2O_2 and 2% $NaBH_4$. However, this count rate was not high enough to consider using the hydride generator coupled to the ICP-MS. Therefore, it was decided that lead would be analysed separately using the ICP-MS, when it would also be possible to look at other elements such as cadmium and chromium (see Chapter 3).

2.5.5. Problems Encountered with the Hydride Generation Method

As already described the application of hydride generation greatly improves the analytical sensitivity for single element standards. When mixed standards of arsenic, selenium, antimony and mercury were made in HCl the response of mercury and selenium was considerably different. This can be seen when the calibration graph of selenium and

mercury in single standards in figure 2.11, is compared with the calibration graph of mixed standards in figure 2.12.

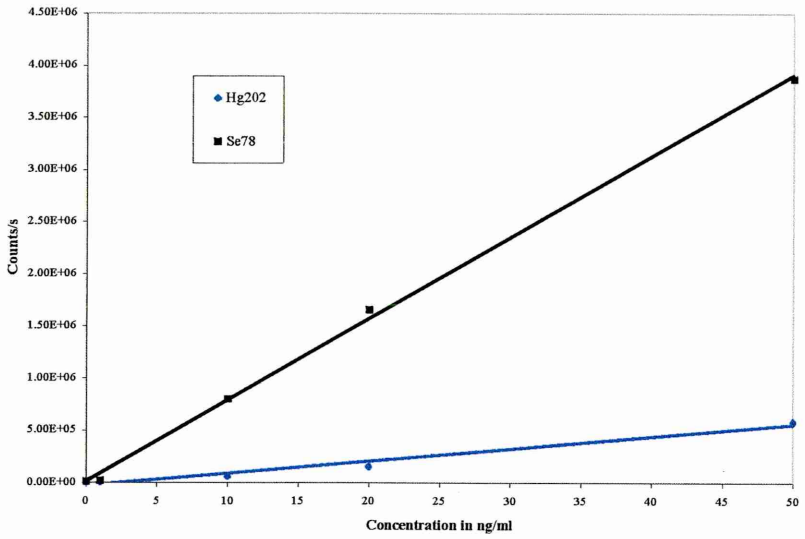

Figure 2.11. Calibration graphs for selenium and mercury using single standards prepared in 2M HCl with 1% $NaBH_4$ in 0.1M NaOH, using HG-ICP-MS optimum conditions.

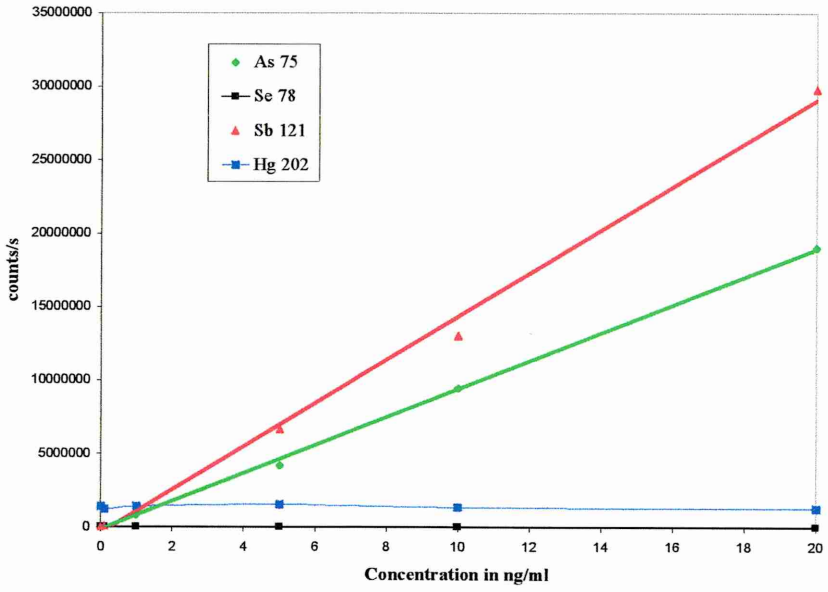

Figure 2.12. Calibration graphs for antimony, arsenic, mercury and selenium using mixed standards prepared in 2M HCl with 1% $NaBH_4$ in 0.1M NaOH.

There was also the additional problem that the mercury was difficult to flush out of the system and there were high background counts as a result of this. When the gas liquid separator was steeped in 5% HNO_3 overnight this reduced the Hg counts and they were further reduced by replacing the tubing. It became part of the procedure to flush the tubing for 30 minutes at the end of the day with 5% HNO_3 and then deionised water. However the determination of mercury in a mixed standard still remained a problem.

The lack of response for selenium and mercury illustrated in figure 2.12 was thought possibly to be due to the reduction of the elements to their elemental states (selenium and mercury need to be in oxidation states +2, to be generated to SeH_2 and elemental Hg^0). Both antimony and arsenic need to be in oxidation state +3 to form SbH_3 and AsH_3. Investigations followed using the oxidising agent, hydrogen peroxide. However, it was clear that mercury showed no improvement. At this stage it was not possible to achieve a calibration graph for mercury in a mixed standard and so it was concluded that it was not feasible to continue with mercury analysis using HG-ICP-MS.

2.5.6. Analysis of Antimony, Arsenic and Selenium in Hair by HG-ICP-MS

The hair digestion were carried out with HCl and H_2O_2 because the counts were higher for antimony, arsenic and selenium and the microwave vials stained with HCl on its own (see Chapter 3 for more details on the digests). Calibrations for selenium for these results were satisfactory, although there was a high blank, possibly as a result of matrix interferences.

Hair digestions were then carried out, where approximately 0.1 g of hair was digested with 2 ml of concentrated HCl and 1 ml of H_2O_2 (either 30% or 15%), using the microwave digestion procedure outlined in section 3.2. Hair samples were spiked with standards, and the recoveries and results are shown in table 2.7.

Digestion Method	Recovery of selenium in hair samples spiked with a 10 ng ml^{-1} mixed standard	Recovery of arsenic in hair samples spiked with a 10 ng ml^{-1} mixed standard	Recovery of antimony in hair samples spiked with a 10 ng ml^{-1} mixed standard
2M HCl +1ml 30% H_2O_2	4.8%	91.8%	7.3%
2M HCl +1ml 15% H_2O_2	4.9%	91.9%	7.7%

Table 2.7. Microwave digested hair samples with hydrochloric acid and 15% or 30% hydrogen peroxide and the recoveries of samples spiked with a mixed standard solution.

The results showed that the recoveries of arsenic were good and quite consistent. However, for antimony and selenium the recoveries were poor. To try and improve this the hydride generation conditions were modified.

2.5.7. HG-ICP-MS with Reductants to Improve Sensitivity

Several studies have looked at the possibility of using potassium iodide within the hydride generation system in order to enhance the signal of the hydride forming elements[13,18,21]. This has been seen to be particularly useful for antimony and it was thought that it would improve the recoveries of the standards in the spiked hair digests. Additionally, iodide is said to be one of the most typical pre-reductants used for reducing arsenate to arsenite[3]:

$$H_3AsO_4 + 3I^- + 2H_3O^+ \longrightarrow H_3AsO_3 + I_3^- + 3H_2O$$

It is also known that the addition of potassium iodide may reduce selenium to a zero oxidation state[18]. In introducing potassium iodide into the system it was decided to react the hair digest with the sodium borohydride first to allow the selenium to form the hydride and then introduce the reducing agent potassium iodide, where it would be unable to reduce the selenium hydride, yet still be able to reduce the antimony that had not formed the hydride because it was in +5 oxidation state[3,13]. With the selenium standards it was hoped that by making/digesting in HCl the selenium would in the +4 state and hence able to form the hydride:

$$HSeO_4^- + 3H^+ + 2Cl^- \rightarrow H_2SeO_3 + Cl_{2(aq)} + H_2O$$

Once reduced with the HCl and KI mixture, the Sb^{3+} can then form a hydride with the excess $NaBH_4$ present in the tubing. This is outlined in figure 2.13.

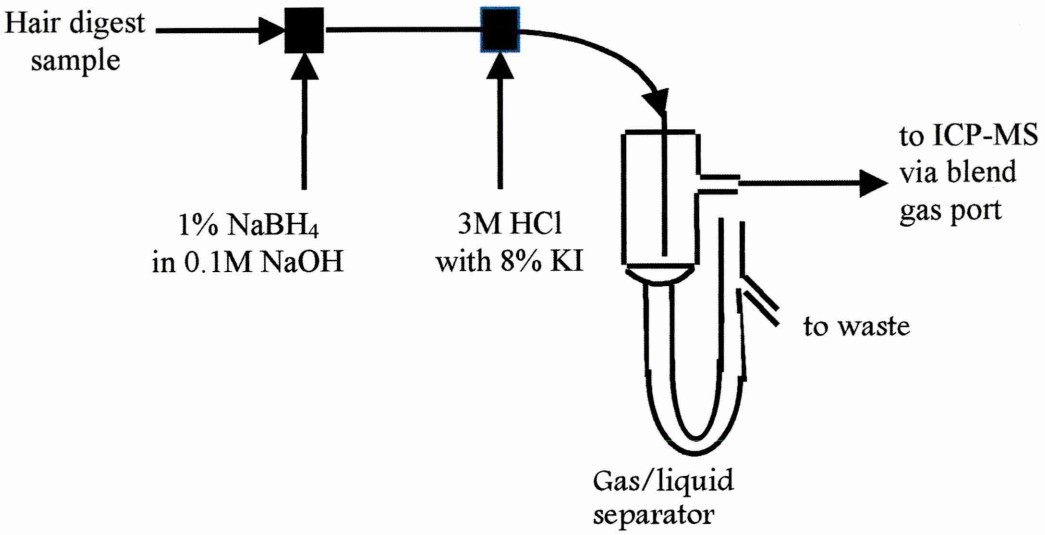

Figure 2.13. Diagram to show the outline of the HG method using potassium iodide.

It was found that the addition of potassium iodide greatly improved the recovery of antimony in the hair samples (see table 2.8), however, the selenium did not work with this method. The graph in figure 2.14 shows the results of varying the concentration of the potassium iodide in the solution.

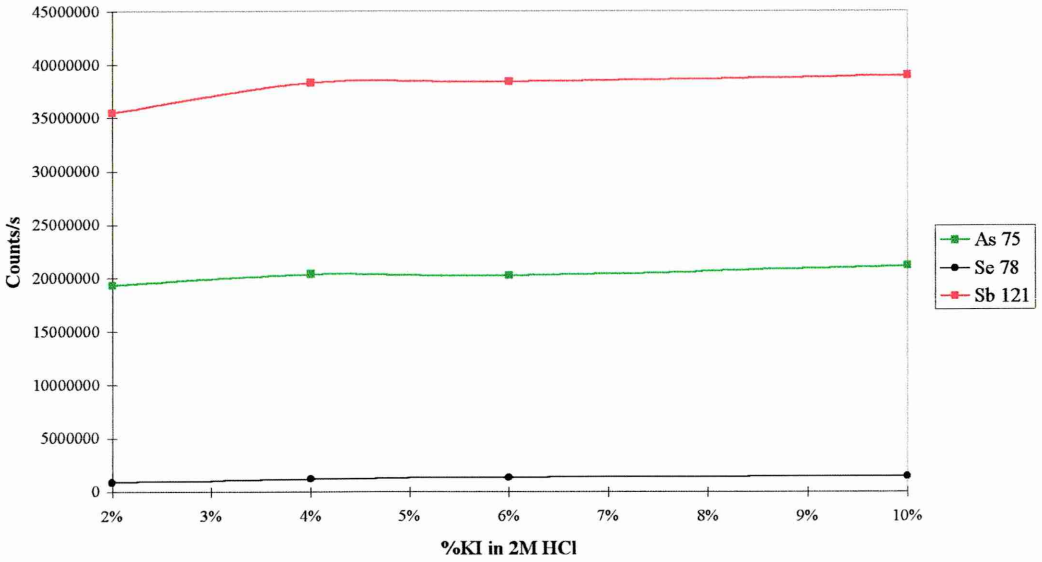

Figure 2.14. The effect of changing the % m/v of KI in 2M HCl with 10 ng ml^{-1} water standard of antimony, arsenic and selenium with 1% $NaBH_4$ in 0.1M NaOH and 2M HCl.

From figure 2.14 it can be seen that the selenium signal was not enhanced with the potassium iodide, whereas the arsenic and antimony gave satisfactorily high count rates. It is also apparent that the count rates did not increase significantly past 4% m/v KI concentration. However, as several studies had investigated this parameter in further detail it was decided for the meantime to continue using 8% m/v potassium iodide[13,18,21]. The selenium response was found not to improve at lower concentrations of potassium iodide and the poor calibration graphs obtained suggested that it would not be possible to obtain selenium hydrides by this method.

Another suggested method for determining arsenic, selenium and antimony by HG-ICP-MS was to use thiourea as a reductant[3,15]. It has been stated that thiourea is a very efficient reducing agent[3] and that it allows the simultaneous formation of SeH_2, AsH_3 and SbH_3[15]. It is believed that although thiourea will reduce Se^{4+} to its elemental state it is a slower reaction than with the potassium iodide.

0.3M thiourea was prepared in deionised water and introduced into the hydride generation system as shown in figure 2.15.

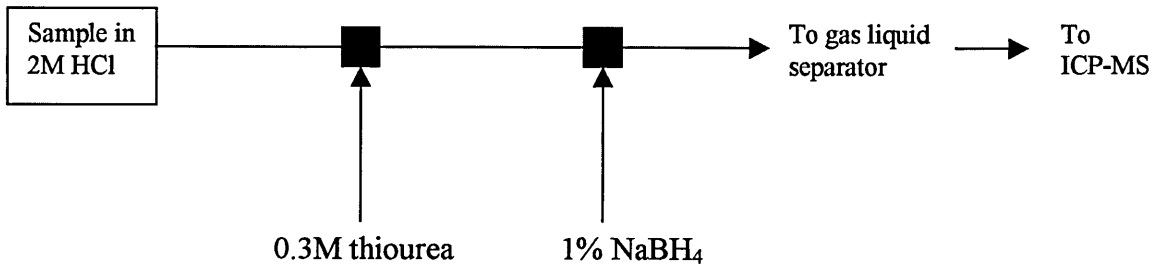

Figure 2.15: Thiourea introduction to HG-ICP-MS

Unfortunately the selenium count rate was not improved significantly by using the thiourea. In fact results showed that the selenium count rate obtained from this method were not very different from that seen when direct aspiration ICP-MS was used.

This study has shown that there are difficulties in achieving multielemental analysis with the HG-ICP-MS for arsenic, selenium, antimony and mercury. The results indicate that arsenic and antimony can be determined under the same conditions. Therefore, it was decided to carry out the analysis of mercury and selenium in hair by direct nebulisation ICP-MS and use HG-ICP-MS for the analysis of antimony and arsenic in hair.

2.5.8. The Analysis of Arsenic and Antimony in Hair Samples by HG-ICP-MS.

The method that worked best for the arsenic and antimony samples was using the potassium iodide system shown in figure 2.13. The concentration of hydrochloric acid in the hydride system was investigated using 1-4M HCl with and without 8% m/v potassium iodide. The results of this can be seen in figure 2.16.

Figure 2.16 shows that the antimony in the standard gave a higher count rate in each HCl/KI mixture compared to the HCl alone, peaking at 3M HCl and 8% m/v KI. The arsenic count rate shows no improvement with the addition of potassium iodide, however, using 3M HCl and 8% m/v KI to maximise the antimony count rate gave a reasonable response from the arsenic.

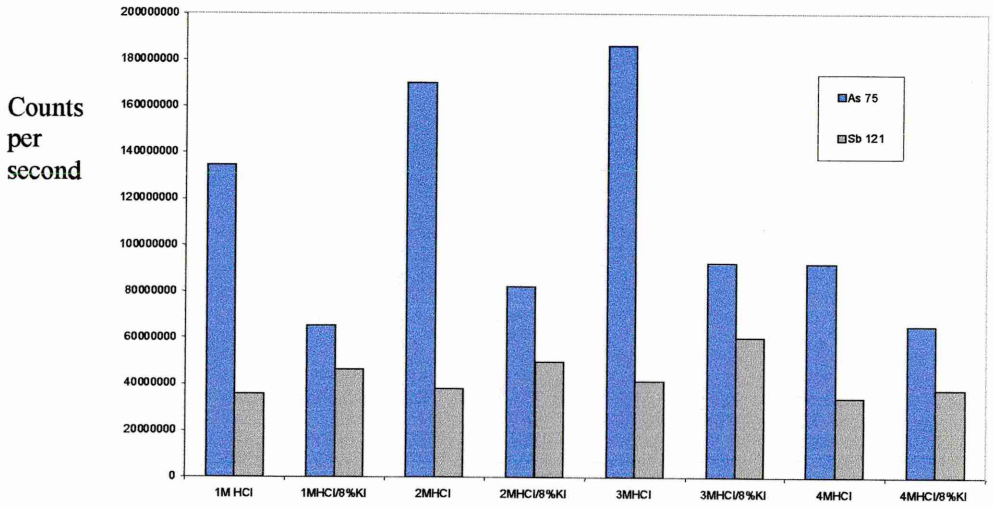

Figure 2.16. Graph to show the count rates of a mixed 10 ng ml^{-1} 2M HCl standard of arsenic and antimony using different molarities of HCl with and without 8%KI with 1% $NaBH_4$ in 0.1M NaOH.

The method adopted for the hydride generation of antimony and arsenic was to use 2M HCl samples, 1% m/v $NaBH_4$ in 0.1M NaOH, 3M HCl with 8% m/v KI, 4.5 mm sample depth and a pump speed of 0.3 rps. The carrier gas was changed to 1.4 L min^{-1}, as the addition of a new torch had meant a higher carrier gas flow rate was now the optimum.

The Rf power optimisation of antimony and arsenic had shown that they would both work at the normal condition of 1200 W.

The optimised conditions were used for the analysis of hair samples and reference materials (approximately 0.1 g) that were microwave digested using 2 ml HCl and 1 ml H_2O_2, after spiking with 1 ml of 100 ng ml^{-1} standard. The results in table 2.8 show that the recoveries of the antimony are much improved (114%), when compared to those obtained without the potassium iodide in the HG system (compare with table 2.7). However, the antimony values obtained were low compared with the certified values. The arsenic values show good recoveries with the spiked hair samples and the hair certified reference material.

Element	Spiked Hair Sample (n=5)	Certified Reference Material GBW 07601 (n=5)	
	Unspiked Hair	Found	Certified value
Arsenic	37 ± 6 ng g^{-1}	312 ± 14 ng g^{-1}	280 ± 40 ng g^{-1}
	Recovery of 10 ng ml^{-1} spike		
	103 ± 5%		
	Unspiked Hair	Found	Certified value
Antimony	48 ± 11 ng g^{-1}	58 ± 6 ng g^{-1}	95 ± 12 ng g^{-1}
	Recovery of 10 ng ml^{-1} spike		
	114 ± 14 %		

Table 2.8. Recoveries of spiked hair samples and certified reference materials using potassium iodide (8% m/v in 3M HCl) as a reductant with HG-ICP-MS.

2.6. Conclusion

It is possible to analyse arsenic and antimony simultaneously in hair by HG-ICP-MS. The carrier gas flow rate was the only ICP-MS parameter to be optimised as no other modifications were necessary. Simultaneous determination of lead, mercury and selenium was not possible because the conditions required for the determination of each element

were incompatible. Mercury appeared to cause long memory effects and the determination would not work when the other elements were present in the sample. It should however, be possible to determine mercury on its own with the cold vapour generation method. Selenium was a very problematical element to determine by hydride generation due to the fact that it did not give reproducible results and did not form the hydride under the same conditions as arsenic and antimony. Other reducing agents should be investigated further in order to optimise the conditions for selenium hydride generation. A similar conclusion was reached in studies carried out by PSA Analytical Ltd, Kent (UK)[37], where hydride generation was used with atomic fluorescence detection to analyse elements in blood samples. They used three different methods in total: one for the determination of arsenic and antimony using potassium iodide, ascorbic acid and digesting in HCl matrices for the hydride generation; the second was for the determination of selenium and bismuth hydrides where the sample was digested in a nitric acid matrix and the hydride generated with HCl and HNO_3 only; and thirdly the method for mercury determination used $SnCl_2$ instead of $NaBH_4$ for elemental mercury[37].

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Chapter 3: Digestion Methods for Hair Analysis

3.1. Sample Digestion Procedures

There has not, to date, been a universal digestion method for hair analysis[1,2]. Many different types of digestion procedures and digestion matrices have been used in previous studies. These include heating the hair on a hot plate[3,4], in a water bath, or in a Teflon bomb[5,6,7], with concentrated acids, such as nitric and perchloric, or alkali digesting with sodium or potassium hydroxide[8,9,10]. Methods have also included simply digesting the hair at room temperature, in a concentrated acid solution[11,12]. Recently, microwave digestion seems to be the preferred technique for the digestion of hair prior to analysis[13,14].

A critical factor in the evaluation of sample digestion procedures is the completeness of decomposition of the original sample matrix. The most common digestion technique is an open beaker with acid(s) on a hot plate and this has been used in many hair analysis studies[10,15,16,17,18,19,20]. Despite their frequent use, hot-plate dissolution procedures are limited by several primary factors: long dissolution times, the potential loss of volatile elements, contamination of the sample by excessive amounts of reagents, airborne contaminants and prolonged contact with vessel materials. To accelerate dissolution, closed systems are used, such as digestion bombs and microwave digestion systems[21].

In 1995, a detailed study carried out in Glasgow, investigated digestion procedures that would allow the analysis of selenium in hair by electrothermal atomic absorption spectrometry[14]. The accurate determination of selenium in hair requires the total destruction of all the organoselenium compounds. These compounds are the acid resistant selenium analogues of the sulphur-containing amino acids, methionine, cysteine and cystine, that are present in the keratin of hair. Nitric acid, used in a closed system was found to give incomplete digestion of the organoselenium compounds. However, closed-vessel microwave digestion of hair with nitric acid, followed by the addition of hydrogen peroxide, gave complete digestion[14].

In recent years, bombs for pressurised acid digestion have found widespread use in the determination of trace metals in organic and inorganic materials. The main advantages of this method are the relatively fast digestion of the sample, the very low reagent blanks and the negligible losses of trace elements even for the volatile elements[22].

Microwave digestion systems also offer additional advantages over pressurised bombs with faster digestion times and more controlled analysis with high reproducibility. A digestion bomb is a moderate or high pressure microwavable vessel without temperature or pressure measurement connection capabilities. A digestion vessel however, has the capability to connect to at least one reaction condition monitoring system and usually has an excess pressure relief system[21].

Microwave sample preparation allows the operator to carry out complete, safe and reliable digestions[21]. Microwave energy is applied to mineral acids, which are directly heated by coupling reagents with the energy source.

3.1.1. Microwave Digestion

Microwave heating involves direct absorption of energy by the sample material being digested. Microwaves are electromagnetic energy, which is a non-ionising radiation that causes molecular motion by the migration of ions and rotation of dipoles, but does not cause changes in molecular structure[21,23]. Microwave energy has a frequency range from 300 to 300 000 MHz.

The laboratory microwave cavity system is based on commercial microwave equipment. However, the resonant cavity and other components of these devices are similar to those of a domestic microwave oven. The laboratory device is equipped with numerous safety features. The microwave system must be capable of venting the fumes without contacting or damaging the control electronics. To prevent acid corrosion, the inner walls of the microwave cavity are made from resistant materials such as stainless steel covered with Teflon. Laboratory microwave units are also equipped with inter-locking door mechanisms, explosion-resistant doors with resealing hinge systems and door shields. These design considerations differ significantly from those applied to domestic microwave systems

A typical laboratory microwave unit consists of six major components: the microwave generator (the magnetron), the wave guide, the microwave cavity, the mode stirrer, a circulator and a turntable. A typical laboratory microwave unit with its vessels in a turntable, is illustrated in figure 3.1.

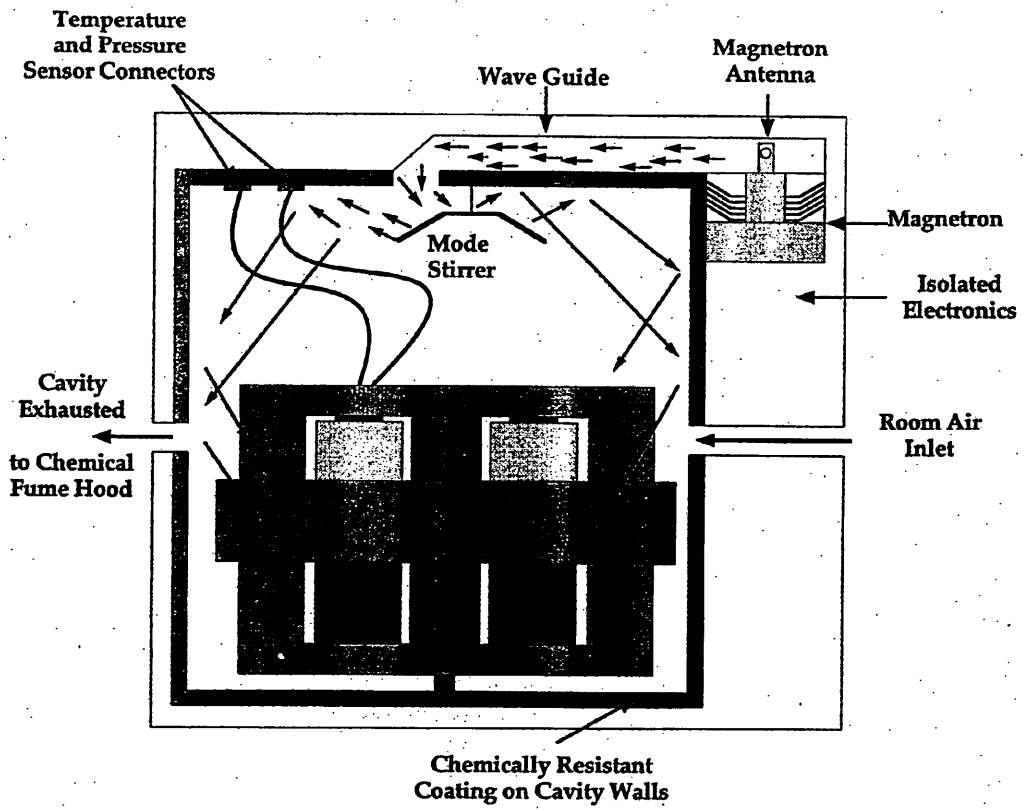

Figure 3.1. The microwave digester[21].

The magnetron produces microwaves that are radiated from its antenna into the wave guide. The wave guide is a reflective metal that directs the waves into the microwave cavity. As the microwaves enter the cavity, they are reflected by the mode stirrer to assist in homogenising the microwave field inside the cavity. Despite the design of the mode stirrer, a non-uniform standing pattern is established inside the cavity. To improve the homogeneity of the microwave field, the samples are rotated through the variable field. Under moderate cavity loading conditions, the entire microwave energy is not absorbed by the materials inside the cavity. The stray energy or the reflected power is reflected back to the wave guide and magnetron, heating the magnetron antenna. This reflection may result in irreproducible microwave energy emission and a shortened magnetron lifetime. In laboratory units, stray microwave energy is absorbed by materials which are large absorbers, typically a ceramic plate within the cavity. Alternatively, the reflected microwave power is shunted down a second wave guide for dissipation[21].

The closed-vessel systems have a number of advantages over open vessel systems. Firstly, the pressure raises the boiling point of the acids, achieving higher temperatures, which in turn reduce the time required for the digestion. Secondly, the possibility of losing volatile elements is virtually eliminated by the closed system. Thirdly, less acid is required because little evaporation of the sample digest occurs. Finally, the potentially hazardous fumes produced are contained within the vessel and the possibility of airborne contamination is eliminated. In addition, samples can be digested in as little as ten minutes, depending on the sample matrix. Furthermore, by using carousels in the microwave unit the sample throughput is increased, because the carousel allows for multiple samples to be digested at once.

3.1.2. Digestion Reagents

Nitric acid is an oxidising acid that will dissolve most metals to form the soluble metal nitrates. It has poor oxidising strength at concentrations less than 2M, but is a powerful oxidising acid in the concentrated form. Nitric acid is the most common acid for the oxidation of organic matrices. Its oxidation potential is directly proportional to increases in temperature. The oxidising strength of nitric acid can be enhanced by the addition of chlorate, permanganate, hydrogen peroxide or bromine. Nitric acid is a more powerful oxidising agent when used in combination with hydrochloric acid[21].

Hydrochloric acid is a non-oxidising acid that exhibits weak reducing properties during dissolution. Many metal carbonates, peroxides and alkali hydroxides are readily dissolved by hydrochloric acid. Some metals can be dissolved by hydrochloric acid, (e.g. cadmium, gold, iron and tin) but dissolution is accelerated by the addition of another acid. Most metals form soluble metal chlorides but there are several notable exceptions: AgCl, HgCl and TiCl are insoluble whilst $PbCl_2$ is only slightly soluble. The complexing nature of hydrochloric acid allows for complete dissolution of metal ions such as Ag^{+}, Au^{2+}, Ti^{3+}, Hg^{2+}, Ga^{3+}, In^{3+}, $Fe^{2+\&3+}$ and Sn^{4+}. Certain metals can be stabilised by combining acid digestion with an oxidising reagent and complexation with chloride ions at temperatures above the acid's atmospheric boiling point. Nearly 70% of antimony can be lost during a typical atmospheric boiling point digestion with an oxidising acid such as nitric acid. However, a mixture of nitric and hydrochloric acid oxidises Sb^{3+} to Sb^{5+} at a reaction

temperature of 175°C and results in the formation of a stable complex ion with chloride ions[21].

When hydrochloric acid is combined with hydrogen peroxide, strongly oxidising chlorine gas is formed. The mixture's oxidising strength is enhanced with increasing acidity. Typically, concentrations of 30-50% hydrogen peroxide are used in digestions. Hydrogen peroxide is usually combined with an acid because its oxidising power increases at higher acidity. Hydrogen peroxide is added to the digestion solution to complete the digestion without the potential safety hazards of reacting a strong oxidising reagent with an easily oxidisable material, in this regard, hydrogen peroxide is used as a replacement for perchloric acid.

Perchloric acid has been used in several hair digestion procedures[11,15,19,24,25,26], however, it was decided that it would not be used for this study. This is because it would involve using a fume cupboard with a perchloric fume collector and also there are risks of explosion and fires during digestion.

3.2. Experimental

3.2.1. Reagents Used

Aristar grade solutions of hydrochloric acid (1.18gr.) (37%), nitric acid (69%), and hydrogen peroxide 30%, and 1000 mg L^{-1} Plasma Emission Standards of chromium (III), lead, antimony, mercury, arsenic, selenium and cadmium in 2% HNO_3 were used, all of which were from BDH (Poole, Dorset, UK). Additional chemicals were Aristar sodium hydroxide pellets and analar grade solutions of sodium borohydride 99%, vanadium pentoxide and potassium permanganate from BDH (Poole, Dorset, UK). The potassium iodide was a Normapur reagent from Prolabo (Fontenay, S/Bois, EEC).

Deionised water was used throughout. The glassware was all cleaned in 5% nitric acid as were the nylon forceps and the pyrex plate. The ceramic blade was cleaned after use with a damp cloth. The microwave vials were purchased for this study from Scientific & Medical Products Ltd (Manchester, UK) and when not in use were stored in a 5% nitric acid bath.

3.2.2. Hair Sampling

Once removed from the head the hair was placed in sterile plastic bags and labelled. The hair was removed either by cutting with stainless steel scissors or shaving. The shavings did not require homogenising in any way. Longer hair samples were cut with a ceramic blade on a pyrex plate and the cuttings were mixed in the sample bags after cutting.

Two certified reference materials were used in this study: CRM 397, which was produced by the Community Bureau of Reference (BCR) Luxembourg; and GBW 07601 from China, which was supplied by Laboratory of the Government Chemist (LGC), Teddington, Middlesex, UK.

3.2.3. The Microwave Digestion System

The laboratory system used was the MLS-1200 MEGA Microwave Digestion System with MDR (microwave digestion rotor) technology from Milestone, Scientific & Medical Products Ltd (Manchester, UK). The ten vials/vessels and their lids in the carousel are made from a PTFE based material, TFM (tetrafluormethaxil). The outside cover (safety shield) for the vials was made from PEEK (made from polyether ether ketone, which has an extremely high mechanical and chemical resistance) as were the adapter plate and spring disk which slotted in on top of the vial. The carousel into which the vials are placed was made from polypropylene.

The vials were cleaned between digestions by adding 5 ml concentrated nitric acid and digesting at 250W for 10 minutes and then cooling for an hour. During intense use the vials were cleaned by adding 4 ml concentrated nitric acid which was digested using the same programme as used for the hair.

The microwave programme used for the digestions is shown in Table 3.1.

Time in minutes	Power Setting in W
5	250
2	0
5	400
2	0
5	600
10	vent

Table 3.1. Microwave programme used for hair digestion.

3.2.3.1. Digestion Procedures

Preliminary studies using nitric, hydrochloric and sulphuric acids and various mixtures with and without hydrogen peroxide were carried out. Oxidative digests with vanadium pentoxide and potassium permanganate were also investigated for the hydride forming elements.

The results from the studies showed that the optimised digestion procedure for the determination of cadmium, chromium, lead, mercury and selenium in hair using direct aspiration ICP-MS, was to add 2 ml of concentrated nitric acid and 1 ml of 30% hydrogen peroxide when digesting 0.1 g of hair. The optimised procedure for the determination of arsenic and antimony in hair by HG-ICP-MS was to add 2 ml concentrated hydrochloric acid and 1 ml of 30% hydrogen peroxide when digesting 0.1 g of hair.

3.2.3.2. Routine Hair Analysis

Approximately 0.1g hair was weighed directly into the microwave vial with nylon forceps and then 2 ml of concentrated nitric acid was added with 1 ml hydrogen peroxide (30%). The vial's lid was put on, and then the spring top and the vials were placed in the protective shield, which was in turn placed in the carousel and into the digester. The samples were digested by the method in table 3.1. Once the digestion was finished the carousel was placed in cold water for 30 minutes and then the vials were opened in a fume cupboard. The digest was transferred to a sterile conical tube with a lid, diluted to 10 ml and stored in the fridge until analysis was carried out. An autosampler was used during the ICP-MS analysis and each sample had 0.1 ml of a 1 $\mu g\ ml^{-1}$ internal standard mix (Rh, Y and Bi) added, to correct for instrumental drift.

3.2.4. Instruments Used

3.2.4.1. ICP-MS

The ICP-MS used was as described in section 2.4.3. The ICP-MS was tuned daily using the recommended manufacturers' solution of 10 $ng\ ml^{-1}$ of Li, Y, Ce and Tl. The dwell time on each of the isotopes used is shown in table 3.2. The ICP-MS was turned on 1h before use and the sample uptake time was 1 minute (30 seconds uptake and 30 seconds for the pump to stabilise) before analysis was carried out.

When using HG-ICP-MS the gas/liquid separator was flushed with water between hair digest runs to remove all of the previous digest. Between 0.5-1h was required to equilibrate the hydride system before use.

The reagents for hydride generation were as described in section 2.4.1.

Metal	Isotope Used	Dwell time (seconds) per point	Dwell time (seconds) per mass
Chromium	52	0.1	0.3
Selenium	82	1	3
Cadmium	114	1	3
Mercury	202	1	3
Lead	208	1	3

Table 3.2. The acquisition times for the isotopes used for each element, in the ICP-MS method for hair digest samples.

3.2.4.2. Scanning Electron Microscopy (SEM)

A Philips XL40 SEM (Cambridge, UK) was used with an Oxford EDX analyser (Oxford Instruments, High Wycombe, Buckinghamshire, UK) and the beam was set at 12 kV. The hair samples were prepared by placing on a platform and coating in carbon powder.

Scanning electron microscopy (SEM) allows the close study of the structure of the hair surface. In this instrument a sharply focused electron beam strikes the specimen and a signal from the surface (secondary electrons) is detected, amplified and displayed on a cathode-ray tube. The strength of the signal from a given point is a function of the surface topography and the chemical composition of the given point. The light intensity at a point on the cathode-ray tube monitor is directly related to the strength of signal generated from the corresponding point of the specimen. The incoming electron beam also produces characteristic X-rays from elements in the specimen, therefore, the instrument can be used simultaneously for elemental analysis[32].

3.2.5. Statistical Tests

Paired t tests were used to determine if there was a significant difference between two methods. The observed t value calculated using equation 1 was compared with the 95%

confidence t values in the t-distribution statistical tables, for the relevant n-1 value. If the observed t value was greater than the statistical t value then the difference was more than 5% significant.

Equation 1: $t = \bar{d}/(s_d/\sqrt{n})$

where t is the observed statistical value where t has (n-1) degrees of freedom, $\bar{d}$ is the average of the differences between the two sets of values, s_d is the standard deviation calculated by equation 2 and n is the number in each group.

Equation 2: $s_d = \sqrt{\sum(d-\bar{d})^2 / n-1}$

where d is the difference between two sets of values[28].

3.3. Results and Discussion

3.3.1 Initial Hair Digests

A comparison of microwave digestions with cold digestions showed that the former produced higher concentrations of chromium, lead and mercury from hair. Nitric acid digestions, with and without the addition of H_2O_2 at various stages of the digestion process, including in a second digest, were examined. Table 3.3 shows the results of these experiments.

Digestion Procedure	Hair chromium concentration (ng g^{-1})	Hair mercury hair concentration (ng g^{-1})	Hair lead concentration (ng g^{-1})
CD with 2 ml HNO_3	1400	160	510
CD with 2ml HNO_3 and 1ml H_2O_2.	1710	120	540
MD with 1 ml HNO_3	1600	240	1080
MD with 2ml HNO_3	1740	270	1200
MD with 2ml HNO_3 and 1ml H_2O_2.	2500	300	1340
MD with 2ml HNO_3 and 1ml H_2O_2. added in 2nd MD	1680	210	1120

Table 3.3. The effect of digestion procedures on the hair concentrations of chromium, lead and mercury using cold digestions (CD) and microwave digestions (MD) with nitric acid and the addition of hydrogen peroxide at different stages of the digestion process.

The results showed that the microwave digestion showed higher concentrations for chromium, mercury and lead than the room temperature (cold) digestion. The addition of hydrogen peroxide to the cold digest did not increase the hair concentrations significantly, however, with the microwave digestion the hair concentrations obtained were increased with the addition of hydrogen peroxide. The results also showed that the digest where the hydrogen peroxide was added at the start of the digest showed higher concentrations than when it was added before a second digest.

Digests containing nitric acid gave poor results for the determination of the hydride forming elements, arsenic and antimony. Evidence for this can be seen in figure 3.2.

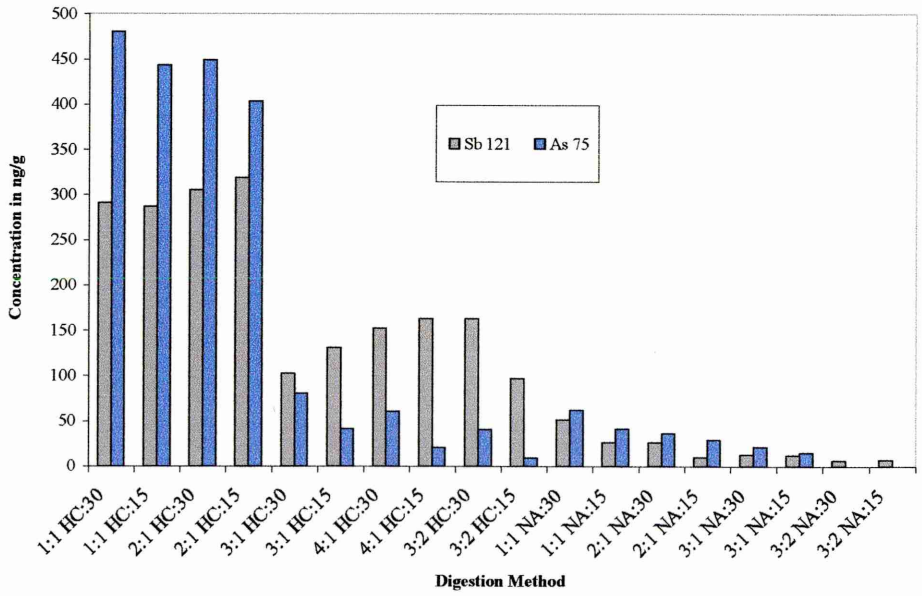

Figure 3.2. The concentrations of antimony and arsenic after hair digestion with mixtures of hydrochloric acid (HC), nitric acid (NA) and 15% v/v (15) or 30% v/v (30) hydrogen peroxide. The ratios denote the volume of each reagent used (in ml). Analysis was carried out by HG-ICP-MS using 1% m/v $NaBH_4$ in 0.1M NaOH, with 3M HCl and 8% m/v KI.

Figure 3.2 shows that the hair samples digested in hydrochloric acid and hydrogen peroxide show higher antimony and arsenic concentrations than with nitric acid and hydrogen peroxide. It should be noted that using more than 2 ml of hydrochloric acid also shows a substantial decrease in the hair concentrations of arsenic and antimony.

A 2:1 $HCl:H_2O_2$ mixture was chosen for the determination of arsenic and antimony in hair. Although the 1:1 ratio gave similar results, the 2:1 ratio of $HCl:H_2O_2$, resulted in the least amount of staining in the microwave vials. The 30% v/v of hydrogen peroxide was used because it produced slightly higher arsenic and antimony concentrations than the 15% v/v solution.

It was then investigated whether more than one application of the 2:1 $HCl:H_2O_2$ digestion would further enhance the arsenic and antimony hair concentrations. Figure 3.3 shows the results of one and two digestions and the effects of the addition of strong hydrochloric acid solutions to the sample after digestion, instead of the water dilution.

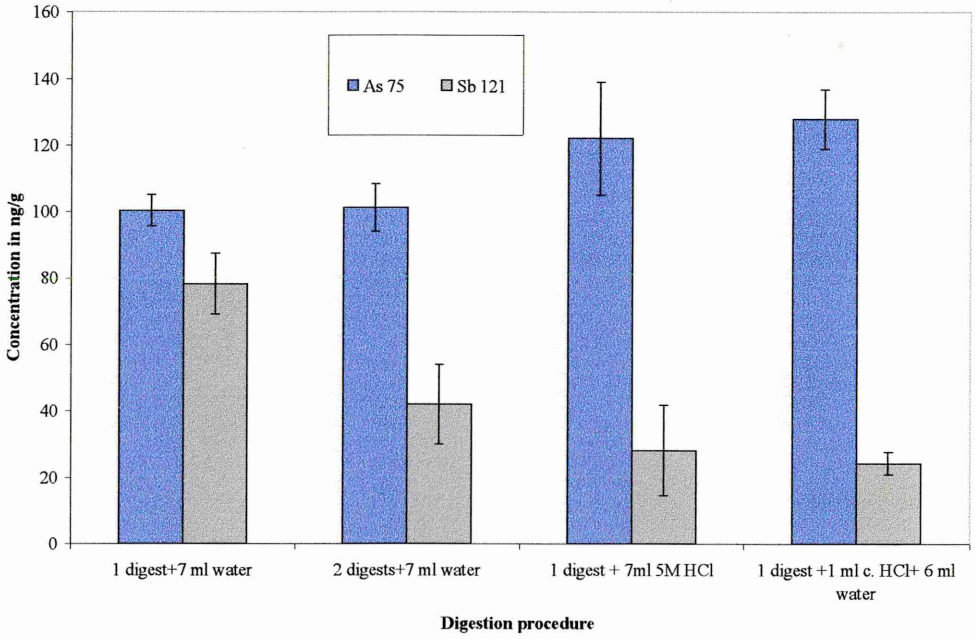

Figure 3.3. Graph to show the hair concentrations of antimony and arsenic in one and two digestions using 2:1 $HCl:H_2O_2$, with and without the addition of HCl solutions to the single digests. Analysis was by HG-ICP-MS using 1% m/v $NaBH_4$ in 0.1M NaOH, with 3M HCl and 8% m/v KI.

The results show that there is no noticeable improvement with the second digest for arsenic and an observed decrease in the antimony concentration. The results also show that although the arsenic concentration might be slightly improved by the addition of 7 ml 5M HCl or 1 ml concentrated HCl and the 6 ml of water, antimony gives better results with the hair digest diluted with water only. The method chosen for the analysis of arsenic and

antimony in hair using HG-ICP-MS was therefore 2 ml HCl and 1 ml H_2O_2, which was then diluted to 10 ml with deionised water.

Other digestions that were investigated used different ratios of acids including 1:1:1 nitric:hydrochloric:hydrogen peroxide and sulphuric acid with and without nitric or hydrochloric acid and hydrogen peroxide addition. The investigation showed that when a hair sample was digested with an acid on its own there were a lot of fumes on opening the vials. The combination of nitric and hydrochloric acid gave high element concentrations for the hair digests, but the concentrations were not higher than those seen for arsenic and antimony with 2:1 $HCl:H_2O_2$ digestions or those seen for chromium, lead and mercury with 2:1 $HNO_3:H_2O_2$ digestions. When hydrogen peroxide was used with a mixture of hydrochloric and nitric acid higher hair concentrations were obtained. Other oxidising agents were investigated, but neither vanadium pentoxide or potassium permanganate gave as good results as the hydrogen peroxide with either nitric or hydrochloric acid.

An investigation was then carried out to see if one digestion method could be used for both the hydride generation work and the determinations using only ICP-MS. Five hair samples from the same donor were digested with 2:1 $HCl:H_2O_2$ and another five samples were digested with 2:1 $HNO_3:H_2O_2$, both of which were spiked with 1 ml of 50 ng ml^{-1} water standard of cadmium, chromium, lead, mercury and selenium. Certified reference material GBW397 (LGC) was also digested in the different matrices. The nitric acid digests were run against standards made in 10% HNO_3 and the hydrochloric acid digests were run against 2M HCl standards.

The calibration graphs in figures 3.4 and 3.5 for chromium and selenium respectively highlight problems that can occur when hydrochloric acid is used as the matrix for determining elemental concentrations in biological matrices. The enhancement of the chromium signal in figure 3.4, arises from interference due to the presence of $^{35}Cl^{16}OH$ at 52 amu and $^{37}Cl^{16}O$ at 53 amu, which occur in hydrochloric acid matrices[27]. This makes it very difficult to determine Cr in matrices containing hydrochloric acid. Figure 3.5 shows a high count rate for the blank solution in the presence of hydrochloric acid. It can be seen in figures 3.4 and 3.5 that the calibrations for selenium and chromium appear better in 10%

HNO_3 than in 2M HCl. This was further confirmed by results from the recoveries of certified reference materials and the spiked samples, seen in table 3.4.

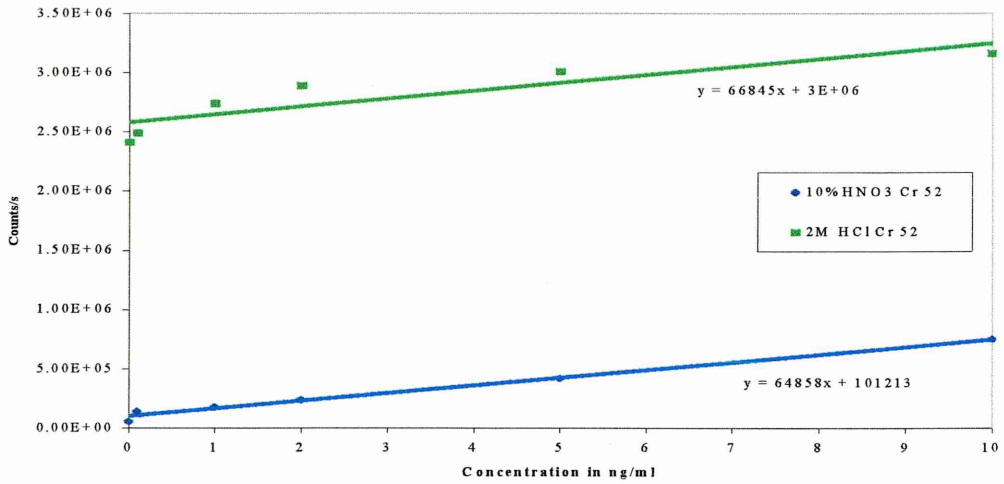

Figure 3.4. Chromium calibration curves in 2MHCl and 10% HNO_3, determined using direct nebulisation ICP-MS.

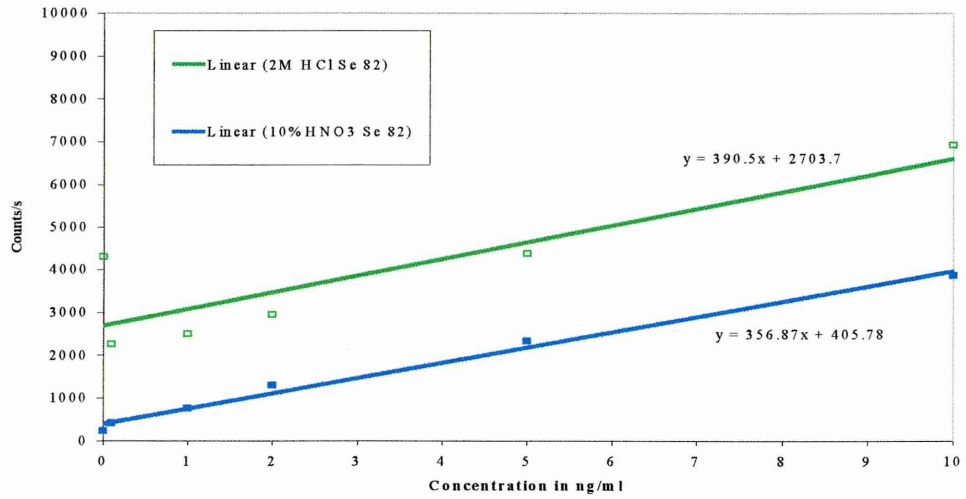

Figure 3.5. Selenium calibration curves in 2MHCl and 10% HNO_3, determined using direct nebulisation ICP-MS.

Table 3.4 shows that better cadmium, chromium, lead, mercury and selenium recoveries are obtained when the hair samples are digested in 2:1 HNO_3:H_2O_2. Chromium could not be determined in the chloride matrix because of the high blanks. The increased blank counts observed with the selenium standard in a HCl matrix was as a result of a high blank

selenium value in the hydrochloric acid matrix, as seen in the calibration graph in figure 3.5. The certified reference materials (CRMs) also show better results with the nitric acid digest.

	Initial Hair Concentration (ng g^{-1}) in samples digested with 2:1 HNO_3:H_2O_2	2:1 HCl:H_2O_2 Digests Recovery of the 5 ng ml^{-1} Spike (ng ml^{-1}) (as a % of recovery) against matrix matched standards	2:1 HNO_3:H_2O_2 Digests Recovery of the 5 ng ml^{-1} spike (ng ml^{-1}) (as a % of recovery) against matrix matched standards
Recovery of 5 ng ml^{-1} standard spiked hair sample	Cr 560 Se 480 Cd 57 Hg 281 Pb 612	Cr none Se 166 ± 12 (3310%) Cd 8 ± 0.3 (153%) Hg 8 ± 0.5 (161%) Pb 9 ± 0.7 (176%)	Cr 6 ± 0.5 (127%) Se 5 ± 0.3 (91%) Cd 5 ± 0.2 (102%) Hg 5 ± 0.2 (92%) Pb 6 ± 0.4 (120%)
	Certified values (ng g^{-1})	2:1 HCl:H_2O_2 Digests Recovery of CRM (ng g^{-1})	2:1 HNO_3:H_2O_2 Digests Recovery of CRM (ng g^{-1})
Recovery of certified reference material GBW 397	*Cr Se 2000 Cd 500 Hg 12300 Pb 33000	 Se 4740 ± 202 Cd 235 ± 45 Hg 18330 ± 3070 Pb 56230 ± 9000	 Se 2170 ± 145 Cd 619 ± 104 Hg 14400 ± 1150 Pb 43420 ± 3900

* No certified value for chromium is provided.

Table 3.4. A comparison of the recoveries of elements from spiked hair samples and certified reference samples digested with nitric and hydrochloric acid with hydrogen peroxide. No value for chromium was seen in the 2:1 HCl:H_2O_2 matrix as a result of the high blanks observed.

Results of these preliminary digestion studies confirmed that it would not be possible to use the same digestion method for ICP-MS analysis of cadmium, chromium, lead, mercury and selenium and HG-ICP-MS analysis of arsenic and antimony. For the determination of arsenic and antimony in hair using the hydride generation technique, it was necessary to use a single hydrochloric acid and hydrogen peroxide digest. From the ICP-MS analysis of cadmium, chromium, lead, mercury and selenium the nitric acid digests with hydrogen peroxide showed better results, with low blanks, good recoveries of spikes and good reproducibility of results which concurred with the certified values in the reference material, which was confirmed using paired t tests.

3.3.2. The Effect of the Matrix on Hair Digestion

It was necessary to test whether the nature of the hair matrix affected the slope of the calibration curves. Standards were prepared in 10% HNO_3 solution and in a digested solution of 2:1 HNO_3:H_2O_2 which was diluted as in the hair digests. Two different hair samples digested with 2:1 HNO_3:H_2O_2 were then spiked with standards made in distilled pure water so that a standard addition curve could be compared with the calibration curves of the standards in 10% HNO_3 and 2:1 HNO_3:H_2O_2. Figures 3.6 – 3.10 show the results of the calibration curves in the different matrices. Table 3.5 shows the linear equations for the calibration curves of each element.

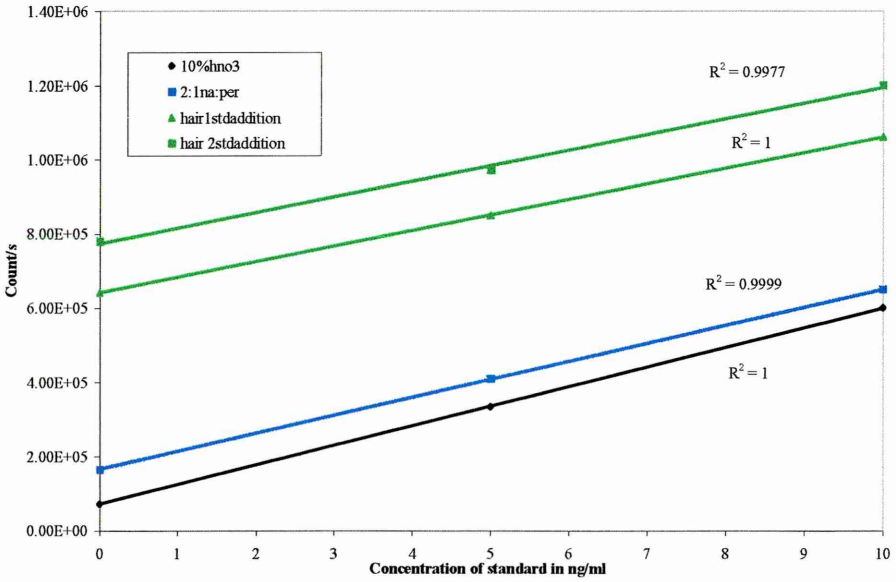

Figure 3.6. Comparison of the slopes of the calibration curves prepared from a chromium standard solution added to the following matrices: 10% HNO_3, 2:1 HNO_3:H_2O_2 and two hair samples digested in 2:1 HNO_3:H_2O_2 with standard addition.

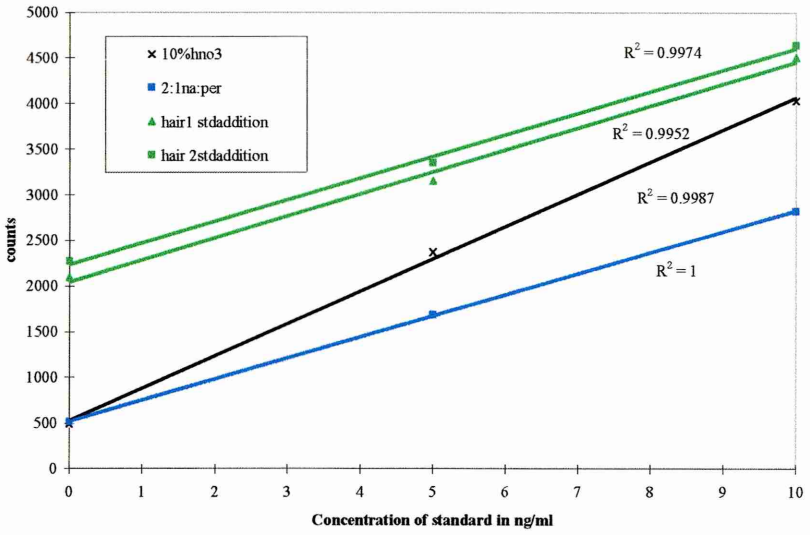

Figure 3.7. Comparison of the slopes of the calibration curves prepared from a selenium standard solution added to the following matrices: 10% HNO_3, 2:1 HNO_3:H_2O_2 and two hair samples digested in 2:1 HNO_3:H_2O_2 with standard addition.

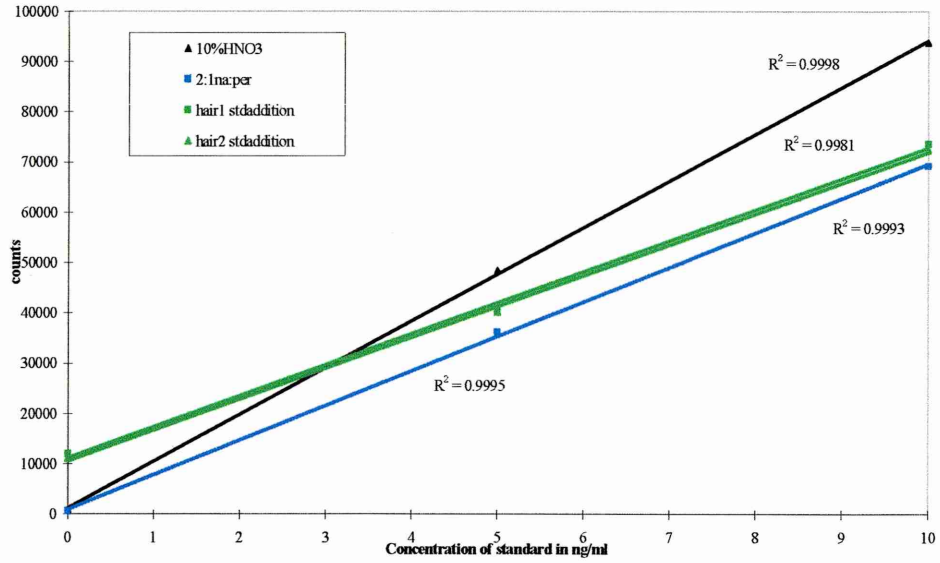

Figure 3.8. Comparison of the slopes of the calibration curves prepared from a cadmium standard solution added to the following matrices: 10% HNO_3, 2:1 HNO_3:H_2O_2 and two hair samples digested in 2:1 HNO_3:H_2O_2 with standard addition.

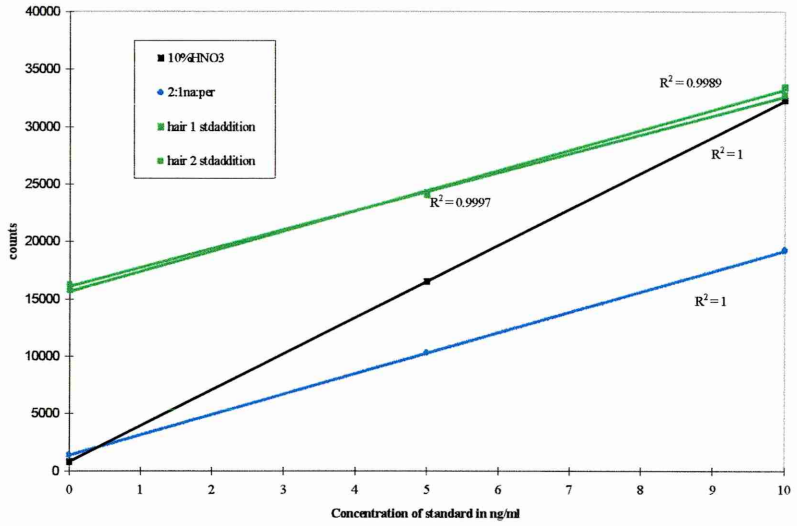

Figure 3.9. Comparison of the slopes of the calibration curves prepared from a mercury standard solution added to the following matrices: 10% HNO_3, 2:1 HNO_3:H_2O_2 and two hair samples digested in 2:1 HNO_3:H_2O_2 with standard addition.

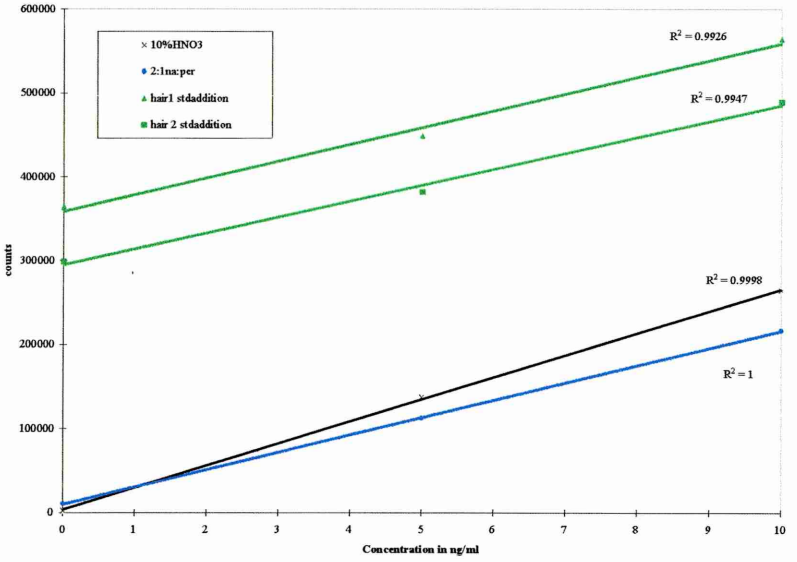

Figure 3.10. Comparison of the slopes of the calibration curves prepared from a lead standard solution added to the following matrices: 10% HNO_3, 2:1 HNO_3:H_2O_2 and two hair samples digested in 2:1 HNO_3:H_2O_2 with standard addition.

	Hair 1	Hair 2	10% HNO_3	2:1 HNO_3:H_2O_2
Cr	y=42100x+773167	y=41900x+640833	y=52870x+71750	y=48600x+165000
Se	y=237x + 2235	y=241x + 2041.7	y=353.9x + 527.5	y=230.6x + 518.33
Cd	y=6170x + 11117	y=6130x + 10317	y=9298.7x+1177.5	y=6859.7x+935.83
Hg	y=1760x + 15633	y=1650x + 16117	y=3149x + 791.67	y=1781x + 1391.7
Pb	y=20000x + 35900	y=19000x + 29500	y=26290x + 3250	y=20626x + 9450

Table 3.5. The line equations for the calibration curves in figures 3.6 – 3.10.

The gradients of the calibration curves, obtained from the line equations in figures 3.6 - 3.10, in table 3.5 clearly show that the hair matrix curves do not compare well with gradients of the 10% HNO_3 standards, especially for cadmium, lead and mercury. However, the gradients of the 2:1 HNO_3:H_2O_2 curves are similar to those in the hair digestion curves. This suggests that the hair matrix does not make a difference to the calibration when the matrix of the standards and the hair digests are matched. This was further confirmed when certified reference material was digested and analysed against the different matrices, i.e. against the standards made in 10% HNO_3, the 2:1 HNO_3:H_2O_2 and by standard addition. The results are outlined in table 3.6. A second CRM (GBW 07601) that gave an indicated value for chromium was used in the digests, where approximately 0.1 g was digested.

Results obtained against the 10% HNO_3 calibration curve give low recoveries for cadmium, lead and mercury. This is improved against the 2:1 HNO_3:H_2O_2 standards. However, the lead levels are still lower than the certified value and although this does improve with standard addition (where the calibration is achieved by adding differing concentrations of standards to the actual digest).

These results show that the standards for further calibrations should be made in 2:1 HNO_3:H_2O_2, the same as the digested samples. This was carried out for the remainder of the study.

Element	Certified Values (ng g^{-1})	Results obtained (ng g^{-1}) for the CRM after the instrument was calibrated with standards prepared in 10% HNO_3 (n=5)	Results obtained (ng g^{-1}) for the CRM after the instrument was calibrated with standards prepared in 2:1 HNO_3:H_2O_2 (n=5)	Results obtained (ng g^{-1}) for the CRM after standard additions (n=5)
Cr	370 ± 50 *	1383 ± 249	1329 ± 204	1990 ± 241
Se	600 ± 30	545 ± 17	642 ± 12	981 ± 126
Cd	110 ± 20	64 ± 3	103 ± 9	136 ± 16
Hg	360 ± 50	192 ± 5	338 ± 10	512 ± 49
Pb	8800 ± 900	5407 ± 227	6629 ± 279	9172 ± 1419

* only a suggested value was stated for chromium.

Table 3.6. Recoveries of chromium, selenium, cadmium, mercury and lead in CRM 07601 against different calibration matrices, as determined by direct nebulisation ICP-MS..

3.3.3. Effect of Hair Sample Mass on the Digestion Procedure

Three different masses of hair from the same donor were digested using the 2:1 HNO_3:H_2O_2 mixture. The experiments were repeated five times using the same hair sample. Table 3.7 summarises the results obtained from the digestions of 0.05 g, 0.1 g and 0.2 g of hair.

To determine whether there was any significant correlation between the mass of hair digested and the elemental concentration, a two tailed paired t test was applied to the results for each mass with respect to the 0.1 g of hair data. The statistical significance was set at a probability of $P \leq 0.05$, i.e. 95% confidence intervals (see section 3.2.5)[28].

Element	Concentration in 0.05 g of hair ($ng\ g^{-1}$) (n=5)	Concentration in 0.1 g of hair ($ng\ g^{-1}$) (n=5)	Concentration in 0.2 g of hair ($ng\ g^{-1}$) (n=5)
Cr	1497 ± 130	1402 ± 136	1281 ± 80
Se	645 ± 33	781 ± 68	861 ± 44
Cd	124 ± 14	170 ± 12	167 ± 8
Hg	1017 ± 66	1094 ± 111	1052 ± 29
Pb	2072 ± 175	2335 ± 145	2214 ± 151

Table 3.7. Concentrations of cadmium, chromium, lead, mercury and selenium in digestions of different masses of the same hair sample.

Chromium, mercury and lead levels in hair were found not to vary significantly when different masses were used. There was no significant difference between either the 0.05 g and 0.1 g masses or the 0.1 g and 0.2 g masses, i.e. for P=0.05 the experimental t value was less than the critical value of t for 4 degrees of freedom (2.78). Analysis of the selenium and cadmium levels in hair showed that the concentrations obtained for the masses 0.1 g and 0.2 g ($P<0.05$) did not vary significantly, whereas the levels for 0.05 g and 0.1 g did vary significantly ($P>0.05$). The t value for P=0.05 for n=5 in a two tailed test is 2.776 and, in the comparison of the concentrations for the 0.05 g and the 0.1 g masses of hair, selenium and cadmium t values were found to be t=5.104 and t=5.20 respectively. It is apparent from these results, therefore, that in order to digest a hair sample and carry out multielemental analysis, the hair must weigh at least 0.1 g, because at a mass lower than this significant variations in the concentrations obtained can be seen.

Digesting the certified reference material at the three different weights was also investigated. The digestions were carried out with 2:1 HNO_3:H_2O_2, using the LGC 397 CRM. The results obtained are shown in table 3.8.

Element	Certified Value (ng g^{-1})	Concentration obtained for digestion of 0.05 g of CRM (ng g^{-1}) (n=3)	Concentration obtained for digestion of 0.1 g of CRM (ng g^{-1}) (n=3)	Concentration obtained for digestion of 0.2 g of CRM (ng g^{-1}) (n=3)
Se	2000 ± 80	1467 ± 57	2161 ± 57	2307 ± 111
Cd	521 ± 24	521 ± 50	652 ± 100	714 ± 81
Hg	12300 ± 500	5264 ± 279	10557 ± 540	11688 ± 645
Pb	33000 ± 1200	22690 ± 1037	31961 ± 2064	30822 ± 1789

Table 3.8. The concentrations of cadmium, lead, mercury and selenium in the CRM digested in 2:1 HNO_3:H_2O_2 at different masses in triplicate.

The information book that accompanied the certified reference material stated that at least 100-200 mg of the CRM should be used for digestion. The reason for this is obvious from the results in table 3.8. The recoveries are much better for all of the elements at the higher masses of CRM. A paired t test confirmed that for lead, mercury and selenium there was a significant difference in the recoveries between the 0.05 g and 0.1 g of CRM. However, all of the elements showed that there was no significant difference in the values acquired between the 0.1 g and 0.2 g masses of CRM. Cadmium showed that the lower mass of 0.05 g was sufficient to achieve the CRM value and that the different masses did not show significantly different values.

3.4. Trends in Actual Hair Samples with the Optimised Digestion Procedure

Ideally the hair samples would have been digested by the two optimised methods: 2:1 HCl:H_2O_2 for the determination of arsenic and antimony hair concentrations by the HG-ICP-MS method; and 2:1 HNO_3:H_2O_2 for the determination of cadmium, chromium, lead, mercury and selenium hair concentrations by ICP-MS. However, there was not enough hair sample to digest all of the samples by the two methods in triplicate. Therefore the method used for the digestion of the hair samples was the nitric acid and hydrogen

peroxide method because it was possible to analyse all of the elements adequately by this method.

3.4.1. The Variation of Trace Element Concentrations in Hair Over Time

Hair from one donor was collected over a period of twenty-one months on a regular basis. The hair was shaved off the scalp and stored in plastic sample bags. The treatment of the hair had not changed over the time period. The digestions for this experiment were all carried out at the same time, after the last sample had been collected. Each sample was digested in triplicate using the 2:1 HNO_3:H_2O_2 mixture. Figures 3.11 & 3.12 show the results in graphical forms.

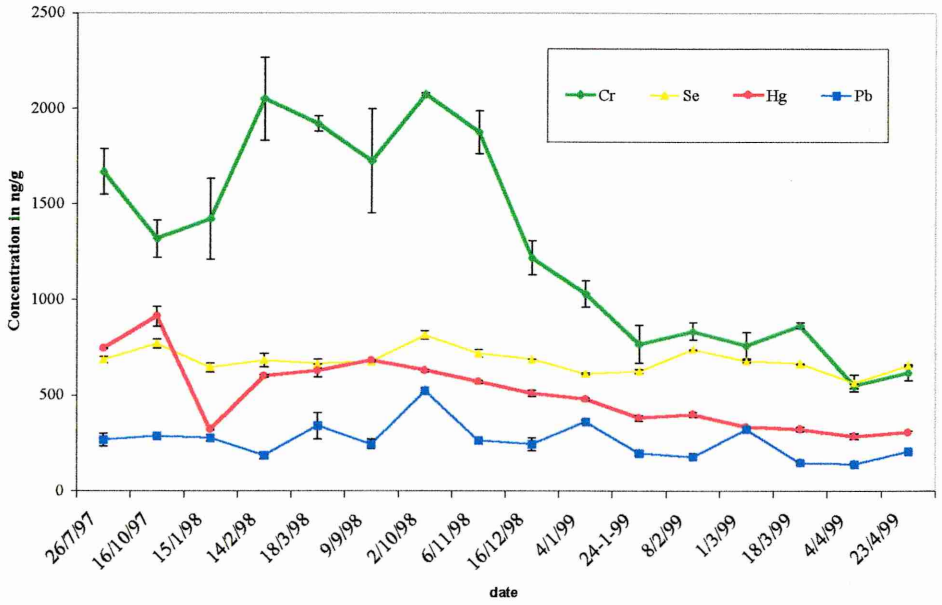

Figure 3.11. Variation of chromium, lead, mercury and selenium in hair samples taken from the same donor at intervals over a period of 21 months.

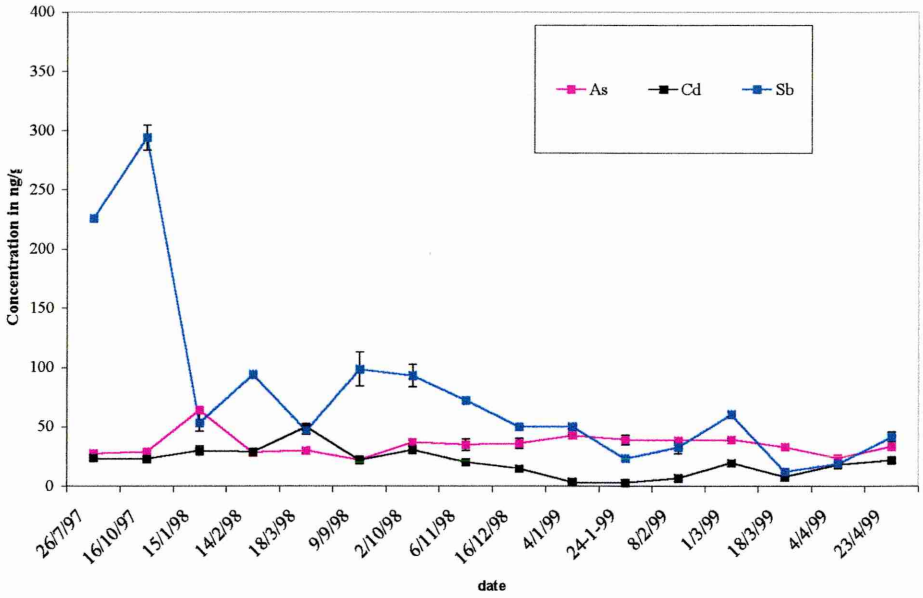

Figure 3.12. Variation of antimony, arsenic and cadmium in hair samples taken from the same donor at intervals over a period of 21 months.

The hair chromium levels show a considerable variation over the time period and in the latter half of the time period a slow decrease in values is observed. It seems unusual that the chromium levels varied so much, especially as chromium is an essential element. The selenium hair levels are relatively stable within the time period, which suggests that the intake of selenium through the diet has not varied. Mercury and lead levels shown in figure 3.11 and arsenic and cadmium levels shown in figure 3.12 did not vary considerably, however the hair antimony levels appeared much higher at the start of the period than at the end of the period.

Results from a similar study, this time involving four donors and hair samples which were collected on two occasions, are shown in figures 3.13 - 3.16. Each of the donors stated that they had not knowingly been exposed to any of the elements being determined. They were also unaware of any changes to their diets, health and environments during the time period of the sample collections.

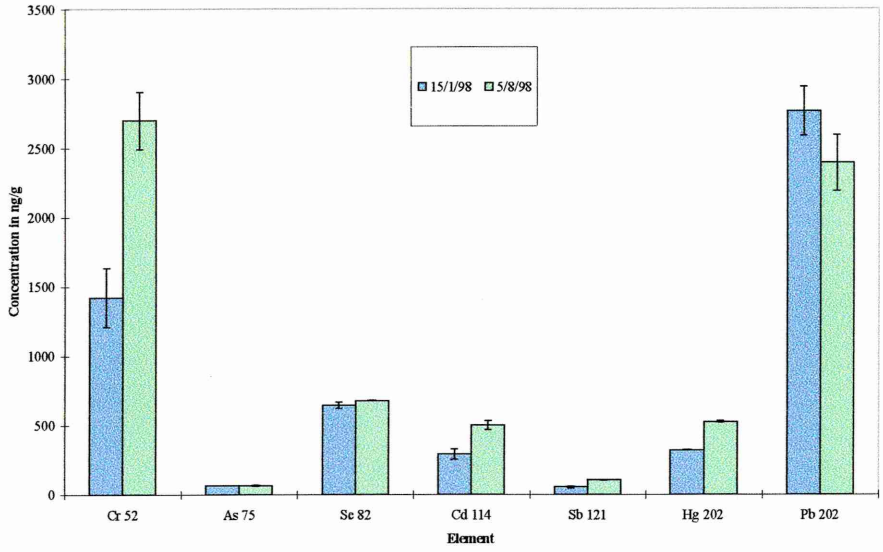

Figure 3.13. Levels of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in two hair samples collected from the same donor seven months apart.

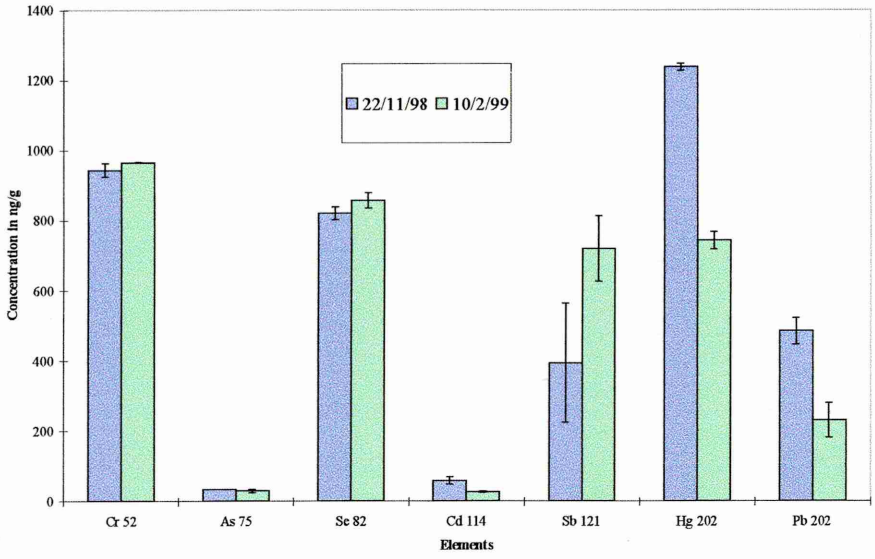

Figure 3.14. Levels of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in two samples from the same donor collected three months apart.

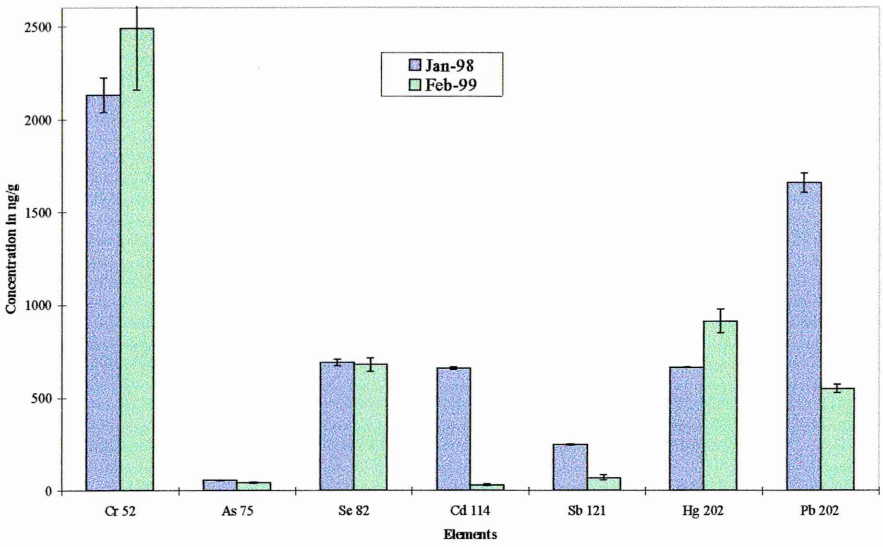

Figure 3.15. Levels of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in two hair samples from the same donor collected thirteen months apart.

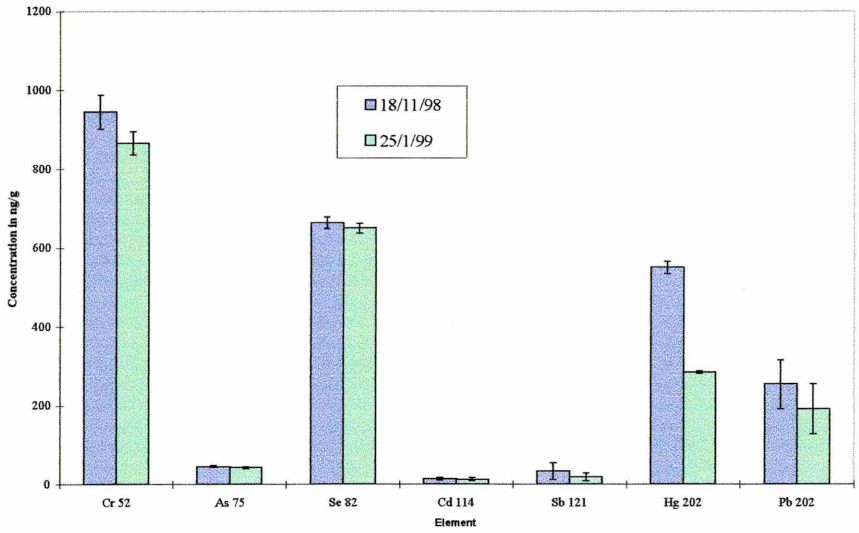

Figure 3.16. Levels of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in two hair samples from the same donor collected two months apart.

From these results it can be concluded that the hair sample results must be interpreted with care, because the concentrations of the elements will change with time. From figures 3.13-3.16 it is apparent that selenium is the one element in hair that does not vary very much (<4%) which confirmed the results shown in figure 3.11.

3.4.2. Analysing Hair from Different Areas of the Head.

Hair samples from the top of the head and the back of the neck were collected from one donor and analysed. The results can be seen in table 3.9.

A two-tailed paired t-test was applied to the data to confirm whether or not the results from the different areas of the head varied significantly. The results showed that while the arsenic, chromium and selenium levels in hair did not vary significantly when P=0.05, the hair levels from the top of the head and the back of the neck for cadmium, lead and mercury did vary significantly (P>0.05). Interestingly the heavy metals, cadmium, lead and mercury were found at significantly higher values at the top of the head than at the back of the neck. This may be due to the fact that the hair was of a slightly longer length at the top of the head than at the back and therefore had been exposed to the elements for a longer period of time, hence the higher concentrations in the longer hair.

Previous studies on trace element variations in the hair suggested that at the top/front of the head, hair would be more susceptible to contamination from the environment, e.g. from running hands through the hair or flicking a hair out of the eyes, than the hair at the back of the head[29]. They also suggested that the hair at the neck is more likely to reflect endogenous levels of trace elements because of sweat excretions and the fact that hair in this area is sometimes protected by clothing[29,30]. This could possibly explain why the levels of cadmium, mercury and lead are higher at the top of the hair. What is clear is that once again when sampling hair the details must be specified to enable accurate interpretation of the results.

Element	Back of Neck Concentration (ng g^{-1}) (n=3)	Top of Head Concentration (ng g^{-1}) (n=3)
Cr	1007 ± 35	846 ± 58
As	50 ± 3	41 ± 0.4
Se	654 ± 16	636 ± 37
Cd	12 ± 1	206 ± 54
Sb	21 ± 6	25 ± 3
Hg	276 ± 11	539 ± 38
Pb	203 ± 34	475 ± 26

Table 3.9. Variations in trace element concentrations in hair collected from the top of the head and the back of the neck of the same donor.

3.4.3. The Variation of Trace Elements in Different Types of Hair.

Two donors collected their beard hair and their head hair. Four samples from each were digested and analysed, with the results being shown in tables 3.10 and 3.11.

Donor 1 had a very short beard and although the shavings used were not from the root, it could be assumed that the length of exposure to the environment was less than that of the hair that was cut from the head. The levels of cadmium, lead and mercury are reduced in the beard hair, suggesting that the increased levels in the head hair came from the environment. Two-tailed paired t tests were carried out between the beard and head hair samples which showed that only the antimony and chromium values showed no significant differences when P=0.05. The beard hair shows lower levels of mercury, lead, selenium and cadmium and higher levels of chromium and arsenic than the head hair.

Elements	Head Hair concentration (ng g^{-1})	Beard Hair concentration (ng g^{-1})
Cr	437 ± 12	543 ± 108
As	34 ± 4	51 ± 5
Se	878 ± 38	675 ± 36
Cd	39 ± 5	16 ± 2
Sb	122 ± 37	120 ± 20
Hg	316 ± 13	220 ± 12
Pb	954 ± 145	553 ± 19

Table 3.10. Concentrations of trace elements in the beard and head hair from donor 1.

With donor 2, the beard that was cut off was as a result of many months growth, hence there were samples from the outside of the beard (the furthest from the skin) and from the inside of the beard (the nearest to the skin). The results of the analyses can be seen in table 3.11. Overall, the levels of the elements in the beard were lower than in the head hair. Interestingly, the beard hair from the inside of the beard i.e. nearer to the skin, has less chromium, cadmium, antimony, mercury and lead, which suggests that as the hair grew longer these elements increased due to external contamination. This is especially noticeable with cadmium, where there was no detectable cadmium present in the beard hair closest to the skin. Similar results were seen in a study comparing scalp and facial hair in 1980, when cadmium, copper, lead and zinc were determined[31]. The scalp hair values were significantly higher than the facial hair values: for cadmium the facial hair was found to be 1900 ng g^{-1}, whereas the scalp hair was 4900 ng g^{-1}, with lead the facial hair level was 4600 ng g^{-1} and the scalp hair level was 8100 ng g^{-1}. The zinc levels in the scalp hair were about five times higher than in the facial hair and the copper levels in the scalp hair were more than ten times higher than in the facial hair.

Elements	Head Hair concentration (ng g^{-1})	Beard Hair concentration (ng g^{-1}) (from the outside)	Beard Hair concentration (ng g^{-1}) (from the inside)
Cr	1813 ± 673	609 ± 6	444 ± 54
As	10 ± 3	3 ± 1	9 ± 1
Se	634 ± 17	793 ± 59	789± 38
Cd	169 ± 29	23 ± 4	<2
Sb	128 ± 49	35 ± 0.4	9 ± 1
Hg	161 ± 26	1181 ± 2	879 ± 5
Pb	3911 ± 436	897 ± 94	340 ± 1

Table 3.11. Concentrations of trace elements in the beard and head hair from donor 2.

3.5. Scanning Electron Microscopy (SEM) of Hair Samples

Scanning electron microscopy was carried out on hair samples of interest in the study, details are described in section 3.2.4.2 This was the case for the beard and head hair samples of donor 2. Figures 3.17 & 3.18 show the SEM images of the beard and head hair of donor 2 (from table 3.11).

The images were taken at the same magnification (x250) and clearly show the difference between the beard and head hair of donor 2. The beard hair is much thicker than the head hair.

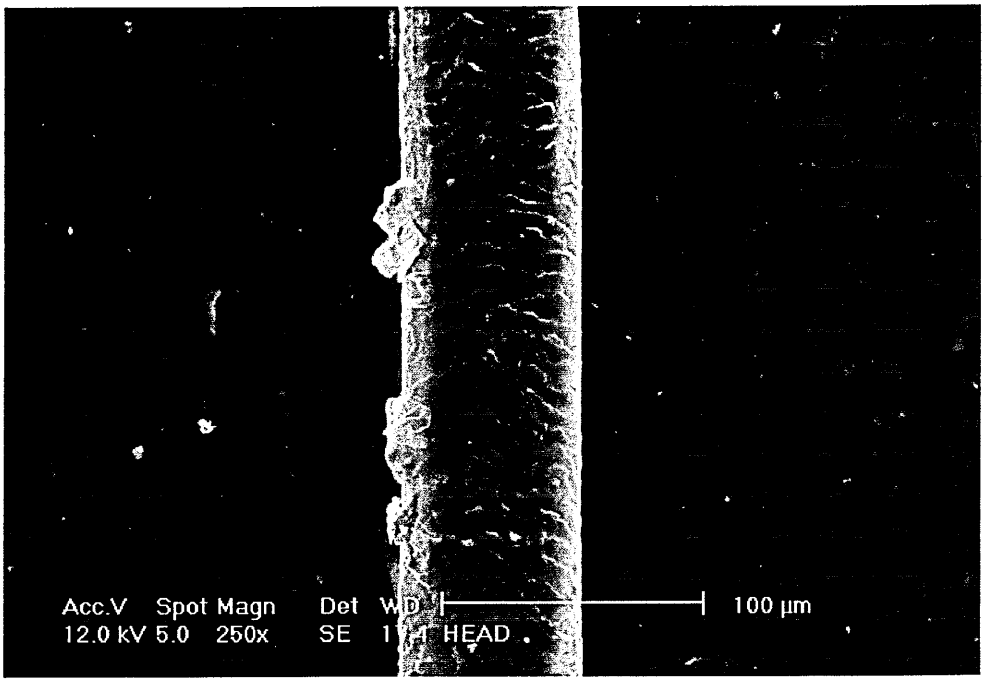

Figure 3.17. Electronmicrograph of donor 2's head hair

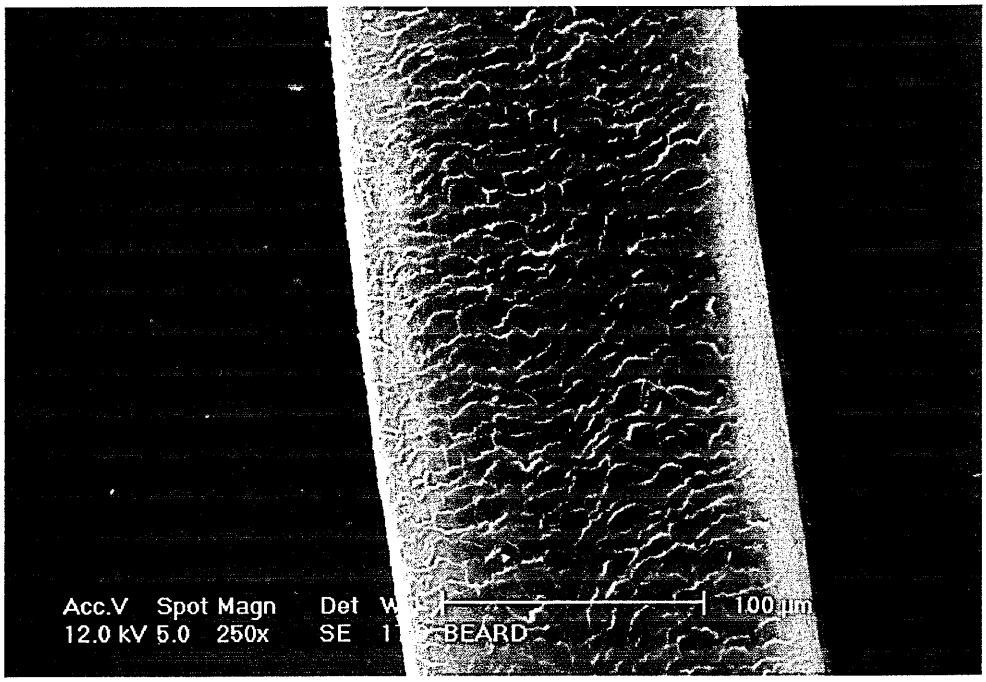

Figure 3.18. Electronmicrograph of donor 2's beard hair

3.6. Establishing Normal Levels for the Exposure Study

Before the exposure studies could be started it was necessary to establish normal levels. Analysis of hair samples from a normal group were carried out extensively and the results are reported in chapter 4, however, an initial study was carried out with ten donors and the results are summarised in table 3.12. The samples were digested and analysed in triplicate.

Element	Concentration Range from the hair of 10 donors (ng g^{-1})	Mean concentration of group (ng g^{-1}) n=10	Sky-Peck[30] (ng g^{-1}) n=24	Lenihan[32] (ng g^{-1})	Iyengar[33] (ng g^{-1}) n=35
Cr	778.8 - 2536.3	1329	1560	1000	60 - 4100
As	1.9 – 61.5	35	550	200	50 - 85
Se	48.0 – 890.1	722	610	1000	200 - 1400
Cd	10.2 – 194.6	89		1000	350 - 2430
Sb	11.9 – 126.6	68		200	
Hg	32.3 – 928.6	508	1170	2000	500 - 12200
Pb	130.6 – 3449.8	912	6410	20000	420 - 5200

Table 3.12. Trace element concentrations from a group of unexposed donors, along with data from comparable studies.

From the results it is clear that a wide range of concentrations are present in the small 'normal group'. The average values do give an idea of the levels expected, however a much larger group is needed, so that the significance of any anomalies may be determined. Normal group levels from Sky-Peck's study[30], shown in table 3.12, are higher than those found in this study, for all elements except selenium. The Lenihan study also found much higher normal values than those found here. Iyengar's study gave ranges found in unexposed hair samples and except for cadmium and arsenic the mean of this study's results fall within the reported ranges of their study. In 1982, a study in which the concentrations of trace elements in the hair of English residents were investigated, found 400 ng g^{-1} for antimony, 500 ng g^{-1} for arsenic and 3500 ng g^{-1} for mercury, all of which are higher than the levels seen in this study[2].

3.7. Conclusion

Two different digestion procedures were required for the total analysis of the elements by ICP-MS and for the HG-ICP-MS method for arsenic and antimony determination. Results from the digestion chosen have shown that the 2:1 nitric acid and hydrogen peroxide digest is reproducible with hair masses as low as 0.05 g, although 0.1 g of hair provides more accurate CRM values. It was necessary to make the calibration standards in the digested solution of 2:1 nitric acid and hydrogen peroxide. It is also apparent that the levels of trace elements in hair do not remain constant with time and that each hair sample is unique to the donor, exhibiting an individual trace element profile. Care must be taken to look at each individual result and the background information acquired from the questionnaires when analysing the data. It is also clear that external contamination of the hair must be investigated to in order to assess its impact on trace element levels in hair.

3.8. References

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4.1. Washing Procedures

The aim of hair washing is to remove externally bound contaminants and thus allow the determination of internally bound analytes. There are no agreed washing procedures for hair and this is reflected in the different recommendations in the literature[1,2,3,4]. The reported methods have been discussed in section 1.7.2.

Several washing procedures were investigated in this study and the most promising methods were used on forty samples which were used to establish the unexposed levels. The methods investigated were all taken from previous methods examined in the literature[5,6,7,8,9,10]. The main agreement in the literature is firstly to remove grease and particulates from the hair with an organic solvent and so this was investigated initially. Physical methods for the washing procedure were also investigated, using mechanical shakers, ultrasonic baths, centrifuges and finally incubator shaking platforms. Once methods were established several studies were carried out looking at the recoveries when hair samples were spiked and then washed following the appropriate methods.

4.2. Experimental

4.2.1. Reagents Used

The acids and standards used were the same as stated in section 3.2.2. Additional chemicals used in the washing procedures were Analar grade EDTA (diaminoethanetetra-acetic acid disodium salt), HPLC grade acetone and isopropanol from BDH (Poole, Dorset, UK). Sodium lauryl sulphate (SLS- dodecyl sulphate, sodium salt) 98% and Triton X-100 (octyl phenoxy polyethoxyethanol) from Sigma-Aldrich Co. Ltd (Gillingham, Dorset, UK) were also used. 1% (w/v) solutions of EDTA, SLS and Triton X-100 were made by adding 1g of substance to 100 ml of deionised water.

Deionised water was used throughout. Glassware, nalgene bottles and nylon forceps were all cleaned in 5% v/v nitric acid.

4.2.2. Sample Collection and Pretreatment

The samples were collected from a local barber's and placed in a sample bag. Each donor completed a questionnaire (see appendix 1). Additional samples were collected from people who worked at Sheffield Hallam University.

Ten different washing procedures were investigated using different reagents and the data obtained compared to that from unwashed hair samples. This was to determine the most appropriate methods for removing externally bound species from the hair. The methods consisted of washing with water[3,4], the IAEA method[11,12,13,14,15,16,17,18], 1% SLS[10,19,20,21], 1% Triton X-100[5,22,23], with and without an organic wash[8,24], 1% EDTA[3,4,25], isopropanol[10], 0.1% HNO_3, 0.1M HCl[5,28] and a commercial shampoo[5,9]. The shampoo was from Alberto Balsam (Albert Culver Co. (UK) Ltd, Basingstoke, England) and contained no selenium compounds.

Each hair sample was weighed directly into a conical sample tube and then 3 ml of one of the reagents listed was added and the lid put on the tube. The samples were shaken in the tubes for the appropriate time and the solvent removed by decanting. The hair sample was then transferred into a large weighing boat and placed in a drying box. The drying box was sealed tightly and contained desiccant to promote the drying of the samples. The samples were left overnight to dry and digested the next day.

All of the hair samples were digested by weighing the sample directly into the digestion vial, then adding 2 ml of HNO_3 and 1 ml of H_2O_2 and then placing them into the microwave carousel. The microwave digestion method is described in section 3.2.3.2. After the digestion, the samples were diluted to 10 ml with deionised water and analysed against 2:1 HNO_3:H_2O_2 standards. Internal standards containing Rh, Y and Bi were used with the ICP-MS samples.

Each of the 'washings' i.e. the solution that had been in contact with the hair and then decanted, were collected. These were analysed directly by ICP-MS. The washings were filtered through a 45 μm filter into an autosampler tube and analysed against standards made the same day in millipore water.

4.2.2.1. Initial Hair Washing Methods

The hair used in the preliminary studies came from the donors who had donated their hair over the duration of the work (see chapter 3). 3 ml of reagent was added to approximately

0.1 g of hair, in a sterile conical tube with a lid. Each washing was spun in a mechanical spinner for about 1 minute, then placed in an ultrasonic bath for 30 minutes and centrifuged for 10 minutes at 4000 rev/min. The solution was then decanted off and then 3ml water was added to the samples and the same procedure repeated. The three water fractions were 'washed' with the hair sample and decanted before the hair was transferred to the drying chamber. Blank solutions of the reagents were subjected to the same procedure as the hair.

Preliminary studies also involved shaking hair samples with 3 ml of reagent for 24h or 3h on a mechanical shaker, followed by washing three times with 3 ml of water for 3h.

4.2.2.2. Optimised Hair Washing Methods

The washing methods used on the normal group of 40 samples consisted of three different methods. These were 1% SLS, 1% EDTA and 0.1M HCl where 3ml of one was added to the hair sample and placed on an incubator platform shaker for 3h, followed by 2 x 4.5 ml of water for 3h. The washings were pipetted off the hair samples and blanks of the reagents underwent the same treatment as the hair samples.

4.2.3. Instrumentation

The mechanical shaker used was a Stuart flask shaker (Essex Scientific Laboratory Supplies Ltd., Benfleet, Essex, UK). The ultrasonic bath was a Branson 1210 Ultrasonic cleaner (Danbury, Connecticut, USA) and the centrifuge used was a bench top Sorvall TC6, (DuPont, Stevenage, Hertfordshire, UK) set at 4000 rev/min.

The incubator platform shaker was a Gallenkamp orbital incubator shaker (Fisher Scientific, Loughborough, UK) which was set at 200 rev/min and 20^{O}C.

The conditions for the ICP-MS were as described in section 2.4.4.

4.2.4. Simulated Sweat Experiments

Calcium sulphate (0.04g), sodium chloride (0.38g), magnesium sulphate (0.0005g), potassium sulphate (0.223g), sulphur (0.003g) and urea (NH_2CONH_2- 0.09g) were dissolved in 100 ml of millipore water. All of the chemicals were of Analar grade and obtained from BDH (Poole, Dorset, UK). The concentrations were based on ranges for electrolytes present in eccrine sweat[26]. Hair samples were spiked with an enriched

cadmium isotope obtained from the Laboratory of the Government Chemist (Teddington, Middlesex, UK) where to enrich the 106 isotope 1.0208g of the cadmium isotope had been added to 6.063g of solution.

4.3. Results and Discussion

4.3.1. Initial Washing Procedures

4.3.1.1. Investigation of Six Different Washing Procedures

Initial studies involved the washing methods outlined in table 4.1. The samples were washed using a mechanical spinner, ultrasound and centrifuge (see section 4.2.2.1. for further details). The results are based on the same hair sample washed with the six different methods three times. The mean for cadmium, chromium, lead, mercury and selenium levels are shown in figures 4.1 – 4.5.

Method 1	3 ml water followed by another 3 ml water
Method 2	3 ml acetone, 3 x3ml water, 3 ml acetone*
Method 3	3 ml 1% sodium lauryl sulphate (SLS), 3 x 3 ml water
Method 4	3 ml 1% Triton X-100, 3 x 3 ml water
Method 5	3 ml 1% Triton X-100, 3 x 3ml water, then 3 ml acetone
Method 6	3 ml 1% EDTA, 3 x 3 ml water

* the IAEA method

Table 4.1. The washing procedures investigated

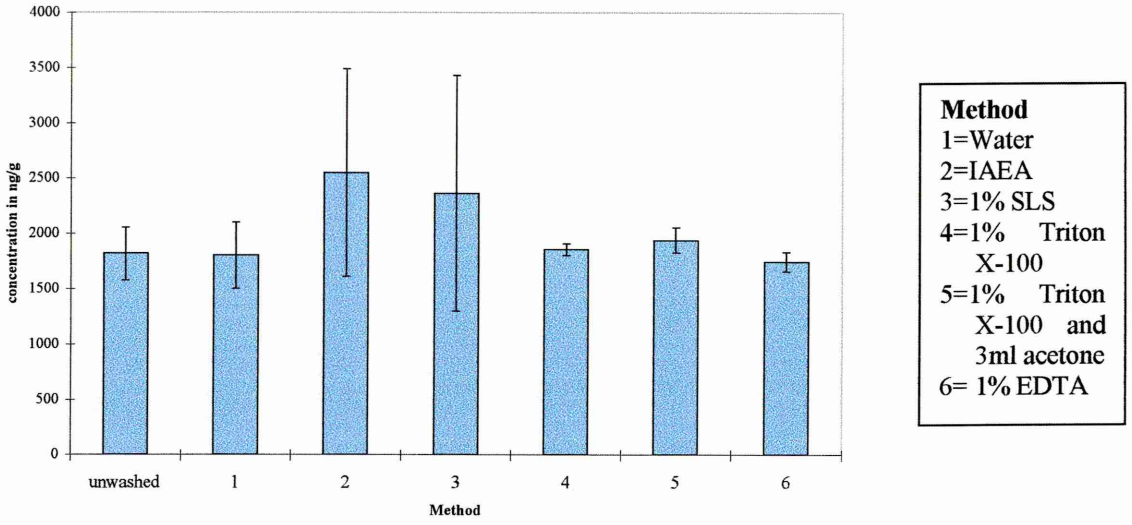

Figure 4.1. A comparison of chromium values in unwashed hair and hair washed by methods 1-6.

The initial results shown in figure 4.1 suggest that overall, chromium does not wash out of the hair. Paired t tests (see section 3.2.5) applied between each of the washed hair samples and the unwashed hair sample showed that none of the washed hair values varied significantly from the unwashed hair values. However, the samples that had been in contact with acetone and SLS show an average chromium value higher than the unwashed sample, after the samples had been blank corrected. This would suggest that chromium had adsorbed to the hair from the reagents, where the blank value for chromium in SLS was around 40 ng ml^{-1} and the acetone showed concentrations of up to 6000 ng ml^{-1}. However, this is not observed with method 5 where the last washing consisted of acetone. With the SLS solution, which produced foam when shaken, it is possible that there was still some of the solution remaining on the hair. Studies have been carried out investigating the adsorption of SLS to hair and have shown that SLS binds substantially to hair[3,27]. The relative standard deviations (RSD) of methods 2 and 3 are higher than with the other methods, 36.9% RSD for the IAEA method (method 2), and 45.1% RSD for the 1% SLS method (method 3). The average %RSD for the other methods was 8.7%.

The use of acetone was proving to be problematical not only due to the chromium levels that were present in the Aristar grade acetone (3-6 μg ml^{-1}), but also because of problems during the microwave digestion procedure. In the methods where acetone was the final

washing stage, the nitric acid from the digestion reacted violently with the hair sample, where there was still acetone present. There were a lot of fumes when the nitric acid was added to the hair sample and even after the fumes were allowed to dissipate before the vial was placed in the digester there were still traces of solvent fumes in the laboratory.

Figure 4.2 shows the results for selenium determination when the six methods were used to wash the hair. Initial results show that selenium in hair does not wash off. In fact, most of the washed results show slightly higher values than the unwashed, which has been noted previously[10]. Paired t tests applied between the unwashed and washed hair samples showed that there was no significant differences between the washed and unwashed hair selenium values. The cadmium results shown in figure 4.3, also show no significant difference between washed and unwashed hair samples when paired t tests are applied. However, the cadmium levels show high variations (mean 27.6% RSD), even for the unwashed hair sample, with the IAEA wash showing the highest RSD at 51.1%.

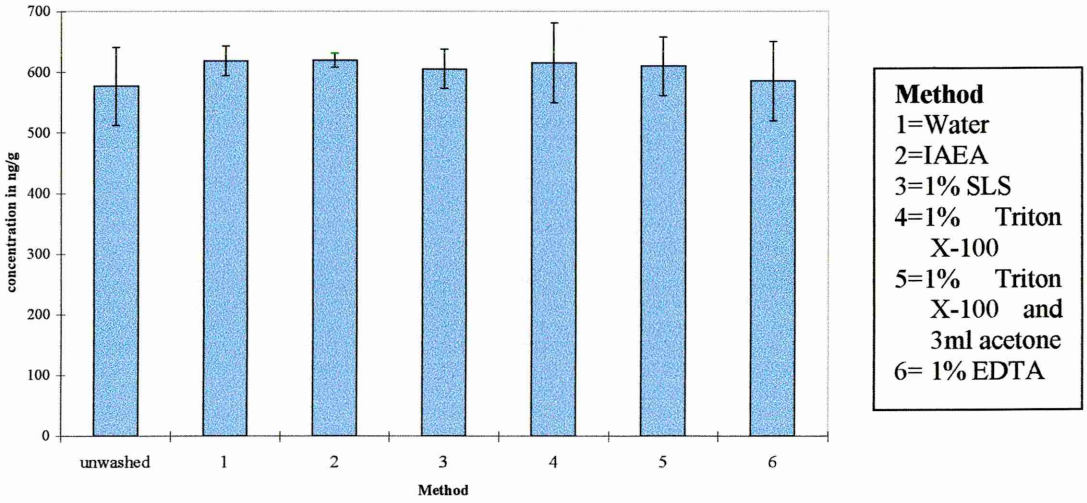

Figure 4.2. A comparison of selenium values in unwashed hair and hair washed by methods 1-6.

The data in figure 4.4 indicate that mercury in hair is not washed out as no significant changes in mercury concentration were observed when a paired t test was used to compare values obtained with washed and unwashed hair. The lead hair values, shown in figure 4.5, indicate that the lead is washing out to some extent, especially with the EDTA

solution. However, comparison of the various lead values using paired t tests, showed there was no significant change in the values obtained by the different methods.

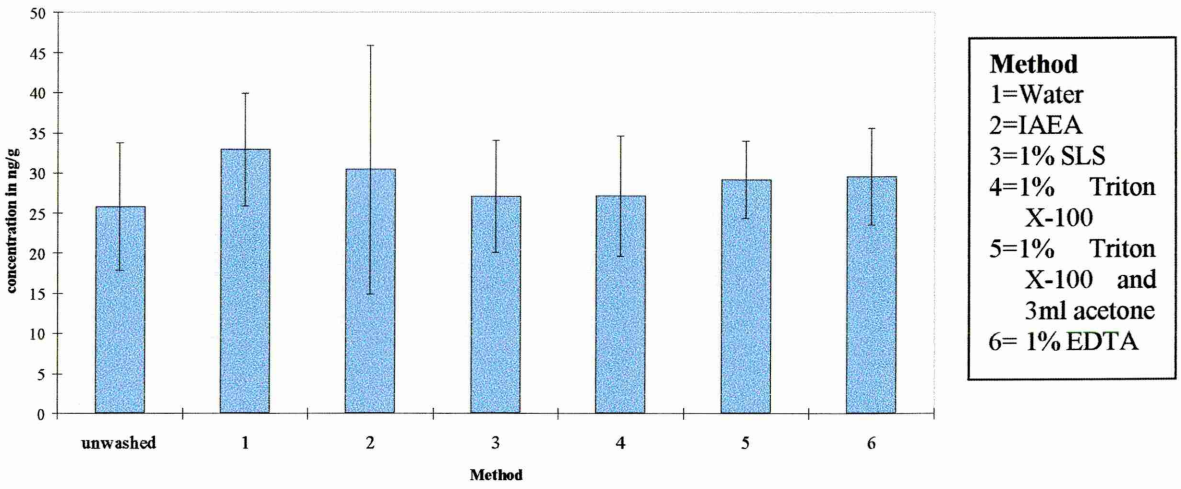

Figure 4.3. A comparison of cadmium values in unwashed hair and hair washed by methods 1-6.

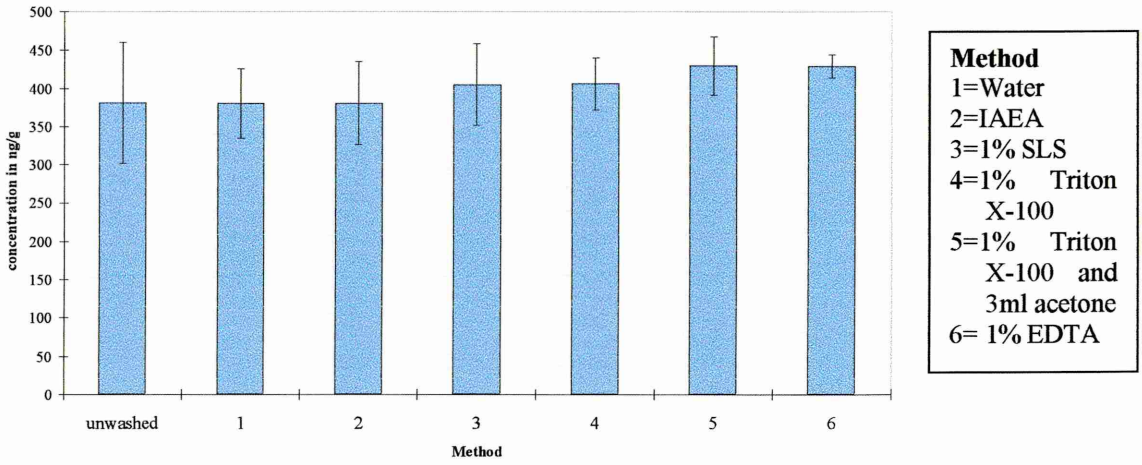

Figure 4.4. A comparison of mercury values in unwashed hair and hair washed by methods 1-6.

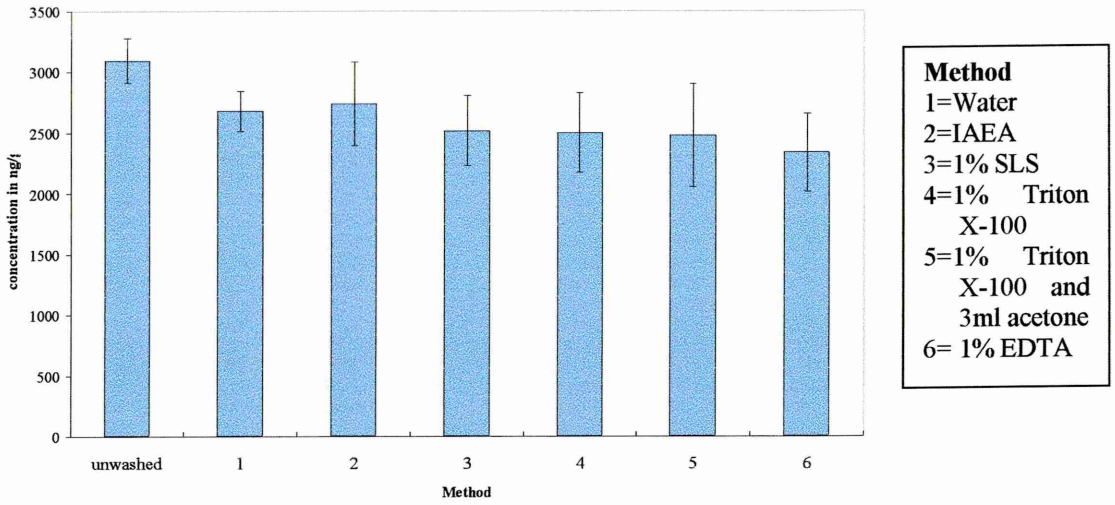

Figure 4.5. A comparison of lead values in unwashed hair and hair washed by methods 1-6.

4.3.1.2. Six Washing Methods over 24h

The washing methods were repeated using a mechanical shaker in order to improve the hair-solvent contact. The hair samples were washed with the different reagents listed in table 4.1, for 24h by shaking with the mechanical shaker. The reagents used for the 24h wash were water, isopropanol (as a alternative to acetone)[10], 1% SLS, 1% Triton X-100, 0.1% HNO_3 and 1% EDTA. Then the hair samples were washed three times with 3 ml of water for 3h on the mechanical shaker to thoroughly remove the reagents. Table 4.2 presents the results of the washing procedures.

The concentrations of the elements of interest in each of the reagents were determined. Isopropanol had 3816 ng ml^{-1} of chromium present whilst in all of the other reagents the concentrations were below 150 ng ml^{-1}. Selenium was only present in 1% SLS at 0.27 ng ml^{-1}, cadmium was present in all the reagents except nitric acid at levels lower than 10 ng ml^{-1}, mercury was not present in any of the reagents and lead was present in all of the reagents at levels less than 100 ng ml^{-1}.

In general the results show that little has been removed from the hair, with paired t tests applied to the data confirming that there were no significant decreases between the unwashed and washed hair values. However, significant increases were observed in some of the washed hair samples. For selenium and cadmium in particular, the Triton X-100 and

the nitric acid wash showed increased values on comparison with the unwashed hair. Neither Triton X-100 nor nitric acid had any detectable selenium in the solutions and only Triton X-100 had cadmium present (1.5 ng ml^{-1}) and so there seems to be no explanation as to why the levels increased by over 200% for selenium and over 1000% for cadmium. Substituting the isopropanol for acetone eliminated the production of fumes during sample digestion. This could also be as a result of the fact that there was now no organic final step, as the isopropanol was only used in the first step which was then followed by the three water washings. The data showed that there was no chromium enhancement, despite the isopropanol containing over 3000 ng ml^{-1} of chromium. After correction for the blank, it appeared that chromium had not adsorbed to the hair from isopropanol as was seen with acetone in the IAEA method in figure 4.1.

Element	Method and Hair Concentration (ng g^{-1})			
Cr		**Unwashed**	**1324 ± 22**	
	Water wash	1305	TX100	1563
	Isopropanol	1309	0.1% HNO_3	1468
	SLS wash	1385	EDTA wash	1344
Se		**Unwashed**	**445 ± 0.6**	
	Water wash	577	TX100	1014
	Isopropanol	483	0.1% HNO_3	931
	SLS wash	461	EDTA wash	471
Cd		**Unwashed**	**52 ± 29**	
	Water wash	107	TX100	691
	Isopropanol	86	0.1% HNO_3	1259
	SLS wash	89	EDTA wash	44
Hg		**Unwashed**	**349 ± 14**	
	Water wash	368	TX100	338
	Isopropanol	318	0.1% HNO_3	357
	SLS wash	399	EDTA wash	447
Pb		**Unwashed**	**2740 ± 39**	
	Water wash	2880	TX100	3351
	Isopropanol	3534	0.1% HNO_3	2762
	SLS wash	2651	EDTA wash	2566

Table 4.2: Results from the hair samples washed with 3ml water, isopropanol, 1% SLS, 1% Triton X-100, 0.1% HNO_3 and 1% EDTA for 24h on mechanical shakers followed by 3 x 3h 3 ml water washes for the sample, compared to the same unwashed sample.

4.3.1.3. Hair from Three Donors Washed by Six Methods.

The next set of washings was also carried out using the mechanical shaker and involved a 3h wash with 3 ml of the reagent followed by 3 ml of water for 3h in triplicate (3 x 3h x 3 ml). The reagents used were water, isopropanol, 0.1M HCl[5,28], 1% SLS, 1% EDTA and 1% v/v shampoo[5,9]. Hair from three different donors was washed with the six methods and the results are shown in figures 4.6 – 4.11.

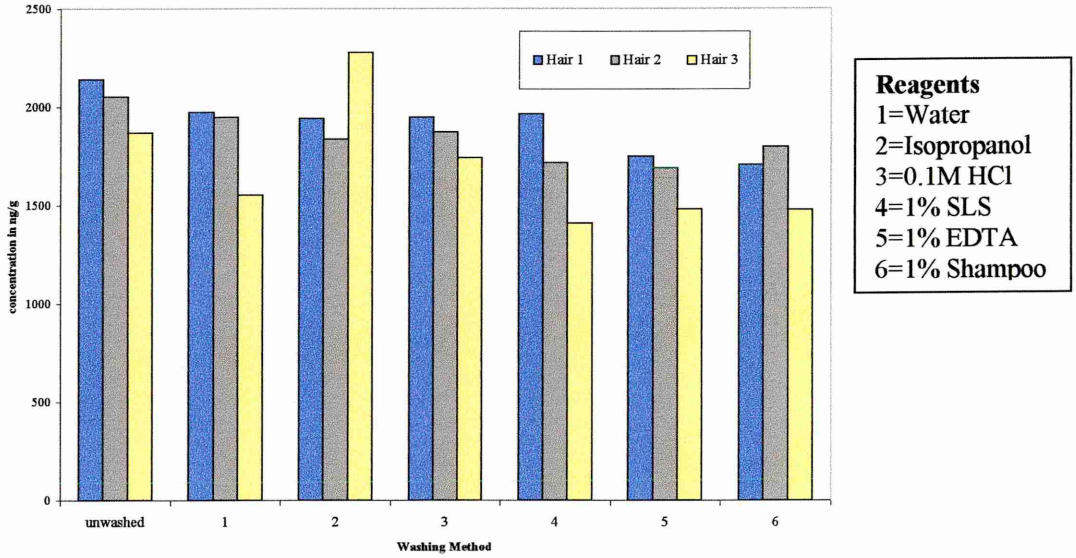

Figure 4.6. Chromium levels in the hair of three donors comparing unwashed hair with hair washed for 3h with 3 ml of reagents 1-6, followed by 3h of 3 ml water in triplicate.

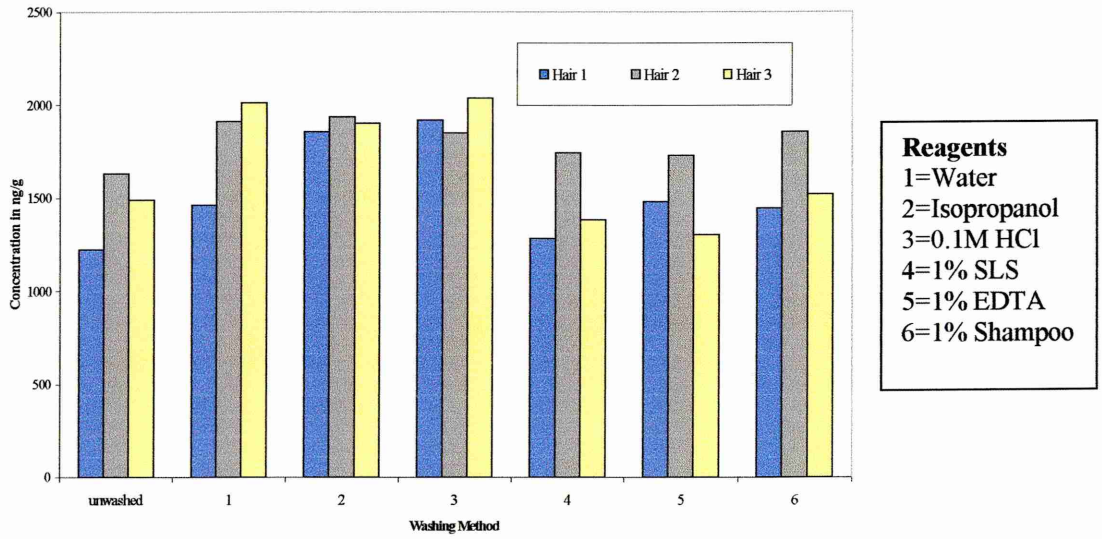

Figure 4.7. Selenium levels in the hair of three donors comparing unwashed hair with hair washed for 3h with 3 ml of reagents 1-6, followed by 3h of 3 ml water in triplicate.

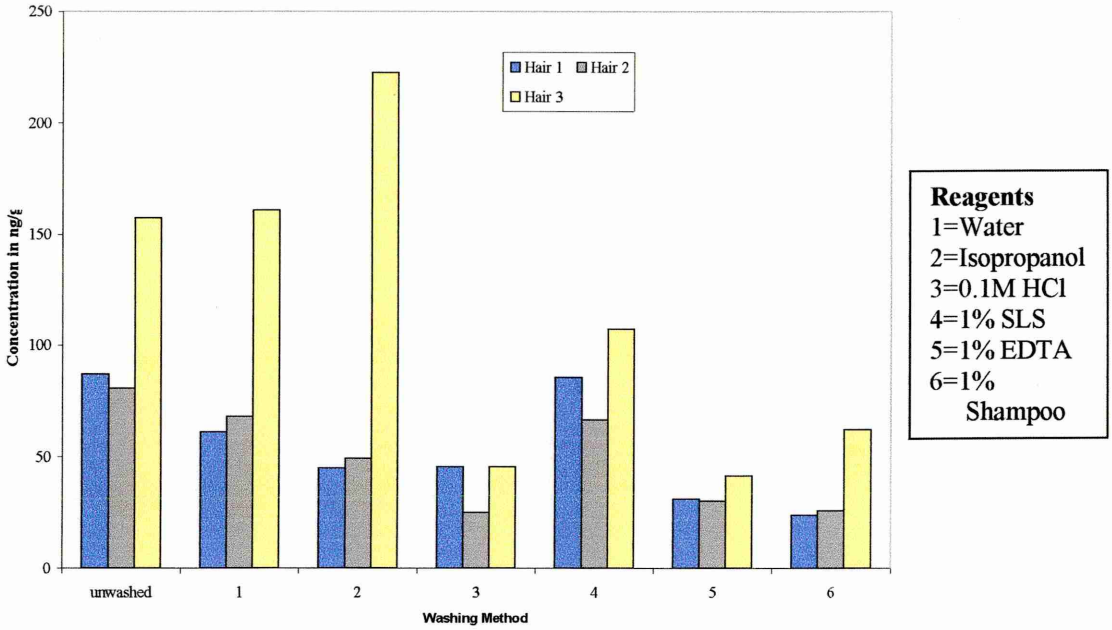

Figure 4.8. Cadmium levels in the hair of three donors comparing unwashed hair with hair washed for 3h with 3 ml of reagents 1-6, followed by 3h of 3 ml water in triplicate.

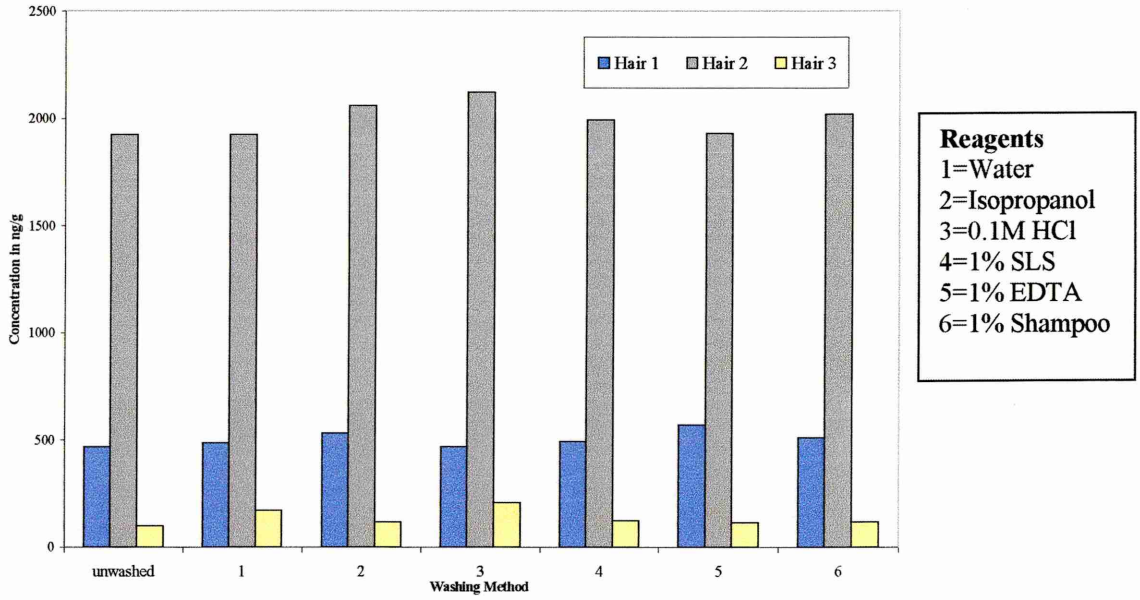

Figure 4.9. Mercury levels in the hair of three donors comparing unwashed hair with hair washed for 3h with 3 ml of reagents 1-6, followed by 3h of 3 ml water in triplicate.

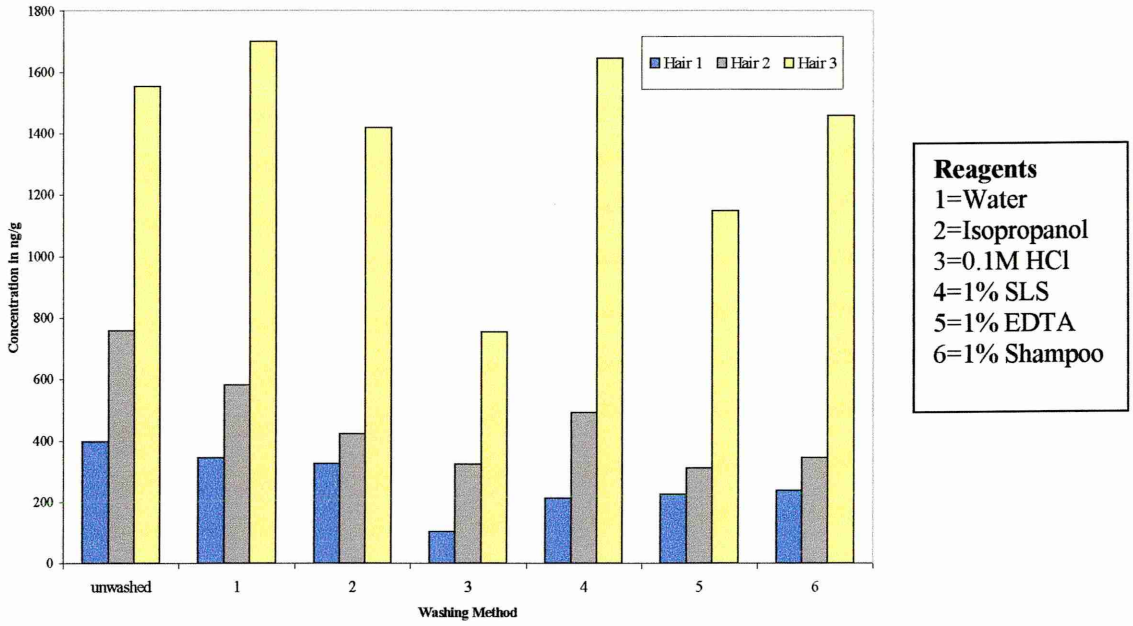

Figure 4.10. Lead levels in the hair of three donors comparing unwashed hair with hair washed for 3h with 3 ml reagents 1-6, followed by 3h of 3 ml water in triplicate.

Inspection of the chromium levels (figure 4.6) shows that up to 18% (with 1% EDTA) of the chromium is washing off. Paired t tests showed that there were significant differences between the chromium levels in the unwashed hair and those in the hair washed with 0.1M HCl, 1% EDTA and 1% shampoo. Paired t tests confirm that selenium is not being removed significantly from the hair by any of the washing procedures (figure 4.7). However, a significant increase ($p>0.05$) in selenium concentration was observed when the hair is washed in isopropanol. Cadmium, as shown in figure 4.8 does wash off the hair sample with all reagents except isopropanol. Paired t tests show that cadmium is removed significantly from the hair by washing with 1% shampoo. Mercury, shown in figure 4.9, is not removed significantly from the hair sample by washing and this was confirmed with paired t tests. In contrast, lead, shown in figure 4.10, is significantly removed from the hair with on average 56% being washed off when the samples were washed with 0.1M HCl.

As selenium is an essential element that is not prevalent in the environment it would be expected mostly to come from within the body and therefore endogenously bound. Mercury also does not wash off, this suggests that all of the mercury is internally bound to the hair structure. It is also possible that mercury that has bound from the environment has

done so strongly due to its high affinity for disulphide groups within the matrix of the hair[3] and hence does not wash off.

From these results it appears that SLS, an anionic detergent, was removing some of the chromium. This suggests that the chromium could either be present in the hair in the cationic form if the sulphonate groups of the SLS were extracting the cations, or in the anionic chromate form if anionic exchange is taking place. The HCl, which is an ionic solution, washed off the lead and cadmium, suggesting that the metal chlorides are being formed with the extracted metal cation. EDTA also removed some of the lead, suggesting that it is complexing the available lead on the surface of the hair, as EDTA is a well known chelating agent for lead.

It is clear from the preliminary results that washing the hair can decrease the concentrations of chromium, cadmium and lead in the hair and thus more studies were carried out to investigate this further.

4.3.2. Optimisation of the Washing Procedures

The SLS, EDTA and HCl methods were used in further washing experiments. It was necessary to examine the length of time the reagent mixed with the hair, to see if this affected the results and to investigate whether or not the water fractions actually removed any further exogenously bound species. Samples of approximately 0.2 g of hair (from the same sample) were washed with 1% SLS, 1% EDTA and 0.1M HCl, for the following four different time periods using the mechanical shaker.

i. 12h with 3 ml of reagent
ii. 12h with 3 ml of reagent and then 3 x 3h with 3 ml of water
iii. 3h with 3 ml of reagent
iv. 3h with 3 ml of reagent and then 3 x 3h with 3 ml of water.

Each method was carried out in duplicate for 1% SLS, 1% EDTA and 0.1MHCl and the results are shown in tables 4.3 – 4.5.

Element	Unwashed hair value ($ng\ g^{-1}$)	Hair washed for 12h with 1% SLS ($ng\ g^{-1}$)	Hair washed for 12h with 1% SLS followed by 3 x 3 ml x 3h water ($ng\ g^{-1}$)	Hair washed for 3h with 1% SLS ($ng\ g^{-1}$)	Hair washed for 3h with 1% SLS followed by 3 x 3 ml x 3h water ($ng\ g^{-1}$)
Cr	1421	1381	1273	2019	1295
Se	1021	1024	1055	1052	1001
Cd	46	226	36	29	49
Hg	1416	1552	1619	1466	1521
Pb	629	472	372	432	352

Table 4.3. A comparison of the levels of cadmium, chromium, lead, mercury and selenium in unwashed hair samples with hair washed in 1% v/v SLS solution for 12h and 3h with and without the water wash of 3 x 3 ml x 3h.

Element	Unwashed hair value ($ng\ g^{-1}$)	Hair washed for 12h with 1% EDTA ($ng\ g^{-1}$)	Hair washed for 12h with 1% EDTA followed by 3 x 3 ml x 3h water ($ng\ g^{-1}$)	Hair washed for 3h with 1% EDTA ($ng\ g^{-1}$)	Hair washed for 3h with 1% EDTA followed by 3 x 3 ml x 3h water ($ng\ g^{-1}$)
Cr	1421	1240	1182	1380	1212
Se	1021	1068	1056	1015	977
Cd	46	84	31	18	12
Hg	1416	1565	1564	1449	1481
Pb	629	303	334	287	201

Table 4.4. A comparison of the levels of cadmium, chromium, lead, mercury and selenium in unwashed hair samples with hair washed in 1% v/v EDTA solution for 12h and 3h with and without the water wash of 3 x 3 ml x 3h.

Table 4.3 shows the results for the SLS washed hair samples. The data show that lead is the only element significantly washing off (up to 45% is removed) and that only 9% of the chromium is removed by this method. Paired t tests show that selenium, cadmium and mercury are not being removed significantly with 1% SLS. The data also show that the amount of element removed is increased when the water washes are carried out after the

washing with 1% SLS. There is no significant difference between the 12h wash followed by the water fractions and the 3h wash followed by the water fractions.

The data in table 4.4 show that the 3h wash with 1% EDTA followed by 3 washes with 3 ml of water for 3h removes more of the elements than the other methods. The addition of the water washes causes more element to be removed. With the 3h 1% EDTA wash and the water wash 15% chromium, 5% selenium, 75% cadmium and 69% lead are removed from the hair. Mercury is not removed from the hair using 1% EDTA, with or without additional water washes.

The data in table 4.5 also show that more elements are removed using additional water washes and that the shorter 0.1M HCl wash of 3h removes more element than the longer 12h wash. With the 3h 0.1M HCl wash and three water washes 8% chromium, <1% selenium, 89% cadmium and 73% lead are removed from the hair. Mercury is not removed with the 0.1M HCl wash, with or without additional water washes.

Element	Unwashed hair value ($ng\ g^{-1}$)	Hair washed for 12h with 0.1M HCl ($ng\ g^{-1}$)	Hair washed for 12h with 0.1M HCl followed by 3 x 3 ml x 3h water ($ng\ g^{-1}$)	Hair washed for 3h with 0.1M HCl ($ng\ g^{-1}$)	Hair washed for 3h with 0.1M HCl followed by 3 x 3 ml x 3h water ($ng\ g^{-1}$)
Cr	1421	1381	1169	1631	1311
Se	1021	1067	1049	1014	1014
Cd	46	6	11	6	5
Hg	1416	1665	1533	1525	1494
Pb	629	183	173	148	169

Table 4.5. A comparison of the levels of cadmium, chromium, lead, mercury and selenium in unwashed hair samples with hair washed in 0.1M HCl solution for 12h and 3h with and without the water wash of 3 x 3 ml x 3h.

As a result of these findings, the method of choice for the washing procedures was a 3h wash with the reagent followed by a further three 3h washes with water to remove the reagent and some more of the elements from the hair. The three methods using 1% SLS, 1% EDTA and 0.1M HCl were continued on each hair sample.

4.3.3. Washing the Hair Samples from the Normal Group

Hair samples from non occupationally exposed group of 40 people were collected as described in section 4.2.2. Sixteen samples were washed by each of the three washing methods but, because there were not enough of each of the samples to allow duplicate analyses, the remaining 24 samples were only washed with 1% SLS and 0.1M HCl, but in duplicate. Hair samples from donors numbered 1-16 were analysed as duplicate unwashed samples and single washed samples by the three methods. Hair samples from donors 17-40 were analysed in duplicate unwashed, with 1% SLS and 0.1M HCl washings. There were no EDTA washes carried out for hair from donors 17-40. To allow the washes to be carried out easily in a working day, the reagent fraction 3 ml was washed for 3h, then 2 x 4.5 ml of water was added for 3h each, instead of 3 x 3 ml x 3h, for each of the washing procedures. Also, the samples were now washed using a incubated platform shaker at 20°C, because the mechanical shakers would only wash 9 hair samples and 3 blanks at once.

A summary of the mean levels of trace elements determined, based on all of the washed and unwashed samples, is shown in table 4.6. Details of all the answers obtained from the normal group questionnaires are in appendix 2.

All of the unwashed hair samples were compared with the washed values to see which elements were being significantly removed from hair. Paired t tests showed that the chromium levels in the SLS and HCl washed hair samples did not show a significant decrease (approximately 12%), however the EDTA washed hair samples did show a significant gain in chromium when p=0.05. The arsenic levels were significantly reduced (by 40%) when hair was washed with 1% v/v SLS and significantly increased when washed with 0.1M HCl. The three washing methods did not remove significant amounts of selenium from the hair. Paired t tests showed that cadmium was removed significantly by all of the washing methods when p=0.05, especially with 0.1M HCl (over 80% washing off). Antimony was also removed significantly from hair by each of the washing methods, 1% SLS (about 43%), 1% EDTA (62%) and 0.1M HCl (about 27%). Using paired t tests mercury and lead were both shown to be removed significantly by washing with 0.1M HCl (with over 70% of lead washed out).

Element	Unwashed ($ng\ g^{-1}$) n=40	Washed in 1% SLS ($ng\ g^{-1}$) n=40	Washed in 1% EDTA ($ng\ g^{-1}$) n=16	Washed in 0.1M HCl ($ng\ g^{-1}$) n=40
Cr	**985** [539 - 4077]	**862** [518 - 1766] (12.5% loss)	**1064** [656 - 1552] (8.0% gain)	**874** [586 - 2123] (11.3% loss)
As	**34** [<1 - 148]	**21** [<1 - 61] (40.0% loss)	**34** [<1 - 50] (0.1% loss)	**44** [<1 - 108] (28.4% gain)
Se	**471** [157 - 704]	**470** [165 - 639] (0.1% loss)	**532** [449 - 701] (12.9% gain)	**460** [157 - 607] (2.4% loss)
Cd	**72** [<0.1 - 834]	**68** [<0.1 – 735] (6.09% loss)	**18** [<0.1 - 55] (74.34% loss)	**9** [<0.1 - 66.7] (86.93% loss)
Sb	**89** [2 – 961]	**62** [9 – 557] (43.2% loss)	**33** [15 – 117] (62.4% loss)	**65** [7 – 325] (27.2% loss)
Hg	**287** [3 – 802]	**300** [0.5 – 928] (4.6% gain)	**324** [<1 – 904] (12.9% gain)	**273** [<1 – 736] (4.9% loss)
Pb*	**1030** [84 – 5863]	**946** [45 – 4408] (8.0% loss)	**619** [281 – 1413] (39.9% loss)	**279** [<0.1 – 1551.6] (72.9% loss)

* lead values calculated were n=39 as one donor had a hair lead level >70000 $ng\ g^{-1}$.

Table 4.6. A summary of the washing procedures and the trace element concentrations determined in the normal group hair samples, showing the mean value in bold, the range of values in square brackets and the amount of element removed as a percentage of the unwashed hair value underneath.

The reproducibilities of the analyses of the 24 (17-40 donor number) samples that were washed in duplicate with SLS and HCl solutions are outlined in table 4.7. The average unwashed and washed values were recalculated for this group and so the losses/gains of the washing procedures vary from those in table 4.6. However, the percentage of relative standard deviations calculated are based on the individual standard deviations between the duplicate results in each sample for the SLS and HCl washes.

The results show that the washing procedures have in some cases up to 40% variation between the duplicate samples. This occurred with the cadmium values determined for the SLS washed samples. This could be due to the low cadmium values as similar variations

with cadmium have been seen throughout the washing study. The hair samples being washed will always vary between duplicate samples as it would be difficult to standardise the amount of element being removed from the surface of the hair. This is because it depends on each strand of hair being washed for exactly the same time with the same amount of solution. Also there was hair lost in the washing procedure from the time of initially weighing out the samples before washing to weighing again when the sample had been washed and dried. However, it can be seen in table 4.7 that the variations of the element concentrations are similar in the two methods. Clearly this varies between samples. One reason for this is that the hair sample being washed generally rolls into a ball/clump and then the inside of the ball/clump is not as exposed to the wash solution as the outside.

Element	Unwashed average ($ng\ g^{-1}$) n = 24	SLS washed value ($ng\ g^{-1}$) and the gain/loss in brackets	HCl washed value ($ng\ g^{-1}$) and the gain/loss in brackets
Cr	996	773 ± 89 (loss 22%)	851± 105 (loss 15%)
As	25	14 ± 4 (loss 42%)	33 ± 6 (gain 33%)
Se	435	436 ± 6 (gain 0.2%)	428 ± 28 (loss 1.6%)
Cd	71	74 ± 43 (gain 4%)	11 ± 7 (loss 84%)
Sb	79	60 ± 10 (loss 25%)	63 ± 12 (loss 207%)
Hg	313	316 ± 27 (gain 0.8%)	292 ± 31 (loss 7%)
Pb	1256	1174 ± 244 (loss 6.5%)	354 ± 59 (loss 72%)

Table 4.7. The reproducibility of the SLS and HCl duplicate washings for the last 24 samples in the normal group of hair samples.

4.3.4. Washing Spiked Samples

Although, in some cases, the elements were apparently washing off, it was necessary to know whether the amounts being removed were equivalent to the exogenously bound species present on the hair.

Hair samples were spiked with standards made in a 'sweat' solution (see section 4.2.4) and an enriched isotopically ratioed cadmium sample. The hair samples were left in the spiked solution for 24h in a sealed conical flask. The solution was then pipetted off and the hair sample transferred to a weighing boat, covered with a sheet of paper and allowed to dry at room temperature in a fume cupboard. The samples were then weighed before and after being washed and digested in the normal way.

4.3.4.1. Sweat Solutions

Eight samples of the same hair sample (approximately 0.1 g) were weighed into conical tubes and 3 ml of 100 ng ml^{-1} spike, made in the sweat solution, was added to the hair samples. The tubes were capped and then left for 24h. Six of the samples were then washed with 3 ml of reagents, two with 1% SLS, two with 0.1% EDTA and two with 0.1M HCl. The unwashed spiked hair samples, the washed hair samples and two unspiked hair samples were then digested and analysed by ICP-MS. The washings were also analysed. The results obtained are summarised in Table 4.8.

The reason for making the standard in the sweat solution was to try and mimic the exchange that may occur when head hair is exposed to sweat. Hair is continuously bathed in sweat from the scalp and trace elements are likely to be adsorbed onto hair[29].

The results show that all of the elements except lead adsorb to the hair, with selenium and mercury taken up to a greater extent. The lead on the hair being spiked actually was removed by the simulated sweat solution which had not been shaken. This suggests that the lead on the hair is not tightly bound at all. The adsorbed chromium and almost all of the adsorbed cadmium washed off with HCl. In contrast, arsenic and selenium did not wash off the hair once spiked. Antimony was only partially washed off, with 34% of the spiked amount being removed in the SLS wash. Up to 30% of the adsorbed mercury washed off with the HCl wash.

Element	Concentration determined in unwashed, unspiked hair (ng g^{-1})	Concentration determined in spiked hair (ng g^{-1})	Concentration determined in hair spiked and washed with 1% v/v SLS (ng g^{-1})	Concentration determined in hair spiked and washed with 1% v/v EDTA (ng g^{-1})	Concentration determined in hair spiked and washed with 0.1M HCl (ng g^{-1})
Cr	487	656	601	501	485
As	4	240	316	326	310
Se	580	3408	3338	3225	3253
Cd	< 5	149	16	31	1
Sb	14	429	283	328	335
Hg	540	2453	2042	2026	1709
Pb	1247	391	454	205	71

Table 4.8. Elemental concentrations determined in hair samples spiked with a standard made in a sweat solution and then washed by the three methods, 1% SLS, 1% EDTA and 0.1M HCl.

In a similar experiment carried out in 1966, with simulated sweat, it was found that the extent of adsorption was pH dependent[30]. The results showed that antimony, arsenic, mercury and selenium were adsorbed to the hair whilst in contrast caesium, potassium and sodium did not adsorb to hair. Washing was carried out using EDTA in the presence of a surfactant 'Kyro EO'. A small amount of selenium was seen to be removed along with partial removal of antimony, arsenic and mercury whereas copper and manganese were almost completely removed in the washing.

In this work, the washing fractions were also collected and analysed as part of the simulated sweat study. Blank solutions were run with the washing fractions and subtracted accordingly. The levels determined in the washings were not seen to tally very well with the losses seen in the washed hair. This is probably due to the reasons discussed previously i.e. it is difficult to accurately quantify the amount of solution analysed or the mass of the hair the solution was in contact with. Also, although the standards were made freshly in water they were not matrix matched to the SLS, EDTA and HCl solutions.

As yet there is no accurate method of quantifying the concentration of the elements extracted from the surface of the hair by the washings.

4.3.4.2. Cadmium Spikes and Washing Procedures

An enriched standard, with a 106:111 cadmium isotope ratio of 1:1, was used to spike the hair samples. The natural cadmium ratio for 106:111 is 0.0976, and the following amount of spike was added to make the ratio 1:1.

Natural abundance		**Spiked abundance**	
Cd 106	1.25%	Cd 106	79.01%
Cd 111	12.8%	Cd 111	2.6%

Six replicates of four hair samples were spiked with 3 ml of the enriched ratio standard (diluted 1 in 4) and left for 24h. Two of the six samples were unwashed, two were washed by the SLS method and two were washed by the HCl method. The samples were then dried overnight and digested in the normal way. Cadmium ratios measured in the samples and the washings are summarised in tables 4.9 and 4.10 respectively.

The ratio results show that the ratio of Cd 106:111, in each of the four hair samples, increases when spiked with the enriched standard and decreases again when washed in SLS and HCl, with the HCl method showing the removal of more of the spike (see table 4.9). This means that the cadmium that washes off the hair is that which has adsorbed from the spiked solution. This is further confirmed in the 'washings' results, shown in table 4.10, where the washings from the different hair samples show significant increases in the ratio of Cd 106:111. The reagent washings show greater increases than the water washings that followed the reagent washings. It is also apparent from analysis of the hair samples that different hair samples adsorb different amounts of cadmium and so each hair sample must be analysed individually. This could be due to several reasons, for example the different concentrations of internally and externally bound species can affect the number of sites available for the cadmium to bind. Additionally the physical structure and condition of the hair may be important in that hair that is broken/torn may adsorb more or less than hair that is in good condition.

	Hair 1	Hair 2	Hair 3	Hair 4
Unspiked Hair	0.3376	0.1699	1.565	0.2711
Spiked sample	0.6193	0.4390	1.991	1.067
Spiked and washed in 1% SLS solution	0.4114	0.2436	1.296	0.5324
Spiked and washed in 0.1M HCl solution	0.3006	0.2745	1.403	0.2253

Table 4.9. The cadmium ratios of isotopes 106:111 in the normal hair sample, the spiked sample and the washed hair samples.

Fraction	Natural ratio 106:111 in blank solution	Hair 1	Hair 2	Hair 3	Hair 4
SLS wash	0.2123	0.8898	0.9279	1.403	0.6997
SLS water	0.1952	0.4464	0.4846	0.6676	0.4501
HCl wash	0.0967	0.7301	0.4005	0.4756	0.7785
HCl water	0.1623	0.1941	0.1741	0.2454	0.2087

Table 4.10. The cadmium 106:111 ratio of the washings of the spiked hair samples

4.3.5. Scanning Electron Microscopy of Washed and Unwashed Hair Samples

The SEM used was as described in section 3.2.4.2. It was not possible to analyse specific elements in the hair using the EDX facility of the scanning electron microscope in this case, as only sulphur and silicon were present in detectable quantities. However, the topography of unwashed hair strands was compared with samples that had been washed with the 1% SLS method, the 0.1M HCl method and the IAEA method. Areas with particulates present on the hair surface are highlighted with a white arrow. Figures 4.11 – 4.14 show the electronmicrographs obtained.

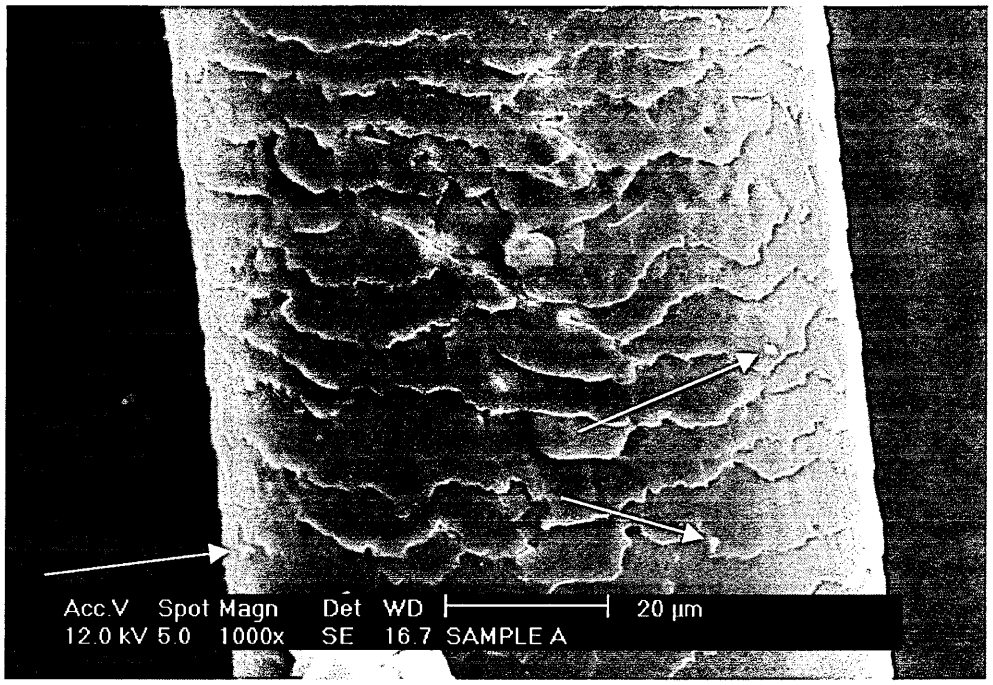

Figure 4.11. Electronmicrograph of an unwashed hair strand.

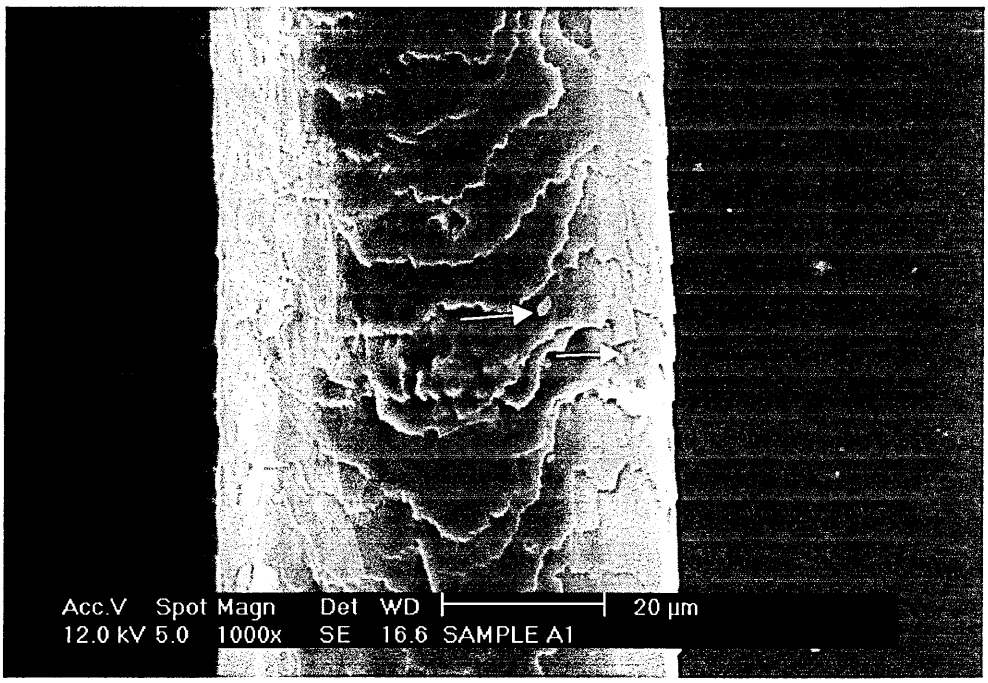

Figure 4.12. Electronmicrograph of a hair strand washed by 1% SLS method.

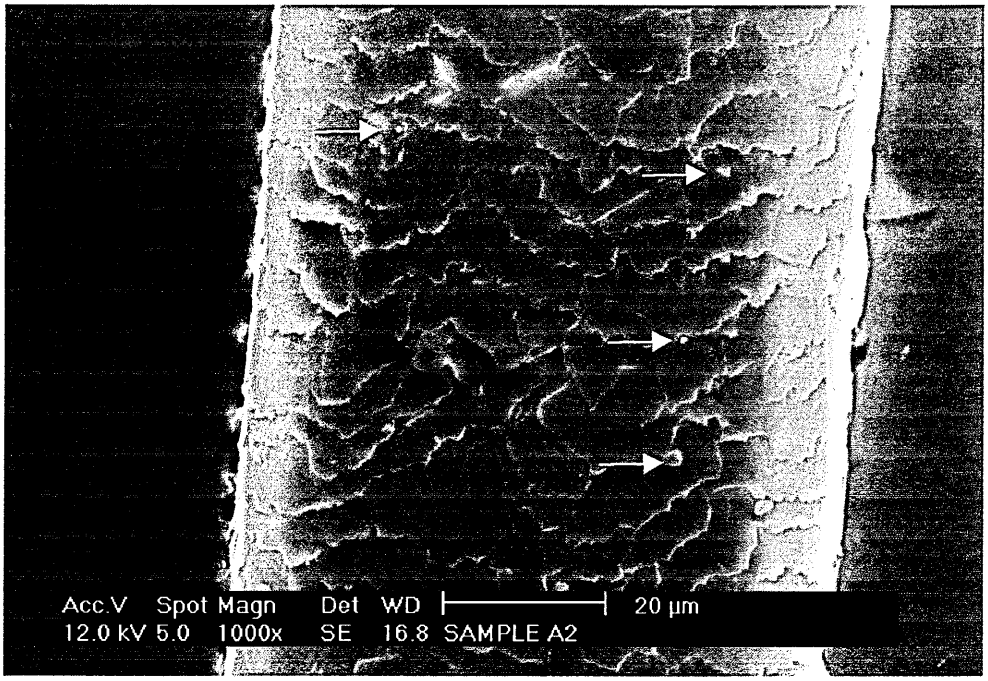

Figure 4.13. Electronmicrograph of a hair strand washed by 0.1M HCl method.

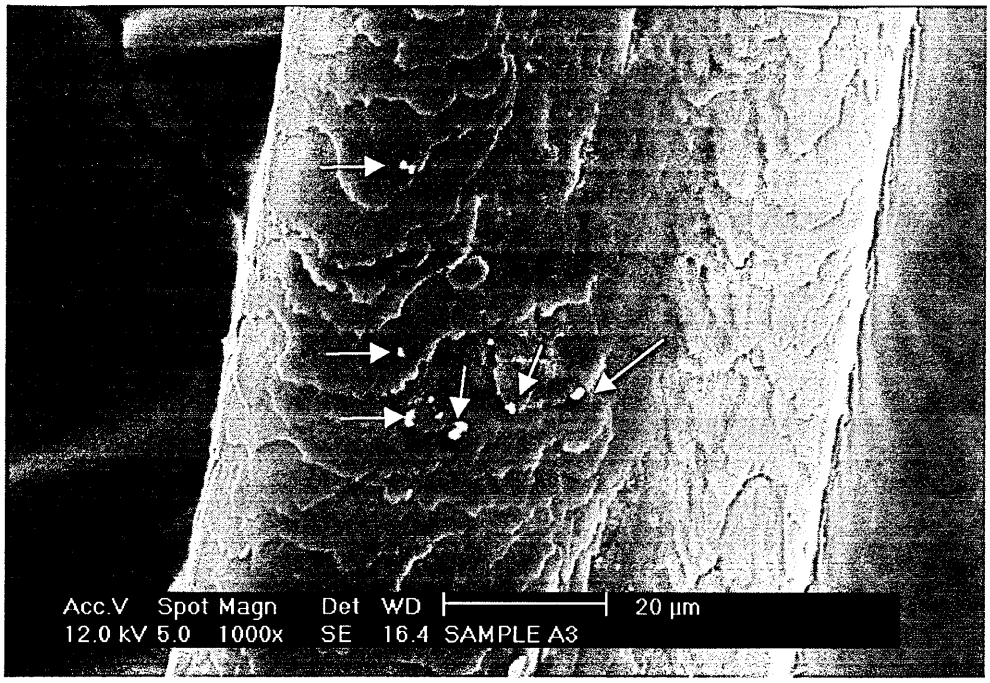

Figure 4.14. Electronmicrograph of a hair strand washed by the IAEA method.

The electronmicrograph of the unwashed hair (figure 4.11) shows particulates on the surface of the hair and, while the hairs washed by the SLS method (figure 4.12) and the HCl method (figure 4.13) still show some particulates on the surface, the hair washed by the IAEA method (figure 4.14) appears to have more particulates on the surface. This suggests that the IAEA method does not remove as much of the surface contamination as the other washing methods. This was further investigated by spiking the hair in the sweat solution mentioned previously in this chapter. The elements of interest, antimony, arsenic, cadmium, chromium, lead, mercury and selenium, were added to the solution so that the concentration was 100 $\mu g\ ml^{-1}$. The solutions were left on the hair for 24h, the hair was then dried and washed using the same three methods as previously. Electronmicrographs of these spiked hairs are presented in figures 4.15 – 4.18. From the electronmicrographs it can be seen that the spiked hair washed with 1% SLS and 0.1M HCl have less particulates on the surface than the unwashed spiked hair sample, suggesting that the particulates have been removed by the washes. However, once again this is not the case for the IAEA method as there seem to be many particles on the surface of the hair.

The final SEM images (shown in figures 4.19 & 4.20) are of donor 17's hair, a fireman, who had been shown to have very high concentrations of metals in his hair. Lead had been determined at levels of 70 000 $ng\ g^{-1}$, however, this was still below the limit of detection of the Oxford EDX analyser. The unwashed hair sample shows that there were a lot of particulates present on the hair. The hair was also damaged in places (see figures 4.19 & 4.20), which may have provided extra surfaces for external particles to bind to.

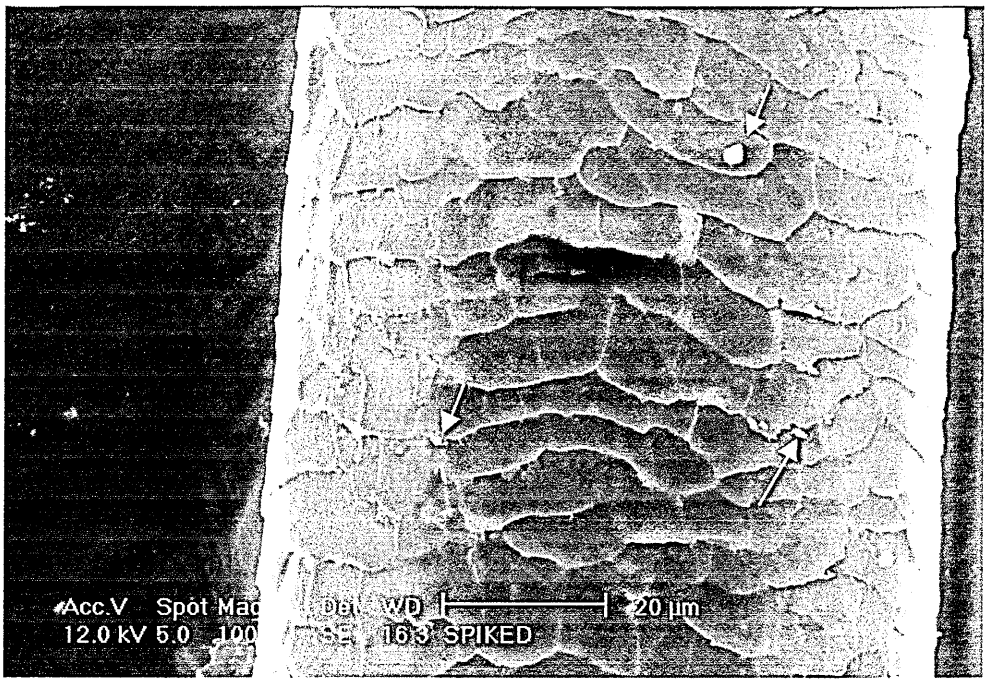

Figure 4.15. Electronmicrograph of an unwashed spiked hair sample.

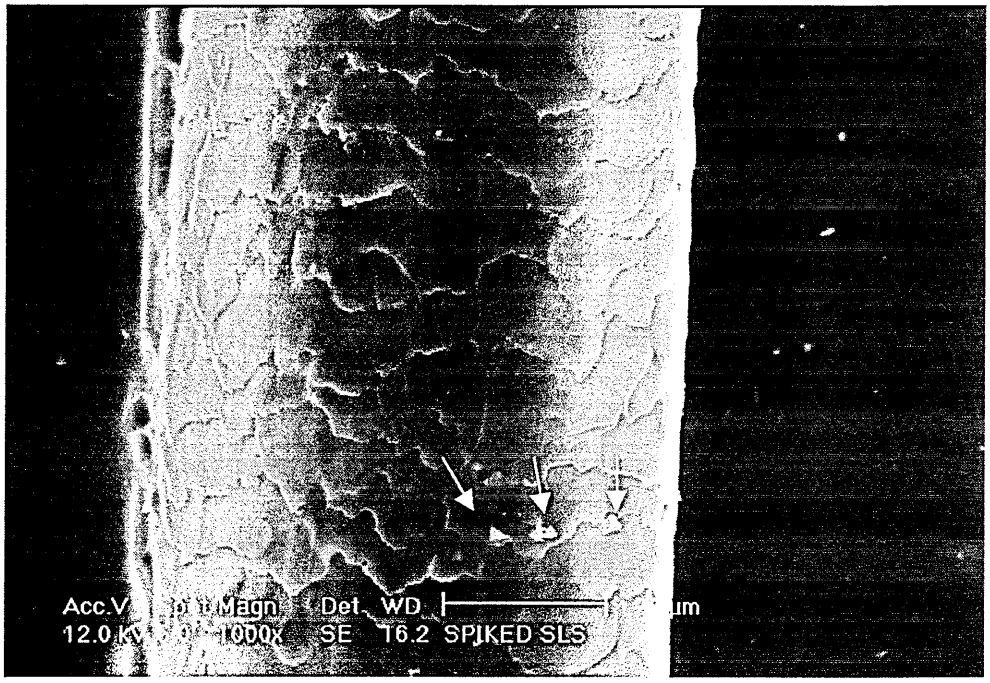

Figure 4.16. Electronmicrograph of a spiked hair sample washed by the 1% SLS method.

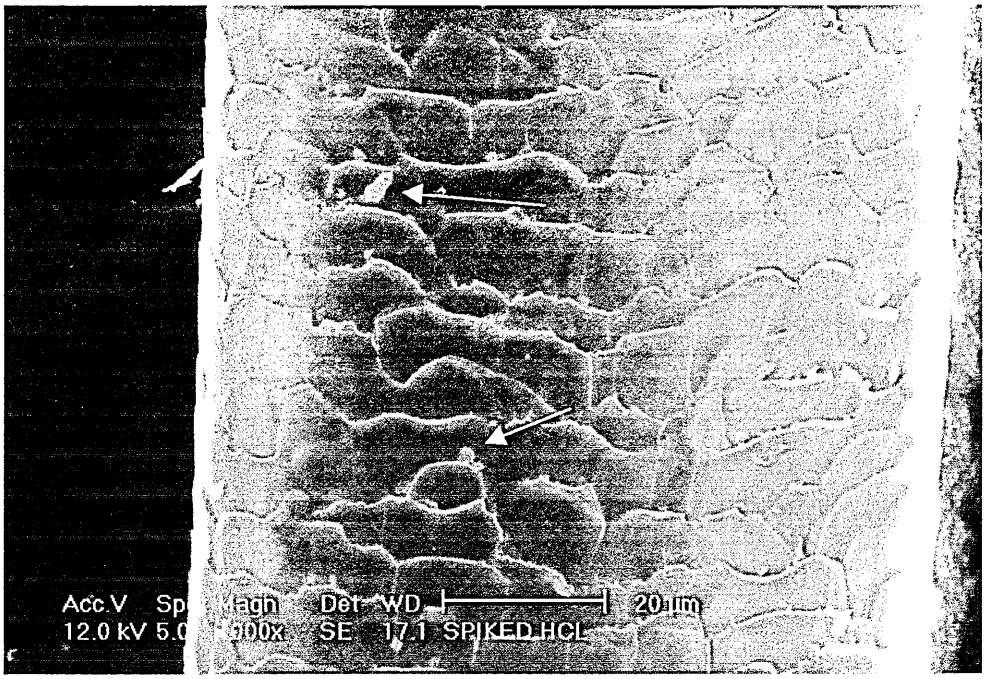

Figure 4.17. Electronmicrograph of a spiked hair sample washed by the 0.1M HCl method.

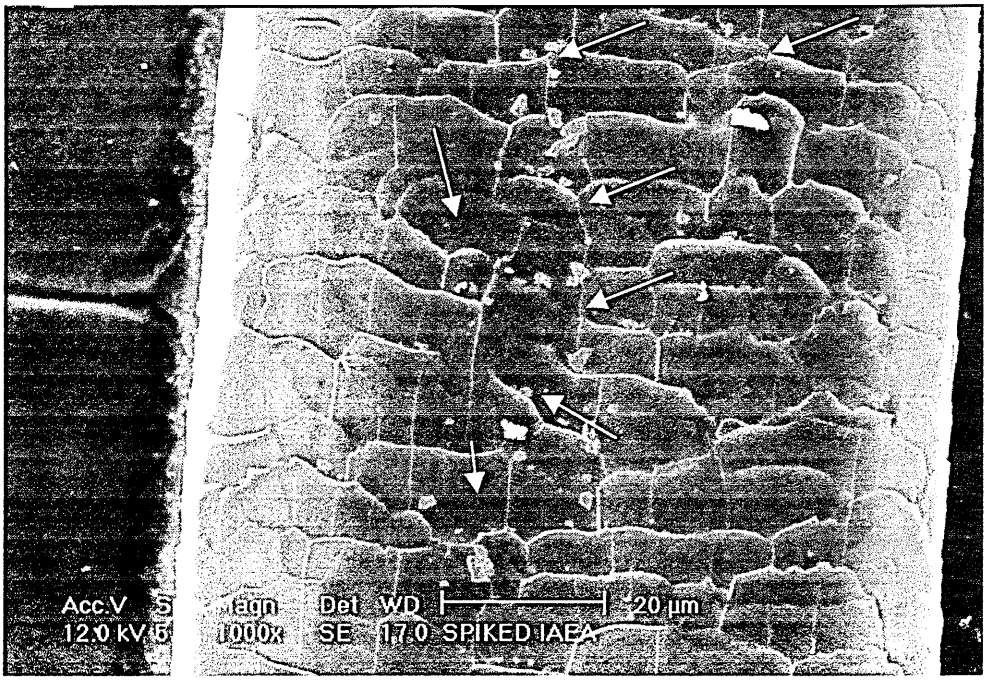

Figure 4.18. Electronmicrograph of a spiked hair sample washed by the IAEA method.

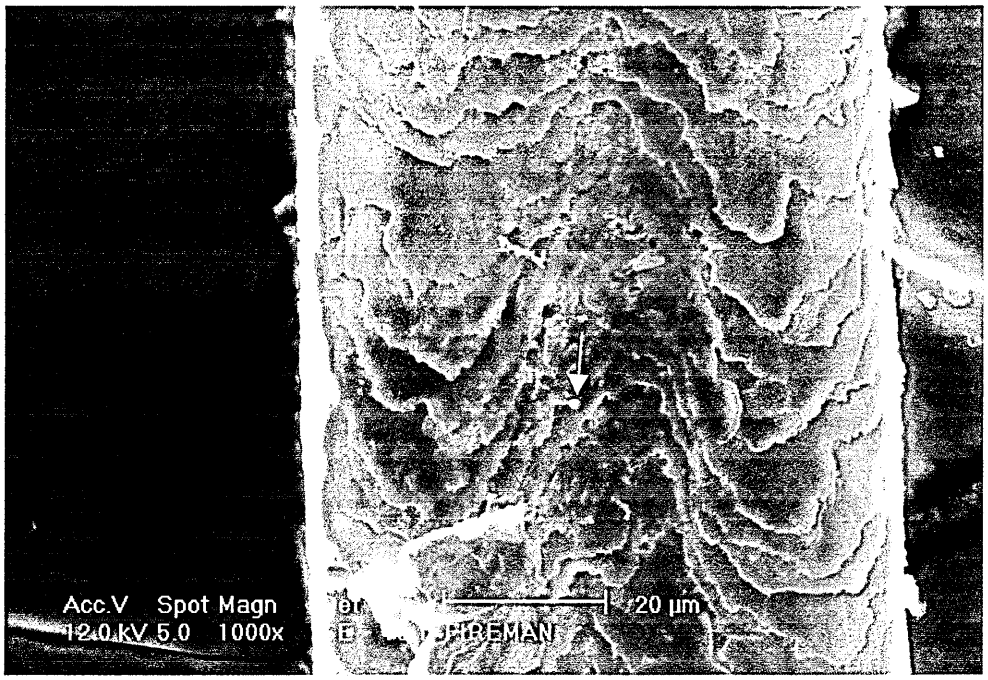

Figure 4.19. Electronmicrograph of Donor 17's hair sample from the normal group (a fireman).

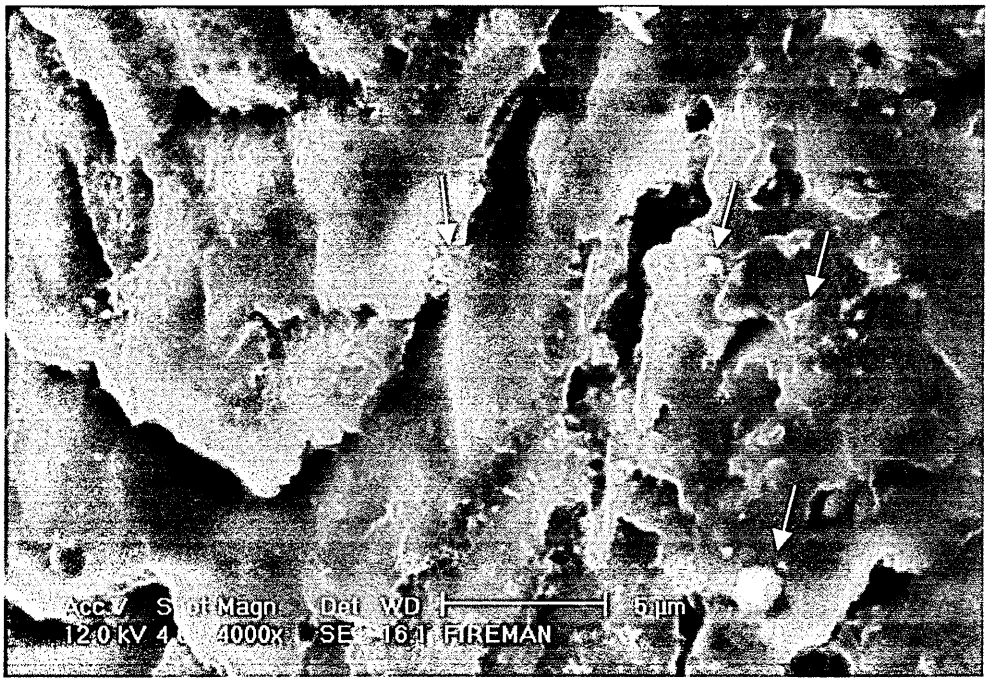

Figure 4.20. Electronmicrograph of Donor 17's hair sample at a higher magnification.

4.4. Conclusion

The elements for whom differentiation between exogenously and endogenously bound species may be possible are chromium, cadmium and lead. Antimony, arsenic, mercury and selenium, did not wash off once adsorbed to the hair sample and for these it is not possible to distinguish between exogenously and endogenously bound species of these elements. However, it is apparent from this work that because antimony, arsenic, mercury and selenium adsorbed to the hair from the spiked solution their presence is a very good indicator of exposure to the elements.

In untreated samples, the only element not to significantly wash off was selenium. The other elements all washed off to some extent. Arsenic would only wash off in 1% SLS, mercury only washed off slightly (up to 7%) in 0.1M HCl, whilst antimony and chromium could be partially removed by both the SLS and HCl methods, (up to 20%). Cadmium and lead washed off the most, (up to 80%), using the 0.1M HCl method.

To summarise the results from this chapter, it seems that it is necessary to wash hair samples to determine accurate levels for all of the elements, except for selenium. It is unclear whether or not the analysis of washed hair gives a value which accurately reflects the body burden of the element. However, to determine whether or not a person has been occupationally or environmentally exposed to these elements, it may be reasonable not to wash the hair but instead to compare the analysis results with those from unexposed subjects.

The preferred methods, of the washing procedures investigated, were the 1% SLS and 0.1M HCl. The IAEA method was not suitable for hair that was being microwave digested or for analysing chromium.

4.5. References

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Chapter 5: The Use of Hair to Assess Selenium Status

5.1. An Introduction to Selenium

Selenium was discovered over 180 years ago[1], although the first description of selenium poisoning in animals was that by Marco Polo, during his travels in West China in 1295[2].

The toxicology of selenium and its compounds is of continuing interest for a variety of reasons. These include: the long established selenium poisoning of animals that consume seleniferous plants[2]; the disorders in humans and plants resulting from deficiencies and excesses[1]; the nutritional essentiality of the element[3]; the protective effect of selenium against metal toxicity[4]; the metabolic interactions between the element and vitamin E and other antioxidants; the reported carcinogenicity as well as anticarcinogenicity[5,6] of selenium; the reported inverse relationship between the dietary intake of selenium and cancer incidence and mortality[7].

In 1957, it was discovered that selenium was an essential element[8] and since then low dietary intake has been associated with cardiomyopathy, cardiovascular disease and cancer[4]. Its most recognised role is as a component of the enzyme glutathione peroxidase, which forms part of the organism's defence mechanisms against free radical damage[9,10]. However, selenium is only an essential element at low levels and can produce toxic symptoms at levels 3 to 5 times higher than those required for adequate nutrition[1,4]. Toxic levels have been suggested at 5 mg of selenium per kg of food[4]. In general the order of toxicity for selenium is organic selenides > inorganic selenides > Se^{4+} > Se^{6+}> Se^{0} and selenites are more toxic than selenates or selenium salts[6].

As well as the overall beneficial nutritional role of selenium it has been established that selenium plays a protective role against trace element poisoning (e.g. by mercury, arsenic and cadmium)[4]. Selenium has been shown to counteract the effects of both inorganic and methyl mercury toxicity in several animal species[3].

Exposure to selenium from the environment occurs mostly through natural geological activity and man-made pollution and although it is widely distributed in the environmental water, soil and air, it occurs generally in concentrations lower than 1 mg l^{-1}. The earth's crust contains an average selenium concentration of 0.05-0.09 mg l^{-1}, which is similar to that of cadmium and antimony and higher than molybdenum, silver, mercury and uranium.

As a metalloid, selenium possesses semi-conducting properties which have led to its use in photocopying machines, light meters, rectifiers and stainless steel production. Its other uses are as additives in pigments for ceramics, in the vulcanisation of rubber, in fertilisers, pesticides and animal feeds[11]. Annual world production of selenium was estimated as 1890 tonnes[1] in 1997.

5.1.1. The Absorption of Selenium

The principal source of selenium is the diet and the uptake of the element depends largely on the chemical form. Selenium can enter the food chain via selenium rich soil or via plants where it is found in the selenium containing amino acids[12]. Selenomethionine has been found to be the most predominant form of selenium in wheat, soyabeans and selenium enriched yeast. The selenium content in plants differs widely and largely depends on the plant's ability to absorb the element e.g. tobacco contains 0.1-1 mg l^{-1} selenium[12]. Fish are known to contain higher levels of selenium than most other foods and thus are a major dietary source of this element[13].

The Ministry of Agriculture, Fisheries and Foods (MAFF) has monitored selenium in the diet throughout the UK for over 25 years. In a survey of 100 different foods carried out in 1995, it was found that the foods with the highest levels of selenium were Brazil nuts (254 μg/100 g), kidney (146 μg/100 g) and crab meat (84 μg/100 g), whereas levels in vegetables and dairy products were much lower (below 2 μg/100 g). Levels in white bread and flour were lower than in previous studies suggesting that the soil selenium levels are decreasing[1]. Observed changes in the UK diet from 1978 to 1995, were that in 1978 50% of the selenium intake came from the consumption of cereals but in 1995 this had fallen to 18%. Conversely, many other food groups contributed more selenium to the diet, e.g. meat had increased from 28% to 40%, fat had increased from 1.7% to 8% and vegetables had increased from 3% to 11%. This study does raise concern with regard to some minority groups such as vegans and vegetarians because, based on the 1995 MAFF study, these groups may not be receiving the recommended level of 40 μg of selenium per day for adults[1]. Soil leaching due to acid rain and intensive farming methods have been blamed for the reduced selenium levels in soil and therefore in the diet[14].

The absorption of selenium from food depends upon the chemical form of the element in the food, the interaction with other elements in the food and the physiological state of the individual. Other dietary factors that affect the absorption of selenium include the presence or absence of adsorption promoters or inhibitors such as ascorbic acid, fibre, sugar, fats and proteins. The metabolism of selenium in the body has been monitored by several studies[1,2,3] and it is has been found that approximately half of the daily intake is excreted in urine and the remainder in faeces. Very little selenium is excreted in sweat or by respiration. Gastrointestinal absorption of organoselenium is more complete than absorption of inorganic selenium. Absorbed selenium is widely distributed by the blood to organs and tissues, with the highest concentrations found in the liver and kidneys. Both inorganic and organic forms of selenium are utilised as nutrients in the production of specific selenoproteins, but only after conversion via reduction to the selenide form.

5.1.2. Selenium Deficiency

Symptomatic selenium deficiency in man is known, but rare. Chronic selenium dietary deficiency, however, is more common and has been associated with various degenerative diseases. The daily dietary intake of selenium by the UK population was estimated in 1995 as 40% of the recommended level and elderly people may consume even smaller amounts[10]. In the People's Republic of China endemic cardiomyopathy[8] and osteoathropathy have been shown to be related to selenium deficiency and have also been shown to be corrected with selenium supplementation[2,15]. Other diseases which have been linked with a low selenium status include sickle cell anaemia, Keshan disease, cystic fibrosis, infertility, cancer, muscular dystrophy, cataracts, hypertension, muscular complaints and degeneration[1].

5.1.3. Symptoms of Selenium Poisoning

The external manifestations of chronic selenium exposure include hair loss, deformity of nails and discolouration of the teeth[16]. It has recently been suggested that doctors should become more aware of the symptoms as more people take selenium tables to supplement the diet. Dizziness and fatigue are associated with occupational inhalation of selenium compounds. It has been reported that workers exposed to selenium aerosols containing

elemental selenium and selenium dioxide developed nasal bleeding, headache and loss of weight[11]. Other similar studies have shown that eczema and conjunctivitis are symptoms of selenium exposure[2]. Episodes of chronic selenosis have been reported in workers exposed to high levels of selenium and in China where food contamination had taken place. Garlic odour on the breath, dermatitis, arthritis and paralysis are some of the symptoms in these cases[2].

5.1.4. Selenium in Hair

There have been several studies looking at the levels of selenium in hair[9,10,15,17,23]. The basis for considering hair to assess selenium status is similar to that of mercury. Both elements readily bind to the sulphydryl groups in thioamino acids, which are abundant components of the hair matrix. Other biological markers of selenium status include the selenium concentrations in whole blood, plasma and urine, all of which reflect recent intake. Determination of the selenium content of the sulphur rich protein, keratin, found in hair offers a marker over a longer period of time[10].

Levels of mercury and selenium in hair have been analysed in new mothers and neonatal scalp hair[9]. New mothers and babies are thought to be more at risk of mercury exposure because, in the UK, dental treatment is free to pregnant women. As a direct result of this, it is thought that this population group is at a higher risk of exposure than normal to elemental mercury vapour released during insertion or replacement of mercury based dental amalgam. Selenium is known to give some protection against mercury toxicity[1], which is based on the fact that the strong affinity mercury has for sulphur in the cysteinyl and histidyl side chains of amino acid residues is decreased as a result of selenium substituting for sulphur in these amino acids. These amino acids are common components of globular proteins which are involved in a range of important biological functions, including those affecting the immune and endocrine systems and nutrient transport. Results of the study[9] showed that the mercury levels of mother and baby were similar at 0.54 and 0.52 $\mu g\ g^{-1}$ respectively, whereas the selenium levels differed with the mother having higher levels than the baby, at 1.02 $\mu g\ g^{-1}$ compared with 0.63 $\mu g\ g^{-1}$.

The interactions of selenium with toxic metals in hair has also been investigated[5]. Rats were fed diets containing selenium and then five groups of rats were separately fed arsenic,

thallium, cadmium, mercury and water. The rats' hair and nails showed lower levels of selenium with the addition of arsenic, as well as lower selenium levels in the kidney and plasma. The addition of mercury also decreased the selenium content of the hair and nails, but increased the content in the plasma, liver, muscle, bone, colon and kidney. Thallium decreased the selenium hair content but increased the liver and kidney content, whereas cadmium had no effect on the hair and nail selenium content but decreased red blood cells. The results showed that the toxic metals interfere with the uptake of selenium in the body and also that hair may not be a reliable indicator of selenium status if there has been exposure to such toxic elements[5].

It has successfully been shown that selenium can be monitored in hair samples[17] and that this can reflect recent selenium intakes[23]. Selenium in hair has been found to exist in organic and inorganic forms at trace levels[17]. A study in Japan investigated the different forms of selenium in hair samples using Hydride Generation Atomic Absorption Spectrometry[17]. The results showed that the majority of selenium in hair was organoselenium. The mean total level of selenium in human hair was found to be 687.14 ± 4.1 ng g^{-1}, the organic level of selenium in hair was 683.19 ± 4.8 ng g^{-1} and the inorganic forms Se^{4+} and Se^{6+} were 0.57 ± 0.05 ng g^{-1} and 3.38 ± 0.07 ng g^{-1} respectively[17]. The Se^{6+} was reported to be determined using solvent extraction which was subtracted from the total selenium to give the Se^{4+} value, whereas the organic selenium was reported to have been leached from the hair via a mix of nitric, perchloric and hydrochloric acids[17].

A significant decrease in selenium hair levels has been reported in women who have had breast cancer, but in contrast only a slight decrease in plasma levels was observed[18,19]. It was also found that selenium was 30% less concentrated in subjects with dyed hair[19]. Hair has also been used to determine occupational exposure to selenium[11,16] and vegetarians have been shown to have lower selenium levels[11].

The selenium content of subjects from different countries has been studied[13] and the values seen are shown in table 5.1. It is believed that the high selenium levels in hair may be as a result of high selenium intake from a predominantly fish diet[8]. In a Chinese study correlation between liver and hair selenium levels was found but in contrast to this no relationship was found between lung and kidney levels[14]. Strong correlation has been reported between hair and whole blood selenium concentrations[9].

Country	Concentration of selenium in hair ($\mu g\ g^{-1}$)
Japan	0.60
France	0.39
Ivory Coast	0.41
Brazil	0.46
Paraguay	0.39
Canary Island	0.45
Papua New Guinea	0.59
Italy	0.41
New Zealand	0.43
Iraq[20]	0.9
India[20]	1.3
Pakistan[20]	1.2
Turkey[15]	Boys 0.29 Girls 0.32
Bulgaria[8]	0.31
Sweden[8]	0.42
China[14]	0.89
USA[10]	0.58-0.76
Venezuela[10]	0.70-2.90
Scotland[10]	Men 0.68 Women 0.53 Overall 0.52

Table 5.1. Hair selenium levels analysed in hair samples from different countries[10,11,13,14,15,20].

5.1.5. Selenium Supplementation

It has been suggested that if humans have the same need for selenium as animals then the requirement to prevent deficiency diseases in man would be 60 to 120 μg selenium per day[3]. In the US, some have cautioned against the use of nutritional supplements containing selenium by healthy persons because the benefits of selenium are not well established and the difference between beneficial and toxic levels of selenium is narrow. In addition to this it is thought that vitamin E may protect against the lack of selenium[3]. In a Welsh study in 1991, fifty subjects took yeast based tablets containing 100 μg selenium on a daily basis for five weeks. The results showed that there was a marked improvement in the mood and general well-being of those who took the selenium tablets compared with

those who took the placebo. The study suggested that the lower the level of selenium in the diet the more reports of anxiety, depression and tiredness[21].

Selenium supplementation has been used to prevent the growth of cancerous tumours[6,22]. It has been shown that people in the US with high to medium selenium levels have significantly lower cancer mortality rates for total cancer and cancers of the lung, colon and rectum, bladder, pancreas, breast, ovary and cervix. Although, it has also been shown that selenium treatment does not protect against the development of basal or squamous cell carcinomas of the skin[22].

Selenium in hair has shown increased levels when the diet is supplemented. Healthy subjects were fed malt cakes that were high in selenium, daily for one year and the selenium hair levels were seen to increase by 194.8% and the serum selenium content increased by 178%[7]. This study also showed that selenium-supplementation protected the DNA repair capacity which would help protect against lung cancer. Hair selenium levels have been found to increase with selenium supplementation where blood concentrations have not. This was observed in a study in the US in 1984, when eight subjects took selenium supplements for six weeks[23] and showed a rise in hair selenium levels from 440 ng g^{-1} to 560 ng g^{-1}. Results from these studies suggest that hair is a valuable tool in assessing selenium status[7,23].

5.2. Experimental Details

The purpose of this study was to assess the selenium status in a sample of the population by hair analysis and to determine whether or not selenium supplementation improved their various symptoms. This study was carried out along with an MSc. project at Sheffield Hallam University[25], whereby the MSc. student collected the samples and the completed questionnaires while the author of this work carried out the hair analysis. The aim of the study was for hair analysis to be carried out before and after the selenium tablets were taken to see if the concentration of selenium in the hair increased within the duration of the supplementation period.

5.2.1. The Selenium Supplement

Yeast based selenium-containing tablets and identical-looking placebo tablets were obtained from Larkhall Green Farm (Putney, London). The selenium tablets contained 100 μg of the element. The placebo tablets were given to the subjects in the first six weeks of the study, in order to avoid any mid clearance period, followed by six weeks selenium supplementation.

5.2.2. The Subject Group

The study started with 41 subjects, but due to illness and non compliance only 25 subjects (8 males and 17 females) successfully completed the trial. The average age of the group was 50 y, with a range of 32-65 years.

Hair samples were provided for analysis by 29 subjects at the beginning and end of the trial, but only 25 of these completed the appropriate questionnaires. Instructions were given for the hair to be taken from the suboccipital region of the head and placed in a plastic sample bag. Hair samples were collected from the same area of the head and cut as close as possible to the scalp, i.e. the most recent growth.

At the beginning, middle and end of the study the subjects received and completed a questionnaire (see appendix 3) and the results were compiled by the MSc. student.

5.2.3 Hair Analysis

5.2.3.1. Reagents Used

Aristar nitric acid (69%), hydrogen peroxide 30% and plasma emission standards of 1000 mg l^{-1} chromium (III), lead, antimony, mercury, arsenic, rhodium, yttrium and bismuth in 2% HNO_3, from BDH (Poole, Dorset, UK) were used.

5.2.3.2. The Digestion of the Samples

The hair was transferred using nylon forceps into the microwave vial and digested as described in section 3.2.3.2. Certified reference materials (CRM) were digested in the same way.

5.2.3.3. The Analysis of the Samples

A Hewlett Packard HP4500 ICP-MS was used as described in section 2.3.3., operating under the same experimental conditions as those used previously (see table 2.3). Table 5.2 shows the isotopes and acquisition details.

The ICP-MS was switched on and allowed to run for 1h before a calibration with matrix matched standards in the range 0-25 ng ml^{-1} was carried out (see chapter 3). In order to monitor experimental drift a 10 ng ml^{-1} standard was analysed after every ten samples. In addition to this 0.1 ml of a 1 mg l^{-1} solution of the following internal standards were added to the samples: yttrium, rhodium and bismuth.

Metal	Isotope Used	Dwell time (seconds) per point	Dwell time (seconds) per mass
Chromium	52	0.1	0.3
Selenium	82	1	3
Yttrium	89	0.1	0.3
Rhodium	103	0.1	0.3
Cadmium	114	1	3
Mercury	202	1	3
Lead	208	1	3
Bismuth	209	0.1	0.3

Table 5.2 The isotopes used for each element in the ICP-MS determination of the hair samples.

5.3. Results and Discussion

5.3.1 The Selenium Levels in the Hair

Hair concentration before selenium supplement (ng g^{-1}) n=29	Hair concentration after 6 weeks of selenium supplement (ng g^{-1}) n=28	Hair concentration up to 8 weeks after selenium supplement (ng g^{-1}) n=13
551 ± 111	598 ± 74	678 ± 130

Table 5.3 : Average selenium hair concentrations, before and after selenium supplements were taken, in 29 subjects.

The average selenium concentrations for all of the donors can be seen in table 5.3 and the details of individual concentrations can be seen in appendix 3. The average increase in selenium from the first sample to the last sample of hair analysed (the second if there were only 2 samples and the third if there were 3 samples collected) was 19.3 %. The original mean value compares well to the Glasgow study[10] of hair carried out in 1995, where the mean found was 520 ng g^{-1}. The increase of selenium in the hair found here is lower than values previously reported[7] where increases of up to 94% were reported. However, in that study the subjects were given higher amounts of selenium (up to 300 μg per day) for longer periods of time (up to 1 year). The results from this study appear to agree better with the results from a similar six week selenium supplement study in 1984 in the US, where a 27% increase in hair selenium levels was observed in eight samples[23].

A paired t test[24] was carried out as described in section 3.2.5., with the selenium hair values before and after the supplementation. The results of this showed that the differences between the before and after results were significant at 99% confidence limits. This was also reflected in the results from the symptomology questionnaire, where significant improvement in symptoms relating to depression ($p<0.02$), anxiety ($p<0.03$) and musculoskeletal pain ($p<0.02$), but not social functioning ($p=0.65$) were found[25].

The increase in the hair selenium values can also be compared to the work in chapter 3 where normal hair samples were monitored over time. It was found that the selenium levels in normal hair do not vary significantly with time, with the standard deviation of selenium concentrations in hair over a period of 21 months being less than 10% of the mean value.

5.3.2. Concentrations of Other Elements Present in the Initial Hair Samples

Chromium, cadmium, mercury and lead were also analysed in the hair samples at the same time as the selenium. The results are presented in table 5.4.

Element	Hair concentration before selenium supplement (ng g^{-1}) n=29	Hair concentration after 6 weeks of selenium supplement (ng g^{-1}) n=20	Hair concentration up to 8 weeks after stopping the selenium supplement (ng g^{-1}) n=13
Cr	929 ± 94	1231 ± 226	593 ± 43
Cd	135 ± 23	99 ± 18	120 ± 19
Hg	513 ± 34	596 ± 42	429 ± 13
Pb	1960 ± 391	2086 ± 629	943± 69

Table 5.4. Average levels of cadmium, chromium, lead and mercury in the hair of the subjects who took part in the selenium supplementation study, before, after and up to eight weeks after finishing the supplement.

The chromium values in table 5.4 show that the levels vary in hair samples over the selenium supplement period, but a paired t test confirmed that this was not a significant variation. As shown in chapter 3, the levels of chromium in hair varied by up to 43% in a study carried out over 21 months. The mean chromium level of 929 ng g^{-1} in this group agrees well with the 985 ng g^{-1} mean found in the normal group in chapter 4. Additionally the chromium levels found in this study fall within ranges reported[26] previously of 650 - 33000 ng g^{-1}.

The cadmium and mercury hair concentrations before and at the end of the study did not vary significantly and this was confirmed using a paired t test. This finding does not agree with previous studies where selenium was seen to reduce the levels of mercury[3,4]. The cadmium mean value in the selenium hair samples was slightly higher than the value for those in the normal group (72 ng g^{-1}), whereas the mercury mean value was significantly higher in the selenium hair samples than that reported in the normal group in chapter 4 (287 ng g^{-1}).

A paired t test showed that the lead levels determined before the selenium supplementation were significantly higher than those obtained after the supplement had been taken. The lead normal group mean value, from chapter 4, was 1030 ng g^{-1}, which appears closer to the values in this group after the supplement had been taken.

5.3.3. Trends Seen With the Hair Values

The increase in hair selenium levels was greater in the women's hair than in the men's hair. The women (n=20) saw an increase of 24.5%, from a mean of 560 ng g^{-1} to 698 ng g^{-1}, whereas the men (n=9) saw a rise of 17.9% from 531 ng g^{-1} to 605 ng g^{-1}. Generally, the selenium levels in the hair of the women were higher than in the men's hair. The numbers of dental fillings were compared with the amount of mercury seen in the hair of the donors but no correlation between the data could be seen.

5.3.4. The Certified Reference Materials (CRMs)

Certified reference materials and spiked hair samples were analysed along with the hair samples. Details regarding the CRMs were given in previous experimental sections. Table 5.5 presents the results from the CRMs and table 5.6 shows the results from the spiked hair samples.

Element	Expected values for CRM 397 ($ng\ g^{-1}$)	Values obtained for CRM 397, mean concentration ± 1 standard deviation ($ng\ g^{-1}$)	Expected values for CRM 07601 ($ng\ g^{-1}$)	Values obtained for CRM 07601, mean concentration ± 1 standard deviation ($ng\ g^{-1}$)
Cr	(90000)	58129 ± 3557	370	845 ± 224
Se	2000	2016 ± 203	600	765 ± 87
Cd	521	865 ± 148	110	109 ± 6
Hg	12300	9555 ± 921	360	231 ± 14
Pb	33000	27571 ± 4276	8800	5625 ± 347

Table 5.5. Comparison of the expected and experimentally determined levels of cadmium, chromium, lead, mercury and selenium in the certified reference materials 397 and 07601, which were digested at the same time as the hair samples.

Donor	Cr	Se	Cd	Hg	Pb
340	118	132	105	105	76
321	111	118	98	94	85
390	98	135	107	95	108
260	102	114	99	94	91

Table 5.6. Spiked recoveries of a 10 $ng\ ml^{-1}$ standard added to duplicate hair samples from four donors in the selenium study as a percantage.

The chromium values determined experimentally for the CRMs do not compare well with the indicated values and the standard deviations appear high, however, in the spiked hair samples the chromium recoveries are better, with a mean value of 107%. The selenium CRM experimental values show good correlation with the CRM stated values while the spiked hair samples show on average a recovery of 25% more than expected. The cadmium and mercury spiked hair recoveries in table 5.6 show good correlation with the known concentration, whereas the lead recoveries are slightly lower. The CRM experimental values for cadmium show a better correlation with the expected value for

CRM 07601, while the values determined for mercury and lead are closer to the expected values for CRM 397.

Variations in the found and expected values for the CRMs show that although higher values than expected were found for chromium and lower values than expected were found for mercury and lead, the values for selenium compared well with the CRMs.

5.4. Conclusion

The study showed that the hair selenium value increased significantly when supplementation was taken for just six weeks. The levels in female hair samples increased more than in the male samples but this could be due to the fact that 20 women and only 9 men took part in the study. The results from the symptomology questionnaires suggested that the subjects' overall moods improved whilst taking the supplements. There is evidence to suggest from this study that the UK population may indeed benefit from taking a selenium supplement.

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In this chapter methods based on high performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) are used for the separation and determination of lead and mercury species in human hair.

6.1. Mercury in Hair

Hair has been shown to be a good indicator of exposure to mercury[1]. Hair mercury levels have been used to assess dietary[1,2,3], environmental[4,5,6,7] and occupational exposure [8,9,10,11]. Incidents of chronic mercury exposure, in Japan and Iraq, showed that mercury levels in hair were good indicators of dietary exposure when either contaminated fish or bread were consumed respectively[1,3]. Hair mercury concentrations of up to 183 $\mu g\ g^{-1}$ were reported in Japan, whereas in Iraq levels as high as 649 $\mu g\ g^{-1}$ were observed, with 73 $\mu g\ g^{-1}$ of this being inorganic mercury[2,3]. Other studies have confirmed that hair mercury levels increase with fish consumption[12]. Airey evaluated mercury concentrations in over 500 hair samples from 32 locations and found a correlation between fish consumption and hair mercury levels[2]. Concentrations of 11.6, 2.5, 1.9 and 1.4 $\mu g\ g^{-1}$ were found for those who ate fish every day, every week, twice a month and once a month respectively[2]. Fish accumulate methyl mercury from sediments and water. In addition, methyl mercury can also be produced by the bacterial methylation of inorganic mercury in fish[20]. Approximately 80% of total hair mercury in fish eating populations is the more toxic methyl mercury form, compared with less than 5% in the hair of those with a low fish intake[13].

Environmental exposure to mercury has also been determined using hair analysis. A study of 225 Chinese, Malaysians and Indians in Singapore showed that mercury hair levels were higher in men than women and that levels varied between the different ethnic groups. This latter variation was thought to be related to the use of herbal medicines containing, for example, cinnabar (HgS)[5]. Occupational exposure to mercury is well known (see section 1.10.1) and hair mercury levels higher than those of unexposed subjects[14] (for example 9.84 $\mu g\ g^{-1}$ compared to 3.52 $\mu g\ g^{-1}$) have been reported in studies of dentists' and dental workers' hair samples. One study showed a 4-7 fold increase of mercury in head hair and fingernails of dental workers when compared to levels in pubic hair and toenails which

was attributed to the fact that the head hair was exposed to external mercury whereas the pubic hair and toenails were concealed and therefore not exposed to external sources of mercury[9].

Analysis of hair from exposed subjects has shown that levels of more than 5 $\mu g\ g^{-1}$ are indicative of mercury intoxication[3]. Correlation between methyl mercury levels in hair and mercury levels in blood has been reported[15], where hair has been found to contain about 300 times more mercury than blood[5,10].

Normal hair mercury concentrations of 1-2 $\mu g\ g^{-1}$ have been reported[14,16], although values up to 3.5 $\mu g\ g^{-1}$ have been reported in an English study[11]. However, the range of 0.5-10 $\mu g\ g^{-1}$ has been used as a guide in several studies[2,3]. Total mercury levels in hair have been shown to be stable over long periods and it has also been found that organomercury levels in hair have remained constant for over 11 years[5].

Different chemical forms of mercury have different toxic effects on the body. High doses of inorganic mercury can lead to kidney damage while methyl mercury can penetrate the blood-brain barrier which can lead to irreversible brain damage[1]. The principle reaction of mercury in the body occurs with thiols, which form mercury mercaptides and the distribution and amount of damage depends on this reaction[17]. Organic and inorganic mercury compounds have a high affinity for sulphydryl groups and so can inhibit a large number of enzymes, precipitate protein and cause cell death[1]. It has been suggested that after the mercuric ion binds to the sulphydryl groups present in hair, as the mercuric ion still contains a free valence, it is capable of binding with some other local group and thus binds more strongly to the hair structure[17]. Mercury compounds can rapidly disperse to all tissues because simple mercurials, such as the methyl mercury halides, are very soluble in lipids (100 times more soluble than in water). Organic mercury is effectively absorbed through the digestive tract and inorganic mercury, in the form of vapour, is absorbed through the lungs and skin[1]. Mercury is excreted in urine, faeces, sweat, breath and hair. Both organic and inorganic forms are excreted through hair[2].

6.1.1. The Determination of Mercury Species in Hair

To date, the methods for determining mercury species in hair have involved separate extractions and determinations of the inorganic and organic forms[6,18,19,20,21,22]. The

methods reported vary, in that some digest the hair sample whilst others extract the species from the hair without digestion.

An early study extracted the mercury from hair samples by shaking with 10 ml of 0.1N hydrochloric acid for 1h[23]. The hydrochloric acid extract was then oxidised with sulphuric acid and 3% v/v potassium permanganate, refluxed with 20% v/v hydroxylamine hydrochloride and analysed using mercury vapour photometry. This study found that not all of the mercury was extracted and the majority of species in the extract were methyl mercury. Recently, a study reported[24] a method for the rapid acid leaching and slurry sampling for the determination of methyl mercury and total mercury in hair by electrothermal AAS (ET-AAS). The methyl mercury was extracted with HCl by mechanically stirring the hair in 4% v/v HCl, for two minutes. Slurries of the hair were then achieved using HCl with palladium reduced by ascorbic acid as a chemical modifier for both methyl and total mercury. The study reported impressive limits of detection of 40 and 100 ng g^{-1} for methyl mercury and total mercury respectively.

Extractions of mercury from hair samples have been reported using chelating agents such as sodium pyrrolidinedithiocarbamates[22]. The hair was compressed into a syringe piston and shaken with 2 ml of the extraction solvent (acetonitrile:water 50:50 v/v, 50 mmol L^{-1} of the carbamate compound at pH 5.5) for 2h. The extraction solution was analysed by a HPLC-UV-CV-AAS system. Inorganic and methyl mercury were separated on a C_{18} column with a mobile phase consisting of 0.5 mmol L^{-1} carbamate dissolved in 65% acetonitrile in water at pH 6.5. After the separation, the mercury species were detected using a CV-AAS system[22]. This method showed that 85% of the mercury species were extracted from the hair and, of this, the ratio between methyl and inorganic mercury was on average 10:1.5. Another study investigated two methods, acid leaching and solvent extraction, for the removal of methyl mercury from hair[6]. Hair was shaken with 2 mol L^{-1} hydrochloric acid for 4h and the methyl mercury in this aqueous phase was determined by AAS. The second method extracted the methyl mercury by heating the hair sample to 90-95°C with 4 ml sodium hydroxide (10 mol L^{-1}) for 30 minutes, followed by addition of water and adjustment of the pH to less than pH 1. Solid potassium iodide and 4 ml benzene were then added and finally, after the addition of sodium hydroxide to the organic phase, the solution was analysed by AAS[6]. A comparison of the results showed that both

of the methods gave similar values for the concentration of methyl mercury in hair. The mean concentrations found by acid leaching were 129 ± 5 ng g^{-1} for methyl mercury, 291 ± 8 ng g^{-1} for inorganic mercury and 420 ± 12 ng g^{-1} for total mercury. The solvent extraction method reported 123 ± 10 ng g^{-1} for methyl mercury. The authors concluded that this meant that the mercury in hair could be determined following either acid leaching or solvent extraction.

Studies where the hair is first digested and then the mercury species are separated use various mercury detection methods. Kratzer et al[21] used a 0.2-1 M solution of sodium or potassium hydroxide to digest the hair samples, which resulted in less volatisation of the mercury species than acid digests. The species in the hair digests were then separated using solvent extraction to extract the methyl mercury and analysed using neutron activation analysis (NAA). The extraction was achieved by adding an aqueous solution of 0.5M potassium bromide (at pH < 10) or 0.5M potassium iodide (at pH < 11), to the hair digest and then extracting the methyl mercury into an organic phase by adding 2 ml benzene. The methyl mercury was then re-extracted into either a cysteine or sodium hydroxide solution. All the methods were checked by using the radio tracers ^{203}Hg and $Me^{203}Hg$. Similar work with mercury species confirmed that neutral halide complexes of the type HgX_2 or CH_3HgX can be extracted into non-polar organic solvents such as benzene, toluene or xylene. It was also shown that at higher concentrations of ligands, inorganic mercury forms non-extractable anionic complexes, whereas methyl mercury remains extractable as an electroneutral complex[25]. The distribution constant of individual complexes increases in the order: chloride< bromide<iodide[21].

In early study, the hair sample was alkali digested with 11.2 mol L^{-1} sodium hydroxide solution and then the total mercury was analysed directly by AAS. To analyse the methyl mercury present however, the digest was extracted in hydrogen bromide and benzene, back extracted in a cysteine solution and acidified with oxalic acid and potassium iodide, then finally extracted with benzene and analysed by gas chromatography (GC)[26]. The same approach was used to analyse hair samples from pregnant women in a Chilean fishing village[19]. After digesting the hair in nitric and perchloric acids total mercury was determined in hair by CV-AAS and methyl mercury was determined using gas chromatography with electron capture detection (GC-ECD). The methyl mercury in this

case was 'trapped' on cysteine impregnated filter paper from the acid digested hair sample, acidified using potassium bromide and sulphuric acid and finally extracted using toluene. However, using such a vigorous digestion method the author of this thesis suspects that a lot of the methyl mercury present may have been destroyed before the separation of species. The results showed that the mercury levels were higher in the hair of the women in the fishing village, where the fish also had high mercury levels, than in the control samples (2440 $\mu g\ g^{-1}$ compared with 400 $\mu g\ g^{-1}$). The methyl mercury content of the exposed women was 75-89% of the total mercury[19].

Other methods incorporated both methyl mercury extraction with hydrochloric acid and digesting the hair to obtain a total mercury value[2,13,18]. One such method was used in the study of exposure in miners who used mercury to extract gold[7]. Methyl mercury from the hair samples was extracted using hydrochloric acid and benzene and the total mercury was determined after digesting with nitric, perchloric and sulphuric acids. The total mercury digest was determined by CV-AAS and the methyl mercury extract was determined by GC-ECD. The study found that the levels of mercury in the hair correlated with the blood levels (hair Hg:blood Hg ratio was 242:1) and that abnormally high levels of total mercury were found in the hair of the fishing village inhabitants, with more than 95% of this being methyl mercury. The hair of the goldminers contained both inorganic and methyl mercury which were both present in equal amounts[7].

Mercury speciation was also achieved using hair samples taken from a woman who had attempted a mercuric chloride overdose[27]. The methods used involved extracting the organic mercury with hydrochloric acid and then determining the inorganic and total mercury using the Magos digesting method[28]. The digests and the organic mercury extract were all analysed by AAS. The amount of mercuric chloride taken (0.9 g), was a lethal dose and yet the Japanese woman survived as a result of a combination of therapies, including chelate therapy using dimercaprol, plasma exchange, haemodialysis and peritoneal dialysis. The inorganic mercury in the hair taken from the patient showed a peak after 30 days and the level of methyl mercury peaked 95 days after the exposure. The Magos method was first reported in 1971 and it allowed determination of inorganic and total mercury in undigested biological samples and calculated the methyl mercury level as the difference between the values. All organic mercury was converted to inorganic

mercury by adding cadmium chloride and tin (II) chloride to a water-cysteine solution containing the biological sample, then sulphuric acid followed by sodium hydroxide was added and the solution filtered and analysed by AAS[28].

6.1.2. Mercury Speciation by LC-ICP-MS

In recent years, mercury speciation has mostly been carried out using HPLC-CV-AAS[29,30,31,32]. The HPLC methods involve the formation of alkyl- or tetra-methylenedithiocarbamate and dithizonate complexes of the mercury species[30] before column separation. When lower detection limits are required ICP-AES[20] or ICP-MS[33] have been used with cold vapour systems to detect mercury.

In a study of the speciation of mercury compounds in contact lens solutions[34], a C_{18} column was used to separate the species with a mobile phase containing 0.06 mol L^{-1} ammonium acetate, 3% v/v acetonitrile and 0.005% v/v 2-mercaptoethanol, at a flow rate of 1 ml min^{-1}. The study used post-column mercury cold vapour generation before mercury detection by ICP-MS and reported that methylmercury acetate, mercury (II) chloride and ethylmercury chloride were separated by this method. However, the chromatogram published shows that the $MeHg^{+}$ and Hg^{2+} peaks were not very well resolved. In addition to this the signal to noise ratio was not very high.

Lead and mercury species in urine have been determined using ion-pair chromatography with a C_{18} column and ICP-MS. The ICP-MS was modified using a direct injection nebuliser (DIN) which was placed inside the ICP torch[35]. The DIN is a microconcentric pneumatic nebuliser and the low dead volume and absence of a spray chamber minimise the postcolumn band broadening and facilitate the use of microscale LC columns and liquid flow rates that are low enough for all the column effluent to be introduced into the plasma[36]. The study used a 5 mM solution of ammonium pentanesulphonate in a 20% v/v acetonitrile mobile phase. The results from this method showed that the resolution was better for the lead species than the mercury, with the inorganic and methyl mercury being very poorly resolved in the urine sample.

Better resolution of mercury species was obtained when the separation was carried out on a reversed phase C_{18} column with a mobile phase of 3% v/v methanol, 1.5% v/v acetonitrile, 0.1% v/v 2-mercaptoethanol and 0.06 mol L^{-1} ammonium acetate, at pH 6.8[37]. Using this

method complete resolution of Hg^{2+}, CH_3HgCl and C_2H_5HgCl was obtained. Bloxham et al, in a study of mercury species in seawater by LC-ICP-MS[38], found that by decreasing the content of acetonitrile to 1% v/v the resolution between the $MeHg^+$ and Hg^{2+} peaks was improved. Additional reagents in the mobile phase included 0.06 mol L^{-1} ammonium acetate and 0.005% v/v 2-mercaptoethanol. However, this method used an offline pre-concentration step using a dithiocarbamate resin to improve the detection limits. Even better resolution between $MeHg^+$ and Hg^{2+} was achieved by Wan et al using a cold vapour generator with sodium borohydride and a mobile phase of 0.5% m/v L-cysteine at pH 5[34].

A study of the problems encountered with mercury speciation by LC-ICP-MS[39] found that the use of stainless steel components in the HPLC system led to adsorption of the mercury compounds. It was also found that the spray chamber temperature must be lowered to reduce the production of organic vapour and also oxygen had to be added to reduce the build up of carbon on the cones and torch. For mobile phases containing 60% v/v methanol the spray chamber temperature had to be reduced to $-17^{o}C$ to maintain a workable vacuum level and the addition of 2.5-2.7% v/v oxygen was advised. The study also examined the effect of the addition of 2-mercaptoethanol to the mobile phase and found that the peak height for methyl mercury doubled and the peak height for inorganic mercury increased five-fold. It was concluded that the best mobile phase for the separation of the two mercury species was 60% v/v methanol, 10 mM tetrabutyl ammonium bromide with 0.01% v/v of 2-mercaptoethanol.

This review of previous work carried out on mercury speciation highlighted several potential problems for the speciation of mercury in hair. High concentrations of organic solutions could not be used without the addition of oxygen to the plasma and to add oxygen to the plasma and work at high organic concentrations a new and expensive torch would be required. Therefore, a less involved method requiring low concentrations of organic solvents was needed. The aims of this study therefore, were to develop a method that would allow separation of mercury species using <20% v/v organic solvent without adding oxygen and is fast, reproducible and can resolve methyl mercury and inorganic mercury.

6.2. Lead in Hair

The toxicity of lead compounds to humans has long been recognised and well documented[40,41,42]. It is also known that the organolead compounds are more toxic than the inorganic forms. Total lead in hair has been reported to increase with exposure and elevated levels have been reported in hair[43,44], blood[45,46] and urine[47], however, little work has been performed looking at the different species in these mediums.

It has also been seen that hair lead values correlate with the overall lead burden in the body. An example of this was a case study that was carried out in South India where a high daily intake of lead occurs[48]. In the community where the study was carried out the exposure to lead came from household vessels that were made of 100% lead and used daily to prepare a soup-like meal. Hair and blood samples were taken from the men in the village and the average blood lead level was 220.93 $\mu g\ L^{-1}$ whilst the hair lead level was 61.68 $\mu g\ g^{-1}$. Both values were more than twice the average levels found in unexposed subjects. In another study, hair lead levels and those of other trace elements were determined in samples from occupationally exposed workers in Pakistan[49]. The hair was washed with acetone and water and the data obtained compared with that from unwashed samples. All samples were digested with 5 ml concentrated nitric acid after which 1 ml perchloric acid was added and the sample was diluted to 50 ml with a 2% nitric acid solution. Cobalt, lead, nickel, potassium, sodium and zinc concentrations were determined using an inductively coupled atomic emission spectrometer (ICP-AES) whilst arsenic and mercury levels were determined with a hydride generator coupled to an atomic absorption spectrometer. Arsenic, lead, mercury and nickel appeared at significantly higher levels in the industrial workers' hair than in the control group and were removed to some extent, by washing the hair. Lead was removed the most, with levels being reduced from 9.6 to 5.6 $\mu g\ g^{-1}$ after washing with acetone and water[49]. Foo et al[50] determined lead in the hair and blood of inorganic lead battery workers (n = 188) by electrothermal atomic absorption spectrometry. The samples were washed in 0.1% triton X-100 solution and rinsed with water before being digested with 1 ml of concentrated nitric acid in a Teflon bomb at 120°C for 2h. The hair lead levels were significantly increased in the exposed workers. The correlation coefficient between the hair and blood levels was 0.85. It was also

observed that the lead concentrations determined were slightly higher in the distal end of the hair, however, this was not statistically significant[50].

6.2.1. Lead in the Environment

Lead has been used by humans for as long as 5000 years[40,51,52]. It was recovered in early times as a by-product in the smelting of silver[40]. Large scale lead smelting was revived at the start of the industrial revolution, 1400 years after the decline of the Roman Empire as a result of lead poisoning[52]. The annual production of lead has grown from 9.25×10^7 kg in 1700, to 3.048×10^9 kg in 1992[51]. Approximately 85% of this amount is consumed in the U.S., Canada, Western Europe and Japan, with the U.S. alone accounting for 40% of the total. Much of the increase in the use of lead is attributed to the vehicles, as approximately 60% of lead used in Japan and the West is committed to the manufacture and operation of cars. The production of lead-acid storage batteries accounts for 60% of the total lead consumption, whilst the other major uses of lead include tetraethyl (TEL), triethylmethyl (TEML), dimethyldiethyl (DMDEL) and trimethyl (TML) organic lead compounds as antiknock agents in fuel. The extensive global use of lead antiknock additives in petrol, for over 50 years, has made lead perhaps the most widely distributed toxic heavy metal in the urban environment. The lead released from vehicle exhausts has accounted for more than half of the lead pollution in the entire earth. Whilst most of the pollution was in the form of elemental or inorganic lead compounds, tetraalkylleads (TAL) and other organic compounds made up as much as 20% of the lead pollution in some urban areas. Most of the uncombusted TAL compounds in the atmosphere rapidly undergo photolytic decomposition to ionic elemental lead which settles out in the soil and becomes bound to the organic soil matter. Other sources of organoleads in the environment are vapours that escape from chemical plants, petrol stations and fuel tanks where spills and leaks from such tanks provide a significant amount of pollution. The sludge that accumulates in the bottom of fuel tanks is another potential source of TALs in the environment. Evidence of environmental pollution is the significant amount of TALs in rainwater[53], soil and road dust, whilst further studies have shown TALs in the brains and organs of pigeons and fish[51].

In the environment the decomposition of TAL compounds is believed to occur in sequential stages as $R_4Pb \rightarrow R_3Pb^+ \rightarrow R_2Pb^{2+} \rightarrow Pb^{2+}$, with the decomposition being faster in light than darkness. The ionic R_3Pb^+ and R_2Pb^{2+} are much more stable in aqueous solutions than the R_4Pb species. It is believed that biodegradation of TAL in natural waters, soil and sediments may be responsible for the presence of R_3Pb^+ and R_2Pb^{2+} in soils.

Lead alkyls are potentially mobile in the environment because they exist as liquids over a very wide range of temperatures. They also tend to be very soluble in non-polar solvents but are relatively insoluble in water. Waste containing more than 5 mg L^{-1} of lead is considered hazardous for disposal purposes, while the maximum level of lead in drinking water is 50 $\mu g\ L^{-1}$

Mutagenicity, carcinogenicity and tetragenicity have been reported for alkyl lead compounds. They are extremely lipophilic and are readily absorbed through the skin, lungs and digestive tract, the rate of absorption depending on the target organism, the lead alkyl involved and the exposure time. TAL are the most commonly encountered alkyl lead compounds and they are intrinsically non-toxic and must be converted to trialkyls before toxicity occurs. In humans the conversion of TAL to trialkyls in the liver is rapid, occurring in a matter of minutes, whilst TAL may also be broken down to trialkyls in the stomach by HCl. Trialkyls are more stable than tetra- or dialkyls and may remain in the body for extended periods of time, whereas once the trialkyl is converted to dialkyl, elimination from the body is rapid. Trialkyl lead compounds can damage the central nervous system and triethyl lead is 10 to 15 times more efficient at the destruction of neuronal tissues than trimethyl lead.

6.2.2. The Speciation of Lead Compounds by LC-ICP-MS.

In order to fully assess the impact of lead on environmental and health issues it is necessary to have speciation information available. The different lead compounds need to be identified and quantified and this must be carried out at low levels because of lead's cumulative nature[54].

The majority of the analyses of TAL and ionic alkylleads to date have used complexing agents such as dithiocarbamates, with instrumentation such as GC, GC-AAS, GC-

AED[52,53], graphite furnace with atomic absorption spectrometry (GF-AAS)[47] and HPLC-AAS/GF-AAS[51]. Additionally, speciation of ionic alkyllead species has been accomplished using ultra violet detection, chemical reaction detectors[55,56], differential pulse anodic stripping voltametry and GC-ECD[54,57]. The most widely used technique has been GC-AAS[54], because of its high sensitivity and selectivity, but none of the aforementioned methods can speciate lead in all sample types.

The use of HPLC-ICP-MS for the speciation of lead compounds has been investigated in a number of studies[35,54,58,59,60,61,62,63,64]. However, there have been no studies carried out looking at the different lead species in hair and so it was necessary to look at analytical methods that had been used for other mediums, such as urine[35], water, soil and sediment[71], blood[62] and wine[61]. The aim was to separate lead compounds in hair by LC-ICP-MS.

Although HPLC systems have not been used widely for the separation of lead alkyls, the advantage of using HPLC over GC is that the compounds do not require a derivatisation step. Separations of lead, mercury and cadmium in aqueous solutions have been carried out using a C_{18} column with an acetonitrile/methanol and water mobile phase, containing the chelating agent dithiocarbamate[65,66,67,68]. Tetramethyl and tetraethyl lead in water were separated by HPLC using a mixture of an aqueous mobile phase of $LiClO_4$ (10%) and a solvent mobile phase of methanol and chloroform (90%) on a Lichrospher 60 column. Ion-pair chromatography has also been used to successfully separate two or three alkyllead compounds on C_{18} columns[69]. In some cases the ion-pair separation has been improved with a gradient system. On-column derivatisation methods have also been developed for the simultaneous separation of organic ionic lead and mercury species, on a C_{18} column, with the addition of methyl thioglycolate[69,70]. Atomic absorption spectrometry (AAS) has been used as the detector for the separation of ionic alkyllead compounds in water by HPLC[71,72].

The main problem encountered in this study with methods involving coupled HPLC and ICP-MS systems was separating the lead species using a mobile phase where the organic concentration of the mobile phase was lower than in previous separations. This can be helped in a LC-ICP-MS system, as explained earlier in this chapter, by increasing the Rf power, cooling down the spray chamber and (possibly) introducing oxygen into the plasma[77].

A summary of the LC-ICP-MS methods and conditions used in previous studies for lead speciation is outlined in table 6.1.

Author and Date	Species separated	Column	Mobile Phase	Comments
Ibrahim et al[58] 1984	$(Me)_3Pb^+$ and $(Et)_3Pb^+$ in water and alcohol	C_2 (12 cm,10 μm)	Butanol:ethanol: water (15:35:50) 1 ml min^{-1}	Used ICPAES 1.5 kW, 15 W (reflective power (rp)), 0.2 L min^{-1} carrier gas, concentric nebuliser.
Al-Rashdan et al[54] 1991	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ In mobile phase standards	ODS-2 C_{18} (25cm x 4.6mm, 5μm)	4 mM sodium pentane sulphonate, 70% v/v methanol pH3	ICP-MS, separation of Pb^{2+} and $Pb^+(CH_3)_3$ was poor. 1.35 kW 0 W rp, 0.67 L min^{-1} carrier gas.
Al-Rashdan et al[54] 1991	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ In mobile phase standards	ODS-2 C_{18} (25cm x 4.6mm, 5μm)	0.1M ammonium acetate, 0.1M acetic acid, pH 4.6, 10% v/v methanol for 6 min and then 70% v/v methanol for 10min.	HPLC-ICP-AES Gradient necessary to elute the triethyllead peak rp < 15 W and 1.75 kW gas flow 0.45 L min^{-1}
Al-Rashdan et al[54] 1991	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ In mobile phase standards	ODS-2 C_{18} (25cm x 4.6mm, 5μm)	0.1M ammonium acetate, 0.1M acetic acid, pH 4.6, 30% v/v methanol	ICP-MS, all three peaks eluted, separation between Pb^{2+} and trimethyllead not complete, isocratic.
Al-Rashdan et al[54] 1991	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ In mobile phase standards	Cation exchange column-adsorbosphere SCX, (25cm, 4.6mm, 5μm)	0.2 M acetate buffer, pH7 and 70% v/v methanol	ICP-AES detection, very poor separation of all compounds.

Author and Date	Species separated	Column	Mobile Phase	Comments
Al-Rasdan et al[60] 1992.	Pb^{2+}, Et_3Pb^+, Et_4Pb and triphenyllead chloride, in water.	C_{18} (5 μm, 25 cm, 4.6 mm)	8 mmol L^{-1} sodium pentane sulphonate, 30-90% v/v methanol, pH3.	ICP-MS, poor separation between Pb^{2+} and Et_3Pb^+. 1.4 kW, rp<20 W, 0.67 L min^{-1} flow rate, spray chamber –10°C.
Brown et al[59] 1994	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ with isotope dilution	C_{18} ODS (25 cm x 4.2 mm, 5 μm)	4 mmol L^{-1} sodium pentane sulphonate, buffer 0.1 mol L^{-1} ammonium acetate, and acetic acid, pH 4.6 with up to 40% v/v methanol	ICP-MS 1.5 kW, r.p. < 5kW, 0.7 L min^{-1} flow rate, -15°C using a super cooled spray chamber built in-house, used gradient elution to separate Pb^{2+} and Me_3Pb^+.
Shum et al[35] 1992	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ in urine.	C_{18} (5 cm x 4.6 mm)	5 mmol ammonium pentane sulphonate, 20% v/v acetonitrile, pH3.4.	ICP-MS, mercury compounds separated also. Used a DIN. 0.4L min^{-1} flow rate, 1.4 kW. Again poor resolution between Pb^{2+} and $(Me)_3Pb^+$
Yang et al[64] 1995.	Pb^{2+}, $(Me)_3Pb^+$ and $(Et)_3Pb^+$ in urban particulate matter	C_8 (30 cm x 3 mm, 3μm)	0.1mol L^{-1} CH_3COOH-$CH_3CCOONH_4$, 8% v/v methanol and pH 4.7.	ICP-MS- also used hydride generation after LC column. Good resolution between Pb^{2+}, $(Me)_3Pb^+$, and separation in less than 7 minutes.

Table 6.1: Review of methods for lead speciation by LC-ICP-MS.

Several of the methods reported used high concentrations of organic solvents and also gradient systems with ICP-MS detection. The Hewlett Packard 4500 ICP-MS used in this study could only run methanol concentrations up to 30% v/v, with the forward power increased from 1.2 kW to 1.4 kW, without the reflective power getting too high, i.e. over

10 W. The ICP-MS was not tolerant of more than 5% v/v acetonitrile[73]. The method of choice for the speciation of lead compounds would use an ion-pair reagent, such as sodium pentane sulphonate. Several studies mention using a gradient system with the ICP-MS and the ion-pairing separation. However, it has been stated that a gradient system is not suitable for use with an ion-pair separation[74,75], because the slow equilibrium of the ion-pair reagent on the column would create an unstable system[76]. Gradient separations can also cause the plasma to be unstable[77].

6.3. Experimental

6.3.1. Reagents

All reagents, standards, water and cleaning methods used were as described in sections 4.2.1 and 3.2.1. All standards for the hair analysis were prepared in same matrix as the hair digest using 2 ml concentrated nitric acid and 1 ml hydrogen peroxide (30% v/v) diluted to 10 ml with ultrapure deionised water. The inorganic mercury was prepared from a stock solution of 1000 mg L^{-1} ICP Aristar standard in 2% v/v HNO_3, supplied by BDH (Poole, Dorset, UK) and a stock solution of 1000 mg L^{-1} of the methyl mercury chloride from Riedel-de-Haen (Seelze, Germany) was made by dissolving 0.125g in 10 ml of 10% v/v HNO_3. The inorganic lead was prepared from a stock solution of 1000 mg L^{-1} (ICP Aristar standard in 2% in HNO_3, BDH, Poole, Dorset, UK) and a stock solution of 1000 mg L^{-1} of the tetraethyllead from Alfa Chemicals (Alfa Aesar, Karlsruhe, Germany) was made by diluting 0.0944 ml in 100 ml methanol under nitrogen. A stock solution of 1000 mg L^{-1} triethyllead also supplied by Alfa Chemicals was made by dissolving 0.1592g in 100 ml of 10% HNO_3.

The sodium lauryl sulphate was prepared daily by adding 0.5 g to 50 ml of deionised water and the 0.1M HCl was prepared from Aristar grade HCl 1.18gr., from BDH (Poole, Dorset, UK) and diluted accordingly with deionised water.

Additional chemicals were HPLC grade methanol (99.9% v/v) and 2-mercaptoethanol (Analar grade) which were also supplied by BDH. Ammonium acetate (99.99%) was obtained from Aldrich Chemical Company (Milw. USA) and, being hygroscopic, was stored under nitrogen gas each time it was opened. The mobile phase was made fresh each

day. Initial mobile phases used 5mM sodium 1-butane sulphonate anhydrous, from Lancaster Synthesis (Morecambe, UK) and acetic acid from BDH.

6.3.2. Safety Precautions

The methyl mercury and alkyl lead compounds were opened and stored in an isolated fume cupboard. The fume cupboard was labelled with the appropriate toxic symbols. The author wore a double layer of latex gloves, a disposable apron and a mask when working with the compounds. The plastic pipette tips and weighing boats used when preparing solutions were disposed of in toxic hazard bins and all used solutions were labelled and sent to be disposed of by an authorised hazards disposal company.

6.3.3. Instrumentation, Column and Mobile Phase

6.3.3.1. ICP-MS

The inductively coupled mass spectrometer used was the Hewlett Packard 4500. The instrumental conditions for mercury speciation are described in table 6.2 and the conditions for lead speciation are outlined in section 6.3.3.2. The instrument was tuned daily with a 10 ng ml^{-1} solution of lithium, caesium, yttrium and tellurium. The conditions for LC-ICP-MS for mercury speciation are also listed in table 6.2.

6.3.3.2. Mercury Speciation

An isocratic liquid chromatography system was used, the pump was a Dionex GPM 2 (Dionex Corporation, Sunnyvale, California, USA) and the samples were injected through a Rheodyne 7125 injector with a 20 µl sample loop. The initial work was carried out using reversed phase C_{18} columns ODS2 and ODS1 'Hypersil' supplied by Supelco (Sigma-Aldrich Co.Ltd, Dorset, UK) (250 x 4.6 mm, 5µm). The column used for the optimised conditions and real sample separations was a Novapak C_{18} reversed phase column (150 x 3.9 mm, 4 µm) which was comprised of dimethyloctadecylsilyl bonded amorphous silica (Waters HPLC Columns, Milford, MA, USA). The column was initially connected with PTFE tubing directly to the pumps on the ICP-MS which in turn entered directly into the nebuliser. However, it was found subsequently that the flow on the LC was sufficient to pump the mobile phase into the nebuliser without the need to use the ICP-MS pumps.

Different mobile phases were tested for their suitability for mercury speciation. Firstly, the ion-pairing method for both lead and mercury using 10% v/v acetonitrile or 10% v/v methanol and sodium butane sulphonate was studied. Then, for mercury speciation, mobile phases containing ammonium acetate with methanol, acteonitrile and 2-mercaptoethanol were used. The optimised mobile phase conditions for mercury speciation are shown in table 6.2. The column was equilibrated with the mobile phase for 1h everyday before use and flushed overnight with 50% v/v methanol in water.

ICP-MS conditions		
	For Total Analysis	For Hg Speciation
Rf Power/W	1200	1200
Carrier Gas Flow Rate L min^{-1}	1.27	1.15
Sample Depth / mm	6.0	6.0
Pump Speed / rps	0.12	0.3
Acquisition / seconds	1	2 using time resolved analysis
LC conditions for mercury speciation		
Column	C_{18} reversed phase (150 x 3.9 mm, 4 μm)	
Mobile phase	0.06 mol L^{-1} ammonium acetate, 5% v/v methanol, 0.1% v/v 2-mercaptoethanol.	
Mobile phase flow rate	1 ml min^{-1}	
Helium pressure	10 psi	

Table 6.2. Instrumental conditions for the determination of mercury and mercury species using ICP-MS and LC-ICP-MS respectively.

6.3.3.3. Lead Speciation

The initial work (using mobile phases 1-5 in table 6.3) was carried out using several reversed phase C_{18} columns (ODS2 and ODS1 'Hypersil', 250 x 4.6 mm, 5 μm particles). The column used for the optimised conditions (mobile phase 6 in table 6.3) and real samples was a 150 x 4.9, 4 μm particles, 'HyPurity Advance' C8 reversed phase column

(ThermoQuest, Chromatography Suppliers, Runcorn, Cheshire, UK). With mobile phase 6 (see table 6.3) a Rf power of 1.35 kW, pump speed of 0.3 rps and flow rate of 1 ml min^{-1} were used.

Parameters on the LC-ICP-MS system that were altered in investigating the lead speciation methods were the Rf power, which was increased to 1.4 kW from 1.2 kW, the carrier gas flow rate, which was reduced from 1.15 L min^{-1} to 0.7 L min^{-1} and the spray chamber, which was cooled from 2°C to -3°C.

	Mobile phase used
Mobile phase 1	10% v/v methanol, 5mM sodium 1-butane sulphonate, pH 4.6 (adjusted with 5% HNO_3).
Mobile phase 2[54]	30% v/v methanol, 5mM sodium 1-butane sulphonate, pH 4.6 , in buffer solution of 0.1 mol L^{-1} ammonium acetate and acetic acid.
Mobile phase 3[59]	The buffer mobile phase consisted of 0.1 mol L^{-1} ammonium acetate and 5mM sodium pentane sulphonate, adjusted to pH 4.6 with concentrated acetic acid.
Mobile phase 4	Separation procedure based on Shum et al[35], a 10% v/v acetonitrile mobile phase was used (they used 20% v/v) with 5mM sodium pentane sulphonate at pH 4.6.
Mobile phase 5	Dithiocarbamates had been used to chelate metal ions in previous studies[67,68] and so it was attempted in this study using a Hypersil C_{18} column. Sodium diethyldithiocarbamate (0.05% w/v) was added to a mobile phase of 20% v/v methanol.
Mobile phase 6	15% v/v methanol, 0.1M ammonium acetate and 0.1M acetic acid. This was using the 'Hypersil Advance' C_8 column.

Table 6.3. Methods investigated using LC-ICP-MS for lead speciation in hair samples.

6.3.4. Hair Samples

Hair samples for mercury speciation studies were cold digested using a mixture of 2:1 HNO_3:H_2O_2 (concentrated HNO_3 and 30% H_2O). The digest was diluted to 10 ml with

deionised water and injected unaltered directly onto the column. For lead speciation studies the hair samples were microwave digested as described in Chapter 3. Occupational samples from Chapter 7 were washed using the 0.1M HCl method as described in Chapter 4.

To study the uptake of mercury species hair was spiked with methyl mercury and inorganic species in both water and simulated sweat solutions. 0.2 g hair was soaked in 3 ml of spiked water or simulated sweat solution for 24h as described in section 4.2.4. The water was deionised, the sweat solution was prepared as described by Hopps[78] and both were spiked with methyl mercury and inorganic mercury. After 24h the hair was transferred to a weighing boat and dried for a further 24h in a fume cupboard. The hair samples were then washed with 1% SLS and 0.1M HCl as previously described (section 4.2.2.2.) and cold digested.

Hair samples from occupationally exposed subjects (see Chapter 7) were analysed by LC-ICP-MS a standard calibration graph was obtained before running the samples and then a standard run every hour. If the peak areas of the standards varied by more than 5% then the system was recalibrated.

6.4. Results and Discussion

6.4.1. Initial LC-ICP-MS Conditions Investigated

Initially a method that would allow the speciation of both lead and mercury compounds, based on the method by Shum et al[35], was investigated, using mobile phases 1 and 4 in table 6.3. Their method had involved ion-pair chromatography, on a microbore C_{18} column (50 x 1.6 mm), with a mobile phase consisting of 20% v/v acetonitrile and the addition of a DIN to the ICP-MS. However, it was found in this work that the ICP-MS would not tolerate 20% v/v acetonitrile, even at the increased Rf power recommended. Using a mobile phase of 10% v/v acetonitrile with 5 mM sodium 1-butane sulphonate at pH 3.4 on an ODS2 C_{18} column no mercury signals were detected when solutions containing Hg^{2+} and Pb^{2+} in 10% v/v HNO_3 were injected onto the column. This was also the case when a mobile phase containing 10% v/v methanol with 5 mM sodium 1-butane sulphonate at pH 4.6 (mobile phase 1, table 6.3) was applied to the LC-ICP-MS system and the same standards injected. Using the latter mobile phase it was apparent that inorganic lead was

easily detectable with the ion-pair reagent and so this was investigated further (see section 6.5).

Inorganic mercury could be detected when 2-mercaptoethanol was added to a methanol mobile phase instead of the ion-pair reagent, as was reported in previous work[37,79]. Unfortunately, no lead was detected using this method. It was seen however, that methyl mercury could be separated from inorganic mercury using this method, with methyl mercury eluting first at approximately 23 minutes, as shown in figure 6.1. The standards used were prepared in a 2:1 HNO_3:H_2O_2 matrix to match the hair sample matrix.

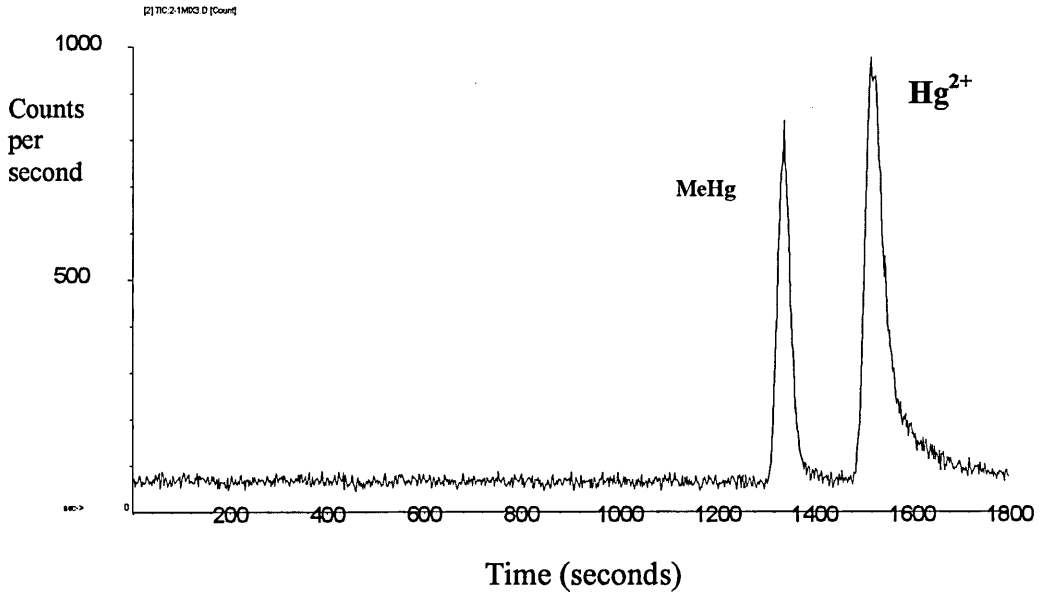

Figure 6.1. Chromatogram of the separation of a mixture containing 100 ng ml^{-1} of Hg^{2+} and $MeHg^{+}$ standards in 2:1 HNO_3:H_2O_2 on an ODS2 C_{18} column with a mobile phase consisting of 3% v/v methanol, 1.5% v/v acetonitrile, 0.1% v/v 2-mercaptoethanol, in 0.06 mol L^{-1} ammonium acetate at pH 6.8 (adjusted with 5% HNO_3).

In order to reduce the retention times, a shorter C_{18} column was then used (Novapak 150 x 3.9 mm). As shown in figure 6.2, the mercury species were resolved and both eluted in less than 13 minutes. The same elution order has been reported by Huang et al[37].

An increase in the reflective power on the ICP-MS was observed with the use of this mobile phase and the plasma was burning green, which is an indication of the presence of

C_2. Others have reported such occurrences but with >50% v/v acetonitrile[35], and so the acetonitrile content in the mobile phase was replaced with 5% methanol.

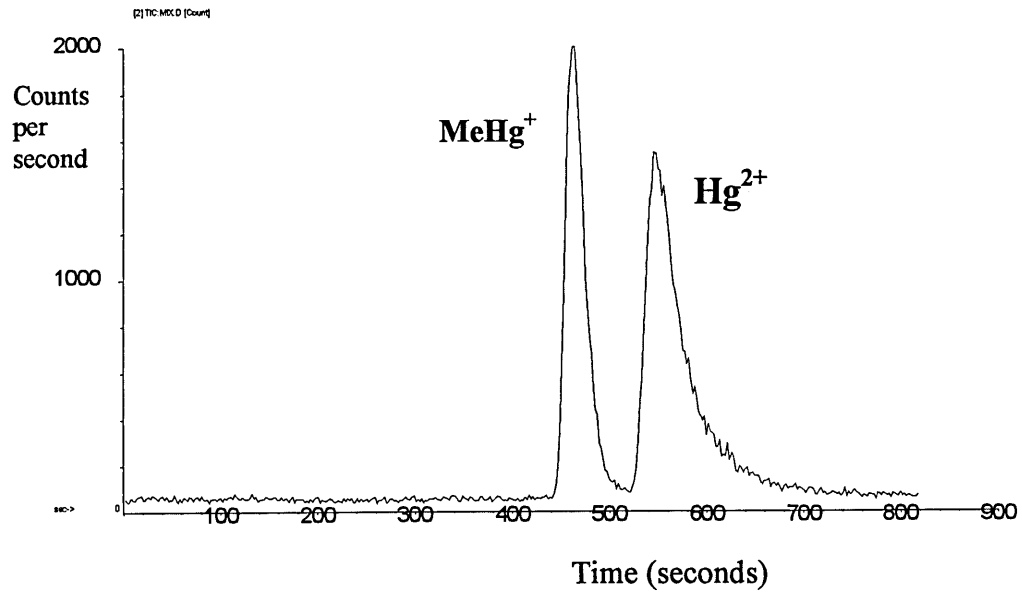

Figure 6.2. Chromatogram showing the separation of 100 ng ml^{-1} of Hg^{2+} and $MeHg^{+}$ standards in 2:1 HNO_3:H_2O_2 with a mobile phase containing 3% v/v methanol, 1.5% v/v acetonitrile, 0.1% v/v 2-mercaptoethanol and 0.06 mol L^{-1} ammonium acetate at pH 6.8. using the Novapak 15cm C_{18} column.

6.4.2. Optimisation of the LC-ICP-MS Conditions for Mercury Speciation

In order to maximise the mercury signal from both species it was necessary to optimise the ICP-MS parameters as the performance of an ICP-MS depends strongly on the operating conditions. The two key parameters are the aerosol gas flow rate and the plasma forward power[37,80]. Therefore a mixed standard was injected using a range of settings for the Rf power and carrier gas flow rates on the ICP-MS, and the results are shown in figures 6.3 and 6.4.

It was not necessary to alter the Rf power as the optimum counts for both species were obtained at the normal setting of 1200 W, as shown in figure 6.3. The carrier gas setting was altered from the normal setting of 1.27 L min^{-1} to the optimised value of 1.15 L min^{-1}, as shown in figure 6.4. The calibration graphs obtained using these conditions were linear

with correlation coefficients (r^2) of 0.9911 and 0.9937 for Hg^{2+} and CH_3Hg^+ respectively, as shown in figure 6.5.

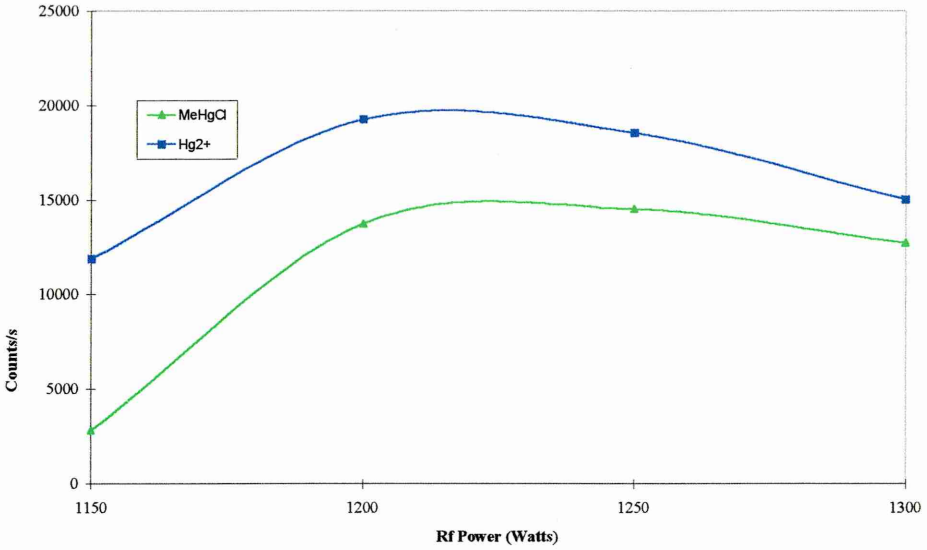

Figure 6.3. Optimisation of ICP-MS Rf power for mercury speciation by LC-ICP-MS with 100 ng ml^{-1} standards of $MeHg^+$ and Hg^{2+} in 2:1 HNO_3:H_2O_2 matrix.

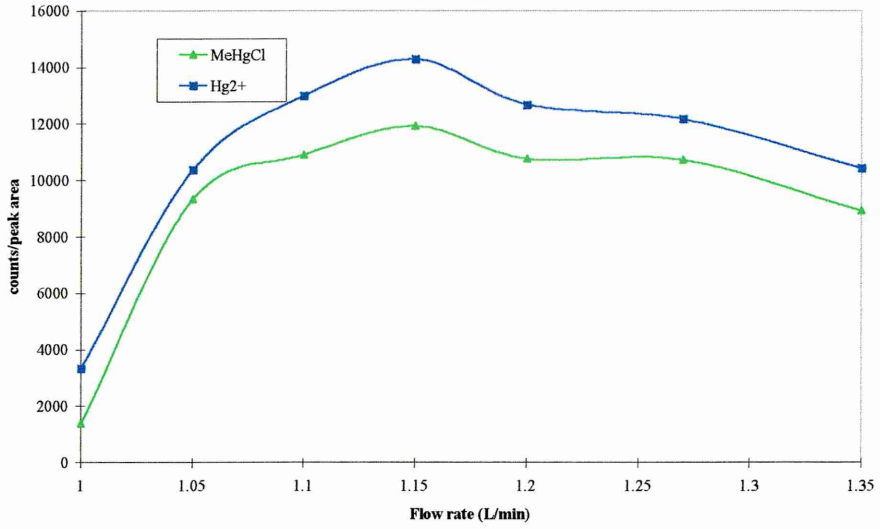

Figure 6.4. Optimisation of carrier gas flow rate for mercury speciation by LC-ICP-MS with 100 ng ml^{-1} standards of $MeHg^+$ and Hg^{2+} in 2:1 HNO_3:H_2O_2 matrix.

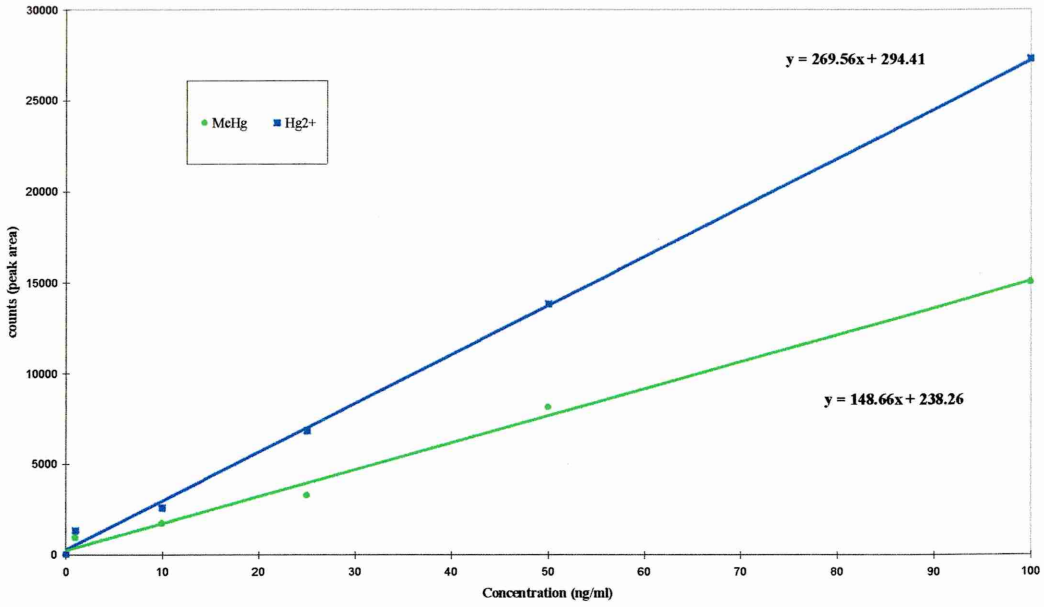

Figure 6.5. Calibration of $MeHg^{+}$ and Hg^{2+} in 2:1 HNO_3:H_2O_2 by LC-ICP-MS using mixed standards and separation on Novapak C_{18} column with a mobile phase of 5% v/v methanol and 0.1% 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate.

Chromatograms of mixed standards containing 25 ng ml^{-1} and 100 ng ml^{-1} $MeHg^{+}$ and Hg^{2+} obtained using the optimised conditions shown in figures 6.6 and 6.7 respectively.

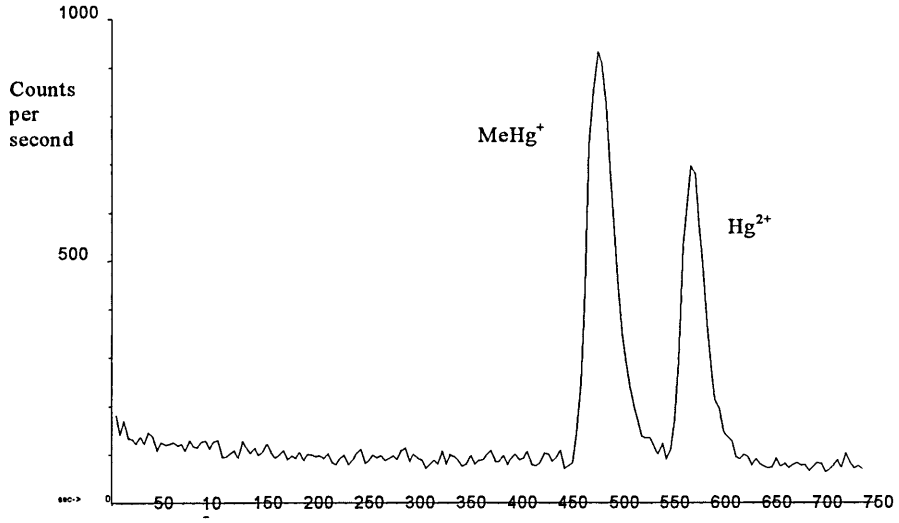

Figure 6.6. Chromatogram showing the separation of 25 ng ml^{-1} Hg^{2+} and $MeHg^{+}$ standards in 2:1 HNO_3:H_2O_2 with a mobile phase consisting of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8, using the Novapak (150 x 3.9 mm) C_{18} column.

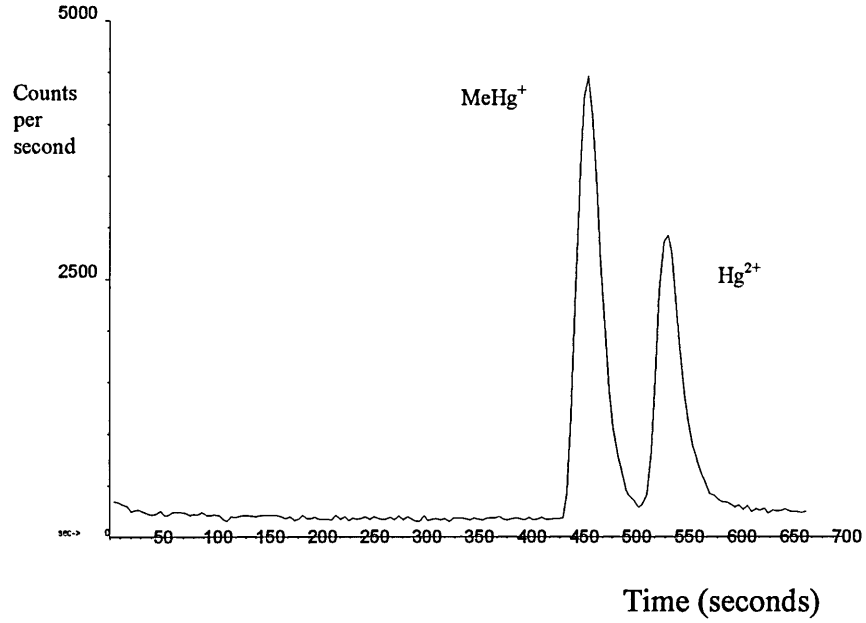

Figure 6.7. Chromatogram showing the separation of 100 ng ml^{-1} Hg^{2+} and $MeHg^{+}$ standards in 2:1 HNO_3:H_2O_2 with a mobile phase consisting of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8, using the Novapak (150 x 3.9 mm) C_{18} column.

6.4.3. Speciation of Mercury in Hair

6.4.3.1. Hair Sample Digests

In order to test whether methyl mercury remained intact on digestion, both cold and microwave digestions of hair samples were performed. The cold digested samples were hair samples digested overnight in a sealed conical tube in the same mixture of 2:1 HNO_3:H_2O_2 used in the microwave digestion studies.

It was observed in all of the hair samples (n=3) analysed that the methyl mercury had been destroyed/converted to Hg^{2+} in the hair digest when the hair had been microwave digested but could be seen after cold digestion. This is apparent from figures 6.8 and 6.9, which show the results from one of the hair samples.

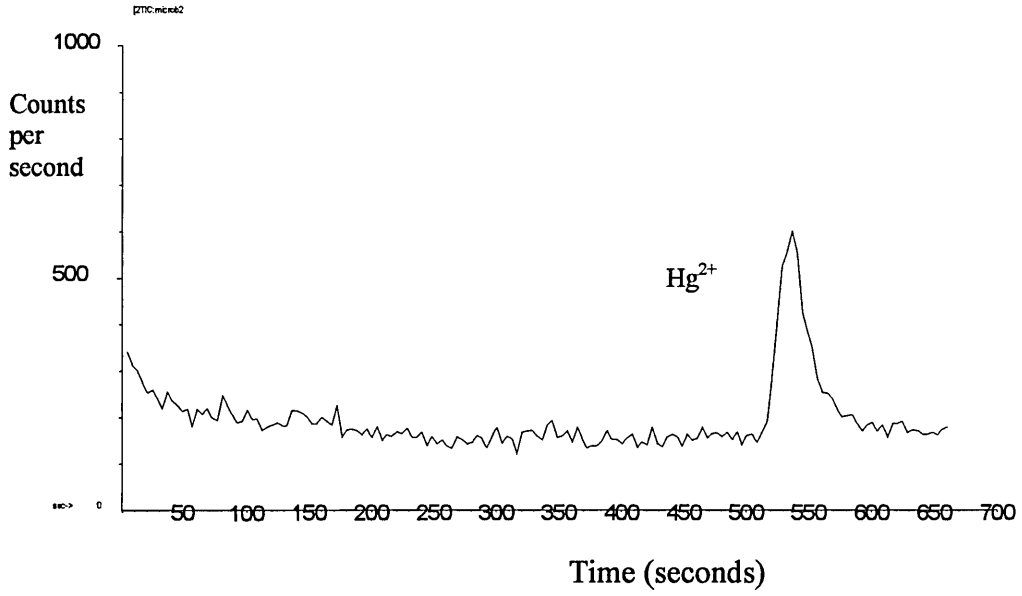

Figure 6.8. Chromatogram of a hair sample microwave digested using 2:1 HNO_3:H_2O_2 and analysed by LC-ICP-MS with a mobile phase consisting of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8, using the Novapak (150 x 3.9 mm) C_{18} column.

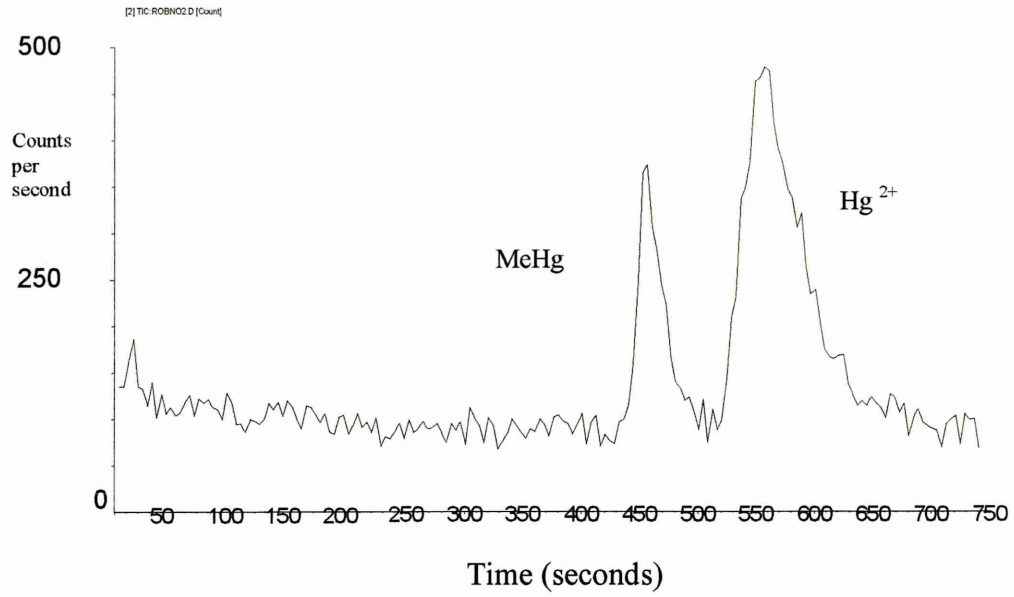

Figure 6.9. Chromatogram of a hair sample cold digested using 2:1 HNO_3:H_2O_2 and analysed by LC-ICP-MS with a mobile phase consisting of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8, using the Novapak (150 x 3.9 mm) C_{18} column.

Both the cold and the microwave digests of the three hair samples were analysed by ICP-MS to compare the total mercury concentrations and the results are shown in table 6.4. The results show that the cold digest total mercury concentrations are slightly lower than the microwave digests. Paired t tests (see section 3.2.5) confirm that only Hair 2 shows a significantly lower total mercury concentration in the cold digest from that in the microwave digest at 95% confidence intervals.

	Total mercury concentration in hair after microwave digestion (ng g^{-1})	Total mercury concentration in hair after cold digestion (ng g^{-1})
Hair 1	1034 ± 20	916 ± 138
Hair 2	767 ± 49	549 ± 91
Hair 3	843 ± 37	799 ± 141

Table 6.4. Comparison of the total mercury concentrations in hair samples after microwave and cold digestion with 2:1 HNO_3:H_2O_2 and analysis by ICP-MS.

As the methyl mercury was destroyed by microwave digestion it was necessary to see whether any degradation was occurring during the cold digestion. A methyl mercury 100 ng ml^{-1} standard was cold digested overnight, using the same method as used for the hair samples and then compared with an undigested standard of methyl mercury. The results of this are shown in figures 6.10 and 6.11. It can be seen that most of the methyl mercury remains intact after an overnight cold digestion, with less than 15% of the methyl mercury standard being degraded to Hg^{2+}.

When a 100 ng ml^{-1} mixed standard of $MeHg^{+}$ and Hg^{2+} was digested overnight in 2:1 HNO_3:H_2O_2 97% of the $MeHg^{+}$ and 100.7 % of the Hg^{2+} were recovered. Hair samples were spiked with the same 100 ng ml^{-1} mixed standard of $MeHg^{+}$ and Hg^{2+} and cold digested overnight with 2:1 HNO_3:H_2O_2. Recoveries of 63.2 ± 35.3% for methyl mercury and 107.6 ± 14.1% inorganic mercury recovery were obtained. Recovery in hair was lower than the value found when no hair was present.

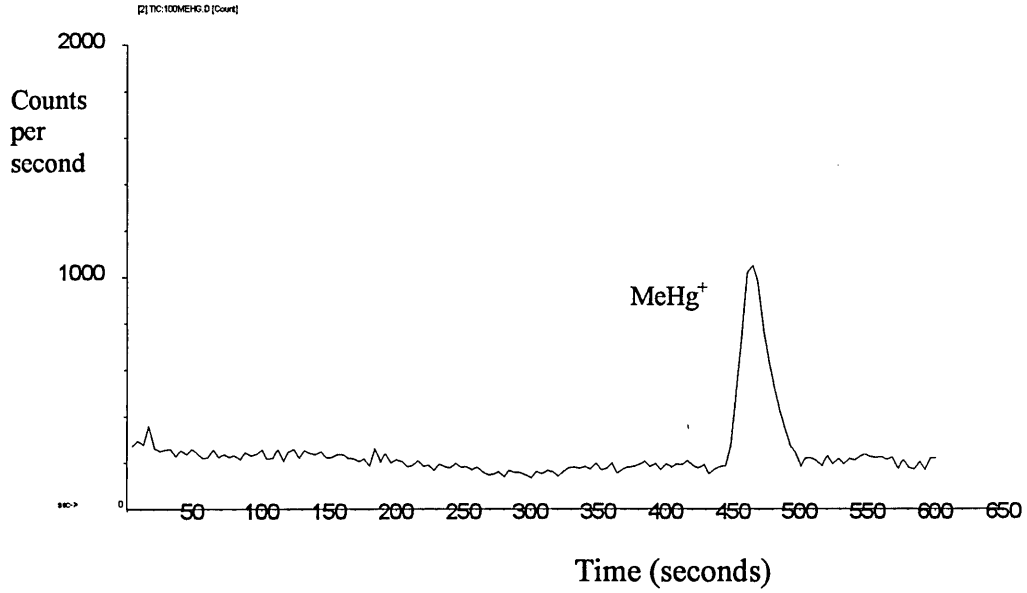

Figure 6.10. A chromatogram of an undigested standard of 100 ng ml^{-1} MeHgCl analysed by LC-ICP-MS with a mobile phase consisting of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8, using the Novapak (150 x 3.9 mm) C_{18} column.

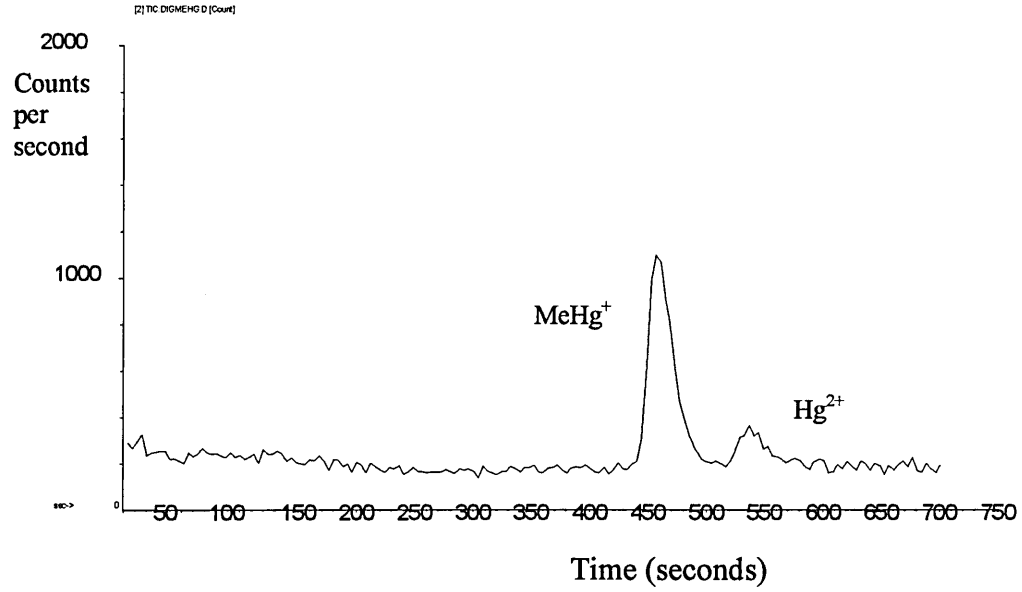

Figure 6.11. Chromatogram of 100 ng ml^{-1} MeHgCl cold digested with 2:1 HNO_3:H_2O_2 and analysed by LC-ICP-MS with a mobile phase consisting of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8, using the Novapak (150 x 3.9 mm) C_{18} column.

6.4.3.1. Reproducibility of LC-ICP-MS Method

The reproducibility of the separations was determined by injecting a cold digested hair sample three times and recording the peak area and retention times. The relative standard deviation for the peak areas was found to be less than 5% for all the species, whilst the relative standard deviation for the retention times was found to be less than 1%.

6.4.4. Mercury Uptake in Hair from Spiked Solutions

6.4.4.1. Water Spikes

From Chapter 4, it is known that mercury binds readily to hair (see table 4.8) and that at best 30% of the adsorbed mercury is removed with 0.1M HCl. It was necessary therefore to investigate whether the mercury being removed was in the inorganic or organic form. Six hair samples from the same donor were spiked with a 10 μg ml^{-1} water standard as described in section 6.3.4. Two of the hair samples were then washed by the 1% SLS method and two by the 0.1M HCl method as described in Chapter 4. The other two hair samples were left unwashed. The samples were then dried overnight and cold digested the

following day. The digested hair samples, which were diluted to 50 ml with water and the washing fractions collected were then speciated directly by LC-ICP-MS. The results of this experiment are outlined in table 6.5.

The results show that both $MeHg^+$ and Hg^{2+} bind to the hair, but more of the Hg^{2+} is adsorbed. With the SLS wash, about 23% of the bound $MeHg^+$ is removed in contrast to 13% of the inorganic Hg^{2+}. More $MeHg^+$ is removed by the HCl (about 45%) than the SLS solution. This is also reflected in the washings, where there is more $MeHg^+$ in the HCl washings compared with the SLS washings. Adsorbed inorganic mercury was hardly removed by the SLS wash (13%) whereas none was removed in the HCl wash.

Hair Samples	$MeHg^+$ concentration ($ng\ g^{-1}$)	Hg^{2+} concentration ($ng\ g^{-1}$)
Unspiked	223	262
Spiked	1907 ± 12	9307 ± 30
Spiked and washed by 1% SLS	1470 ± 66	8079 ± 286
1% SLS wash fraction	167 ± 59	1455 ± 225
Spiked and washed by 0.1M HCl	1051 ± 41	9845 ± 951
0.1M HCl wash fraction	1039 ±648	388 ± 22

Table 6.5. The concentration of methyl and inorganic mercury in the unspiked hair and the hair samples spiked with the water standard which were left unwashed and washed with 1% m/v SLS and 0.1M HCl solutions. The concentration of the mercury species in the SLS and HCl washings are also shown.

6.4.4.2. Simulated Sweat Spikes

The previous experiment was repeated using the sweat solution described in section 6.3.4. The solution was spiked with 1 $\mu g\ ml^{-1}$ of methyl and inorganic mercury. Duplicate samples were used.

The results, presented in table 6.6, suggest that both inorganic and organic mercury bind to the hair and that the 0.1M HCl wash will remove 65% of the inorganic mercury and all of the methyl mercury. It is also apparent from the data in table 6.6 that Hg^{2+} again binds

more readily to hair than $MeHg^+$. The amount of inorganic mercury that has bound to the hair is higher in the sweat solution than in the water solution, even though a lower concentration of mercury species was used in the simulated sweat solution than in the water spike, at 1 μg ml^{-1} compared with 10 μg ml^{-1}. More adsorbed inorganic mercury was removed with the HCl wash in this experiment, possibly as a result of competition for sites with the other doubly charged ions present in the sweat solution. The removal of all of the methyl mercury when the samples were washed with HCl suggests that methyl mercury is bound less tightly to hair than inorganic mercury, which is an indication that methyl mercury binds to hair through ion exchange. This is in agreement with results from other studies[23].

This finding also agrees with the work carried out by Zahn et al[81] who investigated the binding of methyl mercury to the sulphur containing amino acids in hair. They reported that the cystine and cysteine residues of hair bind the iodide form of methyl mercury in the presence of sulphite and urea. In doing so the amino acids can be quantified by the amount of binding that takes place. However, their work does not report that this binding of methyl mercury (see section 1.5) could be reversible.

	$MeHg^+$ concentration (ng g^{-1})	Hg^{2+} concentration (ng g^{-1})
Normal Hair Value (ng g^{-1})	280	353
Spiked hair concentration (ng g^{-1})	476 ± 261	13272 ± 3640
Spiked hair concentration after washing with 1% SLS method (ng g^{-1})	532 ± 185	16462 ± 5633
Spiked hair concentration after washing with 0.1M HCl method (ng g^{-1})	No peak	4757 ± 3448

Table 6.6. Concentrations of inorganic and methyl mercury in hair spiked with 1 μg ml^{-1} of inorganic and methyl mercury in a simulated sweat solution. Hair values are compared for unspiked hair, spiked hair and spiked hair samples washed using HCl and SLS.

The removal of externally bound methyl mercury by HCl was further confirmed when the actual washings were analysed by LC-ICP-MS. The 'washings' were directly injected onto the column. Figure 6.12 shows the chromatogram of the 1% SLS wash fraction where there is a greater amount of Hg^{2+} present than $MeHg^{+}$, whereas the chromatogram of the 0.1M HCl wash fraction in figure 6.13, shows that much more of the $MeHg^{+}$ is washed off using this method.

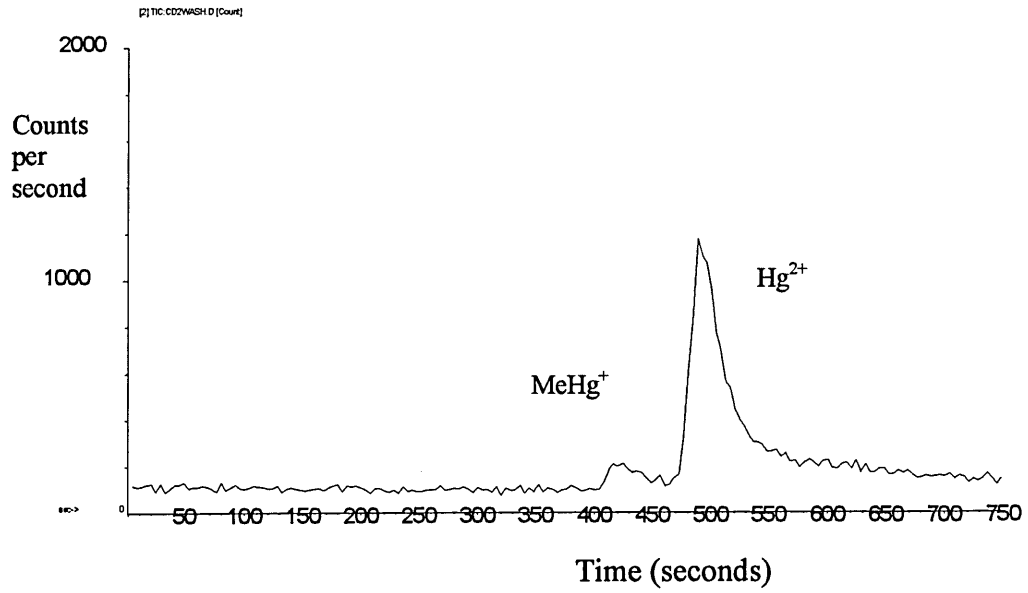

Figure 6.12. Chromatogram of a SLS wash fraction of spiked hair wash, analysed by LC-ICP-MS with a mobile phase of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate.

These results show that it is not possible to differentiate between endogenous and exogenous methyl and inorganic mercury in hair. As inorganic mercury adsorbs readily from the spiked solution and only 64% of this can be removed by HCl it would not be possible to determine how much mercury in hair is externally bound. Although all of the adsorbed methyl mercury from the sweat solution is removed with the HCl, the internally bound species were also removed. Consequently it is not possible to distinguish what is internally or externally bound to the hair.

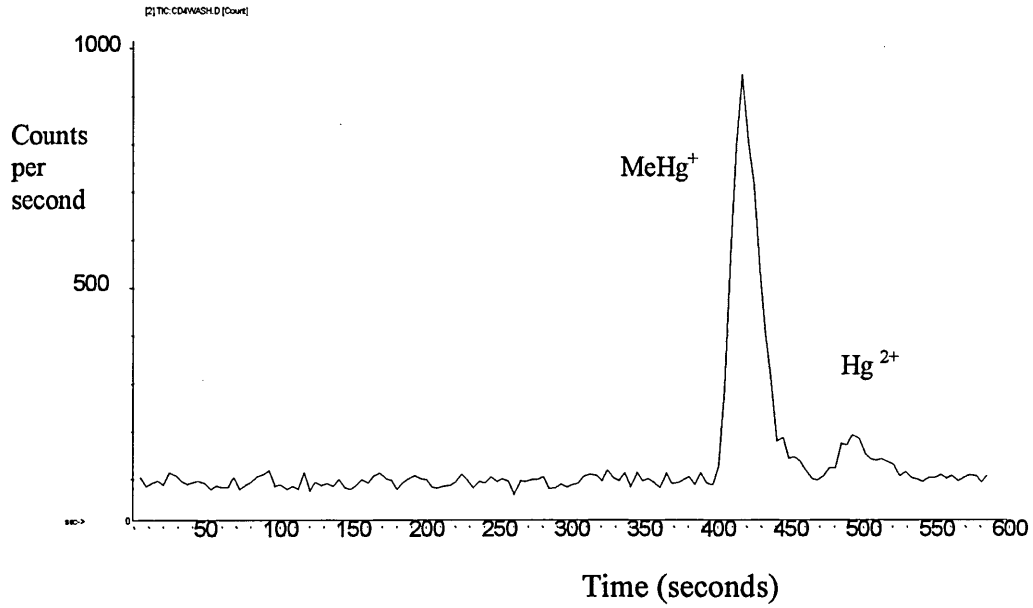

Figure 6.13. Chromatogram of a HCl wash fraction of spiked hair wash, analysed by LC-ICP-MS with a mobile phase of 5% v/v methanol, 0.1% v/v 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate.

6.4.5. Chemical Speciation of Mercury Species in Occupationally Exposed Hair

6.4.5.1. Glasgow Hair Samples

Hair samples were collected from dentists and dental workers (see Chapter 7) and where there was enough hair sample the sample was cold digested with 2:1 HNO_3:H_2O_2 and analysed by the optimised LC-ICP-MS method.

Some occupational samples were provided by the Health and Safety Laboratory in Glasgow. The laboratory in Glasgow had analysed the hair by CV-AAS to determine the mercury concentration and the results in table 6.7 compare their values with the values found in this study by the LC-ICP-MS method.

Chromatograms obtained from samples in this study can be seen in figures 6.14 – 6.16, where it is apparent that only hair samples containing levels of total mercury above 500 ng g^{-1} can be quantified by the LC-ICP-MS method. The sample sizes in this study were not sufficient to analyse reliably i.e. it was not possible to duplicate the results nor was it possible to get total mercury analysis from the ICP-MS.

Mass of hair sample cold digested (g)	Hair mercury concentration (ng g^{-1}) determined by LC-ICP-MS	Hair mercury concentration (ng g^{-1}) determined by CV-AAS
0.0449	887	900
0.0450	1355	1100
0.0271	7049	3500
0.0435	2239	1700
0.0174 *	1847	1300
0.0169 *	943	1100

*digested with 1ml HNO_3 and 0.5ml H_2O_2, and diluted to 5ml due to small sample size.

Table 6.7. Comparison of mercury concentrations in dental hair samples determined by LC-ICP-MS and CV-AAS.

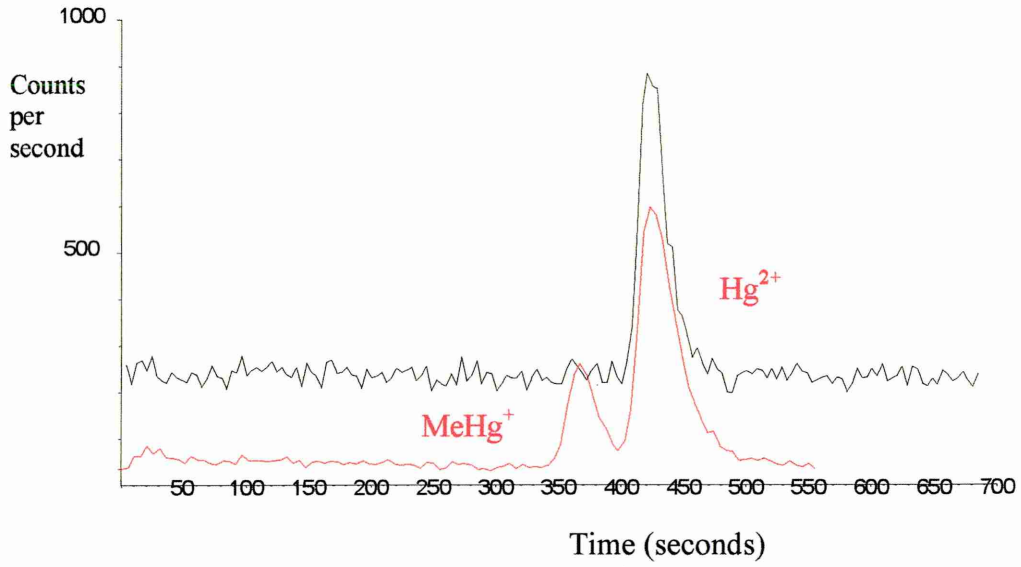

Figure 6.14. Chromatogram from a hair sample from a dentist, with a total mercury concentration of 1300 ng g^{-1} determined by the Glasgow laboratory and a concentration of 1847 ng g^{-1} by LC-ICP-MS. A 25 ng ml^{-1} mixed standard from the day of analysis is superimposed (red line) onto the chromatogram to confirm the identities of the peaks.

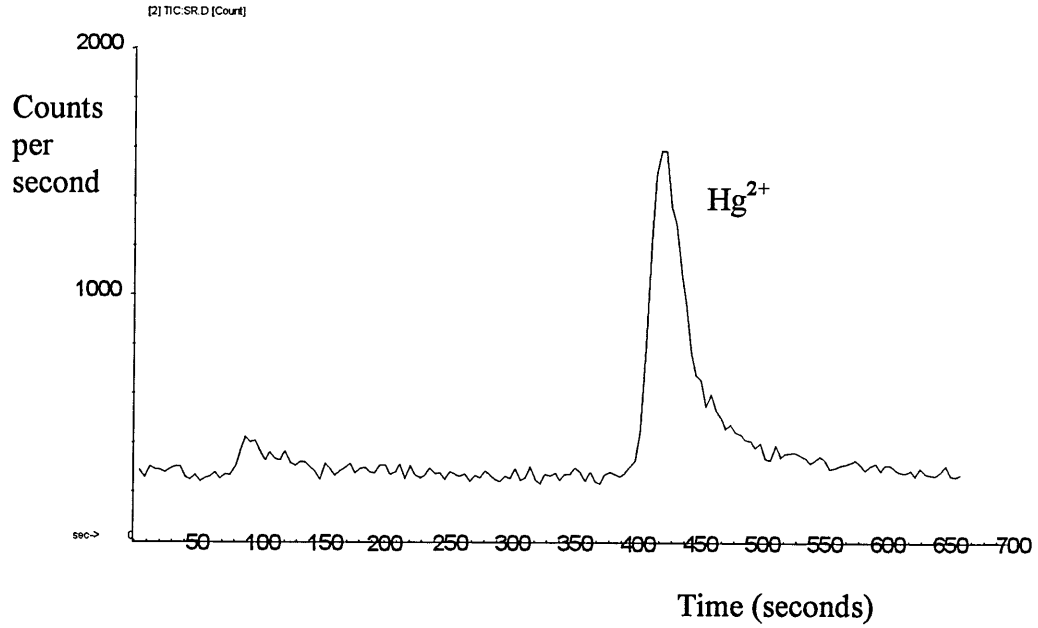

Figure 6.15. Chromatogram from a hair sample from a dentist, with a total mercury concentration of 3500 ng g^{-1} determined by the Glasgow laboratory and a concentration of 7049 ng g^{-1} by LC-ICP-MS.

Although it is possible to distinguish the inorganic mercury peak from the background in a chromatogram form hair sample from a dentist with 400 ng g^{-1} total mercury in their hair, shown in figure 6.16, it was not possible to see a definite peak that could be quantified.

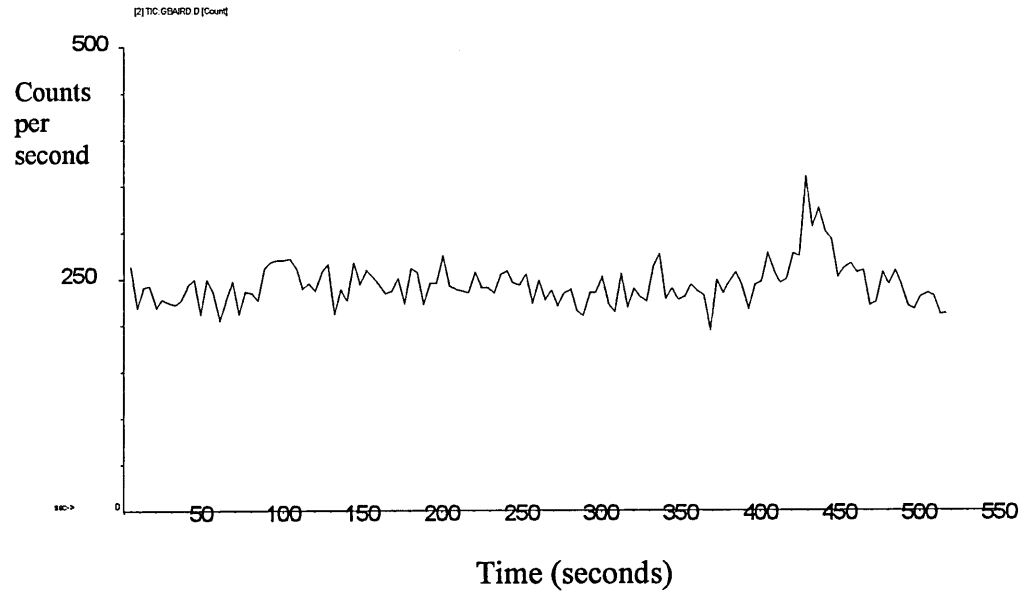

Figure 6.16. Chromatogram of a hair sample with a total mercury concentration of 400 ng g^{-1} determined by the Glasgow laboratory analysed by LC-ICP-MS.

6.4.5.2. Sheffield Hair Samples

The second study of dentists was carried out with samples from eight dentists from Sheffield (details in Chapter 7). The samples were both microwave and cold digested and both of the digests were run on the ICP-MS to give total mercury values, before the cold digests were run on the LC-ICP-MS. Six of the dentists were dental surgeons and did not show high amounts of mercury in the total analysis, which meant that their samples could not be speciated by the LC-ICP-MS. However, one of the two remaining dentists' samples showed a considerable exposure to mercury in both the total analysis and the LC-ICP-MS studies. The total mercury level was 3754 ng g^{-1} by ICP-MS with 501 ng g^{-1} of this being $MeHg^{+}$ and 2850 ng g^{-1} being Hg^{2+} determined by LC-ICP-MS, as shown in figure 6.17.

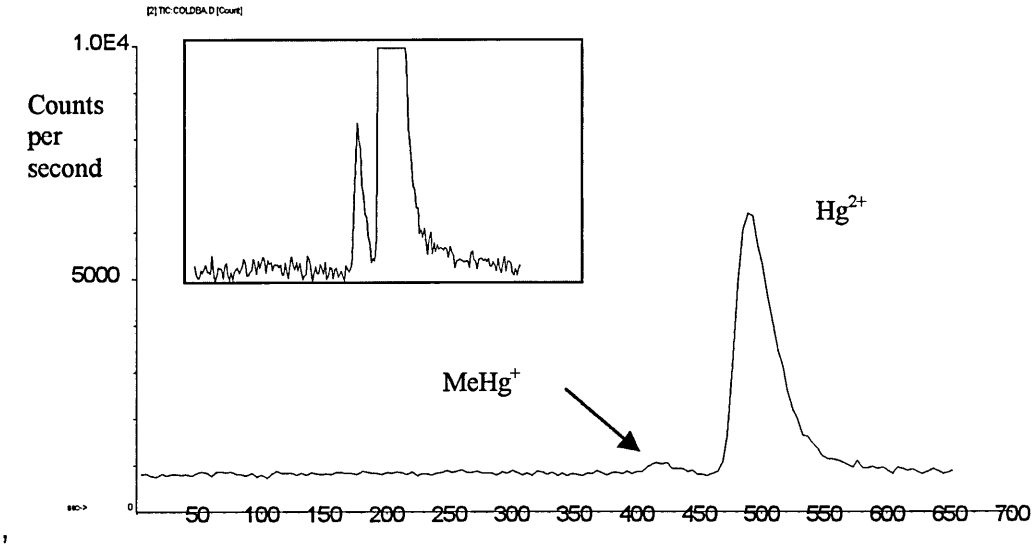

Figure 6.17. Chromatogram from a dentist's hair sample with a total mercury concentration of 3754 ng g^{-1} separated using LC-ICP-MS. The inset is an expansion of the lower area of this chromatogram to show the $MeHg^+$ peak.

It is apparent from the work done that dentists have mercury in their hair at much higher levels than the general public (see Chapter 7 for more details). Also, it is clear from this speciation work that mercury is present mostly in the inorganic form, suggesting that it has come mostly from the elemental mercury present in the amalgams, which may have bound externally.

6.4.6. The Effect of the Different Masses of Hair used in the Cold Digestions on the Speciation Results.

It is evident that the mass of hair used is crucial to the speciation analysis. The chromatogram from a dentist's hair sample, in figure 6.18, shows small peaks for a sample that was seen to have 458 ng g^{-1} of total mercury in the hair. When this is compared to the chromatogram in figure 6.16, where a similar total mercury level had been determined by ICP-MS, it can be seen that the peaks in figure 6.18 are more prominent. This is because the hair sample in figure 6.18 weighed 0.0967 g whereas in figure 6.18 only 0.0164 g was available for the cold digest. However, as shown in figure 6.17, when a dentist's hair

samples containing a high amount of total mercury was cold digested (in duplicate) at masses of around 0.1 g, there was little methyl mercury present in the hair.

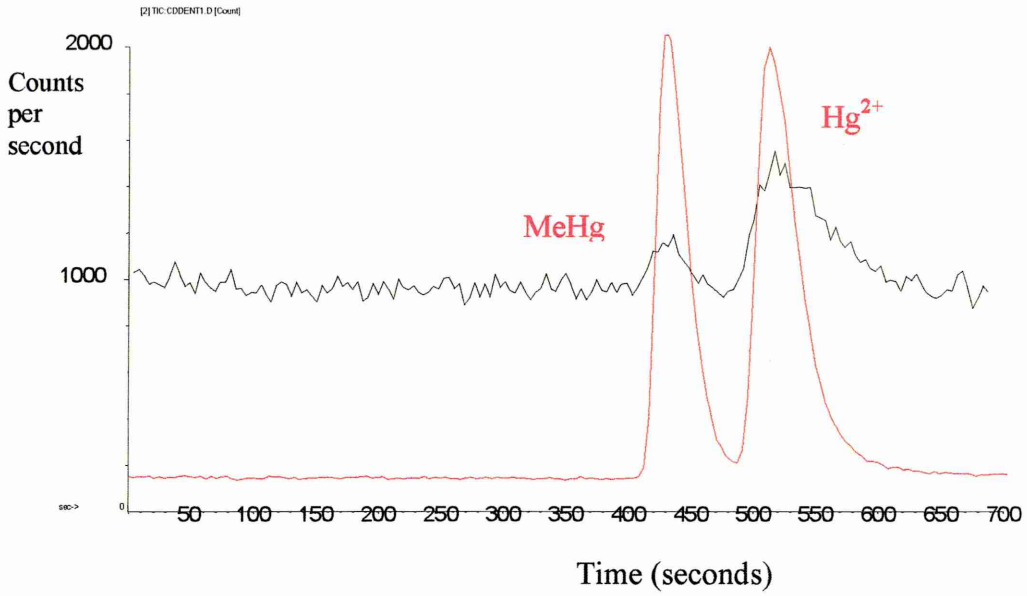

Figure 6.18. Chromatogram from a cold digested sample of a dentists' hair (0.0967 g) containing 458 ng g^{-1} total mercury determined using the ICP-MS. The chromatogram from a mixed 100 ng ml^{-1} standard is superimposed (red line) on the hair mercury peaks to identify the methyl and inorganic mercury.

Another hair sample from a donor in the normal group, previously seen to contain methyl mercury, was digested at various weights to show the effect the mass can have on the analysis. The results are shown in figures 6.19 – 6.22. The sample was known to have a total mercury concentration of 740 ng g^{-1}. The results clearly show that the more hair digested the bigger the peaks, especially for methyl mercury as the method is not as sensitive to this as to inorganic mercury (see calibration graph in figure 6.6).

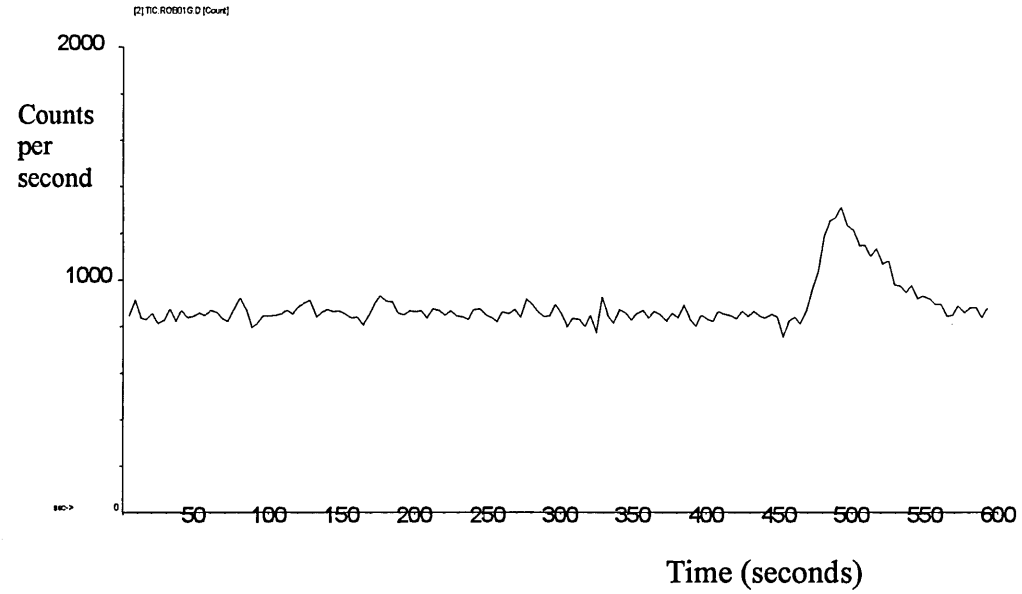

Figure 6.19. Chromatogram from a hair sample of mass 0.0209 g, cold digested with 2:1 HNO_3:H_2O_2 and determined by LC-ICP-MS, using 5% v/v methanol, 0.1% 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8 using the Novapak (150 x 3.9 mm) C_{18} column.

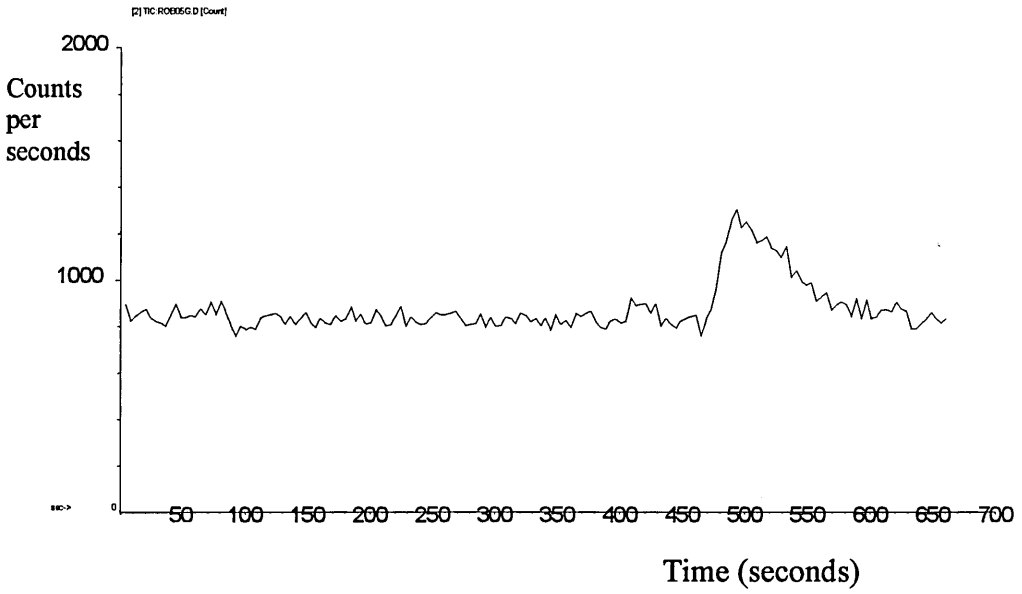

Figure 6.20. Chromatogram from a hair sample of mass 0.0534 g, cold digested with 2:1 HNO_3:H_2O_2 and determined by LC-ICP-MS, using 5% v/v methanol, 0.1% 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8 using the Novapak (150 x 3.9 mm) C_{18} column.

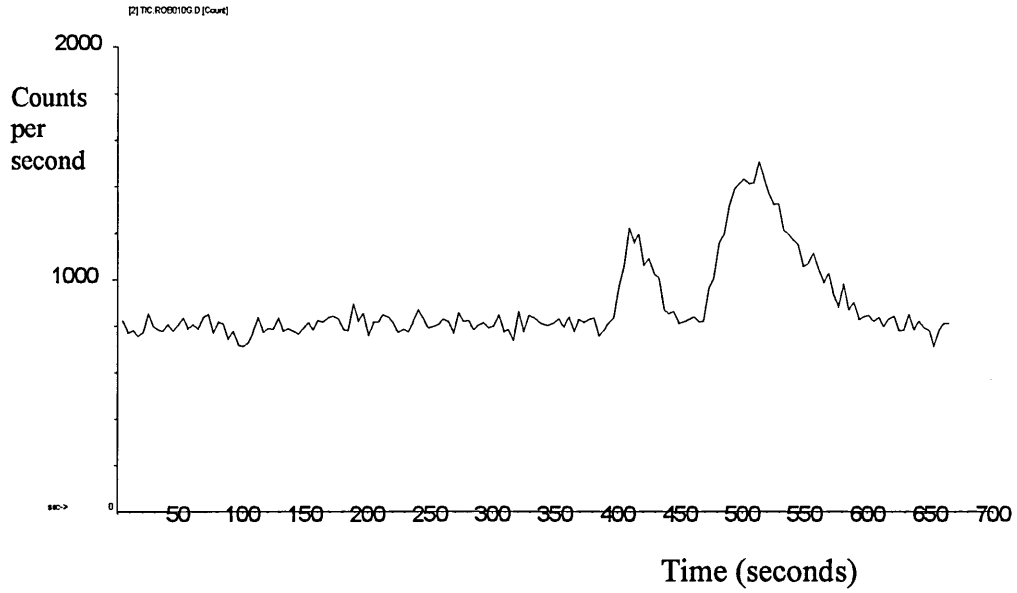

Figure 6.21. Chromatogram from a hair sample of mass 0.0994 g, cold digested with 2:1 HNO_3:H_2O_2 and determined by LC-ICP-MS, using 5% v/v methanol, 0.1% 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8 using the Novapak (150 x 3.9 mm) C_{18} column.

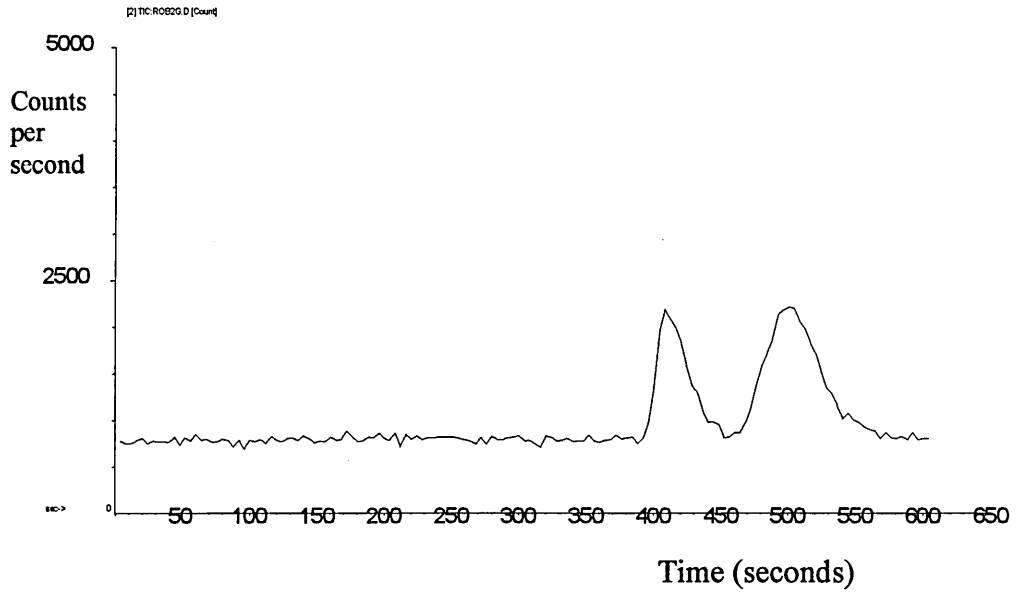

Figure 6.22. Chromatogram from a hair sample of mass 0.2390 g, cold digested with 2:1 HNO_3:H_2O_2 and determined by LC-ICP-MS, using 5% v/v methanol, 0.1% 2-mercaptoethanol in 0.06 mol L^{-1} ammonium acetate at pH 6.8 using the Novapak (150 x 3.9 mm) C_{18} column.

6.5. Investigation of Mobile Phases for Lead Speciation

Initial separations of lead compounds were attempted using a 250 x 4.6 mm, 5 μm particle size, C_{18} column. With organic solvent concentrations of up to 20% it was apparent that $Pb(Et)_4$ was not eluting from the column. It is likely that the organic content was not high enough to elute the compound from the column. Further work concentrated on separating Pb^{2+} and $Pb(Et)_3Cl$ compounds. For the investigation of separations of $Pb(Et)_3Cl$ and Pb^{2+}, the parameters on the ICP-MS were altered in the same way as reported in previous studies[35,54,59,60] (see section 6.3.3.2. for further details).

It was found that none of the mobile phases 1-5 (see table 6.3) eluted two peaks for Pb^{2+} and $Pb(Et)_3Cl$. It was also apparent that acetonitrile could not be used with the ICP-MS as the green C_2 gas was present in the plasma[35].

It was considered then that if the inorganic peak was quantitative then this value could be subtracted from a total value to give an organic lead value. However, the method that gave the best peaks used mobile phase 1 from table 6.3, but it was not quantitative for Pb^{2+}, as the peak areas varied for the same inorganic lead standard. Figure 6.23 shows a typical chromatogram obtained using this method. It was thought that the inorganic lead was adhering to the column and therefore not totally eluting each time. Shum et al[35] recommend the injection of a solution of EDTA onto the column between samples, in order to remove lead adsorbed on the column. However, this did not improve the reproducibility of the method in this case.

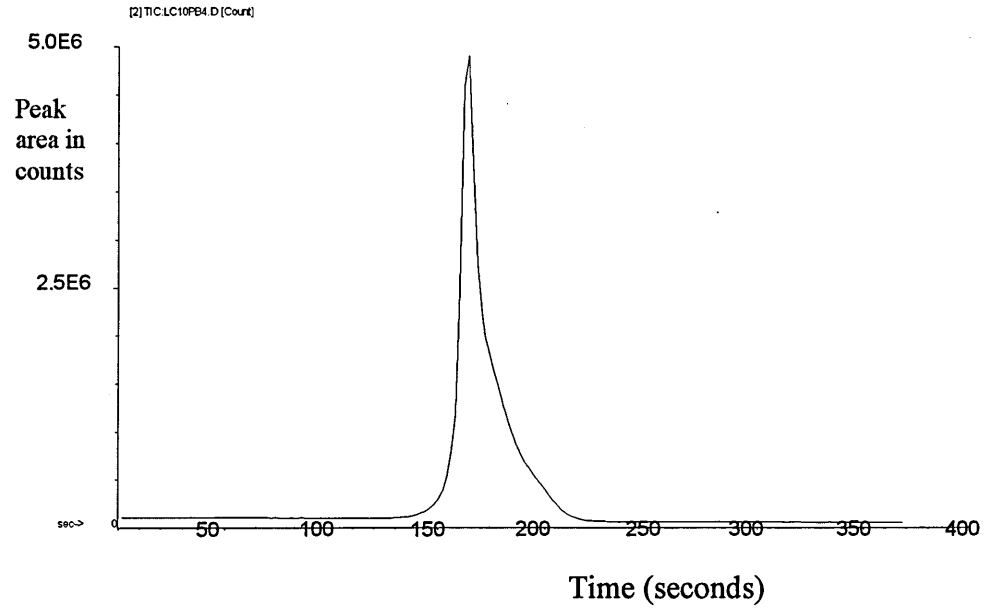

Figure 6.23. Chromatogram of a 10 ng ml^{-1} Pb^{2+} standard, with a mobile phase of 10% methanol, 5mM sodium pentane sulphonate, pH 3.4 with a C_{18} column.

A new type of LC column for the lead speciation work, called 'Hypersil Advance', was then used (see section 6.3.3.2. for details). It consisted of a C_8 column, which had a positive charge on the silanol groups of the stationary phase, which would repel the charge on the lead species and thus elute the compounds. The mobile phase used was 15% v/v methanol (the maximum methanol concentration that could be aspirated into the plasma without increasing the reflective power), 0.1M ammonium acetate, with 0.1M acetic acid and the LC-ICP-MS parameters were as stated in figure 6.24. A peak attributable to Pb^{2+} was eluted but it was apparent that it was necessary to optimise the carrier gas flow rate. This was done by injecting a 25 ng ml^{-1} Pb^{2+} standard in 2:1 HNO_3:H_2O_2 at different carrier gas flow rates. The optimised value of 1.1 L min^{-1} can be seen in figure 6.24. It was also possible to quantify the Pb^{2+} standards and an example of a calibration graph obtained with standards can be seen in figure 6.25.

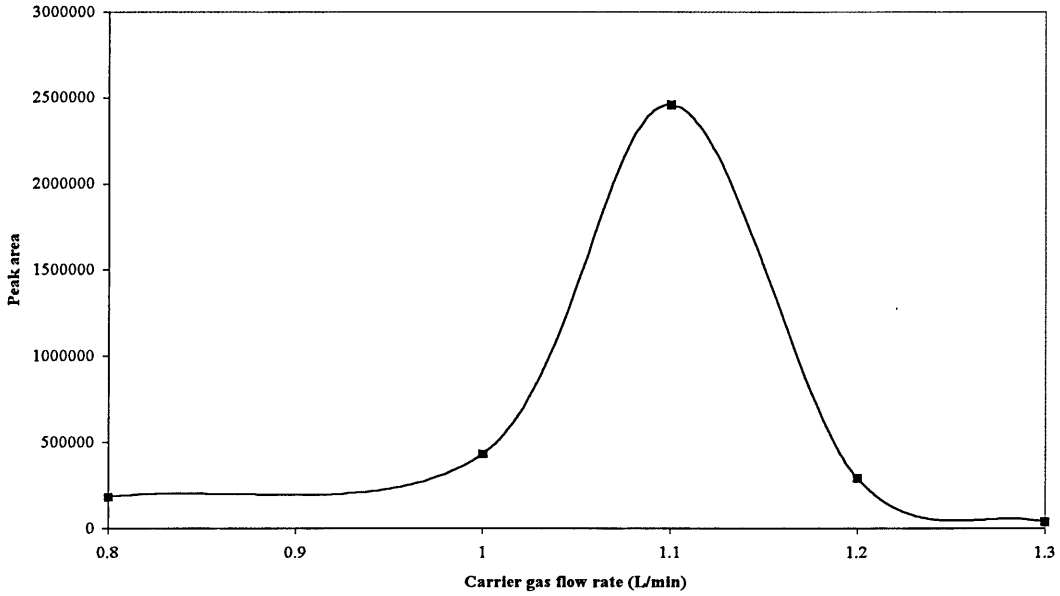

Figure 6.24. The optimisation of the carrier gas flow rate for determination of Pb^{2+} by LC-ICP-MS with a mobile phase of 15% v/v methanol, 0.1M ammonium acetate, 0.1M acetic acid and an Rf power of 1.35 kW, pump speed of 0.3 rps and flow rate of 1 ml min^{-1}. This was using the 'Hypersil Advance' C_8 column.

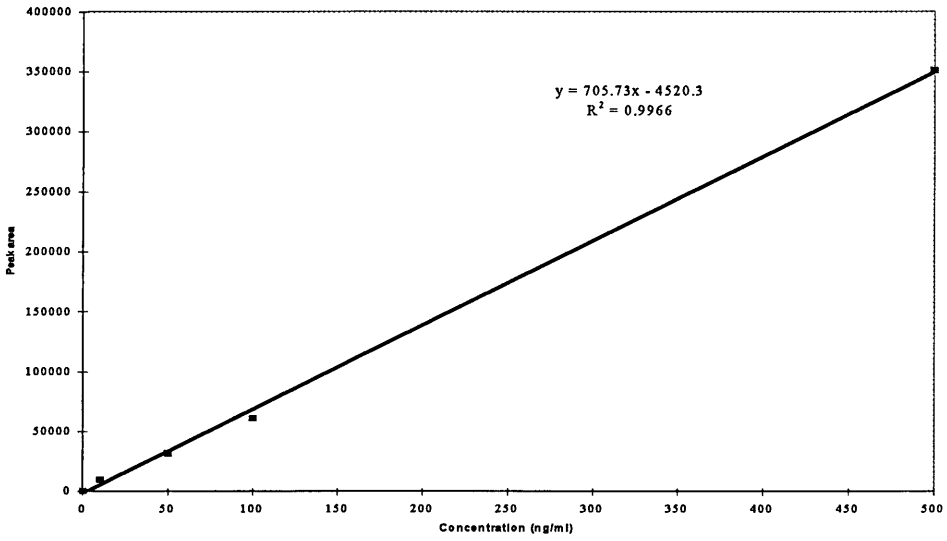

Figure 6.25. Calibration of Pb^{2+} in 2:1 HNO_3:H_2O_2 determined using LC-ICP-MS with a mobile phase of 15% v/v methanol, 0.1M ammonium acetate, 0.1M acetic acid and an Rf power of 1.35 kW, pump speed of 0.3 rps and flow rate of 1 ml min^{-1}. This was using the 'Hypersil Advance' C_8 column.

The reproducibility of the inorganic lead standard peak was then investigated by injecting a 50 ng ml^{-1} standard five times. The mean retention time of the peaks was 130.0 ± 5.5 seconds and mean peak area was of 48036 ± 5398.

6.5.1. Lead Speciation in Hair Samples

After this method had been established the next step was to inject hair samples onto the column to determine the inorganic lead species. Although hair samples could be injected directly onto the column and the peaks obtained and quantified, it was not possible to say with any certainty that the peaks eluting were indeed inorganic lead peaks, because the $Pb(Et)_3Cl$ also gave a peak at this retention time. The presence of $Pb(Et)_3Cl$ in a standard solution was confirmed by mass spectrometry using a VG Quattro 1 (Micromass, Manchester, UK). However, the $Pb(Et)_3Cl$ standard was not reproducible when injected onto this column and mostly only one peak was eluted. When hair samples were injected directly onto the column other peaks were observed but it was not possible to identify them.

Figures 6.26 and 6.27 show chromatograms from a hair sample from a lead foundry worker who was occupationally exposed to lead. The hair sample had been washed with a HCl solution, the washed and unwashed samples were analysed by LC-ICP-MS and total values were determined by ICP-MS. The unwashed hair sample had a total lead value of 114056 ng g^{-1} while the hair washed with 0.1M HCl had a total lead value of 1274 ng g^{-1}. The hair digests were analysed by LC-ICP-MS and the results in figures 6.26 and 6.27 show that a much smaller Pb^{2+} peak is apparent in the washed hair sample than in the unwashed sample, thus confirming the results of the ICP-MS analysis. The actual concentrations obtained with LC-ICP-MS also concurred well with the total values, where the unwashed hair gave a Pb^{2+} concentration of 105400 ng g^{-1} and the washed hair gave a Pb^{2+} concentration of 1300 ng g^{-1}. However, because only the inorganic lead gives a peak with this method it is not satisfactory to carry out lead speciation of hair samples using this method.

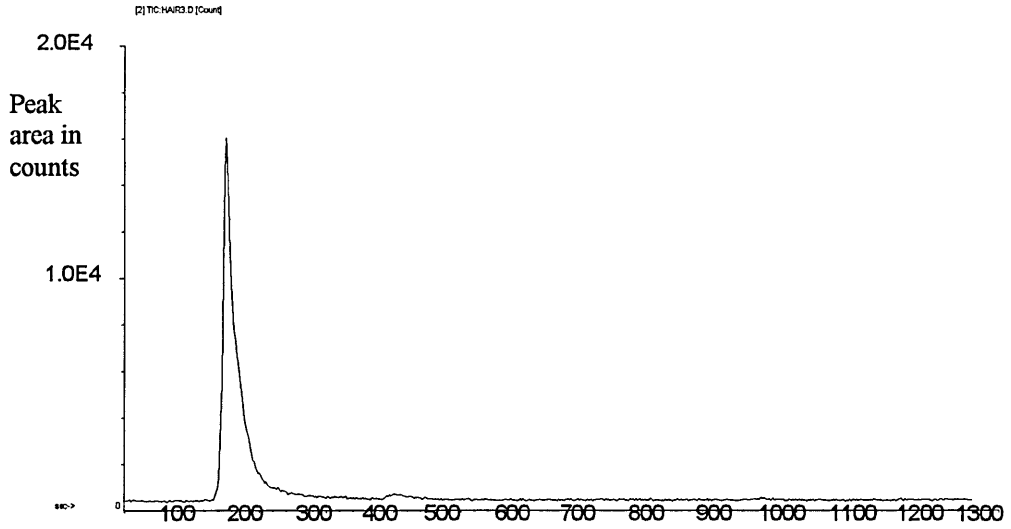

Figure 6.26. Chromatogram of an unwashed hair sample (with total lead concentration 114056 ng g^{-1}), obtained on a Hypersil Advance C_8 column, with a mobile phase of 15% v/v methanol, 0.1M ammonium acetate, 0.1M acetic acid at a flow rate of 1ml min^{-1}.

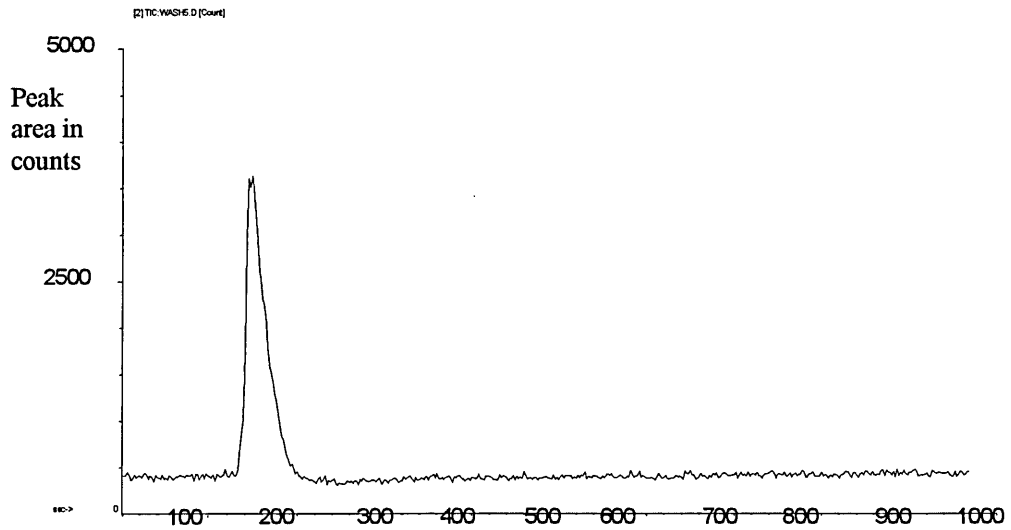

Figure 6.27. Chromatogram from hair in figure 6.26, after washing with HCl, (total lead value determined by ICP-MS was 1274 ng g^{-1}) determined using LC-ICP-MS with a mobile phase of 15% v/v methanol, 0.1M ammonium acetate, 0.1M acetic acid at a flow rate of 1ml min^{-1}.

6.6. Conclusion

The determination of mercury species in human hair by LC-ICP-MS is possible without modification to the existing ICP-MS. The method developed is robust, reproducible and can be used to determine mercury in hair digests, washings and spiked sweat samples.

The results of the studies show that although it is not possible to distinguish between endogenously and exogenously bound mercury species, it is possible to remove the adsorbed methyl mercury in a HCl wash. Methyl mercury could be determined in hair after cold digesting with 2:1 HNO_3:H_2O_2. Clearly when the total mercury concentration is low in the hair sample (<500 ng g^{-1}) a greater mass of hair is required for speciation. Of the dentists' hair analysed most of the mercury found in their hair was in the inorganic form. Further work is required to fully establish this method, using larger numbers of exposed workers and larger samples. The use of cold vapour generation should also be investigated as this may improve the detection limits.

It was not possible to separate lead species using ion pair chromatography with the organic content of mobile phase used in this study. It has been observed previously that the elution of alkyllead species is improved with increasing methanol concentrations[60]. In order to increase the organic content of the mobile phase with the HP 4500 ICP-MS, oxygen would need to be added to the plasma and a torch made specifically for this would be required. The 'Hypersil Advance' C_8 column gave some useful results and showed very high sensitivity to inorganic lead standards. If it were possible to positively identify the peaks seen in the determination of hair samples as being attributable to inorganic lead then it would be possible to use the method optimised here. This could then provide the means to identify endogenously and exogenously bound lead in hair, because approximately 80% of lead is removed from hair by washing with 0.1M HCl.

6.7. References

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Chapter 7: Occupational Studies of Arsenic, Chromium, Lead and Mercury in Hair

Hair from occupationally exposed subjects was examined in order to establish whether total element levels could be used as a measure of exposure. Four elements in hair, mercury, chromium, arsenic and lead, were studied from the following groups of workers: dentists and dental assistants, steel platers and ink analysts, semi-conductor workers and foundry workers, respectively. The hair samples were taken by the workers and placed in sample bags, except for the foundry workers, where the hair samples were collected by the author. Where possible, each donor completed a questionnaire and the information from these forms is compiled in appendix 4 (a sample questionnaire is shown in appendix 1). Using the average hair concentrations of the species of interest from the normal group of 40 donors (reported in Chapter 4) as control values, it was possible, by comparison, to determine the extent of exposure of the occupational samples.

The hair analysis was carried out as previously described in section 3.2.3.2 and, where enough sample was available, the hair was washed following appropriate methods, as described in section 4.2.2.2. In addition to the element of exposure, all of the elements of interest were determined in the hair samples. The sample digests were analysed using the autosampler (Cetac Technology, Macclesfield, UK) for the HP4500 ICP-MS and a standard was analysed every hour to monitor any drift in the instrument (see section 5.2.3.3 for further details). Limits of detection (LOD) were determined as described by Miller et al[1] and were found to be as follows: 1 ng g^{-1} for antimony, cadmium and chromium; 0.7 ng g^{-1} for selenium and arsenic; 10 ng g^{-1} and 8 ng g^{-1} for mercury and lead respectively.

7.1. Hair Mercury Levels

Hair samples from dentists and dental workers were obtained from several sources. The majority of samples were from the Glasgow Health and Safety Laboratory (HSL). These samples had previously been analysed by the Trace Element Unit at the Department of Clinical Biochemistry, Royal Infirmary in Glasgow and so the findings in this study were compared with the values obtained in Glasgow. The Trace Element Unit in Glasgow analysed the hair by atomic absorption spectrometry. Other samples were obtained from

the Dental Hospital at Sheffield University and from dentists in Stoke via the Stoke Occupational Health Department.

7.1.1. Total Mercury Levels in the Hair of Glasgow Dental Workers

Table 7.1 shows a comparison of the total mercury levels values in hair samples obtained in this study with those of Glasgow. The samples had been collected and analysed in the summer of 1998 in Glasgow and were analysed in Sheffield in February and July 1999. Comparison of the two sets of results using a two tailed paired t test (see section 3.2.5)[1], showed that there was no significant difference between the results, at 95% confidence intervals.

The data in table 7.1 was obtained from single digests of samples because for this study there was not enough sample to carry out more than one digestion.

Sample identification	Mass of hair digested in this study (g)	Hair mercury concentration determined in this study (ng g^{-1})	Hair mercury concentration determined by Glasgow laboratory (ng g^{-1})
KY 140	0.0783	1091	6800
KY 141	0.0427	442	400
KY 142	0.0415	164	300
KY 113	0.0235	285	800
KY 171	0.0602	866	800
KY 66	0.0388	956	1000
KY 67	0.0373	589	600
GG 159	0.0277	612	600
GG 158	0.1319	933	1100
GG 139	0.0872	520	900
GG 138	0.0258	1735	800
GG 134	0.0228	1507	1700
DG 14	0.0367	< 10	600
DG 3	0.0467	227	200
DG 5	0.0661	101	<100
DG 10	0.0246	652	500
GG 622	0.0487	771	< 100
215214 H	0.0945	231	< 100
KY 163	0.0817	265	200
KY 166	0.0278	505	500
SFREW	0.0703	265	100
DG 19	0.0259	389	700
KY 181	0.0144	1601	6300
DG 17	0.015	76.9	300
DG 23	0.0423	< 10	< 100
KY 154	0.0483	5321	10700
KY 177	0.0312	388	900
DG 54	0.0358	246	200
DG 52	0.0257	243	500
KA 10	0.0554	252	400
JDWL	0.075	367	400

Table 7.1. A comparison of total mercury levels in the hair of dentists and dental workers determined in this study with the results obtained by the Trace Element Unit, Department of Clinical Biochemistry in Glasgow.

Table 7.2 shows the values obtained for the certified reference material (CRM). The mercury value obtained for the CRM was 87% of the certified value. Using paired t tests (see section 3.2.5) the CRM experimental recoveries showed significant differences to the certified value for selenium, mercury and lead. Spiked samples were also run with the occupational hair samples and they showed that there were no significant differences between the expected and experimental values for all of the elements (see table 7.17 for details of all the spiked hair recoveries analysed).

Element	Expected CRM value ($ng\ g^{-1}$)	Actual value obtained (n=10) ($ng\ g^{-1}$)
Cr	(370)	1121 ± 98
As	280	265 ± 16
Se	600	664 ± 21
Cd	110	120 ± 6
Sb	95	94 ± 4
Hg	360	312 ± 7
Pb	8800	7311 ± 252

Table 7.2. A comparison of the expected and experimental values of the CRM 07601 digested and analysed with the dental workers' samples, detailed in table 7.1. The concentration of chromium is an indicated value and is not certified.

Table 7.3 shows the average hair concentrations of the other metals of interest in this study, determined in the dental workers' samples. These values are compared to the values found in the 'normal group' hair samples, who had not been occupationally exposed to mercury. A two tailed t test was carried out between the hair element concentration in the dentists (n = 31) and the normal group (n = 40), to compare the two means to see if the difference between samples in the two sets varied (for details see appendix 5). The results of the paired t test showed that only the arsenic and mercury values were significantly different at 95% confidence intervals. The mean values show the dentists have more than twice as much mercury (697 ng g^{-1} compared with 287 ng g^{-1}) in their hair than the normal group and that the hair arsenic levels are also higher in the dentists' hair (55 ng g^{-1}

compared with 34 ng g^{-1}). The other elements, apart from lead, appear to have mean values within the normal range and although the lead concentration in the dentists' hair is elevated by approximately 65% of the level in the normal group, this was seen to not be statistically significant at 95% confidence intervals.

The levels determined by the Glasgow laboratory for the same samples show an average mercury concentration of 1235 ng g^{-1}, which is higher than the average value determined in this study. Although the mean hair mercury level in dentists in this study were elevated, they were within the range of values reported for occupationally unexposed subjects[2,3,4,5]. Normal ranges of mercury in hair have been stated from 500-2000 ng g^{-1}, with toxic levels[3] believed to be above 50 000 ng g^{-1}.

Element	Dentists' average hair concentration and range (ng g^{-1}) (n = 31)	Normal group's average hair concentration and range (ng g^{-1}) (n = 40)
Cr	1024 [234 - 1589]	985 [539 - 4077]
As	55 [6 - 112]	34 [<1 - 148]
Se	393 [91 - 765]	471 [157 - 704]
Cd	117 [2 - 925]	72 [<0.1 - 834]
Sb	158 [17 - 1323]	89 [2 – 961]
Hg	697 [<10 - 1735]	287 [3 – 802]
Pb	1705 [196 - 15014]	1030 [84 – 5863]

Table 7.3. A comparison of the element levels in the hair of the Glasgow dentists and the normal group levels, as determined in this study.

It was possible to separate and analyse some of the samples from Glasgow by LC-ICP-MS and thereby calculate how much of the total mercury was methyl mercury and how much existed in the inorganic form, Hg^{2+}. From the data shown in Chapter 6, it was seen that little or no methyl mercury was detected in the dental samples. Table 7.4 compares the concentrations of Hg^{2+} detected with the total mercury levels determined in Glasgow. There was not enough hair to microwave digest the samples and hence only one cold

digestion was carried out for each sample. A two tailed paired t test confirmed that there was a good correlation (i.e. no significant difference at 95% confidence intervals) between the inorganic mercury values and the total mercury values determined in Glasgow. In this study, the sample GG-BAI showed no peak for Hg^{2+}. The mercury concentration determined by the Trace Element Unit, for this sample was 400 ng g^{-1} and further work has shown that the detection limit in this study was 500 ng g^{-1} for hair samples of less than 0.1 g (see Chapter 6 for further details).

Sample identification	Hg^{2+} concentration determined by LC-ICP-MS (ng g^{-1})	Total mercury concentration determined in Glasgow (ng g^{-1})
GG-HUT	887	900
GG-GLE	1355	1100
GG-ROB	3049	3500
GG-ATK	2239	1700
GG-SUT	1847	1300
GG-GRI	987	1100
GG-BAI	No peak	400

Table 7.4. A comparison of the Hg^{2+} values in the dentists' hair samples determined in this study with the total mercury values as determined in Glasgow.

7.1.2. Hair Samples from Sheffield and Stoke Dental Workers

The second set of mercury exposed samples was taken from dental workers in the Sheffield and Stoke areas. The hair samples were collected along with a completed questionnaire. Where there was enough hair, the samples were digested in duplicate and determined by LC-ICP-MS. The results of this analysis can be seen in section 6.4.5.2. Table 7.5 shows the concentration of total mercury found in the hair.

As can be seen in table 7.5, all of the mercury hair levels are higher than the normal group average of 287 ng g^{-1}. However, the results do not correlate with the length of time spent working in the dental profession. The correlation coefficient of the data was $r^2=0.08$. It

would be expected that increased hair concentrations would be indicative of a long term exposure to mercury [3,5,9], however, this is not the case here. It does not appear that mercury has accumulated in the hair of the dental workers except in the case of sample SHF 7 where the dentist who has been in the profession for 20 years has the highest mercury level.

Dentist number	Occupation details and length of time in position	Hair mercury concentration (ng g^{-1})
ST1	Dental assistant (13.5y)	1223
ST2	Dental Nurse (11y)	927
ST3	Dentist (14y)	390
ST4	Dental assistant (10y)	1437
ST5	Dental assistant (>20y)	387
SHF1	Dental surgeon (17y)	454
SHF2	Dental surgeon (16y)	713
SHF3	Dental surgeon (8y)	792
SHF4	Dental surgeon (15y)	848
SHF5	Dental surgeon (15y)	1112
SHF6	Dentist (>20y)	1323
SHF7	Dentist (20 y)	3754

Table 7.5. Total mercury levels in the hair of dental workers in Sheffield and Stoke.

Element	Dental Worker average (ng g^{-1}) n = 12	Normal group average (ng g^{-1}) n = 40
Cr	1063 [496 – 3789]	985 [539 - 4077]
As	35 [4 – 99]	34 [<1 - 148]
Se	518 [375 – 761]	471 [157 - 704]
Cd	82 [8 – 308]	72 [<0.1 - 834]
Sb	57 [0.3 – 131]	89 [2 – 961]
Hg	1113 [387 – 3754]	287 [3 – 802]
Pb	1232 [139 – 2687]	1030 [84 – 5863]

Table 7.6. The hair concentrations of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in the different dental workers compared to the levels from the normal group.

Table 7.6 shows the concentrations of the other elements of interest, along with mercury, found in the hair samples from the dental workers compared with the values found in the normal group. As expected, all of the dental workers show elevated levels of mercury in their hair compared to the value of the normal group. The two tailed t test (see section 3.2.5.) confirmed that there is a significant difference in the mercury values when the mean of the dental hair samples was compared with the mean of the normal group at 95% confidence intervals. The dentists in this group (n=5) show the highest level, with more than six times the value of the normal group, followed by the dental assistants (n=4), with the dental surgeons (n=5) having the lowest mean concentration of mercury in their hair. This result is to be expected as the dental surgeons do not carry out amalgam work on patients, whereas the dentists and the assistants would be exposed to mercury amalgam compounds for dental fillings on a daily basis. The antimony, arsenic, chromium and selenium levels appear consistent with those in the normal group, and while the dental assistants and dentists have slightly higher levels of cadmium and lead, none of the mean values of these elements in the dental samples varied significantly from the mean values of the normal group. However these latter levels are still within the normal group range. Increased cadmium levels may be linked with cigarette smoking, as cigarettes are known to contain up to 1 ppm of both cadmium. However, using the data from the questionnaire (see appendix 4), it was seen that the elevated cadmium and lead levels were not related to the smoking of cigarettes in either the normal group or in this dental group. To confirm this finding a larger study would need to be carried out.

From table 7.6 it can be noted that the essential elements, chromium and selenium, show less variation between the different dental samples and the normal group ranges than the non essential elements, arsenic, cadmium, antimony, mercury and lead. This result is to be expected because the levels of non essential elements in hair will depend on the exposure to the elements, which will vary from sample to sample.

7.1.3. Summary of Hair Mercury Levels in the Dental Samples.

The average mercury levels previously reported[6,10,11,12] in hair of exposed subjects have ranged between 1250-7600 ng g^{-1}. There appears to be only one dental worker in this study to have a hair mercury concentration higher than 7600 ng g^{-1} and that was

determined by the Glasgow laboratory. The sample, KY154 shown in table 7.1, was reported to have a concentration of over 10 000 ng g^{-1}, although more hair would have been required for this study to have confirmed this result.

A study of dental students in Germany showed a significant increase in hair mercury levels after their first exposure to mercury during a six week technical course. The hair mercury levels increased from 470 to 1050 ng g^{-1}, which confirmed that hair is a good indicator of exposure to mercury[7]. The levels reported in the German study fall within the range of values of the exposed Glasgow, Stoke and Sheffield samples examined in this study. Another study[8], which looked at mercury in the hair of dentists, showed that mercury levels in the exposed hair samples ranged from 1790 to 12800 ng g^{-1} which compares well with the dentist samples from Sheffield and Stoke in this study that show an average mercury level of 1822 ng g^{-1} (see table 7.6), indicating exposure at the lower end of the range.

7.2. Hair Lead Levels in Foundry Workers

Hair samples were collected from workers in a 'Brass Foundry' in Sheffield. The samples were collected by the author, to ensure enough sample was taken and blood samples were taken on the same day by the Sheffield Occupational Health Service.

The hair samples were cut from the nape of the neck and placed directly into a plastic sample bag. Many of the workers worked in intense heat and so several of the samples collected were soaked in sweat. Seven of the thirteen samples collected were large enough to analyse both unwashed hair and hair that had been washed in the 0.1M HCl solution (see Chapter 4).

The foundry workers worked with non-ferrous lead based bronzing alloys which contain between 0.025 to 23% w/w lead. On the day the samples were taken, the workers were working on an aluminium-bronze alloy that contained 0.03% w/w lead. The extent of exposure to lead depends on the type of tasks carried out by the workers. At greater risk of exposure are those who work with the molten metal, particularly the furnace men and the moulders.

Table 7.7 shows the levels of lead found in both the hair samples and the blood of the foundry workers. The blood samples were analysed by the Occupational Health Service, Northern General Hospital, Sheffield.

Sample Number	Job Description	Hair lead level (ng g^{-1})	Washed hair lead level (ng g^{-1})	Blood lead (µg%)	ZPP (µmolzpp/ mHe)
1	Manager	2485	812	29.8	61
2	Storeman	111590	7608	19.8	72
3	Moulder	331592	17081	28.0	90
5	Moulder	43737	4208	19.4	66
6	Moulder	17074	1150	20.2	59
7	Moulder	320111	20685	18.6	54
8	Moulder	29192		14.0	54
11	Moulder	16667		15.2	65
13	Moulder	9563		17.8	73
9	Furnaceman	11119		20.4	50
10	Furnaceman	653927		57.8	360
4	Labourer	62259	5162	29.8	69
12	Fettler	7068		16.2	72

Table 7.7: The lead levels in the hair of foundry workers determined in this study and the blood lead levels determined by the Occupational Health Service, Sheffield. The units for the blood lead and ZPP levels are µg/100 ml and µmol of zinc-protoporphyrin per mole of haemoglobin, respectively.

As shown in table 7.7 the mean unwashed hair lead level for the furnacemen was 332523 ng g^{-1}, compared with 109705 ng g^{-1} and 45851 ng g^{-1} for moulders and others respectively. The blood lead levels did not correlate well with the hair levels. Correlation coefficients (r^2) obtained when hair lead levels were compared with blood lead and ZPP levels were 0.62 and 0.69 respectively.

Lead hair levels in excess of 52 000 ng g^{-1} have been reported for people who live in a lead smelting and mining area[9]. The hair samples in that study had been washed with detergent and EDTA, which have been shown in this study not to be effective at removing lead from hair (see Chapter 4 for further details). Unexposed hair lead levels have been reported as 6400 ng g^{-1} in hair before washing with the IAEA method and 5960 ng g^{-1} after washing[10],

17800 - 26700 ng g^{-1} in unexposed persons' hair in the US[11] and 200 ng ml^{-1} in blood[12]. The lead blood levels in this study show an average of 23.6 μg%, or 236 ng ml^{-1} which agrees well with the reported 200 ng ml^{-1} in blood in Lenihan's review of normal levels[12]. In this study, five of the thirteen workers showed hair lead levels above 52 000 ng g^{-1}, the maximum of the normal ranges previously reported, however after washing with hydrochloric acid (0.1M) the hair lead levels were reduced. This would suggest that there is evidence of external contamination, with the average (unwashed) hair lead concentration being over 124 000 ng g^{-1}. A study[8] of exposed battery workers (n = 209) showed hair lead levels between 930 and 3 527 000 ng g^{-1}. In the same study, the blood levels were found to be in the range of 3.33 – 77.4 μg% and these levels correlated significantly to the hair levels. This does not appear to be the case with this study, however, with the low number of donors in this study it is not possible to draw any firm conclusions.

Blood levels of lead above 40 μg / 100ml have been found to cause slowing of the median and ulnar nerves in exposed workers, leading to abnormalities in nerve functions[13]. Levels of zinc-protoporphyrin (ZPP) are the most commonly used indicator of effect in studies of occupational exposure to lead and are determined after oxygenation of the blood[14]. In lead poisoning, ZPP is a product formed in place of heme under certain conditions. It is widely accepted that lead inhibits the reactions in which iron and protoporphyrin IX combine to form heme, by inhibiting the action of ferrochelatase enzyme. At this point zinc, instead of iron, is incorporated into the structure, to form ZPP. There is however a theoretical lag of 120 days, representing the life span of the red corpuscle. Normal levels of ZPP in unexposed workers have been reported at 10.5 μg / 100 ml. In a study where workers were exposed to inorganic lead the highest ZPP level was 57 μg / 100 ml[13]. The results from the brass founder workers in this study show that only one worker had a blood lead level over the permissible limit[14] of 40 μg / 100 ml whilst all of the workers had elevated ZPP levels.

The results from this study also show that the foundry workers have much higher levels of lead in their hair than the normal population, at over 120 times more. However, from table 7.8, it can be seen that up to 94% of this lead is removed on washing the hair in HCl and thus as little as 6% of the total lead in the hair appears to be endogenously bound.

Table 7.9 shows the recoveries of each of the metals in the washings compared to the amount actually washed off the hair.

Element	Percentage of element that was removed from the hair of the foundry workers with the HCl wash (n = 13)	Percentage of element that was removed from the hair of the normal group with the HCl wash (n = 40).
Cr	62	11
As	54	(+8)
Se	5	2
Cd	75	87
Sb	38	27
Hg	13	5
Pb	94	73

Table 7.8: A comparison of the amount of element that 'washed out' of the foundry workers hair with 0.1M HCl and water with the levels removed from the hair of the normal group. The value in brackets indicates an increase in value after the hair was washed.

Element	Percentage of element found in washing when compared to difference in value between unwashed and washed hair (%).
Cr	32
As	206
Se	59
Cd	18
Sb	41
Hg	59
Pb	98

Table 7.9. The percentage of element removed from the hair by washing with 0.1M HCl and recovered in the HCl fraction.

The results shown in table 7.9 suggest that the lead that 'washed off' the hair is present in the hydrochloric acid solution that had been in contact with the hair. The data for the other elements show that the recovery in the 'washing' is not as high, with arsenic showing recovery levels inexplicably higher than the amount actually removed from the hair and the other elements showing less element in the washing than was removed from the hair.

The results in table 7.10 indicate that all of the other elements of interest also appear to be present at high levels in the brass founders' hair. There is approximately twice as much

chromium, ten times as much arsenic, 40% more selenium, thirty times more cadmium and fifty times more antimony present in the hair of the foundry workers than in the unexposed hair of the normal group. Only the level of mercury appears to be similar to that of the normal group. Two tailed t tests of the mean values of the elements in the lead foundry workers hair and the mean values of the normal group confirmed that the levels of antimony, arsenic, cadmium and lead differed significantly at 95% confidence intervals (see appendix 5 for equation). The levels of chromium, selenium and mercury did not differ in the two sets of samples.

Element	Average element concentration and range in the hair of foundry workers (ng g^{-1})	Average element concentration and range in the hair of the normal group (ng g^{-1})
Cr	1886 [642 - 10550]	985 [539 - 4077]
As	318 [21 - 1279]	34 [<1 - 148]
Se	664 [204 - 2463]	471 [157 - 704]
Cd	2629 [94 - 16061]	72 [<0.1 - 834]
Sb	4886 [64 - 14119]	89 [2 – 961]
Hg	373 [72 - 1606]	287 [3 – 802]
Pb	124337 [2485 - 653926]	1030 [84 – 5863]

Table 7.10. Levels of elements of interest in foundry workers' hair compared with levels in the normal group.

7.3. Hair Chromium Levels in Exposed Workers

7.3.1. Levels of Chromium and Other Elements of Interest in the Hair of Steel Platers

Seven hair samples were collected from a small firm of Sheffield steel platers. Chrome plating by dipping stainless steel in a 'Chrome 3' solution and drying the product is one of the many tasks performed by the workers.

The hair samples were collected by the workers themselves and placed in plastic sample bags. Questionnaires were also completed (see appendix 4 for details). There was enough hair in all of the samples to allow duplicate analyses to be performed. The results from the determination of the chromium levels in the hair of the steel platers are shown in table 7.11.

Workers 2 - 7 have higher concentrations of chromium in their hair than the normal group mean of 985 ng g^{-1}. However, the hair chromium levels do not correlate to the amount of time spent in the industry. Table 7.12 shows a comparison of the levels of hair chromium and other elements from the steel platers with those found in the non-occupationally exposed normal group.

Worker	Job description and time in position	Hair chromium concentration (ng g^{-1})
1	electroplater (10y)	743
2	electroplater (10y)	2856
5	electroplater (23y)	999
6	electroplater (15y)	1820
7	electroplater (6y)	6642
3	jigger/unjigger (5.5y)	1176
4	clerk (15.5y)	1272

Table 7.11. The hair chromium levels of the steel plater workers. The length of time in the position is given in brackets.

Element	Chromium platers average hair concentration (ng g^{-1})	Normal group average hair concentration and range (ng g^{-1})
Cr	2201	985 [539 - 4077]
As	197	34 [<1 - 148]
Se	631	471 [157 - 704]
Cd	1828	72 [<0.1 - 834]
Sb	126	89 [2 – 961]
Hg	284	287 [3 – 802]
Pb	3599	1030 [84 – 5863]

Table 7.12. Comparisons of the hair concentrations of the elements of interest for steel platers and the normal group.

The data in table 7.12 show that the chromium levels of the steel platers are on average twice as high as the unexposed group, although this is approximately the same concentration as the elevated levels seen in the lead foundry. The arsenic levels are also increased, the cadmium levels were more than twenty-five times higher than normal, whilst the antimony levels were elevated by about 40%, the selenium levels are increased by more than a third and the lead levels more than three times the value of the normal group. In comparison, the mercury levels appear the same as the normal group. Two tailed t tests showed that only selenium was significantly different when the mean element concentrations of the steel platers were compared with the mean concentrations of the normal group at 95% confidence intervals (see appendix 5 for equation). However, as the arsenic, cadmium and lead levels were higher in the steel platers than the non-exposed group the information regarding smoking, contained in the questionnaires, was compared with the results. It was found that only three of the seven donors smoked cigarettes and that the average arsenic and lead levels were lower in the smokers than in the non-smokers. Donor 2 had a very high level of hair cadmium, at 10 835 ng g^{-1}, which increased the average level of cadmium for the group. Without this sample, the average cadmium level was 326 ng g^{-1} and the smokers showed lower levels than the non-smokers. It is likely that the effect of smoking on the hair cadmium levels is masked by the relatively high exposure to the metal from the work environment.

Non occupationally exposed hair chromium levels range between 130-3650 ng g^{-1} and so the normal value of 985 ng g^{-1} for the normal group in this study is in good agreement with the literature[6,10,12,15]. In a study to determine the level of chromium welders had been exposed to, a hair chromium average of 2470 –5560 ng g^{-1} was reported[5], which is also in good agreement with values obtained in this study where two of the seven workers showed elevated hair chromium concentrations in this range. In a Turkish tannery[16], control chromium hair levels were found to be about 560 ng g^{-1} and the exposed workers had levels as high as 17 400 ng g^{-1}. Interestingly, the office workers in the tannery showed levels of 14 500 ng g^{-1} of chromium in their hair. In this study of workers in a steel platers a smaller increase in the office clerk's hair chromium level was observed when compared to the normal group value, i.e. 1272 ng g^{-1} compared to the normal group value of 985 ng g^{-1}.

7.3.2. Levels of Chromium and Other Elements of Interest in the Hair of Inkjet Chemists

Thirteen hair samples were taken from industrial chemists who analysed ink, containing chromium. The hair samples were collected by the donors and questionnaires completed (for details see appendix 4). Six of the thirteen samples were washed in 1% v/v SLS, in order to determine the amount of exogenously bound chromium (see Chapter 4 for further details). The levels of chromium determined in the washed and unwashed hair are shown in table 7.13.

The results show that the chromium levels of the ink analysts were lower than the mean hair chromium level of 985 ng g^{-1} seen in the normal group. It should be stated, however, that the laboratory where these analysts work maintains a strict level of safety and the workers may not actually be exposed to the chromium present in the inks at significant levels. In addition, the length of time in the job did not correlate with the hair chromium concentration.

The SLS wash solution removed 6% of the total chromium in the ink analysts' hair, in contrast to 12.5% of hair chromium removed by SLS in the normal group (see Chapter 4 for details). This implies that chromium is mainly endogenously bound to the hair.

Donor	Time in position (y)	Hair chromium concentration (ng g^{-1})	Hair chromium concentration after 1% v/v SLS wash (ng g^{-1})
1	2.5	356	331
2	5	733	558
3	4	438	487
4	7	483	403
5	0.75	389	479
6	5	470	449
7	2.5	499	
8	4	588	
9	4	138	
10	4	295	
11	3	313	
12	1.7	341	
13	15	466	

Table 7.13. Chromium levels determined in unwashed hair samples (n=13) and washed hair samples (n=6) with 1% SLS.

Table 7.14 compares the average hair concentrations of the elements of interest in this study for the ink analysts with the mean values from the normal group. The levels of all of the elements of interest are similar to the normal group, except for chromium and antimony, where the ink analysts have approximately half the level of chromium and antimony in their hair than the normal group. Two tailed t tests confirmed that the mean values chromium and antimony in the ink analysts' hair and those in the normal group differed significantly at 95% confidence intervals (see appendix 5 for equation).

Using information from the questionnaires (see appendix 4) it was known that seven out of the thirteen donors smoked up to twenty cigarettes per day. For these seven the arsenic and cadmium levels were higher in the smokers than in the non-smokers; the arsenic level was 29 ng g^{-1} for the non-smokers compared to 35 ng g^{-1} for the smokers whilst the cadmium level was 24 ng g^{-1} for the non-smokers compared to 75 ng g^{-1} for the smokers. The number of dental fillings was also investigated for each donor in the ink analysts group. The donors with less than five fillings (n = 5) had a mean hair mercury level of 378 ng g^{-1} whereas the donors with less than ten fillings (n = 2) had a mean hair mercury level of 757 ng g^{-1}, however, those with more than ten fillings (n = 6) had a mean hair mercury level of 320 ng g^{-1}. In the normal group, those with less than ten fillings had slightly higher hair mercury levels than those with less than five or no fillings, with concentrations of 327, 317 and 244 ng g^{-1} respectively. This would suggest that, in this study, there is not a significant increase in hair mercury levels in those with increased dental amalgam fillings.

Element	Average Hair concentration of ink analysts (ng g^{-1}) n = 13	Average hair concentration and range of values in the normal group (ng g^{-1}) n = 40
Cr	424 [138 – 733]	985 [539 - 4077]
As	33 [8 – 75]	34 [<1 - 148]
Se	465 [160 – 873]	471 [157 - 704]
Cd	53 [12 – 174]	72 [<0.1 - 834]
Sb	41 [18 – 80]	89 [2 – 961]
Hg	410 [102 – 989]	287 [3 – 802]
Pb	989 [20 – 4558]	1030 [84 – 5863]

Table 7.14. Comparison of average hair concentrations of elements of interest in the ink analysts with the normal group.

7.4 Hair Arsenic Levels in Exposed Workers

The hair samples used in this study were collected from semiconductor workers by the Glasgow Health and Safety Laboratory and analysed by the Trace Element Unit, Department of Biochemistry, Royal Infirmary, Glasgow. The values determined in this study were compared to the values obtained in Glasgow. Head and pubic hair were used to determine exposure to arsenic. Table 7.15 shows the head hair analysis results.

The results in table 7.15 show that overall the average head hair arsenic concentration from this study was 96.8% of the Glasgow concentration value of 215 ng g^{-1} and a two tailed paired t test confirmed that there was no significant difference at 95% confidence intervals. The normal group average concentration of arsenic in hair was 34 ng g^{-1} whilst the average value in this study of hair from semi-conductor workers was 208 ng g^{-1}, which is significant at more than six times the normal level of arsenic in hair. Normal ranges of arsenic in hair have been reported to be between 60 and 1200 ng g^{-1} and so it appears that although the exposure is obvious when the exposed samples are compared to the normal group, the exposed group hair levels are still within a normal range[5].

Table 7.16 shows the comparison between the pubic and head hair samples.

The results in table 7.16 confirm that the masses of the pubic hair samples were insufficient for accurate analysis because most of the samples gave lower values than the Glasgow values and consequently the comparison between the Glasgow study and this study's results is poor. The masses of hair that were digested were as shown in table 7.16.

In Chapter 3 it was seen that the minimum mass of hair for digestion was 0.05 g and that reproducibility was improved with a mass of 0.1 g. The pubic hair masses were very low indeed, except for the last sample where 0.0447 g was digested and this gave a similar value to the Glasgow concentration. It is clear from this that to have confidence in the results obtained from hair analysis at least 0.05 g of hair must be used in the digestion.

Sample identification	Mass of hair digested (g)	Head hair arsenic concentration determined in this study (ng g^{-1})	Head hair arsenic concentration determined by Glasgow laboratory (ng g^{-1})
SC 1	0.0642	43	40
SC 2	0.0837	129	80
SC 3	0.0455	172	120
SC 4	0.0226	91	50
SC 5	0.1252	32	<50
SC 6	0.0371	64	<50
SC 7	0.0397	181	80
SC 8	0.0477	2279	1610
SC 9	0.0319	82	<50
SC 10	0.0760	44	<50
SC 11	0.0366	218	200
SC 12	0.0094	30	600
SC 13	0.0419	335	400
SC 14	0.0423	100	200
SC 15	0.0207	58	70
SC 16	0.0165	61	200
SC 17	0.0460	129	100
SC 18	0.0209	49	70
SC 19	0.0366	37	60
SC 20	0.1003	335	300
SC 21	0.1135	303	300
SC 22	0.009	<10	100
SC 23	0.0182	141	300
SC 24	0.132	42	70

Table 7.15. The head hair arsenic levels in semiconductor workers determined in this study and by the Trace Element Unit, Department of Biochemistry, Royal Infirmary, Glasgow.

Sample identification	Head hair arsenic concentration determined in this study (ng g^{-1})	Head hair arsenic concentration determined by Glasgow laboratory (ng g^{-1})	Pubic hair arsenic of pubic hair concentration determined in this study (ng g^{-1})	Pubic hair arsenic concentration determined by Glasgow laboratory (ng g^{-1})
1	218	200	31 (0.0325g)	<50
2	30	600	356 (0.0065g)	500
3	129	100	96 (0.015g)	400
4	<30	100	75 (0.0134g)	100
5			36 (0.0219g)	200
6			111 0.0447g	100

Table 7.16. Arsenic levels in pubic and head hair samples of semi-conductor workers determined in this study and by the Trace Element Unit, Department of Biochemistry, Royal Infirmary. Glasgow. The mass of the pubic hair sample analysed is stated in brackets.

Normal hair arsenic levels reported in another study were in the range of 130 - 3710 ng g^{-1}, which is higher than the level of 34 ng g^{-1} for the normal group in this study[6]. Other studies[10,12,15] have also reported higher normal hair arsenic concentrations of 200 ng g^{-1}, 240 ng g^{-1} and 550 ng g^{-1}. However, a study of exposure to gallium-arsenide, in 1989, reported levels of 60 ng g^{-1} for the normal group, averaged over 100 samples and exposed hair levels ranged from 560 – 13 800 ng g^{-1}, showing an obvious elevated concentration of arsenic[17]. Interestingly in the same study, hair samples from the clerical workers in the same plant were analysed and found to have arsenic levels as low as 10 - 20 ng g^{-1}, which is more in the range of the normal group values in this study. In comparison, hair from semiconductor workers, analysed in San Diego in 1995, showed much higher levels of arsenic in the hair of both the controls and the exposed samples[18]. The non-smoking administrative workers' hair was used as the control, and they were shown to have arsenic levels of 30 000 ng g^{-1} whilst exposed workers showed levels of 45 000 ng g^{-1}. These

values are much higher than previously reported. In a copper smelting town, levels of up to 9100 ng g^{-1} were reported, with the control value being around 300 ng g^{-1}. Another wide ranging study of arsenic levels in West Bengal[19] examined hair and other body tissues and fluids. The control value for the study of arsenic in hair was 204 ng g^{-1} (n = 250), whilst the average exposed value was 8440 ng g^{-1}, with a range of 1000-42 150 ng g^{-1} (n = 7548). The control values of these studies, with the exception of the San Diego study, are approximately ten times the values obtained in the normal group in this study. This could be as a result of there being a significantly less amount of arsenic in the environment in Sheffield, or the fact that in West Bengal[19] or in 1971[9] there were higher levels of arsenic in the environment.

7.5. Recoveries of Spiked Hair Samples in Occupational Sample Analysis

Hair samples taken from three of the groups studied were spiked with multi-elemental standards to give a final concentration of 10 ng ml^{-1}. Good recovery values were obtained for all of the elements in all of the three groups. The recoveries are shown in table 7.17.

Element	Mean recovery obtained from spiked hair samples analysed with dental and semiconductor workers' samples as a percentage of expected value (n=7)	Mean recovery obtained from spiked hair samples analysed with brass foundry samples as a percentage of expected value (n=2)	Mean recovery obtained from spiked hair samples analysed with chromium workers' samples as a percentage of expected value (n=3)
Cr	107 ± 9	104 ± 10	110 ± 11
As	101 ± 3	96 ± 2	95 ± 3
Se	92 ± 6	103 ± 7	99 ± 1
Cd	103 ± 4	93 ± 4	97 ± 2
Sb	117 ± 5	98 ± 3	98 ± 7
Hg	92 ± 4	95 ± 2	91 ± 6
Pb	120 ± 7	91 ± 4	95 ± 4

Table 7.17. Recovery of spiked solutions in hair samples (final concentration 10 ng ml^{-1}) digested and analysed with the occupational samples.

7.6. Conclusion

The studies show that exposed workers do have significantly elevated levels of elements in their hair when compared to levels in a normal group. The level of exposure to mercury amalgam is reflected in the hair mercury levels as the dentists and the dental assistants both had higher mercury levels than the dental surgeons, who still had increased mercury levels. The inorganic mercury levels from the LC-ICP-MS data showed a good correlation with the total mercury values for the same samples, which supports the findings from Chapter 6, that dentists have mostly inorganic mercury in their hair. The lead workers in the foundry had more than 120 times the normal level of lead in their hair, but it was shown that over 90% of this lead is removed from the hair when it is washed with dilute HCl, an indication that the lead is mostly exogenously bound. Overall, the chromium workers in the steel platers did show elevated hair chromium levels, however, the ink analysts showed half the normal hair chromium value. The semiconductor workers showed elevated arsenic levels in their hair. The level of the element in the hair did not correlate with the length of time in the industry and hence it was concluded that it was more likely that the actual job performed and subsequent level of exposure determines the concentration of the element in hair.

It is apparent from this study that the workers may also be at risk from exposure to other metals not necessarily linked to the occupation. The dentists all showed elevated lead levels, the lead workers showed significantly high cadmium and antimony levels whilst the steel platers showed elevated arsenic, cadmium and lead levels in their hair. Results from this study indicate that the workers may need to be monitored for other metals as they might be at risk from unknown hazards. No differences were observed for the normal group, lead workers, dentists and steel platers when the smokers hair levels were compared with the non smokers hair levels. However, in the ink analysts who had been occupationally exposed to relatively low levels of arsenic and cadmium showed increased levels in smokers' hair.

For reliable estimates of occupational exposure it is essential that at least 0.2 g of hair is collected to allow duplicate digestions of the hair prior to analysis. It is also imperative that increased numbers of hair samples are used in further studies so that trends will be more apparent.

7.7. References

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Chapter 8: Conclusion

This study has shown that it is possible to determine the levels of antimony, arsenic, cadmium, chromium, lead, mercury and selenium in head hair. A digestion method based on a mixture of nitric acid and hydrogen peroxide was developed and the digested samples analysed by ICP-MS. It was found that a digestion method based on hydrochloric acid and hydrogen peroxide was required for the determination of antimony and arsenic by HG-ICP-MS. However, it was not possible to simultaneously determine mercury and selenium by HG-ICP-MS by the same method used for antimony and arsenic, which included the use of sodium borohydride and potassium iodide. This study also developed washing methods to remove some externally bound elements from hair and also a novel LC-ICP-MS method for the separation of mercury species in hair.

Investigation of methods for removing the elements from the hair showed that in unexposed hair samples arsenic was significantly removed from hair with a SLS wash and antimony could be removed significantly from unexposed hair samples washed with SLS, EDTA and dilute HCl. With arsenic being removed from hair using an anionic detergent it suggests that either it has bound to the sulphonate groups in the SLS in its cationic form or more likely, that the arsenate/arsenite compounds have been exchanged in the hair for the sulphonate groups in the SLS. The fact that antimony is removed from unexposed hair with all of the reagents suggests that it is not tightly bound to hair. However, as a result of spiking hair samples with simulated sweat solutions it was found that it is not possible to identify endogenously or exogenously bound arsenic or antimony in hair, as both elements irreversibly bound to the hair from the spiked solution and were not removed by washing with any of the reagents. Although, it was possible, with increased levels of arsenic in hair, to identify exposed persons. This was seen in the occupational studies of semi-conductor workers that showed mean arsenic hair levels of 208 ng g^{-1} compared with unexposed levels of 34 ng g^{-1}.

Investigation of methods for removing the elements from the hair showed that selenium was the only element investigated not to be removed from unexposed hair samples after washing, suggesting that any selenium present in hair is endogenously bound. This was further confirmed using simulated spiked sweat solutions where it was apparent that it is

not possible to remove selenium from hair after it has adsorbed and thus it is not possible to differentiate between endogenously and exogenously bound selenium species. It is known that selenium content in the body is reflected in hair and when selenium supplements were taken over a six week period the selenium hair content increased by approximately 19%. This confirmed that hair may be used as a good indicator of selenium levels in the body.

Paired t tests confirmed that chromium was significantly removed from unexposed hair samples using washing solutions of HCl and SLS, although HCl was better at removing exogenously bound species from the hair. This was also seen when the hair was spiked with simulated sweat as all of the bound chromium from the spiked solution was removed when the hair was washed with dilute HCl making it possible to differentiate between endogenously and exogenously bound chromium in hair. Occupational studies of chromium showed that steel platers have significantly higher hair chromium levels than the unexposed group (2201 ng g^{-1} compared to 985 ng g^{-1}), whilst ink analysts have less chromium in their hair than the unexposed group (424 ng g^{-1} compared to 985 ng g^{-1}).

Results have shown that it is also possible to differentiate between endogenously and exogenously bound lead and cadmium in unexposed hair samples when washed with dilute HCl as 73% and 87% respectively was removed when washed with dilute HCl. Using the spiked simulated sweat solutions it was also seen that it is possible to differentiate between endogenous and exogenous cadmium and lead in hair, as the cadmium that was bound to the hair was fully removed after washing with the dilute HCl and the lead spiked solution instead of adsorbing to the hair actually removed some of the weakly bound lead in hair. Studies using a spiked solution of an enriched cadmium isotope confirmed that the cadmium adsorbed to the hair when spiked was then removed when washed with the HCl solution. Occupational studies of hair from lead foundry workers showed significantly higher levels of cadmium and lead in hair and that approximately 80% of each element was removed from the hair after being washed with dilute HCl solution. This suggests that most of the lead and cadmium found in the hair of the lead foundry workers was exogenously bound. Analysing the hair samples of the foundry workers for all of the elements found elevated levels of chromium (1886 ng g^{-1} compared to 985 ng g^{-1} in the control group), arsenic (318 ng g^{-1} compared to 34 ng g^{-1} in the control group), cadmium

(2629 ng g^{-1} compared to 72 ng g^{-1} in the control group), antimony (4886 ng g^{-1} compared to 89 ng g^{-1} in the control group) as well as lead (124337 ng g^{-1} compared to 1030 ng g^{-1} in the control group) which suggests that the workers there need to be analysed for exposure to other elements and not just lead. LC-ICP-MS studies with lead compounds were not successful and it is necessary to carry out more work in this area to confirm which species of lead are being removed from hair.

Mercury in hair is also removed (5%) from unexposed hair samples by HCl, however it is not possible to differentiate between endogenous and exogenous species as mercury adsorbed irreversibly to hair when exposed to the spiked simulated sweat solution and cannot be removed by washing. However, it was possible to determine inorganic and methyl mercury in hair by LC-ICP-MS without any modifications to existing instrumentation. Methyl mercury could only be determined in hair when the hair sample was cold digested with 2:1 HNO_3:H_2O_2 because after microwave digesting the methyl mercury peak was not detected. Results from these speciation studies showed that when hair samples are spiked with both species in a simulated sweat solution the adsorbed methyl mercury can be fully removed when the hair sample is washed with dilute HCl, whereas inorganic mercury is irreversibly bound to the hair. From the LC-ICP-MS studies carried out for hair analysis it is apparent that sample size is a major factor in the quality of the results obtained and perhaps by using a cold vapour generation system between the column and the ICP-MS the detection levels for the mercury species may be improved. Occupational studies involving hair from dental workers showed that only inorganic mercury is present in hair and that dentists have higher levels of mercury in their hair than the normal group used in this study (813 ng g^{-1} compared with 287 ng g^{-1} in the unexposed group).

For further work in this area, more samples and studies are required if hair is to be used as an indicator of occupational exposure to these elements. Results from this study suggest that hair is a good medium to determine exposure and that if further LC-ICP-MS methods were established for other elements, more information regarding the species of elements would be achieved. It would also be necessary to correlate the levels of the elements in other body tissues as this was not possible in this study. Using the LC-ICP-MS method developed for mercury speciation it would be possible to carry out a large sample study,

and this quick and reproducible method would be ideal if there were a lot of samples from people who had been exposed to inorganic or methyl mercury.

Appendix 1

The questionnaire completed by the donors of the normal group and occupational studies

☐ Male ☐ Female

Age ☐ <18

☐ 18-25

☐ 26-35

☐ 35-50

☐ >50

Job Description

Time in position

Colour of hair

Has your hair had any cosmetic treatment?
If yes, please describe the treatment:

What hair care products do you use?

When did you last wash your hair?

How many dental fillings do you have? none ☐ <5 ☐ 5-10 ☐ >10 ☐

When did you last visit the dentist?

Do you eat meat? yes ☐ no ☐
if yes how many times a week, once ☐ twice ☐ more than twice ☐
Do you eat fish? yes ☐ no ☐
if yes how many times a week, once ☐ twice ☐ more than twice ☐

Are you currently taking any vitamin supplements? yes ☐ no ☐
What are the supplements called?

Smoker ☐ or non-smoker ☐
Number of cigarettes smoked per day
<10 ☐ 10-20 ☐ >20 ☐ other ☐

Appendix 2

Information from the questionnaires completed by the donors normal unexposed group.

Donor	Sex m/f	Age range (y)	Colour	Number of days since hair was washed	Number of fillings	Eat meat yes/no	Eat fish yes/no	Take vitamins yes/no
1	m	18-25	black	1	1	y	n	y
2	m	18-25	brown	0	0	y	y	n
3	m	18-25	brown	0	0	y	y	y
4	m	26-35	black	1	1	y	y	n
5	m	35-50	br/grey	0	6	y	y	n
6	m	18-25	brown	1	8	y	y	n
7	m	26-35	brown	0	0	y	n	n
8	m	26-35	bl/grey	0	3	y	y	n
9	m	18-25	blonde	0	4	y	y	n
10	m	18-25	brown	2	1	y	y	n
11	m	26-35	brown	0	10	y	y	y
12	m	18-25	brown	1	10	y	y	n
13	m	18-25	brown	1	0	y	y	y
14	m	18-25	brown	0	1	y	y	y
15	m	18-25	black	0	5	y	y	n
16	m	18-25	brown	1	1	y	y	y
17	m	26-35	brown	2	6	y	y	n
18	m	<18	black	0	0	y	y	n
19	m	18-25	blonde	0	0	y	y	n
20	m	26-35	brown	0	4	y	y	y
21	f	18-25	blonde	0	8	y	y	n
22	f	>50	brown	1	6	y	y	n
23	m	18-25	blonde	0	0	y	y	n
24	f	>50	brown	1	6	y	n	y
25	f	18-25	black	0	0	y	y	n

Donor	Sex m/f	Age range (y)	Colour	Number of days since hair was washed	Number of fillings	Eat meat yes/no	Eat fish yes/no	Take vitamins yes/no
26	m	26-30	black	1	0	y	y	y
27	f	18-25	brown	2	6	n	n	y
28	m	<18	black	1	0	y	y	n
29	m	18-25	brown	1	7	y	y	n
30	m	26-35	brown	2	4	y	y	n
31	m	18-25	brown	1	8	y	n	n
32	m	26-35	brown	0	8	y	y	n
33	m	26-35	brown	1	4	y	y	n
34	f	18-25	black	0	0	y	n	y
35	f	18-25	brown	1	3	y	y	n
36	f	26-35	brown	1	14	n	n	y
37	f	18-25	brown	0	0	y	y	n
38	f	26-35	red	0	2	n	y	n
39	m	26-35	brown	0	0	y	y	n
40	m	26-35	brown	1	2	y	y	n

Appendix 3

Data from the selenium study in chapter 5.

Donor Number	Hair selenium concentration before supplement (ng g^{-1})	Hair selenium concentration after 6 weeks of supplement (ng g^{-1})	Hair selenium concentration up to 8 weeks after supplement (ng g^{-1})
261	449	613	644
110	465	627	662
220	641		654
260	624	628	736
150	536	606	
290	530	622	
41	592	598	
391	537	583	
280	653		661
172	666	639	700
360	635	602	
111	526	697	700
50	804	758	1078
170	364	430	
120	684	460	584
173	457	580	
370	639	559	
60	542	628	
40	508	497	571
430	372	574.2	615
171	521	533	
440	508	617	
380		559	614
420	333	525	
321	583	605	
390	398	563	
410	691	749	
320	635	662	
340	527	630	

The hair selenium concentrations in the hair of the subjects who took part in the selenium study, before, directly after and up to eight weeks after taking the selenium supplements. Questionnaire completed by donors when a hair sample was cut is shown on page V.

Appendix 4

Details from the questionnaires completed by the hair donors in the occupational studies.

1. Data obtained from the questionnaires completed by the Sheffield and Stoke dental workers.

Sample Number	Age	Male/ female	Number of fillings	Days per week eat meat	Days per week eat fish	How many cigarettes?	Take vitamins yes/no
ST1	26-35	F	2	>2	2	0	N
ST2	26-35	F	4	>2	0	<5	N
ST3	35-50	M	0	>2	1	0	Y
ST4	35-50	F	2	>2	1	0	N
ST5	35-50	M	7	2	1	0	N
SHF1	26-35	M	11	>2	1	0	N
SHF2	35-50	M	0	0	0	5-10	N
SHF3	35-50	M	0	>2	1	0	N
SHF4	35-50	M	4	2	0	0	N
SHF5	35-50	M	1	>2	1	0	N
SHF6	26-35	F	3	>2	2	0	N
SHF7	35-50	M					
SHF8	35-50	M					

2. Data obtained from the questionnaires completed by the Steel Platers.

Sample Number	Age	Male/ female	Number of days since hair last washed	Number of fillings	Days per week eat meat	Days per week eat fish	How many cigarettes?	Take vitamins yes/no
1	26-35	m	0	1	>2	2	0	N
2	26-35	f	1	<5	>2	1	10-20	N
3	35-50	f	1	5-10	2	1	0	N
4	26-35	f	2	5-10	>2	1	0	N
5	35-50	m	0	5-10	>2	1	0	Y
6	35-50	m	0	0	>2	1	10-20	N
7	26-35	m	1	5-10	>2	2	5-10	N

3. Data obtained from the questionnaires completed by the Lead Foundry Workers.

Sample Number	Age	Time in position (y)	Number of days since hair last washed	Number of fillings	Days per week eat meat	Days per week eat fish	How many cigarettes?	Take vitamins yes/no
1	>50	6	1	0	>2	2	10-20	Y
2	>50	23	2	0	>2	1	10-20	N
3	>50	42	1	<5	>2	2	0	N
4	35-50	3	0	<5	>2	1	10-20	N
5	>50	45	1	5-10	>2	2	0	N
6	>50	45	2	<5	>2	1	10-20	N
7	35-50	22	2	<5	>2	0	>20	N
8	26-35	18	1	5-10	2	>2	0	N
9	>50	22	1	>10	>2	2	0	N
10	>50	22	2	<5	>2	1	0	N
11	>50	38	2	0	2	2	>20	N
12	>50	15	<5	<5	>2	1	0	N
13	>50	33	2	<5	2	2	0	N

4. Data obtained from the questionnaires completed by the Ink Analysts

Sample Number	Age	Male/ female	Number of days since hair last washed	Number of fillings	Days per week eat meat	Days per week eat fish	How many cigarettes?	Take vitamins yes/no
1	18-25	f	0	9	1	1	<10	N
2	35-50	f	0	4	>2	1	<10	Y
3	18-25	f	2	8	>2	1	0	N
4	26-35	m	1	>10	>2	1	10-20	N
5	26-35	m	0	3	2	<1	0	N
6	35-50	m	0	3	>2	2	0	N
7	18-25	m	1	>10	>2	1	<10	N
8	26-35	f	0	4	2	1	0	N
9	35-50	f	1	>10	>2	1	0	Y
10	26-35	m	0	>10	>2	1	10-20	N
11	35-50	m	3	>10	>2	2	0	Y
12	26-35	f	0	4	>2	>2	<10	Y
13	>50	m	3	>10	>2	1	10-20	Y

SURNAME (Mr/ Mrs/ Miss/Ms) ..... HEIGHT (Inches)..... ....WEIGHT (Lbs).....

FIRST NAMES ..... DATE OF BIRTH .....

Tick box if you use a hair shampoo or conditioner that contains selenium ☐

Tick box if you colour your hair ☐ If yes please specify.....

Cigarettes smoked per day ____, Approximately number of amalgam/silver dental fillings ____

Do you take a dietary supplement that contains selenium ____

Read the questions carefully. **CIRCLE** the number which you consider most appropriate over the time period of the last week or two.

0 = never	1 = occasionally	2 = often	3 = nearly always

Leave blank any questions you do not understand. Add comments if you wish. When you are finished add the number of points in each section as indicated. The score for **Yes** is the number inside the brackets ( ).

PART 1

SECTION A

1. Feel tired or sluggish	0	1	2	3
2. Feel cold	0	1	2	3
3. Hands and feet feel cold	0	1	2	3
4. Tight sensations in neck	0	1	2	3
5. Difficult and infrequent bowel movements	0	1	2	3
6. Dryness or discolouration of skin or hair	0	1	2	3
7. Puffy face, hands and feet	0	1	2	3
8. Swollen upper eyelids	0	1	2	3
9. Eyeballs move involuntarily	0	1	2	3
10. Muscles weak, cramp and/or tremble	0	1	2	3
11. Slow mental processes	0	1	2	3
12. Forgetfulness	0	1	2	3
13. Slow heart beat awareness	0	1	2	3
14. Abdominal swelling	0	1	2	3
15. Unsteady gait, movements	0	1	2	3
16. Gain weight easily	N			Y(5)
17. Swelling about the throat	N			Y(5)
18. Thinning hair on scalp, genitals or face	N			Y(3)

Total Points ____

SECTION B

1. Joint pains	0	1	2	3
2. Joint stiffness after rest	0	1	2	3
3. Creaking or grinding noises from joints	0	1	2	3
4. Aching muscles	0	1	2	3
5. Dry skin	0	1	2	3
6. Difficulty sleeping	0	1	2	3
7. Feel unrefreshed upon waking	0	1	2	3
8. Dry mouth	0	1	2	3
9. Dry and painful eyes	0	1	2	3
10. Blurred vision	0	1	2	3
11. Headaches	0	1	2	3

Total Points ____

PART 2

SECTION A

1. Feel daily work or activities interesting	0	1	2	3
2. Make good and full use of spare time	0	1	2	3
3. Rewarding hobbies or leisure interests	0	1	2	3
4. Manage your daily routine well	0	1	2	3
5. Consider your appearance important	0	1	2	3
6. Manage your finances well	0	1	2	3
7. Keep in touch with family members	0	1	2	3
8. Interact well with immediate family	0	1	2	3
9. Make efforts to socialise	0	1	2	3
10. Full and rewarding social life	0	1	2	3
11. Others seek your company	0	1	2	3
12. Find it easy to express opinions to others	0	1	2	3
13. Enjoy searching for information about things, situations or people to improve understanding	0	1	2	3
14. Involve yourself with community life	0	1	2	3

Total Points ____

SECTION B

1. Feel tense or wound-up	0	1	2	3
2. Feel frightened – as if something bad looming	0	1	2	3
3. Can suddenly feel panicky	0	1	2	3
4. Difficulty sitting down and relaxing	0	1	2	3
5. Feel restless and need to be on the move	0	1	2	3
6. Feel 'butterflies' in the stomach	0	1	2	3
7. Worried or have worrying thoughts	0	1	2	3

Total Points ____

SECTION C

1. Enjoy all the things you used to	0	1	2	3
2. Look forward with enjoyment to things	0	1	2	3
3. Feel cheerful	0	1	2	3
4. Enjoy a good book, radio or TV programme	0	1	2	3
5. See the funny side of things	0	1	2	3
6. Coping with things as well as ever	0	1	2	3
7. Take an interest in your appearance	0	1	2	3
8. Take an interest in other people	0	1	2	3
9. Appetite good	0	1	2	3
10. Sleep well	0	1	2	3

Total Points ____

SECTION D

1. Feel discouraged about the future	0	1	2	3
2. Feel a sense of failure in yourself	0	1	2	3
3. Feel disappointed in yourself	0	1	2	3
4. Feel inferior to others	0	1	2	3
5. Have feelings of guilt	0	1	2	3
6. Feel the need to cry	0	1	2	3
7. Increasing difficulty making decisions	0	1	2	3
8. Easily irritated	0	1	2	3
9. Worry about your health	0	1	2	3

Total Points ____

Signature.....

Date .....

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Please list any medical conditions/drugs overleaf

Appendix 5

Equation for a two tailed paired t test to compare the difference of two means, where the size of the groups vary.

The t value was derived from the following equation[1]

$$t = (\bar{x}_1 - \bar{x}_2)/\sqrt{(s^2_1/n_1 + s^2_2/n_2)}$$

where $\bar{x}_1$ is the mean of the first set of data, $\bar{x}_2$ is the mean of the second set of data, s_1 and s_2 are the standard deviation of the two sets of data and n_1 and n_2 are the number of values in the set of data (number of people in the group).

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1 Miller, J.C., Miller, J.N., Statistics for Analytical Chemistry, 3rd Edition, Ellis Horwood, Chichester, UK, 1993.